POSTER SESSIONS

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Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-01 Basic and Translational Session: Glial and Enteric Neurons in Health and Disease

001

In vivo neural regeneration and neurogenesis promoted by 5-HT4 receptor activation

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Objective: In gastrointestinal diseases, drug discovery targeting 5-HT receptors has been developed focusing on antiemetic and treatment for irritable bowel syndrome targeting 5-HT3 receptors and on gastrointestinal motility activation targeting 5-HT4 receptors. Recently, we found a new pharmacological activity mediated 5-HT receptors showing actions of neural regeneration and neurogenesis.
Methods: In this study, we are focusing on promoting effect of 5-HT4 receptor-activation on *in vivo* neural regeneration and neurogenesis in rodent rectal transection and anastomosis (RTA) and intestinal transection and anastomosis (ITA) models.
Results: In rat and guinea-pig RTA models, local and oral treatment with mosapride citrate (10–100 μ mol L^{-1}) promoted enteric neural regeneration and neurogenesis at the anastomotic site from neural stem cells for 2 weeks after the surgery. In mouse ITA model, oral treatment with mosapride citrate (100 μ mol L^{-1}) promoted enteric neural regeneration and neurogenesis at the anastomotic site for 1 week after the surgery. Especially, in guinea pig model, the recovery of rectal distension-induced internal anal sphincter (IAS) relaxation reflex response was found for 2 weeks after the surgery. 5-HT4 receptor antagonists, GR113808 (10 μ mol L^{-1}) and SB 207266 (10–50 μ mol L^{-1}) suppressed 5-HT4 receptor activation induced neural regeneration and neurogenesis, and the recovery of IAS relaxation reflex response. In addition, we found mosapride citrate enhanced mobilization of neural stem cells by subcutaneous transplantation of gel sponge experiments in rats. This effect contributes to *in vivo* neural regeneration and neurogenesis. Furthermore, we confirmed transplanted embryonic neural stem cells of central nervous system from tail vein mobilized at the anastomotic site in mouse ITA model with oral application of mosapride for 1 week.
Conclusion: Drug discovery based on the evidence of the present study will propose the possibility contributing to pharmacotherapy for defecation dysfunction after surgery and gastrointestinal motility dysfunction due to decreased enteric neurons in diabetic patients, pseudo-Hirschsprung's disease patients and elders.

002

Mechanosensitive myenteric neurons in the guinea pig gastric corpus

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Objective: We recently described mechanosensitive myenteric neurons in the guinea pig ileum. According to their response pattern they were termed rapidly adapting mechanosensitive enteric neurons (RAMEN). RAMEN are multifunctional and multitasking, a concept that we wanted to validate in the guinea pig stomach.
Methods: Activity of myenteric neurons in the gastric corpus was recorded with the voltage sensitive dye Di-8-ANEPPS. Mechanical stimulation was performed via intraganglionic volume injection that evoked a dynamic followed by a sustained deformation.
Results: Mechanical stimulation activated 25% of the neurons. They showed two different response patterns. The majority, namely 61%, fired during the dynamic deformation at 8.7 Hz and rapidly adapted with a sharp decline in spike discharge during the sustained deformation, a property typical for RAMEN. The remaining 39% fired spikes during the dynamic and throughout the sustained deformation at 7.2 and 10.3 Hz, respectively. These neurons behaved like slowly adapting mechanosensitive enteric neurons (SAMEN). Increasing the pressure in four steps gradually increased the percentage of responding neurons (0%, 10.7%, 19.4% and 15.2%) and the spike discharge of RAMEN (0, 1.7, 3.3 and 3.3 Hz) and SAMEN (0, 0.3, 5.3 and 2.2 Hz). Capsaicin evoked desensitization abolished responses in 20% of mechanosensitive neurons and decreased spike discharge from 3.6 to 2 Hz. Blockade of synaptic transmission with low Ca^{++}/high Mg^{++} did not change the number of responding neurons (20% before and after perfusion). Mechanosensitive gastric neurons showed different chemical codes (55% cholinergic, 45% nitrergic, 1.5% Calbindin-IR), an indication of their multifunctionality.
Conclusion: We identified for the first time mechanosensitive enteric neurons in the stomach. Most of them were RAMEN but a substantial proportion was also SAMEN, a population that we did not observe in the ileum. It is likely that RAMEN and SAMEN encode phasic and tonic muscle activity, respectively. Activation of visceral afferents prominently contributed to the response of enteric neurons to ganglion deformation. Results further supported the concept of multifunctional mechanosensitive enteric neurons and at the same time revealed region-specific adaptations in their response patterns.

003

Pancreatic cancer cells secrete CXCL12 which is chemoattractive upon peripheral neural glia: Reversal of a paradigm

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Objective: Neural invasion (NI) in pancreatic cancer (PCa) results from the biological affinity of PCa cells (PCCs) to intrapancreatic nerves. However, the molecular mediators of NI, and particularly the role of chemokines in this chemoattraction between nerves and PCCs, remain largely unknown. Therefore, we aimed at determinig the role of the chemokine CXCL12 and its rececptor CXCR4 in NI in PCa.
Methods: Expression of CXCL12 and CXCR4 was studied in normal human pancreas (NP), PCa tissues, PCC lines, intrapancreatic nerves and human Schwann cells (hSC) via immunohistochemistry, immunoblotting and enzyme-linked-immunosorbent-assay (ELISA). To identify the contribution of CXCL12/CXCR4 axis to NI, 3D-neural-migration and chemotaxis assays were performed under the influence of the CXCR4 chemical inhibitor AMD3100 and recombinant CXCL12.
Results: PCa tissues and PCCs demonstrated an upregulation of CXCR4 and especially of CXCL12 when compared to NP. Interestingly, intrapancreatic nerves and within these especially hSC showed prominent levels of CXCR4. When co-cultivated, hSC migrated in a strictly targeted manner towards PCCs long before these even started with their migratory activity. Pre-treatment of hSC with AMD3100 significantly reduced the cancer-targeted migration of hSC. Correspondingly, recombinant CXCL12 exerted a potent chemotactic effect upon hSC.
Conclusion: Chemokines like CXCL12 which are secreted by PCCs strongly attract glia cells that harbor the corresponding chemokine receptors like CXCR4. Hence, in sharp contrast with the traditional assumption, NI results from the migration of peripheral glia (hSC) towards PCCs. This ''chemokine-mediated migration of nerves towards cancer'' urges for a reversal in our common understanding of NI in PCa.

004

Alpha-synuclein is constitutively secreted from enteric neurons and impacts on intestinal epithelial barrier

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Objective: A growing body of evidence suggests that alpha-synuclein secretion is involved in Parkinson's

disease (PD) pathogenesis. PD is a multicentric disorder that affects the enteric nervous system (ENS), whose involvement may herald the degenerative process in the central nervous system. Whether alpha-synuclein is secreted by enteric neurons has not been studied yet. We undertook the present research to determine whether enteric neurons secrete alpha-synuclein and to study the effects of extracellular on gastrointestinal functions.
Methods: Alpha-synuclein secretion was assessed by western blot analysis and ELISA assay in rat primary cultures of enteric neurons. Recombinant alpha-synuclein was used to study whether extracellular alpha-synuclein impacts on intestinal epithelial barrier (IEB) functions using both an *In vitro* (differentiated caco-2 cells monolayer) and *in vivo* approach (Ussing's chamber). We further studied the signalling pathways regulated by extracellular alpha-synuclein in caco-2 cells.
Results: Alpha-synuclein is constitutively secreted from enteric neurons in an unconventional exocytosis pathway. Extracellular alpha-synuclein decreases IEB permeability without affecting tight junctions proteins expression and regulates protein tyrosine phosphorylation in caco-2 cells.
Conclusion: We showed that alpha-synuclein is secreted by enteric neurons and that extracellular α-synuclein modulates IEB permeability. Our results shed new light on the role of alpha-synuclein in the ENS.

005
Expression of P2X3 subunit during ontogenic development of murine and guinea pig myenteric plexus
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Objective: To further characterize the expression of P2X3 subunit in the enteric nervous system during development.
Methods: We used RT-PCR, single cell PCR and patch clamp in the whole cell configuration.
Results: We found a P2X3 downregulation during the development of the small intestine of both mice and guinea pig at the mRNA level. We report that the difference in P2X3 expression in guinea pig is caused in part by reduction in the number of P2X3 positive myenteric neurons, as showed by the single cell RT-PCR, ranging from 53% in the embryo (E12–E16) to a lower 5% in the adulthood (4–6 weeks). The electrophysiology recordings of guinea pig myenteric neurons show that 18% of the recorded cells from newborn responded with an inward current to the administration of 100 μ mol L^{-1} α,β-methylene ATP whereas none of the myenteric neurons from the adult responded to administration of the same agonist concentration. All recorded neurons responded to the application of 100 μ mol L^{-1} ATP.
Conclusion: These data suggest an active role of P2X3 during myenteric plexus development in cell signaling and neural differentiation.

006
M3- and NK1-receptor facilitation of [3H]-acetylcholine release from myenteric motoneurons depends on extracellular adenosine accumulation acting on prejunctional A2a receptors
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Objective: Endogenous adenosine (ADO) contributes significantly to maintain cholinergic neurotransmission at the myenteric synapse through the activation of prejunctional facilitatory A2A receptors (Duarte-Araújo *et al.*, 2004). Muscarinic M3 receptors facilitate ACh release via a mechanism that depends on ADO outflow leading to activation of excitatory A2A receptors (Vieira *et al.*, 2009). As tachykinins (e.g. substance P) have also been implicated in the facilitation of cholinergic neurotransmission, we decided to investigate the interplay between A2A, M3 and NK1 receptors on stimulation-evoked (5 Hz) [3H]-ACh and ADO release in the longitudinal muscle-myenteric plexus (LM-MP) of the rat ileum.
Methods: Confocal microscopy studies indicate that intramuscular interstitial cells of Cajal possess M3 and NK1 receptors, while A2A receptors localize predominantly on (e.g. VAChT-positive) myenteric nerve terminals.
Results: Activation of A2A, M3 and NK1 receptors, respectively with CGS21680C (3 n mol L^{-1}), oxotremorine (Oxo, 300 μ mol L^{-1}) and s,m-Substance P (s,m-SP, 300 n mol L^{-1}), facilitated [3H]-ACh release and ADO outflow from stimulated LM-MP preparations. The facilitatory effect of CGS21680C (3 n mol L^{-1}, 53 ± 10%, n = 4) was abolished in the presence of (i) adenosine deaminase (ADA, 0.5 U ml^{-1}, n = 8), (ii) the selective A2A receptor antagonist, ZM241385 (50 n mol L^{-1}, n = 4), and (iii) the selective M3 receptor antagonist, J104129 (6 n mol L^{-1}, n = 5). Blockade of NK1 receptors with L732138 (20 n mol L^{-1}) only partially attenuated CGS21680C-induced facilitation (25 ± 5%, n = 4). Oxo (300 μ mol L^{-1})-induced facilitation of [3H]-ACh release (34 ± 4%, n = 3) was prevented by ADA (0.5 U ml^{-1}, -5 ± 8%, n = 6) and by blocking M3 and A2A receptors with J104129 (6 n mol L^{-1}, -1 ± 7%, n = 4) and ZM241385 (50 n mol L^{-1}, -5 ± 8%, n = 6), respectively, but it was kept unaltered in the presence of the NK1 receptor antagonist, L732138 (20 n mol L^{-1}, 28 ± 9%, n = 6). The selective NK1 agonist, s,m-SP (300 n mol L^{-1}), increased [3H]-ACh release by 16 ± 4% (n = 4). This effect was significantly (P < 0.05) enhanced in the presence of (i) CGS21680C (3 n mol L^{-1}, 48 ± 8%, n = 4), (ii) EHNA (50 μ mol L^{-1}, 27 ± 6%, n = 5), which increases ADO accumulation by inhibiting ADA activity, and (iii) forskolin (3 μ mol L^{-1}, 31 ± 6%, n = 4), an activator of adenylate cyclase activity.
Conclusion: Data suggest that facilitation of evoked [3H]-ACh release by M3 and NK1 receptor agonists depends on ADO outflow (possibly from ICC-IM) leading to retrograde amplification of transmitter release via pre-junctional A2A receptors. Work supported by FCT (FEDER funding, PTDC/CVT/74462/2006 and UMIB-215/94.

007
Enteric glial cells from patients with Crohn's Disease misreact to inflammation and induce intestinal epithelial cell permeability
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Objective: Enteric glial cells (EGC), the major constituents of the enteric nervous system, have been identified as key regulators of intestinal epithelial barrier (IEB) homeostasis. Under physiological condition, EGC-derived soluble factors enhance IEB healing, reduce its permeability, and increase its resistance to pathogen aggression. These barrier processes are all dysfunctional in the gut of Crohn's Disease (CD) patients. In the meanwhile, EGC lesions have been observed in CD, but whether EGC participate in IEB lesions or EGC dysfunction is only consequent to the pathology is still unclear.
Methods: To address this question, we have isolated and cultivated EGC from CD patients and have compared their phenotype and functional impact on IEB to control EGC isolated from patients undergoing surgery for cancer.
Results: No significant changes in their proliferation or hypertrophy rates, as well as in the mRNA expression of GFAP, Sox10 or S100B glial markers were observed. In addition, conditioned medium of EGC from CD or control patients diminished the permeability of intestinal epithelial cell monolayers, demonstrating their potential to reinforce IEB. We next determined the functional impact of EGC under a chronic inflammatory stress induced by a four days treatment with Cytomix (TNFα 1 ng ml^{-1} plus Il1β 1 ng ml^{-1}). Following this inflammatory challenge, control EGC still reduced significantly IEB permeability. In contrast, CD EGC challenged by the cytomix failed to reduce permeability and even induced an increase in IEB permeability. We next aimed to validate *in vivo* this observation by grafting into the wall of rat colon, rat EGC control or pre-stimulated four days with Cytomix. Four days after grafting, paracellular permeability of the EGC-enriched area (identified by fluorescent microscopy) was measured in Ussing chambers. Grafting of control EGC did not modify the colon permeability (as compared to mock graft), whereas Cytomix-pre-stimulated EGC decreased colon permeability.
Conclusion: All together, these results show that (i) rat EGC reaction to inflammation is to reinforce the IEB *In vitro* and *in vivo* (ii) human EGC reaction to inflammation also reinforce the barrier *In vitro*, and (iii) EGC from CD patients not only have lost this property, but could also be deleterious for the barrier.

008

Glial cell line derived neurotrophic factor prevents high fat diet induced obesity

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Objective: GDNF is a neurotrophic factor that plays an important role in enteric neuronal survival and differentiation. We have previously shown that GDNF improves β-cell mass. The role of neurotrophic factors in the regulation of obesity is not well known. In this study we investigated the ability of GDNF to protect against high fat diet-induced obesity.
Methods: Six weeks old WT and GDNF transgenic (GDNF-tg) mice were fed a high fat diet (60% cal from fat) or a regular diet (18% cal from fat) for 11 weeks and weight gain, fat pad weight, insulin sensitivity and glucose tolerance tests assessed. Livers from the mice were assessed for fat deposition. The expression in white adipose tissue (WAT) and liver of genes modulating adipogenesis, lipogenesis and fatty acid oxidation was assessed by real-time PCR. *In vitro* the effects of GDNF on adipocyte differentiation were assessed using the 3T3L1 cell line.
Results: GDNF-tg mice resisted high-fat diet-induced weight gain compared to WT mice ($P < 0.001$) and had reduced visceral fat. GDNF-tg mice also resisted high fat diet-induced glucose intolerance, insulin resistance, hyperleptinemia, hyperlipidemia and hypertriglyceridemia ($P < 0.01$). In addition, GDNF-tg mice had lower serum ALT levels, liver weights, hepatic steatosis and liver triglyceride level ($P < 0.001$). Gene expression analyses revealed expression of receptors for GDNF in adipose tissue and liver. We found lower expression of genes influencing adipogenesis such as PPARγ and leptin in WAT and liver of GDNF-tg mice ($P < 0.001$). Expression of genes influencing fatty acid oxidation and metabolism (PRDM1 and UCP1) was increased in GDNF-tg mice ($P < 0.01$). *In vitro* studies revealed GDNF mediated suppression of adipogenesis through a Ret-dependent pathway and involved the MAPK signaling pathway. This was associated with a significant reduction in FABP4, FASN, Srebf1 and PPARγ mRNA levels ($P < 0.001$).
Conclusion: We demonstrate a novel role for GDNF in the regulation of high fat diet-induced obesity through modulation of fatty acid metabolism, adipogenesis, and insulin sensitivity. Together, our studies show that GDNF is protective against the development of metabolic syndrome in mice and that GDNF and its receptor agonists may be potential targets for the treatment or prevention of obesity.

009

Supernatant of human ulcerative colitis biopsies have pro-angiogenic properties: Role of enteric glial cells

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Objective: Inflammatory bowel diseases (Crohn's Disease and ulcerative colitis) remain a major public health problem, especially because of the lack of efficient therapy and their association with an increased risk of colorectal cancer. To date, the cellular components of the inflamed gut presenting tumorigenic, i.e. pro-angiogenic properties, remain to be identified. As emerging evidences suggest that enteric glial cells (EGC) are key regulators of intestinal functions, modulating enteric neuronal activity but also regulating intestinal epithelial barrier, we have analysed if EGC could modify endothelial cell functions and angiogenesis.
Methods: To analyse if EGC could modify endothelial cell functions and angiogenesis, we have measured proliferation, adhesion of HMEC endothelial cells co-cultured with EGC treated with LPS (0.1 μg ml^{-1}) or TNFα and IL1 (Cytomix; 1 ng ml^{-1} and 2 ng ml^{-1} respectively), or culture in the presence of supernatants of human biopsies (SHB) from control patients or patients with ulcerative colitis (UC).
Results: Proliferation of HMEC endothelial cells co-cultured with primary cultured human EGC for 24 h was increased (2.35 ± 0.42 or 1.82 ± 0.23 fold respectively) when EGC were treated with LPS or Cytomix as compared to control (i.e. EGC not treated). In addition, adhesion of HMEC was greatly increased when they were placed in conditioned medium (CM) of EGC treated with LPS or Cytomix (4.2 ± 0.13 or 6.0 ± 1.2 fold, respectively). Boyden chamber assays have shown that EGC co-culture induced HMEC migration with no additional increase in presence of LPS or Cytomix. As expected, the wound closure of HMEC is favoured in CM of EGC treated with LPS or Cytomix. Angiogenic properties measurement in matrigel have shown that EGC not only have pro-angiogenic properties, but also help maintaining capillary like structures in presence of LPS or Cytomix. In the same manner, CM of EGC treated with LPS or Cytomix decreased endothelial permeability. SHB from patients with UC also induced an increase in HUVEC endothelial cells proliferation (8.3 ± 1.22 fold as compared to control SHB, $n = 3$, $P \leq 0.01$).
Conclusion: These results demonstrate that under inflammatory stress, EGC produce soluble factor that have pro-angiogenic properties and could modify vascular integrity.

010

Enteric neuroplasticity of seawater-adapted European eel ''(Anguilla anguilla) experimentally induced to sexual maturation

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Objective: European eel lives most of its life in fresh water until their spawning migration to the Sargasso see. During seawater adaptation the eel modifies its physiology: the digestive system has to force its function to adapt the body homeostasis to the new environment, drinking salt water. During this period, eels interrupt food intake until spawning: the prolonged starving is associated with morphological changes of their body up to death because of the extreme effort. The eel represents a unique model to establish the adaptive changes of its digestive innervation, i.e. the enteric nervous system (ENS), to modified salinity first and starving thereafter.
Methods: We investigated the modification of the eel ENS during prolonged starvation in seawater after hormonal treatment with standardized carp pituitary extract (CPE). Intestinal specimens from control freshwater and starved seawater-adapted female eels were obtained and assessed. Animals were classified as controls, T0, and T4. Control eels ($n = 6$), ageing about 5–7 years, were raised in fresh-water and were normally fed. T0 eels ($n = 8$) were captured in north-Italian salty-water estuaries ready for reproductive migration. T4 eels ($n = 6$) were adapted in captivity to sea-water; animals were treated for 22 weeks with CPE to induce sexual maturity. The density of HuC/D immunoreactive (IR) ENS neurons was assessed by counting ten randomly chosen fields (40x) of control, T0 and T4 eel wholemount preparations.
Results: Control eels showed myenteric plexus (MP) neurons clustered in small ganglia and only a few scattered neurons in the submucosal plexus (SMP). In T0 and mainly in T4 eels, the SMP was full of HuC/D-immunoreactive (IR) neurons which were significantly higher than in control eels. Also, in the T0 (264 ± 120) and T4 (485 ± 78) eels the number of HuC/D-IR MP neurons was higher than controls (162 ± 21).
Conclusion: The marked increase of Hu-IR SMP and MP neurons of starved, seawater-adapted, and hormone-treated eels indicate considerable neuroplastic properties of the ENS. Likely, high salty water and hormone treatment represent stimuli eliciting enteric neuronal changes necessary to balance body homeostasis in this species.

011

Pain and neural remodelling in pancreatic neuropathy are characterized by increased unmyelinated nerve fiber content and selective glial activation

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Objective: Pancreatic neuropathy and pain in pancreatic adenoarcinoma (PCa) and chronic pancreatitis (CP) are associated with decreased sympathetic innervation of the pancreas and ''neural remodelling''. To complete the characterization of pancreatic neuropathy, the degree of myelination and glia content were investigated in intrapancreatic nerves in PCa and CP.
Methods: Intrapancreatic nerves of patients with PCa ($n = 20$), CP ($n = 20$) and normal human pancreas ($n = 10$) were immunolabeled with the neural myelination marker neurofilament-H (NFH), and the glial activation markers Glial-Fibrillary-Acidic-Protein (GFAP) und p75 receptor (p75NTR). The neural immunoreactivity of each marker was correlated to the neuropathic pain sensation, the degree of neural invasion (NI) in PCa and to the degree of pancreatic neuritis in PCa and CP.
Results: PCa, and not CP, is associated with decreased neural immunoreactivity of GFAP, p75 and NFH. PCa patients with pain possess even less GFAP and NFH in their intrapancreatic nerves when compared to those without pain. Intrapancreatic nerves with increasing degree of pancreatic neuritis in PCa and NI harbour higher amounts of NFH and p75. Contrastingly, pancreatic neuritis in CP is mostly encountered around nerves with small NFH content.
Conclusion: Pain in PCa is associated with increased appearance of unmyelinated intrapancreatic nerve fibers and a relative decrease of glia cells. However, pancreatic neuritis and NI in PCa are directed towards

myelinated nerve fibers which are accompanied by activated glia cells. Therefore, pancreatic neuropathy in PCa induces a selective – non-global – glial activation and the dominance of unmyelinated and thus pain-transmitting intrapancreatic nerve fibers.

012

Neurogenesis: Granulocyte colony-stimulating factor facilitates neural stem cell differentiation

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Objective: The continuous generation of neuronal cells throughout life is an indispensable requirement for functions of gastrointestinal tract. The enteric nervous system (ENS) responds quickly to inflammation, which could influence the ENS stem cell niche. During inflammation, granulocyte colony-stimulating factor (G-CSF) is released and the production of granulocytes induced. G-CSF protects neurons from cell death. The capacity of G-CSF to influence the behavior of cultured neural stem cells from mouse ENS compared to subventricular zone cells (SVZ) was investigated.
Methods: Neural progenitors isolated from small intestine (SI) and SVZ were cultivated with increasing G-CSF concentrations (1 pg ml^{-1}–10 ng ml^{-1}). Number and diameter of neurospheres were assessed. Immunofluorescence was performed with neuronal, glial cell and G-CSF receptor (G-CSFR) markers. Neurite length and neurite density were quantified after differentiation. Immunogold labeling of G-CSFR was performed and analyzed using scanning electron microscopy. The expression level of G-CSF, G-CSFR, the anti-apoptotic protein (Bcl-xl) and neuronal marker ßIII-tubulin were investigated after G-CSF treatment using qRT-PCR.
Results: After G-CSF stimulation of neural stem cells, we measured a dose dependent increase in cell proliferation. Proliferation rate of neural stem cells of SI was increased, that of SVZ cells decreased with rising G-CSF concentrations. Differentiation of neural stem cells was enhanced by G-CSF. Density of outgrowing fibers was increased in SVZ cultures by 40%. Neurite length expanded for 70% in SI cultures. Immunogold labeling of G-CSFR indicated localization on neurons and a decreased number after stimulation. G-CSF gene expression is not effected, whereas G-CSFR expression is down-regulated. The expression of Bcl-xl and ßIII-tubulin was up-regulated.
Conclusion: G-CSF is a potent stimulator of enteric and SVZ neuron differentiation with the capability to enhance neurogenesis by increasing neural survival and neural outgrowth. The gene expression pattern of Bcl-xl and ßIII-tubulin reflects the enhancement of differentiation after G-CSF treatment and the protective function of G-CSF against apoptosis. The G-CSFR is located only on neurons which could explain the increase of the neurite length in SI cultures and the increased density of outgrowing fibers in SVZ cultures after G-CSF treatment.

013

Apoptotic action of salsolinol on myenteric plexus neurons in the rat intestine

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Objective: Salsolinol is an endogenous agent formed from aromatic amines precursors that may play a role in the etiology of Parkinson's disease. The aim of the current study was to evaluate the effects of salsolinol on myenteric plexus neurons (MPN) as well as neurons expressing nitric oxide synthase (NOS) and acetylcholine transferase (ChAT) in the rat jejunum and to assess the mechanism of neurotoxic action of salsolinol.
Methods: Male Wistar rats were subjected to continuous intraperitoneal dosing of salsolinol (200 mg kg^{-1} in total) with osmotic mini-pumps for two (S1 group) or four weeks (S2 group). An equivalent group of rats served as the control (C). Intestinal motility was measured by one-hour stool collection. At the end of the experiment animals were euthanized and from fragments of proximal jejunum the longitudinal muscle–myenteric plexus (LMMP) strips were prepared, stained with anti-PGP 9.5, anti-NOS, anti-ChAT and anti-Bax polyclonal antibodies using immunofluorescence method, and assessed by image analysis.
Results: The mean number of MPN was significantly decreased in both salsolinol-treated groups: 103.04 $mm^{-1}2$ ± 37.5 (S1), 103.95 $mm^{-1}2$ ± 26.1 (S2) *vs* 131.40 $mm^{-1}2$ ± 42.3 for the control group. The mean neuron size was significantly decreased in both salsolinol-treated groups: 306.32 μm^2 ± 104.3 (S1) and 231.34 μm^2 ± 81.8 (S2) compared to the control group - 330.55 μm^2 ± 98.9. The area of myenteric ganglia was decreased in both salsolinol-treated groups. The mean number of cells expressing NOS was significantly lower in both salsolinol-treated groups: 32.96 $mm^{-1}2$ ± 12.06 (S1), 29.41 $mm^{-1}2$ ± 11.63 (S2) *vs* 38.99 $mm^{-1}2$ ± 12.75 for control. The number of cells expressing ChAT remained unchanged. Neurons over-expressing Bax protein were present in the myenteric plexus. The mean faecal wet and dry weight was reduced in both salsolinol-treated rats.
Conclusion: Salsolinol evokes neuronal cell death in the jejunum by initiation of apoptotic processes. Impairment of myenteric plexus neurons, especially the nitrergic inhibitory motor neurons, might be responsible for sustained smooth muscle contraction and thus may lead to abnormal intestinal transit.

014

Effects of anti-cancer chemotherapy on gastrointestinal motility and enteric neurons in the mouse colon

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Objective: Diarrhoea, nausea and vomiting leading to malnutrition, dehydration, and rapid weight loss are gastrointestinal side-effects of anti-cancer chemotherapy. The traditional view is that these side-effects of anti-cancer drugs are due to mucosal damage. However, the physiological functions of the gastrointestinal tract such as motility, secretion and nutrient absorption are controlled by the enteric nervous system (ENS) innervating the intestine. This study investigates morphological and functional changes that occur in the ENS and gastrointestinal functions during anti-cancer chemotherapy.
Methods: Oxaliplatin (3 mg kg^{-1} day^{-1}) was administered to Balb/c mice via i.p. injections three times a week for 3 weeks. Segments were collected from oxaliplatin and sham-injected mice at Day 3, 7, 14 and 21 following injections. Peristaltic contractions were induced by increasing intraluminal pressure with physiological solution. Spatiotemporal maps were used to obtain quantitative measurements of the threshold for activation of propagating contractions, their frequencies, duration, speed, distance propagated and amplitudes. Wholemount preparations and cross sections of the distal colon segments were examined histologically and immunohistochemically. Neuronal cells were labelled with PGP9.5, β-Tubulin and NOS, while nuclei were labelled with DAPI. Nerve fibre bundles were labeled with anti-β-Tubulin antibody. The number of nerve bundles was visualized and quantified in mid-villi sections.
Results: Repeated *in vivo* injections of oxaliplatin caused significant neuronal loss and an increased proportion of nitric oxide synthase (NOS)-immunoreactive neurons. Morphological changes in NOS-immunoreactive neurons occurred from Day 3 and continued at Days 14 and 21 following oxaliplatin injections (n = 6 at each time point). Significant axonal damage and axonal loss occurred in neurons projecting to the mucosa at Days 14 and 21 after administration of oxaliplatin. *in vivo* oxaliplatin treatment caused inhibition of motility starting from the day 3 of treatment. Acute *In vitro* application of oxaliplatin (100 n mol L^{-1}) induced changes in electrophysiological properties of neurons in the myenteric plexus.
Conclusion: This study is the first to examine the effects of oxaliplatin on enteric neurons in the mouse colon. Oxaliplatin inhibits neurally-dependent activity in the colon. Repeated exposure to oxaliplatin causes substantial effects on both structure and functioning of the ENS which might underlie functional changes in the gut.

015

Oxytocin hyperpolarizes cultured duodenum myenteric intrinsic primary afferent neurons by opening BKCa channels through IP3 pathway

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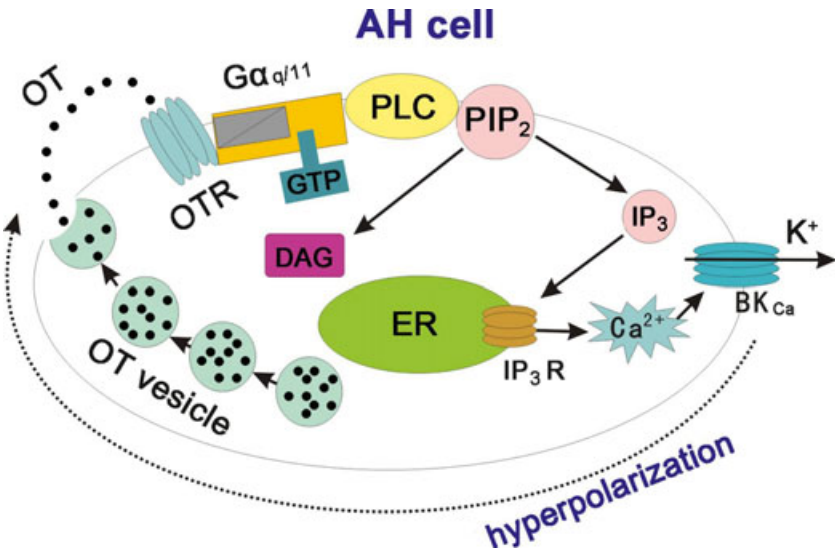
Objective: Oxytocin (OT) is constitutively reduces duodenum contractility. Intrinsic primary afferent neurons (IPANs), whose physiological classification is as AH cells, are the 1st neurons of the peristaltic reflex pathway. We set out to investigate if this inhibitory effect is mediated by IPANs and to identify the ion channel(s) and intracellular signal transduction pathway that are involved in this effect.
Methods: Myenteric neurons were isolated from the longitudinal muscle myenteric plexus (LMMP) preparation of rat duodenum and cultured for 16–24 h before electrophysiological recording in whole cell mode and AH cells identified by their electrophysiological characteristics. The cytoplasmic $Ca2^+$ concentration ($[Ca2^+]i$) of isolated neurons was measured using cal-

cium imaging. The concentration of IP3 in the LMMP and the OT secreted from the LMMP were measured using ELISA. The oxytocin receptor (OTR) and large-conductance calcium-activated potassium (BKCa) channels, as well as the expression of OT and the IPAN marker calbindin 28 K, on the myenteric plexus neurons were localized using double-immunostaining techniques.
Results: Administration of OT (10^{-7}–10^{-5} mol L^{-1}) hyperpolarized the resting membrane potential and increased the total outward current. The OTR antagonist atosiban or the BKCa channel blocker iberiotoxin (IbTX) blocked the effects of OT suggesting that the increased outward current resulted from BKCa channel opening. OTR and the BKCa subunit were co-expressed on a subset of myenteric neurons at the LMMP. NS1619 (a BKCa channel activator) increased the outward current similar to the effect of OT. OT administration also increased [Ca2+]i and the OT-evoked outward current was significantly attenuated by thapsigargin or CdCl2. The effect of OT on the BKCa current was also blocked by pretreatment with the IP3 receptor antagonist 2-APB or the PLC inhibitor U73122. OT also increased the IP3 concentration within the LMMP. Both of the spontaneous and KCl-induced secretion of OT was enhanced by atosiban. Most of OT-immunoreactive cells are also immunoreactive for calbindin 28 K.
Conclusion: OT hyperpolarized myenteric IPANs by activating BKCa channels via the OTR-PLC-IP3-Ca^{2+} signal pathway. OT might modulate IPANs mediated ENS reflex by an autocrine and negative feedback manner.



016
The appendix, a suitable and autologeous neural stem cell source
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Objective: Evidence of neurogenesis in the adult enteric nervous system (ENS) brought new perspective for cell therapy and neural regeneration. Although these cells show a decreased plasticity in the aging gut, they are a potential source for neural stem cells. The appendix might be the appropriate location with a sufficient amount of enteric nervous tissue where these cells could be easily harvested, under the condition that a suitable isolation procedure is provided.
Methods: Tissue samples from adult appendices were collected and separated for neurosphere generation, further differentiation and transplantation into rat brain slices. On average after 7 days freefloating neurospheres were seen in the culture and could be cultivated up to 40 days. R-PCR, immunohistochemical stainings and transplatation experiments into brain slices from adult rat were performed.
Results: mRNA expression of these spheres demonstrated an increase of nestin, suggesting stemcellness. After dissociation and further differentiation an intricate network with glial cells, neurons and interconnecting fibers developed within two to four days. The immunohistochemistry with a panel of neuronal, gilal and stem cell markers (PGP9.5, ß-Tubulin III, GFAP, S-100, Nestin) revealed different cell types, meaning that the neurospheres generated from human postnatal myenteric plexus keep their plasticity to differentiate in neuronal and glial cells. After transplantation into organotypically cultures with rat brain slices, the cells migrate into the cortex, differentiate and network formation was commenced.
Conclusion: Using the appendix as a potential target opens up a new perspective, which might lead to a minimal invasive harvest of neural stem cells without endangering the patient. This neural stem cell pool, delivering cholinergic and catecholaminergic neurons, could be used for the recruitments of cells for the treatment of neurodegenerative diseases.

017
Alterations of colonic cholinergic and tachykininergic motility in a rat model of Parkinson's Disease
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Objective: Parkinson's disease (PD) is characterized by degeneration of nigrostriatal dopaminergic neurons. PD patients can develop gastrointestinal motor dysfunctions and alterations of enteric nervous system. This study examines the patterns of colonic neuromuscular excitatory cholinergic and tachykininergic pathways in rats bearing a neurotoxic lesion of the nigrostriatal pathway reminiscent of PD.
Methods: Nigrostriatal degeneration was induced by stereotaxic injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle of rats. Animals were sacrificed 28 or 56 days after surgery. Colonic longitudinal muscle preparations were set up in organ baths, and connected to isometric transducers to record contractions (g/g tissue) elicited by electrical stimulation (ES, 10 Hz), in the presence of guanethidine and Nω-nitro-L-arginine methylester. L-732,138 (NK1 receptor antagonist) or atropine were used to record contractions driven by acetylcholine or tachykinins, respectively. Contractions elicited by exogenous substance P (SP, 10 μ mol L^{-1}) or carbachol (10 μ mol L^{-1}) were also recorded.
Results: In control preparations incubated with L-732,138, ES evoked cholinergic contractions (49.6 ± 5.3), which were reduced in 6-OHDA lesioned rats at 28 and 56 days (31.3 ± 4.6 and 37.7 ± 2.3, respectively). Carbachol-evoked contractions were enhanced in 6-OHDA lesioned rats both at 28 and 56 days (92.6 ± 3.3 and 91.7 ± 2.4, respectively), as compared with controls (51.7 ± 4.4). In the presence of atropine, electrically evoked tachykininergic contractions were enhanced in 6-OHDA lesioned rats at 56 days, as compared with controls (49.1 ± 4.1 *vs* 28.7 ± 3.8). Contractions elicited by exogenous SP were enhanced in tissues from 6-OHDA lesioned rats both at 28 (45.0 ± 5.3) and 56 days (77.2 ± 4.2), in comparison with controls (27.0 ± 1.9).
Conclusion: Experimental PD, elicited by nigrostriatal dopaminergic degeneration, is associated with changes in neurotransmitter pathways driving the excitatory motor functions of colon: an impairment of cholinergic transmission occurs in concomitance with an enhancement of tachykininergic control. Such a shift takes place along with an up-regulation of contractile responses mediated by muscular muscarinic receptors, which might be compensatory in nature.

018
A D-galactose mouse model of dysmotility: Spatiotemporal motility mapping and intrinsic primary afferent neuron function
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Objective: D-galactose (DG) administration produces oxidative stress and mitochondrial dysfunction. Moreover, such stress has been associated with dysmotility and constipation (1). To determine if chronic DG administration might produce a mouse model of enteric neuropathy-related dysmotility we tested if such administration could alter jejunal migrating motor complexes (MCs) and intrinsic primary afferent neuron (IPAN) physiology.
Methods: Adult mice were IP injected with vehicle (V) or DG daily for 8 weeks. Then mouse distal jejunum segments were placed in recording dishes perfused with carbogenated Krebs and spatiotemporal diameter maps or IPAN whole-cell recordings made (2). MC frequency or velocity were measured at 3 hPa luminal (Krebs) fill-

ing pressure. Resting membrane potential (RMPs), action potential (AP) threshold, and post-action potential slow afterhyperpolarisation (sAHP) duration were measured. *T*-tests were performed on the results.
Results: Control versus DG treatment MC frequencies (mean ± SEM (*n*)) were 41 ± 6.0 (9) *vs* 14 ± 2.0 (8) mHz ($P = 0.001$), and MC velocities were 2.5 ± 0.5 (7) *vs* 4.0 ± 1.5 (7) mm s^{-1} ($P = 0.4$). RMPs V *vs* DG were -56 ± 2(8) *vs* -61 ± 1(10) mV ($P = 0.07$). AP thresholds were 58 ± 15 *vs* 115 ± 17 pA ($P = 0.03$), and sAHP durations following three APs were 19 ± 4 *vs* 30 ± 3 s ($P = 0.03$).
Conclusion: Jejunum MC frequency was decreased by DG treatment and this was correlated with increases in IPAN AP firing threshold and the post-spike refractory period (sAHP). These results suggest that DG treatment may produce a mouse model for neurogenic dysmotility which is possibly relevant to pathologies involving increased oxidative stress as might occur in senescence.
Reference
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019
Enteroglial derived S100B protein modulates differentiation, proliferation and nitrosative stress of human intestinal epithelial cells in a rage-dependent manner
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Objective: In the human gut, S100B protein is specifically expressed by enteroglial cells (EGCs) and, in response to various stimuli, it is released in the extracellular space where it is suggested to participate to intestinal homeostasis. Most of its effects are mediated by the interaction with the receptor for advanced glycation endproducts (RAGE). We aimed to study the effects of different concentrations of S100B on viability, proliferation, differentiation and nitric oxide (NO) release of the epithelial intestinal cells (IECs).
Methods: Caco-2 cells were exposed to nanomolar or micromolar concentrations of S100B (0.005 and 5 μ mol L^{-1}, respectively), for 24 h, in presence or absence of a specific anti-RAGE neutralizing antibody (1/1000 v/v) added 2 h before S100B stimulation. Cells viability, proliferation, differentiation and NO release were respectively studied by MTT vitality test, Bromodeoxyuridine incorporation assay, lactase/sucrase enzymes activity tests and with nitrite assay test. Cells with medium alone were used as control. Data are expressed as mean ± SD.
Results: Both S100B 0.005 μ mol L^{-1} and S100B 5 μ mol L^{-1}, compared to control, did not affect cell viability (0.8 ± 0.3 and 0.7 ± 0.3 *vs* 0.7 ± 0.1 λ540–630; P = ns). Both concentrations of S100B significantly decreased proliferation respect to control (0.17 ± 0.01 and 0.18 ± 0.01 *vs* 0.23 ± 0.01 λ450–540; $P < 0.05$). This antiproliferative effect was not observed when cells were pre-treated with anti-RAGE. Compared to control, S100B 0.005 μ mol L^{-1}, but not S100B 5 μ mol L^{-1}, significantly increased lactase activity (+7.5 ± 0.9, $P < 0.05$ and +1.9 ± 0.5 fold increase versus control; P = ns) and sucrase activity (+2.6 ± 0.3, $P < 0.05$ and +0.7 ± 0.4, P = ns fold increase versus control). The increase in lactase/sucrase activity was not observed when cells were pre-treated with anti-RAGE. Both S100B 0.005 μ mol L^{-1} and S100B 5 μ mol L^{-1} increased NO release (+2.5 ± 0.3 and +3.4 ± 0.5 fold increase versus control; $P < 0.05$) from Caco-2 but not from cells pre-treated with anti-RAGE.
Conclusion: Nanomolar, but not micromolar, concentration of S100B seems to act reducing IECs proliferation and prompting cells towards differentiation, without cytotixic effects. Conversely, both concentrations of S100B increased nitrosative stress in IECs. Very intriguingly, these effects were abolished in the presence of a specific anti-RAGE antibody. Our findings further highlight the ability of EGCs to regulate intestinal homeostasis.

020
Toxoplasma gondii increases the expression of S-100 and GFAP in rat colonic myenteric plexus
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Objective: The aim of this study was to evaluate whether Toxoplasma gondii infection can alter the expression of S-100 and glial fibrillary acidic protein (GFAP) in glial cells of the rat myenteric plexus.
Methods: Ten, 60-days-old, male Wistar rats (Rattus norvegicus) were assigned in a control group, CG, ($n = 5$) and in an infected group, IG, ($n = 5$). IG animals received 500 sporulated oocysts of the genotype II (ME-49) Toxoplasma gondii strain orally. Thirty-six days after inoculation, colon whole-mount preparations consisting of the myenteric plexus adhering to the longitudinal muscle were stained using immunohistochemical technique to label the proteins: S-100 and GFAP. For both labels, we used antibody conjugated with a fluoresceine (S-100: rhodamine-546 nm; GFAP: Cyanine-488 nm). Fifteen images from each whole-mount were photographed on the same fluorescence microscope patterning the light incidence (Exposure: 170 ms to S-100 and 220 ms to GFAP; Saturation: 1, 5; Gamma: 1, 25; and Gain: 1, 0x). All were saved as TIF files for no loss of quality. Only ganglia with clear labeling were assessed to ensure avoiding extreme variations in brightness and artifacts. The images were analyzed, by blinded fashion, for the brightness intensity by the software ImageJ 1.45b in order to infer whether the expression of S-100 and GFAP was altered by T. gondii infection. Data are presented as fluorescence units. The two groups were compared by student test for independent samples considering $P < 0.05$.
Results: Glial cells – labeled both for S-100 as the GFAP protein – showed surrounding the cell bodies of myenteric neurons. Our data analysis showed a significant increase of brightness intensity both for S-100 (CG: 46.94 ± 13.74; IG: 55.27 ± 9.75) and GFAP (CG: 39.77 ± 14.09; IG: 45.21 ± 13.87) in rats infected with Toxoplasma gondii ($P < 0.001$), as illustrated in the Figure 1.
Conclusion: The infection caused by oocysts of the genotype II (ME-49) T. gondii provoked an increase of the immunostaining for S-100 and GFAP in glial cells of the colon myenteric plexus of rats. The increased expression of these proteins may contribute to understanding the pathophysiology of diarrhea observed in some species of animals (especially chickens and pigs) infected with T. gondii as well as the myenteric neuronal changes already revealed in our previous studies.

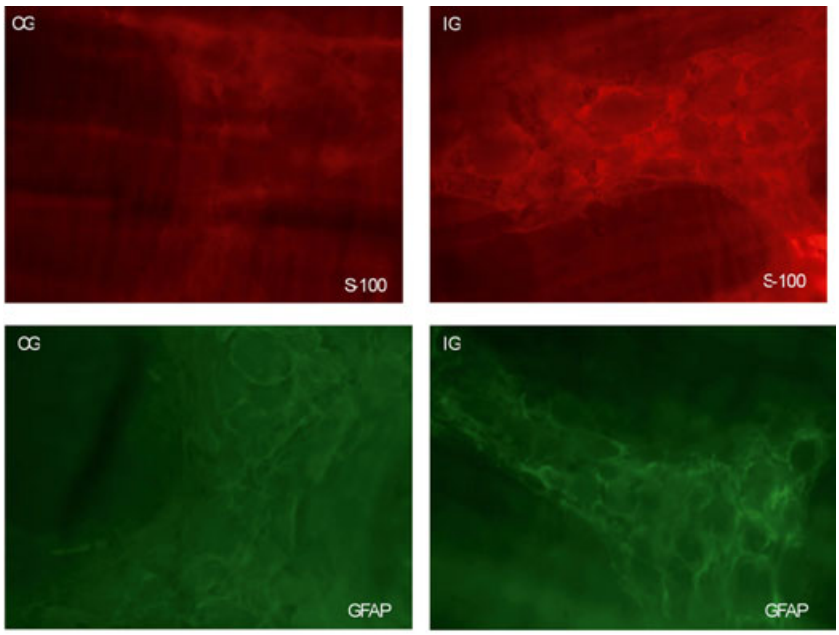

Figure 1 – Photomicrography of immunostaining for S-100 and GFAP protein of glial cells in the myenteric plexus of rats infected with *Toxoplasma gondii* (IG). CG: Control Group.

021

Effects of quercetin supplementation on the myenteric neurons HuC/HuD immunoreactive in ileum of dibetic rats

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Objective: Peripheral neuropathy is a chronic complication of diabetes mellitus and is directly related to the consequences of gastrointestinal disease due morphofunctional alterations of the Enteric Nervous System. Increased oxidative stress and changes in the antioxidative activity, as well as elevated levels of sorbitol observed in diabetic state, have been implicated in the development of enteric neurons damage. The antioxidants have been used in an attempt to minimize the neuropathic damage. Quercetin is a polyphenol with important antioxidant activity, which may play a significant role in the treatment of neurological complications of diabetes. This study aimed to verify the antioxidative activity of quercetin on myenteric neurons HuC / HuD immunoreactive in ileum of diabetic rats.
Methods: The myenteric neurons HuC / HuD immunoreactive in ileum of diabetic rats induced by streptozotocin, supplemented with quercetin, were analyzed in normoglycemic (N), diabetic (D) and diabetic treated with quercetin (DQ) rats.
Results: In group D, there was a reduction of 36.7% ($P < 0.05$) in the density of myenteric neurons than in group N. In the DQ group, there was observed a preservation of 16% ($P < 0.05$) neuronal density compared to group D. It was found that the areas of cell bodies of myenteric neurons of the DQ group were smaller than in group D.
Conclusion: This is probably due to the fact that quercetin can act as potent antioxidant and has the potential to reverse the changes in the gastrointestinal tract caused by diabetes.

022

Enteric neuronal function and colonic motility in PINK1-/- mice: A model for Parkinson's Disease

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Objective: Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by typical motor problems but also non-motor issues like depression, dementia and constipation. The hallmark of PD pathology is loss of neurons in the substantia nigra, however many other neuronal populations in the brain and even the peripheral nervous system, including the enteric nervous system (ENS) are affected. Over the last years accumulating evidence supports an important role for mitochondrial impairment in the onset of PD. Loss of function mutations in the mitochondrial protein PINK1 (PTEN (phosphatase and tensin homologue) induced kinase-1), a protein that interacts with Parkin and is involved in mitochondrial dynamics, were shown to cause recessive hereditary PD.
Aim: To investigate whether neuronal function in the ENS is affected in PINK1-/- mice and whether the motility defects are present in this model for PD.
Methods: Large intestines of control and Pink1 -/- mice (± 14 weeks old) were collected and mounted in an organ bath; video recordings of peristaltic movements and pellet propulsion were analysed in Igor Pro. Immunohistochemical analysis was performed using antibodies against HuC/D and GFAP to compare ENS morphology. Part of the large intestine was dissected to expose the ENS and was loaded with the $Ca2^+$ indicator Fluo-4 used to monitor nerve activity.
Results: The total number of enteric neurons (HuC/D) did not differ significantly between the PINK1 -/- and control mice, nor did the glial (GFAP) cell network in the ganglia. Varicose release sites in the ENS of PINK1 -/- mice displayed lower maximal $Ca2^+$ increases upon stimulation with a 20 Hz electrical stimulus (37 ± 3% *vs* 48 ± 5%; $P < 0.05$; $n = 78–81$ from 3 mice each). However, video analysis revealed no differences in frequency and propulsive force (~ 15 mm $wave^{-1}$) of the colonic peristaltic waves between control and PINK1 -/- mice.
Conclusion: Although some alterations in $Ca2^+$ signaling in the ENS could be detected, PINK1 -/- mice of 14 weeks old displayed no obvious motility problems. It may well be that the gastrointestinal motility problems, that are often associated with PD, occur only much later in the life of these PINK1 -/- mice.

023

An unexpected role of the enteric nervous system on gastrointestinal smooth muscle differentiation

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Objective: The gastrointestinal (GI) tract is a vital organ, highly conserved across species, which motility is ensured by the correct coordination of the visceral smooth muscle cells and the autonomous enteric nervous (ENS) system. Understanding the molecular processes that govern the differentiation of these cell types and their interrelationships during development could offer insight into the mechanisms altered in human GI motility disorders. The GI musculature is initially composed of splanchnic mesoderm. As development proceeds, the splanchnic mesoderm differentiates into four distinct concentric layers among which the smooth muscle. How the radial differentiation of the gut mesenchyme is established during development remains to be clarified. Concomitant with the patterning of the mesenchyme across the radial axis, the GI tract is colonized by the vagal neural crest cells (vNCC). vNCCs originate from the neural tube, invade the foregut, differentiate into neurons and glia and migrate in a rostrocaudal direction to colonize the entire gut to establish its innervation. Previous reports suggested that the coalescence of neurons and glia cells into ganglion plexuses coïncides with the differentiation of smooth muscle. However, whether vNCC has an impact on smooth muscle cells differentiation remains to be studied.
Methods: We used avian model and neural tube ablation at level 3–6 somites to reduce the number of vNCCs and analyze the impact on gastrointestinal smooth muscle differentiation.
Results: Here, we first examined and compared the spatiotemporal expression pattern of smooth muscle and ENS genes during avian GI tract development. We found that in the stomach, smooth muscle differentiation is initiated after vNCC migration. In contrast, in the colon, smooth muscle differentiation is initiated well before vNCC migration. These results suggest that visceral smooth muscle does not follow a rostrocaudal gradient of differentiation along the AP axis. Finally, we show that a reduction in vNCC number by neural tube ablation impairs smooth muscle differentiation in the stomach and alerts the BMP signaling pathway.
Conclusion: Our work demonstrate for the first time the influence of the vagal NCC on gastrointestinal smooth muscle differentiation and highlest a potential rostrocaudal difference in these processes.

024

Histopathology of the severe effects induced by repeated cisplatin in rat digestive tissues

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Objective: To identify the effects induced in the rat intestinal histological structure by the antitumoral drug cisplatin after repeated administration.
Methods: Male Wistar rats received saline or cisplatin (2 mg kg^{-1}, ip) once a week for 5 weeks. One week after the last administration, samples of ileum were processed for histological analysis. Hematoxilin-eosin (HE) staining was used to measure general tissue damage using criteria adapted from Galeazzi et al (1999): loss of mucosal architecture (graded 0–3, absent to severe), the extent of inflammatory cell infiltrate (graded 0–3, absent to transmural), crypt abscess formation (0–1, absent or present), goblet cell depletion (0–1, absent or present), muscular layer thickness (0–1, normal to reduced). Thus, for each section a numerical score of zero to nine was assigned. Inflammatory nodules and thickness of the muscular layers, also stained with HE, were quantified. Van Gieson's staining was used to assess collagen in submucosa. Enterochromaffin cells were counted using an anti-chromogranin A antibody. Globet cells were quantified after PAS staining. Cell proliferation was assessed with Ki-67 antibody.
Results: A general damage of the gut wall was found 1 week after repeated cisplatin. A loss of the normal mucosal architecture was particularly evident. Inflammatory nodules were significantly increased in size and number. Enterochromaffin and globet cells also increased their numbers in a significant manner. Proliferating cells had a broader distribution along the mucosa. On the contrary, submucosa thickness and the width of both muscular layers significantly decreased in cisplatin-treated rats.
Conclusion: One week after repeated cisplatin treatment different severe histopathological alterations throughout the gut wall are patent in the rat. These effects might not completely disappear and might have functional consequences or make the intestines more susceptible to future insults. The long-term effects induced by cancer chemotherapy in the gut wall deserve further investigation.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-02 Basic and Translational Session: Small Intestine, Colon and Anorectum: Physiology and Pathophysiology

025

Characterization of smooth muscle, enteric nerve, interstitial cells of Cajal, and fibroblast-like cells in the gastric musculature of the patients with diabetes mellitus

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Objective: Experimental studies of diabetes in animals suggest that the disease process can affect enteric nerves, extrinsic nerves, ICC and smooth muscle. By contrast, human data are sparse and generally inadequate for the validation of data obtained from experimental models. The aim of this study was to investigate histologic abnormalities in the gastric smooth muscle of patients with diabetes mellitus (DM).
Methods: Full-thickness gastric specimens were obtained from patients undergoing surgery for gastric cancer. To investigate the pathological changes of stomach, H&E stain, Masson Trichrome stain and immunohistochemical examination were done on the sections. Antibodies against protein gene product (PGP) 9.5, neuronal NO synthase (nNOS), vasoactive intestinal peptide (VIP), neurokinin 1, c-Kit, and PDGFRa were used. Immunofluorescent stain and evaluation with confocal microscopy were also done.
Results: Tissues were collected from 26 controls, 21 short duration DM patients and 14 long duration DM patients (39 males/22 females, mean age 61.2 ± 9.6 years). In clinical characteristics, there were no significant differences of age and proportions of gender and upper gastrointestinal symptoms between DM group and control group. Mean BMI in the DM group was higher than that in the control group ($P < 0.05$). The proportions of moderate to severe intercellular fibrosis in muscle layer were significantly higher in DM group than these in control group ($P < 0.01$). On immunohistochemical staining, c-Kit and PDGFRa-positive immunoreactivity were significantly decreased in DM group compared with control group ($P < 0.05$). There were no statistically significant differences in the PGP9.5, nNOS, VIP and neurokinin 1 expression between DM group and control group. On immunofluorescent staining and confocal microscopic examinations, network of ICC and fibroblast-like cells in the muscle layer were greatly decreased in the DM group compared with control group.
Conclusion: Our study suggests that on full-thickness gastric specimens, increased intercellular fibrosis and loss of ICC and fibroblast-like cells are found in the smooth muscle of patients with DM. These cellular abnormalities may contribute to changes of gastric motor activity in patients with DM.

026

Changes in colonic motility in a murine model of Alzheimer Disease

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Objective: Amyloid beta-protein deposits have been described in the intestine (Joachim et al. Nature 1989). However whether AD affects colonic motility remains to be resolved. The aim of the present work is to determine whether AD affects colonic motility in a murine model of AD.
Methods: Male 3–12- and 18-month-old double transgenic (B6C3-Tg) mice that express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) were used in the study. Offspring wild type mice were used as control. Under isofluorane anesthesia the colon was removed and spatiotemporal maps of peristalsis were built by video imaging. *In vitro* contractility was recorded in proximal and distal muscle strips without the mucosa layer (submucosal strips, SM) or the mucosa and submucosal layers (myenteric strips, My) under basal conditions, bethenecol and KCl challenges and electrical field stimulation (EFS).
Results: In the proximal colon, KCl-induced contractions, in both SM and My strips, were higher in 3-month-old (mo) transgenic (TG) mice compared to 3 mo WT, but an excitatory influence showed up in the SM plexus of 12-mo WT that was not present in TG mice and disappeared at 24 mo. Similar results were obtained with bethanechol. Surprisingly, the presence of SM plexus in the proximal colon had inhibitory effects on neurogenic ''on'' responses in both groups of mice at 3 and 12 months of age. This effect was more evident in TG mice and frequencies of 2 and 10 Hz and seemed to be due to an increase in nitregic innervation. In the distal colon of TG mice, SM plexus had an excitatory effect on EFS-induced ''on'' responses. Off responses were always contractile in character and proximal SM plexus decreased them in either WT or TG at 12 months of age. Frequency of peristaltic waves (PW) was higher at 3 months of age in WT but similar frequencies were recorded for 3 and 12 mo TG mice. Subtle increases were found in the propagation speed of PW and in the reduction of colonic diameter caused by the propagating wave (both higher in 12 mo TG).
Conclusion: AD-induced colonic motility changes are mainly restricted to the submucosal plexus, where an inhibitory component, probably nitrergic in origin, reduces neurogenic contractility. This translates into slight improvements in peristaltic activity, which would affect colonic absorption and defecation.
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027

Cholera toxin has a sexually dimorphic effect on colonic migrating motor complexes in mice

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Objective: Despite increasing evidence about enteric neural mechanisms responsible for hypersecretion induced by cholera toxin (CT), the effects of this toxin on motility remain ill-defined. We investigated the effects of luminal CT on colonic migrating motor complexes (CMMCs) in colon isolated from male and female mice (C57Bl/6).
Methods: Video imaging was used to construct high resolution spatiotemporal maps of colonic motor patterns *In vitro*. The full length of the colon was cannulated at each end and mounted horizontally in an organ bath containing physiological saline warmed to 37oC. The proximal end was connected to a reservoir of physiological saline, the distal end to an outflow tube. CT (0.125 μg ml^{-1}, 1.25 μg ml^{-1}, 12.5 μg ml^{-1}) was introduced to the lumen after control recordings with physiological saline and later washed out with physiological saline.
Results: CMMCs recorded in colon isolated from female and male mice did not differ in control conditions ($n > 6$ in each case). However, CT produced a concentration-dependent reduction in the frequency of CMMCs in female mouse colon. This occurred within 15 min of exposure to CT and was sustained for 1 h, but reversed when CT was flushed from the colonic lumen. Luminal CT also reversibly constricted the female colon, an effect that was maximal with the lowest concentration of CT tested (0.125 μg ml^{-1}). Effects of CT on the female colon were abolished by granisestron (1 μ mol L^{-1}, 5-HT3 antagonist) in the bathing solution or the lumen. In contrast, CT (1.25 μg ml^{-1}) did not affect either CMMCs or colonic diameter in male mouse colon.
Conclusion: Male and female mouse colon are markedly different in their motility responses to CT. The male is unaffected, the female shows a 5-HT-mediated inhibition of CMMC generation and is constricted via a related mechanism. Differences in time course and reversibility suggest that CT-induced changes in motility of the female colon result from mechanisms distinct from those that produce hypersecretion.

028

Nerve fibres containing enkephalin are reduced in colon from children with slow-transit constipation

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Objective: Opiates reduce colonic motility and cause constipation. Enkephalin (ENK), an endogenous opiate, is present in myenteric motor and interneurons, with

some ENK-neurons containing substance P (SP). In Slow Transit Constipation (STC) motility is slowed in the colon. In STC adults, ENK-immunoreactive nerve fibres in colonic circular muscle are reduced. One quarter of STC children have decreased SP nerve fibres in colonic circular muscle. Aim
Determine if ENK-nerve fibres are reduced in paediatric STC and if these contain SP.
Methods: Seromuscular biopsies (right transverse colon) from 51 STC children (4–14 years) and two familial adenomatous polyposis control patients, were processed for fluorescence immunohistochemistry to detect SP, ENK, Nitric oxide synthase (NOS) and Vasoactive intestinal polypeptide (VIP) immunoreactivity and viewed with confocal microscope. Biopsies were categorised as low-SP or normal-SP nerve fibre density. Quantitative analysis (area containing fluorescent pixels/circular muscle area, =% area) was performed on 12 STC biopsies (6 normal SP, 6 low SP).
Results: ENK-immunoreactive nerve fibres were abundant in muscle but not mucosa. ENK was present in SP but not VIP or NOS immunoreactive nerve fibres. In circular muscle, there were twice as many ENK nerve fibres as SP nerve fibres. Of the SP- or ENK-containing fibres, 46% were ENK-only, 31% SP-only and 23% ENK/SP nerve fibres. 34% of ENK nerve fibres contained SP and 41% of SP fibres contained ENK. In circular muscle from low-SP STC patients, there was a 50% lower % area of SP-nerves (mean±SEM, 0.25 ± 0.06 to 0.14 ± 0.05, P = 0.01) and of ENK-nerves (0.5 ± 0.12 to 0.28 ± 1.2, P = 0.05) compared to normal-SP patients and controls. The reduction was in ENK-only (% area, 0.36 ± 0.04 to 0.18 ± 0.03, P = 0.03) and SP-only nerve fibres (0.18 ± 0.02 to 0.08 ± 0.01, P = 0.007) but not in ENK/SP nerve fibres (0.16 ± 0.06 to 0.14 ± 0.03).
Conclusion: STC children with reduced SP nerve fibres also had reduced ENK nerve fibre density. Nerve fibres containing SP-only or ENK-only were reduced but fibres containing both SP and ENK were not reduced. Reduced ENK in enteric nerves may contribute to delayed colonic transit.

029

Identification of different patterns of propagating motor activity in isolated human colon

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Objective: Few studies have characterized the different patterns of motor activity that exist in isolated human colon. The aim of this study was to characterize the directionality of propagating contractions in the isolated human colon using high-resolution fibre-optic manometry.
Methods: Segments of human colon collected from laparotomies for colonic malignancy are placed in organ baths up to 120 cm long bathed with warm (36oC) oxygenated Krebs solution. Alligator clips are attached to full thickness colon in close proximity (10 mm) and connected to individual fibre-optic gratings on a high-resolution fibre optic catheter (CSIRO; Sydney). Data is collected over 1 h. Colectomies are divided into two anatomical groups- left and right. Propagating sequences are determined as contiguous contractions detected across three or more sensors, and divided into antegrade (oral-anal) and retrograde (anal-oral) events for analysis.
Results: A total of 21 specimens (14 left hemicolons, 7 right hemicolons) were studied. 10/14 (of left and 6/7 right hemicolons showed propagating sequences. Frequency of propagating events remained constant over time (left, mean: 8.88–9.11, P : 0.99), right, mean:8–12.6, P : 0.67). 3/14 specimens show increased tone over time. There were 24.3 ± 4 antegrade and 14.3 ± 6 retrograde events per hour in left colons, and 19.5 ± 2.7 antegrade and 18.5 ± 9.2retrograde events per hour in right colons. There were 3.5 ± 1.1 antegrade events to every retrograde event in left colons, and 2.2 ± 1 in the right colons (P : 0.68). Terminal ileal phasic contractions were recorded at 5.2 ± 0.7 cycles min^{-1}.
Conclusion: In both isolated right and left hemicolons, propagating anterograde contractions are more prevalent than retrograde contractions. Interestingly, the trend reveals more retrograde contractions to every antegrade contraction in the right hemicolon compared to left hemicolon. These results show that propagating anterograde and retrograde contractions can be preserved in isolated human colon.

030

Psychosocial chronic stress induces intestinal dysmotility and changes in enteric nervous system in proximal colon

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Objective: Chronic psychological stress is involved in the development of gastrointestinal and mental disorders such as irritable bowel syndrome and anxiety and depression. Preclinical studies showed that psychological stress affects homeostatic mechanisms in gut and it leads into alterations in immune response and epithelial transport. Intestinal functions are controlled by enteric nervous system (ENS), although is not well known the effects of chronic stress on ENS. The aim of this study was to investigate in mice the effects of an unpredictable chronic psychosocial stress on intestinal motility and molecular changes on enteric nervous system markers.
Methods: Adult male C57Bl/6j mice were exposed to unpredictable stress (mixed schedule of social defeat (2 h) and overcrowding (24–48 h)) over a 19- days period. Food intake, body weight, fecal pellet output and water content were recorded. Two hours after the last stress session whole-gastrointestinal transit was measured by gavaging carmine red (0.3 mL). Animals were euthanized during dark cycle, plasma levels of corticosterone were assessed by ELISA. Terminal ileum and different segments of colon were removed and quantitative RT-PCR and western blot were used to study enteric nervous system markers expression.
Results: Stressed mice had a significant increased food intake (4.2 ± 0.06 g *vs* 3.716 ± 0.04 g, P < 0.0001) and weight gain (3.7 ± 0.3 g *vs* 1.8 ± 0.1 g, P < 0.0001) compared to control mice. Plasma levels of corticosterone were significant decreased in stress group 55.0 ± 9.0 ng mL^{-1} *vs* 91.88 ± 11.2 ng mL^{-1}, P < 0.05). No significant differences in fecal pellet water content and defecation were seen between both groups, although there was a trend towards decrease in fecal output. Interestingly, whole gastrointestinal transit time was significant increased in stressed mice (192.5 ± 14.6 min *vs* 144.3 ± 7.3 min, P < 0.05), and no correlation was found with corticosterone levels. In stressed mice Tyrosine hydroxylase (TH), Brain-derived neurotrophic factor and Glial cell-derived neurotrophic factor (GDNF) mRNA expression was significant decreased compared to control (P < 0.05) in proximal colon and no changes were observed in terminal ileum and distal colon. 5-HT4 receptor expression was increased in proximal colon (P = 0.052). A significant decreased protein expression in proximal colon was observed in glia cells markers (GFAP and S100b), P < 0.05 and there was a corresponding trend toward a decreased levels of protein gene-product 9.5 (PGP 9.5) in proximal colon.
Conclusion: Unpredictable chronic psychosocial stress induces selective impairment of intestinal motility and disruption of enteric nervous system in proximal colon.

031

Non-invasive fluorescence imaging of intestinal motility

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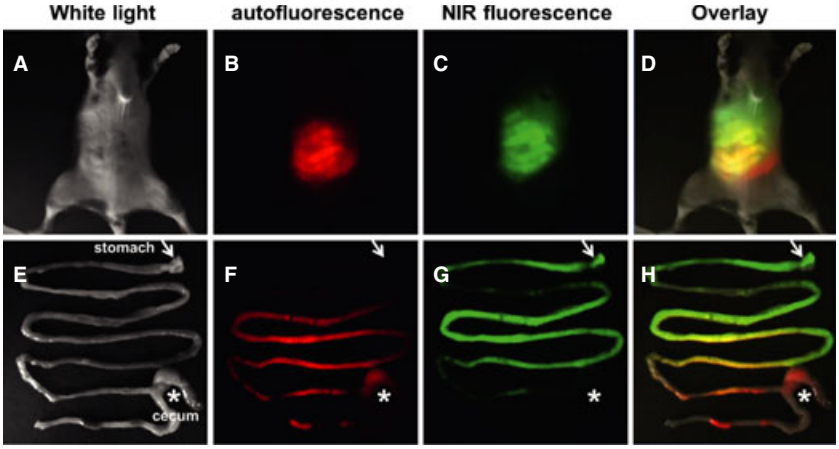
Objective: While several murine animal models have been developed to study gut motility and how it may be impacted by potential pharmacological agents, there remains a need to develop better non-invasive and long-term assessment tools of intestinal motility. Herein, we non-invasively imaged for the first time intestinal motions, such as peristaltic and segmental motions, using two fluorescence imaging techniques; (i) with injection of a near-infrared (NIR) fluorophore, indocyanine green (ICG), which is secreted into bile from the liver via the biliary tracts, enabling fluorescent delineation of the intestine, and (ii) without administration of an exogenous imaging agent using autofluorescence induced by standard murine diet containing chlorophyll.
Methods: Mice were dynamically imaged for 5 min immediately after and up to 24 h after injection of ICG. The mice were illuminated first with 785 nm light and then 660 nm light for acquisition of the NIR fluorescent signal at 830 nm and the autofluorescence signal at 710 nm respectively. NIR fluorescence and autofluorescence imaging data were analyzed to generate a three dimensional spatio-temporal map to quantify intestinal motions.
Results: The secretion of ICG-laden fluorescent bile into the duodenum was observed *in vivo*. Strong and non-uniformly distributed NIR fluorescence was detected throughout the intestines. In addition, mice showed different digestive status at each imaging time point as indicated by autofluorescence imaging. Different patterns of the intestinal motility, such as peristaltic and segmental motions, were dynamically imaged *in vivo* using ICG-fluorescence as well as autofluorescence imaging.
Conclusion: Dynamic fluorescence imaging techniques can provide a tool to monitor intestinal disorders or dysfunction and response to therapeutic agents.



032

High-throughput motility mapping of the whole murine small intestine and analysis with autocorrelation

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Objective: The objective of the study was to measure contraction frequency in the intact small intestine as an assay for the effects of drugs and ionic conditions on pacemaking by interstitial cells of Cajal.
Methods: Contraction frequency was measured using video-based mapping techniques and neuronal influences on motility were blocked by lidocaine. To make the technique high-throughput, several guts were recorded in one bath and the creation of maps and their analysis was automated. To deal with frequency gradients along the length of the intestine, the whole length of the small intestine was used and videoed by multiple webcams. Frequency was measured using time resolved autocorrelation. Other methods - Fourier spectra and peak detection with derivatives were found not to be so useful.
Results: Under control conditions (lidocaine and indomethacin) contractions in the proximal small intestine occurred at a frequency of 35–40 min^{-1} (interval of 1.5–1.7 s). In the distal intestine this abruptly decreased at certain points or contraction was absent. Therefore the proximal small intestine was used for analysis of drug and ionic effects. Some results are presented on the effects of chloride substitution and stilbenes.
Conclusion: The technique can detect quite subtle changes in frequency but there are problems with a small control time-dependent decrease in frequency which complicates statistical analysis.

033

SmoothRec: A poor man's multiple channels recording system

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Objective: Recording at high spatial resolution from a large (>50) number of extracellular recording electrodes has recently become useful in the analysis of many aspects of normal and abnormal propagation of slow waves in the stomach and the small intestines. Unfortunately, the costs of the hardware to record, amplify, and store such large number of electrograms are quite high.
Methods: The aim of this project was to develop a relatively cheap recording system, using off the shelf components and writing the relevant software in a widely available language.
Results: The selected hardware consists of 2–4 x 32-channel amplifier (SCS1X-1303) from National Instruments®, housed in a 4-slot chassis (SCXI-1000). The amplifiers were connected to a Windows-PC via a NI-DAQ PCI card (National Instruments®). The amplifiers, through a terminal block (SCXI-1303), were connected to 64–128 extracellular electrode arrays. Custom made software, written in LabView® v 10.0, was used for controlling and optimizing the amplifiers, for on-line visualizing of the electrograms and for acquiring and storing the signals. Signals were filtered with a 2–400 Hz bandwidth and sampled at 200–1000 s^{-1} sampling frequencies. The (text) files were stored on disc for off-line analysis in Smoothmap (www.smoothmap.org). Several experiments, both in silica and *In vitro*, were performed to determine the feasibility of recordings from large number of extracellular electrodes simultaneously and to check whether the morphology of the recorded waveforms were similar to those acquired with an expensive mapping system.
Conclusion: In the development of this multiple recording system, we have succeeded in (i) assembling off-the-shelf components into a multiple recording system, and (ii) developed the software to drive the amplifiers, display the electrograms on-line and to store them in a text file, for easy access to other software packages. All this was achieved with at least one order of magnitude less costs than current expensive multi-electrode mapping systems. The development of this system could enhance the application of gastrointestinal electrophysiological mapping in research and clinical practice.

034

Angiotensin II positively modulates the spontaneous contractile activity of mouse and human colon via activation of AT1 receptors

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Objective: Angiotensin II (Ang II) is a potent smooth muscle contractile neurohumoral agonist but has not been much investigated with regard to gastrointestinal motor activity. Ang II effects are mediated by specific receptors, the Ang II type 1 (AT1) and the Ang II type 2 (AT2) receptors, which are well expressed in the gut. In this study we evaluated the effects of Ang II on the contractile activity of longitudinal muscle from mouse and human colon and we analysed the subtype(s) of receptors involved in the observed effects.
Methods: Mechanical responses to Ang II, in the absence or in the presence of different drugs, were assessed *In vitro* in colonic longitudinal muscle from mice and humans, as changes in isometric tension.
Results: In the murine proximal and distal colon Ang II induced a concentration-dependent muscular contraction, which was reduced by the AT1 receptor antagonist, losartan, but it was not affected by the AT2 receptor antagonist, PD123319. Pretreatment with TTX, sodium voltage-gated neural channel blocker, partially reduced the contractile response to Ang II in the proximal colon, while abolished it in the distal colon. Atropine, muscarinic receptor antagonist, or SR140333, NK1 receptor antagonist, reduced the TTX-sensitive excitatory effects induced by Ang II in both preparations. On the contrary, hexamethonium, nicotinic receptor antagonists, ondansetron, 5-HT3 receptor antagonist, or SR48968, NK2 receptor antagonist, were ineffective. The contraction induced by a selective NK1 receptor agonist was reduced by atropine, whilst SR140333 did not affected carbachol inducing muscular contraction. Ang II induced a muscular contraction even in the human distal colonic longitudinal muscle preparations. The concentration–response curve was shifted to the right by losartan but it was unaffected by PD123319. TTX and atropine partially antagonized the response to Ang II.
Conclusion: In the longitudinal muscle preparations from mouse and human colon Ang II positively modulates the spontaneous contractile activity via activation of post-junctional and pre-junctional AT1 receptors, the latter located on the enteric nerves and modulating the release of tachykinins and acethylcoline. In mouse tachykinergic neurons and cholinergic neurons are sequentially recruited by Ang II to induce muscular contraction.

035

Γ-amino-butyric acid regulates submucosal cholinergic signalling and secretomotor function in mouse colon

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Objective: γ-amino-butyric acid (GABA) is widely expressed throughout the gastrointestinal (GI) tract where it is a key regulator of GI function including

motility, sensory-perception and gastric acid secretion. However, its role in secretomotor function has to date been understudied. Our aim was to characterize GABA-mediated effects in murine colon, and to determine through which GABA receptor this effect is mediated.

Methods: Following seromuscular stripping, segments of descending colon from male BALB/c mice (20–28 g) were mounted in Ussing chambers, superfused with Krebs buffer solution and bubbled with carbogen gas (95% O_2, 5% CO_2). Tissues were voltage clamped at 0 mV and the short circuit current (Isc) was recorded. To investigate the influence of GABA on enteric secretomotor function, GABA (0.01–100 μ mol L^{-1}) was applied basolaterally. The GABAA and GABAB receptor agonists, muscimol (100 μ mol L^{-1}) and baclofen (100 μ mol L^{-1}) were added to determine through which GABA receptor this effect is mediated. Tetrodotoxin (TTX; 300 n mol L^{-1}) acted as a neural blockade.

Results: GABA had no effect on baseline Isc, however muscimol significantly decreased baseline Isc relative to vehicle (vehicle 0.02 ± 0.1 μA cm^2, n = 6; muscimol -3.7 ± 0.8 μA cm^2, n = 6, P = 0.007). Subsequent responses to bethanechol (BCh; 100 μ mol L^{-1}) were reduced in GABA-exposed tissues relative to vehicle (dH20) and maximal inhibition was observed with 100 μ mol L^{-1} GABA (vehicle, 24.6 ± 7.6 μA cm^2, n = 5; GABA, 6.1 ± 1.0 μA cm^2, n = 7, P = 0.016). However, neither GABA agonist significantly altered BCh-induced ion transport (vehicle 15.4 ± 4.6 μA cm^2, n = 5; muscimol 8.5 ± 2.5 μA cm^2, n = 6 and vehicle 11.9 ± 2.5 μA cm^2, n = 6; baclofen 12.4 ± 5.0 μA cm^2, n = 6), BCh responses were significantly attenuated following TTX (BCh 24.6 ± 7.6 μA cm^2, n = 5; TTX+BCh, 4.3 ± 0.3, n = 5, P = 0.03). Moreover, this diminished response to BCh in the presence of TTX was further reduced by GABA (100 μ mol L^{-1};P=.0001).

Conclusion: Our data suggest GABA significantly reduces cholinergic-induced ion secretion via both neural and epithelial pathways in mouse colon, though neither GABAA nor GABAB receptor agonists mimicked this effect. Moreover, our data indicate that GABAA receptors, in the regulation of basal Isc at least, may be functionally important.

036

Microelectrode array analysis of spontaneous electric activity in the ileum of mice

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Objective: Gut motility is based on the cooperation of electric excitable cells. It is well known that a network of intrinsic neurons simultaneously induce ascending contraction and descending relaxation of smooth muscle, leading to peristaltic movements. In addition, relatively recent studies have revealed that special interstitial cells, referred to as interstitial cells of Cajal (ICC) act as pacemaker cells. In this communication, we show methods to analyze the spatio-temporal properties of slowly oscillating spontaneous electric activity in the gut, using a microelectrode array (MEA) system.

Methods: Mice used were treated ethically. All procedures were approved by the Institutional Animal Care and Use Committee. Isolated Ileal musculatures were mounted on a set of 8 × 8 microelectrodes (50 vm × 50 vm squares) with a distance of 150 vm. The impedance of each electrode was ~30 MΩ at 0.1 Hz. Field potentials over the area of ~1 mm^2 were simultaneously measured using a computer-controlled, multi-channel AC amplifier with a high-pass filter of 0.1 Hz and a low pass-filter of 10 kHz, and were stored in a personal computer via 14 bit A/D converters with a sampling rate of 20 kHz. In data analyses, the arrayed data of field potential recordings were thinned by a 1000-fold time domain, thereby the sampling interval was increased to 50 ms. Spatial characterization of spontaneous electric activity was performed by using spectral power, and phase-shift and peak values of cross-correlation.

Results: The analyses distinguished ileal musculatures obtained from wild-type (WT) and W/Wv mice, the latter serving as a model of impaired ICC network. Especially, in the phase-shift mapping, systematic gradation was observed only in WT mice, showing to propagating electric waves. Application of nifedipine and TTX reduced the phase-shift in the recording area, implying that excitable cells other than ICC support the propagation of pacemaking electric waves.

Conclusion: We hope that with sufficient biophysical and technical consideration, MEA will be utilized in future studies to assess the spatial property of slowly oscillating gut electric potentials.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-03 Basic and Translational Session: Appetite Regulation, Satiety, Obesity and Nutrition

037

Restricted feeding induces inflammation: Role of ghrelin and clock genes?

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Objective: Circadian functions of feeding may depend on a peripheral clock system which is possibly located within the ghrelin-secreting cells of the stomach. Restricted feeding (RF) has been shown to increase plasma ghrelin secretion in anticipation of a regularly scheduled meal. We aimed to study the effect of RF on peripheral clock gene expression and on several functions associated with increased ghrelin signaling.

Methods: Wild-type (WT) and ghrelin receptor knockout (GHSR-KO) mice were fed either ad libitum (AL) or put on RF (access to chow from 12 to 4PM) for 2 weeks. Plasma ghrelin levels were determined by radioimmunoassay. Gastric emptying was determined by the 13C octanoic breath test. *In vitro* contractility changes were measured isometrically in fundic smooth muscle strips.

Results: RF resulted in an increase in plasma octanoyl ghrelin levels in both WT (RF: 556 ± 106 *vs* AL: 271 ± 74 pg ml^{-1}; P < 0.05) and GHSR-KO mice (RF: 974 ± 263 *vs* AL: 149 ± 41 pg ml^{-1}; P < 0.05). Body weight but not food intake was significantly diminished in GHSR-KO compared to WT mice on RF. Gastric half emptying time was significantly decreased after RF in both WT (RF: 56 ± 3 *vs* AL: 104 ± 4 min; P = 10-7) and GHSR-KO mice (RF: 60 ± 4 *vs* AL: 112 ± 5 min; P = 10-7). In WT mice, RF increased the affinity of the contractile response towards ACh and SuP and caused neural hyperexcitability in fundic muscle strips. H&E staining revealed inflammation in WT mice on RF, which was accompanied by an increase in MPO activity (RF: 0.11 ± 0.03 *vs* AL: 0.02 ± 0.01; P < 0.05) and IL-1β expression (RF: 1.43 ± 0.20 *vs* AL: 0.53 ± 0.11; P < 0.05). In GHSR-KO mice, inflammation was subsided and post-inflammatory changes were evident. The antiphasic expression of the clock genes BMAL1 and PER2 in the stomach was significantly decreased and increased, respectively. BMAL1-KO mice did not survive RF.

Conclusion: Ghrelin plays a role in the recovery of body weight after RF, but not in the accelerated gastric emptying. Gastric contractility changes are triggered by RF-induced inflammation which is dampened by ghrelin. Further research is warranted to investigate the role of the clock genes in the observed changes.

038

Impact of diet-induced obesity on motor functions and myenteric neurons in mice distal colon

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Objective: Obesity is associated with altered colonic transit suggesting underlying nerve plasticity that may be linked to obesity-associated chronic low-grade inflammation.

Methods: We therefore investigated functional changes in the distal colon of mice that were made obese with a high fat diet (DIO) before showing signs of metabolic syndrome. We measured additionally acetylcholine (ACh) and serotonin (5-HT) tissue contents and levels of metabolic enzymes. Activity of myenteric neurons was recorded after nicotine and 2-methyl-5-HT (5-HT3 agonist) application. Histological scores and inflammatory markers were measured in colonic and adipose tissues.

Results: DIO mice exhibited higher visceral adiposity with increased pro-inflammatory adipokine expression in mesenteric fat tissue. Colonic histology, macrophage density, mucosal permeability or expression of epithelial pro-inflammatory cytokines were not changed, except for an increased expression of Il-6 and TNF by 106 and 116%, respectively. Together with an enhanced colonic transit, DIO mice had 86% and 48% higher tissue contents of ACh and 5-HT, respectively. This was associated with increased expression of the tryptophan hydroxylase 1 and 2 by 180 and 99%, respectively. In line with this finding we found that the proportion of myenteric neurons that responded to nicotine or 2-methyl-5-HT were increased by 255 and 268% respectively.
Conclusion: The results suggest that DIO impacts on ACh and 5-HT metabolism in the distal colon inducing plasticity in cholinergic and serotonergic myenteric neurotransmissions. This may contribute to the accelerated colonic transit and the increased risk of diarrhea in obese patients. These functional changes are not associated with inflammatory processes in the gut wall but with changes indicative of systemic inflammation. (Supported by DFG Sche 267/8-1).

039

Circadian variations in gastric vagal afferent mechanosensitivity and satiety signalling

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Objective: Many behaviours including food intake vary in level and intensity over the course of the day in circadian/diurnal rhythms (Physiol Behav.1987; 40: 437–66). Disruption of these daily patterns (e.g.shift workers) increase the risk for developing metabolic problems, such as obesity (Science 2010; 330: 1349–54). Gastric vagal mechanoreceptors play an important role in satiety signalling, but information on their sensitivity to mechanical stimuli at different times of the day is unknown. We aimed to determine the: (i) mechanosensitivity of gastric vagal mechanoreceptors at varying times of the day, in mice fed ad libitum, fasted for 6 h or housed for 72 h in darkness; (ii) expression of clock genes in vagal afferents.
Methods: Single fibre recordings of gastric vagal mechanoreceptors were obtained and nodose ganglia collected, for quantification of Bmal1, Per1, Per2 and mRev-erb mRNA by QRT-PCR, at 3 h intervals starting at lights off (1800 h).
Results: In mice fed ad libitum, there was three times more food in the stomach at 2400, 0300 & 0600 h ($P < 0.001$ *vs* 1200 h). The response of tension receptors to 3 g tension was reduced by up to 70% at 1800, 2100, 2400, 0300 & 0600 h, ($P < 0.05$ *vs* 1200 h). Gastric mucosal receptors also displayed circadian rhythm with peak responses to stroking with a 50 mg von Frey hair three times greater at 1200 & 1500 h than the lowest response at 2400 h ($P < 0.05$). There was a significant correlation between stomach content and tension or mucosal receptor mechanosensitivity ($R = -0.77$ and $R = -0.44$ respectively). Similar findings were obtained in mice fasted for 6 h or maintained in continuous darkness for 3 days prior to study. Therefore these changes are not mediated by food intake or the light/dark cycle. QRT-PCR revealed that Per1, Per2, Bmal1 and mRev-erb mRNA are expressed in the nodose ganglia and levels oscillated over a 24 h period. Bmal1 and mRev-erb peak during the light phase and Per1 & 2 peak at the beginning of the dark phase indicating a potential role in modulating vagal mechanosensitivity.
Conclusion: Gastric vagal mechanoreceptors display circadian rhythm. Disruption of these oscillations in satiety signalling may result in over consumption of food and obesity.

040

Detection of the ghrelin activating enzyme ghrelin-O-acyltransferase (GOAT) in the human circulation and expression dependent on body weight

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Objective: Ghrelin is the only known peripherally produced and centrally acting peptide hormone that stimulates food intake in animals and humans. Recently, the ghrelin acylating enzyme ghrelin-O-acyltransferase (GOAT) has been identified in mice and humans. Besides the expected expression in the stomach we also detected GOAT in the circulation of rats and mice and its expression was dependent on the metabolic status with an increase under fasting conditions. To investigate whether GOAT is also present in human plasma and whether expression levels are affected under different conditions of body weight.
Methods: Normal weight (body mass index, BMI 19–25 kg m^{-2}, $n = 4$ women and 5 men, age 48.4 ± 3.8 years), anorexic (BMI <17.5 kg m^{-2}, $n = 9$ women, age 25.4 ± 2.7 years) as well as obese (BMI >30 kg m^{-2}, $n = 4$ women and 5 men, age 46.2 ± 4.4 years) hospitalized patients were recruited from the Division of Psychosomatic Medicine and gave informed consent to participate in the study. Blood was withdrawn in overnight fasted subjects between 07:00 and 08:00 am, collected in EDTA tubes containing aprotinin and plasma was formed by centrifugation, processed for Western blot and stained with an anti-human-GOAT polyclonal antibody (Phoenix Pharmaceuticals). Semiquantitative analysis of pixel intensity was performed using Scion Image 4.0.3.
Results: GOAT protein expression was detected in human plasma as indicated by a strong band at the expected size of 50 kDa. In normal weight patients (mean BMI 22.7 ± 0.8 kg m^{-2}) the intensity of the 50 kDa band was 28361.2 ± 849.6. The expression was significantly lower in anorexic patients (mean BMI 12.6 ± 0.6 kg m^{-2}, intensity: 20733.3 ± 1732.0, $P < 0.001$), while it was significantly higher in the plasma of obese patients (mean BMI 68.9 ± 2.7 kg m^{-2}, intensity: 32532.3 ± 1822.9, $P < 0.05$). Plasma GOAT protein expression showed a significant positive correlation with BMI ($r = 0.59$, $P < 0.01$).
Conclusion: Data show that GOAT protein is also present in human plasma and that GOAT protein levels depend on the metabolic condition with decreased levels in anorexic and increased levels in morbidly obese patients. These data may point towards a contributing role of GOAT in the development or maintenance of these diseases as it is the only enzyme known to acylate ghrelin.

041

Peripheral Glucagon-like peptide 2 administration inhibits food intake in mice: Analysis of the mechanism of action

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Objective: Previously we showed that, in mice, peripheral administration of glucagon-like peptide 2 (GLP-2) or [Gly2]GLP-2, the degradation-resistant analogue of GLP-2, reduces food intake in the short term. The purposes of the present study were to compare the influence of [Gly2]GLP-2 with the anorectic effect induced by glucagon-like peptide 1 (GLP-1) and to analyze the mechanism of action responsible for GLP-2-induced effects.
Methods: Food intake was measured in mice, fasted for 16–18 h, at the first hour following peptide or vehicle intraperitoneally (i.p.) administration. The effects of GLP-2 (3–33), GLP-2 receptor (GLP-2R) antagonist, and exendin (9–39), GLP-1R antagonist were also evaluated. To analyze the mechanism of action the effects of [Gly2]GLP-2 on gastric emptying were determined, and its influence on the food intake was examined in mice with sensory deafferentation induced by capsaicin pretreatment.
Results: Administration of [Gly2]GLP-2 (0.60 $\mu g\ g^{-1}$ b.w.) caused a significant reduction in the food intake when compared with vehicle treated mice, which was blocked by the GLP-2 receptor antagonist, GLP-2 (3–33), or by the GLP-1 receptor antagonist, exendin (9–39). Both antagonists per se did not alter food intake. Also GLP-1 (0.30 $\mu g\ g^{-1}$ b.w.) significantly inhibited food intake and the dose that inhibited the food intake by 50%, was lower than that determined for [Gly2]GLP-2. The effect was antagonized by the GLP-1 receptor antagonist, but not by the GLP-2 receptor antagonist. Coadministration of [Gly2]GLP-2 and GLP-1 did not cause any additive inhibition on the food intake. The rate of gastric emptying was significant decreased in [Gly2]GLP-2 treated animals compared with vehicle treated mice. Sensory deafferentation by capsaicin blocked the effects of [Gly2]GLP-2 peripheral injection, while it did not alter food intake in vehicle treated mice.
Conclusion: Our results suggest that [Gly2]GLP-2 is less potent than GLP-1 in inducing inhibition of the food intake in mice. GLP-1 and GLP-2 appear to act through a common pathway. GLP-2 exerts its effect through peripheral neural mechanisms and requires the functional activity of GLP-1R signaling.

042

Evaluation of the effects of elsiglutide on the toxicity and histological damage induced by Irinotecan in normal fischer rats

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Objective: To confirm that treatment with Elsiglutide, a glucagon-like peptide analogue constituted of 39 amino acids, will prevent and protect from toxicity induced by irinotecan in preclinical Fischer rats model.

Elsiglutide may offer the potential for prevention and treatment conditions in which intestinal tissues are compromised such as Chemotherapy-Induced Diarrhea (CID), requiring dose and treatment delay.
Methods: Fisher rats weighing 150–200 g were treated with Elsiglutide 0.9 and 1.8 mg kg^{-1} dx^{-4} alone and in combination with irinotecan, 150 and 200 mg kg^{-1} dx^{-3} (2x max tolerated dose). Diarrhea, weight loss, lethality, survival, intestinoptrophic and histopathology were assessed as end point of treatment outcome.
Results: While Elsiglutide doses used had no short-term or long-term (up to 3 months of treatment) toxicity, irinotecan induced diarrhea and lethality were dose dependent, 100% and 50% with 200 and 150 mg kg^{-1} dx^{-3}. Elsiglutide offered complete protection from toxicity induced by irinotecan including intestinal damage, weight loss, and diarrhea. 100% protection from irinotecan-induced lethality was observed with both Elsiglutide doses. Histological and measurable toxicity confirmed that Elsiglutide is a highly effective agent in protecting normal tissues from irinotecan-induced toxicity in the rat model system.
Conclusion: Both doses of Elsiglutide were highly effective in protecting against irinotecan induced toxicity in rats including diarrhea, body weight loss and lethality. No score 3 diarrhea was observed with the 1.8 mg/kg Elsiglutide in combination with 200 and 150 mg kg^{-1} dx^{-3}. Hence, no irinotecan dose reduction or delay of treatment were required. The toxicity protection data were supported by histological observation demonstrating drastic protection against small and large intestinal damage. The data strongly supports the use of Elsiglutide in prevention and treatment of Chemotherapy-Induced Diarrhea and intestinoptrophic effects. The data also provides the basis for clinical validation of this agent in patients where diarrhea is the dose limiting toxicity.

043

Cardiac autonomic regulation and cortisol profiles during 48-hour zero-calorie fasting

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Objective: Today fasting is widely used for weight loss, but often inadequate or opposite results reveal that the physiology of fasting and dieting remains poorly understood.
Methods: Sixteen young healthy female volunteers (21.4 ± 2.1 years, BMI 21.6 ± 1.6) underwent a 3-day zero-calorie diet under 24-h medical surveillance at the Department for Psychosomatic Medicine and Psychotherapy, University Hospital Tübingen, Germany. Psychological (PHQ, EDI, FEV, subjective feeling of hunger) as well as physiological data (heart rate variability (HRV) analysis during resting conditions and head-up tilt test, diurnal saliva cortisol profiles) were measured on admission, and after 24- and 48-hours of fasting.
Results: We observed a consecutive weight loss from Day 1 to Day 3 that resulted in significant bodymass index (BMI) reduction across all subjects ($P < .0001$). Slope of the cortisol day profile revealed significant shift towards lower values from baseline to the end of experiment ($P = .002$). Resting standard deviation of the normal-to-normal (NN) intervals (SDNN) and root mean square successive difference (RMSSD) showed significant ($P < .001$) decrease from admission compared to the Day 3 of the experiment with a mild increase after 24 h that did not reach statistical significance. Forty-eight hours of fasting also induced a significant ($P < .001$) decrease of mean IBI, SDNN, RMSSD and logHF power during tilt-test; LFnu have increased during the experiment, but the values did not reach statistical significance due to the correction for multiple comparisons.
Conclusion: Short term (48 h) zero-calorie fasting induced parasympathetic withdrawal with simultaneous sympathetic activation and shift in daily cortisol profile. These changes are similar to those obtained under other types of stress-conditions and further studies need to find out how specific they are for fasting.

044

Diet and irritable bowel syndrome

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Objective: Recent papers highlight the role of diet in irritable bowel syndrome (IBS), but very few population-base studies evaluated this. The aims of the study were to determine the prevalence of IBS in general urban population and to evaluate the type of diet associated with IBS symptoms.
Methods: A randomized sample of subjects ($n = 300$) from a general urban population selected from the family doctors patient lists was invited for interview in the doctor's office. Selected subjects were evaluated for the diagnosis of IBS using Rome III criteria and also for their eating habits and diet using a food frequency questionnaire. Socio-demographic factors and general medical history were also included in interview together with objective evaluation of overweight. Results from logistic regression were presented as odd ratio and 95% confidence intervals.
Results: From the selected sample, 193 subjects (80 males, 113 women, mean age 50.8 ± 16.2 years) have agreed to participate (rate 64.3%). Prevalence of IBS was 19.17% (19.4% for females and 18.7% for males). IBS was associated with older age (1.05; 1.02–1.08; $P < 0.001$) and past history of digestive diseases (5.0; 2.0–12.7; $P < 0.01$). IBS subjects are eating significantly more frequent the following foods: canned food (7.4; 2.2–25.4; $P < 0.01$), processed meat (4.7; 1.6–14.1; $P < 0.01$), pulses (legumes) (4.0; 1.3–16.3; $P < 0.01$), whole cereals (8.7; 2.0–37.8; $P < 0.01$), confectionary (5.7; 1.8–23.2; $P < 0.01$), fruit compotes (canned or not) (7.4; 2.5–23.1; $P < 0.001$) and herb teas (4.0; 1.3–16.3; $P < 0.001$).
Conclusion: This study updated prevalence data and reveal association between diet and irritable bowel syndrome.

045

Ghrelin and glucose homeostasis in perinatal low birth weight and normal weight piglets

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Objective: Ghrelin, the gastrointestinal derived 'hunger hormone', regulates growth hormone (GH) secretion and energy homeostasis. Additionally, a major role of ghrelin in glucose homeostasis independent of GH secretion has been suggested. Its increased plasma levels in intrauterine growth retardated (IUGR) infants suggest a possible relationship between ghrelin and IUGR. IUGR is associated with an increased perinatal mortality and an increased incidence of the metabolic syndrome (MS) in adulthood. In this respect, altered ghrelin levels in IUGR infants could contribute to postprandial catch up growth thereby aggravating the risk of developing MS. Therefore, this study compared both ghrelin and glucose levels in perinatal low birth weight (LBW) and normal birth weight (NBW) pigs.
Methods: Pigs were assigned LBW (birth weight (BW) <1 kg) or NBW (mean BW=1.74 kg). Each age group (day 0, 3, 10 and day 28 of age) consisted of five pairs of pigs. Blood glucose levels and serum ghrelin levels were determined by a glucometer and a radioactive immunoassay (RIA) respectively. The data were analyzed by two way ANOVA. Furthermore, linear regression was used to correlate the ghrelin and glucose levels.
Results: Ghrelin ($P = 0.878$) and glucose levels ($P = 0.499$) showed no BW related differences. However, ghrelin levels did show an age dependent, yet not significant, decreasing trend ($P = 0.195$), whereas glucose levels demonstrated a significant age dependent increasing trend ($P = 0.004$). Hence, linear regression revealed a significant inverse relation between ghrelin and glucose levels ($P = 0.006$, $r = -0.499$).
Conclusion: This study showed no age or BW related differences regarding serum ghrelin levels. This is in accordance with human data, where no difference between preterm and full term newborns as well as between small for gestational age (SGA) and appropriate for gestational age (AGA) infants could be detected. Nevertheless, higher ghrelin levels in IUGR compared to normal weight fetuses and newborns have been reported. Thus, the role of ghrelin in perinatal growth remains unclear. This study did not find a BW related difference in blood glucose levels, whereas other studies have demonstrated insulin resistance in SGA infants. Nevertheless, the relation between ghrelin and glucose levels emphasizes the role of ghrelin in glucose homeostasis in perinatal piglets.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-04 Basic and Translational Session: Gastric Physiology, Pathophysiology

046

Apical NKCC2 in the gastric mucosa involves gastric acid secretion

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Objective: Two isoforms of Na^+-K^+-2Cl- cotransporter (NKCC), NKCC1 and NKCC2, have been detected at exceptionally high levels in the gastric mucosa of several species, prompting speculation that they may play important roles in the gastric secretion. We previously have reported the differential distribution of NKCC1 and NKCC2 in the gastric parietal cells. In the present study, we investigated the potential role of NKCC1 and NKCC2 in the gastric acid secretion.
Methods: Double-label immunofluorescence was used to determine the localization of NKCC1 and NKCC2 in the parietal cells in mouse/human gastric mucosa and primary cultured parietal cells. Real-time pH titration was performed to monitor mouse gastric acid secretion; NKCC protein levels were calculated through Western blot.
Results: NKCC1 and NKCC2 were expressed in the mouse and human parietal cells. NKCC1 immunoreactivity (IR) was mainly observed on the basolateral membrane, while NKCC2 IR was clearly located at the apical membrane. Bumetanide (10 μmol L^{-1}), a well known blocker of NKCC, when added to the apical side, not basolateral side, significantly inhibited forskolin-(n = 13, P < 0.001) and histamine-(n = 5, P < 0.05) induced gastric acid secretion, indicating NKCC2, but not NKCC1, was involved in the secretagogue-induced acid secretion. However, no measurable alteration of NKCC2 protein was observed in the gastric mucosa at resting and histamine stimulation. In the primary cultured parietal cells, NKCC2 and H^+-K^+-ATPase were colocalized in the parietal cells, but resided in the different vesicular pools under resting conditions. Upon histamine stimulation, NKCC2 tended to translocate to the cell membrane, suggesting that the translocation of NKCC2 might be a mechanism involved in the secretagogue-induced acid secretion.
Conclusion: Both NKCC1 and NKCC2 were expressed in the mammalian gastric parietal cells. NKCC2, but not NKCC1, was involved in the secretagogue-induced acid secretion.

047

A novel rat stomach model based on 3D shape context method based strain analysis

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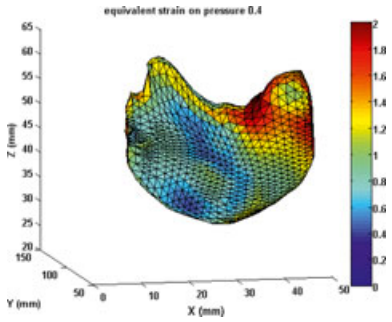
Objective: The stomach has the ability to change its geometry and volume during digestion of its contents. Furthermore, the stomach shape depends on the pressure from adjacent organs. Deformation analysis of the entire organ is therefore important for accurate estimation of the true deformation in this highly non-homogeneous, anisotropic organ. Aims of this study are to describe distension-induced 3D gastric deformation by combining a modified 3D non-rigid image registration method (shape context method) and full-field strain analysis method.
Methods: The geometry of a normal rat stomach was reconstructed from ultrasonic scanning slices obtained at distension pressures from 0.05 to 0.8 kPa. The distension-induced 3D deformation throughout the gastric model with reference to a no-load reference state was computed on the basis of an improved 3D non-rigid image registration method (shape context method) and full-field strain analysis method.
Results: The registered surface showed good agreement with the real deformed surface for all distension states. However, the errors increased with the distension pressure due to increasing dissimilarity between the deformed and the reference surface. The deformation distributions on the stomach surface were non-uniform with the largest deformation in the non-glandular part and the greater and lesser curvature when the pressure was higher than 0.2 kPa (1.67 ± 0.42 *vs* 0.89 ± 0.43 for averaged strain in the non-glandular part and the glandular part at the distension pressure of 0.8 kPa, Fig. 1). The pressure-strain curves in the non-glandular part located right to the curves in the glandular part indicating the softer wall of the non-glandular part.
Conclusion: The modelling method which is closely allied with the non-rigid image registration and deformation analysis provides a kinematically possible deformation mode of the gastric wall. This method can be potentially used for clinical data estimating the kinematical properties of human visceral organs in health and disease.



048

The effect of administration of probiotic bacteria on the composition of the mucosal gut microbiota in IBS patients

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Objective: The gut microbiota is considered a key factor in the aetiology of the Irritable Bowel Syndrome (IBS). An altered commensal microbiota most likely affect the immune responses in the mucosa and the barrier function of the gut. Recent reports indicate that the use of probiotics may alleviate IBS symptoms.
Aim: To investigate the effect of administration of a probiotic mix compared with placebo on the mucosal gastrointestinal microbiota composition in IBS patients.
Methods: In a parallel design 20 IBS patients received a probiotic mix while 16 patients received a placebo. The probiotic therapy consisted of daily administration (dose per strain = 109 bacteria) of: Lactobacillus rhamnosus GG®, Lactobacillus rhamnosus Lc705, Propionibacterium freudenreichii subs. shermanii JS and Bifidobacterium animalis subs. lactis Bb-12®. Results were also compared with healthy controls (n = 10). Sigmoidal biopsies were obtained (without prior bowel preparation) just before (visit 1) and after 4 months of probiotic therapy (visit 2). The mucosal microbiota was determined using the Human Intestinal Tract Chip phylogenetic microarray, which enabled us to analyse the profiles and semiquantitative compositions of the different members of the microbiota. Principal Component Analysis and Redundancy Analysis were applied to analyse the microarray data.
Results: Clear differences were found in the gut microbiota between IBS patients and controls (P = 0.002). The composition of the microbiota in the group of IBS patients differed in time. Between visit 1 and visit 2 there was a decrease in Bacteroidetes and members of the Clostridium Cluster XIVa. An increase in members of the phylum Proteobacteria was detected. Yet, these variations were not correlated to the consumption of the probiotic.
Conclusion: There were differences in the composition of the mucosal microbiota in IBS patients and healthy individuals. Remarkable, the consumption of the probiotic did not modify the composition of the mucosal microbiota during the time of the study.

049

***In vitro* and *in vivo* studies on the effects of oxytocin in a rat model of functional dyspepsia**

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Objective: The vagus nerve plays a significant role in the stress-induced gastric dysmotility observed in functional dyspepsia (FD). The hypothalamic hormone oxytocin (OXY) is recognized as an antistress peptide; activation of OXY pathways to the dorsal vagal complex (DVC) or microinjections of OXY in the DVC itself induce a vagally-dependent gastric relaxation. The aims of the present study were to investigate the *In vitro* and *in vivo* mechanisms of action of OXY in a rodent FD model.
Methods: Ten-day old rat pups of either sex were gavaged daily for 6 days with a solution of 0.1% iodoacetamide. Subsequently, either whole cell patch clamp recordings were made subsequently from identified gastric-projecting DMV neurons in thin brainstem slices or gastric motility and tone were recorded in responses to brainstem microinjections of OXY.
Results: In control rats, OXY had no effect on evoked or miniature GABA currents in any of the neurons tested unless the neurons were pretreated with the stress hormone corticotrophin releasing factor (CRF). Following CRF, OXY altered GABA synaptic transmission in the majority of neurons tested. Conversely, in FD animals, OXY altered GABA neurotransmission in the majority of DMV neurons tested, without the need to for CRF pretreatment. In control rats, OXY microinjections induced a dose-dependent decrease in gastric tone and motility via activation of the vagal inhibitory NANC pathway and subsequent nitric oxide release. Following FD, however, not only did rats become more

sensitive to the gastroinhibitory effects of DMV microinjection of OXY, but the effects were not abolished by the NOS inhibitor L-NAME, implying that the gastric relaxation was likely to result from inactivation of vagal cholinergic excitatory pathways.
Conclusion: These data show that in a rodent model of FD (i) the gastroinhibitory response to brainstem microinjection of OXY is altered and (ii) GABA neurotransmission is modulated by OXY. Indeed, our data suggest that in FD, the OXY-induced gastroinhibition results from engagement of different vagal neurocircuits and neural pathways. Supported by: NIH DK55330.

050
A biomagnetic technique to evaluate the effects of partial gastrectomy on gastric emptying and gastrointestinal transit time in rats
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Objective: The partial gastrectomy is a surgical procedure that has been widely employed and seems to cause changes in some gastrointestinal (GI) properties. The aim of this study was to evaluate the influence of partial gastrectomy in the gastrointestinal transit time in rats employing the AC Biosusceptometry.
Methods: Six male Wistar rats were evaluated before surgery, 1 week after surgery and 2 months later with ingestion of a magnetic pellet. Each period consisted of monitoring the magnetic intensity values on stomach and cecum projections by an ACB sensor positioned on abdominal surface. All signals were analyzed in MatLab® by visual inspection and the statistical moment was calculated. The statistical moment was obtained through the temporal average pondered by magnetic intensity curves, normalized by area under curve. Using this approach, it is appropriate to quantify the following parameters: Mean Gastric Emptying Time (MGET), Mean Cecum Arrival Time (MCAT) and Mean Small Intestine Transit Time (MSITT). Statistical analysis was performed by Student's *t*-test and a *P* value of 0.05 was considered statistically significant.
Results: Values for MGET, MCAT and MSITT obtained before, 1 week after and 2 months later the surgery were: MGET, 168 ± 20 min, 127 ± 6 min ($P < 0.05$ *vs* before) and 110 ± 3 min ($P < 0.05$ *vs* before); MCAT, 327 ± 26 min ($P < 0.05$ *vs* before), 263 ± 15 min ($P < 0.05$ *vs* before) and 221 ± 8 min; and MSITT, 159 ± 15 min, 135 ± 11 min and 110 ± 8 min, respectively.
Conclusion: Our data suggest that gastrointestinal transit parameters were strongly influenced by partial gastrectomy. The widespread use of partial gastrectomy implies in a good understanding about their effects on gastrointestinal motor parameters that only can be reached by noninvasive and repeated measurements provided by biomagnetic techniques. AC Biosusceptometry was able to monitor with accuracy spatial and temporal alterations provoked by this surgical procedure.

051
Long term kaolinite ingestion decreased gastric emptying rate as a consequence of duodenal mucosa remodeling
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Objective: Conflicting results showed accelerated or unchanged gastric emptying after long-term supplementation of the diet with kaolinite, a clay used as a protective compound for the injured gut. We aimed to evaluate in conscious pigs, using scintigraphic imaging, the consequences of 4 weeks ingestion of 5% kaolinite on gastric emptying of a semi-solid meal. Since kaolinite ingestion could modify the absorption of nutrients, hence modulating duodeno-gastric reflex, we tested gastric emptying with and without duodenal infusion of lipids.
Methods: Eight pigs were surgically fitted with a duodenal catheter under general anaesthesia. After 1 week recovery, the animals were divided in two groups depending of the diet: the first group received 5% kaolinite whereas the second get an iso-caloric diet containing silicate microspheres used as an a-caloric control. After 4 weeks, two scintigraphic sessions were achieved 1 week apart; one with and one without Intralipid infusion (1 ml min-1 starting 15 min before the onset of the meal and continued for 2 h) in the duodenum. Gastric emptying was measured by automated analysis of scintigraphic images obtained every 2 min during 2 h postprandial. The test meal consisted in 500 ml porridge labelled with 40 MBq 99 m-Tc-Colloid. At the end of the experiment, the animals were euthanatized and the small intestine collected for histological analysis.
Results: Gastric emptying of porridge in the absence of intralipid was unchanged for the two groups (T1+2; 80 ± 13.7 *vs* 77 ± 10.3 min for kaolinite and microspheres groups, $P > 0.05$). On the contrary, gastric emptying differed between groups in the presence of duodenal intralipid (T1/2; 514 ± 86.8 *vs* 302 ± 57.3 min for kaolinite and microspheres groups, $P < 0.01$). The reduced gastric emptying rate observed after kaolinite ingestion was associated microscopically at the duodenal level only with significant decreases ($P < 0.05$) in the number of goblet cells (0.03 ± 0.002 *vs* 0.05 ± 0.002 μm^2 for kaolinite and microspheres) and crypts length (418 ± 26.1 *vs* 594 ± 43.9 μm for kaolinite and microspheres).
Conclusion: These results suggested that the duodenal structural changes induced by kaolinite resulted in an increased intensity of the afferent limb of the duodeno-gastric reflex.

052
Age-induced changes in gastric myoelectrical activity and the effect of nicotine in ICR mice
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Objective: During aging, there is a reported loss of neurons in the brain and enteric nervous system; a loss of enteric neurons may impact on slow wave activity and motility patterns. Electrogastrography (EGG) is normally used to record gastric myoelectrical activity (GMA) in humans. The aim of the study was to investigate if there are age-related changes in the sensitivity of the gastrointestinal tract to nicotine by recording GMA in mice. It is hoped information from the studies would provide a novel insights into GI motility dysfunction during aging and its responsiveness to drug treatments.
Methods: Forty-one male ICR mice (3, 6, and 12 month old, 28–38 g) were anaesthetized and surgically implanted with telemetry devices (PhysioTel® ETA-F20, Data Sciences International, U.S.A.) with recording wires sutured into the serosal side of the stomach. 7 days later, animals of each age group were randomized to receive vehicle (saline 2 ml kg^{-1}, i.p.) or nicotine (3 mg kg^{-1}, i.p.). Baseline GMA recordings were obtained for 2 h before drug administration and were continued for a further 6 h. Raw data (sampled at 1000 Hz) were processed using Spike2 (Cambridge Electronic Design, U.K.) and analysed using repeated measures 2-way ANOVA (factors: treatments and time (average 10 min)) and Bonferroni post-tests.
Results: There were no age-related differences between the 2 h baseline data of 3-, 6- and 12-month old animals (Dominant frequency, DF: 7.1 ± 0.8 cpm, 7.1 ± 0.8 cpm, and 7.3 ± 0.8 cpm, respectively; $n = 16$, 15, and 10, respectively; $P > 0.05$). Saline had no effect on any of the parameters of the slow waves during the experiment ($P > 0.05$). However, nicotine reduced the DF gradually in the three age groups, producing significant increases in the % power of the bradygastric range (0 to DF-1.5 cpm). The effects of nicotine lasted for 2 h before the DF shifted back to pre-nicotine levels. The % power of normogastric (DF±2 cpm) and tachygastric range (DF+2 to 15 cpm) was not affected by nicotine in any age group ($P > 0.05$). The power in the bradygastric range of 12 month old mouse was significantly higher than that of the 3 month old mouse ($P < 0.05$); no other statistically significant differences were noted.
Conclusion: Nicotine caused bradygastria in three age groups, suggesting an action to predominantly release inhibitory mediators to affect ICC. The inhibitory action of nicotine appeared to increase in the older animals which may reveal age-related changes in the balance of inhibitory/facilitatory control of ICC.

053
Identification of the major site of action for motilin-induced contraction within the suncus stomach
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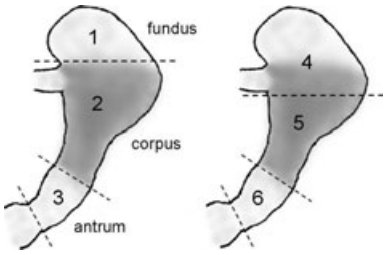
Objective: Motilin, a gastrointestinal hormone released from the duodenum in the fasting state, stimulates gastrointestinal motility. In an earlier study on house musk shrews (Suncus murinus), a motilin-producing small laboratory animal, we showed that motilin significantly evoked stomach contraction in a dose-dependent manner both *in vivo* and *In vitro*. For many years, it has been suggested that a certain site in the stomach is particularly responsive to motilin and that motilin-induced gastric propagated contraction originates at this site. In this study, we hypothesized that an area around the cardia is important for motilin-induced gastric contraction; thus, we compared motilin-induced contraction in each separated suncus stomach parts by using an organ bath system.
Methods: Female suncus stomachs were isolated and separated into three parts as shown in Fig. 1 and were set in an organ bath system in the longitudinal axis. To examine the effect of motilin, different concentrations of motilin (10^{-10}–10^{-7} mol L^{-1}) were used in each

separated stomach, and motilin-induced contractions were measured.
Results: Motilin-induced contractions differed in different parts of the stomach. In the fundus (Fig. 1–1), motilin evoked only slight gastric contractions, whereas in the fundus with the upper corpus (Fig. 1–4), motilin significantly induced gastric contractions even at low concentrations (10^{-9} mol L^{-1}). In contrast, gastric contraction of the corpus (Fig. 1–2) was strongly induced by low concentrations of motilin (10^{-9} mol L^{-1}); however, motilin-induced contractions decreased in the middle to lower corpus (Fig. 1–5). In the antrum (Fig. 1–3 and 1–6), although acetylcholine (10^{-5} mol L^{-1}) strongly induced gastric contractions, motilin only slightly induced gastric contractions.
Conclusion: In this study, we showed that the separated stomach parts retained the ability to react to motilin similar to that observed in the whole stomach *In vitro*. We also found that motilin reactivity differed in different parts of the stomach. These results suggest that motilin reactivity is not consistent throughout the stomach, and an area boundary between the fundus and the corpus, including the cardia, is an important site of motilin-induced contraction.



054

Up-regulated ß1-adrenoceptor mediates the inhibition of norepinephrine on the gastric contractility in 6-OHDA rats

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Objective: Gastroparesis is one of the non-motor syndromes of Parkingson's disease (PD). The mechanism(s) responsible for this abnormality is not well understood. It has been reported that PD patients often manifest sympathetic nerve accentuation. An up-regulation of β1,2,3-adrenoceptors (β-AR) and weakness of gastric motility were observed in the rats with bilateral microinjection of 6-hydroxydopamine (6-OHDA) in the substantia nigra (6-OHDA rats). The present study aims to investigate the role of β-ARs in the gastroparesis in the 6-OHDA rats.
Methods: The expressions of β-adrenoceptors in the stomach were evaluated by immunohistochemistry, RT-PCR and Western blot. The contraction of gastric longitudinal muscle strips from control and 6-OHDA rats was studied by organ bath *In vitro*.
Results: Three subtypes of β-AR, β1, β2 andβ3 were expressed in the gastric muscularis externa of rats.β3 was much higher than the other two subtypes. IC50 (the half maximal inhibitory concentration) of NE was 1.5×10^{-7} mol L^{-1} in 6-OHDA rats *vs* 3.4 × 10-7 mol/L in control rats, reflecting an higher adrenergic reactivity in the 6-OHDA rats. Both β1 and β2 - AR antagonists, Atenolol (1.0×10^{-5} mol L^{-1}) and ICI118551 (1.0×10^{-5} mol L^{-1}), did not affect the inhibition of NE on the strip contraction in normal rats, but β1- AR blockade induced a right shift of the NE concentration-response curve in 6-OHDA rats and normalized IC50 values was increased from 1.9×10^{-7} to 6.1×10^{-7} mol L^{-1} (n = 8, P < 0.05), indicating β1-AR mediates the inhibition of NE on the gastric contractility in 6-OHDA rats. Furthermore, atenolol (20 μg kg^{-1}) relieved the impaired gastric motility of 6-OHDA rats *in vivo*. Although electiveβ3-AR antagonist, SR 59230A (1.0×10^{-5} mol L^{-1}), significantly blocked the inhibition of NE on both normal and 6-OHDA rats, no significant difference was found between the two groups. The mRNA and protein expression of β1- AR in gastric muscularis externa was three and two times higher in 6-OHDA rats than control ones, respectively.
Conclusion: The present study suggested that adrenergic inhibition of gastric contractility is dependent on β3-AR pathways in normal rats, whereas the up-regulated β1- AR could be responsible for the enhanced NE reactivity in 6-OHDA rats, which should be one of the factors leading to the gastroparesis of 6-OHDA rats.

055

The influence of monosodium glutamate on the stability of gastric mucosa to the stress action in rats

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Objective: Well known flavor enhancer – monosodium glutamate (MG), E 621 is widely used in food industries. However, its excessive consumption causes of ''Chinese Restaurant Syndrome''. We chose doses 15 and 30 mg kg^{-1} (correspond to 1 and 2 g for human) because due to the literature 1 g MG has no inauspicious action on the human, but 3 g are hazardous to health. Therefore, the aim of this work was to study the effects of long-term consumption of MG on gastric mucosa (GM) and the stability of GM to the stress action in rats.
Methods: The study was carried out on 42 rats, which were divided into four groups. To the rats of I group during 30 days we injected 0.5 ml water per os. The animals of II group in 30 days of water injection (0.5 ml per os) were subjected to the water immersion restraint stress (WRS). The rats of III and IV group during 30 days received 15 and 30 mg kg^{-1} of MG, consequently and after that they were subjected to the WRS. In 30 days in rats of all groups we investigated the state of GM.
Results: In GM of rats after 30 days injection of MG (15 mg kg^{-1}) ulcers and erosions were developed. The area of ulcers was 4.0+0.22 mm^2 and the length of erosions was 2.57+0.2 mm. Increase of dayly dose of MG to 30 mg kg^{-1} enhanced the damages of GM. WRS in rats of II group evoked the development of ulcers (10.79+2.02 mm^2) and erosions (3.7+0.8 mm). In rats after 30 days of MG injection in dose 15 and 30 mg kg^{-1} WRS evoked more profound destructive processes in GM. So the area of ulcers and length of erosions in GM of rats which received 15 mg kg^{-1} MG were increased respectively by 97% (P < 0.05) and by 168% (P < 0.01) compared with a group of stress-control. Double daily dose of MG showed much stronger effect on GM in rats which were exposed to stress.
Conclusion: (1) Long-term consumption of MG leads to ulceration and erosion in GM;
(2) MG decreases stability of GM to stress action.

056

Transcranial direct current stimulation (TDCS) can modulate esophageal motility in gastroesophageal reflux disease (GERD) patients

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Objective: To evaluate the effects of transcranial direct current stimulation (TDCS) on esophageal manometric parameters in patients with GERD.
Methods: We studied 40 patients with clinical diagnosis of GERD (''Montreal definition'') who previously underwent endoscopy to differentiate erosive reflux disease (ERD) from non-erosive reflux disease (NERD). An esophageal manometry was performed before and during cortical stimulation with TDCS (1, 5 mA) on the right esophageal motor area. Randomly twenty patients were assigned to anodal stimulation, twenty patients to sham stimulation. Distal waves amplitude and number of pathological waves (distal amplitude <30 mmHg or not propagated distal peristalsis) were measured 3 cm over the lower esophageal sphincter (LES) after swallowing a water bolus, for ten subsequent times. LES pressure was obtained as well. A 24-hours pH-metry was further performed to rule out functional heartburn. Mean waves amplitude, number of pathological waves and mean basal LES pressure were compared by paired-samples T-test before and during TDCS in both groups of patients.
Results: Mean distal waves amplitude increased significantly (P = 0.04) and the number of distal pathological waves decreased significantly (P = 0.01) during anodal TDCS, while sham stimulation did not influence both parameters. LES mean pressure was not significantly modified during anodal nor sham stimulation in GERD patients. Comparison between groups (NERD *vs* ERD) showed that significant changes after TDCS occurred only in NERD subgroup.
Conclusion: Our data suggest that TDCS can influence cortical control of esophageal motility and improve pathological motor pattern in NERD patients.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-05 Basic and Translational Session: Probiotics, Pharmacotherapy, Pharmacogenomics and Pharmacology

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Facilitation of cholinergic activity in human isolated stomach by metoclopramide and by the selective motilin receptor agonist GSK962040, but not by domperidone

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Objective: Metoclopramide and domperidone are used to increase gastric emptying in patients with gastroparesis and other disorders. Both antagonise at dopamine D2 receptors, whilst metoclopramide also activates 5-HT4 receptors. Our aims were to examine their abilities to facilitate cholinergic activity in human isolated stomach (representing the functions of the main motor-neurotransmitter regulating gastric motility) and compare their effects to the selective motilin receptor agonist GSK962040, an alternative approach to stimulating gastric motility.
Methods: Human gastric antrum was obtained at surgery following informed consent. After removing the mucosa, strips were cut parallel to the circular muscle and suspended between ring electrodes in tissue baths for isometric recording (Kreb's; 5% CO_2 in O_2; 37°C; 1 g tension). Electrical field stimulation (EFS) was applied at 5 Hz (0.5 ms pulse width, 50 V, 10 s) every 1 min, for sub-maximal responses.
Results: EFS-evoked contractions were predominantly cholinergically-mediated, attenuated by simultaneous nitrergic activation. They were prevented by 1 μ mol L^{-1} tetrodotoxin (n = 3), inhibited by atropine 1 μ mol L^{-1} (n = 3) and facilitated by the nitric oxide synthase inhibitor L-NAME 0.3 m mol L^{-1} (by 57 ± 18% n = 15). Metoclopramide (1 n mol L^{-1}–100 μ mol L^{-1}; n = 3 each concentration) increased the magnitude of EFS-evoked contractions at or above 10 μ mol L^{-1} (Emax=131 ± 36%; EC50 = 13 μ mol L^{-1}) with no effect on baseline muscle tension (P = 0.23). Domperidone (1 n mol L^{-1}–100 μ mol L^{-1}) at concentrations which bind to D2 receptors (Kd 0.42 n mol L^{-1}; Seeman et al 2003, Synapse 49: 209–15) had no effects on baseline muscle tension or on responses to EFS (n = 3 each concentration), except at 100 μ mol L^{-1} where there was a tendency for both EFS-evoked contractions and baseline muscle tension to increase. GSK962040 30 μ mol L^{-1} (a maximally-effective concentration; Broad et al 2012, Br J Pharmacol in press), increased EFS-evoked contractions by 703 ± 264% (n = 8; P = 0.03) and tended to increase baseline muscle tension (by 171 ± 119% EFS; n = 8; P = 0.20).
Conclusion: In human isolated stomach, gastric cholinergic activity was unaffected by dopamine D2 receptor antagonism. However, metoclopramide increased cholinergic activity, possibly by acting at 5-HT4 receptors, an activity likely to explain its gastric prokinetic activity. Facilitation of cholinergic activity was also achieved by GSK962040 but to a much greater level, suggesting greater potential for motilin receptor agonists to increase gastric motility.

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A probiotic treatment attenuates Hypothalamic-Pituitary-Adrenal (HPA) axis response to chronic psychological stress through improvement of cellular proliferation and changes of gene expression in the brain

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Objective: In humans and rodents, a probiotic formulation (L.helveticus R052 and B.longum R175, Probio'Stick®) displays anxiolytic-like activity1 and modulates apoptosis propensity in the lymbic and hippocampic areas in post-myocardia infarction heart failure in rats, associated with depressive behaviour2. Herein we aimed to evaluate the effect of this probiotic formulation on HPA axis response to chronic psychological stress and neuronal plasticity in the brain.
Methods: Eight groups of 10 male 6–8-week-old C57BL/6J mice received orally during 15 days either saline or the probiotic formulation (109 CFU /mice/day). Mice were submitted to a chronic stress (water avoidance stress, WAS) 1 h day^{-1} during four consecutive days or sham-WAS. In the 1st series of experiments, four groups of animals were used, and under general anaesthesia blood was drawn from the abdominal aorta and corticosterone, noradrenaline and adrenaline plasmatic levels were determinated using Elisa kit. Transcriptional analyses of neuronal gene plasticity were investigated on hypothalamus. In the 2nd series of experiments, the brain of animals was removed and free-floating sections were immunostained for Fos (neuronal activity marker), doublecortin (DCX, neuroblast differentiation marker) and NeuN (mature neurone marker) positive cells evaluation.
Results: Corticosterone, noradrenaline and adrenaline plasmatic levels was dramatically increased (P < 0.05) in mice after WAS. Compared to sham-WAS controls, WAS increased (P < 0.05) Fos positive nuclei number in the paraventricular nucleus (PVN) of the hypothalamus, the amygdaloid nucleus (AN) and CA-3 region of hippocampus while in dentate gyrus (DG) of hippocampus, the number of Fos positive nuclei was significantly lower than in sham-WAS group. Further, the number of DCX and NeuN positive cells was reduced (P < 0.05) in comparison with sham-WAS group. A significant impact of WAS was observed on hypothalamus transcriptome involved in the neuronal plasticity. The treatment by the probiotic formulation, decreased (i) corticosterone, noradrenaline and adrenaline plasmatic levels (ii) the number of Fos positive in the PVN, AN and CA-3. In the DG the decrease of Fos positive nuclei induced by the WAS was normalized by the probiotic formulation treatment. Moreover, this probiotic treatment prevents the WAS-induced reduction in the number of DCX, NeuN expressing cells in the DG and abnormal neuronal reorginization in the hypothalamus.
Conclusion: This study shows that a two week treatment by the probiotic formulation Probio'Stick® attenuates HPA axis response to stress as reflected by the decrease of neuroendocrine hormone and prevents the stress-induced reduction of cell proliferation in the hippocampus. We hypothetize that the probiotic promotion of cell proliferation in the hippocampus restores the HPA axis negative feedback.

059

Effect of erythromycin and sumatriptan on meal induced gastric accommodation and maximum nutrient tolerance assessed by a novel nutrient drink test

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Objective: Gastric relaxation in response to a meal, a.k.a. gastric accommodation (GA) is an important physiological mechanism modulating gastric pressure during food intake. GA can be assessed by a nutrient challenge test utilizing high resolution manometry (HRM) (Janssen et al., NMO 2011). This novel approach provides us with an accurate and easy way to study GA. Previously it was shown with barostat studies that administration of erythromycin, a motilin receptor agonist, contracts the stomach and reduces GA. Conversely, administration of sumatriptan, a 5-HT1B/1D agonist, relaxes the proximal stomach and normalizes GA in functional dyspepsia. We aimed to further evaluate intragastric pressure (IGP) as a determinant of meal intake by evaluating the effect of erythromycin and sumatriptan on IGP and meal-induced satiation.
Methods: In fasted healthy volunteers (n = 11) a HRM probe and an infusion catheter was positioned in the proximal stomach. After a stabilization period a nutrient drink (Nutridrink, 1.5 kcal ml^{-1}) was intragastrically infused at 60 ml min^{-1}. At the same time, erythromycin 200 mg (IV) or placebo was administered. The volunteers scored satiation until the level of discomfort at which point the experiment was stopped. After a 1 week washout period the experiment was repeated in a cross-over design. A second set of experiments, again on healthy volunteers (n = 12), were performed in the exact same setting with sumatriptan 6 mg (SC). The IGP was presented as a change from baseline (mean ± SEM) and compared with a paired t-test.
Results: Volunteers scored maximum satiation at 1096 ± 121 *vs* 790 ± 72 ml after placebo and erythromycin respectively (P = 0.003). Similarly, nadir pressure values were significantly higher after erythromycin (-3.52 ± 0.48 mmHg *vs* -2.32 ± 0.47 mmHg; P = 0.002). The area under the curve for pressure over time graphs was significantly lower with erythromycin (2.12 ± 0.35 mmHg•sec *vs* 1.46 ± 0.32 mmHg•sec; P = 0.004). In contrast, sumatriptan had no significant effect on MTV, IGP and AUC values.
Conclusion: We further confirmed that IGP is an important determinant of satiety which can be assessed by HRM during a nutrient challenge. While sumatriptan had no significant impact, erythromycin decreases GA and this is associated with decreased nutrient tolerance.

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Serotonin transporter gene influence on effectiveness of cognitive behavioral therapy for patients with Irritable Bowel Syndrome (IBS)

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Objective: Other researchers have shown that the effectiveness of antidepressant and CBT therapy differs according to erotonin transporter (SERT) gene polymorphism (5HTTLPR). This study examined whether this is also true of a Comprehensive Symptom Management (CSM) therapy for IBS.
Methods: Data are from a previously published study which showed the effectiveness of CSM in reducing IBS symptoms and negative thoughts about IBS and in improving IBS-related quality of life. Mixed model analysis of covariance was used to analyze psychological distress at the three follow-up times while controlling for baseline psychological distress, gender, and race as covariates. The term for interaction between treatment CSM versus Usual care (UC) and SERT (SLC6A4) genotype tested whether the effectiveness of CSM differed by genotype. The primary outcome variables were psychological distress as measured by combining seven symptoms from a daily 4-week diary and by the Brief Symptom Index retrospective questionnaire. Secondary outcomes were a composite GI symptom severity score from 4-week diary and the cognitive symptoms for CSFBD which measures negative cognitions about IBS symptoms.
Results: Follow-up data and genotype were available for 132 subjects, with genotype breakdown 46 l/l, 60 s/l, and 26 s/s. The interaction between treatment group and genotype was significant for psychological distress as measured by diary ($P = .005$) and CSFBD ($P = .019$), with CSM showing a benefit relative to UC in the l/l and s/l genotypes, but not s/s. The interaction term was not significant for psychological distress by the Brief Symptom Index ($P = .652$) nor GI symptom severity ($P = .210$).
Conclusion: Our finding that the s/s genotype is associated with poorer response to CSM is similar to results from studies of major depression showing the s allele to be associated with poorer response to SSRI antidepressants. If these results can be replicated, it would have implications for tailored care.

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Local transdermal delivery of phenylephrine using hollow microneedles as a treatment of fecal incontinence

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Objective: Our previous study presented that locally targeted delivery of phenylephrine (PE) to perianal muscles using solid microneedles could increase resting anal sphincter pressure (J Control Release 2011; 154(2): 138–147) However, topical application of PE on the skin pretreated by solid microneedle array have some limitations. To overcome limitations for clinical application, hollow microneedle system was utilized to deliver predetermined dose of PE through perianal skin (Fig. 1). The objective of this study was to demonstrate that local PE delivery by few administrations around anus using hollow microneedle system can induce elevation of anal sphincter tone with biological safety.
Methods: After administering PE using a hollow microneedle into skin, drug distribution around anus was investigated using fluorescent dye (Rhodamine B) and IVIS (*in vivo* imaging system). The increase in rat anal pressure by administration of PE using hollow microneedles was investigated in terms of concentration of PE and administration methods IV, SC and IM. Anal pressure of sober rats was monitored after administration of drug as function of time using Solenette Urodynamic. To study side effects of locally delivered PE using hollow microneedles, Non-Invasive Blood Pressure system was used to measure the blood pressure of rats regarding dose of PE and administration methods.
Results: After Rhodamine B was injected using hollow microneedle into peri-anal skin area, relatively strong signal lasted for over 6 h and weak signal could be seen until 12 h after injection (Fig. 2). Topical administration of PE using hollow microneedles increased the mean resting anal pressure significantly compared with negative control in a concentration dependent manner ($P < 0.05$, $n = 5$ respectively). In the comparison of efficacy among treatment methods, IV, SC, IM and hollow microneedle injection of PE with same dose of 50 μg, hollow microneedle system enhanced the mean resting anal pressure significantly ($P > 0.05$, $n = 5$ respectively)(Fig. 3).
Conclusion: Hollow microneedle-PE system induced significant contraction of internal anal sphincter at least 6 h after injection. This new administration system could provide potential treatment of fecal incontinence by painless microneedle injection of PE into perianal skin topically. (Experiments of side effects are still under way).

Figure 1. Image of hollow microneedle for fecal incontinence (length < 1mm, outer diameter: 350μm, inner diameter: 200 μm)

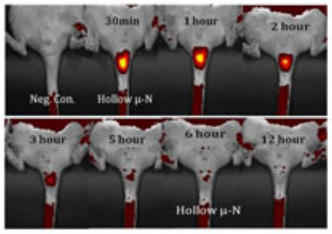

Figure 2. In vivo imaging of Rhodamin B after application using hollow microneedle.
Relatively stable signal lasted for 6 hours and weak signal could be seen until 12 hours after injection

062

Granisetron prevents alterations of gastrointestinal motility in rats repeatedly treated with cisplatin: Radiographic study

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Objective: To study, using radiographic methods, the effect of the 5-HT3 antagonist granisetron on gastrointestinal dysmotility induced in the rat by repeated cisplatin administration.
Methods: Wistar rats received an intraperitoneal (ip) administration of saline or cisplatin (2 mg kg^{-1}) once a week for 5 weeks. Granisetron (1 mg kg^{-1}, ip.) or vehicle (saline, 1 ml kg^{-1}) was administered 20 min before saline or cisplatin each week. Body weight gain was measured throughout treatment. Radiological techniques were used to determine the acute (after first dose), chronic (after last dose) and residual (one week after treatment finalization) effects of cisplatin and/or granisetron on gastrointestinal motility. The size of the stomach was also measured.
Results: Repeated cisplatin induced weight loss which granisetron did not prevent. Gastric emptying was dose-dependently delayed after the first cisplatin administration. Granisetron completely prevented this effect. After weekly administration, cisplatin-induced gastric dysmotility was enhanced and granisetron was not capable of totally preventing this effect. No significant effect was detected 1 week after cisplatin. However, at this time point, when both drugs were given together significant modifications in gastrointestinal motor function were apparent.
Conclusion: Granisetron prevents gastric emptying alterations, but its efficacy is decreased throughout antineoplastic treatment. This might be due to the

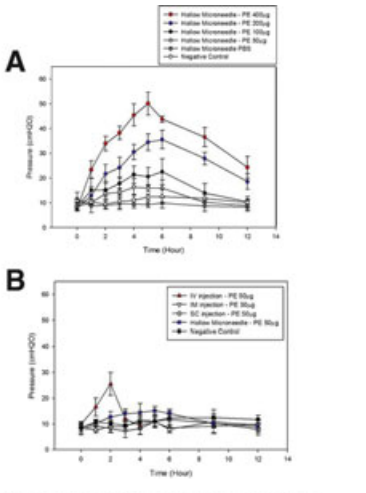

Figure 3. Measurement of resting anal pressure according to different concentrations of phenylephrine(PE) and delivery methods (A) Topical administration of PE using hollow microneedles increased the mean resting anal pressure significantly compared with negative control in a concentration dependent manner ($P < 0.05$, $n=5$ respectively). (B) In the comparison of efficacy among treatment methods, IV, SC, IM and hollow microneedle injection of PE with same dose of 50 μg, hollow microneedle system enhanced the mean resting anal pressure significantly ($P > 0.05$, $n=5$ respectively).

enhanced effect of cisplatin. X-rays are valuable tools to analyse chronic effects of chemotherapy and prokinetic drugs.

063

Novel anti-inflammatory functions for the herbal drug STW 5: A link between cytokine regulation and gastrointestinal inflammation

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Objective: Epidemiological studies show that, in ~25% of patients with irritable bowel syndrome (IBS), bacterial gastroenteritis precedes the onset of the disorder. Subtle inflammation or immune activation has been reported in the results of biopsies and altered peripheral cytokine profile has been shown in a subset of IBS patients. The multi-herbal drug STW 5 (Iberogast®) was effective for treatment of symptoms in patients with functional dyspepsia or IBS. Its mode of action is still not fully elucidated. Modulation of gastric motility and an anti-inflammatory action were hypothesized. These findings provide the basis for investigating STW 5 and its components in inflamed small and large intestinal preparations to identify novel anti-inflammatory pathways.
Methods: The inflammation was induced by intraluminal instillation of 2, 4, 6-trinitrobenzene sulfonic acid (TNBS, 0.01 mol L^{-1}). Contractions were measured isometrically. Gene expression was determined using qRT-PCR.
Results: Preincubation of STW 5 (64–512 μg ml^{-1}) or its components in equivalent concentrations together with TNBS prevented the inflammation-induced disturbances in the muscle wall and hence the depression of acetylcholine (ACh)-induced contractions. In accordance to these findings cytotoxicity testing revealed a cell protective effect of STW 5. The TNBS-induced inflammation in ileum and colon preparations was accompanied by increased TNF-alpha gene expression and without effect on the gene expression of the anti-inflammatory cytokine IL-10. STW 5 inhibited the increased gene expression in ileum but not in colon preparations whereas the gene expression of IL-10 was increased in both preparations by STW 6 (Iberis amara), a component of STW 5. Further experiments indicated that activation of adenosine A2A receptors is involved in the regulation of TNF-alpha gene expression by STW 5, whereas the cucurbitacins E and I in STW 6 could be responsible for activation of the IL-10 pathway.
Conclusion: STW 5 reveals significant anti-inflammatory properties which contribute to the reduction of morphological and contractile disturbances. Its mode of action seems to be twofold, inhibition of the TNF-alpha and activation of the IL-10 pathway. Anti-inflammatory mechanisms and cell protective actions of STW 5 give a clear-cut correlation with symptom improvements in clinical trials and highlight the relevance of STW 5 as a therapeutic approach in IBS.

064

Effect of DA-9701, a novel prokinetic agent, on delayed gastric emptying induced by stress in rats

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Objective: Acute stress is known to alter gastric motility. We aimed to investigate the effects of acute stress on gastric emptying and the possibility of whether DA-9701, a novel prokinetic agent formulated with Pharbitis Semen and Corydalis Tuber, improves gastric emptying altered by stress in rats.
Methods: Animals were fasted for 24 h before experiments. Rats were immobilized in a restrainer and subsequently immersed in water up to xiphoid in a water bath maintained at 18 ± 1°C. Rats of the control group were placed in their home cages without exposure to any stress. DA-9701 (3 mg kg^{-1}) or mosapride, a 5-HT4 agonist, (3 mg kg^{-1}) was orally administered after 2 h exposure to stress. After 45 min, gastric emptying, plasma ACTH and ghrelin levels were measured. Gastric emptying was determined by the phenol red method.
Results: Gastric emptying (64.9 ± 6%) was delayed by 2 h of stress (23.4 ± 4.8%). Delayed gastric emptying induced by 2 h of stress (22.4 ± 4.2%) was significantly restored by DA-9701 (69.8 ± 8.6%). Mosapride also improved the stress-induced delay in gastric emptying up to 64.2 ± 2.1%. In the control group, gastric emptying (64.0 ± 3.8%) was not changed by DA-9701 (66.5 ± 3.4%), but accelerated by mosapride (86.3 ± 5.8%). The level of plasma ACTH in the stress group was significantly higher than that in control group. The level of plasma ghrelin in the stress group was significantly higher than that in control group.
Conclusion: DA-9701 improves delayed gastric emptying induced by stress in rats, suggesting that it may be useful in the treatment of functional dyspepsia associated with delayed gastric emptying.

065

Effect of hyoscine butylbromide (Buscopan®) on cholinergic pathways in the human intestine

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Objective: Hyoscine butylbromide (HBB, Buscopan®) is an antispasmodic drug which is used to treat abdominal pain. While animal studies revealed anti-muscarinic actions of HBB, recent data from cell lines suggest that HBB may also interact with human nicotinic receptors. The aim of this study was to investigate the so far unknown functional consequences of the anti-cholinergic action of HBB in human large intestine.
Methods: For our study we used mucosa/submucosa and muscle preparations from normal tissues derived from 72 patients undergoing abdominal surgery. The secretory activity was measured with the Ussing chamber voltage clamp method, motility was recorded using force transducer. Activation of muscle cells was recorded with the Ca-imaging technique and neuronal effects were assessed by voltage sensitive dye imaging.
Results: We could demonstrate with various approaches the functional relevance of the anti-muscarinic actions of HBB in the human intestine. Firstly, the pro-secretory and prokinetic effects of the muscarinic agonist bethanechol were abolished by HBB (1–10 μ mol L^{-1}). Secondly, HBB dose dependently reduced nerve mediated contractions evoked by electrical field stimulation (EFS) as well as EFS induced mucosal secretion. Thirdly, bethanechol evoked Ca^{++} transients in human colonic smooth muscle cells were inhibited or blocked by 1 μ mol L^{-1} or 10 μ mol L^{-1} HBB, respectively. Fourthly, bethanechol induced spike discharge in enteric neurons was reduced by HBB. In further experiments we found that HBB also modulated nicotinic receptor mediated functions, but only at the highest concentration of 10 μ mol L^{-1}. In the presence of the muscarinic antagonist atropine, HBB reduced EFS induced secretory responses. Nicotine (10 μ mol L^{-1}) induced relaxation in human muscle strips was attenuated by HBB. In addition, HBB reduced nicotine evoked spike discharge.
Conclusion: Our functional *In vitro* experiments demonstrated for the first time the anti-cholinergic actions of HBB in the human intestine. HBB inhibits muscle contractions, epithelial secretion and nerve activity through its antagonistic action on muscarinic receptors. Inhibition of nicotinic responses occurred only at high concentrations. The clinical benefit of HBB as an antispasmodic may be best explained by its anti-muscarinic actions. The contribution of the anti-secretory effect for the spasmolytic efficiency remains to be clarified.

067

Long-term effects of otilonium bromide on tachykinin receptors, substance P and NOS expression in the rat colon muscle coat

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Objective: Otilonium bromide (OB), a quaternary ammonium derivative, orally administered causes a dose-dependent increase of gut transit time and eliminates the exaggerated colonic motor responses in patients affected by irritable bowel syndrome (IBS). OB has a composite mechanism of action, is able to bind to the neurokin-2 receptor (NK2r) and to inhibit NK2r-mediated contraction and NK2r-internalization in the smooth muscle cells (SMC). The excitatory neurotransmitter Substance P (SP), interacting mainly with the neurokinin-1 receptor (NK1r), can both stimulate and inhibit bowel motility by SMC direct activation or by an indirect action through enteric neural circuits. In an IBS rat model, the increased NK1r-mediated colonic motor response is due to decreased nitrergic inhibitory pathways. Recently, a clinical trial showed a long-lasting OB beneficial effect after its discontinuity. Our objective was to test whether repeated OB administration modifies NK1r, NK2r, SP and nNOS expression.
Methods: Male Wistar rats untreated and treated with OB 2 or 20 mg Kg^{-1} day^{-1} for 10 or 30 days were used. OB was dissolved in the tap water. At the end of the treatments, specimens of proximal colon were collected and the expression of NK1r, NK2r, SP and neural and myogenic nNOS were evaluated by immunohistochemistry and Western blot. Results were quantified.

Results: SP expression in myenteric ganglia was found significantly decreased in 10 and 30 days treated rats, and, at 30 days, also in intramuscular nerve fibres. At both time points none of the two NKr showed quantitative changes, while after 30 days the NK1r was concentrated in the SMC cytoplasm. In parallel to SP decrease, the neural nNOS expression increased in all nerve structures and reached the significance after 30 days of treatment. The myogenic nNOS expression was significantly increased at 10 days.
Conclusion: Our findings suggest that the main target of the OB chronically administered is the NO-mediated system, earlier stimulated at the muscular level and later at the neuronal level. We interpret the systemic decrease in the SP expression as consequence of the potentiated NO availability in the ganglia and muscle coat. If true, the late concentration of NK1r in the SMC cytoplasm could represent an attempt to overcome the deficit of SP, the main ligand of NK1r.

068

Effects of Butylscopolamine Bromide on nicotinic acetylcholine receptors in cultured Guinea pig enteric neurons

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Objective: Butylscopolamine bromide (HBB; tradename Buscopan®/Buscapina®) is used since decades to treat painful gastrointestinal spasms. Effects on muscarinic, as well as no nicotinic acetylcholine receptors have been described. This study investigated the effects of HBB on nicotinic receptors in adult guinea pig enteric neurons.
Methods: Primary cultures from the enteric nervous system of adult guinea pigs were used. The cultures were kept for several weeks. Cells were investigated in the whole-cell voltage-clamp configuration of the patch-clamp technique and drugs were applied using a semifast application system.
Results: Nicotine induced inward membrane currents at a holding potential of -70 mV with an EC50 value of 64 ± 4 μ mol L^{-1}, which were inhibited by the well described ganglionic blocker hexamethonium. HBB blocked these currents concentration-dependently and reversibly with IC50 values of 4.1 ± 1, 16.8 ± 8, and 5.9 ± 3 μ mol L^{-1}, respectively, at nicotine concentrations of 20 μ mol L^{-1}, 65 μ mol L^{-1}, and 3 m mol L^{-1}, respectively. Thus, inhibition was not reduced by a high agonist concentration, which suggest non-competitive blockade of the receptor. Currents induced by the application of 100 μ mol L^{-1} acetylcholine were comparably reduced by HBB. Block of nicotine responses had fast onset and offset time constants (in the range from 20 to 50 ms).
Conclusion: The inhibitory effect of HBB on muscarinic receptors as well as on nicotinic receptors has already been published (Tytgat (2007)Drugs 67: 1343–1357; Weiser and Just (2009) Neurosci Lett 450: 258–261). In a neuroblastoma cell line, nicotinic responses were inhibited with an IC50 value of 190 n mol L^{-1}. This study, performed in a more physiological model using enteric neuronal cultures of adult guinea pigs, could show that HBB also inhibits nicotinic receptors in native neurons, but interestingly to a weaker extend (approx. 20x less effective compared to the neuroblastoma cell model). Further investigations will be required to clarify the role of nicotinic receptor blockade by HBB for explaining the clinically proven effectivity for treating abdominal cramping and pain.

069

Analysis of fecal microbiota and effectiveness of a probiotic, VSL#3 in patients with functional constipation: A Korean multicenter study

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Objective: This study is to investigate the characteristics of gut microbiome in patients with functional constipation and the influences of a short-term treatment with probiotic, VSL#3 on the microflora, as well as the degree of improvement of symptom profiles.
Methods: Twenty patients fulfilling Rome III criteria for functional constipation and 20 controls were enrolled. Fecal samples were obtained before and after taking VSL#3 powder (450 billion lyophilizedbacteria/sachet twice a day) for 2 weeks. The changes of stool consistency and spontaneous bowel movement frequency per week(SBMF) of patients were investigated using the Bristol stool form chart. Fecal microbiological analyses for five species (Lactobacillus, Bifidobacterium, Escherichia, Bacteroides and Clostridium spp.) were performed using quantitative real-time PCR.
Results: The means of Bristol chart scores and SBMF(self bowel movement frequency) in constipated patients increased significantly after VSL#3 ingestion, compared with the scores before taking (2.5 ± 1.0 *vs* 4.2 ± 1.1, $P = 0.03$, 2.5 ± 1.4 *vs* 7.1 ± 3.2, $P = 0.001$). Relief of subjective symptoms of stool consistency, SBMF, and abdominal bloating was reported in 79%, 58%, and 53% of the patients. Fecal qPCR analyses demonstrated a significant decrease in fold changes of Bifidobacterium species in fecal specimens from constipated patients when compared to healthy controls($P = 0.03$). After taking VSL#3, the fold changes of Lactobacillus, Bifidobacterium and Bacteroides species increased in controls ($P = 0.01$, $P = 0.07$, and $P = 0.08$, respectively), but not significantly in constipated patients.
Conclusion: Quantitative alteration in GI microbiota of functional constipation were found. VSL#3 could be effective on improving clinical symptoms in constipated patients. However, it was unclear in this study that VSL#3 influence an alteration in gut microbiota of constipated patients. More extensive studies are needed to understand how a short-term treatment with VSL#3 can improve constipation without significantly affecting the gut microflora.

070

Effects of TAK-480, a novel tachykinin NK2 receptor antagonist, on visceral hypersensitivity in rabbits and ricinoleic acid-induced defecation in guinea-pigs

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Objective: TAK-480 4-(Difluoromethoxy)-N-((1R,2S)-2-(((3aR,4R,9bR)-4-(methoxymethyl)-2,3,3a,4,5,9b-hexahydro-1H-pyrrolo[3,2-c]quinolin-1-yl)carbonyl)cyclohexyl)benzamide, is a novel tachykinin NK2 receptor antagonist. In this study, we evaluated *In vitro* and *in vivo* NK2 receptor antagonistic activities of TAK-480 in isolated colon and anesthetized animals, respectively. Further, we investigated its efficacy in animal models of visceral hypersensitivity and stimulated bowel function which were implicated to underlie the symptoms in irritable bowel syndrome (IBS).
Results: TAK-480 showed nanomolar binding affinity for cloned human NK2 receptors (IC50 value: 0.95 n mol L^{-1}), and a 10 000-fold selectivity for NK2 receptor versus NK1 and NK3 receptors. Meanwhile, marked species-difference in binding affinity for NK2 receptors was revealed (higher affinity for human, rabbit and guinea pig, lower for rodents). TAK-480 dose-dependently antagonized colonic contractions induced by the administration of NK2 receptor selective agonist beta-Ala8-NKA(4–10) in anesthetized rabbits. In a rabbit model of intracolonic zymosan-induced visceral hypersensitivity, TAK-480 showed significant dose-dependent inhibition of visceromotor response to colorectal distension, in contrast to moderate inhibition by serotonin 5-HT3 receptor antagonist alosetron. In addition, TAK-480 suppressed ricinoleic acid-induced defecation without affecting spontaneous defecation in guinea pigs, whereas alosetron suppressed both at the same dose. Furthermore, TAK-480 inhibited smooth muscle contractions produced by endogenous tachykinins (substance P, neurokinin A, and neurokinin B) as well as a selective NK2 receptor agonist in an isolated human colon preparation.
Conclusion: In conclusion, a novel NK2 receptor antagonist TAK-480 improved visceral hypersensitivity and accelerated-defecation without causing severe constipation in experimental animals. Further, the potent functional blockade of NK2 receptor in human colon might suggest the potential effectiveness of TAK-480 in IBS patients.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-06 Basic and Translational Session: Neuro-Immune Mechanisms

071

Role of 5-HT7 in intestinal muscle function and host defense in an enteric parasitic infection

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Objective: Alterations in gut motility occurs in a wide range of conditions including infective acute enteritis, inflammatory bowel disease, and functional disorders such as irritable bowel syndrome. Enterochromaffin (EC) cells are dispersed through the gut mucosa and are the main source of serotonin (5-hydroxytryptamine; 5-HT) in the gut. Previous studies have demonstrated an increase in intestinal muscle contractility in murine models of enteric parasite infections. Recently, we have shown an important role for $CD4^+$ T helper 2 cells in up-regulation of colonic 5-HT-expressing EC cells and 5-HT content in an enteric parasitic infection. The 5-HT7 receptor is one of the most recently identified members of the family of 5-HT receptors and is expressed in colon smooth muscle. Here, we examined the effect of the 5-HT7 receptor in the development of intestinal muscle contractility and host defense by using 5-HT7 receptor deficient mice in a model of enteric parasite infection.
Methods: 5-HT7-deficient (5-HT7-/-) and wild-type (5-HT7+/+) mice were infected with Trichuris muris orally and sacrificed on various days post-infection to examine carbachol-induced colonic muscle contractility, worm expulsion, and changes in inflammatory and immune responses.
Results: Expulsion of worms was significantly delayed in 5-HT7-/- mice after T. muris infection and this was accompanied by an attenuation of infection induced colonic muscle contractility. There was reduction in IL-9 levels and an up-regulation of IL-1β production in 5-HT7-/- mice as compared to 5-HT7+/+ mice after infection. There was no significant difference in colonic IL-4 and IL-13 levels between 5-HT7-/- and 5-HT7+/+ mice after infection.
Conclusion: These findings suggest that the 5-HT7 receptor plays an important role in generation of infection-induced intestinal muscle contractility and in worm expulsion in T. muris infection. 5-HT7 mediated signaling seems to play a critical role in alteration of muscle function and modulation of immune responses in the context of host defense in enteric infection.

072

An investigation of the therapeutic potential of chymase-containing mast cells in a preclinical model of irritable bowel syndrome and their translational relevance

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Objective: Mast cell chymase has been extensively studied with respect to its role in the pathophysiology of cardiovascular disease. However, an association between chymase and several inflammatory diseases, including gastrointestinal disorders such as inflammatory bowel diseases (IBD) have been described, though little is known about its role in irritable bowel syndrome (IBS).
Methods: Our aim was to investigate the role of rat β chymase-containing connective tissue mast cells (CTMC) on visceral hypersensitivity and epithelial permeability in an anxiety-prone rodent model of IBS, the Wistar Kyoto (WKY) rat, and to investigate the number and potential role for chymase-containing mast cells in healthy and IBS patient biopsies.
Results: Submucosal rat mast cell protease II (RMCPII)-containing mast cells were found to be significantly increased in WKY colon relative to tissues from normo-anxious Sprague Dawley animals ($P < 0.05$). Consistent with this finding, stimulated RMCPII release was significantly increased from WKY relative to SD tissues ($P < 0.01$). Pretreatment with the putative CTMC stabiliser, disodium cromoglycate (DSCG; 50 mg kg^{-1} i.p.) significantly decreased the number of cumulative pain behaviours ($P < 0.05$) as well as threshold to first pain behaviour ($P < 0.01$) in WKY rats in response to colorectal distension (0–80 mmHg). However, transepithelial resistance (TER) did not differ significantly between WKY and SD colon, nor did DSCG affect TER *In vitro*. In human IBS tissues, significantly more colonic chymase-containing mast cells were observed relative to healthy control subjects ($P < 0.05$). Moreover, in Caco-2 cells we observed a chymostatin-sensitive, chymase-induced, increase in permeability.
Conclusion: Therefore, our data suggest that rodent β chymase-containing CTMC play a role in visceral hypersensitivity but not colonic epithelial barrier integrity, in contrast, however, our identification of increased chymase-containing mast cells in close proximity to the colonic epithelium suggest the opposite may be true in human IBS tissues. Whether targeting chymase-containing mast cells with respect to visceral hypersensitivity in a human context remains unexplored.

073

Eosinophils and eosinophil-regulatory molecules as therapeutic targets for the treatment of intestinal inflammation

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Objective: Eosinophils have a crucial role in the pathophysiology of Inflammatory Bowel Disease. Previous studies suggest that changes in the properties of the enteric neurons following induction of inflammation persist due to ongoing mucosal inflammation indicated by increased eosinophil numbers in the lamina propria after the inflammatory insult (Pontell et al 2009; Nurgali et al 2011). Eosinophil-derived factors activate dendritic cells leading to production of inflammatory mediators and can have neurotoxic effect. Eosinophil accumulation is modulated by a subfamily of chemokines, termed eotaxins, specific chemoattractants for eosinophils. In the present study we investigated effects of the inhibition of eotaxin-1 and its receptors, CCR3, in prevention of functional changes in the inflamed intestine.
Methods: Animal experimental model of trinitobenzene sulphonate (TNBS)-induced colitis was used in this study. Inflammation was induced by injection of TNBS (30 mg kg^{-1} in 30% ethanol) into the guinea-pig colon. Guinea-pigs with TNBS-induced colitis were given CCR3 antagonist intraperitoneally 1 h prior to administration of TNBS to block eotaxin-1 receptor and hence block accumulation of eosinophils. Segments of the colon were collected 1 and 7 days after TNBS administration and used for (i) quantification of eosinophil-derived neurotoxin (EDN)-positive cells as a measure of eosinophil activation and evaluation of their proximity to enteric neurons (immunohistochemistry), (ii) assessment of the level of inflammation and presence of eosinophils within the lamina propria and enteric plexuses (histology) and (iii) examination of changes in colonic motility (video imaging).
Results: Inflammation causes significant inhibition of colon motility. Quantitative immunohistochemical analyses showed that the number of EDN-positive cells significantly increases after the onset of inflammation in the lamina propria and in close proximity to myenteric ganglia. Treatment with eotaxin-1 CCR3 receptor antagonist reduced inflammation in the colon, returned EDN populations to normal level and restored colon motility.
Conclusion: This is the first study to investigate implication of eosinophil accumulation by eotaxin-1 in the pathogenesis of intestinal inflammation. The blockade of eosinophil recruitment with CCR3 receptor antagonist was effective in reducing the symptoms of colitis. Inhibitors of eotaxin-1 and its receptors alleviate functional changes in the intestine that occur following TNBS-induced colitis.

074

UC patients in remission have an altered balance between forskolin and carbachol-stimulated chloride secretion in the proximal colon despite a macroscopically normal mucosa

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Objective: Anion and mucus secretion are important components of the colonic barrier. It is well known that the barrier is impaired in active ulcerative colitis (UC) but epithelial function in UC in remission has not been studied systematically. We therefore measured the secretory capacity of colonic biopsies in control patients and UC patients in remission.
Methods: Colonic biopsies were studied in miniaturized Ussing chambers. Square wave current analysis was used to measure epithelial resistance and membrane capacitance. Anion secretion was measured as stimulated current (Im) and mucin exocytosis was estimated as increase in membrane capacitance (Cp) upon carbachol (CCh) stimulation.Forskolin (10 μ mol L^{-1}) and CCh (10 m mol L^{-1}) were used to activate cAMP

and $Ca2^+$ mediated secretion. Biopsies were obtained from subjects undergoing colonoscopy for other reasons (e.g. bleeding of unknown origin, $n = 31$), and patients with UC in remission ($n = 29$). Biopsies were taken from both proximal and distal colon.
Results: In control patients, both baseline values and the response to CCh and forskolin differed between ascending and sigmoid colon. Ascending colon had higher baseline Im, a larger Im response to CCh and a more rapid Cp response to CCh compared to sigmoid colon. Sigmoid colon had a higher baseline Cp and a larger Im response to forskolin compared to ascending colon. Baseline values did not differ between UC patients and controls. In the ascending colon of the UC patients, the Im response to CCh was reduced by 40% while the Im response to forskolin was increased by 35% ($P < 0.05$). The Cp response to carbachol was instead enhanced in the sigmoid colon in the UC patients ($P < 0.05$). The Im response to both forskolin and CCh in this segment were unchanged in the UC patients.
Conclusion: Despite its macroscopically normal appearance, the colonic mucosa of UC patients in remission seems to change its reactivity to secretagogues. This phenomenon may be a protective mechanism to reduce contact with luminal bacteria and may contribute to so called post-inflammatory IBS-like diarrhoeal symptoms in these patients.

075
Do rats and mice differ regarding intestinal muscularis macrophage activation?
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Objective: Both rats and mice are used in studies of various inflammatory processes and it is generally assumed that no species differences exist in the inflammatory response between these rodents. In some conditions where macrophages in the muscularis externa are involved one needs to consider if rats and mice have comparable immunophenotypes. Macrophages have been shown to be activated both by classical and by alternative signaling pathways. Major histocompatibility antigen II (MHCII) immunoreactivity is expressed by dendritic cells and some macrophages, which seem to be regulated both via classical and alternative activation. Hemeoxygenase-1 (HO-1), which is an inducible antioxidant enzyme with immunomodulatory and anti-inflammatory properties, can be expressed by macrophages and is often associated with alternative activation. In mice, intestinal muscularis macrophages are constitutively $MHCII^+$ and are also described to be HO-1-. However, preliminary studies in rats have indicated that their muscularis macrophages have a different immunophenotype.
Methods: Sprague Dawley rats were used. Whole mounts from the muscularis were exposed to antibodies to detect ED-1 (macrosialin, a macrophage and monocyte marker), ED-2 (macrophage scavenger receptor, CD163), MHCII and HO-1. Both conventional immunostaining and double staining were performed.
Results: Both in serosa and between the muscle layers many ramified cells were $ED\text{-}1^+$ and $ED\text{-}2^+$, whereas only a few cells were $MHCII^+$ and they seemed to have a different morphology. In contrast numerous cells were $HO\text{-}1^+$, i.e. endothelial cells, but also many ramified cells showed co-localization with $ED\text{-}2^+$ macrophages. In mice muscularis macrophages are $MHCII^+$ and $HO\text{-}1^-$.
Conclusion: The immunophenotype of muscularis macrophages in normal rats differs from that of normal mice being $MHCII^-$ and seem to be constitutively $HO\text{-}1^+$, which may indicate that the cells are in a state of alternative activation. This may reflect a different microenvironment and could result in a species difference in the inflammatory response, which should be considered when comparing result from the two rodents.

076
Pannexin expression in human colon: Alterations in Inflammatory Bowel Disease (IBD)
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Objective: Pannexins (PANXs) constitute a new family of gap junction-like proteins. PANXs mainly form hemichannels and participate in cell signalling by release of ATP and ions in response to physiological and pathological stimuli. Three types of PANX proteins, PANX1, 2 and 3, have been identified in human. The aims of the present study were to determine the expression profile of PANXs in the human colon and investigate their potential involvement in the pathophysiology of inflammatory bowel disease.
Methods: Real-time PCR and Western blot were performed to determine the expression of PANX genes and proteins in the colonic mucosa and muscle of control, ulcerative colitis (UC) and Crohn's disease (CD). Immunohistochemistry was conducted to localise the cellular distribution of PANX1 and PANX2.
Results: Genes for PANX1 and 2 but not for PANX3 were present in both colonic mucosa and muscle with PANX1 being expressed at higher levels than PANX2. PANX1 mRNA was significantly down-regulated in UC muscle, but there were no changes in UC mucosa, CD muscle and mucosa. PANX1 immunoreactivity was present in all layers of the colon, mostly on endocrine glands, epithelial cells, varicose nerve fibres of submucosal and myenteric ganglia, endothelium of blood vessels and erythrocytes. The size and number of PANX1 positive blood vessels were greatly increased in the mucosal and submucosal regions of UC and CD, where a significant PANX1 positive lymphocyte infiltration was also seen. In CD muscle, there was an increase in number and size of PANX1 stained blood vessels and an increase in immunoreactive density of the muscle itself. These observations correlated to the Western blotting results, showing that PANX1 protein was upregulated in CD muscle. PANX2 immunoreactivity was localised to the cell bodies of submucosal and myenteric ganglia, epithelial cells, vascular smooth muscle and leukocytes, but was absent from erythrocytes. PANX2 positive lymphocytic infiltration was greatly increased in UC and CD mucosa and submucosa.
Conclusion: These results indicate that PANXs may function as ATP release channels to regulate intestinal blood supply, gut hormone secretion, motility and sensory processing. PANX channels may play a crucial role in the development of intestinal inflammation.

077
Immune factors in Irritable Bowel Syndrome plasma activate rat myenteric neurons
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Objective: Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorders which affects 15–20% of the general population worldwide. It is characterized by chronic, episodic abdominal discomfort with constipation and/or diarrhoea. However, there is a poorly understood underlying pathophysiology and there is a lack of suitable disease-defining biological markers. Differences in plasma cytokine levels have been established in IBS patients compared with control patients.IBS patients have elevated circulating levels of the pro-inflammatory cytokine, interleukin-6 (IL-6), which is produced in response to trauma, infection and immunological challenge. These studies aim to determine if the elevations of IL-6 contribute to the excitability of myenteric neurons.
Methods: Myenteric plexus preparations from adult male Sprague Dawley rats were loaded with the ratiometric calcium indicator, Fura 2AM ($7\ \mu$ mol L^{-1}). Real-time calcium imaging experiments were conducted using a standard epifluorescence imager.
Results: In paired recordings, pooled plasma samples from six healthy controls (diluted, 1 : 250 in Krebs) caused a slight increase in effect on intracellular calcium levels in sub-mucosal neurons (ratio change: 0.05 ± 0.01), whereas IBS plasma induced a significant increase in calcium (0.21 ± 0.02, $n = 43$, $P < 0.0001$). The IBS plasma-induced effect was attenuated by pre-neutralisation of IL-6 ($P < 0.0001$), IL-8 ($P < 0.0001$) and C-reactive protein ($P < 0.05$) while neutralisation of IgG had no effect on the evoked neuronal responses. Inhibitors of downstream signalling molecules, the STAT3 inhibitor WP1006 did not effect the evoked responses, while the MAPK inhibitor, PD98059 attenuated the response to IBS plasma ($P < 0.001$).
Conclusion: These data provide evidence that plasma from IBS patients but not healthy controls evoke excitation of rat colonic myenteric neurons, and that this effect is mediated, in part, by IL-6 and IL-8 signalling and CRP, resulting in downstream activation of MAPK. These findings provide a molecular explanation for observed alterations in the pathophysiological alteration in gastrointestinal motility observed in IBS patients.

078
Involvement of jejunal mucosal immunoglobulin production and plasma cell-nerve proximity in the pathogenesis of diarrhea-irritable bowel syndrome
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Objective: Increased immunoglobulin (Ig) production in the intestinal mucosa is associated with intestinal

inflammation in several gastrointestinal disorders. However, the implication of humoral activity in micro-inflammatory disorders, such as irritable bowel syndrome (IBS) has not been elucidated.
Methods: Peripheral blood, intestinal fluid, and one mucosal biopsy were obtained from the jejunum of healthy volunteers (H, n = 15, 6 men) and age-matched patients meeting diarrhea-IBS Rome III criteria (IBS-D, n = 20, 5 men). The number of B cells ($CD20^+$) in the jejunal mucosa was determined by immunohistochemistry, whereas plasma cell counts and distance to nerve endings were assessed by transmission electron microscopy. The expression of Ig heavy chain (IGH) genes in blood and jejunal mucosa was quantified by PCR, and seric and intestinal luminal concentration of Ig was determined by ELISA. Abdominal pain, the number of bowel movements and the stool form were also recorded.
Results: The jejunal mucosa of IBS-D displayed increased infiltration of both B cells (IBS-D:2.7 ± 0.3; H:1.5 ± 0.3 cells hpf^{-1}) and plasma cells (IBS-D:1,413 ± 243; H:335 ± 77 cells mm^{-2}; $P < 0.05$). Moreover, distance of plasma cells to nerve endings was reduced in patients (IBS-D:1.34 ± 0.3; H:2.94 ± 0.7 μm; $P < 0.05$) and correlated with plasma cell counts (r = -70, $P < 0.01$). Mucosal, but not blood samples, showed increased gene expression of IGHA, IGHG, and IGHE in IBS-D compared to H (1.2–1.6 fold; $P < 0.05$). IBS-D also showed higher luminal, but not circulating, concentration of IgG (IBS-D:3.9 ± 0.5; H:2.2 ± 0.5 $\mu g\ mL^{-1}$, $P < 0.05$). Furthermore, the number of B cells correlated with the frequency of abdominal pain in IBS-D (r = 0.41, $P < 0.05$), and the amount of IgG released to the lumen correlated with the intensity and the frequency of abdominal pain, and with the number of bowel movements (r = 0.62; r = 0.75; r = 0.66, respectively; $P < 0.05$).
Conclusion: Mucosal plasma cell activation and immunoglobulin production may contribute to the pathophysiology and clinical manifestations in IBS-D patients. Whether this mechanism is cause or consequence of intestinal dysfunction in IBS warrants further investigation.

079

Role of A2B receptors in the control of colonic cholinergic motility in the presence of bowel inflammation

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Objective: Adenosine A2B receptors (A2BR) contribute to the physiological regulation of immune responses, intestinal secretive/absorptive functions and colonic motility. However, the involvement of these receptors in enteric motor alterations associated with bowel inflammation is unknown. This study evaluated A2BR expression in rat colon and investigated their role in the control of cholinergic motility in the presence of colitis.
Methods: Colitis was induced by intrarectal 2, 4-dinitrobenzenesulfonic acid (DNBS) in Sprague-Dawley rats. After 6 days, A2BR expression and localization in the colonic neuromuscular layer were examined by RT-PCR and immunofluorescence. Colonic longitudinal muscle strips (LMS) were suspended in organ baths with Krebs solution, containing guanethidine, N-omega-propyl-L-arginine and antagonists of NK 1, 2 and 3 receptors. The effects of MRS 1754 (MRS, A2BR antagonist), NECA (A2BR agonist), dipyridamole (DIP, adenosine reuptake inhibitor) and adenosine deaminase (ADA) were assayed on atropine-sensitive cholinergic contractions evoked by electrical stimulation (ES; 0.5 ms, 28 V, 10 Hz), or by carbachol in the presence of tetrodotoxin.
Results: RT-PCR revealed the presence of A2BR mRNA in normal colon, and showed that the expression pattern did not vary in inflamed tissues. Immunofluorescence displayed a predominant localization of A2BR in the longitudinal muscle layer and myenteric plexus, however, in the presence of colitis, A2BR expression was enhanced at muscular level, but reduced in myenteric ganglia. MRS enhanced ES-induced contractions in normal LMS (+37%), while being less effective in inflamed tissues (+13.2%). Under incubation with DIP plus ADA, to abate extracellular adenosine levels, NECA decreased the motor responses to ES in normal LMS (EC50 = 300 n mol L^{-1}, Emax = -58%), and it was more effective in rats with colitis (EC50 = 275 n mol L^{-1}, Emax = -83.8%). Carbachol-evoked contractions were enhanced by MRS in normal LMS (+27%), but not in inflamed rats, while they were inhibited by NECA in normal and, to a greater extent, in inflamed LMS (-34% and -52%, respectively).
Conclusion: These results suggest that, under normal conditions, adenosine modulates the colonic cholinergic motility via activation of A2BR, mainly located on smooth muscle. In the presence of colitis, this inhibitory control is impaired, despite an up-regulated A2BR expression, probably resulting from a reduced endogenous adenosine availability.

080

Changes in the intestinal wall and myenteric plexus of jejunum in rats induced by the infection by oocysts of Toxoplasma Gondii

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Objective: Evaluate the changes caused by chronic infection with Toxoplasma gondii in the myenteric plexus and muscular layer of the jejunum of rats.
Methods: We used twenty rats (Rattus norvegicus) Wistar male adult, sixty days old, weighing 258.58 ± 13.64 g divided into CG (n = 10) control group and IG (n = 10) infected group. The IG were inoculated with 500 oocysts of a genotype II strain (ME49) of T. gondii. After 36 days of infection, the animals were euthanized. Blood samples were collected, in order to detect serum antibodies (IgG) anti-T. gondii by direct agglutination method. For histological analysis a semi-serial sections of 4 μm were obtained, stained with Hematoxylin and Eosin (HE), 80 measurements were performed in the muscular layer. Total preparations of the jejunum were stained with the Giemsa technique and subject to the histoenzymology technique of NADH-d. The cell body and nucleus area was measured. It was also performed the immunofluorescence technique that shows the varicosities of neurons immunoreactive to vasoactive intestinal peptide (VIP-IR) of the myenteric plexus. It was measured the area of 400 varicosities of each animal.
Results: Infected animals showed loose stools, but no changes were observed in the length, width and area of the jejunum. The muscular layer showed an increase of 32.2% ($P < 0.05$) in IG while the total wall thickness a reduction of 9.2% ($P < 0.05$). Quantitative analysis revealed by the Giemsa technique showed a loss of 32.8% ($P < 0.05$) while in the NADH-d technique there was no significant quantitative difference. Both the neurons of the total population and the subpopulation of NADH-dp underwent hypertrophy of the cell body and nucleus. The cytoplasm area in the total population showed a 7.6% increase while in the subpopulation NADH-dp there was a decrease of 5.3%. In the analysis of VIP-IR varicosities there was an increase in varicosities that had a smaller area as well as the intensity of brightness.
Conclusion: The infection caused the death and hypertrophy of the myenteric plexus neurons, alteration in the VIP-IR nerve fibers and in the intestinal wall into the jejunum of rats.

081

Proinflammatory cytokines from patient's with Chron's disease affect cultured enteric neurons

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Objective: The Enteric Nervous System (ENS) appears to be essential for the maintenance of gut homeostasis and mucosal integrity. Inflammatory diseases of the gut, such as ulcerative colitis and Crohn's disease (CD) also affect the ENS. CD is a chronic spontaneously relapsing disorder of unknown origin. The characteristics of CD include transmural inflammation affecting all layers of the bowel wall, including the ENS.
Methods: Serum from patients suffering with Crohns disease in acute and remission phase were collected and analyzed concerning their cytokine content (Multiplex Elisa). Primary myenteric cultures of neurons and glial cells were treated with serum from patients with Crohn's disease, healthy adults and defined media. After 24 h immunostainings for neuronal (PGP 9.5) and glial (S100) markers, as well as live dead assays, using calcein and propidium iodide were performed. Total neurite outgrowth of the individual neurons was measured.
Results: The sera from the three groups investigated showed significant alterations in their cytokine content. The primary enteric cultures harvested with the patient's serum either in Crohn's disease or healthy revealed a decreased survival of neurons compared to defined media. Only cultures, where the Crohn's patients serum was diluted revealed a stimulation in neuronal survival and total neurite outgrowth compared to healthy serum.
Conclusion: A mild inflammation in Crohn's disease is able to stimulate neuronal outgrowth and survival, which is overcome by increasing inflammation. Serum changes in the cytokines in Crohn's disease might influence the regeneration potential of enteric neurons.

082

The expression of immediate early genes of herpes simplex virus type 1 contributes to the neuroplasticity induced by viral infection on the enteric nervous system

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Objective: Recently, we have shown that the neurotropic Herpes simplex virus type 1 (HSV1) establishes latency in rodent enteric nervous system (ENS), leading to gut dysmotility with no signs of illness, suggesting that may have an etiological role in gastrointestinal motor disorders (GIMD). Considering that ICP27 is an immediate early gene involved in HSV1 DNA replication, this study aimed to assess the effects of HSV-1 replication on ENS integrity and gut contractility in mice infected with wild-type (WT) and ICP27-null (KO) HSV1.
Methods: Male C57Bl/6 mice (3 months old) were inoculated with WT or KO-HSV-1 intranasally and after 4 weeks (W) intragastrically (IG). After 1–8 W IG infection, in isolated ileum segments, changes in muscle tension were recorded by isometric transducers following electric field stimulation (EFS, 1–40 Hz). ENS neuroplasticity in whole mount preparations of ileal longitudinal muscle-myenteric plexus, immunostained with the neuronal markers HuC/D, peripherin and beta-tubulin, was assessed by confocal microscopy. Acetylcholinesterase biochemical assay and nNOS and substance P (SP) immunohistochemistry were performed to evaluate neurochemical coding.
Results: In the ENS of WT-HSV1 infected mice an altered number of HuC/D$^+$ and nNOS$^+$ cells at 1 W (-37 ± 12% and +60 ± 20%, respectively) and 8 W (-32 ± 10% and +99 ± 21%, respectively) was found after IG infection whereas KO-HSV1 infection determined a reduction of HuC/D$^+$ cells at 1 W PI (-43 ± 10%) with no changes in nNOS$^+$ neurons. beta-tubulin or peripherin labeling highlighted an irregular distribution in MG after 4 and 8 W of WT-HSV1 infection, respectively. AChE staining revealed a significant reduction of cholinergic processes after 1 W of WT-HSV1 infection. In KO-HSV1 infected mice beta-tubulin immunoreactivity was increased at 8 W PI whereas cholinergic network was significantly reduced at 1 W and increased at 4 and 8 W PI. SP immunoreactivity was reduced at 1 W post WT-HSV1 infection and increased at 4 and 8 W post KO-HSV1 inoculum. Neurally-mediated contractions induced by EFS (20 Hz) were significantly reduced at 1 W (-52 ± 17%) and 8 W (-25 ± 20%) post WT-HSV1 infection. Similar contractile alterations were found but less pronounced in KO-HSV1 infected mice.
Conclusion: HSV1 infection determines functional and morphological alterations of the ENS. Viral ICP27 protein-dependent replication appears to essentially influence ENS neuroplasticity.

083

Up-regulation of IL-6-expressing macrophages in human colonic mucosal biopsies

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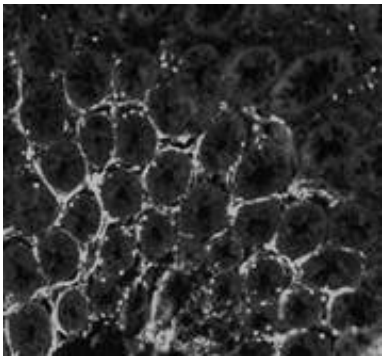
Objective: Irritable bowel syndrome (IBS) is a common disorder affecting between 15 and 20% of the world's population and is characterized by episodes of abdominal pain, altered bowel habit and bloating and/or distension. Dysfunction in the bidirectional signalling axis between the brain and gut is thought to underlie this debilitating disorder, however support for the importance of immune activation and an aberrant stress response is gaining momentum. Indeed, we have demonstrated a role for peripheral corticotropin-releasing factor (CRF) R1 and interleukin (IL)-6 in altered gastrointestinal function. These studies compare expression of CRFR1, IL-6 and IL-6Rs in mucosal biopsies from IBS patients and healthy controls.
Methods: Fixed human recto-sigmoid mucosal biopsies were cryostat-sectioned (10 μm), blocked with 1% donkey serum, permeabilized with 0.1% triton X-100 and stained with human anti-IL-6 (1:100), anti-IL-6 receptor (IL-6R, 1 : 100), anti-CRFR1 (1 : 300) or CRFR2 (1 : 300). Dual labelling was carried out with antibodies against α smooth muscle actin (SMA), DC SIGN, langerin and CD68.
Results: CRFR1 staining appeared to be primarily in epithelial cells and the intensity of staining was similar in IBS and healthy samples. The intensity of IL-6 and IL-6R staining was also comparable between IBS and controls. However, the patterns of IL-6 and IL-6R expression were distinctly different. All IBS patient biopsies examined (n = 25/25 sections from 5 biopsies) displayed a honeycomb-like pattern of IL-6 staining, with brightly stained cells surrounding the base of the crypts and in the lamina propria. Just 2/25 healthy biopsy sections displayed this staining pattern. Similar patterns but with lower intensity were observed for IL-6R expression. Dual-labelling displayed no co-localisation with αSMA, DC SIGN or langerin. However, co-localisation of the IL-6 positive cells and CD68 was observed.
Conclusion: IL-6-expressing CD68-positive macrophages were detected in the subepithelial space surrounding the colonic crypts of IBS biopsies. This may reflect a specific mechanism of immune response in IBS patients.



084

Proinflammatory cytokine gene polymorphisms in Greeks with irritable bowel syndrome

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Objective: Irritable bowel syndrome (IBS) is a multifactorial functional gastrointestinal disorder, characterized by recurrent abdominal pain and altered bowel habits. The genetic predisposition and influence of environment may underlie in the pathogenesis and/or pathophysiology of irritable bowel syndrome (IBS). Earlier studies focused on candidate genes of neurotransmitters, cytokines, and growth factors. Among them, some studies but not all studies revealed association between phenotypes of IBS and 5-hydroxytryptamine (5-HT)-related genes, serotonin related genes, and cytokine genes. The aim of the study was to assess the potential association between proinflammatory cytokines, interleukin-6 (IL6) and tumor necrosis factor-alpha (TNFα), polymorphisms and IBS in Greeks.
Methods: A total of 124 patients with IBS diagnosed according to the Rome III criteria and 238 healthy individuals were included in the study. IL6 (C/G -174), and TNFα (A/G -238), gene polymorphisms were genotyped using polymerase chain reaction-based methods.
Results: It was shown that the frequencies of the GG genotype and G allele of the IL6 (C/G -174) polymorphism were significantly associated with IBS (P = 0.0343 and P = 0.0131, respectively). TNF-α GG genotype (-238) in the IBS group was also significantly overrepresented (P < 0.0001). None of the clinical symptoms analyzed was significantly associated with the polymorphisms tested.
Conclusion: The results suggest that IL6 and TNFα gene polymorphisms might have a role in pathophysiology of disease.

085

Neuronal plasticity of lumbosacral DRG in a model of post-infectious gut dysfunction in rats

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Objective: Inflammation of the gut can lead to the remodeling of extrinsic neuronal circuitries to the gastrointestinal tract, including innervation of regions not directly affected by the insult. This neuroplasticity comprises sensitization of dorsal root ganglia (DRG) neurons, providing neural substrata for the functional alterations observed in the inflamed gut. Neurotrophins, mainly nerve growth factor (NGF), are likely to play a role on this process.
Aim: To characterize morphological changes and NGF/TrkA receptor expression in lumbosacral (LS)-DRG neurons in a model of Trichinella spiralis (TS)-induced post-infectious gut dysfunction in rats.
Methods: Rats received TS (7500 larvae/rat, PO) or vehicle. At the post-infective phase (day 30 ± 2), jejunal and colonic samples and LS (L2-S2)-DRG were obtained. Immune activation was assessed by real-time

Neuronal size and relative content of TrkA and NGF in lumbosacral DRGs from TS-infected and non-infected rats.

	Non-infected				TS-infected			
	Area≤500 μm^2	Area 500-1000 μm^2	Area 1000-1500 μm^2	Area >1500 μm^2	Area ≤500 μm^2	Area 500-1000 μm^2	Area 1000-1500 μm^2	Area >1500 μm^2
% of neurons	51.4±2.5	33±1.2	9.3±1.4	6.3±1.1	40.1±3 *	34.2±1.5	11.2±0.6	14.5±2 *
TrkA immunorreactivity (Relative Intensity)	97±6	96±8	97±6		73±7 *	73±7	89±10	
NGF immunorreactivity (Relative Intensity)	100±7	100±8	100±3		76±5 *	68±7 *	70±5 *	

Mean±SEM (900 cells from 18 DRGs from 4-5 animals per group). *P<0.05 vs. respective non-infected group.

PCR in jejunum and colon: rat mast cell (MC) protease II (RMCPII), IL13, iNOS, IL6 and IL10. Remodeling of DRG neurons was assessed by determining the cell surface area and the NGF/TrkA content by immunofluorescence.

Results: Gut inflammation was confirmed by enhanced expression levels of RMCPII and IL6 (4- and 2-fold increase versus vehicle, respectively; $P < 0.05$) in jejunum and colon; other markers remained unaltered in the colon although the jejunum showed increased IL13 expression. At the histopathological level, inflammatory-related changes were limited to a MC infiltrate within the mucosa/submucosa. In LS-DRG, TS infection increased the mean neuronal cell surface area (vehicle: 632 ± 25 μm^2; TS: 805 ± 44 μm^2; mean from 900 cells from 18 DRGs, n = 4–5; $P < 0.05$)(Table). In control conditions, NGF/TrkA immunoreactivity was particularly abundant in small/medium-sized (area≤1500 μm^2) DRG neurons; while immunorreactivity was absent in large neurons (area>1500 μm^2). After TS infection, NGF/TrkA labeling within small/medium neurons was reduced by 24% (Table).

Conclusion: During TS post-infective phase in rats, there is a persistent inflammatory-like response within the gut, characterized by a MMC infiltrate and the overexpression of inflammatory markers. In these conditions, LS-DRG neurons, likely innervating jejunum and colon, show neuroplastic changes associated to altered expression of NGF/TrkA. These observations suggest that the inflamed gut suffers remodeling of the extrinsic innervation, likely through a neurotrophin-related mechanism. This represents a potential neural substrata for the functional changes observed in post-inflammatory gut dysfunctions.

086

Role of immune-mediated mechanisms in psychological stress-induced colonic barrier dysfunction

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Objective: Recent studies have shown an increase in gut permeability and alterations in tight junction (TJ) protein expression in patients with irritable bowel syndrome (IBS). Although psychological stress is known to worsen IBS symptoms, the mechanisms by which stress enhances gut permeability and alters TJ protein expression remains to be determined. It is our hypothesis that psychological stress activates the release of proinflammatory cytokines which alter TJ proteins to promote increased gut permeability.

Methods: Two groups of male Fischer-344 rats were subjected to 1-hr of water avoidance stress (WAS) or SHAM stress for 7 days. Following the stress protocol, the rats were anaesthetized and the colon was isolated for histological, electrophysiological and molecular analysis. The histological appearance of the colonic segments was assessed via haematoxylin and eosin staining. Colonic permeability was measured electrophysiologically via measurements of transepithelial electrical resistance (TEER) and the macromolecular flux of horseradish peroxidise (HRP) in modified Ussing chambers. Colonic segments were also assayed for TJ proteins [claudin-2, JAM-A and zonula occludens-1 (ZO-1)] via Western blotting. In tissue isolated from rats exposed to the stressor or SHAM stress, an inflammatory cytokine panel was quantified via qRT-PCR.

Results: A significant decrease in TEER (SHAM= 0.37 ± 0.02; WAS= 0.30 ± 0.02 mΩ cm^{-2}; $P < 0.01$) and a significant increase in the macromolecular flux of HRP (SHAM=0.07 ± 0.03; WAS= 0.37 ± 0.10 ngHRP h^{-1} cm^{-2}; $P < 0.05$) was observed in response to WAS. Compared to SHAM controls, the expression of claudin-2, JAM-A and ZO-1 proteins was significantly decreased, 21.9 ± 3.0% ($P < 0.01$), 14.6 ± 2.7% ($P < 0.05$) and 27.8 ± 4.4% ($P < 0.01$) respectively, following WAS. In the absence of overt inflammation as defined histologically, we found that the cytokine profiles from WAS animals revealed a 2.77 ± 1.35 fold increase in IL-1β ($P < 0.05$) and a 3.44 ± 1.36 fold increase in TNF-α ($P < 0.05$), whereas levels of all other cytokines remained unaffected by WAS. Subsequent analysis revealed a significant correlation between TNF-α and gut permeability ($P < 0.05$; $r^2 = 0.62$) as well as TJ expression ($P < 0.05$; $r^2 = -0.64$).

Conclusion: Our findings suggest that psychological stress increases colonic permeability potentially via sub-inflammatory cytokine-mediated remodelling of tight junction protein expression.

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Impairement of the adenosine fine-tuning control of acetylcholine release from myenteric motoneurons in the inflamed rat ileum

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Objective: There is an increasing interest on the role of adenosine (ADO) to control intestinal activity, since ADO receptors were implicated in the pathophysiology of intestinal disorders like inflammatory bowel diseases, ischemia and post-operative ileus (Antonioli et al., 2008). Recently, we resolved the localization and function of ADO receptor subtypes at the longitudinal muscle-myenteric plexus (LM-MP) of the rat ileum (Vieira et al., 2011). The homeostatic significance of inhibitory (A1) and facilitatory (A2A and A3) ADO receptors to control cholinergic neurotransmission in the myenteric plexus, prompted us to investigate the changes on ADO neuromodulation in the inflamed rat ileum.

Methods: Inflammation of the ileum was induced by intraluminal injection of 2, 4, 6-trinitrobenzenesulfonic acid (TNBS, 50 m mol L^{-1}) in adult Wistar rats. Chronic inflammatory infiltrates were detected by hematoxylin and eosin staining at post-operative day 7.

Results: Motility, evaluated by the progression of methylene blue dye gavage along the GI tract, was significantly increased in the TNBS group (70 ± 2%, n = 6) *vs* the sham group (43 ± 7%, n = 4). Inflammation increased the levels of adenine nucleosides (ADO+inosine) in LM-MP superfusates analyzed by HPLC. In TNBS-injected samples, blockade of high-affinity A1 and A2A receptors failed to reproduce the characteristic effects of the blockers on evoked [3H]-ACh release in control animals, which were respectively for DPCPX (10 n mol L^{-1}) - 8 ± 13% (n = 4) *vs* 43 ± 12% (n = 3) and for ZM241385 (50 n mol L^{-1}) 21 ± 3% (n = 4) *vs* -37 ± 10% (n = 4). Activation of A1 receptors with R-PIA (300 n mol L^{-1}, 11 ± 9%, n = 3) and of A2A receptors with CGS21680C (3 n mol L^{-1}, 9 ± 7%, n = 5) also failed to modify evoked [3H]-ACh release in TNBS-injected samples, whereas R-PIA (300 n mol L^{-1}) inhibited and CGS21680C (3 n mol L^{-1}) facilitated respectively by 36 ± 4% (n = 4) and 57 ± 10% (n = 3) release in controls. Activation of the low-affinity A3 receptor with 2-Cl-IBMECA (3 n mol L^{-1}) also failed to increase [3H]-ACh release (-17 ± 2%, n = 3) in TNBS-injected preparations; this agonist facilitated by 17 ± 3% (n = 4) ACh outflow in control animals. The A3 receptor antagonist, MRS1191 (10 μ mol L^{-1}), was devoid of effect in both animal groups.

Conclusion: These results suggest that fine-tuning control of evoked [3H]-ACh release by ADO is severely affected in the inflamed myenteric plexus, regardless of the high endogenous ADO levels attained in the tissue.

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Connective remodelling in the colonic neuromuscular compartment of patients with ulcerative colitis

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Objective: Chronic inflammatory conditions may progress towards tissue remodelling processes, which are often characterized by an enhanced collagen deposition. As far as inflammatory bowel diseases are concerned, consistent levels of fibrotic remodelling have been identified and investigated in Crohn's disease, while scarce attention to such a tissue rearrangement has been paid to ulcerative colitis (UC). The present study evaluated the distribution of collagen and elastic fibers in the colonic neuromuscular compartment of patients with UC.

Methods: Full-thickness left colonic samples were obtained from 10 patients with established, severe and pharmacologically unresponsive UC, who underwent bowel resection. The colonic neuromuscular compartment was evaluated by routine histology, histochemistry and immunohistochemistry in paraffin cross-sections. The distribution of collagen and elastic fibers was evaluated by both histochemical (Van Gieson, orcein, Verroheff staining) and immunohistochemical (anti-collagen I and III, anti-elastin) assays. For comparison, the same evaluations were performed in normal colonic control samples from 10 subjects, who underwent surgery for uncomplicated colon cancer.

Results: Histochemical and immunohistochemical analysis of the inflamed colon showed a significant

increase in collagen fibers and a decrease in elastin content within the neuromuscular compartment, as compared with normal controls. In particular, increments of collagen deposition (mainly collagen type III) were found at level of the outer layers of longitudinal muscle (serosal side; arranged as bunches of fibers intermingled with bundles of smooth muscle cells), within the circular muscle layer (arranged as tangles of fibers along the longitudinal axis of smooth muscle cells), and in perivascular connective tissue. By contrast, elastic fibers were significantly and homogeneously reduced throughout the whole neuromuscular compartment, with particular regard for the myenteric ridge.
Conclusion: The present findings indicate that a significant degree of fibrotic remodelling occurs in the neuromuscular compartment of the inflamed colonic wall in patients with UC. This rearrangement of the connective tissue, taken together with the known alterations affecting the myenteric ganglionic cells and interstitial cells of Cajal, likely contributes to the development of enteric dysmotility, leading to serious digestive symptoms in patients with UC.

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Pancreatic Polypeptide (PP) delays Gastric Emptying (GE) of a radio-opaque liquid meal in conscious rats
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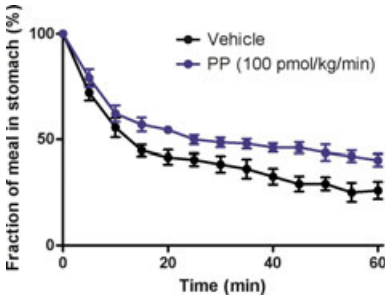
Objective: It has been shown that PP delays GE after intraperitoneal injection in rats, as measured using the phenol red method. We aimed to confirm the effect of PP on GE in conscious rats in a cross-over study using X-rays when PP is administered as a continuous intravenous infusion.
Methods: After an overnight fast, 6 Wistar HAN rats were placed in a Bolmann cage to minimize movement artefacts. The abdomen was visualized as a dorsal projection using X-ray imaging (29 kV, 40 mAs). 100 pmol kg^{-1} min^{-1} PP or vehicle (saline) was administered as a continuous intravenous infusion. 15 min after the start of the infusion, rats were gavaged with 3 ml of a liquid test meal either containing 1% hydroxypropyl methylcellulose (HPMC) and 0.5 g ml^{-1} $BaSO_4$ (0 kcal) or Nutridrink® (Nutricia) and 0.5 g ml^{-1} $BaSO_4$ (4.5 kcal). To estimate the meal fraction in the stomach, X-rays were taken every 5 min and analyzed using Image J software to outline the stomach and calculate the stomach surface area. The surface area directly after gavage of the meal was set at 100% and GE was expressed as the fraction of the original stomach area over time. The area under the curve over 60 min was compared. Data were represented as mean ± SEM and compared using a paired *t*- test ($P < 0.05$ was considered significant). Validation experiments showed that control experiments in the same animals were reproducible and that 1 mg kg^{-1} atropine significantly delayed GE, confirming the literature.
Results: Immediately after gavage, GE was initiated. In a first phase GE was relatively fast, followed by a phase during which GE was constant at a slower rate. The Nutridrink meal slowed GE with 24.1 ± 4.36% compared to HPMC ($P = 0.002$). PP inhibited GE of the HPMC meal with 17.7 ± 6.95% compared to vehicle ($P = 0.046$). When the Nutridrink meal was administered, GE was attenuated with 22.2 ± 4.97% during PP treatment, although significance was borderline ($P = 0.054$).
Conclusion: We confirmed that PP delays GE of a liquid (caloric) meal in conscious rats. The present method allows to reliably assess GE in rats in a continuous non-invasive manner.



Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-07 Basic and Translational Session: Brain-gut-axis and Stress Mechanisms

090
Chronic intermittent stress-induced visceral pain: Evidence for region- and cell-specific epigenetic regulation of Glucocorticoid Receptor (GR) in nociceptive DRG neurons
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Objective: Chronic stress alters behavior via methylation of the glucocorticoid receptor (GR) in the central nervous system. We examined the hypothesis that chronic intermittent stress-related visceral hyperalgesia is linked to epigenetic regulation of GR receptor in colonic DRG neurons via down-regulation of CB1 and up-regulation of TRPV1.
Methods: Male rats were exposed to 1-hour water avoidance stress daily for 10 consecutive days. GR siRNA was administrated in situ to L6-S2 DRGs in a separate control group. The visceromotor response (VMR) to colorectal distension was used to measure visceral pain response. Chromatin immunoprecipitation and methylated DNA-specific qPCR were used to measure DNA methylation and DNA-protein interaction. Parallel *In vitro* studies were performed in isolated control DRGs ± corticosterone (CORT; 10 μmol L^{-1}) and RU-486 (500 n mol L^{-1}).
Results: Stressed rats demonstrated 3-fold increase in the methylation of CpG sites of GR receptors in L6-S2 (innervating the colon) but not L4-L5 DRGs (sciatic nerve distribution) compared with the controls ($n = 3$). The binding to DNA methyltransferase 1 (DNMT1) to GR promoter regions was significantly increased in L6-S2 DRGs in stressed rats ($n = 3$; $P < 0.05$). GR mRNA and protein levels were significantly decreased in L6-S2 but not L4-L5 DRGs in stressed rats, which was prevented by RU-486 ($n = 4$; $P < 0.05$). In stressed rats, GR expression decreased 30% in small (nociceptive) DRG neurons but not in large-sized neurons ($P < 0.05$). GR decreased nearly 50% in cytosolic fractions while it increased in nucleus in L6-S2 but not in L4-L5 DRGs compared with controls ($n = 4$). Moreover, knockdown of GR receptors in L6-S2 DRGs in situ decreased expression levels of CB1 in control rats and enhanced VMR response at 40 and 60 mmHg distension pressures. Treatment of control L6-S2 DRGs *In vitro* with CORT decreased CB1 and increased TRPV1 expression levels that were prevented by RU-486 ($P < 0.05$).
Conclusion: These data support the novel and provocative interpretation that: 1. Chronic intermittent stress induces region- and cell-specific epigenetic regulation of glucocorticoid receptor in primary nociceptive neurons; and 2. The glucocorticoid receptor represents a potential target for treatment of chronic stress-induced visceral hyperalgesia.

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Corticotropin-releasing hormone receptor 2 activation in mesenteric adipose tissue leads to activation of MAP kinases and release of proinflammatory cytokines
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Objective: Corticotropin-releasing hormone (CRH) is a hormone/peptide synthesized both centrally and peripherally. CRH is a known mediator of stress, but it also mediates several GI responses, including motility and intestinal inflammation. Abdominal adipose tissue hyperplasia is a hallmark of active Crohn's disease, and increasing evidence suggests that adipocyte-derived molecules play a role in the pathophysiology of intestinal inflammation. Isolated human visceral and subcutaneous adipocytes express CRH receptors (CRHR) 1 and 2, but expression in the mesenteric fat, including mesenteric adipocytes has not been evaluated. Our objectives were to determine whether the CRH peptide family and CRH receptors are expressed in mesenteric adipose tissue and isolated preadipocytes and determine whether intestinal inflammation is associated with mesenteric adipose tissue CRH signaling.
Methods: Human mesenteric preadipocytes were isolated from surgical specimens of patients undergoing abdominal procedures and grown in culture. Mesenteric preadipocytes were stimulated for 4 h with CRH, or the CRHR2-specific peptide Urocortin 2 (Ucn2). Cells were also treated with antalarmin or astressin 2B, antagonists of CRHR1 and CRHR2, respectively. Following stimulation, cell supernatants were collected for ELISA and protein array, RNA was extracted for real-time quantitative PCR, and cell lysates were processed for Western blot. Real-time quantitative PCR was also performed on mouse adipose tissue harvested from C57/BL6 mice following acute or chronic trinitrobenzene sulfonic acid (TNBS)- or dextran sulfate sodium (DSS)-induced colitis.
Results: CRHR1 and CRHR2 mRNA was detected in human mesenteric preadipocytes and mouse mesenteric adipose tissue. Stimulation of human mesenteric adipocytes with CRH or Ucn2 increased IL-8,

TIMP2 and RANTES mRNA and protein levels. Ucn2 stimulation of human preadipocytes also phosphorylated p38, ERK1/2 and JNK. In acute TNBS and DSS-colitis, CRH, Ucn2 and Ucn3 mRNA levels were dramatically increased, while CRHR2 and growth hormone mRNA levels were increased in chronic TNBS-colitis.

Conclusion: This is the first demonstration of CRH receptors in human mesenteric preadipocytes which upon ligand binding stimulate release of proinflammatory cytokines and activate the MAP kinase pathway. We also show increased adipose tissue CRH/CRHR2 expression during experimental colitis. We suggest that such CRH-CRHR interactions in mesenteric adipose tissue may participate in the pathophysiology of intestinal inflammation.

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Acute water avoidance stress induces visceral analgesia when tested non-invasively - differential role of opioid-dependent mechanisms in male and female rats

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Objective: To assess non-invasively in rats the effects of acute water avoidance stress (WAS) on the visceromotor response (VMR) to colorectal distension (CRD), the role of opioids in stress-related pain modulation and whether there are sex differences.

Methods: Male (M) and female (F) Wistar rats (7–8 weeks, 2–4/cage) were monitored for VMR to CRD (10, 20, 40, 60 mmHg, 20 s duration, 4 min intervals) using a novel manometric technique (Larauche et al., 2009). The 1st CRD (day 0) served as baseline, then rats were exposed to 1 h WAS. One group (F11, M10) received no injection. Two other groups were injected subcutaneously with naloxone (1 mg kg^{-1}) (F10, M9) or saline (0.3 ml) (F12, M12) 10 min before WAS. The 2nd CRD was monitored 45–50 min after WAS (day 1) and the 3rd CRD, 24 h after WAS (day 2). The VMR after WAS was expressed as percentage of the respective baseline. Data were analyzed using 2-way ANOVA and Bonferroni *post-hoc* test.

Results: On day 1, females exhibited an analgesic response to CRD compared to baseline at 40 and 60 mmHg (52.9 ± 11.5% *vs* 111.8 ± 24.6%, $P < 0.001$ and 69.1 ± 1.1% *vs* 100.0 ± 0.0%, $P < 0.05$), while males showed a decrease in VMR to CRD only at 40 mmHg (47.6 ± 8.6% *vs* 88.6 ± 10.7%, $P < 0.001$). On day 2, the VMR was back to baseline values in females and males. Saline-injected females exhibited visceral analgesia at 60 mmHg on day 1 and an increased VMR at both 40 and 60 mmHg compared to baseline on day 2 ($P < 0.05$), while saline-injected males VMR was unaffected on both days. In female rats, naloxone pretreatment prevented the immediate visceral analgesia on day 1 at 60 mmHg, and abolished the delayed visceral hyperalgesia on day 2. Naloxone did not affect the VMR of males.

Conclusion: When monitored non-invasively, acute WAS induces visceral analgesia in both female and male rats. Subcutaneous injection as an additional noxious stress stimulus alters visceral sensitivity to CRD in a time and sex-dependent manner. Only in females, opioid-dependent mechanisms participate in the modulation of immediate visceral analgesia and delayed visceral hyperalgesia. Supported by NIH P50 DK-64539 (YT), 1K01DK088937 (ML) *ML, AM-equal contribution.

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Anxiety and sympathetic nervous system activation are associated with the development of human esophageal pain hypersensitivity

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Objective: Experimental acid infusion in the distal esophagus leads to secondary hyperalgesia in the proximal esophagus in most healthy subjects but 30% remain resistant. The factors that mediate differences in sensitization to acid are unclear and their study may help to understand risk factors for esophageal hypersensitivity in gastro-esophageal reflux disease. The aim is to determine the psychophysiological factors which predict the development of esophageal pain hypersensitivity (EPH) to acid infusion in healthy subjects using a validated model.

Methods: Forty-eighgt healthy volunteers (mean age 29 y range 19–49 y, 33 male) underwent psychological profiling for, anxiety, depression and personality type. Baseline pain threshold (PT) to proximal esophageal electrical stimulation was measured using a visual analogue scales; before HCl acid infusion (0.15 mol L^{-1}) in the distal esophagus for 30 min. This was followed by esophageal PT measurements in the proximal, unexposed esophagus at 30, 60 and 90 min. Parasympathetic (Cardiac Vagal Tone - CVT) and Sympathetic Nervous System (RR interval) responses were monitored throughout the study. Volunteers were classified as sensitizers if the proximal PT fell ≥6 mA after esophageal acidification.

Results: Thirty-five subjects (73%) sensitized to acid, and 13 subjects (27%) did not sensitize. There was no difference in the age, sex or in personality domains between sensitizers and non-sensitizers ($P > 0.05$). Spielberg trait anxiety comparison indicated a trend, $P = 0.08$ for sensitizers to be more anxious than non sensitizers. At baseline there was no difference in CVT and RR interval between the two groups. During acid infusion the key difference in autonomic nerves system response was that in the sensitizers the RR interval was shorter in sensitisers than non sensitisers (sensitisers: Δ1.662 ± SE 48.9 *vs* non sensitisers: Δ66.13 ± SE 25.65. ΔRR interval difference 64.4 ms (SE ± 37.3) $P = 0.002$; see Fig. 1). Trait anxiety demonstrated a negative correlation with the RR interval $r = -0.35$ ($P = 0.04$).

Conclusion: Our results suggest that higher anxiety and SNS activation has a pro nociceptive effect on the development of post-acid infusion esophageal sensitization. This may explain why individuals with anxiety or stress at the time of visceral injury or inflammation are more likely to go onto develop EPH.

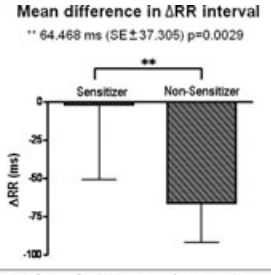

Figure 1: *shows the ΔRR interval (sympathetic autonomic response) comparison of the sensitizer & non-sensitizer groups during acid exposure. The mean difference between groups were 64.468 ms (SE±37.305) p=0.0029.*

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Chronic stress potentiates descending inhibition to colonic circular smooth muscle of mice and humans

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Objective: The post-infectious state is often accompanied by altered nociception and colonic motility. We have shown that post-infectious peripheral nociception is enhanced by stress. However, the mechanisms of stress action on colonic motility are unclear. Current study was to investigate effects of stress and stress hormones on colonic descending-inhibition.

Methods: Distal colonic segments from C57BL control and chronically-stressed (9 days water avoidance stress) mice were placed in an oxygenated tissue bath at 36oC. Balloon-distension (BD) was performed proximally, while descending inhibitory junction potentials (IJP) were recorded from circular smooth muscle (CSM) distally. Nerve stimulation (NS) (4 pulses, 20 Hz, 0.5 ms duration) was applied between distension and recording sites. One preparation of human colonic CSM was studied to test whether mouse data were replicable.

Results: Water-avoidance stress increased motility (initial pelleting increase: 2.6-fold versus control). CSM resting membrane potentials were unaltered in stressed mice (-42.7 ± 2.1 mV, $n = 5$ controls; -46.5 ± 1.4 mV, $n = 6$ stressed) as were power spectra of spontaneous IJPs (1.234 ± 0.293 mV^2 Hz^{-1} controls; 1.567 ± 0.293 mV^2 Hz^{-1} stressed). In controls, NS and BD induced fast IJPs (fIJP) of 32.0 ± 2.2 mV and 13.1 ± 3.1 mV, respectively. These were increased to 37.3 ± 2.2 mV ($P = 0.04$) and 22.7 ± 3.6 mV ($P = 0.02$), respectively, by epinephrine (5 n mol L^{-1}) plus corticosterone (1 μ mol L^{-1}) (E+C), which also increased the spontaneous IJP power spectrum to 2.648 ± 0.710 mV^2 Hz^{-1}. Similarly, IJPs were significantly larger in colons from chronically stress animals ($P = 0.04$). MRS-2500 (1 μ mol L^{-1}) abolished the BD-induced IJP, but converted the NS-induced IJP into a complex of an EJP, fIJP and slow IJP (sIJP) (amplitudes 3.2 ± 0.1 mV, 10.9 ± 1.8 mV and 6.8 ± 2.3 mV, respectively). Atropine (3 μ mol L^{-1}) and L-NAME (200 μ mol L^{-1}) abolished the EJP and sIJP. In human colonic CSM, NS (1 s, 20 Hz) produced a biphasic IJP (fIJP followed by sIJP), with amplitudes and half-amplitude durations of 9.8 mV and 2254 ms, and 6.6 mV and 3156 ms, respectively. E+C increased the fIJP and sIJP to 24.5 mV and 8.9 mV in the same CSM cell.

Conclusion: These data suggest: (i) NS- and distension-elicited descending inhibition to CSM is mainly purinergic in murine distal colon; (ii) Chronic stress potentiates this descending inhibition and may provide a mechanism for stress-induced acceleration of colonic transit; (iii) This stress potentiation of neurotransmission to the CSM is replicable in human colon.

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Gastrointestinal inflammation and environmental enrichment alter stress-induced activation of the corticolimbic system

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Objective: Stress has a negative impact on many disease processes including gastrointestinal inflammation and nociceptive signaling from the gut to the brain. Environmental enrichment (EE) is thought to reduce stress reactivity and promote stress resilience in laboratory animals. It is not known, however, how visceral inflammation and EE affect the central stress circuitry. Thus the first aim of this study was to test whether stress-induced neuronal activation is altered by gut inflammation and/or EE. The second aim of the study was to determine whether EE has an impact on gut inflammation.
Methods: Mice housed under standard or enriched environment for 10 weeks were submitted to psychological stress (water avoidance) after a 1 week treatment with either iodoacetamide to induce gastritis or dextran sulfate sodium to induce colitis. Post-stress c-Fos expression was measured immunohistochemically in brain circuits implicated in the stress response. Gut inflammation was assessed by a disease activity score (DAS) as well as gastric and colonic myeloperoxidase (MPO) levels.
Results: Colitis and to a lesser degree gastritis selectively decreased stress-induced neuronal activation in hippocampal, amygdalar and cortical brain regions. Most remarkably, EE caused a 2.14-fold increase in the number of stress-induced c-Fos expression within the granular cell layer of the dentate gyrus ($P < 0.01$) and a 2.08-fold decrease in the number of c-Fos expressing cells within the central nucleus of the amygdala ($P < 0.01$) of control animals. These effects were abolished completely in animals suffering from colitis and partly in animals with gastritis. Neuronal activation within the infralimbic cortex was also decreased by gastritis ($P < 0.01$) and colitis ($P < 0.01$) in standard-housed mice, but only by colitis ($P < 0.05$) in the EE group. Colitis, but not gastritis, was aggravated by EE as shown by a rise of the DAS from 2.7 ± 1.1 to 3.6 ± 1.0 ($P < 0.1$) and a 1.65-fold increase in colonic MPO levels ($P < 0.1$).
Conclusion: These data indicate that visceral inflammation and EE alter the central stress response within distinct neuronal circuits and that EE increases the severity of colitis. Our findings reveal new aspects of the interaction between environmental factors, external psychological stressors and internal physical stressors (gastrointestinal inflammation).

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Enteric intrinsic primary afferent neurons may modulate the gut-brain axis by synaptic transmission to extrinsic mesenteric afferent fibres

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Objective: The gut possesses intrinsic primary afferent neurons (IPANs) within the enteric nerve plexus as well extrinsic sensory afferent neurons2. Anatomical evidence for synapses between myenteric neurons and extrinsic sensory afferent fibres3 raises the novel idea that the extrinsic fibres may also have an interneuron role with intrinsic primary afferent neurons (IPANs) relaying or gating certain gut related information that reaches the brain. We tested this idea by recording mesenteric nerve activity in response to gut distension or to pharmacologically exciting inhibiting IPANs when intramural synaptic transmission was blocked or not. Synaptic neurotransmission is a calcium dependent process where N- and P/Q-type calcium channels are predominantly involved1.
Methods: We used suction pipettes to record extracellular multi and single-unit activity from the mesenteric afferent nerve bundles innervating ex vivo segments of mouse jejunum.
Results: Constitutive firing frequency was reduced from (mean±SE) 15.5 ± 3 to 5.8 ± 1 Hz, $n = 8$, $P = 0.01$ or from 28 ± 3 *vs* 23 ± 2 Hz, $n = 8$, $P = 0.03$ by adding 400 μ mol L^{-1} CdCl2 or 500 n mol L^{-1} each of w-conotoxinMVIIC plus w-conotoxinGVIA respectively. Toxin's effects still in the presence of 3 μ mol L^{-1} nicardipine to minimise muscle contraction. However, nerve excitation evoked by gut distension (intraluminal 14 hPa) was unaltered by synaptic blockade. The IPAN excitant and KCa 3.1 channel blocker TRAM-34 increased the constitutive firing frequency, while the IPAN inhibitor and IKCa channel opener DCEBIO reduced it. Applying the conotoxin mixture reduced the number of single fibers that responded to TRAM-34 from 75% to 29%, and abolished the TRAM-34 evoked increase in multi-unit firing frequency.
Conclusion: Our results provide strong evidence for gut intramural synaptic transmission from the enteric nervous system to extrinsic afferent fibres.
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Perinatal high fat diet modulates GABAergic transmission in central brainstem circuits regulating gastric functions

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Objective: Obesity results from a combination of both environmental and genetic factors, but several lines of evidence have shown that the perinatal environment is critically important in the development of the neural circuits responsible for energy homeostasis and the integration of autonomic reflexes. Several previous studies have demonstrated that vagal efferent motoneurons innervating the stomach are regulated mainly by tonic GABAergic synaptic inputs from the adjacent nucleus of the tractus solitarius (NTS). The aim of the current study was to investigate whether exposure to a high fat diet (HFD) during the perinatal period alters GABAergic synaptic transmission to gastric-projecting dorsal motor nucleus of the vagus (DMV) neurons.
Methods: Sprague-Dawley rats were exposed to a HFD (60% kcal from fat) in utero from embryonic day 13. Control rats received normal chow (18% kcal from fat). Whole cell patch clamp recordings were made subsequently from gastric-projecting DMV neurons in thin brainstem slices from rats aged 35–60 days of age. The effects of brainstem microinjection of the GABAA receptor antagonist, bicuculline (25–100 pmol) on gastric motility and tone were assessed in rats at 10–12 weeks of age.
Results: In DMV neurons from rats fed the control (18% kcal from fat) diet, electrically evoked GABAergic synaptic currents had an amplitude of 230 ± 28 pA and a decay time of 39 ± 5.6 ms ($n = 13$). In contrast, while evoked GABA currents in DMV neurons from perinatal HFD rats had a similar amplitude (174 ± 19 pA; $n = 10$; $P > 0.05$), the decay time was significantly longer (121 ± 38 ms; $P < 0.05$). In rats fed the control diet, brainstem microinjection of bicuculline produced a dose-dependent increase in gastric tone, viz., 0.06 ± 0.008 g at 25 pmol, 0.108 ± 0.005 g at 50 pmol, and 0.159 ± 0.019 g at 100 pmol ($n = 7$–10). The ability of bicuculline to increase gastric tone was significantly decreased in rats fed HFD, viz., 0.053 ± 0.004 g at 50 pmol and 0.09 ± 0.003 g at 100 pmol ($n = 3$).
Conclusion: These results suggest that exposure to a HFD during the perinatal period increases GABAergic neurotransmission to gastric-projecting DMV neurons. This results in a decrease in DMV neuronal excitability, and an inhibition of vagal efferent outflow to the stomach.

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Sexually dimorphic effects of unpredictable early life adversity on visceral pain in a rodent model: Importance of estradiol

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Objective: Symptoms of irritable bowel syndrome (IBS) fluctuate with the menstrual cycle, suggesting a role for ovarian hormones in the development of abdominal pain and abnormal bowel habits. A high proportion of women with IBS also report a history of early life adversity (ELA). The goal of the current study was to test the hypothesis that ELA induces sexually dimorphic changes in abdominal pain reporting that are mediated via estradiol. Although current experimental models of ELA, such as maternal separation, mimic parental neglect they do not address ELA as a result of abusive relationships. Therefore, in the current study, we used a novel rodent model of ELA, which experimentally reproduces attachment to an abusive caregiver.
Methods: Neonatal rats were exposed to different pairings of an odor and a shock to control for trauma predictability. In adulthood, visceral sensitivity was assessed via a visceromotor response (VMR) quantified by the number of abdominal contractions during colorectal distension (CRD). In a separate group of rats, the VMR to CRD was assessed following ovariectomy (OVX) and with subsequent estradiol replacement. Cycling females were grouped according to estrus cycle.
Results: Adult female rats that received unpredictable ELA exhibited an exaggerated VMR to CRD as compared to all other groups, which was observed at both low and high levels of ovarian hormones. In contrast, the VMR to CRD was not significantly different between groups in adult males (Table 1). OVX reversed the effects of unpredictable ELA on visceral hypersen-

Number of Abdominal Contractions at 60 mmHg

	Cycling Females	Low Ovarian Hormones	High Ovarian Hormones	Males
Unpredictable	30.2±2.8***	26.5±3.7**	27.8±2.7**	16.8±2.1
Odor Only	19.4±1.8	17.8±2.3	18.2±2.4	14.5±1.7
Predictable	18.0±2.6	10.9±1.0	18.8±3.4	12.4±0.9

** p<0.01 vs. all other groups
***p<0.001 vs. all other groups

sitivity in female rats (Unpredictable: 16.17 ± 2.4, Odor-Only: 14.5 ± 1.3), and estradiol replacement induced visceral hypersensitivity in the unpredictable group at 60 mmHg (Unpredictable: 21.8 ± 1.2, Odor Only: 14.5 ± 1.5; $P < 0.05$).
Conclusion: Our data demonstrate that unpredictable neonatal adversity produces visceral hypersensitivity that is sexually dimorphic and dependent on the activational effects of estradiol.

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Microstructural brain changes in patients with Diabetes Mellitus and gastrointestinal symptoms

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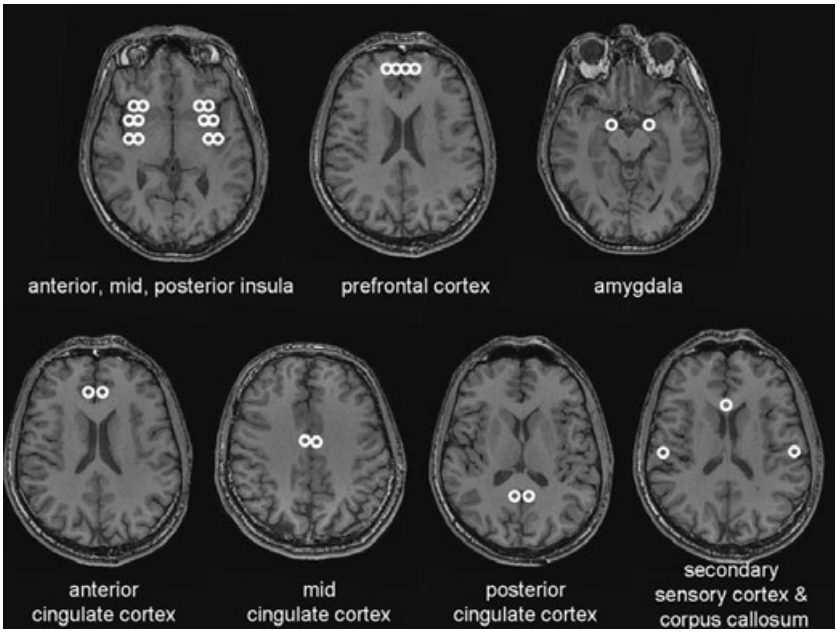
Objective: In patients with longstanding diabetes mellitus (DM) there is increasing evidence of abnormal processing of gastrointestinal sensations in the central nervous system. Using magnetic resonance diffusion tensor imaging, we characterized brain microstructure in areas involved in visceral sensory processing and correlated these findings to clinical parameters.
Methods: Twenty-six patients with DM and gastrointestinal symptoms and 23 healthy controls were studied in a 3T MR scanner. Apparent diffusion coefficient (ADC) (i.e. diffusivity of water) and fractional anisotropy (FA) (i.e. organization of fibres) values were assessed in the ''sensory matrix'': cingulate cortex, insula, prefrontal and secondary sensory cortex, amygdala and corona radiata. Corpus callosum served as control area.
Results: Patients had decreased FA values compared to controls in (i) anterior ($P < 0.001$), mid ($P = 0.002$) and posterior ($P < 0.001$) cingulate cortex, (ii) prefrontal cortex grey matter ($P < 0.001$), (iii) corona radiata ($P < 0.001$), (iv) secondary sensory cortex ($P = 0.005$), (v) anterior white matter ($P = 0.048$), anterior grey matter ($P = 0.013$) and posterior grey matter ($P < 0.001$) insula. No difference between the two groups were found in corpus callosum ($P > 0.05$). The microstructural changes were for some areas correlated to the clinical parameters such as bloating (in anterior insula), mental wellbeing (in anterior insula, prefrontal cortex, mid cingulate and corona radiata), autonomic function (posterior insula and anterior cingulate), and the presence of gastroparesis (in anterior insula).
Conclusion: Our findings suggest that microstructural changes of brain areas involved in visceral sensory processing are correlated to autonomic dysfunction and might be involved in the pathogenesis of gastrointestinal symptoms in diabetes patients.



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Psychological stress induces anxiety and pain hypersensitivity through glucocorticoid and CRF-Mediated Mechanisms in the Amygdala

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Objective: In irritable bowel syndrome (IBS), functional imaging during rectosigmoid distension reveals a hyperactivation of the amygdala, a key central nucleus involved in the autonomic and neuroendocrine response to stress. Our previous studies have shown that local exposure of the central nucleus of the amygdala (CeA) to elevated levels of corticosterone (CORT) induces anxiety-like behavior and pain hypersensitivity, resembling the hallmark features of IBS. The overall goal of this study was to investigate whether the CeA is involved in anxiety and pain hypersensitivity in a rodent model of psychological stress induced by repeated water avoidance stress (WAS). Here we test the hypothesis that glucocorticoid receptors (GR) and corticotrophin releasing factor (CRF) expression increase in the CeA in response to repeated WAS.
Methods: Groups of male Fisher 344 rats were subjected to 1-hr of WAS or SHAM stress (control) for 7 days. The following phenotypes were investigated: anxiety-like behavior was assessed using an open field (OF) test; visceral sensitivity was measured as abdominal contractions induced by isobaric colorectal distension (CRD) (20–60 mmHg); and somatic sensitivity was quantified using Von-Frey filaments. In all rats, fecal pellet output (FPO) and plasma CORT verified the extent of the stressor. In separate groups, brains were collected under anaesthesia immediately upon stress termination (Day 7) or 24 h. following (Day 8) WAS or SHAM stress to measure GR and CRF expression in the CeA with immunohistochemistry and qRT-PCR.
Results: Repeated WAS induced multiple phenotypic behaviors that are summarized in Table 1. Within the CeA, WAS decreased GR staining on Day 7 and 8 by 27% and 21% ($P < 0.05$ *vs* SHAM), respectively. This change in protein content was accompanied by a 0.3 fold decrease in GR expression ($P < 0.05$) and a 2.4 fold increase in CRF expression ($P < 0.05$) in the amygdala on Day 8.
Conclusion: We have shown that reciprocal alterations in GR and CRF expression within the CeA are likely involved in anxiety and pain hypersensitivity induced by repeated psychological stress. This study highlights the importance of abnormal amygdala activity, specifically within the CeA, in rodent models with IBS-like sequelae.

Table 1: Results of Behavioral Testing

Phenotype	Measure	SHAM (n)	WAS (n)
Stress Reactivity	Plasma CORT (ng/ml) (Day 7)	130±50 (2)	1330±130 (2)**
	Plasma CORT (ng/ml) (Day 8)	140±20 (6)	140±30 (5)
	Daily FPO	1.0±0.4 (8)	5.4±0.9 (8)**
Anxiety-like Behavior	% Time in Center of Open field	32.8±3.0% (5)	16.2±4.2% (3)*
Somatic Sensitivity	Von-Frey Filament Withdrawal Threshold (g)	90.1±5.9 (6)	62.8±4.1 (4)**
Visceral Sensitivity	CRD @ 60 mmHg (Day 7)	14.1±1.1 (6)	20.1±1.9 (6)**
	CRD @ 60 mmHg (Day 8)	13.2±2.0 (6)	21.7±2.5 (6)**

* P < 0.05, ** P < 0.01 vs. SHAM, Unpaired Student's *t* test

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Changes in peptidergic neurotransmission with VIP and Substance P during postoperative ileus in rat are associated with c-Fos immunoreactivity in the brainstem

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Objective: Our aim was to study changes in neurotransmission with vasoactive intestinal polypeptide (VIP; inhibitory) and substance P (excitatory) in rat small intestine and to determine whether these changes are associated with brainstem activation during postoperative ileus (POI).
Methods: Jejunal circular muscle strips (n = 8/rat) were obtained from male Sprague Dawley rats. Groups (n = 6/group): Naïve controls (NC), rats 12h (SC12h) and 3d (SC3d) after laparotomy, and rats 12h (P12h) and 3d (P3d) after laparotomy and standardized small bowel manipulation to induce POI. Intestinal transit was measured by charcoal gavage ([%] small bowel passed). Dose-responses to exogenous VIP (10^{-10}–10^{-7} mol L^{-1}) and Substance P (3×10^{-10}–3×10^{-7} mol L^{-1}) were studied. Effects of endogenously released neurotransmitters were studied by electrical field stimulation (EFS; 20 V, 4 ms, 3 Hz) ± L-Nitroarginine (L-NNA; 10^{-4} mol L^{-1}), VIP antagonist ([D-p-Cl-Phe6,Leu17]-VIP; 10^{-6} mol L^{-1}), or Substance P antagonist ([D-Pro2,D-Trp7,9]-Sub P; 10^{-6} mol L^{-1}). Studies were performed under non-adrenergic, non-cholinergic conditions (propranolol 5×10^{-7} mol L^{-1}, phentolamine 10^{-5} mol L^{-1}, atropine 10^{-7}M). c-Fos positive cells were counted in nucleus tractus solitarii (NTS). Data: mean ± SEM.
Results: Intestinal transit was delayed in POI groups and SC12h (P12h 27 ± 2; P3d 40 ± 3; SC12h 48 ± 2; all P < 0.05 *vs* NC 60 ± 3%). VIP caused inhibition in all groups (P < 0.05). Inhibition was more pronounced in P12h, P3d, and SC3d (P < 0.05). Substance P caused excitation in all groups (P < 0.05), which was reduced in P12h and increased in P3d (P < 0.). EFS induced inhibition in all groups, which was more pronounce in P12h (-67 ± 8% *vs* NC -33 ± 8; P < 0.05). VIP and Substance P antagonists had no effect on EFS responses (P = NS), while L-NNA prevented EFS-induced inhibition in all groups (P < 0.05). c-Fos expression in the NTS was increased in all postoperative groups (P12h 1.9 ± 0.4; P3d 6.7 ± 0.9; SC12h 2.6 ± 0.5; SC3d 2.7 ± 0.5; P < 0.05 *vs* NC 0.3 ± 0.1), particularly in P3d (P < 0.05 *vs* all other groups).
Conclusion: Early POI is associated with an increased inhibitory effect of VIP, reduced excitatory effect of Sub P, and increased release of inhibitory transmitters (potentially nitric oxide). After 3d, increased sensitivity for VIP persists, while the excitatory effect of Sub P is now increased. As c-Fos is upregulated in all postoperative groups, the observed changes appear not to be related to brainstem activation. DFG KA2329/5-1.

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Longitudinal assessment of colitis in the adoptive transfer mouse model using colonoscopy and μPET/CT

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Objective: Techniques allowing longitudinal assessment of colonic inflammation over time are indispensable for *in vivo* research in inflammatory bowel diseases. This study evaluated colonoscopy and micro-positron emission tomography/computed tomography (μPET/CT) to follow-up colitis in an adoptive transfer mouse model.
Methods: Colitis was induced in immunodeficient SCID mice by adoptive transfer of $CD4^+CD25^-CD62L^+$ T lymphocytes, isolated from BALB/c mice; controls were injected with PBS (n = 8 in each group). Mice were monitored at weeks 2, 4 and 6 for clinical outcomes (body weight, stool consistency, mobility and piloerection) and for the severity of colitis using colonoscopy and μPET/CT. During colonoscopy, bowel wall thickening, alterations of vascular pattern, stool consistency and adherence of fibrin to the colon wall were scored. The uptake of 2-deoxy-2-[18F] fluoro-D-glucose (FDG) was measured by μPET/CT to detect colonic inflammation. At week 6 mice were sacrificed for post-mortem colonic macroscopic, microscopic and myeloperoxidase (MPO) assessment.
Results: In colitis mice, body weight significantly decreased over time (17% at week 4 and 22% at week 6), whereas the clinical score significantly increased over time from 0.6 ± 0.5 to 4.2 ± 0.5 and 4.3 ± 0.6 at weeks 2, 4 and 6 respectively. Colonoscopy showed signs of mild mucosal inflammation in the distal colon from the 4th week on and the colon remained inflamed at week 6 as evidenced by a significant increase in colonoscopic score from 0.8 ± 0.4 at week 2 to 5.5 ± 1.1 and 4.1 ± 1.0 at week 4 and 6 respectively. The FDG activity was increased in colitis mice to 1.9 ± 0.3 at week 2, 3.0 ± 0.6 at week 4 and 3.0 ± 0.4 at week 6. None of these parameters changed over time in the control mice. The colonoscopic and μPET/CT findings correlated with each other and with the macroscopic score, the microscopic score and MPO activity, reaching significant R-values between 0.60 and 0.93.
Conclusion: Our findings suggest that adoptive transfer of $CD4^+CD25^-CD62L^+$ T lymphocytes in SCID mice results in a reproducible, mild colitis. Colonic inflammation can be monitored over time by both colonoscopy and μPET/CT where μPET/CT picks up inflammation at an earlier time-point than colonoscopy. Both techniques represent reliable and safe methods without the need to sacrifice animals.

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An equivalent of Hypothalamic–Pituitary–Adrenal (HPA) axis exists in the colon: Expression, cellular location and regulation in mice

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Objective: We showed that corticotropin releasing factor (CRF) and CRF receptor type 1(CRF1), respectively the initiator and effector for HPA signaling, are expressed in the rat colon and regulated by immune stress (Neurogastroenterol Motil. 2007, Peptides. 2010). A local HPA equivalent system has been described in the skin (Physiological Rev, 2000), cochlea in mouse ear (J Neurosci, 2011) and human retinal pigment epithelium cells. The activation of this local HPA equivalent system leads to local production and release of cortisol (J Endocrinol 2007). In this study, we detect the elements of the HPA axis in the mouse colon. (i) Gene expression, (ii) Cellular localization, and (iii) Regulation by CRF overdrive in transgenic CRF overexpressing (CRF-OE) mice.
Methods: Proximal colonic tissue samples were collected from adult male C57Bl/6 (7–12 weeks, n = 5), CRF-OE and wild-type littermate mice (3–6 months, n = 6–8). The gene expression and regulation of CRF, CRF1, proopiomelanocortin (POMC), prohormone convertases PC1 and PC2, melanocortin 2 receptor (MC2), key glucocorticoids synthesizing enzymes CPY11A1, CPY11B and glucocorticord receptor (GR) were detected by using RT-PCR and quantitative real time PCR. Immunostaining for CRF, CRF1 and β-Endorphin was performed in the colonic tissue sections and whole mount preparations.
Results: Transcripts of CRF, CRF1, POMC, PC1, PC2, MC2, CYP11A1, CYP11B1 and GR were detected in the colon although with a lower level (except PC1/PC2) than positive controls such as the mouse cerebral cortex and hypothalamus for CRF and CRF1; the mouse pituitary for POMC, PC1 and PC2; the mouse adrenal gland for MC2, CYP11A1 andCYP11B1. CRF immunoreactivity was located in cells scattered in the epithelia, crypts, lamina propria, submucosal and myenteric neurons while CRF1 and β-endorphin were only located in the enteric neurons. Compared to wild type littermates, CRF-OE mice showed a significantly elevated CRF mRNA level not only in the hypothalamus but in the colon as well.
Conclusion: These data support the existence of a local equivalent of the HPA axis in the colon and suggest possible implications in the modulation of colonic response to local stressors which will help to clarify the local neuro-endocrine mechanisms involved in the stress-related bowel disorders such as IBS and IBD.

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Stress reactivity in children with functional abdominal pain and healthy controls

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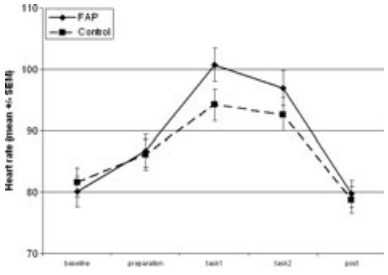
Objective: There is a high prevalence of functional gastrointestinal disorders in school age children which

seem to be related to stress. Children with functional abdominal pain (FAP) report more daily stressors and stressors are predictive for bodily complaints in these children. In experimental studies stress induced higher heart rates and systolic blood pressure in children with FAP compared to healthy controls, but little is known about gastric reactions to stress in these children.
Methods: Stress reactivity of 14 children with FAP and 14 healthy children, matched for gender and age (7–12 years, 9.46 ± 1.5 years; 50% female), was compared by using the Trier Social Stress Test (TSST). The TSST induces psychosocial stress during an anticipation period (10 min) and a test period (10 min) during which subjects have to perform a free speech and mental arithmetics in front of an audience. Outcome measures were subjective assessments of anxiety and symptoms as well as objective physiological outcomes such as the ECG, electrogastrogram (EGG) and saliva cortisol levels.
Results: Children with FAP responded with higher heart rate changes during the stress tasks (P = .022) (Fig). Myoelectrial activity of the stomach was different from healthy children at baseline with fewer normogastric myoelectrical activity (P = .018). Normogastric activity did not respond to stress but tachygastria increased with stress. Furthermore, most physiological reactions were related to perceived anxiety at baseline.
Conclusion: These preliminary results show that children with FAP exhibit altered cardiac as well as gastrointestinal responses to stress as compared to healthy children indicating higher sympathetic activation. The study needs to be continued because of the small sample size.



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Restraint stress elevates CRF expression and increases CRF release in the rat stomach
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Objective: Corticotropin releasing factor (CRF) has been implicated in stress-induced delayed gastric emptying via activating the CRF2 receptors in the brain as well as in the stomach. Stress has been shown to increase CRF expression in several brain regions. The stomach also produces CRF locally. The aim of the present study was to test a hypothesis that stress elevates CRF expression and increases CRF release in the rat stomach.
Methods: Male adult Sprague Dawley rats were placed under restraint stress for 1 h. Controls were allowed to move freely in their cages without restrained. Rats were euthanized at different time points (immediately, 4, 8, and 24 h) after stress. The stomach was removed. Real time RT-PCR was used to study changes in CRF mRNA levels in the stomach. Whole-mount myenteric plexus was dissected and used for immunofluorescence staining of CRF. Enzyme immunoassay was used to measure CRF release in the stomach.
Results: CRF mRNA levels markedly increased (2.27 ± 0.94 fold) immediately after the restraint stress, reached to the highest level (18.16 ± 11.35 fold) 8 h after stress, and remained elevated 24 h after stress. Restraint stress significantly increased the number of CRF-immunoreactive (IR) neurons/myenteric ganglion in the rat stomach (control: 1.75 ± 0.11; immediately after stress: 4.31 ± 1.59, $P < 0.05$; 4 h after stress: 2.77 ± 0.44; $P < 0.05$). The number of CRF-IR neurons/myenteric ganglion returned to normal level 8 h after stress. Restraint stress also increased CRF release in the rat stomach (control: 0.26 ± 0.03 pg mg^{-1} ml^{-1}; immediately after stress: 1.27 ± 0.18 pg mg^{-1} ml^{-1}, $P < 0.001$; 4 h after stress: 0.73 ± 0.22 pg mg^{-1} ml^{-1}, $P < 0.01$). The amount of CRF release in the stomach returned to normal level 8 h after stress.
Conclusion: Restraint stress elevates CRF expression and increases CRF release in the stomach. Elevated CRF levels and CRF release in the rat stomach may contribute to the slowdown of gastric emptying and fullness in the stomach in times of stress.

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The effect of high caloric food on chronic stress responses in rat
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Objective: The purpose of this study was to elucidate whether high caloric food has a beneficial effect on the chronic variable stress induced behavioral and neurochemical changes.
Methods: Twenty-seven adult male rats, weighing about 290 g, were divided into 5 groups: control(CON), restraint stress with regular food (Res A), restraint stress with sweet food (Res B), chronic variable stress with regular food (CVS A), chronic variable stress with sweet food (CVS B). Before and after the experiment, all rats were evaluated and analyzed their exploring behavioral activity using activity monitor system. The weight of body adrenal gland, epididymal fat and the levels of the plasma ACTH and corticosterone were measured. Plasma levels of pro- and anti-inflammatory cytokines (TGF-β, IL-2 and interferon-γ) were also investigated.
Results: All stress groups showed significantly increase in the relative adrenal weight compared to the control group. However, even though there is no significant difference in the plasma corticosterone concentration between all groups, the levels of the plasma ACTH concentration in CVS A group is significantly decreased compared to Res A and Res B groups. In open field test before the experiment, there were no activity differences between all groups. However, after the experiment, CVS B group showed significantly increase their exploring activity compared to CVS A and control group. In western blotting assays, IL-2 and interferon-γ, were significantly increased in CVS A group only and there is no difference in TGF b, anti-inflammatory cytokine expression between all groups.
Conclusion: These results suggest that there is a relationship between non-adaptable stress and the production of the proinflammatory cytokines and that also high caloric food ingestion can reduce the stress-induced proinflammatory cytokine productions but has no effect on the antiinflammatory cytokine productions in rats.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-08 Basic and Translational Session: Extrinsic Neural Pathways, Hypersensitivity and Pain

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Serum from diet induced obese mice inhibits excitability of mouse vagal afferent neurons
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Objective: Recent work has demonstrated that diet induced obesity impairs responses to satiety mediators in vagal afferent neurons. This effect was not limited only to those vagal afferents innervating the GI tract, therefore we hypothesized that circulating factors may play a role. Therefore we set out to determine the effects of serum from obese mice on the excitability of vagal afferents.
Methods: Mice were fed diets composed of 60% kCal from fat (obese) or 10% (lean) for 12 weeks. At 12 weeks serum was collected. Leptin levels were measured by ELISA. Nodose ganglion neurons from control mice were harvested, and incubated overnight with lean or obese mouse serum. Patch clamp studies were performed and excitability of the neurons assessed.
Results: 60% fat fed animals were obese and hyperleptinemic. Serum from obese mice significantly increased rheobase (32.7 ± 3.8 pA (n = 15, 10%) *vs* 78.6 ± 16.1 pA (n = 14, 60%, p,0.02, $P < 0.05$)), decreased input resistance (471.8 ± 39.1 MΩ (n = 15, 10%) *vs* 345.6 ± 45.7 MΩ (n = 14, 60%, $P < 0.05$)) and reduced number of APs at twice rheobase (4.0 ± 0.6 (n = 15, 10%) *vs* 1.5 ± 0.2 (n = 14, 60%, $P < 0.05$)). To determine the possible role of leptin in these changes we incubated neurons with 100 n mol L^{-1} leptin overnight. Leptin treatment significantly increased rheobase (52.3 ± 7.8 pA (n = 13, control) *vs* 100.8 ± 18.6 pA (n = 12, Leptin)) and reduced action potential number (3.7 ± 0.5 (n = 13, control) *vs* 1.6 ± 0.3 (n = 12, Leptin, $P < 0.05$). Leptin also significantly inhibited the number of neurons responding to CCK and 5-HT (assessed by calcium imaging).
Conclusion: Circulating factors present in obsese mouse serum inhibit excitability of vagal afferents. Leptin can reproduce some of these effects, suggesting a possible role for hyperleptinemia in these changes.

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Identification of the classes of spinal afferents activated during spontaneous motor activity of the murine large intestine

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Objective: The functional role of the different classes of visceral afferents that innervate the large intestine is poorly understood. In this study we developed a novel preparation that allowed us to determine which classes of spinal afferents running in the rectal nerves respond during naturally occurring colonic migrating motor complexes (CMMCs) and induced contractions of the smooth muscle.

Methods: Isotonic or isometric transducers were used to record changes in length or force in the rectum and in the distal colon (25 mm orally) in full thickness flat sheet preparations of the whole mouse colorectum (80–90 mm). Extracellular recordings were made from rectal nerves entering the colorectum.

Results: The low threshold muscular and muscular-mucosal rectal afferents were reliably activated by low levels of circumferential stretch induced by increase in length (1–2 mm) or by load (1–3 g). Majority of these units (25 of 34) were also activated by spontaneous CMMC contractions with a mean frequency of 1.53 ± 0.23 Hz (n = 8) under isotonic conditions and 2.52 ± 0.36 Hz (n = 17) under isometric conditions. In a small proportion of cases, some low threshold stretch-sensitive afferents (5 out of 34 units) decreased their firing rate during the peak of the CMMC contractions only when the preparation was under maintained (4 min) circumferential stretch. Physostigmine (0.1 μ mol L^{-1}) enhanced the amplitude of CMMC contractions (by 75 ± 30%, n = 5, P < 0.05) and CMMC-induced firing of low threshold mechanoreceptors (by 115 ± 51%, n = 5, P < 0.05) compared with controls. Bethanechol (100 μ mol L^{-1}) induced strong tonic contractions followed by activation of low threshold afferents under both isotonic (n = 10) and isometric (n = 7) conditions. Intramural high threshold (''serosal'') afferents (n = 6) were activated by intense levels of circumferential stretch (10–20 g) but not during spontaneous CMMC contractions nor intense contractions induced by bethanechol.

Conclusion: Low threshold wide dynamic range muscular and muscular-mucosal rectal afferents are activated by spontaneous CMMC contractions. No activation of high threshold rectal afferents was detected during CMMCs or intense contractile activity in naïve mouse colorectum.

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Mechanical and chemical stimulation of human visceral afferents

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Objective: We have developed *In vitro* electrophysiological recordings of afferent fibre activity in isolated human intestine. This human model of visceral pain has potential usefulness in translational studies on experimental therapeutics. To investigate the utility of our model we examined the response to the algogenic mediators bradykinin (BK) and adenosine triphosphate (ATP) and to mechanical probing alone and in the presence of a TRPV4 agonist.

Methods: All experiments were performed in accordance with UK human ethics regulations [NREC 09/H0704/2]. Surgically resected human colon, ileum and appendix were obtained from consenting patients undergoing right hemicolectomy, subtotal colectomy, or panproctocolectomy as part of their standard surgical treatment. Mesenteric nerve bundles were dissected free and afferent activity was recorded using suction electrodes. Receptive fields were located by probing the serosal surface of the tissue with a 2 g von Frey hair (VFH). A stimulus response curve was generated using 70–4000 mg VFHs. Peak responses to repeat 2 g VFH were compared before and after superfusion of the TRPV4 agonist GSK1016790A (10 μ mol L^{-1} in 10 ml krebs n = 3). BK was superfused into the bath (1 μ mol L^{-1} or 10 μ mol L^{-1} in 10 ml Krebs, n = 5). ATP was either superfused (10 m mol L^{-1} in 10 ml Krebs, n = 2; 1 m mol L^{-1} in 20 ml Krebs, n = 2) or directly applied to the tissue (10 m mol L^{-1} in 500 μl or 1 ml Krebs, n = 2). Peak firing was determined before and after BK application.

Results: Few fibre recordings were made from 11 tissues (5 colon, 4 appendix, 2 ileum). Mechanosensitive receptive fields were found on the serosal surface at the bifurcation of blood vessels. Incremental strength VFH probing produced a stimulus response curve. Application of GSK1016790A produced a significant increase in the response to probing with 2 g VFHs (before 41.3 ± 4.7 *vs* after 71.7 ± 2.7 spikes/20 s, P < 0.05). BK produced a significant increase in nerve activity (before 51.6 ± 29.8 *vs* after 128.2 ± 44.4 spikes/20 s, P < 0.05). ATP elicited an increase in afferent firing (before 14.6 ± 7.0 *vs* after 42.5 ± 9.8 spikes/20 s, P < 0.05).

Conclusion: These data confirm the feasibility of *In vitro* electrophysiological recordings from human visceral afferents. Preparations respond to both mechanical and chemical stimuli. Furthermore, the data demonstrates an important role for TRPV4 in human visceral afferent mechanosensitivity.

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Ageing and gastrointestinal sensory function: Altered colonic mechanosensory and chemosensory function in the aged mouse

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Objective: Ageing has a profound effect upon gastrointestinal function through mechanisms that are poorly understood. In this current study we investigated the effect of ageing upon colonic sensory signalling pathways in order to address this question.

Methods: An *in vitro* mouse colon preparation with attached lumbar colonic nerves, inferior mesenteric ganglion and lumbar splanchnic nerves was used to study mechanosensory and chemosensory afferent function in young (3 month) and old (24 month) mice. Mechanosensitivity was investigated by saline-induced ramp distensions of colonic segments (to an intraluminal pressure of 60 mmHg), while chemosensitivity was determined by superfusion of agonists into the bath. SERT and TPH-1/2 expression were analysed by real-time PCR, and EC cell numbers were determined using anti-5-HT immunohistochemistry. Data presented as mean ± SEM (n ≥ 6). Data analysed by one or two way ANOVA or by Students t-test. P < 0.05 was taken as significant.

Results: Ramp distensions of colonic segments caused an increase in afferent discharge via the activation of distinct subtypes of mechanosensitive afferents. We classed these afferents as low threshold (LT), high threshold (HT) and wide dynamic range (WDR) according to their stimulus response properties. Ageing affected colonic afferent mechanosensory function. Total afferent discharge in response to ramp distension was attenuated in 24 month animals (P = 0.016 versus 3 month) in which the HT afferent response was significantly blunted (P = 0.006 versus 3 month), while LT and WDR afferent responses were unaffected. Ageing also affected colonic afferent chemosensory function. The peak afferent response to 10 μ mol L^{-1} 5-HT was similar in 24 month and 3 month animals; however the latency and duration of the afferent response profile elicited by 5-HT was attenuated in 24 month animals (P < 0.001 versus 3 month). In contrast 300 μ mol L^{-1} 2-Me-5HT elicited an afferent response profile and peak afferent response that were significantly reduced in 24 month animals (P < 0.0001 and P < 0.05 versus 3 month respectively). EC cell numbers were moderately elevated in the distal colon, while SERT and TPH1 expression levels were significantly increased.

Conclusion: Ageing is associated with decreased colonic afferent mechanosensitivity in which the HT mechanosensitive afferent response appears most affected. Chemosensitivity to 5-HT is reduced in the ageing colon and may reflect altered 5-HT bioavailability.

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Neurophysiological, cellular and environmental regulation of visceral hypersensitivity in ulcerative colitis like inflammation

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Objective: Hypersensitivity of the afferent and dorsal horn neurons induced by inflammation is at the root of neuropathic and somatic pain. The tacit assumption is that inflammation in ulcerative colitis and Crohn's disease patients induces visceral hypersensitivity, which contributes to the symptom of pain. However, clinical studies did not consistently find visceral hypersensitivity (VHS) in response to colorectal distension (CRD) in ulcerative colitis patients. We tested the hypothesis that dextran sodium sulfate (DSS)-induced colonic inflammation that mimics the inflammation in ulcerative colitis patients does not induce VHS. However, concurrent chronic psychological stress dramatically enhances VHS to CRD.

Methods: We induced colonic inflammation by oral administration of 5% dextran sodium sulfate for 5 days. A 9-day heterotypic intermittent chronic stress (HeICS) protocol comprised of water avoidance, forced swimming and cold restraint stressors induced chronic stress in rats starting on day-6 of inflammation. Rats receiving regular water or HeICS alone served as controls.

Results: We found that DSS inflammation did not induce VHS to CRD (DSS-rats). However, HeICS, significantly increased VMR to CRD (HeICS-rats) (P < 0.05). HeICS during DSS inflammation (DSS+HeICS rats) synergistically enhanced the VMR to CRD

that was greater than by HeICS alone ($P < 0.05$). The responses of single unit fibers of decentralized dorsal root neurons to CRD from control, DSS-rats and DSS+HeICS rats were similar to those of VMR to CRD. DSS and HeICS significantly increased the recruitment of fibers that responded to CRD ($P < 0.05$). HeICS+DSS significantly increased the proportion of HT fibers versus those in control. We investigated whether the two stressors altered the expression of prominent nociceptive proteins in L6/S1 DRG. We found that DSS or HeICS alone did not affect the expression of nociceptive proteins BDNF and NGF. However, DSS+HeICS significantly enhanced both protein expressions in DRG. We also found that TRPA1 receptor antagonist, HC-030031 (0.5 mg kg^{-1} i.p.) blocked the effects of DSS and HeICS on VHS.
Conclusion: We conclude that ulcerative colitis-like inflammation induced by DSS in rats does not induce visceral hypersensitivity to colorectal distension. However, concurrent chronic stress synergistically enhances the VHS during inflammation that may contribute to the symptom of pain in ulcerative colitis patients.

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Dose-dependent antihyperalgesic effect of somatostatin (sst) receptor subtype agonists in a rodent model of visceral hypersensitivity induced by repeated noxious colorectal distension

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Objective: To investigate whether the stable pan-sst1-5 agonist, ODT8-SST or new selective sst1, sst2, or sst4 peptide agonists injected intraperitoneally (IP) modulate visceral hyperalgesia induced by repeated noxious colorectal distension (CRD) in mice.
Methods: Adult male C57Bl/6 mice were monitored for visceromotor response (VMR) to phasic noxious CRD using a novel non-invasive technique (Larauche et al., 2010). Mice (7–14/group) were injected IP (0.1 ml) with ODT8-SST, sst1, sst2 or sst4 agonists (3 or 10 μg/mouse), vehicle (saline) or received no injection. CRD consisted of 4 sets of distensions (each set: 3 CRDs at 55 mmHg). Immediately after recording a baseline VMR during the 1st CRD set, peptide or saline was injected IP and 30 min later three consecutive CRD sets were performed. The mean VMR value for each consecutive CRD set was expressed as percentage of the respective baseline. For comparison between groups 2-way ANOVA with Bonferroni post-test was used.
Results: A significant increase above the baseline VMR was observed during the three consecutive CRD sets in non-injected (160 ± 9%, 153 ± 15%, 143 ± 11%, $P < 0.01$) and in IP saline-injected (141 ± 9%, 126 ± 11%, 138 ± 10%, $P < 0.05$) mice. ODT8-SST prevented the development of noxious CRD-induced visceral hypersensitivity only at the highest dose (VMR at 10 μg: 92 ± 3%, 80 ± 4%, 90 ± 7%, $P < 0.05$). The antihyperalgesic action of selective sst2 agonist was equally potent at both doses (VMR at 3 μg: 93 ± 9%, $P < 0.01$; 85 ± 12%, $P < 0.05$; 86 ± 16%, $P < 0.001$; 10 μg: 84 ± 7%, $P < 0.001$; 85 ± 10%, $P < 0.01$; 80 ± 9%, $P < 0.001$). The effect of selective sst1 agonist was time and dose-dependent as it prevented the development of hyperalgesia only during the 1st CRD set after injection at 10 μg dose (VMR: 94 ± 5%, $P < 0.001$). The selective sst4 agonist exerted no effect.
Conclusion: The hypersensitivity induced by repeated noxious CRD in mice is prevented by the peripheral injection of the stable pan-sst1-5 agonist - ODT8-SST, selective sst2 or sst1 agonists in a time and dose-dependent manner. Sst1 receptor may represent a novel target to modulate visceral pain in addition to sst2.
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TRPV1 receptors and visceral hypersensitivity in colitis-induced motility and sensitivity disorders

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Objective: Patients with inflammatory bowel disease (IBD) often suffer from gastrointestinal motility and sensitivity disorders. These disturbances have a major impact on quality of life and can present a significant therapeutic problem.
Methods: We evaluated gastrointestinal motility in rats with trinitrobenzene sulphate (TNBS)-induced colitis, focussing on the upper gut. To measure colorectal sensitivity we performed *in vivo* electrophysiological measurements of sensory nerve activity at the dorsal root level as well as VMR (visceromotor reflex) measurements.
Results: Experimental colitis inhibits gastric emptying. This colitis-induced gastroparesis was reversed by hexamethonium, an inhibitor of synaptic neurotransmission, suggesting the involvement of a neuronal pathway between colon and stomach. Fos expression studies indicated that colitis induced hyperactivation of extrinsic afferent nerve fibers within the colonic pelvic nerve. Pelvic nerve section normalized gastric emptying in TNBS rats, showing that colitis sensitized pelvic afferent neurons thereby activating a cologastric inhibitory reflex leading to gastroparesis. TRPV1 receptors are known as integrators of various inflammatory stimuli at the level of the afferent terminal. Pretreatment with the specific TRPV1 receptor antagonist BCTC dose-dependently improved colitis-induced gastroparesis. In agreement, rats with colitis showed a significant increase in TRPV1 receptor expression in the pelvic afferent neurons innervating the inflamed colon. To verify that TNBS colitis sensitized pelvic afferent nerve fibers, we performed *in vivo* electrophysiological measurements of sensory nerve activity at the dorsal root level. Colitis increased both spontaneous and the colorectal distension-induced activity of unmyelinated C-fibers. In addition, colitis increased the VMR to colorectal distension. The TRPV1 receptor antagonist BCTC normalized the sensitized afferent response to colorectal distension of C-fibers. BCTC also partially reversed the colitis-induced increased VMR responses to colorectal distension, both after intraperitoneal and after intrathecal injection. These results confirm the efficacy of TRPV1 blockade in our *in vivo* motility studies and the crucial role of TRPV1 signalling in colitis-induced motility and sensitivity disorders.
Conclusion: TRPV1 inhibition could be a valuable approach in the management of inflammation-induced gastrointestinal motility and sensitivity disorders such as post-infectious irritable bowel syndrome and IBD-associated dysmotility.

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Involvement of the spleen in the anti-inflammatory effect of vagus nerve stimulation in a rat model of colitis

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Objective: Low frequency vagus nerve stimulation (VNS) has been successfully experimented on animals to cure digestive inflammation. The peripheral anti-inflammatory effect of VNS, is due to the release of acetylcholine (Ach) of the vagus nerve which interacts with the α-7 nicotinic Ach receptor of macrophages inhibiting the release of pro-inflammatory cytokines. However, different pathways implicating other immune cells could also have an important role. Indeed, recent data have shown a crucial role of the spleen in the peripheral anti-inflammatory effect of VNS. The aim of the study was to better understand the involvement of different splenic immune cells in the anti-inflammatory effect of VNS during the initiation of inflammation in a rat model of Crohn's disease.
Methods: Rats were divided into 5 experimental groups (n = 10/group): colitis-VNS, colitis-no VNS, saline-VNS, saline-no VNS, control. Colitis was induced by intra-colonic instillation of trinitrobenzene sulfonic acid (TNBS). The stimulation electrode was fixed on the cervical portion of the left vagus nerve. The stimulation parameters were: 0.5 mA, 5 Hz, 30 s ON, 5 min OFF, during 3 h. At the end of VNS, animals were euthanized; the spleen, the distal and proximal colon were removed to analyse the different lymphocytes sub-populations with flow cytometry as well as cytokines' secretion. Myeloperoxidase was also measured in the colonic samples.
Results: VNS had an effect on splenocytes' functionality; it increased the secretion of the anti-inflammatory cytokine IL-10 in cultured activated splenocytes, especially $CD4^+$ lymphocytes. An anti-inflammatory effect of VNS was observed in the proximal colon with a decrease of MPO and a slight decrease of pro-inflammatory TNF for the colitis-VNS group compared to the colitis-no VNS group.
Conclusion: VNS, which has an anti-inflammatory effect on the digestive tract, also has an effect on splenocytes' functionality, especially on splenic $CD4^+$ lymphocytes in a rat model of TNBS colitis. An increase of the anti-inflammatory cytokine secretion IL-10 was measured in the supernatants of cultured splenocytes from the VNS groups compared to the no VNS groups. These data have potential therapeutic implication for the use of VNS in patients with Crohn's disease.

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Effects of DA-9701 on responses of substance P, c-Fos and pERK in spinal cord and dorsal root ganglion to colorectal distension in Sprague-Dawley rats

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Objective: One of the important pathophysiologic mechanisms of functional gastrointestinal disorders is visceral pain. It is transmitted via various pain-related substances such as substance P (SP), phophorylated

extracellular signal-regulated kinase (pERK), and c-Fos. DA-9701, standardized extract of Pharbitis Semen and Corydalis Tuber, is known to be a new prokinetic agent and to exert analgesic effects on the abdomen. The aim of the study was to investigate whether DA-9701 modulates pain-related substances such as SP, pERK, and c-Fos in experimental model of visceral pain induced by colorectal distension (CRD).
Methods: Total eighteen male Sprague-Dawley rats were used. Rats were divided into three groups; the negative control group (A) received no CRD or drug, the control group (B) received CRD but no drug, and the treatment group (C) received both CRD and drug. CRD was produced by inflating the balloon to 60 mmHg four times for 20 s with a 5 min rest interval. The drug (DA-9701) was administered orally and the dosage was 60 mg kg^{-1}. Activations of SP, pERK, and c-Fos in the dorsal root ganglion (DRG) and spinal cord (L5S1) were determined by immunohistochemical stain and western blot in all groups.
Results: Immunohistochemical stains for substance P, pERK and c-Fos in the DRG and spinal cord showed the increase in the number of activated neurons in B group than in A group. The number of activated neurons of p-ERK in C group decreased than that in B group. There was no difference between C group and B group in substance P and c-Fos. Western blot results for p-ERK in the spinal cord showed the increase of intensity in B group than in A group and the decrease of intensity in C group than in B group.
Conclusion: We found that substance P, pERK and c-Fos in the dorsal root ganglion and spinal cord were activated in response to CRD and that the activation of p-ERK was decreased by DA-9701. We demonstrated that DA-9701 can decrease visceral pain mediators in CRD-induced visceral pain model. These data suggest that DA-9701 may play a role in the management of functional GI disorders.

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Associations between visceral and somatic pain in healthy volunteers
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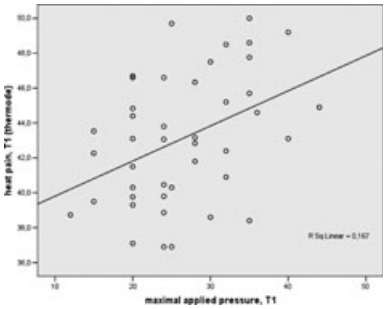
Objective: Patients with irritable bowel syndrome (IBS) experience reduced pain thresholds for visceral (intetsinal) stimuli but with respect to somatic pain data are conflicting. The association between somatic and visceral pain has rarely been investigated in healthy subjects.
Methods: We included healthy male and female subjects (n = 24 and 21, resp.) between 20 and 35 (25.8 ± 3.8) years to measure pain thresholds with rectal distension and with temperature stimulation at the non-dominant hand. Visceral pain sensitivity (in mmHg) was assessed by means of a barostat (G&J Barostat, Missisauga, ON, Canada) using a double-random staircase methods. Somatic pain was assessed by a termode (TSA II, Medoc Ltd, Ramat Yishai, Israel) with triple threshold measurement by ascending methods of limits for heat and cold (in °C). Thresholds were compared by Peason's correlation coefficient.
Results: Pain thresholds for heat, cold, and pressure were normally distributed and well within the published norms (43.1 ± 3.7 °C, 11.9 ± 6.5 °C and 26.7 ± 8.3 mmHg, resp.). Heat and cold pain thresholds were highy and negativel correlated (r = -.685, P < .001), and the pain thresholds for distension pressure were associated both with the heat pain (r = .408, P = .007) (Fig.) as well as cold pain (r = -.339, P = .024). No influence of age and gender was found on these associations.
Conclusion: In healthy subjects pain sensitivity varies largely but is independent of the modality and body system in which it is assessed. Hyperalgesia in pain patients such as in IBS therefore should be independent of the modality tested and may indicate a general (central) dysfunction of processing of nociceptive stimuli. However, in case of a dissociation between two or more modalities this may indicate a pathology of a peripheral organ system. (Supported by a grant from DFG, En 50/30-1 in FOR 1328).



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TRPA1 activation leads to the murine small mesenteric afferent signaling
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Objective: Transient receptor potential A1 (TRPA1) expressed on the sensory neurons is a molecular target for noxious cold, pungent irritants and endogenous inflammatory mediator. It plays a major role in mediating inflammation and pain (Jordt et al. 2004, Nature; Schwartz et al., 2011, Gastroenterology). However, its action on the rodent visceral afferent discharge has not been fully characterized yet. Thus we aimed to examine the contribution of TRPA1 to the mesenteric afferent sensitivity in murine intestine.
Methods: Extracellular recording was made from mesenteric nerve bundles attached to a 2–3 cm segment of adult male mice (C56/BL6) small intestine *In vitro*. The gut was cannulated to allow distension with Krebs up to 60 mmHg at 15–20 min intervals. Once reproducible responses to distensions had been established, the segments were challenged with a selective and potent TRPA1 agonist allyl-isothiocyanate (AITC) at various concentrations (0.1, 0.3 and 1 m mol L^{-1}) for 2 min. The time interval between any two consecutive applications was more than 20 min. Data presented as mean ± SEM (n = 5–10) and were analysed by One or Two-Way ANOVA or unpaired Student t-test as appropriate. P < 0.05 is considered was significant.
Results: AITC stimulate afferent activity concentration-dependently. The latency was reduced (69 ± 7 *vs* 43 ± 7 *vs* 32 ± 4 s, P < 0.005) whereas the peak discharges was significantly increased (10 ± 3 *vs* 37 ± 6 *vs* 55 ± 7 spikes/s, P < 0.005) with higher AITC concentration. The response period was also markedly prolonged (0.1 *vs* 0.3 m mol L^{-1} AITC: 153 ± 12 *vs* 598 ± 53 sec, P < 0.0001). 1 m mol L^{-1} AITC-evoked afferent excitation lasted for >20 min. Surprisingly, the afferent response profile to intraluminal distension up to 60 mmHg was not altered by AITC at any chosen concentration, implying TRPA1 may not be primarily involved in the visceral mechanosensitivity of either the low or high threshold afferent subpopulations.
Conclusion: We demonstrate that TRPA1 agonist has a profound effect on mesenteric afferent firing, consistent with its expression on extrinsic sensory nerve terminals in the gut wall. However, the prolonged nature of the response to AITC may indicate its indirect effects following TRPA1-mediated release of mediators from the mucosal epithelium which we have recently revealed a strong TRPA1 gene expression. Supported by the BBSRC.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-09 Basic and Translational Session: Microbiota in Health and Disease

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Stimulation of colonic Toll-Like Receptors induces a local immune response without histological signs of inflammation or changes in bacterial wall adherence in rats
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Objective: Within the gut, host-bacterial interactions are mediated through epithelial toll-like receptors (TLRs). Altered host-bacterial interactions, implying a deregulation in TLR-dependent signaling, might be a pathogenic mechanism leading to intestinal inflammation and secretomotor alterations, as observed in functional gastrointestinal disorders. AIM: We assessed if stimulation of colonic TLRs alters local immune responses, leading to inflammation, and/or host-bacterial interactions, through the modulation of bacterial wall adherence, in rats.
Methods: Adult male SD rats were treated intracolonically with the TLR7 agonist imiquimod (IMQ, 0.1 mg/rat, n = 12), the TLR4 agonist LPS (E. Coli O55:B5, 0.2mg/rat, n = 12), or vehicle [0.5% hydroxypropyl methylcellulose or saline (0.2 ml/rat, n = 8 each)]. Rats were treated with a single dose or daily for 5 consecutive days and euthanized 24 h after the

last treatment. Fluorescent in situ hybridization (FISH) served to characterize bacterial wall adherence of commensal microbiota (Enterobacteria, Bacteroides spp, Lactobacillus spp, Clostridium spp, Bifidobacterium spp and Verrucobacteria). Colonic gene expression of TLR2, 4, 5 and 7 and markers of immune activation (IFNα1, IL-6 and IL-12p40) were quantified by RT-PCR.

Results: Repeated treatment with IMQ or LPS up-regulated inflammatory markers within the colon (IL-6, IFNa1 and IL-12p40; $P < 0.05$ *vs* vehicle), suggesting a local immune activation. Gene expression changes were particularly evident after repeated LPS. After a single dose, only LPS induced immune activation. However, no alterations consistent with the presence of colonic inflammation were observed upon macroscopical or histopathological analysis, regardless the treatment considered. Repeated administration of IMQ up-regulated TLR7 and 4 (TLR7>TLR4), while treatment with LPS enhanced all TLRs assessed (TLR2>TLR7>TLR5>TLR4). A single dose of LPS was enough to up-regulated TLRs expression (TLR2>TLR7>TLR5). The only bacterial group significantly attached to the colonic wall was Clostridium cluster XIVa (incidence of attachment: 50–100%). Neither single nor repeated treatment with either LPS or IMQ affected bacterial wall adherence.

Conclusion: These observations suggest that persistent activation of colonic TLRs leads to a local immune activation, including changes in TLRs expression, without structural alterations; a similar situation as that described in patients with functional gastrointestinal disorders. Altered gut commensal microbiota-TLRs-dependent signaling might be part of the underlying pathophysiological mechanisms of functional gastrointestinal disorders.

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Microbial colonization of germ-free mice induces marked change in behaviour that is independent of bacterial diversity

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Objective: There is growing evidence that the intestinal microbiota can influence the function of the Central Nervous System (CNS). Although the exact pathways of microbiota-gut-brain communication are unknown, neural, metabolic and immune mediated mechanisms have been proposed. Several recent studies have shown marked differences in behavior and brain biochemistry between germ-free and mice colonized with a complex microbiota. The objective of this study was to determine whether mono-colonization of germ-free mice induces similar changes in behavior and immune responses as colonization with a complex microbiota.

Methods: Swiss Webster germ-free mice obtained from Axenic Gnotobiotic Unit of McMaster University were colonized with (i) complex specific pathogen-free (SPF) microbiota, (ii) Altered Schaedler Flora (ASF, combination of 8 commensal bacteria), or (iii) mono-colonized with E. coli JM83 and compared to germ-free controls. Mouse behaviour was assessed before, 2 weeks and 4 weeks post-colonization by light/dark preference test using custom designed automated system placed within sterile flexible film isolator. Colon samples were collected to analyze immune responses using MPO assay and cytokine cytometric bead array (CBA). Cecal contents were analyzed for microbiota using combination of molecular (DGGE, PCR and immunofluorescence) and culture techniques.

Results: We confirmed the absence of bacteria in GF control group, and the monocolonization with E. coli JM83 or the presence of ASF and SPF microbiota in colonized mice. Both mono-colonized mice and mice with complex microbiota exhibited decreased exploratory and more anxiety-like behaviour compared to germ-free mice as they spend less time in the illuminated area at 2 and 4 weeks post-colonization. MPO and cytokine analyses showed no evidence of overt intestinal inflammation in any experimental group at 2 and 4 weeks.

Conclusion: Our results syndicate that bacterial colonization regardless of its diversity induces persistent change in mouse behaviour. We hypothesize that maturity of the immune and physiological systems rather than inflammation induces the observed behavioral changes following bacterial colonization.

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Local activation of Toll-like Receptor 7 (TLR7) enhances colonic epithelial barrier function in rats

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Objective: Toll-like Receptors (TLRs) are implicated in bacterial recognition within the gut and participate in the local neuroimmune control of gut homeostasis, including epithelial barrier function (EBF). AIM: To assess changes in colonic EBF associated to the local stimulation of TLR7 in rats.

Methods: Adult male SD rats were treated intracolonically with the TLR7 agonist imiquimod (100-300 μg/rat, in 0.2 ml) or vehicle (0.5% hydroxypropyl methylcellulose, 0.2 ml/rat); 5 h later colonic samples were obtained. Mucosal sheets were used to assess EBF *In vitro* (Ussing chambers). Epithelial electrical parameters (short-circuit current; potential difference and conductance) in basal conditions and after neural blockade with tetrodotoxin (TTX, 1 μ mol L^{-1}) and paracellular permeability to 4 kDa fluorescein isothiocyanate-dextran (FD4) were assessed. Colonic expression of tight-junction-related proteins [occludin, claudin-3 and zonula occludens 1 (ZO-1)] was assessed by RT-PCR.

Results: Imiquimod, at 300 μg/rat, showed a tendency to decrease conductance, while potential difference and short-circuit current were not affected (Table). Regardless the treatment considered, TTX reduced short circuit current ($\mu A/cm^2$) in a similar proportion (change in short circuit current; vehicle: 2.82 ± 1.37; imiquimod-100 μg: 3.70 ± 0.99; imiquimod-300 μg: 1.83 ± 0.35, n = 5–8; $P > 0.05$). Imiquimod, reduced in a dose-related manner the flux of f FD4 (Table). Direct addition of imiquimod to the Ussing chambers (apical side, 10 μg) did not affect EBF. In imiquimod-300 μg-treated animals, gene expression of occludin and claudin-3 was increased by 2.5-fold and 0.5-fold, respectively, versus expression levels in vehicle-treated animals (n = 3). The expression of ZO-1 was not affected. No macroscopical or histological signs consistent with an inflammatory state were observed after Imiquimod treatment.

Conclusion: Local activation of colonic TLR7 with imiquimod enhanced epithelial barrier function, as indicated by the reduction in conductance and permeability to macromolecules. These functional changes correlated with an increased expression of tight-junction-related proteins (occludin and claudin-3). Activation of TLRs by luminal factors might lead to an increase in epithelial tightness. Since TLRs are activated preferentially by the gut microbiota, increased epithelial tightness by activation of TLR7 might represent a local defensive mechanism generated by dysbiosis and directed towards the prevention of bacterial translocation.

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Up-regulated expression of TLR4 and TLR9 in small bowel mucosa of patients with Irritable Bowel Syndrome (IBS)

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Objective: Toll-like receptors (TLR) are pattern recognition receptors detecting microbial cellular components. The aim of our study was to compare between patients with irritable bowel syndrome (IBS) and healthy controls the expression of TLR2, TLR4, TLR5 and TLR9 in small and large bowel mucosa. These toll-like receptors are the primary mucosal sensors of bacterial patterns and the reasons for doing this study was that low-grade inflammation and immunological alterations have been implicated in the development of IBS and that microbial products are important in the

Effects of local activation of TLR7 with imiquimod on colonic EBF

	Conductance (ms/cm^2)	Short circuit current ($\mu A/cm^2$)	Potential difference (mV)	FD4 flux (% 1h)
Vehicle	22.63 ± 1.17	-21.70 ± 1.50	1.10 ± 0.09	0.029 ± 0.011
	n=28	n=28	n=28	n=9
Imiquimod (100 μg/rat)	22.17 ± 1.07	-26.16 ± 1.96	1.33 ± 0.10	0.024 ± 0.005
	n=26	n=26	n=26	n=12
Imiquimod (300 μg/rat)	18.85 ± 1.01 #	-22.93 ± 2.26	1.32 ± 0.12	0.010 ± 0.003 *
	n=15	n=15	n=15	n=6

Data are mean±SEM. n=number of colonic sheets evaluated. *: P<0.05 vs. vehicle. #: P=0.08 vs. vehicle.

activation of immune and non-immune cells of the intestinal mucosa.
Methods: We analyzed capsule biopsies from the jejunum and endoscopic biopsies from the sigmoid colon of 20 patients (17 females) with IBS aged 18-(39)-66 years and 14 healthy volunteers (12 females) aged 22-(42)-61 years. Eight patients had constipation-predominant IBS (C-IBS), 7 had diarrhea-predominant IBS (D-IBS) and 7 had non-C-non-D-IBS. TLR gene expression was analyzed by RT-PCR. Statistical analysis utilized the unpaired Student's *t*-test for independent samples and the non-parametric Mann–Whitney *U*-test.
Results: We found increased expression of TLR4 ($P < 0.05$) and TLR9 ($P < 0.01$, driven by a higher expression of TLR9 in patients with C-IBS) in the small bowel mucosa of patients with IBS compared to controls. No significant difference was found between cases and controls regarding TLR gene expression in colon mucosa.
Conclusion: Up-regulation of TLR4 and TLR9 suggests the involvement of pathogenic bacteria or dysregulation of the immune response to commensal flora. Our findings suggest that disturbances in the small bowel can be more important than those in the large bowel for developing of IBS.

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Intestinal microbiota in the neuro-immune regulation of behaviour: Exploring role of vagal-microbiota signalling
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Objective: Emerging evidence suggests the intestinal microbiota plays role in modulation of behaviour. In the present study we aimed to investigate the role of vagal-microbiota signalling in the behavioural response to mild colitis.
Methods: AKR male mice were divided into four treatment groups: (A) sham/water ($n = 14$); (B) sham/DSS (3%, 3 cycles 5 days/7 days, $n = 10$); (C) Vagotomy (Vx)/water ($n = 14$); (D) Vx/DSS ($n = 14$). Behaviour was assessed by step-down test. Changes in the community structure of intestinal microbiota were measured by 16S rDNA-pyrosequencing.
Results: 16S rDNA-pyrosequencing demonstrated that the microbial communities in the ceca of mice with either DSS or Vx were distinct from controls. DSS alone induced a higher variability of microbial community composition with increased Firmicutes at phylum level and a 3-fold increase in Lactobacillus (genus) and a decrease in Lachnospiraceae (family). Within the Bacteroidetes phylum, Alistipes (genus) were also increased by DSS administration. These changes were different to the profile in mice with both DSS and Vx. Vx alone induced mild variability of microbial community composition with decreased Actinobacteria (class) and Lachnospiracea (family) and increased Bacteroidales (order) when compared to control mice. DSS reduced exploratory behaviour increasing step-down latency compared to controls (59 ± 9s *vs* 174 ± 16 s; $P < 0.05$). The DSS-increased step-down latency was reduced to control levels by previous Vx (44 ± 5 s, $P > 0.05$). Vx did not alter exploratory behaviour in mice without DSS (41 ± 6s, $P > 0.05$).
Conclusion: These data show that both Vx and chronic mild colitis are independently associated with changes in composition of intestinal microbial communities. The behavioural and microbiota changes induced by colitis were dependent on vagal integrity. Vx alone induced mild changes in the microbiota composition but did not affect behaviour. These data suggest that Vx-associated microbiota alterations do not affect behaviour in normal animals but may contribute to behavioural changes during chronic, mild gut inflammation.

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Non-absorbable oral antibiotic treatment in mice affects multiple levels of the microbiota-gut-brain axis
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Objective: A change in the composition of the gut microbiota is believed to contribute to bowel disorders, changes of systemic immunity and psychiatric comorbidities. Against this background we hypothesized that antibiotic-induced perturbations of the intestinal microbiota have a direct or indirect impact on gastrointestinal, immune and brain function.
Methods: Groups of six to eight male C57BL/6N mice received either normal (sterile) drinking water or sterile water containing neomycin (10 mg ml^{-1}), cefoperazone (1 mg ml^{-1}), or a triple treatment containing neomycin (5 mg ml^{-1}), cefoperazone (0.5 mg ml^{-1}), and ampicillin (2 mg ml^{-1}). On day 7 of the antibiotic treatment, locomotor and anxiety-related behavior was examined with the open field test (OFT), and on day 8 learning/memory was assessed with the novel object recognition test (NORT). Body weight was assessed before and after antibiotic treatment, and cecum and spleen weight determined after sacrifice.
Results: Unlike cefoperazone, neomycin and the triple treatment stopped the gain of body weight seen in control animals. Cecum weight was increased significantly in all treatment groups. Additionally neomycin and the triple treatment led to a decrease of spleen weight. In the OFT neomycin reduced locomotion as shown by a decrease of the total traveling distance, while the triple treatment seemed to be anxiolytic as it tended to increase the time spent in the central area. In the NORT the triple treatment had an adverse effect on learning/memory as it abolished the preference for the novel object.
Conclusion: The present results reveal that oral treatment with different non-absorbable antibiotics leads to differential local and systemic effects via the microbiota-gut-brain axis. While cefoperazone increased cecum weight only, neomycin and the triple treatment decreased spleen weight indicative of systemic immune alteration. Behaviorally, neomycin alone impaired only locomotion, whereas the triple treatment impaired learning/memory and tended to decrease anxiety. This phenotype is reminiscent of that of germ-free mice which show memory impairment and a decrease of anxiety-like behavior. Our observations indicate that antibiotic-induced perturbations of the intestinal microbiota affect multiple levels of the microbiota-gut-brain axis.

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Role of gastrointestinal dysmotility on intestinal bacterial overgrowth and translocation following burn injury
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Objective: In severe burns, increased intestinal permeability facilitates bacterial translocation (BT) resulting in systemic endotoxemia and multi-organ failure. The role of burn-induced gastrointestinal dysmotility (BIGD) in facilitating BT has not been explored. Here, we investigated (i) the role of BIGD in promoting BT following burn injury, and (ii) whether ghrelin intervention is protective in this process.
Methods: SD rats receiving either sham burn or 60% total body surface area scald burn were fed a phenol red-marked meal and were euthanized after 30 min to measure gastric emptying (GE) and intestinal transit (IT). For intervention studies, animals received ghrelin or vehicle at 1 and 8 h after burn. GE was calculated as the percentage of phenol red recovered from the stomach. IT was determined by measurement of geometric center (GC)of labeled probe. Mesenteric lymph nodes (MLN), liver, spleen, lung and distal small intestine were collected and homogenized for bacterial studies. Serially diluted tissue homogenates were plated onto MacConkey agar and colony forming units (CFU) were determined per gram of tissue.
Results: Scald burn was associated with a significant delay in GE (62 ± 4 *vs* 74 ± 4%; $P = 0.02$), and this trend continued into the small intestine (GC: 5 ± 0.1 *vs* 5.8 ± 0.2; $P = 0.09$). This delay in transit was associated with a significant increase in small intestinal bacterial overgrowth "IBO" (6×10^5 *vs* 2×10^5 CFU g^{-1}; $P = 0.05$), and BT to MLN (2×10^2 *vs* 4×10^1; $P = 0.03$). A significant negative correlation between GE and IBO (rs = -0.61; $P = 0.002$) and between IT and BT (rs = -0.63; $P = 0.004$) was found suggesting that both GE and IT regulate IBO and BT from the small intestine. Ghrelin administration significantly accelerated GE following burn injury (91 ± 3 *vs* 62 ± 4%; $P = 0.00002$), reducing IBO and completely inhibiting BT to MLN (0.0 *vs* 5×10^2; $P = 0.01$).
Conclusion: Our work shows for the first time a significant correlation between BIGD and the systemic translocation of gram negative gut bacteria that are implicated in multiple organ failure in burn patients. Consideration of therapeutic interventions that restore gastrointestinal dysmotility following burn injury is thus warranted to prevent such bacterial translocation.

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Mechanism of opioid tolerance in bowel dysfunction

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Objective: Opioids are one of the most efficacious analgesics used for treatment of moderate to severe pain. Repeated administration results in tolerance development to many of its effects including analgesia and respiratory depression. Clinical evidence suggests that tolerance however does not develop to morphine-induced constipation, severely hampering quality of life. We have previously shown that tolerance to morphine develops in the isolated ileum but not the colon from mice and guinea pigs (Ross et al., JPET 327 (2008); Kang et al., JPET 340 (2012)). The canonical pathway of opioid tolerance involves β-arrestin2 as its deletion prevents antinociceptive tolerance. The objective of this study was to examine the role of β-arrestin2 in the mechanism of opioid tolerance in these tissues.
Methods: Opioid-induced contractions were recorded from circular muscle preparations from wild type C57/Bl6 (WT) and β-arrestin2 knock-out mice (KO) in organ bath. Primary cultures of myenteric neurons were prepared from mouse ileum and subjected to patch-clamp recordings. β-arrestin2 expression was determined in whole-mount and isolated neuron cultures by immunohistochemistry.
Results: Repeated administration of ED90 for morphine (3 μ mol L^{-1}), DAMGO (3 μ mol L^{-1}), Fentanyl (0.1 μ mol L^{-1}) and etorphine (0.01 μ mol L^{-1}) resulted in tolerance development in the isolated ileum of both WT and KO circular muscle. However, only etorphine produced tolerance in the colon of WT whereas all opioids produced tolerance in the colon of KO mice suggesting ligand bias. The expression of β-arrestin2 was localized to neuronal cell bodies within myenteric plexus. The cellular mechanism of opioid effects was examined by patch-clamp of isolated mouse neurons. Two types of action potentials were observed. In 21/33 neurons a 7.5 ± 0.7 mV afterhypepolarization was evident (AH neurons) while 12/33 fired multiple action potentials (S neurons) upon current injection. Morphine (3 μ mol L^{-1}) reduced excitability of AH neurons with a shift in rheobase. This was reversed upon prolonged exposure (2 h) demonstrating tolerance in single neurons. Voltage-clamp analysis revealed inhibition of Na^+ currents by morphine.
Conclusion: Our findings suggest that unlike antinociceptive tolerance, the deletion of β-arrestin2 in enteric neurons promotes opioid tolerance in the gastrointestinal tract. Inactivation of Na+ channels downstream may underlie the mechanism of reduced gastrointestinal function.

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Inhibition of Fatty Acid Amide Hydrolase (FAAH) activity by PF-3845 reduces diarrhoea and alleviates abdominal pain in mice

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Objective: The endocannabinoid system (ECS) consists of cannabinoid receptors (CNR1 and CNR2), endogenous ligands (anandamide; AEA and 2-arachidonoylglycerol; 2-AG) and metabolic enzymes (fatty acid amide hydrolase, FAAH; monoacylglyceride lipase, MAGL). The components of ECS are widely distributed in the GI tract and play an important role in the regulation of GI motor function and sensation of visceral pain. The aim of this study was to characterize the effect of the novel highly selective FAAH inhibitor, PF-3845 on GI motility and visceral sensitivity in mouse models designed to mimic the key symptoms of the irritable bowel syndrome (IBS).
Methods: Pharmacological effects of PF-3845 on GI motility were studied *In vitro*, using organ baths and *in vivo*, by measuring colonic propulsion, whole GI transit (WGT) and stool output in mice in physiological and stress or drug induced diarrhoea. The effects of PF-3845 on visceral sensitivity were characterized in behavioral mouse models of pain. Endocannabinoid levels were established in ileum and colon samples obtained from control and PF-3845-treated mice using LC-MS methods. Finally, FAAH mRNA expression was quantified in biopsies obtained from IBS patients.
Results: PF-3845 (10^{-12}–10^{-6} mol L^{-1}) inhibited the EFS-stimulated smooth muscle contractions in mouse colon in a concentration-dependent manner and this effect was blocked by the CNR1 antagonist AM 251 (10^{-7} mol L^{-1}), but not the CNR2 antagonist AM 630 (10^{-7} mol L^{-1}). PF-3845 had no effect on ileal contractions *In vitro. in vivo*, PF-3845 (30 mg kg^{-1}, ip) slowed colonic bead expulsion and WGT in mice, and improved stool output in animals with GI hypermotility and diarrhea. The effect of PF-3845 on colonic bead expulsion was absent in FAAH-/- mice. The novel FAAH blocker also significantly reduced the number of pain-induced behaviors in mouse models of visceral hypersensitivity. PF-3845 increased AEA and decreased AEA metabolite levels in colon, but not ileum samples from mice with chemically induced IBS-mimicking symptoms. Interestingly, our preliminary studies showed that FAAH mRNA levels in biopsies from IBS patients were significantly lower compared with healthy controls.
Conclusion: Blocking FAAH activity may be a promising approach to the treatment of increased intestinal motor function and visceral hypersensitivity, principal symptoms of diarrhea predominant IBS.

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Achalasia in mice lacking NO-sensitive guanylyl cyclase

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Objective: Esophageal achalasia is known to result from impairment of nitrergic inhibitory neuromuscular transmission. To date, the exact mechanism of nitric oxide (NO)-induced smooth muscle relaxation is still controversial. NO-sensitive guanylyl cyclase (NO-GC) acts as the main target of NO and has been demonstrated to be an important physiological mediator of nitrergic relaxation of the lower esophageal sphincter (LES).
Methods: Recently, we have generated mice lacking NO-GC (GCKO). We showed that deletion of NO-GC resulted in the impairment of GI motility concomitant with a totally abolished NO responsiveness of gastrointestinal and vascular smooth muscle. To clarify the contribution of NO-GC in different cell types to LES relaxation we generated cell-specific knockout mouse lines lacking NO-GC in smooth muscle cells (SM-GCKO) and interstitial cells of Cajal (ICC-GCKO) as well as in both cell types (SM/ICC-GCKO).
Results: We applied esophageal manometry to study the functionality of the LES in the cell-specific knockouts. Isometric force studies on LES were performed to monitor the responsiveness to exogenous NO.
Conclusion: Here we show that the nitrergic inhibitory neuromuscular transmission is mediated by NO-GC in at least two cell types (SMC and ICC) of the LES. In addition, we recently started to investigate the involvement of a third cell type (fibroblast-like cells) which expresses high amounts of NO-GC.

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Comparison of peptidergic neuromuscular transmission in the wildtype and VIP-/- murine internal anal sphincter

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Objective: We have previously provided evidence that VIP contributes to inhibitory neuromuscular transmission (NMT) in the mouse IAS. These studies also revealed an additional non-cholinergic excitatory component that may be due to tachykinins. To further examine the role of peptides in NMT in the mouse IAS we compared responses to nerve stimulation in wildtype (WT) mice to those in VIP-/- mice.
Methods: Contractile and electrical responses to electrical field stimulation (EFS) of nerves were measured in the absence and presence of various blockers including the NOS inhibitor L-NNA (100 μ mol L^{-1}), the P2Y1 receptor antagonist MRS2500 (1 μ mol L^{-1}), the NK-1 receptor antagonist L-733,060 (3 μ mol L^{-1}) and

the NK-2 receptor antagonist MEN10, 627 (1 μ mol L^{-1}) (all under NANC conditions).
Results: Short trains of EFS (4 s) gave rise to frequency (0.1–10 Hz) dependent relaxation and hyperpolarization in WT and VIP-/- mice. Responses were not different between mice under control conditions, nor in the presence of MRS2500. However, they were abolished with combined addition of MRS2500 and L-NNA, suggesting that both NO and purines contribute to inhibitory NMT. These data also indicate that nitrergic NMT does not require VIP. With longer stimulus trains (5 Hz, 30–60 s; 20 Hz, 4–30 s) a slowly developing nitrergic and purinergic-independent relaxation and hyperpolarization were observed in WT but not VIP-/- mice, suggesting that these responses were due to VIP. In WT mice, the slow component was blocked by the VIP receptor antagonist VIP6-28 (30 μ mol L^{-1}); further supporting this hypothesis. Non-cholinergic EFS-induced contraction was observed in VIP-/- mice in the presence of L-NNA and MRS2500. Comparable responses were elicited in WT mice with the further addition of VIP6-28. These contractions were significantly reduced but not abolished with combined addition of L-733,060 and MEN10, 627.
Conclusion: These data provide additional support for VIP as an inhibitory neurotransmitter in the mouse IAS. They also suggest a role for tachykinergic NMT. Both peptidergic pathways require higher levels of EFS whereas only the VIP pathway persists well beyond the period of nerve stimulation. Grant support: DK078736.

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Dopaminergic signaling in mouse duodenum and postnatal developmental changes
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Objective: In this study, we examined in neonatal versus adult mice the functionality of the dopaminergic systems in the regulation of duodenal contractility, since enteric neurons, similar to other biologic systems, are immature at birth and may change with age. In mouse, transcripts for all G-coupled dopaminergic receptors (D1-like family, D1 and D5 receptors, and D2-like family, D2, D3, and D4 receptors) can be detected at each age.
Methods: Mechanical responses to dopaminergic receptor activation were examined *In vitro* in duodenal longitudinal muscle from postnatal and adult mice as changes in isometric tension.
Results: In postnatal preparations, dopamine evoked a TTX-insensitive muscular contraction, reduced by SCH 23390, D1 receptor antagonist, but not by domperidone, D2 receptor antagonist, and mimicked by a D1 receptor agonist. In adults, dopamine response changed to a TTX-insensitive muscular relaxation reduced by domperidone, and, to a lesser extent, by SCH 23390. D1 or D2 receptor agonists mimicked dopamine responses. Contractile responses to dopamine were shown before day 15, and then only relaxations could be detected, being comparable to adult around day 20. Analysis of the response mediated by D1 receptor activation showed that in neonatal mice the excitatory effects were antagonized by U-73122, phospholipase C (PLC) inhibitor, whilst in adults the inhibitory effects were blocked by DDA, adenylyl cyclase inhibitor.
Conclusion: In mouse gut, dopaminergic transmission undergoes to postnatal change in the pattern of receptor functionality. In postnatal period, the responses to dopamine are mediated exclusively by D1-like receptors, likely D5 receptors, linked to activation of PLC leading in turn to muscular contraction. In adults, the response to dopamine changes in a muscular relaxation as the consequence of a recruitment of D2 receptors and of a shift of the effects induced by D1-like receptors, likely D1 receptors, via activation of cAMP pathway. Remain to determine the physiological importance of the switch in the dopamine response in the mouse duodenum, but it may be related to the marked changes in the gastrointestinal tract associated with weaning from maternal milk to solid food.

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The involvement of sympathetic nerve activation in decreased ghrelin production and secretion in urocortin-1 induced stress model
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Objective: Previously, we demonstrated that urocortin (UCN)-induced anorexia may be partly caused by decreased gastric ghrelin secretion via central CRF2 receptor activation. However, the detailed mechanisms have not been elucidated. To have a better understanding of the detailed mechanisms decreasing ghrelin secretion during stress condition, we examined the roles of adrenergic receptors on the decreased plasma ghrelin levels in UCN-treated stress rats.
Methods: Twenty-four h fasted male SD rats were given an intracerebroventricular (ICV) injection of UCN or pPBS, and their blood samples were collected for ghrelin assay. The test drugs were administered 15 min before UCN injection or simultaneously. Plasma acyl and des-acyl ghrelin levels were measured by enzyme-linked immunosorbent assay. To examine fos expression, the brains were fixed by fixative perfusion and removed, and then the fixed whole brains were cut in the coronal plane. The central expression of c-fos mRNA and protein were evaluated by in situ hybridization and immunohistochemical staining, respectively.
Results: Increased expression of both c-fos mRNA and protein were clearly observed in the solitary tract nucleus of rats under UCN-induced stress. They were also observed in several other areas, including the supraoptic nucleus, paraventricular nucleus of the hypothalamus, central nucleus of amygdala, locus coeruleus, and the ventrolateral medulla. They were barely observed in the dorsal motor nucleus of the vagus. Decreased plasma acyl and des-acyl ghrelin levels in rats under UCN-induced stress were inhibited by treatment with a α-adrenergic receptor antagonist (phentolamine: 5 mg kg^{-1}, i.p.), an α2-adrenergic receptor antagonist (yohimbine: 5 mg kg^{-1}, i.p.), a β-adrenergic receptor agonist (isoproterenol: 5 mg kg^{-1}, i.p.) and a β1-adrenergic receptor agonist (Denopamin: 0.1 mg kg^{-1}, i.p.). However, the treatment of a β-adrenergic receptor antagonists (propranolol: 10 mg kg^{-1}, i.p.), a α1-adrenergic receptor antagonists (prazosin: 5 mg kg^{-1}, i.p.) and β2-adrenergic receptor agonist (Sabutamol: 0.1 mg kg^{-1}, i.p.) failed to inhibit the decrease in ghrelin level.
Conclusion: This study indicates that sympathetic efferent nerves may be involved in the inhibition of gastric ghrelin secretion, and that α2-adrenergic receptor signaling activation and β1-adrenergic receptor signaling reduction are involved in reduced ghrelin secretion in rats under UCN-induced stress.

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Influence of CORM-A1 and resveratrol on TNF-a-induced oxidative stress and apoptosis in murine MODE-K intestinal epithelial cells
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Objective: Carbon monoxide (CO)-releasing molecules (CORMs) were shown to markedly reduce oxidative stress in the small intestinal mucosal layer and to restore intestinal transit in a mouse postoperative ileus model (De Backer et al., Gut, 2009). Resveratrol, a cytoprotective bioactive polyphenol present in red wine, exerts its neuroprotective effect against stroke through induction of heme oxygenase (HO)-1, which metabolizes heme to CO, biliverdin and ferrous iron (Sakata et al., 2010). We therefore investigated the effect of CORM-A1 and resveratrol on TNF-α-induced oxidative stress and apoptosis in murine Mode-K intestinal epithelial cells.
Methods: For the preliminary optimization experiments, confluent Mode-K cells (passage 10-35) were exposed to TNF-α/cycloheximide (CHX) for up to 6 h. To investigate the effects of CORM-A1 and resveratrol, the cells were pretreated with the test agents for 1 h followed by its co-treatment with TNF-α/CHX for 3–6 h for different experiments.
Results: TNF-α/CHX increased reactive oxygen species (ROS) production (carboxy-H2DCFDA fluorescence intensity measurement), reduced the level of the antioxidant glutathione (bioluminescence assay), increased caspase-3/7 activity (bioluminescence assay), and induced apoptosis (subdiploid DNA analysis by flow cytometry) in a concentration-dependent manner (0.1 to 1 ng ml^{-1}; 10 μg ml^{-1} CHX). The effects at 1 ng ml^{-1} TNF-α were similar to those at 20 ng ml^{-1} TNF-α, classically used in cell studies. CORM-A1 (100 μ mol L^{-1}) and resveratrol (75 μ mol L^{-1}) were tested against the effects of 1 ng ml^{-1} TNF-α. The decrease in reduced glutathione level by 1 ng ml^{-1} TNF-α, was only very partially prevented by pre- and co-treatment with CORM-A1 and resveratrol. The TNF-α-induced increase in ROS production was partially reduced by CORM-A1, but was completely abolished by resveratrol. The increase in caspase-3/7 activity by TNF-α was only marginally influenced by CORM-A1 while it was markedly decreased by resveratrol. The TNF-α-induced apoptosis was partially reduced by CORM-A1 and resveratrol. Basal HO-1 protein expression (Western blot) was decreased by 1 ng ml^{-1} TNF-α. CORM-A1 did not change basal HO-1 protein levels, nor did it prevent the decrease by TNF-α. While resveratrol per se

increased HO-1 protein expression, it also did not prevent the decrease by TNF-α.
Conclusion: Reduction of oxidative stress by CORM-A1 and resveratrol might contribute to their protective effects on intestinal epithelial cells.

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Mechanisms underlying hydrogen sulphide relaxation in the rat colon
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Objective: Hydrogen sulphide (H2S) is an endogenous gaseous signalling molecule with putative functions regulating colonic motility. We have recently demonstrated that H2S inhibits propulsive contractions without affecting ripples using video-recording of motility and construction of Spatio-Temporal maps. The aim of this study was to investigate the mechanism/s underlying H2S effects in the rat colon.
Methods: Microelectrode and muscle bath studies were performed on colonic strips (full thickness and strips without submucosa). Sodium hydrogen sulphide (NaHS) was used as H2S source.
Results: In strips devoid of submucosa, NaHS dose-dependently hyperpolarized smooth muscle cells (IC50 = 111.6 μ mol L^{-1}, n = 3). NaHS (0.3 m mol L^{-1}) induced hyperpolarization was reverted by a cocktail of potassium channel blockers [apamin(1 μ mol L^{-1}), TRAM-34(1 μ mol L^{-1}), glibenclamide(10 μ mol L^{-1}) and charybdotoxin(0.1 μ mol L^{-1})]. Furthermore, tissue incubation with L-NNA (1 m mol L^{-1}) and MRS2500 (1 μ mol L^{-1}), a P2Y1 antagonist, revealed an atropine sensitive excitatory junction potential (EJP) reaching in presence of nifedipine 3–4 mV in amplitude. NaHS (1 m mol L^{-1}) inhibited cholinergic EJP (<1 mV, n = 4). Carbachol (10 μ mol L^{-1}) caused an atropine sensitive contraction (5.7 ± 0.8 g min^{-1} AUC; n = 5) that was inhibited by a 52.4 ± 4.6% by NaHS (1 m mol L^{-1}). Slow wave activity was recorded in full thickness strips at a frequency of 11.1 ± 0.8 cpm(n = 4). NaHS (1 m mol L^{-1}) caused a smooth muscle hyperpolarisation (-7.1 ± 1.8 mV) without a major change in slow wave frequency (11.0 ± 1.3 cpm; n = 4).
Conclusion: We conclude that (1) smooth muscle hyperpolarization and post-junctional cholinergic inhibition might be responsible for the inhibition of the propulsive activity induced by NaHS and (2) the lack of effect on slow wave activity driven by pacemaker interstitial cells of Cajal associated with the submuscular plexus explains the lack of effect of NaHS on ripples.

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Assessing the molecular target for hydrogen sulphide in the mouse small intestine and sensory ganglia
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Objective: Evidence suggests that hydrogen sulphide (H2S) plays a role in the development of chronic visceral hyperalgesia and pain (Rong et al., 2011, Inflamm Allergy Drug Targets). A number of different molecular targets has been suggested based on a direct action at the level of the sensory nerve endings, including T-type $Ca2^{+}$ channels, KATP channels and TRPA1 (Streng et al., 2008, Eur Urol; Nagasawa et al., 2009, J. Neurochem; Smith 2009, Pain Physician). However, previous work from our laboratory has suggested that the effect of H2S on gut afferent firing is secondary to mediator released from the mucosal epithelium and has implicated a role for ATP despite lacking any direct effect of H2S on HEK cells expressing P2X2-7 receptors. Indeed, these studies using heterologously expressed receptors and ion channels have identified TRPC and TRPM channels as potential targets for H2S activation. In the present study we have examined the expression of these and other TRP channels in the intestinal mucosa and compared this to expression in dorsal root ganglion (DRG) neurons.
Methods: Total RNA was isolated from small intestinal mucosa and thoracolumbar DRGs (T10-L2) of C57/B6 adult male mice. Reverse transcription polymerase chain reaction (RT-PCR) was used to examine gene expression for TRPA1, TRPV1, TRPC4, 5 and 6, TRPM2 and 4. Products were separated by gel electrophoresis and detected with ethidium bromide.
Results: A bright TRPV1 band was present in DRG but not mucosa. TRPA1 was strongly expressed in both mucosa and DRG. Its expression intensity was comparable to TRPV1. TRPC6 and TRPM4 were also relatively strong expressed in mucosa but weaker in DRG compared with TRPA1. Transcripts for TRPC4, 5, and TRPM 2 were also detected in both mucosa and DRG but their expression levels were relatively weaker compared with TRPC6 and TRPM4.
Conclusion: These data demonstrate that molecular targets for H2S exist both at the level of the intestinal mucosa and sensory nerve endings in the gut wall. Functional studies are underway to determine the relative contribution of direct and indirect mechanisms to the sensory signals generated by H2S. Supported by the BBSRC.

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Purinergic (P2Y1) and Nitrergic neuromuscular transmission in the human small intestine
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Objective: Based on our previous results neuro-muscular transmission is purinergic (P2Y1-mediated) and NO-mediated in the human colon. The pharmacological approach has been recently validated with P2Y1 KO mice. Accordingly, we wanted to investigate neuromuscular transmission in the human small intestine and to establish whether P2Y1 receptors play a role in purinergic transmission.
Methods: Jejunum (n = 6) and ileal (n = 6) samples were studied using sharp microelectrodes and muscle bath techniques. None of the samples showed histological neuro-muscular abnormalities.
Results: The frequency of spontaneous contractions was lower in the ileum (6.5 ± 0.44 cpm.) compared to the jejunum (9.17 ± 0.39 cpm; P < 0.01), whereas no difference in the amplitude of contractions was observed. TTX (1 μ mol L^{-1}) and L-NNA (1 m mol L^{-1}) increased spontaneous contractions in the ileum (ANOVA P < 0.05), but not in the jejunum (n.s.).Resting membrane potential was slightly more depolarised in jejunum (-40 ± 1.5 mV) compared to the ileum (-45 ± 2 mV). Inhibitory junction potentials were elicited with 3 protocols of electrical field stimulation. Protocol 1. Single pulses at increasing voltage of stimulation elicited a fast IJP progressively higher in amplitude reaching -30mV in the jejunum and -20mV in the ileum. Both responses were totally blocked by MRS2179 (10 μ mol L^{-1}). Protocol 2. 5 consecutive pulses at 1 Hz elicited 5 fast IJP that were totally blocked by MRS2179 (10 μ mol L^{-1}). Protocol 3. EFS at 5 Hz during 5 seconds elicited a fast followed by a sustained IJP. MRS2179 (10 μ mol L^{-1}) antagonised fast IJP while L-NNA antagonised sustained IJP. EFS induced a mechanical relaxation that was antagonised by L-NNA and MRS2179 (1 and 10 μ mol L^{-1}). Exogenous addition of Sodium Nitropusside (1 μ mol L^{-1}) and ADPßS (1 μ mol L^{-1}) induced a smooth muscle hyperpolarization (about 10 mV) with cessation of spontaneous motility. ADPßS responses were inhibited by MRS2179 10 μ mol L^{-1}. All these mechanisms were similar in the jejunum and ileum.
Conclusion: We conclude 1.Purinergic (via P2Y1) and nitrergic neuromuscular transmission is the predominant inhibitory mechanism in the human small intestine 2. Differences between jejunum and ileum are consistent with (i) a gradient in pacemaker frequencies and (ii) the presence of a nitrergic neural tone in the ileum but not in the jejunum. Our data demonstrate a common mechanism controlling smooth muscle relaxation throughout the human small and large intestine.

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Absence of purinergic neuromuscular transmission in the antrum and caecum of P2Y1 knocked out mice
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Objective: Previous studies performed in the human colon using selective agonists and antagonists of the P2Y1 receptor demonstrated the crucial role of this receptor in neuromuscular transmission. Recently, studies performed in the mouse colon of P2Y1-/- knock out (KO) mice confirmed that P2Y1 receptors are essential to accomplish purinergic neuromuscular transmission. The aim of the present study was to test whether P2Y1 receptors are or are not involved in purinergic neurotransmission in other areas of the gastrointestinal tract using P2Y1-/- KO mice.
Methods: Microelectrode recordings were performed in strips from antrum and caecum of wild type (WT) (n = 4) and P2Y1-/- KO mice (n = 5).
Results: No differences in the resting membrane potential were observed between groups neither in the antrum nor in the caecum. In the caecum of WT animals, electrical field stimulation (Single pulses and trains of 1, 5 and 20 Hz) revealed an IJP (reaching 24.7 ± 2.8 mV at 20 Hz) that was abolished by the P2Y1 receptor antagonist MRS2500 (1 μ mol L^{-1}). In contrast in P2Y1-/- KO mice the IJP was reduced (6.0 ± 1.9 mV at 20 Hz) and was totally abolished by L-NNA (1 m mol L^{-1}). ''Spontaneous'' IJP (i.e. ongoing

release of neurotransmitters), MRS2500 sensitive, could be recorded in WT but not in P2Y1-/- animals. MRS2365 (1 μ mol L^{-1}), a P2Y1 agonist, caused a smooth muscle hyperpolarization in WT animals, that was blocked by MRS2500. In contrast, MRS2365 did not modify smooth muscle resting membrane potential in P2Y1-/- KO mice. In the antrum, slow wave activity (9.1 ± 2.3 mV) was observed at 7.2 ± 0.6 cpm in WT animals. Slow waves were observed (n.s.) in P2Y1-/- KO mice. In WT animals EFS (Single pulse) induced an IJP (8.5 ± 1 mV) that was abolished by MRS2500. In contrast in P2Y1-/- KO mice EFS (Single pulse) induced a small IJP (1.6 ± 0.6 mV) that was L-NNA sensitive.
Conclusion: We conclude that P2Y1 receptors mediate purinergic neurotransmission in the caecum and antrum demonstrating a common mechanism of purinergic inhibition in the gastrointestinal tract. P2Y1-/- KO mouse is a useful animal model to study the role of purinergic neurotransmission in patho-physiological motor functions in the gut.

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Early gastrointestinal motor disturbances induced by Lipopolysaccharide (LPS) in sheep may be initiated through activation of COX-2 in pulmonary and hepatic macrophages

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Objective: The aim of this study was to investigate the involvement of cyclooxygenase (COX) and peripheral macrophages in the gastrointestinal motor alterations induced by LPS.
Methods: Myoelectric activity was recorded in conscious ewes with electrodes implanted in rumen, gastric antrum, duodenum and jejunum. Body temperature was continuously monitored with an intraperitoneal thermistor. In addition, groups of 3 ewes were treated intravenously (iv) with saline or LPS (0.1 μg kg^{-1}) from Escherichia coli and slaughtered 1 or 4 h later. Samples of rumen, antrum, duodenum, liver and lung were collected for immunohistochemistry (IHC) and western blotting (WB) studies.
Results: LPS increased body temperature, inhibited antral and intestinal myoelectric activity and increased MMC frequency. These effects were completely abolished by the iv pretreatment with the selective COX-2 inhibitor nimesulide (1 mg kg^{-1}) or with tyloxapol (200 mg kg^{-1} h^{-1}, 1 h), an agent that desensitizes endotoxin-recognizing receptors in macrophages. In control animals, COX-1 and COX-2 were expressed in the vascular endothelial cells of all tissues. Furthermore, positive COX-1 immunostaining was observed in the cytoplasm of epithelial cells of the rumen, antral glands and intestinal goblet and endocrine cells. In the enterocytes, COX-1 staining showed the same perinuclear localization than the cis-Golgi protein GM130. COX-2 was present in the cells of intestinal circular muscle layer and in the muscularis mucosae of antrum and intestine. COX-1 and COX-2 were detected in endocrine cells in the epithelium of the bile ducts and bronchial tubes. In the liver, COX-2 was observed in CD163 (ED2) positive cells (macrophage marker) located in the sinusoids and in the portal space. In the lung, COX-2 was localized in some cells scattered throughout the parenchyma. LPS administration strongly increased the number of the hepatic macrophages expressing COX-2. In the lung, LPS induced the appearance of groups of rounded cells related with blood vessels that were positive to COX-1 and COX-2 and coexpressed the ovine macrophage marker VPM32. WB showed bands of 70 kDa (COX-1) and 72 kDa (COX-2) in all tissues. LPS increased the intensity of the COX-2 band only in the liver.
Conclusion: Our data suggest that LPS alters gastrointestinal motility and induces fever in sheep through the release of peripheral prostaglandins, probably produced by COX-2 in hepatic and/or bronchial macrophages.
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Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
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Regulation of excitation-contraction and excitation-transcription coupling by caveolin-1 via microRNA133a and transcription factors, Foxo4 and Elk1 in gastrointestinal smooth muscle

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Objective: We have previously shown that in gastrointestinal muscle, disruption of caveolae or suppression of its constituent protein, caveolin-1 inhibits muscarinic m3 receptor-mediated RhoA/Rho kinase signaling and contraction. The RhoA/Rho kinase pathways have been associated with regulation of smooth muscle-specific proteins and their transcriptional factors, SRF and myocardin. Myocardin activity is inhibited by phosphorylation of Elk via ERK1/2 and stimulated by phosphorylation of Foxo4 via Akt.
Aim: To examine whether absence of caveolin-1 affects the expression of contractile proteins in gastric smooth muscle.
Methods: Mouse gastric smooth muscle cells were cultured in DMEM-10 and caveolin-1 siRNA was transfected for 48 hours. Rho kinase and ERK1/2 activity was measured by immunokinase assay, and phosphorylation of Elk1 and Foxo4 forkhead transcription factor was measured using phospho-specific antibody. Expression of microRNA133a (miR133a), Akt1, transforming growth factor β1 Induced transcript 1 (TGFβ1I1), serum response factor (SRF), myocardin, smooth muscle myosin heavy chain (smMHC), caldesmon and calponin were measured by real time PCR in control and caveolin-1 siRNA transfected cells as well as in gastric muscle from control and caveolin-1 knockout mice.
Results: In muscle cells transfected with caveolin-1 siRNA: (i) Acetylcholine (ACh)-induced Rho kinase activity was significantly inhibited, (ii) basal and ACh-induced ERK1/2 activity, phosphorylation of EGF receptor and Elk1, and expression of microRNA133a were significantly augmented, and (iii) phosphorylation of Foxo4, and expression of Akt1, SRF, myocardin and the smooth muscle-specific proteins, smMHC, TGFβ1I1, caldesmon, and calponin were significantly reduced. Transfection of miR133a precursor, which increases miR133a expression, mimicked the effect of caveolin-1 siRNA on basal and ACh-induced ERK1/2 activity and phosphorylation of EGFR, Elk1 and Foxo4. In contrast, transfection of miR133a antagamir, which decreases mir133a expression, or treatment of cells with the selective EGFR inhibitor reversed the effect of caveolin-1 siRNA. Similarly, overexpression of caveolin-1 decreased miR133a expression and inhibited ACh-induced ERK1/2 activity and EGFR phosphorylation. Expression of TGFβ1I1, Akt1, caldesmon and calponin, and ACh-induced Rho kinase activity and contraction were also inhibited in muscle cells from caveolin-1 knockout mice.
Conclusion: We conclude that caveolin-1 facilitates smooth muscle contractile phenotype by regulation of miR133a expression, and by stimulation of Foxo4 phosphorylation via Akt and inhibition of Elk phosphorylation via ERK1/2.

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Platelet-Derived Growth Factor Receptor a positive (PDGFRa+) cells in mice colonic smooth muscles are the primary site of purinergic inhibitory motor neurotransmission

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Objective: Inhibitory motor neurotransmission in the GI tract consists of nitrergic, purinergic and peptidergic components. Stimulation of inhibitory neurons (single pulses to <10 Hz) elicits inhibitory junction potentials (IJPs) that consist of a fast, transient component (fIJP)

that is purinergic and a slower component (sIJP) that is nitrergic. The purinergic component is mediated by P2Y1 receptors. We sought to compare responses of PDGFRα+ cells and smooth muscle cells (SMC) to purinergic (P2Y1) stimuli to determine which cells might mediate purinergic responses *in vivo*.

Methods: Currents and voltage-transients elicited by P2Y1 agonists (ATP, ADP, β-NAD and MRS2365) were measured in PDGFRα$^+$ cells obtained from colonic smooth muscles of Pdgfratm11(EGFP)Sor/J heterozygote mice with constitutive labeling on PDGFRα$^+$ cells via expression of a histone/eGFP fusion protein and SMC, in patch clamp using either voltage- or current-clamp conditions.

Results: PDGFRα$^+$ cells displayed large amplitude, $Ca2^+$-sensitive outward currents when exposed to P2Y1 agonists. The outward current reversed at about -80 mV, close to the equilibrium potential for K^+, and displayed a linear I-V relationship between -80 and 0 mV. These currents were blocked by SK channel blocker apamin or P2Y1 antagonist, MRS2500. In current clamp (I = 0) condition, P2Y1 agonists elicited fast, transient hyperpolarization responses in PDGFRα+ cells with a peak at about -80 mV (i.e. EK). These responses were blocked by MRS2500. In contrast, P2Y1 agonists failed to elicit similar hyperpolarization in SMC.

Conclusion: In colonic muscles, the purinergic component of post-junctional responses (fIJP), mediated *in vivo* by P2Y1 receptors, is a fast, transient hyperpolarization. SMC did not hyperpolarize from resting membrane potentials in response to P2Y1 agonists, but fast, transient hyperpolarizations were elicited in PDGFRα+ cells. These data suggest that PDGFRα+ cells are the primary site of membrane potential responses to purinergic motor neurotransmission in mice colonic muscles (Supported by DK41315).

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Functional characterization of Ca2+-activated Cl- conductance in murine colonic ICC

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Objective: Immunohistochemistry studies demonstrated that ANO1 (Tmem16a) expressed in interstitial cells of Cajal (ICC) of murine colonic smooth muscle. Spontaneous electrical activity of murine colon is composed of slow depolarization with spike action potential complexes. Spike action potential complexes are due to activation of L-type $Ca2^+$ channels. However, little is known about ionic conductance for slow depolarization which leads the activation of L-type $Ca2^+$ channels. It has been proposed that the spontaneous generation of slow wave may be due to activation of ion conductance in ICC. In this study we investigated the functional role of $Ca2^+$-activated Cl- conductance (CaCC) in relation to slow wave generation in murine colonic smooth muscles.

Methods: Conventional microelectrode recordings were performed in murine colonic smooth muscle after removal of mucosal and submucosal layer. Whole-cell patch clamp experiments were conducted to characterize the functional expression of CaCC using transgenic mouse with a bright green fluorescent protein (copGFP) constitutively expressed in ICC to facilitate study of these cells in mixed cell dispersions.

Results: Murine colonic smooth muscle displayed spontaneous membrane potential oscillations (slow depolarization and spike action potential complexes). Niflumic acid (NFA, up to 100 *v* mol L^{-1}) caused membrane hyperpolarization and abolished spontaneous membrane potential oscillations. Since NFA has non-specific effects on various ion channels, we tested a more specific CaCC blocker, 5-nitro-2-(phenylpropylamino)-benzoate (NPPB, up to 10 *v* mol L^{-1})) on spontaneous membrane oscillation. NPPB inhibited slow depolarization without affecting the resting membrane potential and remained spike action potential complexes. These data suggest that slow wave depolarization may be due to activation of CaCC. In patch clamp experiments, colonic smooth muscle cells did not reveal any CaCC currents. However, copGFP+ cells (ICC) functionally expressed CaCC currents. Reversal potentials of CaCC followed ECl. CaCC currents were also inhibited by NPPB, cyclopiazonic acid and extracellular free $Ca2^+$.

Conclusion: In conclusion, CaCC is expressed in colonic ICC and involves in the generation of spontaneous slow depolarization in colonic smooth muscle. (Supported by NIDDK 41315).

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Changes of enteric nerve, interstitial cells of Cajal, and fibroblast-like cells with age in the human stomach

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Objective: Aging-associated changes in the gastric motor function include changes in gastric emptying time, postprandial peristalsis and gastric contractile force. Neuromuscular regulation of gastrointestinal muscles is controlled by a dynamic interaction between different cell types, including smooth muscle, enteric nerves, and interstitial cells of Cajal (ICC). The aim of this study was to investigate the effect of aging on number of ICC and fibroblast-like cells (PDGFRa+ cells) in the human stomach.

Methods: Full-thickness gastric specimens were obtained from patients undergoing surgery for gastric cancer and they were screened to ensure that they did not have diabetes or other gastrointestinal diseases. To investigate the pathological changes of stomach, H&E stain and immunohistochemical examination were done on the sections. Antibodies against protein gene product (PGP) 9.5, neuronal NO synthase (nNOS), vasoactive intestinal peptide (VIP), neurokinin 1, c-Kit, and PDGFRa+αwere used. Immunofluorescent stain and evaluation with confocal microscopy were also done.

Results: Tissues were collected from 26 patients (15 males/11 females, mean age 59.7 ± 10.4 y). On immunohistochemical staining of antrum and gastric body, number of c-Kit positive ICC significantly decreased with age ($P < 0.05$). Number of PDGFRa-positive cells, PGP9.5, nNOS, VIP and neurokinin 1 expression did not significantly decrease with age. On immunofluorescent staining and confocal microscopic examinations, network of ICC in the muscle layer were greatly decreased in the older patient compared with younger patient.

Conclusion: Our study suggests that on full-thickness gastric specimens, loss of ICC cells are found in the smooth muscle of elderly patients. These cellular abnormalities likely reduce the functional reserve of the human stomach and may contribute to changes in gastric motor activity in elderly.

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Activation of Ca2+ transients by purines in Platelet Derived Growth Factor Receptor a positive (PDGFRa+) cells in situ

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Objective: The fibroblast PDGFRα+ cells are a novel class of interstitial cells distributed throughout GI tissues. PDGFRα+ cells are in close proximity to motor nerves and have the necessary machinery to mediate purinergic electrical responses. The aim of this study was to establish a functional role of PDGFRα+ cells as targets /mediators of purinergic signalling in situ.

Methods: Immunostaining of GI muscles was used to characterize the specific distribution of PDGFRα+ cells in murine colon and fundus. Real-time quantitative PCR was used to characterize P2Y receptor expression in PDGFRα+ cells. Genetically engineered mice, that express a histone 2B–eGFP fusion protein in the nuclear region of PDGFRα cells, were used in combination with fluorescent $Ca2^+$ imaging (Oregon Green 488 BAPTA-1 AM, 10 μg ml^{-1}) to allow measurements of intracellular $Ca2^+$ transients in identified PDGFRα+ cells.

Results: Immunohistochemistry demonstrated PDGFRα+ cells were in close proximity to enteric motor neurons in muscle layers. q-PCR data demonstrated that P2Y1, P2Y2 and P2Y13 receptor subtypes are expressed abundantly in comparison to other purinergic receptors in PDGFRα+ cells. $Ca2^+$ fluorescence measurements in PDGFRα+ cells revealed spontaneous ongoing $Ca2^+$ transients in many cells under control conditions. Tetrodotoxin (TTX; 1 μ mol L^{-1}) decreased spontaneous $Ca2^+$ transients significantly. Brief exposures to exogenous purines (ATP, ADP, UTP, and β-NAD 100 μ mol L^{-1}) in the presence of L-NNA (100 μ mol L^{-1}) and atropine (1 μ mol L^{-1}) greatly increased intracellular $Ca2^+$ transients in PDGFRα+ cells. $Ca2^+$ transients were also elicited by the P2Y1-specific agonist N)-methanocarba-2MeSADP (MRS-2365), and inhibited by P2Y1-specific antagonist (MRS-2500). P2Y1-/- animals demonstrated attenuated responses to MRS2365 and β-NAD but partial responses to ATP and ADP were retained in muscles of these animals. Purine responses appear to be mediated through a $Ca2^+$ release mechanism because cyclopiazonic acid (10 μ mol L^{-1}) and thapsigargin (1 μ mol L^{-1}) completely abolished $Ca2^+$ transients elicited by purines.

Conclusion: The present study shows the close proximity of PDGFRα+ cells to motor neurons and the ability of these cells to generate $Ca2^+$ transients in response to purines. Release of $Ca2^+$ from intracellular stores is likely to activate SK3 channels that are abundantly expressed by these cells. Our data are consistent with a role for PDGFRα+ cells serving as targets for purinergic enteric inhibitory neurotransmission in GI muscles.

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Sensory nervous system input into small intestine ICC-MP calcium transients

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Objective: The present study was to investigate potential communication between AH sensory neurons and the ICC-MP network of the small intestine.
Methods: To determine the functionally communication from enteric neurons to ICC network, ICC network was loaded with the calcium dye Fluo-4. Neuron was impaled with sharp electrodes and verified by electrophysiology and morphology. A series of stimulus was provided on the neuron to study possible effects on a nearby network of ICC; To exam a structural basis for the functional communication between enteric neurons and ICC network, a morphological relationship between enteric neuron processes and ICC was examined by double labelling the neurobiotin avidin-D-Texas-Red reaction in enteric neuron with c-KIT immunofluorescence staining for ICC network.
Results: Stimulation of AH sensory neurons, evoking action potentials in these neurons, caused a marked increase of ICC intracellular calcium concentrations with increasing of calcium oscillations in ICC-MP.
Conclusion: AH neurons, which sense the contractile state of the musculature, have the potential to modify gut pacemaking through action on calcium transients.

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Effect of age on mucosal serotonin signalling and circular muscle contractions in the murine rectum

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Objective: Increasing age is a risk factor for a range of gastrointestinal disorders including constipation and faecal incontinence. The rectum is known to play a key role in the storage of faecal matter, however there is limited information about its pharmacology and how it alters with age. This study examined the age-related changes in mucosal serotonin (5-HT) signalling and spontaneous circular muscle contractions.
Methods: *In vitro* electrochemical mapping was utilised to identify the rectum from C57BL/6 mice. Amperometric recordings were conducted to measure changes in 5-HT release and reuptake. The numbers of 5-HT-containing enterochromaffin (EC) cells and their 5-HT content were determined using immunohistochemistry and HPLC, respectively. The activity of the serotonin transporter (SERT) was measured by determining the 5-HIAA:5-HT ratio. Measurements of circular muscle contractility were carried out on *In vitro* tissue segments under control conditions and in the presence of 1 μ mol L^{-1} fluoxetine and 100 μ mol L^{-1} L-NNA.
Results: There was an age-related increase in mucosal 5-HT release ($P < 0.01$, $n = 6$) and a decrease in reuptake between 3 and 24 month old animals ($P < 0.001$, $n = 6$), which led to an age-related increase in 5-HT availability in 24 month old animals ($P < 0.001$, $n = 6$). No changes in 5-HT availability were observed between 3 and 18 months. The increases in 5-HT release were not correlated with EC cell number, which remained constant with age. The ratio of 5-HIAA:5-HT, a marker for turnover, was greater in 12 and 18 month rectum when compared to 3 month rectum ($P < 0.05$, $n = 6$), however no differences were observed in 24 months. The amplitude of circular muscle contractions decreased between 3 and 24 month old animals ($P < 0.05$, $n = 4$). The circular contractions were altered by the presence of fluoxetine and L-NNA in 3 month rectum, but not 24 month old rectum.
Conclusion: Overall we have found that 5-HT availability increases with age and rectum circular muscle contractions are reduced with age and are insensitive to fluoxetine. Thus mucosal signalling to the myenteric plexus is impaired, resulting in reduced contractility. The data from this study could explain the increased incidences in faecal impaction, which is observed in the elderly.

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Effects of transient receptor potential channel blockers on pacemaker activity in interstitial cells of cajal from mouse small intestine

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Objective: The interstitial cells of Cajal (ICCs) are pacemakers in the gastrointestinal tract and transient receptor potential melastatin type 7 (TRPM7) is a candidate for pacemaker channels.
Methods: The effect of the 5-lipoxygenase (5-LOX) inhibitors NDGA, AA861, MK886 and zileuton on pacemaking activity of ICCs was examined using the whole cell patch clamp technique.
Results: NDGA and AA861 decreased the amplitude of pacemaker potentials in ICC clusters, but the resting membrane potentials displayed little change, respectively. Also, perfusing NDGA and AA861 into the bath reduced both inward current and outward current in TRPM7-like current in single ICC, respectively. But, they had no effects on $Ca2^+$ activated Cl- currents. The 5-LOX inhibitors MK886 and zileuton were, however, ineffective in pacemaker potentials in ICC clusters and in TRPM7-like current in single ICC, respectively. A specific TRPC3 inhibitor, pyrazole compound (Pyr3), and a specific TRPM4 inhibitor, 9-phenanthrol, had no effects in pacemaker potentials in ICC clusters and in TRPM7-like current in single ICC.
Conclusion: These results suggest that, among the tested 5-LOX inhibitors, NDGA and AA861 modulate the pacemaker activities of the ICCs, and that the TRPM7 channel can affect intestinal motility.

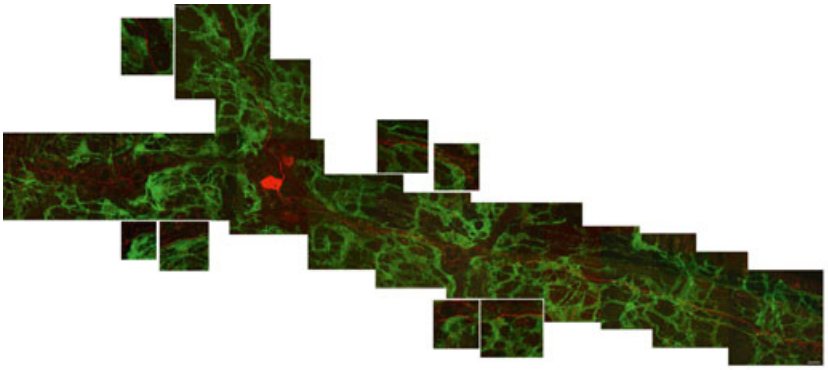

Figure AH neuron with c-KIT-immunoreactive ICC in the myenteric plexus.

Dogiel type II neurons labelled with neurotin-avidin-Texas red reaction were identified as AH neurons. In the figure, an AH neuron sent out two circumferential and one longitudinal projections. All processes were closely associated (within several micrometers) to c-Kit-immunorecative ICC in the myenteric plexus. Enlarged figures (insets) indicated the close association of nerve varicosities with ICC-MP. Scale bar = 20 μm.

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Toward the biophysical basis of monophasic and biphasic slow wave extracellular potentials

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Objective: In the gastrointestinal tract, ICC generate and propagate electrical activities known as slow waves, which are then conducted to smooth muscle cells (SMC). Extracellular and intracellular electrical recordings are used to record slow waves, but the fidelity of these recordings varies and depends on the method of electrode attachment. In this study we acquired in-vivo slow wave recordings using suction

and surface contact attachment methods and modeled extracellular potentials in relation to simulated membrane potentials.
Methods: Suction and surface contact recordings were performed simultaneously in three pigs. Slow waves were recorded from adjacent sites on gastric corpus using a glass capillary (diameter: 1.2 mm) electrode with gentle suction on the serosa, and contact electrodes (diameter: 0.28 mm). Modeling: Slow waves were modeled over a 10 mm virtual tissue strip. Membrane and extracellular potentials were simulated using a validated mathematical ICC-SMC model. To simulate effect of suction pressure on tissue, extracellular conductivity was decreased (contact: 9 mS mm^{-1}; suction: 4 mS mm^{-1}), while intracellular conductivity was unchanged (0.4 mS mm^{-1}); Cell surface-to-volume ratio was increased (contact: 1.5 mm^2 mm^{-3}; suction: 50 mm^2 mm^{-3}); and the resting membrane potential was depolarized to -58 mV.
Results: Experimental and simulation results demonstrated close concordance across a range of extracellular signal characteristics. Suction electrode recorded monophasic potentials (n = 50); recording ± S.D. versus simulation: amplitude: 861 ± 93 *vs* 804 μV; upstroke: 988 ± 297 *vs* 1210 $\mu V\ s^{-1}$; frequency: 3.8 *vs* 3.8 cycles per minute (cpm). Contact electrode recorded biphasic potentials (n = 50); amplitude: 545 ± 194 *vs* 584 μV; down-stroke: -1273 ± 423 *vs* -1460 $\mu V\ s^{-1}$; frequency: 3.8 *vs* 3.8 cpm. The timing of the simulated trough of the internal deflection in the monophasic potential correlated with the middle of the down-stroke in the biphasic potential, which in turn correlated with the middle of the upstroke phase in the membrane potential.
Conclusion: Initial findings suggest that a volume conductor effect could relate simulated extracellular potentials to experimental recordings. Future studies are needed to include simultaneous intracellular and extracellular recordings, detailed measurements of tissue conductivities, and ion channel level contributions to the extracellular potentials to further define the biophysical basis of extracellular potentials.

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The effect of aging on proximal and distal colonic muscle contractility in F344 rat

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Objective: The effect of aging on gastrointestinal (GI) tract is not well established. One of major age related GI effects could be impaired colonic smooth muscle contraction. In the previous experiment we found that the number of ICC and nNOS positive neuronal cells decreases during the aging process in rat proximal colon. The aim of this study was to investigate changes of aged colonic contractility in terms of cholinergic and nitrergic response in the aged rat colon.
Methods: Fischer 344 rats at two ages (30 weeks, young; 84 weeks, senescent) were sacrificed (n = 5, each). Lipid hydroperoxide and salt soluble collagen were measured in the proximal colon for the indicator of aging. Isometric force measurements were performed in proximal and distal colon colonic muscle strip with longitudinal direction. In addition, spontaneous contractile activity by area under the curve (AUC) was measured at the several concentrations (10^{-9}–10^{-4} mol L^{-1}) of acetylcholine (ACh), sodium nitroprusside (SNP), and N(G)-nitro-L-arginine methyl ester (L-NAME).
Results: Lipid hydroperoxide increased in the senescent rat than the young rat (5.32 ± 1.75 *vs* 2.03 ± 0.88 μ mol L^{-1} mg^{-1} wet weight, P < 0.05). Salt soluble collagen also increased (young 3.33 ± 0.74 *vs* senescent 5.52 ± 0.75 μg mg wet weight, P < 0.05). In resting state, spontaneous contractile responses were significantly decreased in distal colon (young 6.30 ± 3.16 *vs* senescent 3.77 ± 2.08 mN) but not in the proximal colon (young 12.65 ± 7.95 *vs* senescent 9.14 ± 4.05 mN). The responses to Ach were proportionally increased with increasing concentration (10^{-9}–10^{-4} mol L^{-1}) in both of young and senescent rat in the proximal and distal colon. The contractile responses to various concentrations of SNP under ACh (10^{-4} mol L^{-1}) were significantly relaxed in both of young and senescent rat. In addition, the relaxation responses to SNP in the senescent rat were more prominent than those in the young rat. The relaxation responses to SNP under L-NAME (10^{-4} mol L^{-1}) were observed at concentration of SNP 10^{-6} mol L^{-1} or more, however, the responses were similar in both ages.
Conclusion: The overall resting contractility was decreasing tendency in the senescent rat colon. In contrast, the relaxation response to SNP under the condition of ACh-induced contraction was more prominent in the senescent rat colon. These results suggest that impairment of colonic motility in the senescent rat could be originated from changes of nitric oxide responsiveness due to decreased nNOS. Additional functional experiment is undergoing to evaluate this presumption.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-12 Clinical Session: Esophagus: Clinical

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A comparison of multichannel intraluminal impedance and pH monitoring in patients with gastroesophageal reflux related laryngitis and non erosive reflux disease

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Objective: Today, there is no gold standard for diagnosis of gastroesophageal reflux disease (GERD). This problem is even bigger in patients with extraesophageal reflux symptoms, therefore overdiagnosis in this group is a common problem. The use of Multichannel Intraluminal Impedance and pH (MII-pH) monitoring has increased sensitivity in the diagnosis of GERD by 24-hour esophageal pH monitoring. We aimed to compare MII-pH monitoring outcomes of patients referred to outpatient reflux clinic considering GER-related laryngitis as a result of laryngoscopic examination and patients has normal esophagogastroduodenoscopy but typical reflux symptoms. Also aimed to show MII-pH monitoring has whether a contribution of the diagnosis of GER-related laryngitis or not.
Methods: Consecutive patients with GER-related laryngitis and patients with non erosive reflux disease (NERD) were enrolled. The lower esophageal sphincter (LES) level was determined by esophageal manometry in all patients then impedance catheter with a pH sensor placed five cm above the LES. After 24-hour measurement, the catheter is removed and record was analyzed manually. Between two groups acid, weakly acid, non acid, liquid, gas and mixed reflux, bolus clearance time and reflux episodes with proximal extend were analyzed for statistical significance.
Results: Fourty NERD, fifteen GER- related laryngitis patients were included. Age, sex, and BMI were not significantly different between the groups.In GER-related lariyngitis group total reflux episodes(median: 47 *vs* 98, P < 0.02), total mild acid reflux episodes (median: 22 *vs* 71, P = 0.03) percentage of proximally extend reflux episodes (24 ± 19 *vs* 35 ± 13, P = 0.02) were significantly lower than those with NERD group. Between the groups in terms of acid reflux, liquid, gas and mixed reflux statistically significant differences were not found.
Conclusion: Acid reflux episodes seems to be more effective at development of clinical symptoms than weakly acid and non-acid reflux in GER related laryngitis patients. Being liquid or gas reflux episodes were not significantly different between two groups. According to these data,the use of multichannel intraluminal impedance-pH monitoring instead of 24-hour esophageal pH monitoring has no additional contribution for diagnosis of patients with GER-related laryngitis.

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Altered taste perception in patients with gastroesophageal reflux disease

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Objective: Patients with gastroesophageal reflux disease (GERD) may experience an altered taste perception, which is likely associated with a direct damage of the taste buds within oral cavity by refluxate. Our aim is to study the taste perception in GERD patients in the absence of ear-nose-throat diseases.
Methods: Twenty-four clinically and instrumentally diagnosed GERD patients (18 males and 6 females, age range 25–69 years) and twenty gender and age matched healthy subjects (HS) were studied. All subjects underwent a standardized taste-testing to evaluate the ability of each subject to identify sweet, bitter, salty, umami and sour taste. The following substances served as specific taste agonists: acesulfame K (30 m mol L^{-1}), quinine (10 m mol L^{-1}), NaCl (120 m mol L^{-1}),

monopotassium glutamate+ inosine monophosphate, (30+0.5 m mol L^{-1}), and citric acid (50 m mol L^{-1}), respectively. In addition each subject was asked to score the intensity of each taste by using a 100 mm line-visual analogue scale (VAS).

Results: The percentage of overall taste misperception was significantly higher in GERD than in HS (23% *vs* 13, respectively, $P = 0.003$), with salty representing the most frequent misperceived taste (40% *vs* 13, $P = 0.009$). In the subset of GERD, but not in HS, a gender difference in the ability to correctly identify the taste (M 73% *vs* F 90 $P = 0.004$) was also found. GERD patients compared to HS, reported a significant higher perception for acid (70 ± 21 vs. 58 ± 22 mm, $P = 0.009$) and lower perception for salty (53 ± 19 vs. 66 ± 20, $P = 0.001$) respectively.

Conclusion: GERD patients showed a significant degree of taste misperception. The ability of patients to score acidity more than healthy subjects may suggest a possible sensitization of sour taste receptors. Translating this hypothesis in esophagus, a similar effect could be supposed to participate to symptoms generation in GERD patients.

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Modulation of oesophageal afferent pathways by 5-HT3 receptor inhibition

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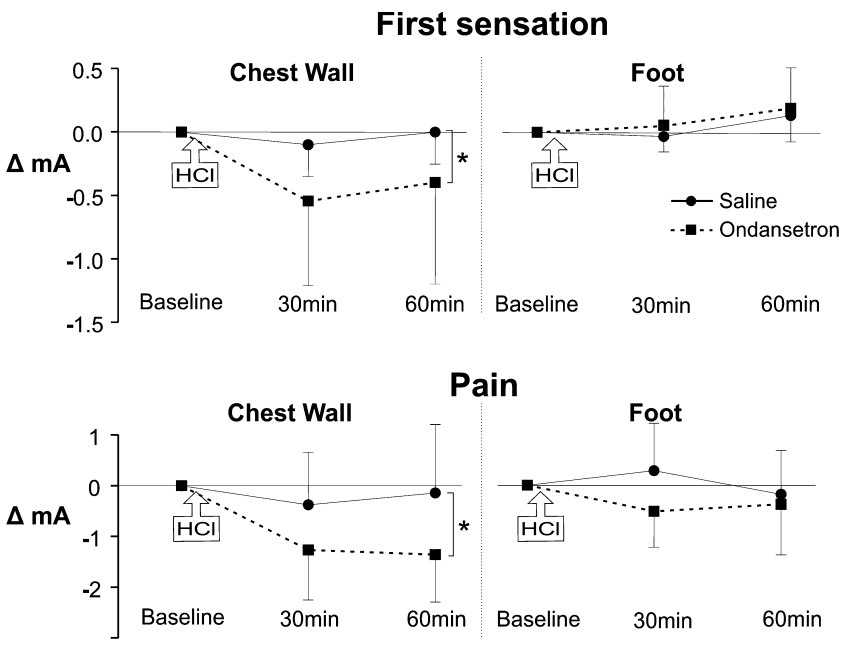
Objective: A high density of 5-HT3 receptors are found in the dorsal medulla on vagal afferent terminations, implying that 5-HT3 mediates vagal reflexes at a central level. Although the specific role of 5-HT3 containing nerves or receptors on oesophageal afferents is not clear, it is thought to modulate sensory pathways. To examine the effect of 5-HT3 blockade on the distension-induced upper oesophageal sphincter (UOS) reflexes and oesophageal sensitivity to acid and electrical stimuli.

Methods: Effect of 5-HT3 blockade with ondansetron on the distension-induced UOS reflexes and sensory perception were investigated in two separate double blind cross-over trials in healthy participants. In Study I ($n = 9$) oesophageal sensory and pain thresholds to electrical stimulation were measured in the oesophagus, the chest wall and the foot, before subjects were randomized to receive either Ondansetron (8mg i.v.) or NaCl (0.9% w/v). HCl (0.15 mol L^{-1}, 30 min) was then infused into distal oesophagus and electrical thresholds were reassessed. Following electrical sensory threshold testing, subjects received another infusion of HCl. In Study II ($n = 10$) frequencies of distension-induced UOS relaxation responses were scored before and after treatment with Ondansetron (8 mg i.v.) and NaCl (0.9% w/v).

Results: In Study I, Ondansetron had no effect on oesophageal sensitivity to HCl or acid-induced sensitisation. Blockade with 5-HT3 did reduce somatic pain thresholds in the viscerosomatic referral area. The mean baseline somatic pain thresholds mid-sternum were 4.21 ± 1.8 mA and 5.36 ± 1.78 mA in the NaCl and Ondansetron arms respectively. Sixty minutes after oesophageal acid exposure pain thresholds were significantly lower in the ondansetron arm (mean Δ-1.36 ± 0.4 mA) when compared to NaCl (mean Δ-0.14 ± 0.58 mA) ($P < 0.05$). In Study II, 5-HT3 blockade with Ondansetron had no significant effect on UOS relaxation responses.



Conclusion: This study does not support the hypothesis that in health, 5-HT3 receptors play a significant role in oesophago-UES reflexes and oesophageal sensitivity to acid or electrical stimulation. It does provide new evidence for involvement of 5-HT3 receptors in viscerosomatic sensitisation.

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Detection of laryngopharyngeal reflux (LPR) using 24-hour esophageal and pharyngeal impedance-pH monitoring: Normal values off and on PPIs and inter-observer reproducibility

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Objective: Combined pH and impedance monitoring can detect all types of reflux episodes within the esophageal lumen and the pharynx. The aim of this multicenter study was to establish normal values of esophageal and pharyngeal impedance-pH monitoring and to determine the inter-observer reproducibility of this technique.

Methods: Twenty-four hour pH-impedance recordings were performed using a bifurcated probe positioned according to UES and LES location determined with manometry. Impedance data were obtained at 3 and 5 cm above the LES (distal esophagus), 2 and 4 cm below the UES (proximal esophagus) and 0.5 and 1 cm above the UES (pharynx). pH values were obtained from the distal esophagus and the pharynx. LPR events were considered present with retrograde bolus transit across all ring sets that ultimately reached the hypopharynx and characterized as acidic or weakly acidic. The subjects were studied twice, off therapy and on esomeprazole 40 mg bid taken for 14 days. The inter-observer agreement (2 experimented physicians for impedance analysis) for the detection of pharyngeal reflux events was determined in 20 subjects randomly selected. Results expressed as median (IQ range).

Results: Forty-six asymptomatic subjects were included (22 males, mean age 46.3 yrs, extr 18–78). Preliminary results in 37 subjects are available. Off therapy, the total number of pharyngeal reflux events was 1 (0–2), including 0 (0–0) acidic and 1 (0–1) weakly acidic reflux episodes. On therapy, the total number of pharyngeal reflux events was 0 (0–0), including 0 (0–0) acidic and 0 (0–0) weakly acidic reflux episodes. Pharyngeal acid exposure was 0% (0–0) and 0% (0–0) on and off therapy, respectively. Pharyngeal bolus exposure was 0% (0–0) and 0% (0–0) on and off therapy, respectively. The pharyngeal clearance was 1.2 s (0.7– 9) and 1.2 s (0.6–5.5) off and on therapy, respectively. Inter-observer agreement for pharyngeal reflux was poor: among 22 episodes detected, only 3 (13.6%) were detected by both observers. The median concordance per subject was 63% (0–100).

Conclusion: This study provides normal values of pharyngeal reflux detected by 24-hour impedance-pH monitoring off therapy and on esomeprazole 40 mg bid. However, analysis of pharyngeal events is difficult and poorly reproducible. This limitation has to be taken into account in further studies.

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Sensitivity of high resolution manometry for detection of esophageal motility disorders: Comparison with 24H-manometry

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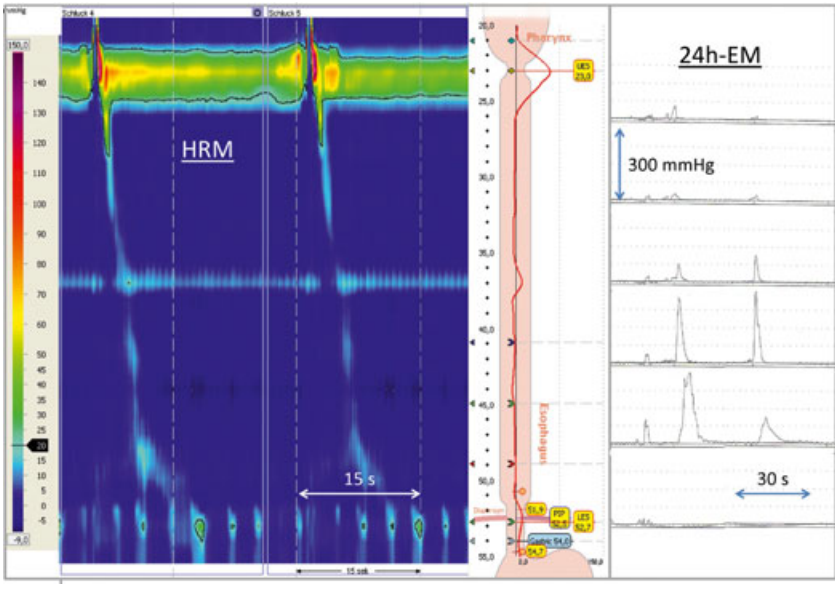
Objective: Sensitivity of conventional short time esophageal manometry for detection of hypertensive

esophageal motility disorders is limited. It was our aim to compare high resolution manometry (HRM, analysis of data using the Chicago classification (Bredenoord et al. 2012)) with 24 h-esophageal manometry (24 h-EM) and to test whether HRM improves sensitivity of short term esophageal manometry.
Methods: Data from 20 patients with non-obstructive dysphagia (unrevealing esophagogastroduodenoscopy) and/or non-cardiac chest pain were analyzed retrospectively. All subjects received HRM (catheter with 36 pressure sensors) and 24h-EM (catheter with 6 pressure sensors at 5 cm distance, sleeve sensor at tip of the tube). Diagnosis of HRM was obtained using the Chicago classification. For evaluation of 24h-EM the number of contractions per 24 h, mean amplitude and duration of contractions at 5 and 15 cm above the lower esophageal sphincter and percentage of contractions with normal or abnormal propagation were analyzed. Moreover, maximal contraction amplitude and duration were recorded and abnormal motor events were analyzed visually and correlated with symptoms.
Results: HRM resulted in diagnosis of achalasia in 3 subjects (type 1 in 1, type 2 in 2), diffuse esophageal spasm (DES) in 4, hypercontractile esophagus in 2, weak peristalsis in 5 and frequent failed peristalsis in 2 subjects. It gave normal results in 4 patients. 24 h-EM gave compatible results in 10 subjects. However, all subjects with normal HRM and 3 out of 7 patients with weak or frequent failed peristalsis showed evidence of hypertensive peristalsis (n = 4) or DES (n = 3) in 24h-EM (fig 1: Patient with frequent failed peristalsis in HRM but DES with markedly hypertensive contractions exceeding 350 mmHg during 24h-EM). Moreover, both subjects with type 2 achalasia according to HRM showed marked esophageal spasms during 24h-EM. Vice versa, in one subject with weak peristalsis according to HRM, 24h-EM was normal.
Conclusion: Hypertensive esophageal motility disorders are frequently overlooked or underestimated by short term esophageal manometry. This is also true when using HRM and the Chicago classification for data acquisition and analysis. Sensitivity of HRM could probably be increased by prolongation of recording time and/or inclusion of provocation tests.



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Modality specific alterations of esophageal sensitivity caused by longstanding diabetes mellitus
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Objective: Abnormal visceral sensory function has been demonstrated in patients with diabetes mellitus with neuronal changes located in the enteric, peripheral and/or central nervous system (REF). Diabetic autonomic neuropathy seems to be involved in the development and progression of gastrointestinal tract dysfunction. The possibility of multimodal (e.g. mechanical, electrical, thermal and chemical) stimulation in e.g. the esophagus has developed visceral pain research. The major advantage is involvement of distinctive receptors, various sensory nerves and different pain pathways mimicking clinical pain that favours investigation of central pain mechanisms involved in allodynia and hyperalgesia. Hence the rationale of the presents study was to explore the nervous system by assessment of esophageal sensitivity to multimodal stimulations.
Methods: Throughout an euglycemic clamp, 31 healthy volunteers (age 44.3 ± 10.6 (mean ± sd) years; 11men) and 31 patients (age 46.3 ± 11.7 years; 10 men) with insulin dependent diabetes mellitus (duration 31.3 ± 13.1 years) were included in the study. By use of a multimodal oesophageal probe, sensitivity to heat, mechanical distension and electrical stimulation was assessed in the lower oesophagus.
Results: For heat stimulation patients had increased sensitivity in the sensory range with shorter stimulus duration until pain tolerance threshold (122 ± 3.8 vs. 136 ± 3.7 s; P = 0.006) and larger area under the temperature curve (2380 ± 1847 vs. 1409 ± 1450; P = 0.03). There were no differences between groups for mechanical stimulation (maximum pressure (39 ± 57vs. 24 ± 48 mmHg; P = 0.3; maximum volume 59 ± 21vs. 62 ± 25 ml; P = 0.56). As an overall finding, patients tolerated higher electrical stimulation intensities (P = 0.02), dominated by discrimination in the sensory range: At sensory detection threshold (VAS1) 21.1 ± 12.4 vs 16.3 ± 5.5 mA(P = 0.03); at moderate sensation (VAS3) 27.5 ± 13.3 vs. 21.5 ± 5.0 mA (P = 0.03) however at pain detection threshold (VAS5) 31.6 ± 13.1 vs. 28.8 ± 5.2 mA; the trend was unsignificant (P = 0.3).
Conclusion: Patients with insulin dependent diabetes mellitus had modality-specific alterations of esophageal sensitivity. Heat stimulation activates selectively mucosal receptors whereas on the contrary electrical stimulation depolarizes the free nerve endings non-selectively. Hence, due to the different sensitivity profile, the esophageal neuropathy is most likely a result of both peripheral and central neuropathy caused by longstanding diabetic neuronal damage.

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Silent aspiration after Traumatic Brain Injury (TBI)
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Objective: Patients affected by brainstem (mesencephalic, bulbar and pontine) injuries have high risk of aspiration due to swallowing alterations. Aspiration is suspected when coughing while eating and drinking but it may be also clinically silent. The relationship between brain site injury and altered swallowing has been reported in patients with vascular accidents but not in patients after traumatic brain injury (TBI) without involvement of the brainstem. Aim of the present study is to assess the presence of silent aspiration in patients after TBI and without brainstem lesions.
Methods: Seventy-seven patients (62M, age26 y±8) with TBI (time from injury: 6–12 months) not affecting brainstem as assessed at brain computerized tomography underwent a videofluoroscopic study with videotaping of the swallowing act (VFSS). VFSS was performed in the antero-posterior and lateral upright position for the assessment of the oro-pharyngeal phase. During this study, videorecording of swallowing of a variety of boluses with different consistencies (semiliquid, semi-solid of increasing volumes, liquid and solid) was performed for subsequent analysis. The presence of aspiration with or without coughing (silent aspiration) was evaluated for each patient.
Results: Aspiration was present in 27% of patients and coughing reflex was absent in 15%. The table summarizes the presence of aspiration in relation to the lesion site.
Conclusion: Patients with TBI with lesions not involving the brainstem may have aspiration regardless of encephalic lesion localization. About 70% of the cases of aspiration occurs without reflex coughing. These observations suggest to perform a dynamic VFSS study of the swallowing act in all patients with TBI, even in those not presenting symptoms of aspiration.

	Aspiration (n 21, 27%)		
Lesion site	With coughing (n 6, 29%)	Silent (n 15, 71%)	p
Frontal	3 (14%)	8 (38%)	Ns
Parietal	1 (5%)	2 (9%)	Ns
Temporal	1(5%)	10 (48%)	Ns
Occipital	1(5%)	3 (14%)	Ns

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Neuronavigated repetitive magnetic cerebellar stimulation induces long-lasting changes in human swallowing motor pathways

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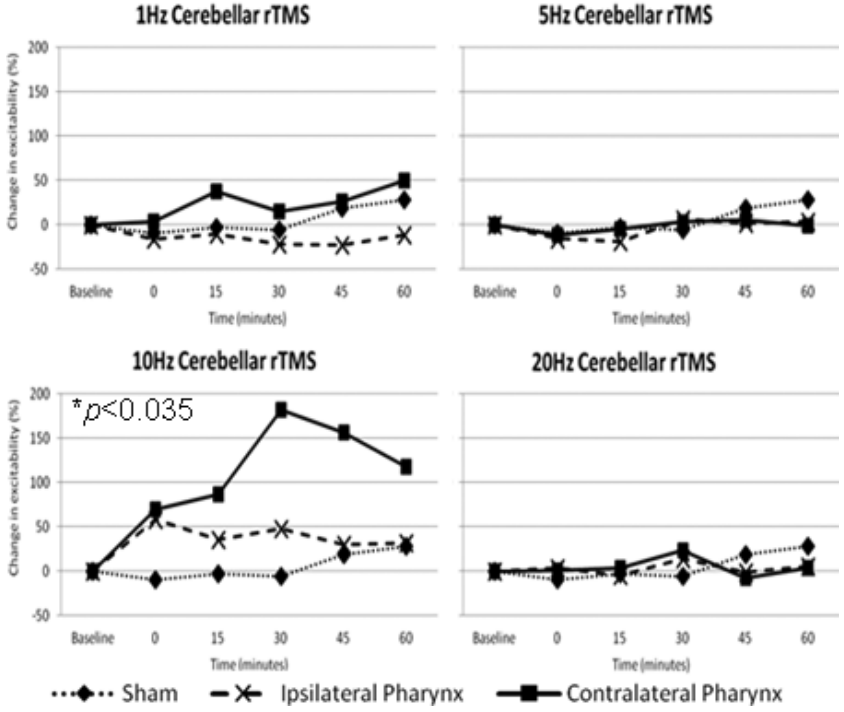
Objective: Functional neuroimaging studies consistently show cerebellar activation during human swallowing. Recently, we have shown that single-pulse transcranial magnetic stimulation (TMS) of the human cerebellum can directly evoke muscle responses in the pharynx and induce short-term excitation of pharyngeal motor cortex (V Jayasekeran et al., Neurogastroenterol Motil 2011:23). We aim to provide proof of concept evidence that cerebellar conditioning with longer trains of repetitive TMS (rTMS) can induce long-lasting, frequency-dependent changes in pharyngeal motor cortex excitability that may have therapeutic potential in the treatment of swallowing disorders (dysphagia) after stroke.

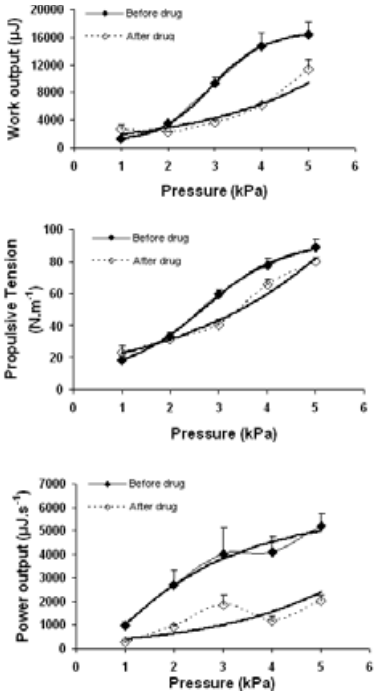
Methods: In this preliminary report of 5 healthy adults (2 male, 23–61 years old), anatomical brain scans were acquired. During subsequent experimental sessions, performed at least a week apart, participants were randomised to receive one of 5 frequencies of neuronavigated cerebellar rTMS (Sham, 1, 5, 10 and 20 Hz). During each session, pharyngeal motor cortex excitability (in both hemispheres) was measured using TMS and pharyngeal electromyography (recorded via a swallowed intraluminal catheter) at baseline and for up to one hour post cerebellar rTMS. Abductor pollicis brevis (APB) recordings were used as control. Data were compared using repeated measures ANOVA and *t*-tests.

Results: Cerebellar rTMS was tolerated well and delivered at an average intensity of 42% of rTMS output. Compared to Sham, 10 Hz cerebellar rTMS significantly increased pharyngeal motor cortex excitability from baseline in the hemisphere contralateral to cerebellar stimulation (max 181%, $P < 0.035$) (Fig. 1). In contrast, 1, 5 and 20 Hz rTMS cerebellar conditioning did not significantly alter pharyngeal motor responses when compared to Sham. APB motor responses after all four active rTMS interventions were not significantly different to Sham.

Conclusion: Our preliminary observations from this proof of concept study show for the first time that sustained changes in swallowing motor pathways can be induced through cerebellar conditioning using 10 Hz rTMS, and may also extend to the other frequencies tested with greater participant numbers. Cerebellar rTMS may therefore have novel therapeutic potential in the treatment of dysphagia after stroke.

Figure 1: Effects of rTMS conditioning on swallowing motor pathways





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Distension evoked motor response analysis in human esophagus

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Objective: The major function of the esophagus is to transport food from the mouth to the stomach by peristaltic muscle action. However, only few techniques exist to evaluate characteristics of motor activity and mechanical energy output of the esophagus *in vivo*. The aim of this study was to use distension combined with manometry and impedance planimetry for assessing the mechanical energy based esophageal peristaltic motor function analysis by using pressure-cross-sectional area (P-CSA) recordings.

Methods: Distension evoked rhythmic esophageal peristalsis was analyzed using P-CSA curves at distension pressures up to 5 kPa. The probe with a bag and electrodes for CSA measurements was positioned 7 cm above the lower esophageal sphincter in sixteen healthy volunteers before and after butylscopolamine administration. The P-CSA, work outputs (area of the tension-CSA curves), propulsive tension, and power output were analyzed.

Results: The rhythmic wave-like peristalsis resulted in P-CSA and tension-CSA loops that could be subdivided into relaxation and contraction behavior. The averaged positive work (from 1311 ± 198 to 16330 ± 1845 μJ before drug administration vs. from 2615 ± 756 to 11404 ± 1335 μJ during the drug administration), power (from 978 ± 175 to 5214 ± 534 μJ s^{-1} vs. from 265 ± 47 to 2033 ± 239 μJ s^{-1}), and propulsive tension (from 18.7 ± 1.9 to 88.5 ± 5.5 N m^{-1} vs. from

23.1 ± 3.9 to 79.5 ± 3.3 N m^{-1}), increased with the distension pressure level, and with significantly lower values after butylscopolamine administration (Fig. 1, $P < 0.01$).
Conclusion: Dynamic esophageal muscle properties during peristalsis can be assessed *in vivo* in terms of mechanical output parameters. Butylscopolamine administration impaired the muscle contraction which could be detected quantitatively. The analysis can likely be used as advanced esophageal motor function parameter analysis for studying the mechanical consequences of esophageal contractions *in vivo*.

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High-resolution impedance manometry criteria in the sitting position indicative of incomplete bolus clearance

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Objective: This study used high-resolution impedance manometry (HRM) to determine the length of breaks on the isobaric contour predictive of incomplete bolus clearance with patients in the sitting position.
Methods: A total of 651 swallows in 71 consecutive patients with esophageal symptoms were studied using a solid state HRM system in the sitting position. Patients with abnormal esophageal anatomy, impaired esophagogastric junction relaxation and hiatal hernia by HRM criteria were excluded. Each swallow was classified as complete or incomplete bolus clearance by impedance criteria, and the peristaltic integrity was evaluated using the 20 and 30 mmHg pressure topography isobaric contours. Each isobaric contour plot was characterized by the location and length of the breaks in the isobaric contour. Correlation between the length of the breaks for the 20 and 30 mmHg were analyzed.
Results: Complete bolus clearance was observed in 83.3% (542/651). Breaks of less than 3 and 7 cm were associated with a bolus clearance of 96.8% on the 20 mmHg and 94.7% on the 30 mmHg isobaric contour respectively ($P < 0.001$). The area under the ROC curve (AUC) for the 20 and 30 mmHg isobaric contour was 0.900 and 0.950 respectively. The sensitivity and specificity for complete bolus clearance was 75.61%, 89.34% for breaks less than 3 cm on the 20 mm Hg isobaric contour and 87.90%, 78.69% for breaks less than 7 cm on the 30 mmHg contour ($P < 0.0001$). There was no difference between the ROC curves for the 20 and 30 mmHg isobaric contour ($P = 0.4405$).
Conclusion: Breaks <3 cm in the 20 mm Hg isobaric contour or <7 cm in the 30 mmHg isobaric contour are associated with complete bolus clearance. The threshold for breaks in the sitting position was greater than previous reports using the supine position, and longer breaks predicted incomplete bolus clearance.

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Esophageal contractions and transit in patients with excessive belching

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Objective: Excessive belching is the consequence of the elimination of gas coming from the stomach (gastric belching) or esophagus (supragastric belching). Our hypothesis is that esophageal function may be affected by the excessive presence of gas.
Methods: We studied the esophageal contractions and transit of 16 patients who complained of excessive belching (10 women, mean age: 46.1 ± 8.2 years) and 15 normal volunteers (9 women, mean age: 46.2 ± 7.8 years). The patients had at least three episodes of belching a day, 3 days per week in the last 3 months, with duration of the complaint for more than 6 months. The normal volunteers had no complaint. We used a system that registered simultaneously the esophageal manometry and impedance at 5, 10, 15 and 20 cm from the lower esophageal sphincter (LES). The recording of spontaneous belching over a period of 20 min showed no gastric belching in either group, no supragastric belching in the normal volunteers and 1 to 11 episodes of supragastric belching in the patients. Esophageal motility and transit were evaluated with five swallows of a 5 ml saline bolus.
Results: There was no difference between patients and normal volunteers in amplitude, duration and area under the curve of contractions. The bolus head advance time was longer for patients than volunteers in the proximal esophageal body (patients: 0.7 ± 0.8 s, volunteers: 0.1 ± 0.1 s) and middle esophageal body (patients: 1.6 ± 2.6 s, volunteers: 0.3 ± 0.1 s) ($P = 0.01$), and the bolus presence time was longer for volunteers (6.0 ± 1.1 s) than patients (4.9 ± 1.2 s) in the distal esophagus ($P = 0.04$). There was no difference in total bolus transit time (patients: 6.2 ± 1.8 s, volunteers: 6.2 ± 2.3 s) or in segment transit time.
Conclusion: We conclude that patients with supragastric belching have a slower progression of the bolus head to the distal esophageal body but a normal total duration of bolus transit. The slower bolus progression in the proximal esophagus is not a consequence of changes in esophageal contractions.

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Diagnosis of Gastro-esophageal Reflux Disease (GERD): A prospective analysis based on prolonged, ambulatory pH-studies

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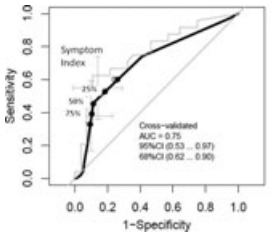
Objective: GERD diagnosis by endoscopy or esophageal acid exposure time (AET) on pH-studies do not discriminate patients that present with reflux symptoms from ''healthy controls (HCs)'' (Labenz AJG04). GERD can be established also by a positive association between reflux and symptoms; however existing metrics (e.g. symptom index (SI)) have limitations, including high day-to-day variability, and suboptimal prediction of treatment response (Taghavi Gut05). Building on a retrospective assessment of 4-day studies in GERD patients (Fox DDW11), a prospective study was performed to identify those measurements that discriminate HCs and GERD patients and predict PPI response.
Methods: Reflux symptom severity (Eraflux) was assessed in HCs and patients with typical reflux symptoms off medication and after 2-weeks twice-daily PPI. Endoscopy documented mucosal disease. Wireless pH-studies (Bravo®, Given) documented acid reflux and symptoms over 4-days. Overlap of confidence intervals identified parameters that discriminate health and disease. Receiver Operating Curve (ROC) assessed prediction of PPI response. For each prediction 80% of patients were randomly selected as the training set, the remaining 20% constituted the test set. This was repeated 200 times producing an average ROC with standard errors.
Results: Complete data was available from 25/33 HCs (18F, age 20–56) and 70/108 eligible patients (31F, age 18–77), >320 days data in total. Esophagitis was present in 9 HVs (32%: Grade A) and 26 patients (33%:A = 19, B = 2, C–D = 5). AET was elevated (>5.6%) in 3(12%) HVs and 35(50%) patients. Eraflux off-PPI was >25 (consistent with GERD) in 60/63 patients and fell by 7 (95%CI 5–10) on PPI, 46% reported positive response (>3 fall). Endoscopy, AET and reflux-symptom association (SI, SAP) did not discriminate, but the number of reflux-associated symptoms (RS/Day) covered different ranges in health and disease. Bootstrap validation identified that >3RS/Day identified GERD patients. Clinical parameters and AET did not predict PPI response but 96hr SI (9.2 vs 30.2, $P = 0.0023$) and nRS/Day (1 vs 2.6, $P = 0.012$) were higher in responders. SI ROC had an AUC of 0.73 (CI 0.51 to 0.92) to predict outcome and SI>25 was the optimal cut-off for identifying responders (figure). Prediction from 24 h studies had lower AUC (0.69) and wide confidence intervals.
Conclusion: In contrast to existing measurements, a simple count of reflux associated symptoms (RS/Day) on pH-studies discriminates HCs from GERD patients. A lower SI than applied currently (SI>25) provides the single best prediction of PPI response; however, prolonged pH-measurement is required to predict treatment outcome with confidence.



SI	sens	spec
0.00	1.00	0.00
5.56	0.92	0.32
11.11	0.80	0.54
16.67	0.71	0.68
22.22	0.62	0.81
27.78	0.62	0.88
33.33	0.44	0.93
38.89	0.28	0.93
44.44	0.25	0.93
50.00	0.21	0.93

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Comparative study for the high-resolution manometric observation in globus sensation, gastroesophageal reflux disease patients and normal controls

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Objective: Globus is a non-painful sensation of a lump or foreign body in the throat without dysphagia, odynophagia, esophageal motility disorders, or gastroesophageal reflux. There was no clearly accepted etiology. Previous studies suggested that increased upper

Table. Comparison of HRM parameters in globus, GERD, and normal controls

	Normal	Globus	GERD	P value
UES pr (mmHg)	84.50 (57.70~93.60)	59.40 (40.95~80.25)	60.15 (43.42~82.10)	0.171
CFV (cm/sec)	3.20 (2.90~4.00)	3.40 (2.90~4.00)	3.35 (2.92~4.47)	0.865
PCI (in 30mmHg)	112.82 (103.84~307.60)	126.00 (49.50~231.30)	130.70 (25.30~211.39)	0.923
DCI (in 30mmHg)	1030.78 (849.50~1388.32)	786.00 (447.50~1335.50)	758.78 (447.73~1090.36)	0.299
Transition zone (cm)	2.26 (1.22~2.92)	4.26 (2.30~5.85)	5.91 (3.97~7.62)	0.001

Median (Interquartile range)

esophageal sphincter pressure, gastroesophageal reflux or hypertonicity of esophageal body is a possible etiology of globus. This study was to quantify the upper esophageal sphincter pressure (UES pressure), contractile front velocity (CFV), proximal contractile integral (PCI), distal contractile integral (DCI), and transition zone (TZ) in patient with globus, and gastroesophageal reflux (GERD) patient without globus, and normal controls for suggesting the correlation specific high resolution manometric findings and globus.

Methods: Total 88 patients (57 globus patients, 24 GERD patients, and 7 normal controls) were studied with 36 channel high-resolution esophageal manometry (HRM) since 2009. We reviewed the HRM reports of all enrolled patients, and selected 5 swallowing plots suitable for analysis in each report, then analyzed each individual plot with ManoView (Sierra Scientific, Los Anglels, USA). The 5 parameters (UES pressure, CFV, PCI, DCI, TZ) gained from swallowing plots in 57 globus patients were compared with that of 24 GERD patients and 7 normal controls.

Results: There was no significant difference in the UES pressure, CFV and body tonicity (PCI, DCI). But, transition zone (spatial gap between the termination of the proximal contraction and the initiation of the distal contraction using a 30mmHg isobaric contour) in globus showed significant difference compared with normal controls and patient controls (GERD patients). The median values of TZ were 4.26(2.30~5.85 IQR) in globus, 5.91(3.97~7.62 IQR) in patient controls, and 2.26(1.22~2.92 IQR) in normal controls (P = 0.01).

Conclusion: HRM analysis suggested that UES pressure, CFV, and esophageal tonicity (PCI and DCI) were not associated with globus sensation, But, prolonged length of transition zone may be correlated with globus sensation. Further study comparing HRM analysis in globus group with larger normal control population needs to confirm their correlation.

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Prolonged, direct observation of esophagogastric junction opening patterns using capsule endoscopy for 12 hours

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Objective: The exact role of the lower esophageal sphincter(LES) in gastroesophageal reflux disorder (GERD) remains to be fully elucidated.The objective of this study was to watch transient lower esophageal sphincter relaxation (TLESR) and deglutitive LES relaxation (dLESR).

Methods: The LES opening patterns were observed for 12 h in five healthy subjects by capsule endoscopy (CE; MiroCam). The CE was placed above the esophagogastric junction using 7Fr endoscopic nasobiliary drainage (ENBD) tube. High-resolution manometry was performed simultaneously for 1 h, and pH monitoring was performed for 24 h.

Results: The CE did not induce any esophageal motor activity. The LES openings were well observed during the TLESR and the dLESR with the CE. The episodes of TLESR were correlated with abrupt falls in esophageal pH. Esophageal shortening was observed all the time when the TLESR took place.

Conclusion: Prolonged LES observation with the CE can be applied to the assessment of the motility of esophagogastric junction.

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Role of the Esophago-Gastric Junction (EGJ) in GERD patients: Reflux esophagitis spectrum

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Objective: GERD is a prevalent disease in most populations around the world. Mainly, GERD pathogenesis depends on EGJ competence and its' mechanical properties. Different changes of this complex anatomical zone (such as hiatus hernia (HH) or previous surgery) could lead to deterioration of the antireflux mechanism. It could be a reason of the severe clinical course and frequent exacerbation of GERD. For developing and implementing the patient management plan it is crucial to determine the extent of the problem.

Methods: The aim of the study was to analyze the prevalence and severity of RE in patient with normal GEJ in comparison with those who have HH and previous gastric surgery (total gastrectomy). A retrospective review of 9,456 pts (mean age 54.9 years, SD=15.8) with normal GEJ who had visited our hospital for routine check-up was performed. We also reviewed the endoscopic findings in 367 pts (mean age 57.1 years, SD=12.3) with hiatus hernia, 191 pts (mean age 64.2 years, SD=10.2) with previous total gastrectomy. In all of pts with previous gastrectomy gastric cancer was the indication for surgery. The presence of erosive reflux esophagitis (RE) was based on the Los Angeles (LA) Classification. Additionally, minimal changes of mucosa (such as hyperemia and edema) observed under endoscopic investigation (Olympus GIF H180, GIF 160Z, GIF H260Z) were recorded as a manifestation of non-erosive RE.

Results: The prevalence of RE was 3.13% in pts with normal GEJ, 44.96% in pts with HH and 22.5% in pts after total gastrectomy. In particular, minimal changes were found in 27.7%, 26.6% and 4.65%, LA grade A – 29.7%, 21.81% and 9.3%, LA grade B - in 37.5%, 41.2% and 30.2%, LA grade C - in 4.05%, 5.4% and 37.2%, LA cases D – in 1.01%, 4.8% and 18.6% in pts with normal GEJ, HH and pts who had previous total gastrectomy respectively. The prevalence of severe forms of RE (grade C and D) was only 5.06% in pts with normal GEJ and 10.2% in pts with HH, but it was 55.8% in pts after total gastrectomy.

Conclusion: These results suggest that risk of RE in pts with HH increases 15.6-fold in comparison with pts with normal GEJ. Total gastrectomy significantly increases the risk of RE 7.2-fold. The prevalence of severe forms of RE (grade C and D) was higher in pts after gastric surgery. However, the prevalence of minimal changes of RE was similar in pts with normal GEJ and HH.

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High resolution manometry and PH-impedance monitorung in patients after gastrectomy

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Objective: Esophageal lesions (erosions and ulcers) and also complaints of dysphagia are frequently identified in patients with gastrectomy.

Methods: Esophageal high-resolution manometry (HRM) was performed in 35 patients after total gastrectomy and in 28 patients after partial gastrectomy. ''Solar'', pH-impedance monitoring was performed by «Omega» («MMS», Netherlands) in 2011. The basis for interpretation of results derived manometry classification of primary esophageal motility disorders, Castell DO, 2001. Analyzed parameter pH-impedance monitoring - Bolus clearance time (time of refluxate exposition in a distal segment of esophagus).

Results: 60% of patients complained of dysphagia. Dysmotility of esophagus was found in 89% of patients after total gastrectomy, esophageal motility disorders were more severe in comparison to patients with partial gastrectomy (P = 0,01) (Table 1). In patients with ineffective esophageal motility after total gastrectomy the percentage of non-transmitted and dropped contractions was higher than in patients after partial gastrectomy (P = 0,05). Functionally, this pattern is associated with impairment of esophageal clearanceBolus clearance time in patients with esophageal lesions was higher than normal levels. The strong correlation between percentage of non-transmitted and dropped contractions to the distal esophageal segment and bolus clearance time was detected (r = 0.87, P = 0,00). High frequency of non-transmitted and dropped contractions increases the period of refluxate contact with esophageal mucosa, leading to inflammatory and destructive lesions.

Conclusion: Dysphagia occurs in 60% of patients after gastrectomy and is not always associated with

The results of manometry in patients after gastrectomy with and without esophageal lesions.

Results of esophageal manometry	Total gastrectomy		Partial gastrectomy		p (between the groups)	p_2 (total gastrectomy)	p_3 (partial gastrectomy)
	Normal n = 8	Esophagitis n = 27	Normal n = 14	Esophagitis n = 14			
Normal motility n = 12	2 (25%)	2 (7%)	4 (29%)	4 (29%)	0,13	0,43	0,67
Esophagospasm n = 14	4 (50%)	6 (22%)	2 (14%)	2 (14%)	0,26	0,27	0,58
Ineffective esophageal motility n = 25	**0**	**16 (59%)**	3 (21%)	6 (43%)	**0,01**	**0,01**	0,36
mixed disorders n = 12	2 (25%)	3 (11%)	5 (36%)	2 (14%)	0,43	0,67	0,36

organic lesions or dysmotility of the esophagus. It may be the result of sensitivity disorders after vagotomy. After total gastrectomy ineffective esophageal motility in conjunction with esophageal lesions is observed more often in comparison to partial gastrectomy.

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Role of upper esophageal sphincter and pharynx in the development of swallowing abnormalities in patients who received prolonged mechanical ventilation

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Objective: Swallowing dysfunction and pulmonary aspiration can occur in patients after prolonged mechanical ventilation. This is a descriptive pilot study to evaluate swallowing abnormalities in patients intubated longer than 48 h using high resolution solid state esophageal manometry (HRM) and fiberoptic endoscopic evaluation of swallow (FEES).
Methods: Intensive Care Unit patients older than 18 years of age, intubated for a minimum of 48 h were considered for the study. Exclusion criteria: preexisting swallowing abnormalities/dysphagia; neuromuscular disorders, obstructive lesions in the esophagus/pharynx, INR > 2.0, platelets <50 000, altered mental status. Within 72 h of extubation, patients underwent HRM (Sierra Scientific Instruments, Los Angeles, CA) and FEES.
Results: One hundred and twenty one patients were intubated longer than 48 h between April 2011 and April 2012. Twelve patients met all inclusion/exclusion criteria. Four patients (3 males and 1 female), mean age 67.25 (range: 57 – 82 y.o.) underwent study procedures. Length of intubation was 73 – 237 h. Group 1 - three patients with abnormalities on FEES: one with silent aspiration after swallowing juice and symptomatic aspiration after swallowing gelatin with penetration of juice and gelatin into the laryngeal vestibule. The second patient had silent aspiration, laryngeal penetration with gelatin, pooling of gelatin into the hypopharynx. The third patient had pooling of juice, nectar and gelatin in the hypopharynx. Group 2 – 1 patient with normal findings on FEES. All patients had normal residual UES pressure (<12 mmHg) with dry and 5 cc swallows (Table 1). Peak pharyngeal pressure was low (<265 mmHg) in all patients with 5 cc swallow. Patients with abnormalities on FEES had shorter duration of UES recovery time with 5 cc swallows (85 ms vs 641.6 ms), shorter UES relaxation duration (173 ms vs. 1341.6 ms), longer pharyngeal contraction duration (1093.8 vs. 576.7 ms) and longer recovery time of pharyngeal contractions (767.3 ms vs. 278.3 ms).
Conclusion: Prolonged endotracheal intubation with mechanical ventilation does not affect residual upper esophageal sphincter pressure, but affect strength, duration of pharyngeal contractions, its recovery time and UES relaxation time. This study is ongoing and enrollment of a larger group of patients is necessary to confirm these findings.

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Utilizing intrabolus pressure and EGJ relaxation pressure to predict esophageal bolus transit in dysphagia patients

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Objective: High-resolution manometry (HRM), with a greatly increased number of recording sites and decreased spacing between them, is sufficient to resolve the dynamic simultaneous relationship between intrabolus pressure (IBP) and EGJ relaxation pressure. We aimed to determine whether IBP overcoming integrated relaxation pressure (IRP) is predictive of esophageal bolus transit.
Methods: Twenty-two dysphagia patients with normal EGJ relaxation were examined with a 36-channel HRM assembly. Each of the ten examinations was performed with 20 mmHg and 30 mmHg pressure topography isobaric contours and findings were categorized based on the Chicago classification. We analyzed the relationships between peristalsis pattern and the discrepancy between IBP and IRP.
Results: Twenty-two patients were classified by the Chicago classification; one patient with normal EGJ relaxation and normal peristalsis, eight patients with intermittent hypotensive peristalsis, and thirteen patients with frequent hypotensive peristalsis. A total of 220 individual swallows were analyzed. There were no statistically significant relationships between peristalsis pattern and the discrepancy between IBP and IRP in the 20 mmHg and 30 mmHg isobaric contours.
Conclusion: We hypothesized that when IBP overcomes IRP, bolus transit may occur. However, the discrepancy between IBP and IRP was not associated with peristalsis pattern. We need to determine another way to evaluate bolus transit such as the impedance method.

Table 1. High resolution esophageal manometry results in 3 patients with abnormalities on FEES (group1) and in one patient with normal findings on FEES (group 2).

Type of swallows	UES residual pressure, mmHg *	UES relaxation time to nadir, msec	UES relaxation duration, msec	UES recovery time, msec	Peak pharyngeal pressure 2.0 above mid UES, mmHg	Contraction time to peak 2.0 above mid UES, msec	Contraction duration 2.0 above mid UES, msec	Recovery time at 2.0 above mid UES, msec
Group 1 5 cc swallow	2.9 (2.1–4.4)	88.3 (29 –180)	173.6 (70-280)	85.0 (10-140)	202.0 (161-252)	326.1 (290-380)	1093.8 (920-1340)	767.3 (550-970)
Group 2 5 cc swallow	9.6 (7.5 – 11.7)	700.0 (450-860)	1341.6 (1190-1500)	641.6 (530-940)	182.0 (147 – 229)	298.3 (280–370)	576.7 (520-680)	278.3 (180 – 300)
Group 1 1 cc swallow	4.5 (0–17.4)	177.0 (90-300)	567.2 (370-850)	315.7 (140-460)	206.9 (24.9-319)	364.3 (270-910)	718.6 (400-1780)	354.3 (130-870)
Group 2 1 cc swallow	3.7	560.0	1000	440	315.7	270	420	150

* All results are presented as mean (range).

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GERD symptoms severity is explained by familiar segregation independently of BMI

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Objective: Obesity is a risk factor for GERD symptoms; both conditions separately have familiar segregation but there are no studies analyzing a possible common cause, such as low grade chronic inflammation. In this study we analyzed at which extent the variance of GERD symptom severity is explained by familiar BMI and therefore chronic inflammation markers.
Methods: Fifteen families with at least 13 members each living in urban areas close to Mexico City. GERD symptom severity was assessed by ReQuest® questionnaire, an analog visual scale of six dimensions. BMI, serum concentration of fibrinogen serum concentration were recorder. A mixed model with random and fixed factors was used to calculate GERD variance components. Families were selected only by number of members; as GERD and obesity are highly prevalent in Mexico no proband or other criteria were used.
Results: A total of 277 subjects were included: 59.6% women, age 42.3 ± 16 years, BMI 28.4 ± 6. Variance attributable to the family of origin was the most important determinant for total ReQuest® score (7%, $P < 0.05$), adjusted by gender, age, BMI and fibrinogen.

ANCOVA size effects (eta2) showed that family influenced the variance of all Request® dimensions: sleep disorders (9.6%), chest pain (11.4%), upper abdominal discomfort (9.2%) and lower abdominal discomfort (11.4%). While negligent influence was observed from fibrinogen (<0.2% for sleep disorders and chest pain) or BMI (<0.6% for any Request® dimension).
Conclusion: The present study supports that familiar segregation explains GERD symptoms severity, independently from BMI or chronic inflammation. Is still not clear if familiar/genetic factors can explain functional disturbance that determine hypersensitivity of GERD symptoms, like non erosive GERD. Further studies will be directed using polygenetic equations and linkage to clarify this association.

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Effect of an acidic bolus on oral, pharyngeal and esophageal transit in normal subjects

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Objective: Oral, pharyngeal and esophageal transit is influenced by the characteristics of the bolus swallowed. We evaluated by the scintigraphic method the influence of an acidic bolus and a neutral bolus on oral, pharyngeal and esophageal transit.
Methods: We evaluated 43 normal volunteers aged 23–71 years (median: 49 years), 21 men and 22 women. The scintigraphic evaluation of swallowing was performed in the sitting position with swallows in random order of a bolus of 5 ml of water (neutral, pH: 6.8) and a bolus of 5 ml of 3 g of concentrated lemon juice diluted in 50 ml of water (acidic, pH: 3.0), both labeled with 37 MBq of 99mTechnetium coupled with phytate.
Results: There was no difference between the acidic and neutral bolus in the duration of oral transit, pharyngeal transit or pharyngeal clearance, and in the amount of residues in the mouth and pharynx. In the proximal esophagus the transit and clearance duration was shorter with the acidic bolus (transit: 0.85 ± 0.07 s, clearance: 1.17 ± 0.07 s)| than with the neutral bolus (transit: 1.09 ± 0.11 s, clearance: 1.40 ± 0.11 s, $P < 0.04$). In the distal esophagus the transit and clearance duration was longer, and the amount of residues was higher, with the acidic bolus (transit: 5.27 ± 1.03 s, clearance: 10.66 ± 0.87 s) than with the neutral bolus (transit: 2.50 ± 0.63 s, clearance: 6.60 ± 0.58 s, $P < 0.02$).
Conclusion: We concluded that an acidic bolus causes a faster transit in the proximal esophageal body and a longer transit in the distal esophageal body, which are associated with a longer permanence and an increased amount of residues in the distal esophagus.

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The inclusion of solid boluses in high resolution manometry has high diagnostic yield

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Objective: High resolution esophageal manometry (HRM) is important in assessment of dysphagia and acid reflux symptoms. HRM is generally performed with water boluses, although symptoms may occur only with solids. The value of adding solid boluses to a manometric protocol is unknown. The objective of the study was to evaluate the diagnostic yield of including solid boluses in esophageal manometric testing.
Methods: Consecutive manometric reports for patients undergoing HRM in the Motility Laboratory at the Repatriation General Hospital for assessment of previously undiagnosed dysphagia and/or reflux symptoms were reviewed. Patients with diabetes mellitus, achalasia, cardiomyotomy or incomplete clinical data were excluded. Detailed swallowing history was taken prior to procedure. Esophageal manometry performed using a 16-channel water-perfused catheter. Ten 5ml water boluses were administered in right lateral (RL) and upright (UR) postures, as well as 5 solid boluses (2cm square piece bread) in UR posture. Each swallow was classified according to standard criteria for amplitude and organisation. Studies were categorised ''normal'' if ≥70% liquid and ≥60% solid swallows were normal. Ineffective esophageal motility (IEM) and spastic esophageal motility (SEM) diagnosed if characteristic amplitude and migratory abnormalities were present in ≥50% liquid and/or ≥60% solid swallows. Studies classified as mixed if both IEM and SEM characteristics were present.
Results: Data were obtained for 100 patients (39M; 53.4 ± 1.6 years), 47% of whom reported dysphagia during swallowing history prior to study. When analysing liquids alone, IEM was classified in 34% of patients. With the addition of solid boluses, this increased to 45%. Furthermore, analysis of both solid and liquid boluses altered SEM classification from 2 to 12% and mixed from 0 to 9%. Conversely, normal diagnosis decreased from 64 to 34%. Overall the inclusion of solid boluses to manometric assessment altered classification in 35% of patients. The analysis of both liquid and solid boluses altered the classification of IEM (36%), SEM (83%) and mixed (100%) motility disorders (Table). During the motility study, dysphagia was reported most frequently with bread boluses (77%); 1/3 of these did not report a history of dysphagia.
Conclusion: These data suggest inclusion of bread boluses in manometric assessment alters the diagnosis of motility disorders in 35% of patients. Without the use of solids, a significant proportion of this patient cohort would have been inaccurately diagnosed with normal esophageal motility.

	Classification		
	Final (n)	Altered (n)	Altered (%)
IEM	45	16	36
SEM	12	10	83
MIXED	9	9	100

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Increased in recent years, the diagnosis of Eosinophilic Esophagitis in patients with Refractory Gastroesophagial Reflux

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Objective: Eosinophilic esophagitis is a chronic esophageal inflammatory disease(1). Because signs and symptoms of the disease are similar to the gastroesophagial reflux (GER) disease many patients are treated as GER disease. However unresponsiveness to the GER disease treatement and increasing number of patients in the last decades make eosinphilic esophagitis more important(2).
Methods: Ninety-eight patients with reflux symptoms unresponsive to treatment were included in this study. Forty-two of these patients were diagnosed with eosinophilic esophagitis by endoscopy. These patients were included in the study diagnosed with eosinophilic esophagitis.
Results: Fifty-seven percentage of patients was male, mean age 7.24 ± 3.8. Skin prick test was applied to the patients. The factor that may cause allergies could be detected in 1/2 of them. We applied gastroscopy to all patients and nodular appearance was seen in 90% of them. Red furrows and longitudinal shearings were detected in 25% of the cases.Biopsy specimens of all cases had at least 15 eosinophils infiltration and had no signs of peptic esophagitis. A number of patients before addmission and some during the follow-up period was taking PPI and H2 blocker for 3 months however their symptoms were going on desipite the treatment. Average follow-up period was 6 ± 3 months. Food elimination was performed to 12 patients whose allergic factor was detected by skin pirck test. Antihistaminic treatement was applied to 12 cases and four of them had systemic steroid treatment. 8 cases were treated by montelukast and topical steroid treatment. Complaints of thirty eight patients who did not have systemic steroid treatment were relieved in the end of first month however four patients that had to use steroids relieved in the end of the second month.
Conclusion: Eosinophilic esophagitis is a chronic inflammatory disease of the esophagus whose incidence is increasing everyday. complaints and the findings are similar to much of GER disease however ıt should be considered first in patients whose complaints are going on desipite the GERD treatment. Treatment costs and the quality of life is high in terms of early response to treatment in pediatric patients which is recognizable form of the disease is gaining importance.

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A 24-hour ambulatory simultaneous multichannel intraluminal impedance-pH monitoring in patients with doubtful laryngopharyngeal reflux

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Objective: Assessment of laryngopharyngeal reflux (LPR) is difficult and there are few studies in patients with LPR using multichannel intraluminal impedance-pH (MII-pH) monitoring. The aims of this study are to provide the useful data of 24-hour MII-pH monitoring in patients who suspected laryngophaynggeal reflux (LPR), and to clarify the differences of clinical characteristics between the patients with LPR and those with non-LPR revealed by 24-hour MII-pH monitoring.
Methods: A total 70 patients with laryngopharyngeal symptoms of gastroesophageal reflux disease (GERD) who underwent endoscopy, manometry and 24-hour MII-pH monitoring from January 2008 to May 2011 were enrolled for this study. We evaluated all parame-

ters of 24-hour MII-pH monitoring retrospectively and compared clinical and laboratory findings between the patients with LPR and without LPR.
Results: Twenty-seven patients (38.6%) had LPR. At 70 patients, 41.5% (894/2154) of acid reflux episodes and 58.5% (1260/2154) of nonacid reflux episodes were detected at the distal esophagus. At 27 patients with LPR, 52.1% (542/1041) of acid reflux episodes and 47.9% (499/1041) of nonacid reflux episodes were detected at the distal esophagus. The numbers of acid LPR were 64. The median and 95th percentiles numbers of LPR were 0, 4 at 70 patients and 3, 6.2 at 27 patients with LPR. Compared between LPR and non-LPR patients, most parameters of all reflux episodes and acid reflux episodes in LPR patients were considerably greater than those of non-LPR patients. However, nonacid gas reflux episodes of the distal esophagus in upright position were increased in patients with LPR. (P = 0.028).
Conclusion: Of all patients with LPR manifestations, acid reflux episodes detected by pharyngeal pH monitoring were 38.6%. Nonacid gas reflux episodes of the distal esophagus in upright position were more common among patients with LPR compared to non-LPR patients.

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An evidence of esophageal deconditioning in patients with achalasia in the view of its subtype

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Objective: Achalasia is characterized by improper peristalsis and abnormal relaxation of lower esophageal sphincter. It is categorized into classic and vigorous type. In recent studies, vigorous achalasia represents acute form, and classic achalasia is considered as progressed, chronic form. We hypothesized that as resting lower esophageal pressure rises, esophageal transit would be hindered, and to pass food through, body pressure would increase. Another assumption is esophageal body will be gradually exhausted and deconditioning would occur, in turn widening esophageal lumen.
Methods: We retrospectively reviewed newly diagnosed 48 patients from January 2006 to October 2011. Patients with mean body pressure higher than 37 mmHg were defined as vigorous type. Esophageal manometry was done to obtain resting lower esophageal sphincter (LES) pressure and esophageal body pressure. Esophageal width ratio was calculated by dividing maximal diameter from transverse planes formed by barium column by minimal width of resting esophagogastric junction to evaluate dimensions of esophagus. Esophageal transit scan was done to measure T1/2, time required for radioactivity to half, and R30, residual radioactivity after 30 s.
Results: Resting LES pressure did not affect body pressure in both type of achalasia. Esophageal width ratio was in positive correlation with resting LES pressure (P = 0.023) in classic achalasia. Mean esophageal body pressure and resting LES pressure did not affect esophageal transit in both type. Instead, T1/2 and R30 were in positive correlation with esophageal width ratio in vigorous achalasia. (P = 0.003 and 0.018 respectively).
Conclusion: Higher resting LES pressure made wider lumen in classic, but not in vigorous. This implies, as disease progresses, compliance of body would decrease and deconditioning would occur. Absence of such relationship in vigorous type could be explained by still dynamic state and relatively remained neuromuscular activity of the body.

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Nutritional profile of children with Acid Gastroesophageal Reflux Disease and its association with the severity of disease

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Objective: Few pediatric studies related to nutritional status (NS) in patients with Gastroesophageal Reflux Disease (GERD) were reported. Objective: To describe nutritional profile of children with GERD and its association with the severity of the disease.
Methods: Sample: 40 children, 1 to 12 years, with diagnosis of acid GERD with refractory respiratory symptoms with dual channel 24 h pH probe, providing mild severity (5–10% reflux index, RI), moderate (RI 10–15%) and severe (RI >15%). Study period: 01-01-10 to 31-12-2010. Z scores were estimated for weight, height and BMI according to WHO references adapted by the Argentine Ministry of Health. The NS was established according to BMI: normal-No (Pc 10 to 85), overweight/obesity-Ov/Ob (>Pc 85) and underweight-Un (<Pc 10).
Results: 42.5% female, 57.5% male, mean age 5y 4m ± 2y 8m. Diagnosis: a) Mild GER (70%), Moderate (12.5%), Severe (17.5%), with significantly higher percentages of Moderate-Severe GERD in children >5 years (45%) rather than with lower age (11.1%) (Fisher P = 0.03) and also among Ov/Ob children (50%) than No and Un (13.6%) (Fisher=0.017); b)No NS 47.5% (95%CI 32-62), Un 7.5% (95%CI 0-15), Ov/Ob 45% (95%CI 29.5-60) without gender differences, with average height z-score of -0.46 ± 1.1. The averages weight z-score and BMI z-score were significantly higher in children with mild reflux (weight z-score 1.22, BMI z-score 2.06) and severe (weight z-score 1.74, BMI z-score of 1.98) for those with mild reflux (weight z-score -0.12, BMI z-score 0.43) (Anova P = 0.003 and P = 0.008)
Conclusion: Nutritional profile of the population studied shows high prevalence of overweight. Severity of GERD in children depended on age group and NS, being more affected over 5 years and those with overweight or obesity. The average weight and BMI z-scores were higher in children with greater severity of disease.

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An unusual cases of laryngopharyngeal squamous cell carcinoma mistaken as globus

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Objective: Globus is common functional symptom as a non-painful sensation of a lump or foreign body in the throat in the absence of organic cause. Some malignancy of laryngopharynx can produce the nature of symptoms that mimic globus. Here, we report two cases, laryngopharyngeal (hypopharyngeal and laryngeal) squamous cell carcinoma without exophyting mass lesion, initially mistaken as globus, then diagnosed after tissue biopsy.
Methods: A 47-year-old man presented with sensation of foreign body in the left throat for 3 months visited ENT dept. OPD. A stroboscopic finding was just left arytenoid mucosal swelling, outside endoscopy was normal, and neck CT findings was diffuse enhancing mucosal thickening on left pyriform sinus. He took conservative treatment as pharyngitis for 2 weeks. Without improvement of symptoms, He was refred to GI OPD. A barium swallow study, endoscopy, and high-resolution manometry showed normal results. His symptom was not improved despite of PPI treatment for 4 weeks as NERD or globus. Finally, He received a excisional biopsy. The pathology of tissue biopsy revealed SCC at the left pyriform sinus.
Results: In the other case, a 73-year-old woman presented with globus for 1 year visited ENT and GI dept. OPD. A stroboscopic exam showed mucosal color change on the base of epiglottis. Neck CT scan reported the mucosal enhancement on the base of epiglottis. Biopsy was done at the discolored site. Final diagnosis was laryngeal SCC.
Conclusion: We suggest that laryngopharyngeal malignancy without exophyting mass lesion should be considered as a rare cause of sensation of a lump or foreign body in the throat. The hypopharynx and larynx shoud be observed carefully during endoscopy for such reason.

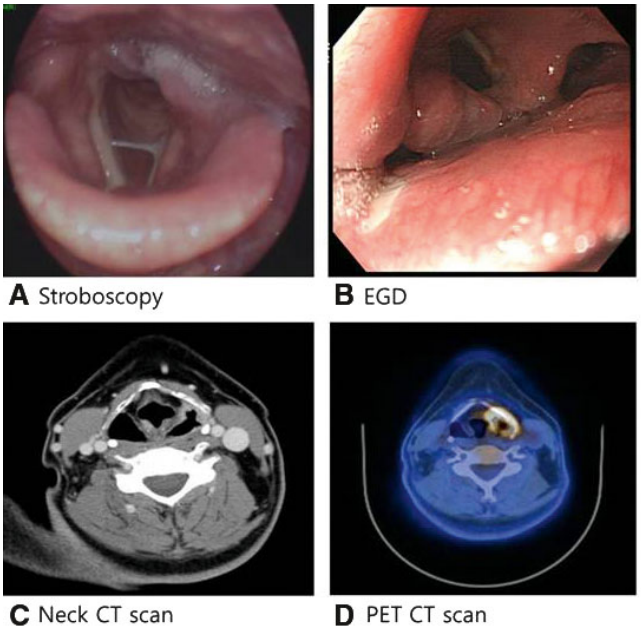

Figure 1. A 47-year old man's Images

Left arytenoid mucosal swelling in stroboscopy (A)

Left arytenoid mucosal Swelling in EGD (B)

Neck CT shows just diffuse enhancing and mucosal thickening on left pyriform sinus witout enlargement of regional lymph nodes (C)

Diffuse hypermetabolism (SUV 8.8) on left pyriform sinus in PET CT scan (D)

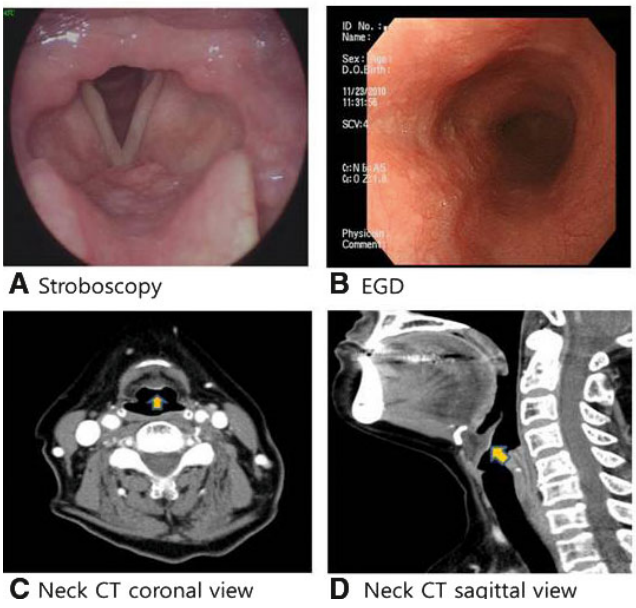

Figure 2. A 73-year old Woman's Images

Stroboscopy shows mucosal color change on the base of epiglottis (A)

EGD was normal (B)

Neck CT shows mucosal enhancement on the base of epiglottis (see the yellow arrow) (C) (D)

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The epidemiology of achalasia of the lower esophageal sphincter in children in San Diego County, California

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Objective: To determine the incidence and examine the epidemiology of achalasia of the lower esophageal sphincter in children in San Diego County, California.
Methods: The 4 regional pediatric surgical centers in San Diego County were asked to provide demographic and epidemiological data on cases of childhood achalasia from 2000 to 2009. Incidence rates were calculated from San Diego County childhood population estimate from the 2010 US Census.
Results: Between 2000 and 2009, 18 children (61% male, mean age at diagnosis was 9.5 ± 5 years) were diagnosed with achalasia of the lower esophageal sphincter. The population of children was estimated to be 726 048 children in 2010. The incidence of achalasia during this time period was 0.24/100 000/children/year (95% CI 0.11—0.28). Of the 18 children, 9 (50%) were Latino-Caucasian; 6 (33%) were non-Latino Caucasian; 2 (11%) were non-Latino Asian; and 1 (6%) was non-Latino African American. Pneumatic dilatation was the primary treatment rendered to 15 (83%) children and cardiomyotomy was the primary treatment in 3 (17%). All but one child managed by pneumatic dilation required repeat procedures to control symptoms (median 3, range 1–7). One child initially treated by cardiomyotomy also required a single pneumatic dilatation to control symptoms.
Conclusion: This is the first study to evaluate the epidemiology of achalasia in children in the US population. The mean incidence of achalasia in San Diego County from 2000 to 2009 is at least 0.24/10 000 children/year. This number is similar to the incidence of achalasia published for the UK over a similar time period.

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The pathogenetic implication of secondary oesophageal peristalsis impairments in patients with gastroesophageal reflux disease

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Objective: To determine the pathogenetic implication of secondary oesophageal peristalsis impairments in patients with gastroesophageal reflux disease (GERD).
Methods: One hundred and forty five patients with GERD and 22 virtually healthy individuals were evaluated. The diagnosis of GERD was based on characteristic clinical data and endoscopic signs of reflux oesophagitis, as well as on results of 24-hour oesophageal pH monitoring using ''Gastroscan-24'' device. Oesophageal motility was studied in all study subjects by means of intracavitary impedance monitoring with a Rheogastrograph RGG9-01 device(Saint Petersburg) working at frequency of 10 Hz, in dynamic mode, before and after functional acid test. An impedance monitoring probe connected in advance to a polyvinylchloride tube(diameter, 1mm) was introduced into the oesophagus.We used the tube to inject 5 mL of 0.1 n hydrochloric acid solution pre-heated to 370 °C into the lower one-third of oesophagus.
Results: Oesophagogastroscopy revealed endoscopy-negative GERD in 5.7% of study subjects. Reflux oesophagitis grade A was found in 63.0% of patients,grade B in 22.7%,grade C in 7.0%, grade D in 1.7%. Barrett's oesophagus was seen in 3.8% of cases, and bleeding in 0.4%. Results of 24-hour oesophageal pH monitoring were utilized to divide all study subjects into two groups according to distal oesophageal acidity (proportion of time with distal oesophageal pH less than 4 units within 24 h). Normal 24-hour proportions of pH<4 time were obtained for 20.7% of GERD patients, while those exceeding 4.5% were observed in 79.3%. Secondary oesophageal peristalsis (SOP) was preserved in 31.7% of patients with GERD, while it was undetectable in 17.2%. Increased baseline oesophageal motility was observed in 30.4% of subjects. The most severe grades of reflux oesophagitis (A, B, C) were more frequently ($P < 0.05$) observed in GERD patients with SOP impairment. Extra-oesophageal manifestations of the disease (pharyngitis, laryngitis, cough) were identified only in patients with pathological gastroesophageal reflux, more commonly ($P < 0.05$) in patients with abnormal SOP.
Conclusion: Impairment of secondary oesophageal peristalsis is one of the most important pathogenetic mechanisms in the development of extra-oesophageal clinical manifestations and pathomorphological abnormalities of oesophageal mucous membrane in patients with GERD and Barrett's oesophagus.

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Segmental changes in smooth muscle contraction as a predictive factor of the response to high-dose proton pump inhibitor treatment in patients with functional chest pain

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Objective: High-dose proton pump inhibitor (PPI) treatment leads to relatively little symptomatic improvement in patients with functional chest pain (FCP). This study was to evaluate the use of smooth muscle segmental changes in esophageal contraction as measured by topographical plots of high resolution manometry (HRM) as predictive factors of the response to high-dose PPI treatment in FCP patients.
Methods: Thirty patients diagnosed with FCP were treated with rabeprazole 20 mg twice daily for 2 weeks and classified as positive and negative responders based on symptom intensity score. HRM topographical plots were analyzed for segment lengths, maximal wave amplitudes, and pressure volumes of the proximal and distal smooth muscle segments.
Results: A positive response was observed in 23.3% of the patients. While the pressure volume of the proximal segment was significantly higher in the positive responders than the negative responders (900.4 ± 91.5 mmHg cm^{-1} s^{-1} *vs* 780.5 ± 133.3 mmHg cm^{-1} s^{-1}, $P = 0.017$), the pressure volume of the distal segment was significantly lower in the positive responders (1914.0 ± 159.8 mmHg cm^{-1} s^{-1} *vs* 2140.5 ± 276.2 mmHg cm^{-1} s^{-1}, $P = 0.014$). A prominent shifting in pressure volume to the distal segment was observed in the negative responders compared to the positive responders (segmental ratio of pressure volume (SRPV): 2.9 ± 0.5 *vs* 2.1 ± 0.1, $P < 0.001$), and 2.39 was found to be the SRPV that best differentiated positive and negative responders.
Conclusion: A low SRPV was associated with a positive response to high-dose PPI treatment in patients with FCP.

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Prevalence and characteristics of eosinophilic esophagitis in rural versus urban populations

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Objective: Eosinophilic esophagitis (EoE) has been associated with exposure to aeroallergens. Living in different locations (urban versus rural) could potentially expose individuals to different environmental factors. Currently, there is limited data in the literature. We postulated that such varied exposures could have an effect on the prevalence of EoE. AIM: The primary aim of this study was to determine the prevalence of EoE in an urban versus rural population and compare demographic characteristics.
Methods: Esophageal biopsies were obtained from a cohort of patients who presented with symptoms of dysphagia, odynophagia, globus sensation, and heartburn during a 10-year period. Patients that had biopsies from the distal and mid esophagus with ≥20 eosinophils/HPF were diagnosed with EoE. Urban population was defined as >1000 people/square mile and rural population was defined as ≤1000 people/square mile (U.S Census Bureau). Demographic data from each group was analyzed for age, sex, body mass index, duration of symptoms, and tobacco use. Chi-square analysis was used for frequencies with statistical significance defined as $P \leq 0.05$.
Results: A cohort of 19 172 patients with esophageal biopsies was evaluated. A total of 167 patients (M/F:113/54, median age = 27 years) had biopsy proven EoE (100 rural, 67 urban). There was a statistically significant difference between BMI in an urban setting as compared to rural (27 + 9.3, 24.4 + 6.6, respectively, $P = 0.05$). There was no difference in duration of symptoms prior to diagnosis between the groups. Smoking was compared only in the adult population (≥18 years old, $n = 113$) of which 15 were smokers (5 rural, 10 urban) and there was no statistical significance.
Conclusion: EoE is more common in the rural population. Rural EoE patients had a significantly lower BMI as compared to the urban EoE population with no difference in duration of symptoms prior to diagnosis. Although not statistically significant, rural EoE patients tend to be younger and less likely to be smokers.

Characteristics of Eosinophilic Esophagitis Patients

Variables	Rural (n=100, 59.8%)	Urban (n=67, 40.2%)	p-value
Age, yrs, median (Q1,Q3)	24.5 (13, 40.5)	31 (21,40)	0.08
Males, n (%)	64 (64%)	49 (73%)	0.21
BMI, mean ± SD	***24.4 (6.6)***	***27 (9.3)***	***0.05***
Duration of symptoms, months, median (Q1, Q3)	24 (8,72)	24 (7.5, 90)	0.80
Smokers, n (%)*	5/61 (8.2%)	10/52 (19.2%)	0.08

*pertains only to adult patients (≥18 years of age) (n=113)

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Impaired bolus transits and esophageal peristaltic dysfunction may play an important role in patients with globus

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Objective: Globus sensation is common in general population but etiology is unknown. Gastroesophageal reflux disease(GERD) is considered to be a possible cause of globus sensation, but proton pump inhibitor(PPI) has a little effect on patients with globus sensation. We aimed to explore the new mechanisms of globus sensation by high resolution impedence-manometry(HRiM) and Chicago classification and evaluate the role of impaired esophageal bolus transit and peristaltic dysfunction of esophageal swallow.
Methods: A total of 24 patients with globus sensation were evaluated at the Samsung Medical Center, Seoul, Korea, from June 2010 to May 2011. They were performed the HRiM, impedence-pH monitoring test, and upper GI endoscopy. Normal bolus transit group was defined as ≥70% liquid swallows with complete bolus transit. And impaired bolus transit group was defined as >30% liquid swallows with incomplete bolus transit. Using HRiM, contractile front velocity (CFV), distal contractile integral (DCI), distance (transitional zone), and integrated relaxation pressure (IRP) were measured in all patients. We used the Chicago classification to evaluate the esophageal peristaltic movements of NCCP patients.
Results: Among 24 patients, 14 were women and 10 were man. There mean age was 53.48 years. 8(33%) patients and 6(25%) patients complaint heartburn and acid regurgitation. Upper GI endoscopy revealed reflux esophagitis in one patient and the others showed normal. Total 5(21%) patients with pathologic acid and bolus exposure were diagnosed as GERD by HRiM, impedence-pH monitoring test. 7(29%) patients were classified as normal bolus transit, 17(71%) patients were classified as impaired bolus transit. According to Chicago classification, 2 patient showed normal peristalsis but 11(46%) patients showed peristaltic dysfunction (hypoperistalsis or aperistalsis). We divided patients to two groups: GERD-related globus group ($n = 5$) and non GERD-related globus group ($n = 19$). There was no significant difference in age, sex, underlying disease, peristaltic dysfunction of esophagus, bolus transit between two groups.
Conclusion: Many patients with globus sensation showed an impaired bolus transit in HRiM and they were combined with an esophageal peristaltic dysfunction. But only a few patients was diagnosed as GERD and it doesn't seems to explain the mechanism of globus sensation. Impaired bolus transit and esophageal peristaltic dysfunction may play an important role in globus sensation.

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GERD symptoms and PPI response in the patients with erosive oesophagitis, non-erosive reflux disease or functional heartburn

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Objective: To compare GERD symptoms and response to proton pump inhibitor (PPI) in the patients with erosive oesophagitis, nonerosive reflux disease (NERD) or functional heartburn (FH) using GERD impact scale (GIS) questionnaire.
Methods: 126 patients with GERD symptoms, enrolled prospectively, were diagnosed as erosive oesophagitis ($n = 62$), NERD ($n = 34$) and FH ($n = 30$) by endoscopy, Bernstein test and 24-hour esophageal pH testing. Risk factors were evaluated, and GIS

questionnaire for GERD symptoms and quality of life (QoL) were performed before and 8 weeks after PPI.
Results: The proportion of men was higher in erosive erophagitis group than in NERD or FH groups ($P < 0.001$). Erosive oesophagitis group had also frequent alcohol consumption, smoking, hiatal hernia, BMI ≥25 (kg m^{-2}), triglyceride levels (≥150 mg dL^{-1}) than other two groups (all $P < 0.05$). On the other hand, both psychiatric treatment and psychopharmaco therapy were more frequent in patients with FH than erosive oesophagitis and NERD (both $P < 0.05$). Among GERD symptoms, chest pain was frequent in FH group than erosive oesophagitis and NERD groups ($P < 0.05$). In terms of QoL, eating problem and limitation of productive daily activities frequently occurred in FH group than in erosive oesophagitis and in NERD group. GIS after PPI treatment showed improvement of chest pain in FH ($P = 0.031$) and acid regurgitation in NERD groups ($P = 0.034$). In terms of QoL, PPI treatment improved eating problem in the FH ($P = 0.021$) and limitation of productive activity in the NERD group ($P = 0.026$).
Conclusion: GIS questionnaire could be useful in the follow-up after PPI therapy among patients with erosive oesophagitis, NERD or FH.

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Impact of the manometric esophageal alterations and the exposure to acid in the incidence of Barrett's Esophagus

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Objective: To determine if the presence of manometric alterations and the exposure of the esophagus to acid are related with a higher incidence of Barrett's esophagus.
Methods: Retrospective analysis of 228 patients with clinical manifestations of GERD studied with esophageal manometry and 24 h pH monitoring in Agencia Sanitaria Costa del Sol. According to the endoscopic findings, the patients were divided into three groups: histologically confirmed Barrett's esophagus, erosive esophagitis without the presence of Barrett and non-erosive reflux disease (NERD).
Results: Thirty-nine patients had Barrett's esophagus (17.1%), 151 patients had NERD (66.2%), and 38 patients had erosive esophagitis (16.6%) according to the endoscopic findings. Barrett's esophagus was three times more frequent in men ($P < 0.001$). 12.5% of the patients with Barrett's esophagus had a normal-pressured lower esophageal sphincter, vs 36.7% who had severe hypotonic lower esophageal sphincter ($P = 0.051$). There was no relationship between the presence of lower body motility and the presence of Barrett's esophagus. ($P = 0.446$). The presence of Barrett's esophagus and erosive esophagitis was related to a more severe acid exposure, especially in severe mixed GER ($P = 0.007$), with a longer exposure to acid, and a higher score of DeMeester ($P < 0.001$). Hiatal hernia is more frequent in erosive forms and in Barrett ($P < 0.001$). There was no significant relationship between de body mass index and the presence of Barrett's esophagus.
Conclusion: Our results of the relationship between the appearance of Barrett's esophagus and low-pressured inferior esophageal sphincter, male sex and a longer exposure to acid coincide with the literature. On the other hand, there was no significant relationship with peristaltic alterations of the esophagus' body or with the body mass index. We believe that further prospective studies with a larger sample size that allow for a multivariable analysis are needed.

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Esophageal combined multichannel intraluminal impedance-pH monitoring and its clinical value in the diagnosis of various types of gastroesophageal reflux disease

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Objective: Multichannel intraluminal impedance-pH monitoring is a novel method that allows to estimate different characteristics of gastroesophageal reflux. However, its diagnostic yield compared to routine 24-hours pH-metry has not been evaluated yet.
Aim: To evaluate the effectiveness of the esophageal combined multichannel intraluminal impedance-pH monitoring in the diagnosis of various clinical types of gastroesophageal reflux disease (GERD).
Methods: Forty nine patients (18 men, 31 women, age 47 ± 2.3) with gastroesophageal reflux disease were enrolled to the study. The diagnosis was based on the presence of typical symptoms (heartburn, acid regurgitation) at least two times a week. Depending on the presence of esophagitis, all patients were divided into two groups. The first group consisted of 31 patients with nonerosive reflux disease (NERD) (11 men, 20 women, age 43 ± 3), the second group - 18 patients with erosive (ERD) (7 men, 11 women, age 53 ± 4). Multichannel intraluminal impedance-pH monitoring (MMS, the Netherland) was performed to all the enrolled patients, using disposable 8-channel impedance, 2-channel pH catheters.
Results: In patients with NERD the average number of refluxes recorded by pH sensors was 26 ± 4, the average number of refluxes detected by impedance was 47 ± 5 ($P = 0.01$). Number of refluxes that were not taken into account with the pH sensor in this group was 44%. In ERD group number of refluxes detected by pH sensors was 53 ± 10 compared to 64 ± 10 ($P > 0.05$) detected when impedance sensors were used. The results of main pH-metric parameters obtained in ERD and NERD groups are shown in the Table 1. Due to the lower number of the gastroesophageal refluxes detected by 24-hours pH-metry the DeMeester index didn't reach the diagnostic cut-off values in the NERD group and probably cannot be used as a solely diagnostic criterion to confirm the presence of GERD.
Conclusion: Combined esophageal multichannel impedance-pH monitoring is significantly more effective in the diagnosis of the presence of gastroesophageal refluxes in patients with different forms of GERD compared to the routine 24-hours pH-monitoring.

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Identification of esophageal dysfunction with combined impedance-manometry: A 6-year single-center experience

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Objective: Combined impedance and esophageal manometry (MII-EM) has been widely accepted as a valuable tool for assessment of esophageal function. The aim of this study was to determine and analyze functional defects in subjects referred for MII-EM from a single center.
Methods: All subjects underwent combined MII-EM including 10 liquid and 10 viscous swallows during a 6-year period. Ineffective esophageal motility (IEM) was defined as >30% liquid swallows with contraction amplitude <30 mmHg in the distal esophagus. Esophageal transit abnormalities were defined as < 30% of liquid swallows with complete bolus transit and/or < 30% of viscous swallows with complete bolus transit.
Results: During a 6-year period, 286 patients (150 women; mean age, 51 year; range, 19–83 year) had completed MII-EM studies, of which IEM was identified in 82 patients (29%) and abnormal bolus transit was seen in 116 patients (41%). Other motility diagnosis included achalasia in 30 patients (11%), nutcracker esophagus in 8 patients (3%), and isolated lower esophageal sphincter dysfunction in 17 patients (6%). In 179 patients with symptomatic gastroesophageal reflux disease (GERD), IEM occurred in 57 patients (32%) and abnormal bolus transit was found in 83 patients (46%), and 42 patients (24%) had both IEM and impaired bolus transit. IEM increased with the severity of GERD, whereas abnormal bolus transit occurred similarly among GERD patients with different severity. In 79 patients with dysphagia, IEM occurred in 20 of those patients (25%) and abnormal bolus transit was present in 26 of those patients (33%).
Conclusion: Out data suggest that esophageal dysmotility occurred paralleling with the severity of GERD. A significant proportion of subjects referred MII-EM studies appear to have normal esophageal function in term of esophageal motility by manometry and bolus transit by impedance.

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Association between smoking and eosinophilic esophagitis

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Objective: Several studies have shown a positive correlation between smoking and GERD, whereas others have failed to show a correlation. There have not been any studies evaluating the association between smoking and eosinophilic esophagitis (EoE). AIM: The aim of our study was to evaluate the impact of smoking on the incidence of EoE.
Methods: Data was obtained from a cohort of patients who presented with symptoms of dysphagia, odynophagia, globus, and heartburn during a 10-year period. Patients that had >20 Eosinophils / HPF from the distal and mid esophagus were diagnosed as having EoE. Data regarding smokers was limited to patients >18 years old. Smokers versus non-smokers were compared in regards to age at diagnosis, sex, BMI, and duration of symptoms. A non-normal data distribution of continu-

ous variables was used. Wilcoxon-rank sum testing was used when comparing groups. The prevalence of Iowan smokers was obtained from Center for Disease Control (2007–2008 survey).
Results: Among 113 adult patients with EoE, 15 (13.2%) were smokers at time of diagnosis. as compared with 18.8% smokers in the general Iowan adult population. There was a non-significant trend for a shorter duration of symptoms prior to diagnosis in smokers [13 months, (2, 72)] compared to non smokers [48 months (12 120)] (P = 0.08). There was no significant difference between non-smokers and smokers in regards to age at diagnosis, sex, or BMI. (Table 1)
Conclusion: According to our preliminary data, we could not establish a direct correlation between smoking and EoE. However, smokers with EoE tend to present earlier to health care providers.

Table 1: Prevalence of smoking among EoE patients

Variables	Non-smokers (n=98)	Smokers (N=15)	p-value
Age (yrs), median (Q1,Q3)	35 (26,46)	31 (26, 49)	0.80
Males, n (%)	62 (63.3%)	9 (60%)	0.80
BMI (kg/m^2), median (Q1, Q3)	26.5 (23.5, 31)	25.9 (24.8, 32.3)	0.63
Duration of symptoms (months), median (Q1, Q3)	48 (12, 120)	13 (2, 72)	0.08

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Comparison of anxiety and depression in Gastroesophageal Reflux Disease (GERD) patients who failed Proton Pump Inhibitor (PPI) therapy versus those who fully responded to it

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Objective: It has been suggested that patients with refractory Gastroesophageal Reflux Disease (GERD) are more likely to have psychological co-morbidity and Irritable Bowel Syndrome (IBS) than those who respond to treatment with proton pump inhibitors (PPIs). However, there is still scant data on the frequency IBS and of psychological co-morbidity in these patients. The aim of the study was to assess whether psychological co-morbidity and/or co-morbid IBS are associated with response to PPI in GERD patients.
Methods: GERD patients receiving PPI once or twice daily were evaluated by a personal interview regarding their demographics and clinical characteristics. Patients completed the Hospital Anxiety and Depression Scale (HADS), the Rome III diagnostic questionnaire, a validated subjective tool for diagnosing IBS, and the Satisfaction With Life Scale (SWLS) questionnaire, a scale that measures global life satisfaction. The HADS includes 14 items (scored on a 0–3 scale) that compose the HADS-Anxiety and the HADS-Depression sub-scales (7 items each). According to the scores of each sub-scale, patients are considered to have: normal (0–7); mild (8–10); moderate (11–14) and severe (15–21) level of anxiety or depression. The PPI failure groups were defined as patients who continued to report classic GERD symptoms (heartburn and acid regurgitation) while on PPI once daily or twice daily, at least three times a week for the last 3 months. The patients were divided into 3 groups: Patients who fully responded to PPI once daily (Group A, n = 112), patients who failed PPI once daily (Group B, n = 78) and patients who failed PPI twice daily (Group C, n = 56).
Results: Total of 246 patients (59.3% females, 52 ± 17.2 years) participated in this study. There were no differences between groups for the prevalence of co-morbid IBS (3.6% *vs* 6.4% *vs* 3.6%, P = NS); mean HADS-Anxiety score (6.0 ± 4.27 *vs* 6.53 ± 4.4 *vs* 6.3 ± 4.3, P = 0.76), mean HADS-Depression score (5.7 ± 4.0 *vs* 5.7 ± 3.97 *vs* 5.4 ± 2.9, P = 0.99) and mean SWLS score (25.3 ± 7.5 *vs* 25.4 ± 6.6 *vs* 23.0 ± 7.7, P = NS). There were no differences between groups for the distribution of the different levels of anxiety or depression (P = NS). In addition, there were no differences among the groups in age, sex, ethnicity, education, psychiatric medications, diagnosis of major depression, or drug and alcohol use.
Conclusion: The success or failure of PPI therapy in GERD is not associated with global life satisfaction, psychological co-morbidity or IBS co-morbidity. A larger study will be required to confirm our findings.

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Short term treatment of achalasia by dark chocolate

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Objective: Pneumatic dilatation or myotomy for achalasia are usually efficient. Pharmacological treatment (Ca channel blockers or nitrates) is usually minimally helpful and accompanied by side effects.
Aim: to test the efficacy of dark chocolate (containing flavonol – a nitric oxide donor) in improving achalasia symptoms and esophageal physiology.
Methods: Consecutive naive patients diagnosed as suffering from primary achalasia (diagnosed by gastroscopy and/or barium meal plus manometry). All patients were randomized into two groups – treatment arm used dark chocolate, 75% cacao solids and compared to placebo arm - patients using ''white'' chocolate. All underwent repeat manometry 15 min after chewing and melting 21 g of chocolate to test the immediate effects of the chocolate on esophageal function. All patients were discharges with dark chocolate for a period of 2 weeks and were instructed to chew and melt 7 g of chocolate three times a day, 15 min before each meal. All patients filled a daily symptom diary and health related quality of life questionnaire (HRQL).
Results: Twenty-seven patients were recruited for the study. Four omitted because of subsequent refusal. Nineteen agreed to undergo two manometries before and after chocolate consumption (10 study group, 9 controls) all patients were followed by symptom and HRQL questionnaires. No significant changes in LES pressure before and after treatment were observed for each group and no significant differences between the two groups (+6.8 mmHg *vs* -1.38 mmHg). Esophageal body pressures declined more in the study group, however, not statistically significant. After 2 weeks of treatment, statistically significant changes were observed in the sense of food limitation, solid food dysphagia and regurgitations (P < 0.01). No significant changes were observed for liquid dysphagia, vomiting, nausea, chest pain and heartburn.
Conclusion: In this small pilot study, chocolate was able to modify some symptoms related to dysphagia, however, it did not show efficacy in changing physiological parameters.

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Use of GpH-MII/pH for the evaluation of GERD in patients with refractory symptoms. How to optimize the PPI treatment?

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Objective: Adequate gastric acid suppression is of upmost importance for effective gastro esophageal reflux disease (GERD) treatment. Multichannel intraluminal impedance and pH (MII-pH) monitoring allows to distinguishing between weakly acidic and weakly alkaline reflux episodes. To evaluate the usefulness of a double dose PPI treatment in GERD patients with refractory symptoms, by using a combined intragastric pH and MII-pH monitoring (IGpH MII/pH) to evaluate treatment efficacy.
Methods: Thirty-seven patients with erosive esophagitis (grades A and B, LA), received 8 week treatment w/ pantoprazol magnesium (PMg) 40 mg o.d (T1). After a 10 day washout period, patients w/persistent GERD symptoms (reflux questionnaire), received a second treatment regimen (T2), consisting on a 4 week treatment w/PMg 40 mg b.i.d. Five healthy subjects were included as a control group. GpH-MII/pH was performed before and after T2. For statistical analysis, Student t test and ANCOVA adjusted by sex and age were performed. The group with GERD refractory symptoms was contrasted using Sidak Test.
Results: Twelve patients responded to the conventional T1, while 25 (10m, 15f; age 37.6 years ±10 year old), presented refractory symptoms and were submitted to T2. Basal GpH MII/pH confirmed the presence of GER in these patients (mean time percentage with intragastric pH<4 was 81.9%). After T2, 13 out of 25 patients (52%) continued with symptoms and were considered as non responders. According to GpH MII/pH results, they were classified into three groups: (i) GpH-MII$^+$/pH$^-$,SI$^+$ (n = 5), (ii) hypersensitive esophagus, GpH-MII/pH$^-$, SI$^+$ (n = 4), and (iii) functional heartburn, GpH MII/pH$^-$ and SI$^-$ (n = 4). In the control group, the basal GpH-MII/pH showed values in the normal range.
Conclusion: Sixty-eight percent patients required a double dose PPI treatment. Forty-eight percent had a good medical response but still presented symptoms of functional/hypersensitive disorders. In these patients, a combination of PPI and a tryciclic antidepressant or a similar drug would be probably needed to control symptomatology. GpH-MII/pH is a useful tool to evaluate GERD patients refractory to conventional treatment.

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The long-term outcome in patients with primary achalasia according to the balloon dilatation or intrasphincteric botulinum toxin injection

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Objective: The aims of this study are to evaluate the long term outcome and find predictive factors for

remission in patients with after balloon dilatation and intrasphincteric botulinum toxin injection in primary achalasia.
Methods: The 37 patients who underwent balloon dilatation(BD) or intrasphincteric botulinum toxin injection(Botox) between January 1988 and July 2011 were enrolled. Among the 58 patients who could be taken care of more than 10 months after the operation, 37 patients were targeted for this study who were available for telephone interview. All patients underwent clinical, manometric, radiographic and endoscopic evaluation to confirm primary achalasia. Review of medical records and telephone interview as predetermined order by questionnaire including Eckardt score was carried out.
Results: The median age of the patients was 56 (range 19–89) for man 22, woman 15 patients. The number of patients who underwent Botox was 14, Botox-BD was 11 and BD was 12. On long-term outcome of telephone interview, the remission group is 23 patients. The recurrence group is 12 patients and the treatment failure group is 2 patients. The low esophageal sphincter pressure before treatment was median 32.2 mmHg (range 5.1–98.0 mmHg). The low esophageal sphincter pressure after treatment was 22.8 mmHg (0.8–40.0 mmHg). The difference of low esophageal sphincter pressure between before and after treatment was 18.4 mmHg (1.0–65.0 mmHg). The remission duration of patients who underwent Botox was 10 months (1–96 months). The remission duration of patients who underwent BD was 56 months(8–62 months), which was significantly long comparing to others ($P = 0.001$). On univariate analysis, if the difference of low esophageal sphincter pressure was 15 mmHg or higher, remission duration was significantly longer ($P = 0.01$). On multivariate analysis, only treatment method was the significant predictive factor. Patients underwent BD had significantly long-term success ($P = 0.001$).
Conclusion: BD is associated with better long-term outcome than Botox. If the difference of low esophageal sphincter pressure was 15 mmHg or higher, remission duration was significantly longer suggesting usefulness of manometric follow up after treatment. For predictors of long-term remission in patients with primary achalasia, future prospective randomized study is needed.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
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Digestive, cognitive and hedonistic responses to meal ingestion
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Objective: Previous studies have demonstrated that sensory-motor gut dysfunctions may be associated to digestive symptoms. We hypothesized that the gut can also originate pleasant sensations, and wished to investigate the hedonistic component of digestive function. The specific aim of this study was to establish the relation of gastric accommodation and the cognitive-emotive responses to meal ingestion.
Methods: In 22 healthy subjects 240 mL of either clear soup (5 kcal/100 ml) or water, as inert meal, were administered at controlled temperature (55 °C) and standard ingestion rate (60 mL min^{-1}). The following measurements were performed during a 9-min pre- and a 60-min post-prandial period: gastric accommodation (as isotonic changes in the volume of air within an intragastric bag) by a computerized tensostat, the cognitive response (epigastric fullness) by a 0 to 10 scale and the hedonistic dimension (comfort/discomfort) by a+5 to -5 scale. Cross-over studies were performed in random order on separate days.
Results: Soup ingestion induced a gastric relaxation (gastric volume increased by 154 ± 28 ml *vs* 67 ± 11 ml with water; $P = 0.01$). The digestive response was associated to perception of epigastric fullness (3.7 ± 0.8 score *vs* 1.1 ± 0.5 with water; $P = 0.01$), and interestingly, this conscious sensation had a pleasant dimension (digestive comfort increase by 1.5 ± 0.6 score vs 0.8 ± 0.7 decrease with water; $P = 0.02$).
Conclusion: Proper gut responses to a meal induce digestive comfort and pleasant sensations.

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Gastroparesis: An emerging epidemic
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Objective: The epidemiology of gastroparesis is largely unknown. The past decade has witnessed little progress in the management of gastroparesis. We sought to characterize the time trends in the proportion of patients hospitalized with gastroparesis in United States.
Methods: Using the Nationwide Inpatient Sample database, temporal trends in hospitalisations for gastroparesis was performed from years 1994 to 2009. All patients admitted to hospital between 1994 and 2009 with a primary discharge diagnosis of Gastroparesis, identified by the International Classification of Diseases, Ninth Revision procedure codes were included (536.3). Temporal trends in the change in the hospital charges, length of stay, proportion of Emergency department visits admitted as inpatients over the selected time period were evaluated. Statistical analysis was done using STATA 12.0 MP after applying appropriate weights for the propensity score as previously determined by AHRQ recommendations.
Results: Between 1994 and 2009, the absolute number of Gastroparesis hospitalizations increased from 918 to 16738, ($P < 0.01$). There was a significant increase in charges from $14571 to $29481 in 2009 ($P < 0.05$). Also of note there has been a linear increase in the proportion of patients admitted to inpatient service from the emergency department – 43% to 65% ($P < 0.05$).
Conclusion: Over the last decade, the percentage of patients hospitalized for a primary diagnosis of gastroparesis has exponentially increased.

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A psychological status analysis in patients with functional dyspepsia
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Objective: To analyze the psychological status in men and women suffering from functional dyspepsia (FD).
Methods: A total of 68 patients with FD(37.6% of them men and 62.4% women) were evaluated; the mean age at the time of study enrollment was 41.6 ± 6.24 years. The psychological status assessment complex included the Zung Self-Rating Depression Scale, MMPI, Hamilton Depression Rating Scale (HDRS), and Spielberg - Hanin personal and situational anxiety questionnaire.
Results: According to the MMPI questionnaire data, the neurotic overcontrol (78.5 ± 13.4) and depression (77.1 ± 11.9) scores in the personal profile of men exceeded respective scores in women (71.2 ± 10.1 and 72 ± 12.8, respectively), while the emotional lability scores were virtually the same in men (66.9 ± 11.9) and in women (66.2 ± 9.8). In the female group, subjects generally had higher scores of impulsivity (T 70.6), rigidity (T 66.6), individualism (T 71.3), and optimism (T 56.2). Our analysis of the average female profile demonstrated sthenic-type traits in the personality structure of study subjects, and suggested a somewhat different type of response and a broader spectrum of psychological protection mechanisms. Clinically, 46.4% of study subjects had depressive disorders: mild depression was detected in 15.1% of FD patients, moderate depression in 24.1% of them; 7.2% of patients had a severe depressive disorder. Somato-vegetative manifestations prevailed in the depression structure (such as weakness, epigastric pain, nausea, palpitations, headache, dizziness, and hyperperspiration). Hypochondria manifested itself in overestimation of the disease severity and excessive self-observation. Stable sleep disturbances were identified: difficulty falling asleep and early waking. A certain role was also played by asthenic symptoms manifested by excessive lacrimation, fatigue, and weariness. Besides, patients with FD had increased levels of personality (54.8 ± 3.6) and, in particular, reactive anxiety (62.7 ± 4.1).
Conclusion: Patients with functional dyspepsia were found to be frequently affected by anxiety-depressive disorders. Women typically had higher activity levels, as compared with men, with a trend to involve a

broader spectrum of mechanisms for psychological protection and counteracting the high level of anxiety and emotional tightness.

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Effects of RM-131 in healthy volunteers and type 2 diabetics with documented delayed gastric emptying: Pharmacokinetics and pharmacodynamics

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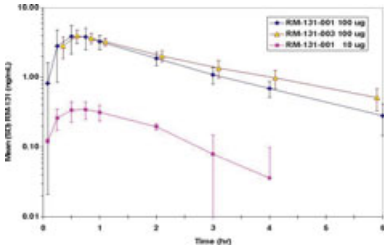
Objective: Ghrelin, the natural GHS-1a receptor ligand, stimulates gastric emptying (GE). RM-131, a pentapeptide synthetic ghrelin agonist, has a longer plasma T1/2 and >100X greater potency than native ghrelin in reversing ileus in animals. In healthy humans, the estimated EC50 for RM-131 acceleration of GE T1/2 was 0.2–0.5 ng mL^{-1}.
Aim: To investigate the pharmacokinetics (PK) of RM-131 and the pharmacodynamics (PD) and safety in response to a single dose of RM-131 in type 2 diabetes mellitus (T2DM) patients with prior documentation of delayed GE (DGE).
Methods: Randomized, double-blind, single-dose, two-period, crossover study of RM-131, 100 μg s.c., or placebo with 7-day washout in 10 DGE patients. GE by scintigraphy with solid-liquid meal ingested 30 min after dosing. Safety was assessed by adverse events, vital signs, glucose, plasma growth hormone, prolactin, cortisol and insulin. Statistics: Paired t-test or Wilcoxon signed rank test (2-sided $\alpha = 0.05$). Coefficient of variation of GE solids T1/2 was 24%, justifying study of 10 patients. Pharmacokinetics were assessed in this cohort and in three healthy volunteers (HV).
Results: At screening of DGE patients, symptom load was low (total GCSI-DD score 1.32 ± 0.21 [SEM]); HbA1c 7.2 ± 0.4; age 51.8 ± 2.5 year; and BMI 31.1 ± 1.8 kg m^{-2}. RM-131 had a significant treatment effect on GE T1/2 of solids (placebo 127.8 ± 18.6 min; RM-131 59.5 ± 7.9; $P = 0.011$). The estimated mean treatment difference [Δ] in solid GE T1/2 was 68.3 min [95% CI 20–117], a 66.1% Δ relative to overall mean. There were numerical differences in GE T1/2 liquids (21.7 ± 4.1 *vs* 13.3 ± 4.0; $P < 0.14$). Higher 120 min blood glucose ($P = 0.07$) reflected more rapid GE T1/2 with RM-131; no significant effects on insulin were noted. Increased 30–90 min AUC levels of GH, cortisol and prolactin were as expected with single dose RM-131 (all $P < 0.02$). There were no significant adverse effects; only light-headedness was reported more on RM-131. PK was similar in DGE patients and HV; after single 100 μg s.c. dose, Cmax was ~4 ng mL^{-1} in both groups (figure).
Conclusion: PK of RM-131 is similar in DGE patients and HV, and is higher than the estimated EC50 for accelerating GE T1/2 from PK-PD model in HV. RM-131 greatly accelerates GE of solids in T2DM with documented DGE.



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Use of erythomycin and related macrolides for the treatmant of gastroparesis: Systematic review

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Objective: To evaluate the effectiveness and safety of erythromycin or its derivatives in reducing symptoms and in improving mechanical gastric emptying in adults with gastroparesis.
Methods: Search methods- Our search included Medline (1950 to 2011 December) and EMBASE (1980 to 2011 December) databases. The Cochrane Central Register of Controlled Trials was also screened for any relevant studies. Selection criteria-Randomized controlled trials were included where Erythromycin or any other macrolide/ derivatives were compared with a control [placebo or other prokinetic, antiemetic] in the treatment of gastroparesis. Quality of studies was evaluated using criteria of masking of randomization/intervention/ outcome assessment and completeness of follow-up. Data collection and analysis - Data was abstracted independently by two authors. Statistical methods included calculation of standardized mean differences, odds ratios (OR) and their 95% confidence intervals when appropriate. Data of individual studies were not combined due to clinical heterogeneity. These results are reported separately by the type of comparator group in the control arm and also by the causality of gastroparesis.
Results: Twenty studies and 426 patients were included in this review. Based on the available evidence, erythromycin or any of the derivatives (mitemcinal, KC 11458, ABT-229) does not significantly improve symptoms related to delayed gastric emptying (nausea, vomiting, early satiety, gastric fullness/pain as a composite outcome) when compared to a placebo within a 7–28 day period. Also no statistically significant difference was noted between erythromycin derivatives compared to placebo in reported serious adverse events. The general trend of studies showed significant improvement in mechanical emptying over 1 day to 8 weeks, when erythromycin or its derivatives (were compared to either placebo or metoclopramide.
Conclusion: From the available evidence erythromycin and other derivatives seem improve gastric emptying up to 8 weeks without tachyphylaxis. This has potential for exploration in patients where mechanical improvement would offer improved outcomes. The trend in evidence for symptom improvement of gastroparesis does not favour erythromycin or other derivatives although a more formal assessment is warranted.

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This abstract has been withdrawn.

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This abstract has been withdrawn.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-14 Clinical Session: Small Bowel: Clinical

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Mitochondropathy: A rare case of selective enteric myopathy

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Objective: Intrinsic neuropathies and myopathy are causes of severe derangement of digestive functions. Among the different mechanisms responsible of enteric myopathies mitochondropathies have been recently identified. Aim: to evaluate clinical features and small bowel motor activities in patients with different forms of mitochondropathy.
Methods: Nine patients with severe digestive symptoms (resembling chronic intestinal pseudo-obstruction in 2 cases) with laboratory and histologically confirmed mitochondropathy were consecutively referred to the Laboratory of Functional Gastrointestinal Disorders of the St Orsola-Malpighi Hospital, University of Bologna to undergo a small bowel stationary manometric test. Relevant issues of health status were recorded for each patient according to predefined, validated questionnaires at entry. Three patients refused small bowel manometry so that six patients (5 M, 41 ± 12.6 years) were included, affected respectively by MNGIE ($n = 3$), SANDO (1), MERRF (1). MELAS (1).
Results: The most frequent digestive symptom was diarrhea. A BMI lower than normal due to insufficient nutrition was also almost invariably present. Patients with MNGIE had similar motor abnormalities represented by repetitive high pressure contractions (HPC). These abnormalities were also present, although less clearly expressed in the patient with SANDO, but were not recorded in the remaining two cases. All patients affected by MNGIE died during the follow-up (2–4 years). The remaining patients remained stable over time (follow up 1–4 years).
Conclusion: MNGIE, compared to other mitochondropathies (MELAS, MERRF), showed more severe clini-

cal features associated with a specific motor pattern of the small bowel.

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High prevalence of carbohydrate malabsorption in patients with unclear abdominal discomfort

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Objective: Carbohydrate malabsorption can lead to increasing end-expiratory hydrogen (H2) concentrations and often causes gastrointestinal symptoms. It seems to be frequent in patients with functional gastrointestinal disorders and also in the general population, but there is a lack of comparative studies in large study populations. To investigate the prevalence of carbohydrate malabsorption by means of hydrogen breath test in a large cohort of patients who sought healthcare because of their gastrointestinal discomfort including symptoms like bloating, pain or changes in bowel habits. Abdominal symptoms were evaluated by the in-house standardized symptom questionnaire.
Methods: 2390 patients (mean age: 49.6 years, 806 male, 1584 female) with abdominal discomfort underwent combined H2 breath testing with 50 g of lactose and fructose. Subjects with a pathologic early rise in both tests were additionally tested with 50 g of glucose. Subjects with pathologic 50 g fructose breath hydrogen values underwent another test after a 25 g fructose load. The breath tests followed standard protocols. Concurrent abdominal symptoms under the carbohydrate load were documented. The population was investigated regarding prevalence of carbohydrate malabsorption, correlation of symptoms with a significant H2 increase and prevalence of small intestinal bacterial overgrowth (SIBO).
Results: 35.5% of all patients with unclear abdominal discomfort were lactose intolerant while 76.1% showed fructose malabsorption. A combined carbohydrate malabsorption was found in 34.7%. The comparison of maximal H2 concentrations in the breath test with 50 g fructose showed that subjects with pathologic H2 breath test with 25 g fructose, classified as severe fructose malabsorbers, exhaled significantly higher H2 concentrations than patients with negative H2 breath test with 25 g fructose, defined as moderate fructose malabsorbers ($P < 0.001$). An additional glucose breath test was performed in subjects with an early H2 increase in the lactose and fructose breath test ($n = 576$). Of these, 103 patients showed an early significant H2 increase after the glucose load, indicative of SIBO in 4.3% of the whole study population. Patients with SIBO were significantly older than patients without SIBO ($P < 0.001$).
Conclusion: This is the largest study cohort presenting with abdominal discomfort describing the prevalence of carbohydrate malabsorption which is a frequent but under-estimated condition in this patient population. Studying the prevalence of carbohydrate malabsorption in the normal population without abdominal symptoms and elimination diet trials will determine the relevance of these findings in daily life. Diagnosis of carbohydrate malabsorption can be easily confirmed by H2 breath test which should be more often utilized.

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Diagnosis of enteric dysmotility: Are manometric disturbances predicable of hystopathological findings?

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Objective: Currently, the diagnosis of small bowel motor disorders relies on the identification of intestinal manometric abnormalities defined by predetermined criteria and/or detection of neuromuscular abnormalities at histopathology. However the correlation between these two diagnostic methods remains unknown. Objective: To establish putative relations between the criteria of abnormality in intestinal manometry and histopathological disorders.
Methods: Patients referred for evaluation of severe symptoms suggestive of small bowel motor dysfunction between 2004 and 2011. Mechanical intestinal obstruction and macroscopically recognizable gut lesions were previously excluded by a thorough diagnostic workup. Conventional small bowel manometry was performed in 320 patients (78M, 242F; age range: 15–78 years). Manometric diagnosis of abnormal motility was established using previously published criteria (Gut 1987; 28: 5–12): 83 patients manifested neuropathic type dysmotility pattern, 17 a myopathic pattern, 38 an occlusive pattern, 182 did not meet criteria of dysmotility. Twenty one patients (7M, 14F; age range 14–55 years) with primary intestinal dysmotility defined by the manometric criteria as neuropathic pattern in the absence of recognizable organic, systemic, and metabolic diseases underwent surgical full-thickness biopsies. Intestinal specimens were processed for evaluation by traditional staining and a panel of immunohistochemical markers for muscle, interstitial cells of Cajal, glia and neurons.
Results: Of the twenty one patients who presented with a manometric pattern of neuropathy, seven exhibited intestinal histopathological features consistent with enteric neuropathy characterized by altered neuron specific enolase immunoreactive pattern and visible neuronal abnormalities Seven patients had a remarkable c-Kit+/tryptase immunoreactive mast cell infiltration of the gut wall. Three other patients showed abnormal smooth muscle features as reflected by abnormalities of smooth alpha actin immunoreactive pattern in the circular and/or longitudinal layers Four remaining patients had normal histology.
Conclusion: Although the manometric expression of a histological lesion in the small bowel is heterogeneous, patients with a neuropathic pattern at intestinal manometry show a high probability of morphological abnormalities (mainly inflammatory) in the gut wall detectable by histopathological analysis.

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Impaired gastrointestinal motility in a novel cluster of patients with familial mediterranean fever

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Objective: Familial Mediterranean Fever (FMF) is a rare autosomal recessive autoinflammatory disorder associated with serositis, amyloidosis and profound influence on gastrointestinal (GI) motility during attacks. We performed genetic, serological, and motility studies in FMF patients from Apulia-Basilicata regions.
Methods: Twenty-nine FMF patients (M:F = 13:16, age 39 ± 3 SEM years) displayed the following MEFV gene mutations: E148Q/R761H (compound heterozygous state, 57%), K695R (heterozygous state, 21%), E148Q (heterozygous state, 10%), R202Q (heterozygous state, 3%) and R202Q+M694V (3%). We investigated fasting and postprandial gastric, gallbladder emptying (functional ultrasonography) and orocecal transit time OCTT (H2-breath test, Lactofan® - Italchimici, IT) in the asymptomatic phase (Test meal: 200 ml Nutridrink®, Nutricia added with 10 g lactulose) during 270 min. Age- sex- BMI-matched healthy subjects served as control ($n = 142$; M:F = 67:75, 38 ± 1 years).
Results: Mean age at symptom onset was 19.2 ± 8.4 years, with definite diagnosis reached after 15.4 ± 9.7 years. Human serum amyolid protein (SAA) was increased in 33% of patients (all same E148Q/R761H mutation). FMF patients, compared to controls, showed in the stomach similar fasting antral areas (FMF 3.3 ± 0.2 *vs* 3.2 ± 0.1 cm^2), significantly ($0.000001 < P < 0.001$) increased max postprandial area (14.1 ± 0.4 *vs* 11.6 ± 0.2 cm^2), residual postprandial area (4.3 ± 0.2 *vs* 3.5 ± 0.1 cm^2) and delayed emptying time (43.2 ± 1.2 *vs* 26.6 ± 0.5 min). In the gallbladder FMF showed comparable fasting (FMF 19.8 ± 2.1 vs 22.4 ± 0.5 ml) and postprandial residual vol. (5.4 ± 0.4 vs 5.5 ± 0.2 ml), with a trend to increased residual volume (28.9 ± 1.6 FMF vs 24.8 ± 0.7%, $P = 0.059$) and comparable emptying time (23.2 ± 1.7 vs 21.0 ± 0.5 min). OCTT was longer in FMF (132.3 ± 10.5 vs 99.5 ± 1.6 min, $P < 0.000001$). Motility defects existed in spite of SAA levels.
Conclusion: A novel cluster of FMF patients has been characterized between Apulia and Basilicata. Final diagnosis appears to be consistently delayed. GI motility appears perturbed in FMF patients between attacks, mostly found at the level of stomach and small intestine, but not gallbladder. Studying GI motility in FMF patients might provide additional clues on the natural history of this rare disorder.

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Neuroradiological and neurometabolic characterisation of CIPO patients

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Objective: The main objective of this ongoing multidisciplinary study is to validate brain and skeletal muscle MR protocols in the diagnostic work-up of patients with chronic idiopathic intestinal pseudo-obstruction (CIPO). The specific objective is to identify integrated patterns of changes suggestive for mitochondriopathies, using MR imaging (MRI) and spectroscopy (MRS), an advanced MR technique, that can identify non invasively tissue oxidative phosphorylation deficit.
Methods: MR studies were performed in a 1.5 T GE whole-body scanner. The brain MR protocol included: a) high resolution T1- and T2- weighted sequences and b) diffusion tensor imaging (DTI); in order to detect signal intensity and volume changes of cortical and subcortical structures; c) brain proton MRS for the evaluation of ventricular lactate; and d) phosphorus MRS at rest and during an aerobic exercise to evaluate skeletal muscle bioenergetics.
Results: From 1 January 2008 to 1 April 2012 we evaluated with a standardized MRI protocol 34 patients with CIPO. In 3 patients a severe leucoencephalopathy and an associated dysfunction of energetic metabolism were suggestive for mitochondrial neurogastrointestinal encephalomiopathy (MNGIE), as confirmed by the findings of mutations in the thymidine phosphorylase gene. In other 3 patients with variable combination of clinical signs such as palpebral ptosis, ophthalmoparesis, hypoacusia or microsomia and brain and/or skeletal muscle bioenergetics defect, genetic analysis disclosed different pathogenic mtDNA mutation: 8344A>G (Myoclonic Epilepsy with Ragged Red Fibers, MERFF, $n = 1$), mutation in the mtDNA polymerase gamma (POLG) gene ($n = 1$), and A1555A>G ($n = 1$). In 7 patients with abnormal muscle and/or brain bioenergetics the mtDNA screening is still ongoing. Other brain MR features included metabolic encephalopathy ($n = 2$); deposition of manganese in basal ganglia structures likely secondary to long-term parenteral nutrition ($n = 5$). One patient presented clinical and skeletal muscle MR alterations compatible with myofibrillar myopathy, as confirmed by biopsy.
Conclusion: Our preliminary results show a high prevalence of mitochondrial disorders in patients with CIPO and suggest that the screening for mitochondrial defects should be included in the clinical work up of these patients.

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An epidemiologic survey of chronic intestinal pseudo-obstruction (CIPO) and evaluation of the newly proposed diagnostic criteria

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Objective: Chronic intestinal pseudo-obstruction (CIPO) is an intractable disease in which clinical symptoms of intestinal obstruction appear without mechanical cause. The diagnosis for CIPO is often delayed owing to the lack of clear diagnostic criteria, and low recognition rate of this disease. The delay for correct diagnosis leads to useless and potentially dangerous surgical procedures. Therefore, we proposed diagnostic criteria to facilitate the diagnosis of this rare intractable disease. The purpose of this study was to evaluate the usefulness and validity of our diagnostic criteria.
Methods: Our diagnostic criteria consist of following four mandatory requirements; (i) onset of one or more symptoms of bowel obstruction at least 6 months prior to the diagnosis, (ii) having at least one of abdominal pain and abdominal bloating for the previous 12 weeks, (iii) dilatation and/or air-fluid levels of the bowels on abdominal X-ray, echo and/or CT imaging, and (iv) exclusion of any organic obstruction of the gut lumen. To investigate the sensitivity of our diagnostic criteria, a questionnaire, asking about the disease duration, clinical symptoms, and radiological imaging findings, was sent to 378 institutions belonging to the Japanese Society of Gastroenterology (JSGE) during the period between December 2009 and February 2010. We summarized the returned data and performed statistical analysis of the data.
Results: In all, 216 (57.2%) of the 378 institutions responded to our questionnaire, and of these, 200 (92.6%) were aware of CIPO as a distinct disease entity, and 103 (51.5% of those that were aware of CIPO as a distinct disease entity) had encountered cases of CIPO. A total of 160 cases were accumulated, and 141 cases (88.1%) fulfilled the criterion of disease duration of more than 6 months, and 157 cases (98.1%) fulfilled the criterion of the clinical symptoms of abdominal pain and/or bloating. Furthermore, 154 cases (96.2%) fulfilled the criterion of the imaging findings. Eventually, 138 cases (86.3%) fulfilled all the criteria.
Conclusion: The proposed diagnostic criteria were useful with a high sensitivity of 86.3% for Japanese patients. Improved recognition of CIPO and practical use of the criteria are desired. The criteria should be appropriately modified by many researchers to make them more practical and internationally applicable.

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Morphological differences in jejunum mucosa between patients with IBS and healthy controls

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Objective: There have been reports on quantitative changes of different inflammatory and motility-associated cells in gut mucosa of patients with irritable bowel syndrome (IBS). Data are mainly from colon and rectum specimens and there is little data on small bowel findings in IBS. There is also a paucity of knowledge regarding normal quantities of different cell types in intestinal mucosa. As part of their function in the gastrointestinal tract, enteroendocrine cells produce serotonin, one of several transmitters involved in the control of intestinal motility. Since IBS is thought to be an intestinal motility disorder, changes in the amount of enteroendocrine cells (EC) and therefore the amount of serotonin and other signalling substances might have a role in the pathogenesis of IBS. The aim of this study was to determine quantitatively the amounts of EC in normal jejunum mucosa and to compare these measures with those obtained in jejunum mucosa from patients with IBS.
Methods: We studied 30 healthy volunteers in whom the presence of any FBD had been excluded and 19 patients with IBS defined by Rome II and Rome III. Biopsies from jejunum mucosa were taken using a Watson capsule. We used immunohistochemistry to identify enteroendocrine cells (Chromogranin-A). EC were counted as numbers per 100 epithelial cells and as number per crypts using light microscopy.
Results: The mean value (±SD) of EC/100 epithelial cells in healthy controls was 9.3 ± 2.2 and for IBS patients 7.3 ± 2.4 ($P < 0.01$). Number of EC per crypts in healthy controls was 2.3 ± 0.6 and for IBS patients 1.8 ± 0.6 ($P < 0.01$).
Conclusion: Patients with IBS exhibit reduced numbers of EC in jejunum mucosa. Reduced numbers of EC may indicate that the enteroendocrine system has a role in the pathogenesis of IBS.

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The clinical characteristics and natural history of colonic pseudo-obstruction in Korea: Based on nationwide multicenter database

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Objective: Colonic pseudo-obstruction (CPO) is a rare motility disorder, associated with high morbidity and symptoms of mechanical obstruction in the absence of occluding lesions. It has been reported as small number of case series. Therefore, it was difficult to fully understand its clinical characteristics and natural history. We aimed to investigate the clinical findings of CPO based on the nationwide multi-center database in Korea.
Methods: The CPO patients who were diagnosed in tertiary care centers in Korea were identified from 2000 to 2011. Their radiological, clinical, and surgical findings were reviewed and analyzed.

Results: A total of 95 patients (M:F = 46:49) were identified from 15 centers. Mean age at the first diagnosis was 46 ± 17 years-old, and their main symptoms were constipation (40.0%), abdominal pain (30.5%) and abdominal distension (23.2%). The previous history of chronic constipation was found to have in 56 out of 95 patients (65.9%). However, there was no significant difference in failure rates to medical treatment or operation rates between constipated and non-constipated groups. All of them showed the air-fluid level by simple X-ray or CT scan. Transition zone by radiologic exam was found in proximal to descending colon (56.6%). Seventy four patients out of 95 were refractory to medical treatment, and underwent surgical treatment at the mean follow-up of 7.4 months (0.5–61). Final surgical pathology was available in 36 out of 74 cases, and they showed neuropathy (88.9%) and myopathy (11.1%).
Conclusion: Considerable CPO patients might need surgical treatment rather than medical treatment. A prospective study based on large number of patients might be warranted to investigate its clinical characteristics and natural course precisely.

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Characterization of bowel habit in IBS patients using the Bristol Stool Form Scale

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Objective: The Rome III committee recommends subtyping of IBS based on report of stool pattern using the Bristol Stool Form (BSF) Scale. However, the majority of existing studies are hampered by using retrospective assessment of bowel habit and/or small number of included subjects. Our aim was to characterize bowel habit in IBS patients by using diary cards and to assess the agreement between IBS subgrouping based on information from diary cards over time.
Methods: We included 842 patients with IBS according to the Rome III criteria (621 females, 221 males; mean age 39 (18–79) years). The patients completed diary cards during 1 week, where stool frequency and consistency were recorded daily using the BSF. A proportion of the patients (n = 81) completed diary cards during 1 or 2 weeks at baseline and an additional diary card during 1 or 2 weeks 1–4 weeks later. Subgrouping of the IBS patients was done according to Rome III recommendations (cut-off: BSF: 25% hard/ lumpy stools and/or loose/watery stools).
Results: Based on the Rome III criteria, 224 patients were defined as IBS-constipation, 347 IBS-diarrhea, 52 IBS-mixed and 219 IBS-unsubtyped. The majority of stools had normal consistency (BSF 3-5), whereas hard/ lumpy stools (BSF 1-2) and loose/watery stools (BSF 6-7) were less commonly reported (Table 1). Increased stool frequency (>3 stools/day) at least one day was seen in 325 patients (39%). Of these, the majority (194 patients) only reported this one or two days during the week, and normal stool frequency the other days. The stool form on days with increased stool frequency varied greatly (hard/lumpy: 15%; normal: 40%; loose/watery: 44%). Only 12 patients had less than three bowel movements/week (1.4%). The agreement between subgroups over time was fair to moderate with 54% remaining in their original classification in the group with one week diary data (kappa = 0.33; $P < 0.0001$) and 61% in the group with two weeks diary data (kappa = 0.42; $P < 0.0001$).
Conclusion: A considerable proportion of IBS patients report normal bowel habits when using diary cards. Change of IBS subtype over time is commonly seen. Using two-weeks rather than one-week BSF diary cards seems to increase the subtype stability.

Hard	Lumpy	Normal	Normal	Normal	Mushy	Watery
BSF 1	BSF 2	BSF 3	BSF 4	BSF 5	BSF 6	BSF 7
9.4%	10.3%	14.5%	19.1%	17.8%	21.3%	7.6%

Table 1. Proportion of stools in the different consistency categories based on BSF.

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The clinical course of postinfectious Irritable Bowel Syndrome (IBS) after shigellosis: A 10-year follow-up study

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Objective: The incidence of post-infectious IBS (PI-IBS) was reported to be in the range of 7–31%, but few studies have reported long term follow-up results. The aim of this study is to investigate the long term clinical course, risk factors and prognosis of postinfectious IBS.
Methods: A Shigellosis outbreak had occurred in hospital employees at December 2001, due to contaminated food of the hospital employee-cafeteria in Gangnam Severance Hospital, Seoul, Korea. A Cohort of Shigella-exposed group was comprised of 124 hospital employees who had been treated for Shigellosis, and responded to initial follow-up symptom questionnaire. A control group was comprised of age, sex-matched, 105 hospital-employees at the same period, who had not been infected, nor exposed to causal food. Follow-up surveys for bowel symptoms of both groups were accomplished at 1, 3, 5, 8 and 10 years after the initial recruitment.
Results: The incidence of IBS in Shigella group was significantly higher than control group at 1 year (13.8% *vs* 1.1%, adjusted OR = 11.90, 95% CI: 1.49–95.58) and 3 years (14.9% *vs* 4.5%, adjusted OR = 3.93, 95% CI: 1.20–12.86) after infection. In 5-year follow-up survey, the prevalence of IBS was slightly higher in Shigella group than control group (20.8% *vs* 12.2%), but the difference was not significant (adjusted OR = 1.88, 95% CI: 0.64–5.54). At 8 years after infection, the difference in prevalence of IBS was not significant (adjusted OR = 2.57, 95% CI: 0.82–8.08). The 10-year follow-up survey was completed in 86 cases of Shigella group and 76 of control group, and showed no difference in the prevalence of IBS(23.3% in Shigella group and 19.7% in control group, adjusted OR = 1.61, 95% CI: 0.70–3.69). The analyzed risk factors for PI-IBS at various time points of surveys included the duration of diarrhea, younger age, and previous history of functional bowel disorder.
Conclusion: This long-term follow-up survey revealed that the relative risk of IBS symptom was significantly higher until 3 years after Shigellosis, but became not evident since 5 years after infection. The duration of diarrhea as an index of severity of initial illness and younger age were risk factors of PI-IBS.

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Upregulation of wide dynamic range afferent pathways in hypersensitive patients with Irritable Bowel Syndrome (IBS): A role for psychosocial factors?

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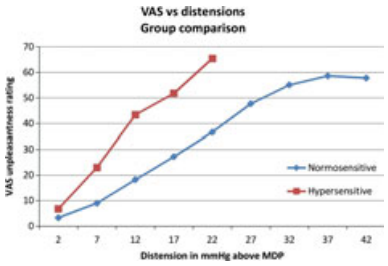
Objective: Rectal hypersensitivity is frequent in IBS. Isolated sensitization of nociceptive pathways in hypersensitive (HS) patients should only give rise to increased ratings of painful sensations during rectal distension; whereas sensitization of wide dynamic range (WDR) pathways should give rise to increased ratings of both painful and non-painful sensations. The aim was to test the hypothesis of sensitization of WDR pathways in HS IBS patients and to investigate whether this sensitization is influenced by psychological factors.
Methods: An ascending method of limits rectal distension protocol (5 mmHg steps) was performed in 138 IBS patients. At every distension step, unpleasantness was rated on a visual analogue scale (VAS). Anxiety, depression, somatization and gastrointestinal-specific anxiety (GSA) were assessed with questionnaires. Mixed models were used to analyze the influence of rectal sensitivity and psychological factors on the evolution of unpleasantness ratings upon increasing distension levels.
Results: Sixty IBS patients (43.5%) were HS based on pain thresholds. The evolution of unpleasantness ratings upon increasing distension levels in normo- and hypersensitive patients is shown in Figure 1. Significant main effects of distension level and sensitivity status were found (both $P < 0.0001$), indicating a linear increase of ratings with every distension level as well as a higher average unpleasantness rating in HS patients over all distension levels. Adding psychological covariates (in separate models) did not reveal a main effect of any of them; neither did this change the significance of the other main effects. However, significant sensitivity-by-distension level ($P < 0.0001$) and anxiety-by-distension level ($P = 0.006$) interaction effects were found, indicating that anxiety and hypersensitivity are independently associated with a more rapid increase in unpleasantness ratings upon increasing distension levels (i.e. a higher linear slope of the curve).
Conclusion: Wide dynamic range pathways rather than pain-selective pathways are sensitized in hypersensitive IBS patients. Rectal hypersensitivity and anxiety are both associated with a more rapid increase in

unpleasantness ratings upon increasing distension level, but the effect of hypersensitivity was found to be independent of psychological factors.



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Ileocolorectal inflammation and psychopathological status in patients with diarrhea-predominant functional bowel disorders

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Objective: To evaluate microscopic ileocolorectal abnormalities, either specific (i.e. microscopic colitis) or non-specific, and psychopathological variables in patients with symptom-based diagnoses of diarrhea-predominant functional bowel disorders (FBD).
Methods: Sixty-six consecutive patients (37F; age range 20–69 years) and 21 asymptomatic subjects (11F; age range 42–67 years), with normal serum biochemistry, CBC, thyroid function, anti-TG2 and ileocolonoscopy were evaluated. Patients matched the Rome III diagnoses of irritable bowel syndrome with diarrhea (IBS-D, $n = 35$), functional diarrhea (FD, $n = 10$), and unspecified FBD (UFBD, $n = 21$). Biopsies of the terminal ileum ($n = 2$), cecum ($n = 1$), ascending ($n = 1$), transverse ($n = 1$), descending ($n = 1$), sigmoid ($n = 1$) colon, and rectum ($n = 1$) were collected and stained with hematoxylin-eosin for microscopic assessment by a pathologist unaware of the clinical diagnoses. The SCL-90-R questionnaire was used to assess psychopathological variables by means of the global symptom index (GSI) and 9 subscales.
Results: Control subjects showed no significant histological abnormalities. Microscopic colitis (either lymphocytic or collagenous) was present in 7/66 (10.6%) patients, three of whom were IBS-D or UFBD. Non-specific microscopic inflammatory changes, such as altered villous architecture, cryptic microabscesses, crypt distortion, increased eosinophils and lymphoplasmacells, were found in further 10/66 (15.2%) patients, 2 of whom were FD. The prevalence of ileal, colonic and/or rectal microscopic abnormalities was significantly higher in FD than in IBS ($P < 0.01$). The psychopathological status, assessed by means of the GSI, was significantly better in FD than in IBS ($P < 0.01$) and UFBD ($P < 0.05$). In FD subjects, also SCL-90-R somatization, anxiety and psychoticism subscales were significantly lower. When comparing patients with and without microscopic inflammatory changes, the former had a lower, yet not significant, GSI and scored significantly lower on the somatization, anger/hostility and phobic anxiety subscales ($P < 0.05$).
Conclusion: A significant proportion of patients with diarrhea-predominant FBD have microscopic inflammation of the ileum, colon and/or rectum. FD patients are characterized by a less impaired psychopathological profile. Patients without microscopic inflammation show a higher severity of general psychopathology and of somatic distress, hostility and phobic fears. Multiple ileocolonic biopsies on endoscopically healthy mucosa may be useful in patients with any diarrhea-predominant FBD. Intestinal microscopic inflammation and psychopathology appear to be independently associated with diarrhea-predominant FBD.

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Regional grey matter volume in right Brodmann's Area 40 correlates with symptom severity and GI specific anxiety in patients with Irritable Bowel Syndrome (IBS)

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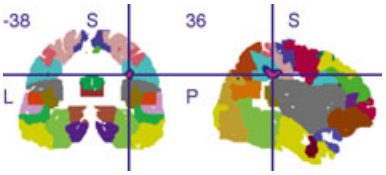
Objective: Previous research has suggested that visceral stimuli elicit an altered activation of the inferior parietal lobe (IPL) in IBS patients, compared to healthy controls (Kwan et al, Neurology, 2005). Right Brodmann's Area (BA) 40 in the IPL has been implicated in attention and emotion processing, i.e. functions relevant for IBS. GI-specific anxiety directs attention to visceral sensations, and has the potential to influence GI symptoms. In this study we explore possible correlations between IBS symptom severity, and GI specific anxiety, with local grey matter volume in the IPL.
Methods: Thirty subjects with IBS (19F/ 11M, mean age 32 ± 8.8 years) completed validated questionnaires on IBS symptom severity and GI specific anxiety. High resolution T1-weighted structural brain MR-images were obtained and segmented into grey matter (GM), white matter and cerebrospinal fluid. Subsequently, GM tissue segments were normalized to MNI space by applying high-dimensional transformations and smoothed. Correlations between local GM volume and IBS symptom severity / GI specific anxiety scores were assessed using a multiple regression model with age entered as a covariate. Since our hypothesis focused on right BA 40, we used this area for small volume correction of the results. That is, within right BA 40 results were thresholded at $P \leq 0.05$ (FWE corrected).
Results: Significant positive correlations between local GM volume and both IBS symptom severity scores ($P = 0.003$ small volume corrected, kE 258, MNI 30, -42, 43) and GI specific anxiety scores ($P = 0.037$ small volume corrected, kE 106, MNI 30, -40, 40) were observed. Interestingly, both clusters overlapped as shown in Figure 1. Neither symptom severity, nor GI specific anxiety scores showed any negative correlation within this region. We also noted a significant correlation between GM volume in left IPL and IBS symptom severity.
Conclusion: The finding that GM volume in the BA 40 correlated with both GI specific anxiety and symptom severity in IBS patients underlines the existing notion that attention focused on visceral sensations contributes to symptom generation in IBS.



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Altered white matter integrity in Irritable Bowel Syndrome (IBS)

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Objective: IBS is characterized by alterations in brain-gut interactions, yet few structural abnormalities in this axis have been reported. At the brain level, IBS patients have been shown to have functional and gray matter abnormalities in multiple brain regions, including those associated with interoception, attention, and emotion. We hypothesized that corresponding changes would be seen in the white matter structure.
Methods: Diffusion tensor images were acquired from 93 healthy controls (HCs) subjects (77% female) and 33 Rome III positive IBS subjects (64% female). Diffusion weighted images were acquired on a Siemens 3 Tesla scanner using either 61 or 64 directions. The FMRIB Diffusion Toolbox was used to perform motion and eddy current correction, then the diffusion tensor was constructed using nonlinear least squares regression. Fractional anisotropy (FA) and mean diffusivity (MD) images were derived and registered to an FA atlas using linear and nonlinear registration. AFNI was used to perform voxel-wise ANCOVA group comparisons with age, sex and BMI as covariates in white matter (Avg FA>0.6). Regions with minimum cluster size of 1 mL, level of significance $P < .05$, and false discovery rate $Q < .05$ were retained.
Results: Mean subject age was 30 and 33 years old respectively for HCs and IBS. Mean BMI was 24 in HCs and 25 in IBS. IBS subjects had a mean symptom severity in the past week of 11 on a 20 point visual analog scale and an average symptom duration of 13.9 years. IBS showed reduced FA in bilateral corticospinal tracts, thalamic radiation, internal capsule, and superior longitudinal fasciculus. IBS showed increased FA in the splenium of the corpus callosum, Increased MD was also observed bilaterally and diffusely throughout the internal capsule.
Conclusion: Based on findings in this large sample of subjects, IBS patients show widespread reduction of white matter integrity in tracts associated with connectivity of the thalamus, prefrontal cortex, and widely though the internal capsule and primary somatosensory pathways. The precise neuroanatomical changes underlying these findings remain to be determined, but suggest widespread remodeling of the brain in IBS.

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Cookie Test: An easy, reliable cheap alternative method for measuring of colonic transit time

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Objective: Measurement of colonic transit time (CTT) is one of the tests which are used for subgroup determination of functional constipation (FC). Capsules which contain 24 radyopaque markers will be given and abdominal X-ray should be taken after 120 h. Numbers of markers in the X-ray over 20% (>5) is considered in favor of long CTT. This study aims to develop a new method for measurement of CTT, which is easy to apply as well low cost and avoided from X-ray.

Methods: Thirty eight patients with a diagnosis of FC were included who referred to constipation-incontinence outpatient clinic between 2008–2010. Twenty four radioopaque markers containing capsuls were given to patients for measurement of CTT. Three pieces of green cookies which are approximately 15 g weigh, containing green food dye and prepared compatible with Turkish Food Codex were given in addition to capsuls. Patients were asked to note the date and time of eating green colored cookies as well as first green colored defecation. With this cookie test CTT is calculated as; CTT = (First green defecation time)–(time of eating the green cookies). According to abdominal X-ray which is taken after 120 h; number of markers ≤5 are evaluated as normal and >5 are considered as long CTT.

Results: Twenty eight out of 38 patient (74%) were women, mean age was 46 ± 16 years. CTT was determined as normal in 26 (68%) and long in 12 (32%) patients while it was 29 ± 13 and 85 ± 47 h with cookie test, respectively. The difference between two groups were statistically significant ($P < 0.0001$). %50 percentile median value is 31 h. With this data, if cut-off value is taken 31 h, the test sensitivity was calculated 100% and specifity was 77%.

Conclusion: Cookie test is more cost-effective, easier in terms of application, avoided from radiation exposure compared to capsule test. This test can be applied commonly because of aforementioned advantages. Due to the reliability of test dependent on the observation of patient and feedback, defect of eyesight and mental aberration can limit the usage.

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Gastrointestinal transit patterns from nuclear transit scintigraphy (NTS) of paediatric chronic constipation

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Objective: Constipation may be a part of a generalized gastrointestinal (GI) tract disorder. Radio-nuclear transit scintigraphy (NTS) identifies the site of slowing in the stomach, small bowel, colon and anorectum. The purpose of this study was to determine different colonic and rectal transit patterns in children with chronic constipation (CC) and to evaluate the association with upper GI tract disorders.

Methods: This was a retrospective analysis of NTS at the Royal Children's Hospital, Melbourne from 1999–2011 to investigate children with intractable chronic constipation. Children with palpable faecaloma were not sent for NTS. The 48-hour NTS protocol involved Gallium-67 citrate milk drink with acquisition of images (anterior and posterior) at 0–2 h for gastric emptying study and at 6, 24, 30 and 48 h for small bowel transit and colonic transit studies. The geometric centre calculation was based on % of radioactivity in each region of interest (ROI). Six regions of interest were employed (1 = pre-colonic, 2 = ascending colon, 3 = transverse colon, 4 = descending colon, 5 = rectosigmoid colon and 6 = toilet).

Results: A total of 955 NTS was performed from 1999 to 2011, with 288 repeat studies and 667 new studies. In the 603 children (284 female, 2–23 years, mean 8.5 ± 4.1 years) included for this study, 114 (19%) had normal colonic transit (NT), 314 (52%) slow colonic transit (ST) and 175 (29%) rapid proximal colonic transit (RT, Table 1). In 209 children with AR, about 1/3 had NT, ST and RT respectively. About 20% of children had delayed gastric emptying associated with all 3 patterns of colonic transit, 21% of children had delayed small bowel transit in both NT and ST, but only 3% of children with RT had delayed small bowel transit. Two percent of children with NT, 2% of RT and 5% of ST had delay in both gastric emptying and small bowel transit.

Conclusion: There are three distinct colonic transit patterns in children with CC: normal, slow & rapid. About 1/3 of the children with CC had AR at 48 h. AR was associated with NT, ST and RT. In addition, 21–24% children with CC had upper GI tract transit disorders.

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Symptomatic spectrums of chronic constipation: Multi-centered stratified clinical study in China

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Objective: Understanding the symptomatic spectrums of chronic constipation (CC) might improve the diagnostic path and should guide subsequent therapies. Previous studies have shown that constipated symptoms of CC varied among different ethical patients and different study centers. This study was designed as nationwide project to explore the symptomatic spectrums of CC in Chinese patients.

Methods: Five study centers were selected as representative of different regions in Mainland of China, each center consists of one tertiary, two secondary and two primary hospitals (one from urban, one from rural). The assignment of study task for tertiary, secondary and primary hospitals was decided according to the estimation for patients' visiting to each class of hospital in 50%, 30% and 20% respectively. CC was diagnosed with Rome III criterion. The questionnaire was fulfilled by well trained physicians or investigators in face to face interview manner. The constipated symptoms include the items listed in Rome III (Rome III symptoms) or not listed in Rome III diagnostic criterion (non-Rome III symptoms).

Results: A total of 921 patients with CC were surveyed, 909 of them were eligible and analyzed in final database. Male to female ratio was 1 : 2.52, mean age was 48.84–18.75 years. The constipated symptoms reported by patients were showed in Table 1. Straining is the most common symptom for CC patients with moderate to severe degree in 64.9% patients. Of 92.7% patients fulfilled at least three items among Rome III criterion, co-existing with non-Rome III constipated symptoms worsen the patients' feeling to troublesome defecation. There were regional differences in symptomatic spectrums. Patients seeking care to primary hospitals were more likely to use manual maneuvers for defecation assistance (78.9% vs 58.3% in tertiary hospitals, $P < 0.01$).

Conclusion: This multi-centered stratified clinical study presented the entire profiles of constipated symptoms for CC in Chinese patients. Mechanically using Rome III criterion for functional constipation in clinical practice might ignore patients' perception to CC, more comprehensive therapies are needed for CC patients, especially for whose seeking care in primary hospitals. (Supported by grants of 2007BAI04B01, 2010AA023007).

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Increased number of double positive CD3+ CD8+ CD4+ lamina propria T lymphocyte in gut mucosa of post infectious IBS patients compared to healthy controls

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Objective: Irritable bowel syndrome (IBS) developed after a gastroenteritis is denoted post infectious IBS

Table 1 – Colonic transit patterns in 603 children with chronic constipation and their association with delayed gastric emptying and delayed small bowel transit – number (%)

Proximal colonic transit	Gastric emptying		Small bowel transit		Distal colonic transit	Anorectal transit	
	Normal	Delayed	Normal	Delayed	Slow	Normal	Retention (AR)
Normal (NT)	91 (15)	23 (4)	82 (14)	32 (5)	-	62 (10)	52 (9)
Slow (ST)	251 (42)	63 (10)	221 (36)	93 (16)	-	237 (39)	77 (13)
Rapid (RT)	135 (22)	40 (7)	156 (26)	19 (3)	21 (4)	74 (12)	80 (13)
TOTAL	477 (79)	126 (21)	459 (76)	144 (24)	21 (4)	373 (61)	209 (35)

Table 1 Symptomatic Spectrums of Chronic Constipation in Chinese Patients

Rome Clinical Symptoms	%	Non-Rome Symptoms	%
Straining during def.	93.4	No awareness to def.	71.9
Lumpy or hard stools	84.2	Unproductive call for def.	53.6
Sensation of anorectal obstruction	33.6	Def. on setting time	19.0
Sensation of incomplete evacuation	48.7	Excessive time for def.(>10min)	75.5
Manual maneuvers to facilitate def. *	65.8	Less stool weight per day	48.0
Infrequent stools(<3 times/week)	76.6		

def. =defecation; *including use of enema

(PI-IBS) and is thought to represent a specific pathophysiological entity. Naive $CD8^+CD45RA^+$ cytotoxic T lymphocytes recognize antigen derived from intracellular bacteria and viruses. Naive $CD4^+$ $CD45RA^+$ helper T lymphocytes are activated by the antigen from extracellular microorganisms with the help of antigen-presenting cells. Activated $CD4^+CD45RO^+$ helper T lymphocytes help phagocytes to kill microbes and activate naive B lymphocytes.

Aim: To characterize subsets of mucosal lymphocytes in PI-IBS with flow cytometry analysis as a lead in identifying new therapeutic methods.

Methods: 5 PI-IBS patients and 6 healthy individuals were recruited. We performed a distal colonoscopy without bowel cleansing or other preparation. Lamina propria lymphocytes (LPL) and intra-epithelial lymphocytes (IEL) were isolated from sigmodal biopsies and sub classified by CD3, CD4, CD8, CD45RO and CD45RA with flow cytometry.

Results: We observed a significant deference ($P < 0.01$, two-tailed Mann–Whitney test) in double positive $CD3^+CD8^+CD4^+$ LPL between PI-IBS patient and healthy controls. We also observed a trend towards increased frequency of $CD3^+CD4^+$ LPL in PI-IBS patients. Additionally, PI-IBS patients showed an increased frequency of $CD3^+CD8^+$ LPL and IEL. The proportion of memory / activated $CD45RO^+CD4^+$ LPL was higher in PI-IBS patients, and its proportion of the $CD8^+$ population was lower compared with the healthy controls. On the contrary the proportion of naive $CD45RA^+CD4^+$ LPL was lower in PI-IBS patients compared with healthy controls. However, we found no differences in the distribution naïve / activated $CD4^+$ and $CD8^+$ IEL between PI-IBS patients and healthy controls.

Conclusion: The difference in double positive (DP) $CD3^+CD8^+CD4^+$ LPL seen between PI-IBS patient and healthy controls is consistent with findings of increased number DP T cells in target organs in immuno-inflammatory conditions. Our analysis revealed that the normal gut has a greater variation in the prevalence of $CD3^+$ $CD4^+$ and $CD4^+$ $CD45RO^+$ LPL than that of PI-IBS patients. These findings confirm aberrant mucosal subsets of lymphocytes. However, more subjects need to be included in the study in order to draw firm conclusions.

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Effects of linaclotide on abdominal and bowel symptoms over the first seven days of treatment in patients with Irritable Bowel Syndrome (IBS) with constipation

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Objective: Linaclotide (LIN) is a minimally absorbed, 14-amino-acid peptide which improved abdominal and bowel symptoms in two Phase 3 trials in IBS-C. The objective of the current analyses was to assess changes from baseline in abdominal symptoms in patients with IBS-C over the first 7 days of treatment with LIN compared to placebo (PBO).

Methods: Patients meeting Rome II criteria for IBS-C were randomized to oral once-daily 290-μg LIN or PBO in two Phase 3 trials. Using pooled data from the first 7 days of the Treatment Period, the mean daily percent change from baseline in abdominal pain and bloating for patient subpopulations with baseline score ≥3 for each parameter (rated on a 0-10 point scale) were determined. The percentage of patients experiencing at least one spontaneous bowel movement (SBM) and the percentage of patients experiencing at least complete SBM (CSBM) on each of first 7 days of treatment and the mean number of SBMs and CSBMs during the first week of treatment were calculated. Median times to first SBM and CSBM were calculated using Kaplan-Meier methodology.

Results: The pooled ITT population included 797 PBO- and 805 LIN-treated patients. LIN (vs. PBO) statistically significantly improved abdominal bloating on day 1 and abdominal pain by day 2 (Table). The percentages of patients reporting at least one SBM or CSBM were statistically significantly higher for LIN vs PBO on each of the initial 7 days of treatment (Table). The median time to the first SBM was 2 days for LIN and PBO patients; the median time to the first CSBM was 5 days for LIN patients and 20 days for PBO patients ($P < 0.0001$). LIN-treated patients reported an average of 6.6 SBMs and 2.4 CSBMs during week 1, vs. 3.5 and 0.9 for PBO-treated patients, respectively ($P < 0.0001$).

Conclusion: Compared to PBO, LIN was associated with statistically significantly greater improvement in abdominal pain and abdominal bloating by day 2; improvement in abdominal symptoms progressively increased over the first week. Stool frequency (SBM and CSBM) statistically significantly increased in LIN compared to PBO by day 1, and was maintained through the first week of treatment.

Table Improvement in Abdominal and Bowel Symptoms over Days 1-7.

Mean Percent Improvement in Abdominal Symptoms, Days 1-7

	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
Parameter	PBO	LIN	PBO	LIN	PBO	LIN	PBO	LIN	PBO	LIN	PBO	LIN	PBO	LIN
Pain (%) (N=1602) [a]	8.4	7.2	7.3	11.8*	10.4	17.2*	12.4	19.6*	13.3	19.9*	11.7	22.1*	11.5	24.0*
Bloating (%) (N=1571)[a]	4.4	7.6*	4.3	12.3*	7.3	15.2*	8.7	18.2*	9.8	18.0*	7.5	19.6*	8.5	18.9*
Percentage of Patients with ≥ 1 SBM or CSBM for Each of Days 1-7														
SBM (%) (N=1602)	23.7	48.6†	39.9	56.8†	41.5	54.3†	39.5	54.9†	39.4	54.7†	39.4	53.9†	39.4	50.4†
CSBM (%) (N=1602)	5.5	20.2†	8.9	23.1†	10.9	23.2†	10.3	23.5†	11.4	21.9†	11.2	22.2†	13.0	20.5†

Mean percent improvement for each of the first 7 days of treatment is presented.

* p<0.05 (LIN vs PBO, ANCOVA) favoring LIN; †p<0.0001 for LIN vs PBO; Cochran-Mantel-Haenszel test comparing LIN versus PBO in the % of patients having a SBM/CSBM for each day.

[a] Patients with baseline score ≥ 3.

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A pooled analysis of two phase 3 trials to determine the effects of linaclotide treatment on patient-reported ratings of change for symptoms of Irritable Bowel Syndrome with Constipation (IBS-C)

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Objective: To examine the effects of linaclotide, a minimally absorbed guanylate cyclase-C agonist (GCCA), on patient response to patient-ratings-of-change (PRC) questions and the extent of improvement in individual IBS-C symptoms within these patient-reported ratings-of-change categories.

Methods: In two Phase 3 IBS-C trials of linaclotide, patients reported daily ratings for abdominal symptoms (pain, bloating, fullness, cramping, discomfort) and bowel symptoms (spontaneous bowel movement [SBM] and complete SBM [CSBM] frequency, straining, stool consistency, unsuccessful BM attempts) via an interactive voice response system diary (pooled data). Patient-reported ratings of change included relief or improvement for each specific abdominal and bowel symptom, respectively, for the prior 7 days, at study visits during weeks 2, 4, 8, and 12 of the Treatment Period, compared to before the trial began, using a 7-point balanced ordinal scale (1 = completely improved/relieved, 4 = unchanged, 7 = as bad as I can imagine). Overall Degree of Relief of IBS symptoms also was assessed weekly using the same 7-point balanced scale. P values were obtained from the Cochran-Mantel-Haenszel (CMH) row-mean score tests comparing linaclotide to placebo.

Results: For all of the patient-reported ratings-of-change parameters, significantly more linaclotide vs placebo patients were completely/considerably relieved (Table); additionally, more placebo than linaclotide patients reported their symptoms as unchanged, somewhat worse or considerably worse/as bad as I can imag-

Table. Patient-Reported Ratings of Change (Week 12), Percentage of Patients in Each Category by Treatment Group and Mean Change for Each Category

	Completely/Considerably Relieved % (Mean symptom change)		Somewhat Relieved % (Mean symptom change)		Unchanged % (Mean symptom change)		Somewhat Worse % (Mean symptom change)		Considerably Worse/as Bad as I Can Imagine % (Mean symptom change)	
	PBO	LIN	PBO	LIN	PBO	LIN	PBO	LIN	PBO	LIN
Degree of Relief of IBS Symptoms (N = 1569)*	22.8	46.3	25.2	28.8	40.9	18.7	7.9	5.0	3.2	1.3
Abdominal Symptoms (N = 1579)										
Pain*	24.6 (–3.3)	48.2 (–3.7)	23.2 (–1.7)	25.4 (–1.6)	40.6 (–0.7)	17.6 (–0.9)	7.0 (–0.1)	6.0 (–0.5)	4.6 (0.3)	2.8 (–0.3)
Bloating*	19.3 (–3.7)	38.1 (–4.2)	23.7 (–1.7)	26.8 (–2.3)	41.1 (–0.6)	24.3 (–0.9)	10.2 (–0.5)	7.4 (–0.4)	5.7 (0.1)	3.4 (–0.7)
Fullness*	18.9 (–3.5)	40.3 (–4.2)	24.6 (–2.0)	26.2 (–2.3)	42.4 (–0.7)	24.4 (–0.8)	8.6 (–0.4)	6.2 (–0.9)	5.6 (0.1)	3.0 (–0.1)
Cramping*	23.7 (–3.3)	48.2 (–3.6)	22.2 (–1.6)	22.4 (–1.7)	43.0 (–0.7)	21.5 (–1.0)	7.7 (–0.2)	6.4 (–0.2)	3.4 (0.6)	1.5 (–0.1)
Discomfort*	22.6 (–3.6)	46.3 (–4.0)	25.1 (–1.7)	24.8 (–1.9)	38.8 (–0.7)	18.4 (–0.7)	8.7 (–0.3)	8.3 (–0.6)	4.9 (0.1)	2.3 (–0.3)
Bowel Symptoms (N = 1579)[a]										
CSBMs*	19.5 (2.4)	43.9 (3.9)	19.8 (1.0)	21.6 (1.2)	47.8 (0.1)	27.4 (0.6)	6.9 (0.0)	4.3 (1.7)	6.0 (–0.0)	2.8 (2.1)
SBMs*	23.9 (2.7)	52.7 (5.2)	21.9 (1.4)	20.3 (2.5)	39.8 (0.1)	19.6 (1.3)	7.9 (0.7)	4.4 (2.7)	6.5 (–0.3)	3.0 (2.1)
Straining*	21.3 (–1.4)	51.7 (–1.8)	21.2 (–0.9)	21.4 (–1.2)	43.9 (–0.5)	21.0 (–0.6)	7.4 (0.1)	3.1 (–0.9)	6.3 (0.0)	2.8 (–0.5)
Stool Consistency*	19.4 (1.1)	48.8 (2.4)	27.2 (0.7)	23.9 (1.7)	43.0 (0.5)	18.5 (1.0)	6.4 (0.1)	5.7 (2.0)	4.1 (0.1)	3.1 (3.0)
Unsuccessful	22.3	49.2	18.1	18.1	50.3	27.3	6.0	3.1	3.3	2.3
BM attempts*	(–5.6)	(–7.3)	(–6.1)	(–7.0)	(–4.2)	(–4.2)	(–4.7)	(–4.7)	(–5.6)	(–0.8)

ITT population.
PBO = Placebo; LIN = Linaclotide 290 µg.
[a]Bowel symptoms were rated as "improved" rather than "relieved."
*P<0.0001 Week 12 (LOCF), LIN vs PBO, CMH row-mean score test for percentage of patients in each category.

ine. Although patients on linaclotide, in general, reported greater improvement in symptoms across all categories of relief/improvement, patients who had greater relief/improvement ratings tended to report greater changes in symptom score for the corresponding symptom, regardless of treatment group assignment.

Conclusion: In clinical studies, linaclotide was approximately twice as likely as placebo to provide complete or considerable relief for overall and individual abdominal and bowel symptoms in IBS-C patients. Nearly half of IBS-C patients randomized to linaclotide reported complete/considerable relief of their IBS symptoms compared to less than a quarter of placebo patients. This analysis provides perspective on the magnitude of change in patients' individual symptoms that corresponds to reporting of complete/considerable relief of their individual and overall IBS-C symptoms.

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Management of pediatric patients with refractory constipation who fail cecostomy

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Objective: Antegrade continence enema (ACE) is a recognized therapeutic option in the management of children with severe constipation. Several techniques have been reported. We have performed laparoscopic-assisted percutaneous endoscopic cecostomies (LAPEC) for the past 10 years. Data on the long-term outcome of patients that fail to improve after an ACE procedure is lacking. The aim of this study is to describe the rate of LAPEC bowel management failure in pediatric patients with refractory constipation, and the management and long term outcome of these patients.

Methods: Study design-Retrospective analysis of medical records of a cohort of patients that underwent LAPEC and had at least 3-year follow-up. Detailed analysis of subsequent treatment and outcome of those patients with a poor functional outcome was performed.

Results: Seventy-six patients underwent LAPEC (study period 2003–2011). 12(16%) failed successful bowel management after LAPEC requiring additional intervention. Mean age was 14.7 ± 1.2 SEM (range 11–23 years). Mean follow-up was 66.3 months (range 35–95 months) after the ACE procedure. All patients had chronic refractory constipation. Additionally, 1 had non-retentive fecal incontinence, and 1 had surgically-corrected colonic atresia as a neonate. Colonic motility studies demonstrated absence of both, gastrocolic reflex and high amplitude propagating contractions (HAPCs) consistent with colonic neuropathy in 7 patients, partially propagated-HAPCs suggestive of abnormal motility in 4 patients, and abnormal left-sided colonic motility in 1 patient. All 12 patients were ultimately treated surgically. 6(50%) underwent laparoscopic total abdominal colectomy with ileorectal anastomosis. 2(17%) had laparoscopic colectomy with ileo-distal sigmoid anastomosis. 2(17%) underwent laparoscopic left-hemicolectomy with colorectal anastomosis (CRA). 1(8%) underwent laparoscopic subtotal colectomy with CRA, and 1(8%) had laparoscopic sigmoid resection with CRA for short-segment dysfunction. 9 patients (75%) had marked clinical improvement after surgery while 3(25%) have continued issues including fecal incontinence, gastroparesis and conversion to an end ileostomy.

Conclusion: In this small cohort of patients with bowel management failure after ACE-procedure, colonic resection led to improvement or resolution of symptoms in the majority of patients. However, this is a complex and heterogeneous group and some patients will have continued issues. Motility studies did not appear to predict long-term clinical outcome.

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Factor analyses of patient-reported outcome measures in Irritable Bowel Syndrome with Constipation (IBS-C): Results from two randomized, double-blind, placebo-controlled phase 3 trials of linaclotide

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Objective: Factor analyses of patient-reported outcome (PRO) data from two Phase 3 clinical trials of linaclotide for the treatment of irritable bowel syndrome with constipation (IBS-C) were conducted to support previous Phase 2b quantitative work that suggested symptoms elicited from patient interviews belong to one of two underlying domains - abdominal symptoms and bowel symptoms.

Methods: 1602 patients with IBS-C were analyzed in two Phase 3 multicenter, randomized, double-blind, placebo-controlled, trials of linaclotide (290 µg linaclotide or placebo) administered orally once daily for 12 and 26 weeks, respectively. Ten daily PRO measures addressing abdominal symptoms (pain, discomfort, bloating, fullness, cramping) and bowel symptoms (spontaneous bowel movement [SBM]/complete SBM [CSBM] frequency, unsuccessful bowel movement [UBM] frequency, stool consistency, straining) were assessed using interactive voice response system technology. Principal component analyses (PCA) and exploratory factor analyses (EFA) explored the correlational structure of the PRO symptoms in one random subsample of the dataset, followed by confirmatory factor analysis (CFA) in the second random subsample. Factor loadings and fit statistics were computed to evaluate the models.

Results: PCA of the 10 PRO measures suggested two emergent dimensions accounting for 71.8% of the total variance. The factor loadings and fit statistics from the EFAs indicated that the two-factor model fit the data relatively poorly. Because the standard errors and residual variances associated with UBM Frequency were larger than those associated with the other variables, and because factor loadings for UBM Frequency were relatively small, this variable was removed from the analysis and new EFAs conducted. With this revision, a two-factor EFA model provided the most interpretable solution. When the two-factor CFA model was applied to the correlation matrix without UBM Frequency, and with select correlated error variances, the fit of the model to the data was very good, and the factor loadings were satisfactory (Table). One factor comprised abdominal symptoms: pain, discomfort, bloating, cramping, and fullness; the other factor was bowel symptoms: CSBM frequency, SBM frequency, stool consistency, and straining.

Conclusion: The present factor analyses confirm earlier findings that the IBS-C symptoms could be grouped into two factors, or clusters: abdominal symptoms and bowel symptoms.

Table. Standardized Factor Loadings (Standard Errors) and Model Goodness of Fit Statistics – IBS-C PRO Measures

	Exploratory Factor Analysis		Confirmatory Factor Analysis	
IBS-C PRO Measures	**Factor 1**	**Factor 2**	**Factor 1**	**Factor 2**
CSBM Frequency[b]	0.79 (0.02)	−0.07 (0.02)	0.70 (0.03)	—
SBM Frequency[b]	0.88 (0.02)	0.09 (0.01)	0.56 (0.04)	—
Stool Consistency[c]	0.63 (0.03)	−0.03 (0.03)	0.52 (0.04)	—
Straining[d]	−0.47 (0.04)	0.34 (0.03)	−0.79 (0.03)	—
Abdominal Pain[a]	0.03 (0.02)	0.94 (0.01)	—	0.95 (0.01)
Abdominal Discomfort[a]	0.02 (0.01)	0.99 (0.01)	—	1.00 (0.00)
Abdominal Bloating[a]	−0.02 (0.02)	0.92 (0.01)	—	0.89 (0.01)
Abdominal Cramping[a]	−0.01 (0.02)	0.84 (0.02)	—	0.87 (0.01)
Abdominal Fullness[a]	−0.04 (0.02)	0.90 (0.01)	—	0.86 (0.01)
Goodness of Fit				
χ^2 (all $P < 0.05$)	1478.304 df = 19		118.60 df = 20	
Comparative Fit Index	0.82		0.99	
Tucker-Lewis Index	0.66		0.98	
RMSEA	0.31		0.08	
SRMR	0.04		0.04	

CSBM = complete SBM; df = degrees of freedom; IBS-C = irritable bowel syndrome with constipation; PRO = patient-reported outcome; RMSEA = root mean square error of approximation; SBM = spontaneous bowel movement; SRMR = standardized root mean square residual.

Note: The confirmatory factor analysis solution includes correlated residuals between the following: CSBM Frequency and SBM Frequency; Straining and Stool Consistency; Abdominal Pain and Bloating; Abdominal Pain and Cramping; Abdominal Pain and Abdominal Fullness; Bloating and Abdominal Fullness.

[a] 11-point numeric response scale: 0 = "none" to 10 = "very severe."

[b] These were derived from daily PRO items about bowel symptoms: number of BMs, use of rescue medication, and completeness of evacuation.

[c] 7-point Bristol Stool Form Scale (BSFS).

[d] 5-point ordinal scale: 1 = "not at all" to 5 = "an extreme amount."

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Home-based transcutaneous electrical stimulation (TES) improved symptoms and reduced laxative use in children with slow-transit constipation (STC)

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Objective: Children with slow-transit constipation (STC) are often resistant to medical treatments. Some children require appendicostomy for antegrade enemas to improve symptoms. Transcutaneous electrical stimulation (TES) is a novel therapy that has helped to improve symptoms and speed up colonic transit. We aimed to assess laxative use in STC children after home-based TES and their response to treatment. We hypothesized TES would improve symptoms and reduce laxative use.

Methods: This was a prospective study (2009–2011) of eligible STC children treated with TES after diagnosis by nuclear transit scintigraphy (Ethics30116A& 30059A). Home-based TES was administered 1-hour daily for 6 months after training by a clinician. Daily continence diary and laxative use were recorded before and throughout treatment. Appendicostomy for antegrade enemas was offered if TES failed to improve symptoms at the end of 6 months. Statistical analyses performed with STATA 12: paired t-test & chi-square, with $P < 0.05$ considered significant.

Results: Sixty-two STC children (34 female; ages: 2–16 years, mean: 7 years) self-administered home-based TES successfully. Symptoms improved significantly in 56 (90%) STC children: soiling reduced (from mean ± SD, pre-4.6 ± 2.4 days soiling/wk vs post-0.7 ± 1.1 days soiling/wk, $P < 0.001$, *t*-test), defecation frequency increased (pre-1.6 ± 1.6 bowel actions/wk vs post-3.5 ± 1.9 bowel actions/wk) and abdominal pain reduced (pre-1.7 ± 1.9 days abdominal pain/wk vs post-0.2 ± 0.5 days abdominal pain/wk). Laxative use was significantly reduced with 15 (24%) children stopped laxative, 30 (48%) reduced laxative ($P < 0.001$, Pearson chi-square), 15 (24%) remained on same laxative dose and 2 children who stopped laxative prior to TES had symptom improvement without further laxative use. Six children (10%) required appendicostomy for antegrade enemas.

Conclusion: Home-based TES is a promising treatment for children with treatment-resistant STC. TES treatment improved symptoms and stopped laxative use in ¼ and reduced laxative use in half of these children. Long-term follow-up is required to determine the duration of symptom improvement and whether further intervention is necessary.

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Laparoscopically assisted subtotal colectomy for slow transit constipation

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Objective: Laparoscopically assisted surgery has gained wide clinical acceptance as a practical alternative for the surgical treatment of colorectal diseases. However, its role in the operation for slow transit constipation has yet to be established. The aim of this study was to evaluate the feasibility, safety, and potential benefit of laparoscopically assisted subtotal colectomy (LASC) for slow transit constipation.

Methods: Between 2007 and 2011, the 43 consecutive patients were diagnosed as slow transit constipation according to the Roman III criteria. There were 6 males and 37 females aging from 20 to 66 years old. Sitz marker study showed prolonged colon transit time. The patients were divided into two groups (OpenSC and LASC), and their background and postoperative data were compared between two groups.

Results: Nineteen OpenSC and 24 LASC cases were included. The two groups were comparable in terms of age, gender, body mass index, and indications. The median operative time was not significantly longer for LAP (230 min; range, 174–310 min) than for surgery (220 min; range, 162–315 min). The blood loss was significantly less with LASC (180 ml; range, 125–270 ml) than with open surgery (338 ml; range, 260–670 ml). Shorter duration of postoperative hospital stay was recorded in patients with LASC (6.5 day; 5–7 day) than that in the OpenSC group (7.8 day; 7–9 day). No difference was seen in postoperative complications, and no mortality occurred in the series. During the follow-up ranging from 3 to 46 months, defacatory function was excellent in 18, good in 19, and moderate in 6 patients.

Conclusion: The findings showed that LASC for slow transit constipation are feasible and safe. The LASC procedure currently seems potentially beneficial for patients by its less invasiveness.

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A pooled analysis of two phase 3 trials assessing the percentage of days linaclotide improved abdominal symptoms and stool frequency in patients with IBS-C

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Objective: Linaclotide, a minimally absorbed guanylate cyclase-C agonist (GCCA), has been shown to improve abdominal and bowel symptoms in patients with IBS-C in two large Phase 3 trials. The objective of the current analyses was to determine the percentage of days patients reported improvements in abdominal symptoms and bowel movements during treatment with linaclotide or placebo.

Methods: In two Phase 3 trials, patients meeting Rome II criteria for IBS-C were randomized to oral once-daily 290-μg linaclotide or placebo. Using post-hoc analyses of pooled intent-to-treat (ITT) data from the first 12 weeks of these trials, the following were determined for the subpopulations of patients with average baseline score ≥3 on an 11-point numerical rating scale for each respective parameter: percentage of days patients had ≥30% improvement in abdominal pain, discomfort, bloating, cramping, and fullness; and percentage of days patients had a spontaneous bowel movement (SBM) or a complete SBM (CSBM).

Results: This pooled ITT population included 797 placebo- and 805 linaclotide-treated patients. The mean percentages of days that patients experienced an SBM and CSBM during the 2-week Baseline Period were 24% and 3%, respectively; baseline abdominal symptom scores were 5.6 (pain), 6.1 (discomfort), 6.6 (bloating), 5.3 (cramping), and 6.6 (fullness). The percentage of days patients experienced ≥30% improvement in abdominal symptoms was statistically significantly greater for linaclotide vs placebo for each abdominal

Table. Percentage of Days Patients Experienced ≥ 30% Improvement in Abdominal Symptoms and Percentage of Days with an SBM or CSBM

Parameter (N)	Placebo	Linaclotide	Difference	P-value
% of Days with ≥ 30% Improvement in Abdominal Symptoms				
Abdominal Pain[a] (N=1602)	41	55	13	< 0.0001
Abdominal Discomfort[b] (N=1589)	38	52	14	< 0.0001
Abdominal Bloating[b] (N=1574)	33	48	15	< 0.0001
Abdominal Cramping[b] (N=1457)	42	55	13	< 0.0001
Abdominal Fullness[b] (N=1583)	33	49	15	< 0.0001
% of Days with an SBM or CSBM				
SBM (N=1602)	37	54	17	< 0.0001
CSBM (N=1602)	12	26	14	< 0.0001

SBM = BM in the absence of laxatives, suppositories, or enemas, CSBM = SBM accompanied by sensation of complete evacuation. P-values for linaclotide vs placebo were determined using a Wilcoxon Rank Sum test.
[a] Patients randomized were required to have a mean abdominal pain score ≥ 3 during the Baseline Period.
[b] Patients with average baseline score ≥ 3 for that particular symptom.

symptom (Table 1). Linaclotide-treated patients met the ≥30% improvement threshold for abdominal pain for 55% of days compared with 41% for placebo ($P < 0.0001$). Similarly, for the other measured abdominal symptoms, patients met the ≥30% improvement criterion for approximately 50% of days during the Treatment Period (placebo-treated patients met the threshold between 33% and 42% of days). The percentage of days patients experienced an SBM or CSBM was statistically significantly greater with linaclotide vs placebo (Table).
Conclusion: Linaclotide treatment resulted in at least 30% improvement in abdominal symptoms, including pain, discomfort, bloating, fullness, and cramping, for approximately 50% of treatment days. Likewise, linaclotide significantly increased the percentage of days with an SBM or CSBM. Thus, linaclotide relieved key symptoms of IBS-C and increased the percentage of days with symptom improvement.

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Multidisciplinary management of chronic pediatric abdominal pain: Initial single center experience
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Objective: Chronic abdominal pain (CAP) a common diagnosis for which patients are referred to a pediatric gastroenterologist. Most patients continue to seek other medical tests and procedures looking for an organic pathology, often undergoing multiple surgical procedures without relief. The aim of our clinic was to evaluate patients with CAP, look for possible organic causes, if none found, help them understand and accept their diagnosis; and guide treatment. The aim of this study is to compare patients with CAP before and after being seen and treated in the multi-disciplinary clinic.
Methods: Retrospective chart reviews were performed for patients seen in the CAP clinic from 2010 and 2011. All patients were evaluated by atleast one prior pediatric gastroenterologist. All had failed previous medical therapy. All patients were evaluated by a pediatric pain certified, a pediatric psychologist and a pediatric gastroenterologist. If needed school services, physical therapist and social work was also involved. The visit lasted over 3 h, at the end of the visit, a conference was held between the team and the families to discuss the diagnosis and plan. Data on pain, school and social functioning was collected and compared to scores at the first follow up visit.
Results: Thirty-nine patients were initially seen. Average initial pain score was 2.9/5. 10 patients were initially home schooled; while patients were nonfunctional in their social life. Twenty patients were seen at a follow up visit. Average follow up time was 2.8 months. Average pain score decreased to 0.9/5; only 2 patients were now in home bound school; 2 children were nonfunctional. Other diagnoses found included ulcerative colitis, Cystic fibrosis, scar neuroma, constipation, eosinophilic esophagitis and depression.
Conclusion: Going through the mutli disciplinary clinic for CAP resulted in decreased pain scores and better school and social functioning. We hypothesize that spending enough time with this group of patients in their initial visit; mutli disciplinary assessment and management and explaining the diagnosis leads to improved outcomes.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-16 Clinical Session: Anorectal: Clinical

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External Anal Sphincter (EAS) muscle morphology reassessed using Ultrasound (US), Magnetic Resonance Imaging (MRI) and Diffusion Tensor Imaging (DTI)
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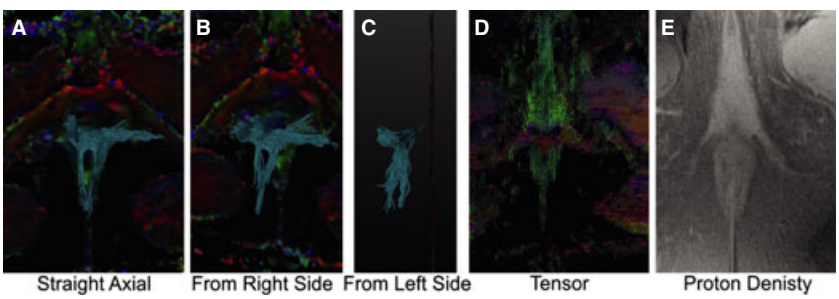
Objective: Morphology of EAS has intrigued many investigators; current thinking is that it is a ''dough-nut'' shaped structure. We hypothesize that instead of ''dough-nut'' configuration, EAS has a ''purse-string'' morphology which is formed by anococcygeal raphae, EAS, perineal body and transverse perenei muscles.
Methods: Fourteen nullipara women were imaged with 3D transperinal US imaging that provided axial sections every 1 mm along the length of anal canal. Proton density MR images, 2.5 mm thick, perpendicular to the subject and perpendicular to the anal canal yielded high resolution morphology. MR imaging was combined with high resolutions DTI to determine anatomical structure of the entire EAS region in 3 dimensions. DTI is a relatively novel technique; it enables mapping of the anisotropic diffusion of water molecules in fibers, and therefore allows tracking of muscle fibers *in vivo*. Three Tesla MR scanner (GE Medical Systems) was used to determine fractional anisotropy of water molecules and the diffusion tensor at each voxel to track the fibers in different muscles of the EAS complex.
Results: The high resolution US and 3D volume rendering of the high resolution MR images revealed that the EAS muscles, as one followed them from the right and left lateral sides merge into the perineal body and transverse perinea muscles so that the cranio-caudal extent of these muscles are identical. DTI revealed that Fractional Anisotropy, a intra-voxel measure of the symmetric nature of the diffusion of motion of water molecules (value of 0 for sphere and 1 for infinitesimal capillary) revealed a higher value (0.36 + 0.04) in the lateral and posterior aspects compared to that in the perennial body thus raising the possibly of fiber crossing in the perineal body and continuing as transverse perinea muscle to be attached to the pubic ramus.



Conclusion: Multiple imaging techniques strongly suggest that EAS is configured as a ''purse-string'' rather than a ''dough-nut''. Our finding has significant relevance to the effects of lateral episiotomy on the EAS function and surgical reconstruction of the EAS to treat anal incontinence.

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Distensibility of the anal canal in patients with systemic sclerosis: A study with the functional lumen imaging probe

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Objective: Systemic sclerosis (SSc) is a generalized connective tissue disease affecting smooth muscle cells. Patients with SSc often have fecal incontinence caused by fibrosis of the internal anal sphincter (IAS). The Functional Lumen Imaging Probe (FLIP) is a novel method allowing dynamic evaluation of segmental biomechanical properties within the anal canal. The aim of the present study was to compare segmental biomechanical properties of the anal canal in incontinent SSc patients and healthy controls. We hypothesized that FLIP could demonstrate weaknesses of the IAS in SSc.
Methods: We performed FLIP distensions, endoanal ultrasonography (ULS) and standard anal manometry on 14 incontinent SSc patients (11 women, median age 60 [range 35 to 80]) and 15 healthy volunteers (12 women, median age 54 [range 33 to 67]). For biomechanical analysis the anal canal was divided into three parts: upper (surrounded by the IAS and the puborectalis), middle (surrounded by the IAS and external anal sphincter) and lower (surrounded by the external sphincter only).
Results: In all subjects the middle anal canal was the segment most resistant to distension, but in SSc patients it was less resistant than in controls ($P < 0.01$). Correspondingly, ULS showed that the IAS of SSc patients was thinner than normal ($P < 0.05$) and anal resting and squeeze pressures were lower ($P < 0.05$). Only minor differences in distensibility were found in the upper anal canal. No changes were found in the lower anal canal.
Conclusion: Fecal incontinence in SSc patients is associated with poor internal anal sphincter function causing increased distensibility of the middle anal canal.

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Evaluation of anorectal function in healthy adults with 3-D high definition manometry (HDAR-3D)

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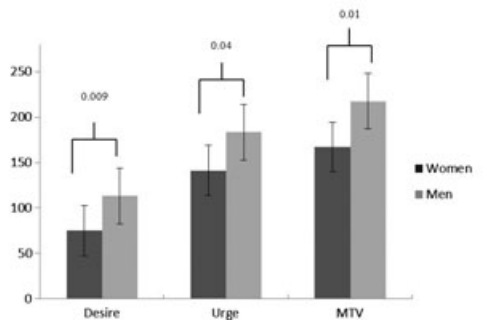
Objective: Recently, high resolution and 3-D topographic studies have provided a new dimension to our understanding of anorectal function and may reveal new pathophysiology. However, normative data is essential to understand abnormal pathophysiological and avoid discrepancies in data interpretation. Our aim was to perform a comprehensive evaluation of anorectal function in healthy adults using High Definition manometry.
Methods: We placed a 10-mm diameter probe containing 256 circumferentially arrayed sensors and a standardized rectal balloon (Given Imaging, Yoqneam, Israel) in 28 healthy subjects controlled for age and gender. Probe was held in place by an operator throughout the study. Subsequently, subjects were asked to squeeze, bear down and blow into party balloon twice each, followed by assessments of rectal sensation and rectoanal reflexes assessed simultaneously using intermittent phasic balloon distentions. 3 patients had repeat testing two weeks apart. Standard manometric and sensory data were measured and compared between genders, and correlations performed for reproducibility.

	All (n=28)	Female (n=16)	Male(n=12)
Length of anal sphincter (mean, cms)	4.2 (3.9-4.4)	4.1 (3.8-4.5)	4.2 (3.9-4.5)
Max Anal Rest Pressure (mean, mmHg)	74.9 (67.5-82.2)	72 (61-83)	79 (67-90)
Max squeeze pressure (mean, mmHg)	219 (191-247)	199 (164-233)	247 (200-294)
Sustained Squeeze (mean, mmHg)	124 (105-194)	104 (82-127)	151 (120-182)*
Squeeze duration (mean, s)	29 (27-31)	28 (26-31)	30 (27-33)
Rectal Rest Pressure (mean, mmHg)	11 (6-14)	10 (6-13)	15 (13-16)
Rectal squeeze Pressure (mean, mmHg)	16 (11-22)	14 (7-21)	19 (7-31)
%Increase in anal sphincter during squeeze	63 (58-68)	61 (53-69)	66 (58-73)
Rectal pressure during party balloon inflation (mmHg)	52 (43-51)	46 (37-54)	61 (42-80)
Anal pressure during party balloon inflation (mean, mmHg)	139 (123-155)	132 (107-156)	149 (126-172)
Rectal pressure Bear Down (mean, mmHg)	49 (38-59)	45 (34-55)	56 (30-81)
Anal Residual pressure during Bear Down (mean, mmHg)	57 (45-70)	52 (39-55)	65 (38-92)
Defecation Index	1.1 (0.7-1.5)	1.1 (0.5-1.7)	1.1 (0.3-1.9)
Defecation Index 60 cc	1.9 (1.5-2.3)	2 (1.3-2.7)	1.7 (1.2-2.2)

* p<0.05



Results: In men, sustained squeeze pressure was higher ($P < 0.05$). All other parameters were similar to women. Distinct thresholds for first sensation, desire to defecate and urgency were identified with significantly higher values in men ($P < 0.05$) compared to women. Dyssynergic pattern was present in 12/18 (67%) (F7:M5) subjects during attempted defecation and in 6/18 (33%) subjects (F4:M2) during attempted defecation with 60 cc inflated rectal balloon. Test-retest evaluations showed good correlation (r^2 0.9). The study was well tolerated and without adverse events.
Conclusion: This is the first comprehensive, gender controlled assessment of anorectal function using high definition manometry. Gender influences some parameters of anorectal function. The high prevalence of dyssynergic pattern may be due to artifact that is either probe-related or position-related and hence is less ideal for assessing dyssynergic defecation. Test-retest shows excellent reproducibility. Our results could serve as a valuable resource of normative data using HDAR-3D.

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Do IBS patients without rectal hypersensitivity adapt to repeated aversive rectal distensions?

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Objective: We have previously shown that the functional MRI blood oxygen dependent (BOLD) response to rectal distensions differs between IBS patients with/without rectal hypersensitivity. Specifically, hypersensitive subjects have more activation in brain regions involved in processing of afferent input from the gut. To further investigate whether these differences in central response were influenced by adaptation or sensitization to repeated stimuli over the course of the experiment, we evaluated the response to stimuli given early versus late. We hypothesized that normosensitive subjects would exhibit a decrease in BOLD response regions associated with interoception and emotional arousal in the later inflations, while hypersensitive subjects would not.
Methods: BOLD response from 15 hypersensitive and 18 normosensitive female IBS patients with similar disease duration, IBS symptom severity score, anxiety and depression symptoms, was acquired during 20 cued aversive distensions (45 mmHg) and the time series was divided into two equal parts (early and late phase). SPM8 was used to perform paired *t*-tests for comparison of early and late phase with correction for multiple comparisons (significance $P = 0.05$). Regions of interest included regions associated with emotional arousal and regions associated with processing of visceral sensations.
Results: Normosensitive subjects showed greater activation of right anterior insula ($P = 0.004$), left posterior insula ($P = 0.04$), right dorsolateral prefrontal cortex ($P = 0.03$), and right ventrolateral prefrontal cortex ($P = 0.02$) in early compared to late phase. Hypersensitive subjects activated the left pregenual anterior cingulate cortex ($P = 0.03$) more during late than during early phase.
Conclusion: Normosensitive subjects engage areas associated with processing of the emotional content as

well as the primary interoceptive cortex more during the early phase, suggesting adaptation to aversive visceral stimulations during the later phase. This adaptation was not seen in hypersensitive subjects and may be associated with an inability to downregulate the perception of a repeated visceral stimulus.

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Assessment of obstructive defecation by high resolution anorectal manometry compared to magnetic resonance defecography

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Objective: Patients with obstructive defecation may have abnormalities of anorectal function or structure. Standard investigation by manometry shows only fair agreement with patient symptoms and defecography. Agreement between investigations has been recommended for definitive diagnosis. Anorectal high resolution manometry (HRM) may improve diagnostic accuracy by differentiating pressure effects caused by contraction vs. straining and by avoiding artifacts caused by movement of the catheter relative to the sphincter during straining. This study compared HRM findings with magnetic resonance (MR) defecography in the clinical assessment of patients presenting with symptoms of obstructive defecation defined by Rome III criteria as straining at stool with the sensation of incomplete evacuation, blockage sensation or digital facilitation.
Methods: HRM was performed by a solid state catheter with 10 circumferential sensors at 6 mm separation across the anal canal and 2 placed 5 cm proximal in the rectum (Manoscan AR 360, SSI, USA). Resting tone squeeze pressure and dynamic pressure activity during bearing down were analyzed. Findings were referred to MR defecography (1.5T, Philips, NL) performed after insertion of 250 ml water based gel labeled with Gd-DOTA paramagnetic contrast as reference standard for pelvic floor anatomy and function.
Results: One hundred and eighty eight consecutive patients (155 female;age 19–93) referred with symptoms of obstructive defecation had full investigation. MRI diagnosis revealed anorectal dyssynergia with paradoxical contraction in 59 and structural pathology in 124 patients (rectocele (n = 68) and rectocele with intususception (n = 33), pelvic floor descent with enterocele (n = 28) or prolapse (n = 7), pelvic floor descent (n = 87) most patients had multiple pathologies. Five patients were excluded because of inconclusive MRI findings. Compared to patients with dyssynergia, those with structural pathology had lower resting (65 vs. 86 mmHg; P < 0.003); and squeeze pressure (150 vs. 181 mmHg; P < 0.011). On simulated defecation a negative rectoanal pressure gradient was more evident in dyssynergia than structural pathology (P < 0.0001). In patients with dyssynergia on MRI, HRM showed paradoxical contraction or failure to increase abdominal pressure without anal relaxation (sensitivity 100% (59/59); specificity 94% (117/124)). One patient with normal findings on MRI but paradoxical contraction on HRM had an anal fissure on examination under anaesthesia. A pattern of high intra-rectal pressure with a steep, positive pressure gradient indicating outlet obstruction in the anal canal. was observed on HRM in n = 26 patients with rectocele with intussusception on MRI.
Conclusion: Diagnostic agreement between anorectal HRM and MR defecography is high and pressure measurements accurately identify recto-anal dyssynergia. Additionally, a steep intra-anal pressure gradient is indicative of intra-anal outlet obstruction by structural pathology as a cause of obstructive defecation.

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Quality of perception of the desire to defaecate and viscerosomatic referral patterns differ between patients with chronic constipation and healthy subjects

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Objective: The ability to sense stool in the anorectum is essential to normal defaecation. Constipation is known to be associated with impaired rectal sensation (predominantly hyposensitivity), and some available evidence indicates that patients with constipation report either altered, or indeed an absent call to stool. However, whether an alteration in viscerosomatic referral area or quality of sensation exists has not been robustly tested. This study aims to objectively assess pre-defaecatory sensations in patients with chronic constipation (CC) in comparison to healthy, asymptomatic volunteers (HV).
Methods: Twenty-two patients presenting for investigation of CC and 20 HV were sent a standardized questionnaire, completed over a three day period, which assessed the quality and perception of the desire to defaecate and associated areas of viscerosomatic referral. The questionnaire included a stylized drawing where the area of sensation experienced immediately prior to defaecation was shaded, a list of descriptive terms/ word triggers for the subject to select, and space for free text to ensure all potential sensory descriptors were captured.
Results: All subjects completed the questionnaire. Three patients (14%) did not report any urge sensation to defaecate, and evacuated 'out of routine', usually with the assistance of laxatives/enemas. By contrast, all HV evacuated following the urge to defaecate. Ninety-five percent of healthy individuals and 50% of CC (P = 0.002) located the urge to defaecate as either a perianal or suprapubic sensation, most frequently described as pressure/fullness (73% vs. 80% respectively). Patients were also more likely to identify greater than one location in which sensations arose (i.e. right iliac fossa, periumbilical, left iliac fossa, left flank, epigastrium etc.) than HV (82% vs. 55%; P = 0.09). Patients also used multiple, more varied descriptive terms (e.g. heaviness, butterflies, cramping, sickness/nausea, aching, bloating) than healthy individuals (86% vs.50%; P = 0.01).
Conclusion: This study confirms altered perception of the call to stool in constipated patients, indicating that aberrant afferent signaling or attenuated sensory processing may contribute to the development of their symptoms. A larger study is currently underway, examining subgroups of constipated patients stratified by rectal sensory status, which may shed light on the clinical significance of this finding.

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Prevalence and risk factors of fecal incontinence in male gulf-war veterans

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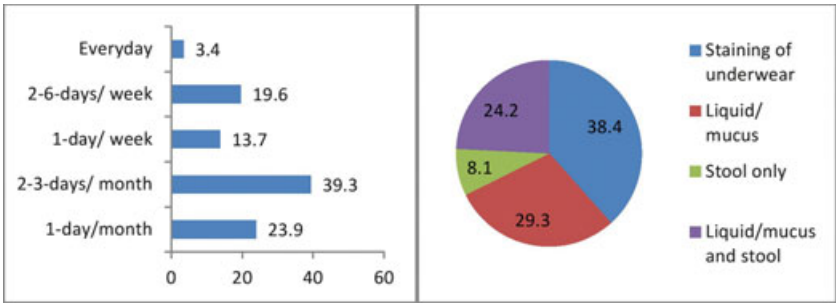
Objective: There is very limited knowledge about the prevalence and risk factors for fecal incontinence (FI) in men. The aim of this study was to determine the prevalence and risk factors for FI in male Persian-Gulf (PG) War Veterans.
Methods: Veterans (1990–1991) registered in the PG-War registry at two Medical Centers were mailed the Talley Bowel-Disease-Questionnaire inquiring about their bowel habits. FI was defined as uncontrolled passage of fecal material at least once a month in the last three months. Diarrhea, constipation and irritable bowel syndrome (IBS) and its subtypes were defined using Rome-III criteria. Veterans were asked about their stool consistency using the 7 descriptions from the Bristol Stool-Scale. Stool consistency was categorized as Bristol stool ratings 1–2 (hard/ lumpy), 3–5 (normal), or 6–7 (loose/watery).
Results: Data were analyzed from 334 male (mean age 48 years) Veterans enrolled in the PG registry. IBS was reported by 34.4%, constipation 32.8%, and diarrhea by 36.9% of Veterans. Normal-stool consistency was reported by 51%, hard/ lumpy stool 10% and loose-stools by 39% of Veterans. FI was reported by 35.0% (n = 117). The most common composition of leakage was staining of underwear (38.4%) (Fig). FI was reported by 27.3% of Veterans with hard-stool, 28.9% with normal and 45.3% with loose-stool whereas only 7.8% of Veterans with FI reported hard stool. FI was not associated with age (P = 0.18). Underlying presence of loose/ watery stool (OR 4.49, 95% CI 2.76–7.29, P = 0.01), IBS-



D (OR = 5.68, CI 2.93–11.02, P = 0.01), and feeling of incomplete evacuation (OR = 2.46, 95% CI 1.54–3.93, P = 0.01) were independently associated with FI.
Conclusion: FI is common among male PG-Veterans. Most of the Veterans report staining of underwear. Presence of liquid stool and IBS are significantly associated with FI. The high prevalence of FI in PG-Veterans may be because of high co-morbidity of diarrhea and IBS in this population. PG-Veterans who report diarrhea and IBS should be questioned about FI to potentially better manage this disorder.

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Prospective comparison of balloon expulsion, anorectal manometry and evacuation proctography for the diagnosis of evacuatory dysfunction

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Objective: Evacuatory dysfunction (ED) is prevalent, and may be subclassified on the basis of specialist tests. The discriminatory abilities of contemporary tests were assessed by anorectal manometry (ARM); balloon expulsion, using water-filled balloons distended to 50ml (BE50) and to individualised urge volume (BEurge); and evacuation proctography (EP).
Methods: Prospective study of 76 consecutive adults undergoing assessment for ED (Rome III – functional defecation disorder). Diagnostic yields and levels of agreement were calculated.
Results: Diagnostic yields for ED were: ARM 70%, EP 37%, BE50 35%, BEurge 21% and ARM ± BE50 23%. For the diagnosis of ED, agreement was good between balloon tests (86%; Kappa = 0.65), fair between EP and BE50 (67%; Kappa = 0.29), but poor and discordant between ARM and EP (46%; Kappa = 0.03) and between ARM and BE50 (42%; Kappa = -0.04) respectively. There was very poor agreement between ARM and other tests for diagnosing subtypes of ED (e.g. for pelvic floor dyssynergia between ARM and EP, agreement 70%, Kappa = 0.01). Of 28 patients (37%) with abnormal EP, 17 had significant anatomical abnormalities (none had coexistent functional abnormalities). A further 31 patients had structural abnormality in EP but the size did not reach significance.
Conclusion: ARM has the highest diagnostic yield for ED, but poor levels of agreement with other tests. EP is the only test that can diagnose all subtypes of ED. Clinical utility of tests, and diagnostic criteria should be reappraised.

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The spinal anal-external sphincter continence reflex

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Objective: To evaluate the mechanism controlling faecal continence.
Methods: Anal electro sensitivity, anorectal pressures and rectal pressure-volumetry were measured in 17 control subjects before and after superficial local anal anaesthesia, one subject before and after injected local anaesthesia and six control subjects before and after spinal anaesthesia. The same investigations were done with three patients with spinal cord lesions at the level of Th3-L3.
Results: After superficial local anaesthesia, anal electro sensitivity decreased but basal anal pressure did not change. Squeeze pressure decreased and rectal filling sensation levels remained present. Superficial local anal anaesthesia reduced anal pressure recorded in the distal anal canal during progressive rectal filling. The same happened, in a more explicit way, after injected local anaesthesia. After spinal anaesthesia, the anal canal became insensitive to electric stimulation, but basal and squeeze pressure values strongly decreased and anal pressure increase during balloon retaining test disappeared completely. With spinal cord lesions the external sphincter could not squeeze on command, but during the balloon retaining test the anal sphincter did autonomously squeeze with more than 300 mmHg.
Conclusion: These results support the hypothesis that faecal continence by contraction of the external sphincter is depending on an spinal anal-external sphincter continence reflex without influence of the brain. The afferent receptors of this reflex are located in the distal anal canal and are contact receptors who are superficially subepithelial located. A not functioning continence reflex results in faecal incontinence.

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Obesity does not impact the prevalence of dyssynergic defecation in adults with chronic constipation

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Objective: Dyssynergic defecation (DD) is characterized by the inability to coordinate the series of events necessary to achieve evacuation of stool from the rectum. On anorectal manometry (ARM), DD is identified by paradoxical contraction or inadequate relaxation of the anal sphincter during simulated defecation (Sim Def). By balloon expulsion testing (BET), DD is defined as the inability to pass a water filled balloon within a prescribed period of time. No studies have evaluated the impact of obesity on the prevalence of DD in adults with chronic constipation (CC). Therefore, the aim of this study is to assess the prevalence of DD in obese adults compared to normal weight adults through an analysis of ARM and BET results from patients with CC.
Methods: We reviewed results from ARMs and BETs performed in all adult patients (18 years of age and older) with CC referred to a single tertiary care center from 2002 to 2011. Data collected included age, gender; height, weight, Sim Def response during ARM (normal or abnormal); and BET (normal = able to pass a 50 cc water filled balloon in <60 sec). We considered patients with DD to have abnormal results on both ARM and BET. The prevalence of DD was determined in normal weight (BMI <25 kg m^{-2}), overweight (BMI ≥25 or <30 kg m^{-2}), and obese (BMI ≥30 kg m^{-2}) patients with CC. Comparisons of dichotomous variables were performed using a chi-square test.
Results: Data from 401 ARMs and BETs was analyzed. Demographic data is presented in table 1. 104 adults were defined as obese and 173 adults were defined as normal weight. The remaining 124 adults were defined as overweight. Compared to normal weight adults with CC, there was no significant difference in the prevalence of DD in either overweight or obese adults with CC.
Conclusion: BMI does not appear to influence the prevalence of DD in a constipated adult population.

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New high-resolution anorectal manometry parameters for predicting balloon expulsion in patients with chronic constipation on the basis of a three-dimensional integrated pressurized volume of spatiotemporal plot

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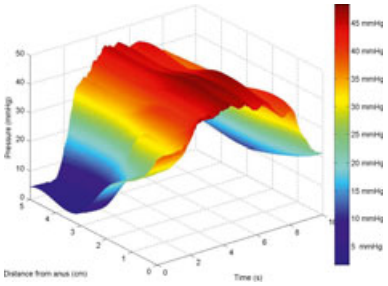
Objective: Anorectal manometry during simulated evacuation (SE) and the rectal balloon expulsion (BE) test, based on 2-dimensional linear waves, has limitations in predicting the results of the BE test, and the relationship between pressures and BE is unclear. The newly developed high-resolution anorectal manometry (HRAM) can show a spatiotemporal plot based on 3-dimensional pressurization. We aimed to develop new parameters for predicting BE test on the basis of three-dimensional integrated pressurized volume (IPV).
Methods: A total of 62 constipated patients (M:F = 26:36, mean age = 62 ± 14) who visited the tertiary care center from October to November of 2011 were identified. BE was done using 50 ml of water and an elapsed time of more than 1 min was regarded as failure. HRAM during SE was performed using circumferential sensors with 23 channels (Sandhill, Highland Ranch, CO) in 6-cm lengths. These profiles were then converted to ASCII files and analyzed with MATLAB™ program (The Math Works, Natick, MA) by an experienced bioengineer. A three-dimensional IPV was plotted after transforming the data to a cubic spline interpolation followed by resampling the manometry position at 0.1 cm intervals.
Results: Twenty two (35.5%) out of 62 patients failed the BE test. The mean IPVs of the upper 2-cm channels and the lower 4-cm channels were
385.13 ± 271.44 mmHg s cm and
563.07 ± 304.75 mmHg s cm, respectively. The mean IPVs of the upper 3-cm channels and the lower 3-cm channels were 401.16 ± 270.61 mmHg s cm and 606.36 ± 330.37 mmHg s cm, respectively. After a receiver-operator characteristic curve analysis, it was

Table 1: Demographic data for BMI Categories

	Normal BMI (< 25 kg/m^2)	Overweight (BMI ≥ 25 or < 30 kg/m^2)	Obese (BMI ≥ 30 kg/m^2)
N	173	124	104
Gender	88% female	87% female	84% female
Avg Age (SD)	46.6 ± 18.9	48.7 ± 16.4	48.7 ± 13.6
% with DD	23.1%	21.0%	17.3%

found that a higher IPV in the upper 2-cm channels as compared with the lower 4-cm channels during SE (area under curve: 0.75, 95% CI: 0.63–0.88, cut-off: -192.70, sensitivity: 0.75, specificity: 0.73, $P < 0.01$) and a higher IPV in the upper 3-cm channels as compared with the lower 3-cm channels during SE (area under curve: 0.71, 95% CI: 0.58–0.84, cut-off: -114.63, sensitivity: 0.55, specificity: 0.91, $P < 0.01$) proved to better discriminate a successful BE test from a failure.
Conclusion: The newly developed IPV method based on a three dimensional spatiotemporal plot appears to be clinically useful for predicting a successful BE test.



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Ano-rectal Dysfunction (ARD) in Functional Bowel Disorders (FBD): A systematic bio-mechanical evaluation - Clinical pilot study
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Objective: Functional Bowel Disorders a collection of symptom Complex with disabling clinical state without any organic etiology. Prevalence is global with increasing morbidity affecting Quality of life Score and compromised daily life style. Ano-rectal dysfunction with dysmotility is a salient clinical manifestation. This study evaluates Ano-Rectal Physiology in FBD.
Methods: Hundred (n = 100) patients were recruited with Group A control (n = 50) Male:Female(25:25), Group B ROME 3 proven IBS with B1 Diarrheal (n = 15) male:female (6:9), B2 Mixed (n = 17) male:female (7:10) and B3 Constipation (n = 18) male:female (5:13). All under went Stool, Lab, to exclude Infection, Systemic Disorders, Proctoscopy, Pre and Post Ano rectal Manometry and Anorectal Electromyography(EMG). Exclusion: Diabetes mellitus, Pregnancy, Thyroid dysfunction,drugs affecting GI Motility, known GI or Systemic Dysmotility Pelvic floor dysfunction Anal fissure Or Rectal incompetence.
Results: Group A, 4/50(8%) high sphincter pressure, 48/50(96%) normal compliance, RAIR normal. Group B1: 4/15(26%) altered squeeze pressure, 14/15(94%) normal compliance, 14/15(94%) RAIR normal. Group B2: 4/14(28%) altered Maximum Squeeze pressure, 15/17(88%) normal Compliance, 16/17(94%) normal RAIR. GroupB3: 9/18(50%) elevated resting pressure, 6/18(33%) altered compliance, 2/18(11%) altered RAIR. All subjects had normal EMG.
Conclusion: This study reveals Altered Ano rectal Dynamics in FBS Constipation type. Detailed neuro physiological study needs to validate.

Characteristics	Group A	Group B1	Group B2	Group B3
Sphincter Pressure	High (8%)	Normal	Normal	Normal
Compliance	Altered (4%)	Altered (6%)	Altered (11%)	Altered (33%)
RAIR	Normal	Altered (6%)	Altered (6%)	Altered (11%)
Squeeze pressure	Normal	Altered (26%)	Normal	Normal
Maximum squeeze pressure	Normal	Normal	Altered (28%)	Normal
Resting Pressure	Normal	Normal	Normal	Elevated (50%)
EMG	Normal	Normal	Normal	Normal

Table Thickness and length of anal sphincters measured by 3D-EAUS (mm, mean ± SD)

	Male (n=15)	Female (n=31)	Total (n=46)	t-test
Gender	44.8 ± 14.3	47.4 ± 12.4	47 ± 13	0.536
Thickness				
- EAS subcutaneous part	7.5 ± 0.6	7.6 ± 0.6	7.5 ± 0.6	0.587
- EAS superficial part	8.1 ± 1.3	6.9 ± 0.9	7.3 ± 1.1	0.001
- PRm	8.7 ± 1.3	9.0 ± 1.5	8.9 ± 1.4	0.605
- IAS	1.7 ± 0.4	1.8 ± 0.3	1.8 ± 0.3	0.095
Length				
- EAS subcutaneous part	13.2 ± 3.3	11.2 ± 1.3	11.9 ± 2.32	0.005
- EAS superficial part	24.1 ± 4.7	19.6 ± 3.1	21.1 ± 4.3	0.0001
- PRm	12.4 ± 3.6	12.2 ± 2.8	12.2 ± 3.1	0.84
- IAS	28.5 ± 4.5	25.3 ± 4.4	26.3 ± 4.6	0.03
- Entire anal canal	38.6 ± 6.6	34.0 ± 4.3	35.5 ± 5.6	0.007
- anal canal, anterior	35.9 ± 8.4	27.7 ± 5.4	30.4 ± 7.5	0.0001
- anal canal, posterior	38.3 ± 6.5	34.77 ± 5.0	35.9 ± 5.7	0.045

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Normative anatomy of anal sphincter detected by 3D-endoanal ultrasonography: A study in Thai population
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Objective: Understanding anatomy of anal sphincter complex is crucial in treatment of anorectal disorders. With 3D-endoanal ultrasound (EAUS), it is possible to define layers of tissue with less operator-dependency. We introduced EAUS to King Chulalongkorn Memorial Hospital since 2009. Normative character of our population has not been established.
Methods: Forty-six healthy subjects (M:F 15:31, mean age ± SD, 47 ± 13 years) without bowel symptom were enrolled. All had no previous anorectal or abdominal surgery other than simple appendectomy. EAUS was performed with mechanically rotated probe (1850 B&K Medical, Sanhoften, Denmark) which produce 360o view of the entire anal canal in 3D box. Series of scan composed of frequency ranges of 9, 10, 13 and 16 MHz. Measurement of thickness and length at 3, 6, 9 and 12 o'clock were performed for external anal sphincter (EAS), internal anal sphincter (IAS) and entire anal canal. For puborectalis muscle (PRm), thickness at 3, 6 and 9 o'clock and length at 6 o'clock were measured. Mean thickness and length of each character were defined. Differences between genders were analyzed using Student's t-test.
Results: Data are shown in table. By EAUS, EAS was divided into the lower subcutaneous part and the upper superficialis part. At upper anal canal, the PRm constitute the highest and thickest part of the sphincter complex. EAS superficialis part was significantly thicker in male than in women. No difference between genders found for thickness of EAS subcutaneous part, IAS and PRm. EAS superficialis is the longest part of voluntary sphincter. Length of EAS subcutaneous part, superficial part, IAS and entire anal canal were significantly longer in male than female. Length of anal canal was shorter in the anterior than the posterior.
Conclusion: We presented details of thickness and length of anal sphincter portions in healthy subjects shown by EAUS. This knowledge will guide diagnosis and treatment of disorders involving anal sphincter. Here, EAS subcutaneous part consists of at least 33.5% of anal canal length. Thus, division of this portion may lead to impaired voluntary sphincter. Anal sphincter length was shorter in female than male, thus, the same degree of injury may lead to more severe symptoms.

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Prevalence of dyschezia in healthy infants 0 to 6 months
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Objective: Infant dyschezia (IDZ) is a functional gastrointestinal disorder (FGD) unrecognized by the pediatrician and pediatric gastroenterologist. The Rome III Criteria defined IDZ in an infant younger than 6 months of age of at least 10 minutes of straining and crying before successful passage of soft stools with no other health problems. No data is available in the pediatric literature on its prevalence in healthy infants. Objective: To demonstrate the prevalence of IDZ in

healthy children less than 6 months attending an outpatient pediatric care center.
Methods: A retrospective sample of 372 surveys done to mothers of infants who attend the outpatient pediatric care center. A questionnaire that assessed the presence of FGD by Rome III Criteria were used. Study period: 01-03-11 to 31-05-11. The following variables were analyzed: age, sex, presence of IDZ and type of food. Measures of association and risk were established.
Results: The overall prevalence of IDZ was 34.1% (boys 54.3%, girls 45.6%) with statistically significant differences by sex (X2 $P = 0.046$). Mean age 2.75 months (range 0.13–5.98) with no difference between children younger than 3 months respect to others (X2 $P = 0.610$). Type of feeding: exclusive brestfeeding (EBF): 64.2%, mixed feeding (brestfeeding + infant formula=MF): 25.5% and exclusive infant formula (EIF): 10.2% with statistically significant differences between brestfeeding compared to complementary feeding (X2 $P = 0.038$) demonstrating an increased risk (OR 1.63) of IDZ between the second group.
Conclusion: IDZ is a prevalent FGD in healthy infants during the first six months of life. Formula-fed infants are at increased risk (OR 1.63) to present IDZ respect to exclusively brestfed infants. Recognizing this entity avoids the unnecessary use of therapeutics strategies, such as anal stimulation and laxatives, addressing the treatment of an apparent nonexistent constipation.

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Recto-anal manometry: Comparison between type 2 diabetics and healthy individuals

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Objective: Diabetes influences the motility of many parts of gastrointestinal tract. However, its influence on the recto anal motility is not consensual. It is our aim to compare recto-anal manometric characteristics between diabetics and healthy individuals.
Methods: A recto-anal manometry was performed to 33 type 2 diabetics of both gender, without signs of autonomic neuropathy, with a mean age of 58.82 years, and 34 healthy individuals with a mean age of 57.03 years. Pressure is in mmHg. Student t Test was used and data are mean ± standard deviation.
Results: In diabetics vs non diabetics, the resting pressure in the external sphincter was 56.61 ± 21 vs 48.0 ± 17.1, $P = 0.07$. In the internal sphincter, the anal channel and in the recto, pressures were similar. The relaxation, during the recto anal inhibitory reflex was 48.52 ± 26.3% vs 38.10 ± 23.1%; $P = 0.06$. The minimum rectal sensibility and the maximum tolerable volume registered similar values. During the squeeze the pressure increase was 86.4 ± 61.1 vs 125.67 ± 57.0, $P = 0.008$; average squeeze pressure 55.75 ± 41.6 vs 82.29 ± 40, $P = 0.01$; amplitude under zero 137.45 ± 68.6 vs 187.14 ± 67.6, $P = 0.004$ and area under curve 622.1 ± 270. vs 9813.3 ± 301.1, $P = 0.008$. In the squeeze endurance the values were: pressure increase 103.3 ± 60.7 vs 132.5 ± 55.8, $P = 0.03$; average squeeze pressure 61.6 ± 22.0 vs 82.0 ± 43.3, $P < 0.05$, area under curve 1515.9 ± 1160 vs 2127.0 ± 1116, $P = 0.03$. The pressure after squeeze was similar.
Conclusion: 1 – The pressures augment were significantly higher in non diabetics during the squeeze and the squeeze endurance. 2 - The resting pressure in the anal sphincters, in the anal channel and in the recto was similar. 3- The rectal sensibility thresholds and the recto anal inhibitory reflex did not revealed significant differences.

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Medium term outcomes following sacral nerve stimulation in patients with severe rectal evacuatory dysfunction allied to rectal hyposensitivity

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Objective: Sacral nerve stimulation (SNS) is an evolving treatment for constipation. A subgroup of patients with rectal evacuatory dysfunction (RED) at the Royal London Hospital had been involved in a trial to investigate the effects of SNS on rectal sensation. This follow-up study presents the medium term clinical outcomes of patients who underwent permanent SNS from this cohort.
Methods: Fifteen patients with severe RED and demonstrable rectal hyposensitivity (elevated thresholds to balloon distension in comparison with age- and sex-matched controls), but normal colonic transit, entered the original double blinded placebo controlled crossover trial. SNS was performed by the standard 2-stage technique (temporary then permanent implantation). Medium term clinical outcomes of all these patients were studied. Reportable events were collected prospectively on 'open label'. Study endpoints were selected from a previous study by Maeda et al. [1] of clinical outcomes and reportable events in SNS patients treated for faecal incontinence. Outcomes were classified into three ordinal categories: good, acceptable and suboptimal. Reportable events included suboptimal therapeutic responses, adverse events and other events related to the SNS that required additional clinical management.
Results: 12 of 15 patients who originally underwent peripheral nerve evaluation had further permanent implantation of the device. At median follow up of 28 (16–44) months, 4 (33%) had a good outcome; 6 (50%) had an adequate outcome; 2 (17%) had a suboptimal outcome. At last follow up, 9 (75%) implantable devices were in situ. Two devices had been explanted for suboptimal outcomes and one device removed for a patient requiring MRI scanning for an unrelated problem. 31 reportable events requiring intervention by the team were documented. This led to 35 clinical interventions (28 conservative interventions and 8 operative interventions). Operative interventions included: 3 repositioned leads, 3 device removals, 1 stimulator exchange and 1 procedure to reposition the lead and revise the stimulator site within the same operation. Of all these interventions 5 patients had complete resolution of their reportable events.
Conclusion: Although our series contains only 12 patients, the findings suggest that SNS therapy for patients with RED have a similar medium term efficacy profile and clinical outcome to those treated for faecal incontinence. 1. Maeda, Y., et al., Suboptimal outcome following sacral nerve stimulation for faecal incontinence. Br J Surg, 2011. 98(1): p. 140–7.

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Top down medical treatment for dis-impaction of patients in emergency department setting

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Objective: In recent years, the idea of high-dose, top-down medical treatments has been discussed. This study investigated the success of colonic dis-impaction using high levels of laxatives at a public hospital emergency department (ED). Current practice for dis-impaction in adults within the facility is step-wise increase with paraffin oil first line treatment followed by enema if patient is distressed. For paediatric patients macrogol/electrolyte solutions via NGT combined with daily suppository.
Methods: Eleven patients (5–68 years) with faecal impaction presenting to a suburban public hospital ED over seven days were triaged by senior nursing staff and a specialist ED medical practitioner. Primary complaint was chronic constipation unresponsive to current treatment. Patients were given a dis-impaction regimen of Movicol (polyethylene glycol) 8 sachets plus 15–25 Dulcolax drops over a 4 h period within the ED. Patients were subsequently discharged home with Day 2 regimen (same medication) and followed up by telephone next day by continence nurse. Patients were directed by referral to community continence nurse for follow up. Patients were encouraged to be participants and in control of their own treatment.
Results: Patients mean (SEM) time to defecate was within 10 h with reported output average 3–5 cups. All patients expressed considerable relief and cessation of abdominal pain within 6 h. Patients continued to defecate daily and their maintenance dosage of Movicol and Dulcolax SP. Patients reported that there were no episodes of soiling following dis-impaction or during maintenance (6 weeks). All patients on follow-up with continence nurse practitioner avoided repeat ED presentation and had no further constipation (6 weeks).
Conclusion: Use of high doses of Movicol and Dulcolax is effective in dis-impaction of patients with chronic constipation presenting to ED. Success is quick and worked well in all patients in this small group. Patients avoided hospitalisation which includes naso-gastric washouts and rectal interventions. Cost savings for the hospital averaged $1500 (AUD) per day per patient. Time commitment from ED and families was considerably lower than current interventions. Success and compliance were high.

Friday, 7 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-17 Clinical Session: Miscellaneous

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Autonomic dysfunction and gastrointestinal symptoms in the joint hypermobility syndrome

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Objective: The Joint Hypermobility Syndrome (JHS) is a hereditary connective tissue disorder characterised by hyperflexibility of the skin and joints as well as musculoskeletal and gastrointestinal (GI) symptoms. Autonomic dysfunction occurs in 'functional' GI disorders, and also in JHS. Our objective was to determine whether autonomic dysfunction contributes to the presence of GI symptoms in JHS.

Methods: In a cross-sectional study in consecutive referrals to GI clinics over 21 months, 43 patients (age range 16–70, 95% female) with JHS were compared to 327 patients (age range 16–70, 54% female) without JHS. The validated Bowel Disease Questionnaire assessed for GI symptoms. The validated COMPASS questionnaire was used for autonomic symptoms in the domains of: constipation, diarrhoea, gastroparesis, orthostatic intolerance, vasomotor, reflex syncope and urinary, with higher scores indicating worse symptoms. Logistic regression analysis was performed for each GI symptom using age, gender, JHS status, orthostatic intolerance, reflux syncope, vasomotor and urinary autonomic scores as covariates.

Results: In comparison to non JHS patients, those with JHS had increased autonomic scores for all domains except for reflux syncope. After adjusting for age and gender, JHS patients had significantly increased prevalence of alternating bowel habit, globus, dysphagia, postprandial fullness, early satiety, regurgitation and bloating vs non JHS patients. When the autonomic scores were added to the regression model, the strength of the association between GI symptoms and JHS was reduced (image). Urinary autonomic scores were associated with globus(P : 0.002) and dysphagia (P : 0.002), and orthostatic intolerance scores with postprandial fullness (P : 0001), early satiety (P : 0.002), regurgitation (P : 0.04) and bloating (P : 0.04).

Conclusion: Autonomic symptoms are associated with both JHS and with GI symptoms. The strength of the association between JHS and GI symptoms is reduced by the presence of orthostatic and urinary autonomic symptoms suggesting that they mediate the association between the two. This may point to a potential autonomic mechanism for GI symptoms in JHS patients.

Symptom	OR (adjusted for age and gender)	OR (adjusted for age, gender, autonomic score)
Alternating bowel habit	4.10 (P:000)	2.93 (p:0.02)
Globus	3.24 (p:0.001)	1.21 (p:0.67)
Dysphagia	3.39 (p:0.003)	1.27 (p:0.64)
Postprandial fullness	4.0 (0.001)	2.28 (p:0.08)
Early satiety	2.9 (p:0.003)	1.42 (p:0.41)
Regurgitation	2.8 (p:0.01)	1.3 (p:0.59)
Bloating	6.08 (p:0.000)	3.32 (p:0.03)

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Clinical dimensions of bloating in functional gastrointestinal disorders

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Objective: Although most previous studies in Western indicate that bloating is related with lower gastrointestinal (GI) symptoms, including irritable bowel syndrome (IBS), a few studies have attempted to classify bloating as upper GI symptoms in Asia. We conducted this to assess the prevalence of bloating with and without overlapping FGIDs and to compare symptom grouping in this population.

Methods: Participants in a comprehensive health screening cohort were prospectively enrolled. They completed the structured questionnaires, including demographics, GI symptoms, medical and social histories, and also about their bowel habits. Principle component factor analysis with varimax analysis was used to identify symptom factors. Abdominal bloating was defined as recurrent feeling of bloating or visible distension at least 3 days a month in the last 3 months.

Results: Among a total of 1149 subjects (mean age, 44.8 ± 10.2 years), the prevalence of abdominal bloating was 26.9%. Abdomen bloating related with female gender (P = 0.009), age below 50 year (P = 0.029), somatization (P = 0.007) and FGIDs such as IBS(P < 0.001), functional dyspepsia(P < 0.020), functional conspitation(P < 0.001), functional diarrhea(P = 0.006) by multiple regression analysis. Factor analysis, with orthogonal rotation, resulted in 5 component structure with factor 1 including postprandial fullness, early satiation, epigastric pain, heartburn, acid regurgitation and bloating, factor 2 including hard stool, straining during defecations, sensation of incomplete evacuation, sensation of anorectal obstruction, manual maneuvers to facilitate of defecation, factor 3 and 4 including symptoms compatible to IBS-D and diarrhea, factor 5 including fecal incontinence.

Conclusion: Abdominal bloating is very prevalent and frequently overlapped with FGIDs. It was classified into the upper GI symptoms group, which are composed of postprandial fullness, early satiety, epigastric pain, heartburn and acid regurgitation by factor analysis. FGIDs as described in the validated Rome III criteria are possibly too much categorized, therefore it may be modified when it applies to variable regional environments.

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Patients with chronic constipation attending outpatient clinics in India and China may present as functional dyspepsia and receive inappropriate treatment

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Objective: We have observed that doctors in Asia frequently make only cursory enquiry of bowel symptoms, attribute any abdominal complaint to dyspepsia, and prescribe proton pump inhibitors as first line treatment. Therefore we set out to describe the symptom reporting and treatment experience of patients with constipation criteria presenting to outpatient clinics in India and China.

Methods: A survey was conducted of primary and secondary care patients presenting to two outpatient gastroenterology clinics in India and China, using a culturally adapted and translated version of the Rome 3 FBD Questionnaire that had been locally validated. Patients who were found to have organic disease during investigation were excluded. Constipation was identified by applying the Rome III criteria for functional constipation (FC) and irritable bowel syndrome with constipation (IBS-C).

Results: A total of 291 (171M, 120F) consecutive patients presenting with non-organic diagnoses were recruited; with a predominance of male patients from India (M 63/141, 44.7%; age mean 38.53 year, SD14.83) and female patients from China (F 46/150, 30.7%; age mean 40.25, SD 12.02). Constipation criteria was met by 108 patients (FC 57, IBS-C 51); prevalence 37.1% (India 41.1%, China 33.3%) (M41.5%, F30.8%). The most frequently reported symptoms were bloating 73.2%, straining 66.7%, incomplete evacuation 53.7% and hard stool 50.0%. Most commonly reported as most bothersome symptoms (>1 symptom allowed) by patients were bloating 50.9%, incomplete evacuation 47.2%, straining 47.2%, indigestion 26.2%, unable to enjoy food 17.6%, heartburn 11.1%. Consistent with their primary care status, 28 (25.9%) had no previous visits to doctors. Of the 80 secondary care patients, only 26.2% reported satisfaction with their previous treatment. On their previous treatment 45.0% felt some improvement, 46.3% felt no change, and 7.5% felt worse. Previous treatment consisted of proton pump inhibitor (PPI) 81.3%, laxatives 71.3%, gastrokinetics 61.3%, anti-spasmodics 35.0%, traditional herbs 18.8%, fibre supplement 13.8%, probiotics 12.5%, anxiolytic 11.3%.

Conclusion: The common presentation with bloating and its reporting as the most bothersome symptom in the majority, together with the extremely high rate of PPI prescription suggest that a substantial number of these patients with chronic constipation may have been labelled as having dyspepsia. The low prevalence of heartburn and indigestion would suggest that the majority of these patients do not truly have dyspepsia. These factors could explain the low satisfaction with previous treatments received.

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The effect of acute axos colonic fermentation on the interdigestive upper gi-tract motility and hunger ratings in man

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Objective: Prebiotics are non-digestible, fermentable food ingredients affecting the host by selective stimulation of growth and activity of colonic bacteria. In particular arabinoxylan-oligosaccharides (AXOS) increase bifidobacteria counts and colonic fermentation (CF) in man. CF may affect upper GI motility and upper GI interdigestive motility (UGIM), namely phase 3, is one of the main trigger of hunger regulation. It is unclear whether the interplay between UGIM and hunger is affected by acute CF by AXOS administration.
Methods: Thirteen fasted HV (6M; 32.9.8 ± 2.9 years; BMI= 21.6 ± 0.8 kg m^{-2}) underwent two antroduodenal high-resolution manometry studies in a randomized single-blinded order after 20 g oral AXOS or placebo (maltodextrin (M)), at least 1 week apart, and simultaneously 13C-lactose ureide and H2 breath tests to assess oro-cecal transit-time (OCTT) and CF, together with hunger VAS questionnaire.
Results: Acute AXOS administration significantly increased CF without affecting the OCTT (786 ± 179 vs 158 ± 31 H2 ppm*min, AXOS vs M, $P < 0.05$; OCTT 351.5 ± 30 vs 351.3 ± 19.5, AXOS vs M, P = NS). A total of 23 and 24 phases 3 (F3) were registered in the M and AXOS arms respectively (P = NS). The number of gastric (G) F3s were not significantly affected by AXOS (6/23 vs. 8/24, P = NS) nor those of small bowel (SB) origin (18/23 vs. 16/24, P = NS), such as the duration of G and SB F3 (2 vs 2 and 6 vs 8 min, both P = NS). AXOS did not change the number of phase 1 (F1), phase 2 (F2) (18 vs 21 and 31 vs 31, both P = NS) and their durations (23 vs 17 and 180 vs 137 min for F1 and F2 respectively, both P = NS). The hunger and appetite scores 60 min before F3s tended to be higher after AXOS (91 ± 18.6 vs 69 ± 17.5 and 108.6 ± 16.2 vs 80.6 ± 16.6, both P = 0.06). The hunger peaks number did not differ between AXOS and M (9 vs 8, NS), nor their duration (9.25 ± 1 vs 10 ± 1.9 min, NS) and interval (8 vs 10 min, NS).
Conclusion: AXOS does not affect interdigestive gastrointestinal motility but tends to increase the hunger ratings in the fasting state in man.

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Cerebral cortical excitability is abnormal in patients with painful chronic pancreatitis

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Objective: In patients with painful chronic pancreatitis (CP) there is increasing evidence of abnormal pain processing in the central nervous system. To further elucidate these abnormalities, we investigated the cerebral response to experimental pain stimuli in CP patients.
Methods: Contact heat evoked potentials (CHEPS) were recorded in 15 patients with CP and in 15 healthy volunteers during repetitive stimulation of the upper abdominal region (pancreatic ''viscerotome'') and the right forearm (heterologous area). Three sequences of painful stimuli were applied at each site. Subjective pain scores were assessed by a visual analogue scale. Habituation was calculated as the relative change in CHEPS amplitudes between the first and the third stimulation sequence.
Results: As expected pain scores decreased in healthy volunteers during successive stimulations at both sites (i.e. habituation), while in the CP group they remained unchanged. The cerebral response consisted of an early-latency, low-amplitude response (N1, contralateral temporal region) followed by a late, high-amplitude, negative–positive complex (N2/P2, vertex). During successive stimulation of the pancreatic area N2/P2 amplitude increased 25% in CP patients, while they decreased 20% in healthy volunteers (P = 0.006). After stimulation of the forearm N2/P2 amplitudes increased 3% in CP patients compared to a decrease of 20% in healthy volunteers (P = 0.06).
Conclusion: Taken together, CP patients had an abnormal cerebral response to repetitive thermal stimuli. This was most prominent after stimulation of the upper abdominal area. As this area share spinal innervation with the pancreatic gland, these findings likely mirror distinctive abnormalities in cerebral cortical pain processing.

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Maladaptive brain changes contribute to gastrointestinal symptoms in diabetes mellitus patients

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Objective: Longterm diabetes mellitus (DM) have been associated with neuronal changes in the enteric, peripheral and/or central nervous system (CNS). Moreover abnormal visceral sensation and gastrointestinal (GI) symptoms is seen in up to 75% of patients. To explore the role of diabetic autonomic neuropathy (DAN) in patients with long-standing DM, we investigated psychophysical responses and neuronal activity recorded as evoked brain potentials (EP's) and dipolar source modelling.
Methods: Fifteen healthy volunteers and 14 type-1 DM patients with DAN were assessed with a symptom score index characterizing upper GI abnormalities. Multi-channel (62) electroencephalography was recorded during painful electrical stimulation of the lower oesophagus. Brain activity to painful stimulations was modelled by use of Brain Electrical Source Analysis (BESA).
Results: Diabetic patients had higher stimulus intensities to evoke painful sensation ($P \leq 0.001$), longer latencies of N2 and P2 components (both $P \leq 0.001$), lower amplitudes of P1-N2 and N2-P2 complexes ($P \leq 0.001$; P = 0.02). Inverse modelling of brain sources showed deeper bilateral insular dipolar source localization (P = 0.002). Symptom score index was negatively correlated to the depth of insular activity (P = 0.004) and positively correlated to insular dipole strength (P = 0.03).
Conclusion: DM patients show neuroplastic changes in the CNS. The role of abnormal insular processing may explain the development and persistence of upper GI-symptoms related to DAN. This enhanced understanding of DAN may have clinical and therapeutically implications.

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Gastrointestinal dysfunctions in Parkinson's Disease and other parkinsonian disorders

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Objective: To comparatively investigate the frequencies of various gastrointestinal (GI) symptoms in patients with Parkinson's disease (PD), parkinson-plus syndromes, vascular parkinsonism and drug induced parkinsonism, and to evaluate the relationships between GI symptoms and the severity of PD.
Methods: A 188 consecutive patients with parkinsonism (123 PD, 29 parkinson-plus syndrome, 21 vascular parkinsonism, and 10 drug-induced parkinsonism) who had visited movement disorders clinic between January and March 2012 at Boramae Medical Center, were screened for GI symptoms using a structured questionnaire regarding drooling, dysphagia, gastroesophageal reflux disease (GERD), and constipation. Drooling was evaluated by SCS-PD, dysphagia by SDQ, and GERD by GERD questionnaire. Constipation was screened according to ROME-III criteria.
Results: The frequencies of drooling, dysphagia, GERD, and constipation was found in 42.9%, 25.2%, 18.1%, and 66.4% in PD; 27.6%, 20.7%, 28.6% and 65.5% in parkinson-plus syndrome. In PD, the unified Parkinson disease rating scale (UPDRS) score was significantly correlated with SDQ and GERD questionnaire scores (Pearson's correlation coefficient = 0.325 and 0.231, respectively, all P-values < 0.05); the severity of these 2 symptoms showed similar correlations with Hoehn and Yahr (HY) stage. Constipation was the most common among the GI symptoms from its early stage (73.7% in HY stage 1) but it was not further increased by advanced stage. Drooling was more severe in vascular parkinsonism than in primary parkinosonian disorders [SCS-PD score, 4.0 ± 5.0 vs. 1.9 ± 3.8; age and gender-adjusted odds ratio, 1.15 (1.04–1.28); P = 0.007], but there were no significant differences in the frequencies of each GI symptoms between synucleinopathies and tauopathies or between lewy body diseases and non-lewy body diseases.
Conclusion: GI dysfunction is also common in other parkinsonian disorders as in PD. Constipation, a lower GI dysfunction, is common in early stage of the diseases, whereas dysphagia, drooling and GERD symptoms, which are upper GI symptoms, seem to appear in later stages. Further studies are warranted to reveal the differential involvement of GI systems according to the subtypes and severities of parkinsonian disorders.

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Overlapping upper and lower gastrointestinal symptoms suggestive of additional functional gastrointestinal disorders in IBS: Rome II versus Rome III

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Objective: IBS patients often complain of a wide range of symptoms, including symptoms from other parts of the gastrointestinal (GI) tract than the bowel. We eval-

uated the presence of symptoms suggestive of other functional gastrointestinal disorders (FGIDs) in IBS according to Rome II or III criteria, and assessed factors of importance for widespread GI symptoms.

Methods: We included 670 patients with IBS - 308 Rome II positive IBS patients 2002–2007 (mean age 40 years, range 18–73; 226 females), and 362 Rome III positive IBS patients 2007–2011 (mean age 37 years, range 18–73; 285 females). The patients completed the Rome II or III Modular Questionnaire, respectively. Symptoms suggestive of functional esophageal, gastroduodenal and anorectal disorders were included in the analyses. Presence of anxiety and depression was assessed with the Hospital Anxiety and Depression scale.

Results: 76 (Rome II) and 68% (Rome III) of the patients had symptoms compatible with at least one additional FGID. Symptoms suggestive of functional esophageal, gastroduodenal or anorectal disorders were all prevalent (Table). Using the Rome II criteria, functional anorectal disorders were most prevalent and proctalgia fugax was the single most common FGID, whereas functional gastroduodenal disorders were most prevalent when Rome III criteria were used, and the single most common FGID was functional dyspepsia. Esophageal symptoms were also common, with heartburn being reported by the highest number of patients. The number of additional FGIDs besides IBS was higher in patients with anxiety (P = 0.001 for Rome II; P = 0.002 for Rome III) or depression (P = 0.005 for Rome II; P = 0.04 for Rome III). With Rome II subtyping, patients with alternating bowel habits were more likely to report additional FGIDs vs. other subtypes (P = 0.03), whereas patients with unsubtyped IBS were less likely than other Rome III IBS subtypes to report additional FGIDs (P < 0.0001). No consistent effect of age or gender on the number of additional FGIDs was seen.

Conclusion: A majority of IBS patients also report symptoms compatible with other FGIDs, according to both Rome II and III criteria. This implies a more widespread gastrointestinal disturbance, and this is related to psychological factors, as well as type of IBS symptoms.

		Rome II (n=308)	Rome III (n=362)
Esophageal	Globus	5.4%	5.2%
	Rumination syndrome	2.7%	NA
	Chest Pain	8.5%	9.1%
	Heartburn	26.4%	16.6%
	Dysphagia	3.0%	3.0%
Gastroduodenal	Dyspepsia	49%/6.5% *	47.5%
	Aerophagia/Belching**	5.8%	24.3%
	Vomiting	1.3%	0.8%
	Nausea	NA	17.1%
	Cyclic Vomiting	NA	3.3%
	Rumination	NA	1.1%
Fecal incontinence	Total	22.4%	13.0%
	Soiling	14.4%	NA
	Gross incontinence	8.0%	NA
Anorectal pain	Total	53%	36.2%
	Proctalgia Fugax	41.8%	24.3%
	Levator ani syndrome	11.2%	NA
	Chronic proctalgia	NA	11.9%

*Patients with dyspeptic symptoms/patients fulfilling Rome II criteria for functional dyspepsia. ** Belching only included in this category in Rome III.

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Generalized transit delay, hypomotility, and absent gut reflex activity activity with spinal cord injury: Comparison to controls and slow transit constipation using wireless motility capsules

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Objective: Patients with spinal cord injury (SCI) commonly report symptoms of gastrointestinal dysmotility including constipation, pain, bloating, and distention. Isolated regional gastric and colon transit delays have been documented in SCI, but studies of generalized transit, contractility, and gut reflex impairments are limited. Wireless motility capsule (WMC) methods show altered gastric, small bowel (SB), and colon transit and pressure and blunted colon responses to meals in dysmotility syndromes, but have not been employed in SCI.

Methods: WMC (SmartPill Corp.) data were compared in 6 SCI patients (3 female; 2 cervical, 3 thoracic, 1 lumbar; 3 complete) vs. a database of 52 healthy controls and 15 slow transit constipation (STC) patients. Gastric emptying (normal <5 h), SB transit (normal <6 h), colon transit (normal <59 h), and numbers of contractions and areas under curves (AUC) >25 mmHg h^{-1} were calculated for 1 h before and after gastric emptying and during colon transit. Gastrocolonic reflexes (GCR) were measured for 15 min before meals and every 15 min for 2 h.

Results: 3/6 (50%) SCI patients had slow colon transit. 3/6 (50%) SCI patients had delayed gastric emptying and 2/6 (33%) had delayed SB transit vs. 1/15 (7%) and 4/15 (27%) of STC patients. Gastric emptying was more prolonged with SCI than STC or controls; SB and colon transit were slower with SCI vs. controls (Table). Gastric contraction numbers trended lower, while SB values were not decreased with SCI vs. controls. Compared to controls and STC, colon pressure parameters in SCI were reduced. GCR activity was modestly blunted with STC but was absent with SCI.

Conclusion: Spinal cord injury patients exhibit delayed colon transit similar to individuals with slow transit constipation, and additionally show prominently delayed gastric emptying and small bowel transit. These are associated with colonic hypomotility and trends to antral hypomotility, rather than normal to increased contractility in slow transit constipation. Gastrocolonic reflexes after meals are more profoundly impaired with SCI vs. slow transit constipation. Future prospective investigations will relate these generalized motor impairments to symptom profiles, comorbidities, and level and severity of spinal injury.

Region/Reflex	Pressure Parameter	SCI Patients	Healthy Controls	STC Patients	P Value SCI vs. Controls	P Value SCI vs. STC Patients
Gastric	Emptying (h)	22.1±10.3	3.5±0.1	4.8±1.3	<0.0001	0.02
	Contraction numbers/h	6.8±3.0	13.2±1.2	16.5±3.9	0.10	0.15
	AUC	28.5±9.9	34.6±3.6	43.4±8.2	0.60	0.32
SB	Transit (h)	6.2±1.9	3.9±0.2	4.7±0.5	0.004	0.27
	Contraction numbers/h	52±47	21±3	24±7	0.10	0.38
	AUC	61±56	23±4	29±7	0.08	0.38
Colon	Transit (h)	67.2±22.5	23.4±2.0	91.0±5.5	<0.0001	0.16
	Contraction numbers/h	11.5±4.8	34.7±3.0	33.7±2.6	0.01	0.0004
	AUC	34.5±18.0	94.0±10.2	126.3±13.4	0.06	0.001
GCR	Contraction numbers/h	-0.7±1.9	2.4±0.7	1.0±0.4	0.05	0.18
	AUC	-9.6±5.6	9.0±2.5	3.7±1.7	0.0006	0.003

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Impairment of gastrointestinal motility and evacuation in patients with liver cirrhosis of different aetiologies

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Objective: The socio-economic and medical importance of neurogastroenterology makes this science one of the most topical areas in modern medicine because of the considerable prevalence of gastrointestinal motility impairment. Of enormous interest is studying gastrointestinal motility and evacuation abnormalities in patients with liver cirrhosis developing due to different conditions. Liver cirrhosis is a disease that has many causes and is characterized by very different clinical manifestations, which result from hepatocellular insufficiency, portal hypertension syndrome, and complications of the latter. A detailed study of gastrointestinal motility and evacuation abnormalities was conducted in patients with liver cirrhosis developing due to different background conditions at the Department of Gastroenterology of the Republican Clinical Hospital in the city of Ufa (Republic of Bashkortostan).

Methods: A total of 100 patients with liver cirrhosis were randomized in this study. The patient distribution by cirrhosis aetiology was as follows: viral in 33 subjects, viral-toxic (alcoholic) in 16, primary biliary cirrhosis in 14, alcohol-induced in 32, autoimmune in 3, and due to Budd – Chiari syndrome in 2 patients.

Results: Comparing the occurrence rates of dyspeptic complaints between liver cirrhosis patients with different aetiotropic factors, we found out that 87.5% of patients with viral-toxic liver cirrhosis experienced abdominal flatulence and right hypochondrial heaviness, while 81.25% of patients in this group made complaints of heartburn and belching with the acidic content of the stomach, significantly more frequently compared with viral liver damage: 36.36% of the latter patients experienced abdominal flatulence; 45.45% of them had pain and heaviness in the right hypochondrium, and heartburn was reported only by 36.36% of patients with viral cirrhosis. All patients with primary biliary cirrhosis 100% experienced heartburn; abdominal flatulence was reported by 85.71% of patients in this group, statistically significantly more frequently compared with viral infection-induced disease, 36.36% (P = 0.0055). Nausea was felt by 92.86% of biliary cirrhosis patients, significantly more frequently

compared with viral-toxic liver cirrhosis, 18.75% $P = 0.0002$.
Conclusion: In patients with Budd-Chiari syndrome, alcoholic and primary biliary cirrhosis, symptoms of the motor-evacuation function occur significantly more often and require medical treatment.

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This abstract has been withdrawn.

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Change in functional bowel symptoms after prostatectomy: A case-control study
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Objective: Irritable bowel syndrome (IBS) is a chronic functional bowel disorder that up to 20% of the population is suffering from it. Also benign prostatic hyperplasia (BPH) is a common problem that approximately 90 percent of men may be affected by this condition until the eighth decade of their life. Prostatectomy as a surgery and pelvic intervention can cause IBS.
Methods: It was a case-control study including 66 patients in 2 case groups and 66 patients in 2 control groups. Case groups were patients who underwent open prostatectomy and transurethral resection of the prostate (TURP) and control groups were patients who were candidate for prostatectomy.
Results: Ten patients in case groups and five patients in control groups had IBS. There was no significant difference in IBS between control and case groups ($P = 0.117$).
Conclusion: This is the first forward study regarding bowel symptom changes following prostatectomy. The main positive finding of this study is that open prostatectomy was followed by significant increase in diarrhea and bowel habit alternation associated with onset of abdominal pain. Specifically the change was found after open operation but not after TURP. Prostatectomy whether in form of open or transurethral may cause onset of abdominal discomfort and bowel habit change.

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Influence of immunosuppressive therapy on gastrointestinal transit after renal transplantation evaluated by AC Biosusceptometry
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Objective: Gastrointestinal (GI) complications after renal transplantation are often associated with immunosuppressant therapy; however, motility disorders have been scantily studied. The aim of this study was to employ the Alternating Current Biosusceptometry (ACB) to investigate the influence of immunosuppressants on GI transit after renal transplantation.
Methods: The protocol has been approved by local Ethics Committee. Twelve renal allograft recipients and 12 healthy volunteers were enrolled in the study. All the patients were receiving triple immunosuppressive therapy: prednisone (PRED), azathioprine (AZA) and tacrolimus (FK). After an overnight fast, subjects consumed a standard 500 Kcal breakfast and 4 g of magnetic markers. ACB sensor was used to monitor gastric and colonic abdominal projections at 10 min intervals for at least 8 h. Magnetic images were obtained to quantifying GI transit parameters. From the gastric emptying (GE) and colonic arrival (CA) time-intensity curves, mean GE time (MGET), mean CA time (CAT) and mean small intestinal transit time (MSITT) were calculated. The results were expressed as mean ± standard deviation. Differences were evaluated by ANOVA and P value <0.05 was considered statistically significant.
Results: MGET obtained for FK and controls was 44 ± 33 min and 166 ± 52 min, respectively. MGET was significantly faster ($P < 0.001$) in FK group than healthy controls. MSITT for FK and controls was 183 ± 93 min and 196 ± 66 min, respectively ($P = 0.52$). MCAT was 229 ± 101 min and 363 ± 55 min for FK and controls, respectively ($P = 0.002$).
Conclusion: Since patients were taking concomitant drugs, we propositioned that the differences may be due to the use of FK which is a macrolide and can stimulate the gastric motility. There are still gaps in our knowledge regarding the influence of immunosuppressants on GI tract and versatile tools such as ACB sensor is able to monitoring GI transit under several conditions.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-18 Basic and Translational Session: Glial and Enteric Neurons in Health and Disease

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Identification of purinergic signaling in the esophageal enteric neurons
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Objective: ATP is an important neurotransmitter in the enteric nervous system; it mediates synaptic as well as postsynaptic events to control motility and secretion. Numbers of studies have investigated purinergic signaling in the control of small and large intestine motility in animals and humans but limited information is available in the esophagus. We investigated whether esophageal enteric neurons functionally express purinergic receptors, and if so, to determine the mechanism of activation.
Methods: The enteric neurons were freshly isolated from the esophagus of postnatal rats (~5 days) and grown in primary culture for 7 days. Morphological, immmunocytochemical and functional properties of the neurons were then studied. Cytosolic free Ca2+ concentrations ([Ca2+]cyt) in the neurons were determined using a digital calcium imaging system.
Results: After primary culture for 7 days, dendrites and axons appeared in the esophageal neurons. In immmunocytochemical studies, over 90% of the cells were positively stained for PGP 9.5 or Milli-MarkTM FluoroPan Neuronal Markerneuron mark antibody, two neuron markers. In functional studies, KCl (100 m mol L^{-1}) was used to depolarize the membrane potentials, and ATP (10 μ mol L^{-1}) to stimulate purinergic receptors. Amplitude of $[Ca^{2+}]$cyt signaling in response to purines was seen to occur in the following order; AT P = AD P > UTP. ATP (10 μ mol L^{-1}) induced transient Ca^{2+} signaling in the absence of extracellular Ca^{2+}, and sustained Ca^{2+} signaling in the presence of extracellular Ca^{2+}. ATP-induced $[Ca^{2+}]$cyt signaling in the neurons was blocked by the nonselective purinergic receptor antagonist pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt hydrate (PPADS), and phospholipase C inhibitor U73122. Furthermore 2-APB (inhibitor of IP3 receptors (IP3R) and blocker of store-operated channel, (SOC)), and KB-R7943 (a selective inhibitor of the Ca^{2+} entry mode of Na^{+}/Ca^{2+} exchanger (NCX)) inhibited ATP-induced $[Ca^{2+}]$cyt signaling in the neurons.
Conclusion: Our results provide direct evidence for functional purinergic signaling in the esophageal enteric neurons. We also demonstrate that purinergic signaling acts through P2Y1 receptors, leading to intracellular Ca^{2+} release through IP3R on the ER, and extracellular Ca^{2+} entry through SOC and the Ca^{2+} entry mode of NCX.

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This abstract has been withdrawn.

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TRPV4 channel activation stimulates enteric glia in the mouse colonic myenteric plexus
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Objective: Enteric glia are a unique type of peripheral glia that surround enteric neurons within enteric ganglia and, similar to astrocytes in the brain, are thought to actively modulate enteric neurotransmission. However, when, and how, enteric glia are activated during physiological processes in the gastrointestinal tract are poorly understood. Normal gut activity physically distorts the nerve plexuses within the gut wall. In the brain, recent evidence suggest that astrocytes respond to cell swelling in response to hypo-osmotic stress through TRPV4 channels. Given the numerous similarities between enteric glia and astrocytes, we hypothesized that enteric glia could respond to cell stretch through TRPV4.
Methods: We tested this hypothesis in whole-mount preparations of the myenteric plexus from the male mouse colon using immunohistochemistry to reveal the expression of TRPV4 in relation to neurons and glia and Ca^{2+} imaging to assess the consequence of TRPV4 activation.
Results: We find TRPV4 immunoreactivity throughout the myenteric plexus, localized to the membranes of both neurons and enteric glia. However, using Ca^{2+}

imaging to monitor neuron and glial activity, we find that activating TRPV4 channels in situ with the agonist GSK1016790A (GSK; 100 n mol L^{-1}) primarily stimulates responses in enteric glia. Following a 50 s bath application of GSK, asynchronous $Ca2^{+}$ responses throughout the enteric glia network persist for 392 s on average. Glial responses to GSK are mainly restricted to glial processes, but propagate to glial cell bodies after summation.
Conclusion: Our data suggest that stretch-sensitive TRPV4 channel activation in the myenteric plexus activates enteric glia. Whether glial responses reflect direct activation of glial TRPV4 or TRPV4-mediated neuron-to-glia communication will be the subject of further investigation.

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Distribution of TMEM100, a novel marker of the mouse and human enteric nervous system
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Objective: Transmembrane protein 100 (TMEM100) is a 134-amino acid protein that is expressed in arterial endothelium of mouse embryos. Its function and systemic distribution have not been reported. TMEM100 mRNA is reported as present in the mouse muscularis propria. The aim of this study was to determine the distribution of TMEM100 in the mouse and human gastrointestinal tract.
Methods: Gastrointestinal tissues were collected from 3 Balb/c mice. Normal human stomach (n = 3), jejunum (n = 3) and colon (n = 3) samples were obtained from patients undergoing surgery for gastrointestinal cancers or having bariatric surgery. Immunoreactivity (IR) for TMEM100 was examined by immunofluorescence labeling of 15 μm cross sections and whole mount preparations of the muscularis propria. Double immunolabeling for PGP9.5, Kit, and S100β was used to determine co-localization of the TMEM100-IR respectively with nerves, interstitial cells of Cajal (ICC) and glia in the muscle and sub-mucosa. TMEM100 distribution was confirmed using antibodies derived from two different species. Preabsorption of these antibodies with their respective immunogens decreased IR. Labeled structures were imaged by confocal microscopy. Total internal reflection fluorescence (TIRF) microscopy of HEK293 cells transfected with TMEM100-GFP plasmid was used to determine the cellular localization of TMEM100.
Results: TMEM100-IR expression was restricted to enteric nerve cell bodies and nerve fibers of both mouse and human tissues and co-localized with PGP9.5-IR. TMEM100-IR was not found in glia or smooth muscle. In mice, TMEM100 positive neuronal structures were closely associated with ICC in the deep muscular plexus of the jejunum but other populations of ICC were negative for TMEM100. All nerve cell bodies examined in mouse and human stomach, jejunum and colon were positive for TMEM100. TMEM100-IR nerve fibers were present in the myenteric and submucosal plexuses, in the longitudinal and circular muscle layers, and in the submucosa. TIRF microscopy showed that TMEM100 is specifically located in the plasma membrane of transfected HEK293 cells.
Conclusion: TMEM100 is a membrane protein expressed in the mouse and human enteric nervous system and is a pan-neuronal marker. Grant Support: DK57061, DK52766

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High fat diet induces murine colonic neuronal cell loss and enteric dysmotility: Role of macro-autophagy and ER-stress
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Objective: High fat diet (HFD) and has been associated with gastrointestinal motility disturbances and altered colonic habits. Saturated free fatty acid palmitate has a major role in the detrimental effects of the HFD and hyperlipidemia. Our aim was to investigate the underlying mechanisms of the HFD induced gastrointestinal motility disorders and enteric neuronal cell death with focus on the role of endoplasmic reticulum (ER) stress and macro-autophagy.
Methods: Eight week old CF1 mice were fed a HFD (60% kcal from fat) versus regular diet (RD, 18% kcal from fat) for 12 weeks. 00Neuronal sub-population was determined in colonic myenteric plexus by histochemical staining. In-vitro studies were performed on enteric neuron cell line and primary enteric neuronal cells. Neuronal cells were exposed to different doses of sodium palmitate for 24 h before studying. Knock down of two macro-autophagy pathway key proteins (ATG-7 and Beclin-1) were performed to assess the role of macro-autophagy in palmitate mediated cell damage.
Results: Mice fed the HFD for twelve weeks showed significant weight gain compared to the RD group (54.23 ± 5.18 *vs* 41.01 ± 2.49 g). Serum cholesterol, LDL and triglyceride were significantly higher in HFD group compared to RD. HFD group developed a constipation phenotype including lower stool water content, lower wet and dry stool weight, slower whole gut transit (P < 0.01) and colonic motility (P < 0.05). Whole mount staining confirmed a decrease in the number of nitrergic and cholinergic neurons in the colon of HFD group compared to RD group. Exposure of enteric neuronal cells to 0.1, 0.5 and 1 m mol L^{-1} Palmitate resulted in a dose dependent reduction in cells viability and increase in cleaved caspase-3 protein level, while LC3B protein level and autophagy flux as two markers for macro-autophagy were significantly increased (Fig. 1A). Autophagy knock down decreased the level of cleaved caspase-3 protein after exposure to palmitate (Fig.1B). Finally, palmitate increased ER stress marker, CHOP (Fig.1C), and decreased in the mitochondrial marker, COX IV (Fig.1D).
Conclusion: High fat diet reduces the colonic motility, which is associated with enteric neuronal loss. Enteric neuronal damage is caused by autophagy mediated activation of apoptotic cascade and is associated with mitochondrial damage and ER stress. This is the first report to highlight the role of ER stress and macro-autophagy in mediating the palmitate induced enteric neuronal apoptotic cell death. Our results warrant further investigations to protect neuronal cell damage in hyperlipidemia by targeting ER stress and macro-autophagy pathways.

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Mechanisms involved in the therapeutic properties of mesenchymal stem cells on colon damages: Application to radiotherapy side effects
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Objective: Patients who undergo pelvic or abdominal radiotherapy may develop high incidence of undesirable acute and/or chronic gastrointestinal complications resulting from radiation induced damages around the tumor. The growing number of cases declared each year and the specific complex symptoms have led some specialists to talk about a new disease termed ''pelvic-radiation disease''. Symptoms can also be aggravated following a radiotherapy accident such as an overdose. Treatments applied to manage complications of radiotherapy are only symptomatic. In this study we address the question of the use of mesenchymal stem cell therapy to manage intestinal complications after radiotherapy. We tested regenerative capability of mesenchymal stem cells (MSCs) after radiation-induced severe colonic epithelial ulceration and dysmotility.
Methods: Rats SD were subjected to a 27 Gy colorectal irradiation. At this dose, irradiation induces histological and functional alterations of distal colon as described in patients developing chronic side effects after radiotherapy. Bone marrow derived-mesenchymal stem cells (5.106) were intravenously administered directly (preventive effects of lesion development) or three weeks (curative effects) after radiation exposure.
Results: We demonstrated in this model that MSCs reduce radiation effects on acute colonic disorders by enhancing epithelium renewal. Our *in vivo* and *In vitro* experiments showed an involvement of Wnt pathway activation in MSCs effects on epithelial cell proliferation. The colonic motility dysfunction participating in the process of radiation-induced chronic disorders is also restored by MSCs infusion. This therapeutic effect is partially dependent of MSCs immunosuppressive properties and their abilities to reduce oxidative stress via modulation of glutathione (GSH). We also demonstrated that MSCs restore neurally-induced muscle activity. MSCs treatment reduces radiation-induced neurochemical changes of the myenteric nervous system. Indeed, radiation-increased nitric oxide synthase immunoreactive neurons and enteric glial cells is reduced after MSCs treatment.
Conclusion: Our results suggest that MSC infusion could be used as a therapeutic treatment to limit acute and chronic radiation-induced gastrointestinal damages.

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Endothelial and neural stem cells in the gut: A successful team
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Objective: Enteric neurons and blood vessels form an intricate network throughout the gut. Anatomical sim-

ilarities and equivalent molecular mechanisms suggest interdependence. This study investigated the interrelationship between developing ganglia and blood vessels in the gut. To examine a possible functional interaction on a cellular level, enteric precursors (ENS-cells) and mesenteric vascular cells (MVCs) were used.
Methods: The stimulatory effect of factors released from ENS-cells on the migration of MVCs was quantified with a transmembrane-migration assay as well as a scratch assay. The effect of factors released from MVCs on the proliferation and differentiation of ENS-cells was studied in co-cultures using transwell-chambers followed by immunofluorescence staining against neuronal and glial markers. To monitor the migration of ENS-cells and MVCs, a spheroid confrontation assay was performed. From both cell types spheroids were generated, picked and confronted in a three-dimensional collagen matrix. The mutual interaction was analyzed using multicellular spheroids consisting of MVCs and ENS-cells. Immunofluorescence staining against neuronal and glial markers to determine the nature of migrating cells followed both assays. The *in vivo* situation of blood vessels and nerves in developing gastrointestinal tract was observed via whole-mount staining of embryonic gut (E12) of a Nestin-positive GFP-mouse against the embryonic marker CD31.
Results: In the transmembrane-migration assay as well as in the scratch assay the migration of MVCs significantly increased when cultured in ENS-conditioned medium. Co-cultures of ENS-cells with MVCs lead to an increased ENS cell proliferation and neuronal differentiation. Obviously in the confrontation assay both cell types migrated straight towards each other. In immunofluorescence staining a neuronal outgrowth was observed. Multicellular spheroids showed a parallel outgrowth of MVCs with glial cells as well as neuronal fibers. Whole mount staining of Nestin-GFP transgenic mouse embryonic gut indicated a close localization of Nestin- positive cells to blood vessels.
Conclusion: An emerging stimulatory effect of ENS-cells and MVCs towards each other considering migration as well as proliferation and neural differentiation could be examined. This supports the hypothesis of an interdependence of the developing vascular and nervous system in the gut.

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Wnt pathway in post-natal enteric nervous system

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Objective: Wnt signaling is essential for the development of the nervous system. So far research has dwelt much on the role of this pathway in the development and maintenance of the central nervous system, as well as on its crucial role in the origin of peripheral nervous system from neural crest. The present work investigates Wnt signaling in the post-natal enteric nervous system (ENS) especially in combination with LPS, as a central mediator in intestinal inflammations.
Methods: ENS cells were isolated from post-natal rat gut and maintained in culture according to Schaefer et al. (1997). Long term LPS and rhWnt3a stimulation studies were performed on ENS cells for 7 (T7) and 14 (T14) days. Specific neuronal and glial markers expression were immunohistochemically investigated. Calcium imaging analysis were performed using LPS and/or rhWnt3a stimulation on ENS cells cultured for 7 and 14 days. In parallel the activation of β-catenin depending Wnt signaling was investigated by transfection (Rotifect-plus) with a newly designed and developed GFP reporter plasmid. Transfected cells were stimulated with 5 μg ml^{-1} LPS or 20 ng ml^{-1} rhWnt3a for 24, 48 and 72 h. An EGFP plasmid was used as transfection positive control. β-catenin expression were evaluated by FACS.
Results: Immunofluorescence stainings of ENS cells stimulated with LPS and rhWnt3a shows an increase of neuronal outgrowth compared to control as demostrated by βIII-tubulin and neurofilament expression. At T14 a colocalization of Nestin and PGP9.5 positive cells is observed. LPS and rhWnt3a stimulated cultures showed higher β-catenin expression than control samples. Calcium imaging has shown that at T7 and T14 20% of all neurons respond to rhWnt3a with a calcium influx, whereas less than 1.5% reacted with a calcium decrease. After pre-treatment with LPS for 24 h, 10% (T7) and 7% (T14) of neurons respond to Wnt3a with a calcium influx. Moreover, it has been observed 10 fold increase of neurons responding to rhWnt3a with a calcium efflux compared to control. GFP reporter plasmid permits to detect cytoplasmic β-catenin accumulation in ENS transfected cells. In particular a significant accumulation of β-catenin was detected in rhWnt3a and LPS stimulated samples. At 72 h β-catenin accumulation is still observed only in rhWnt3a treated cells.
Conclusion: Wnt signaling is active in post-natal ENS cells. On the basis of our experimental data, we hypothesize that LPS and rhWnt3a promote neurite outgrowth through non-canonical wnt pathway. Additionally, LPS modifies the activity of the enteric neurons.

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Myenteric intrinsic primary afferent neuron function correlates with altered motility in aged mice

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Objective: Altered gut motility may contribute to constipation in the aged. Myenteric intrinsic primary afferent neurons (IPANs) modulate migrating motor complexes (MCs). IPANs may be particularly vulnerable to damage in old age1. We measured MC amplitudes and frequencies in segments of jejunum (J) or distal colon (C) taken from young (2–5 mo) or old (24–30 mo) mice. Patch clamp recordings were made using matching gut segments.
Methods: Segments of gut from young or old mice were placed in a recording dish perfused with carbogenated Krebs and intraluminal pressure2 or IPAN whole cell recordings made3. MC amplitudes or frequencies were measured at 3 hPa luminal (Krebs) filling pressure. Resting membrane potentials (RMP), action potential (AP) thresholds, no. of AP fired at 2x threshold (No. AP) and post action potential slow afterhyperpolarisation (sAHP) AUP were measured during neuron recording. IPANs were identified a posteriori by shape. Bonferroni's multiple comparison tests were performed on the results.
Results: Parameters for young and old mice were in mean ± sd (n). MC amp. for J young vs old were; 11 ± 2.7 (12) vs 9.1 ± 4.8 (10) hPa ($P > 0.05$), and for C young vs old were, 51 ± 3.4 (12) vs 48 ± 3.0 (8) hPa ($P > 0.05$). MC freq. were; J: 13 ± 1.6 vs 13 ± 1.3 mHz ($P > 0.5$) and C: 8.6 ± 1.7 vs 3.0 ± 2.2 mHz ($P < 0.01$). 12 of 50 old IPANs could not be fired. RMP young vs old were; J: -55 ± 7 (32) vs -58 ± 10 (19) mV ($P > 0.5$) and C: -55 ± 8 (21) vs -61 ± 7 (19) ($P > 0.5$). AP thesh. were; J: 163 ± 29 vs 173 ± 36 pA ($P > 0.5$), and C: 178 ± 47 vs 228 ± 32 pA ($P < 0.01$). No. AP were; J: 2.2 ± 0.6 vs 1.8 ± 0.6 ($P > 0.5$), and C: 2.0 ± 0.5 vs 0.9 ± 0.5 ($P < 0.01$). sAHP AUP were; J: 29 ± 6 vs 29 ± 4 mV.s ($P > 0.05$) and C: 27 ± 6 vs 48 ± 5 mV.s ($P = 0.001$).
Conclusion: MC frequency in colon but not jejunum was lower in old than young mice which observation correlated with a greater sensory neuron post-AP refractory period (sAHP). The enhanced sAHP may be one of the determinants of the reduced MC frequency and could be a potential target for treating old age related constipation.
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Myogenic and neurogenic properties of three independent motor patterns in the whole rat colon

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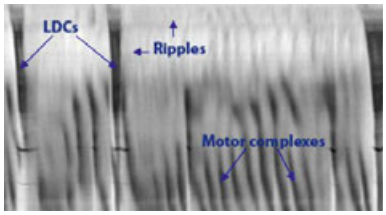
Objective: Spatio-temporal mapping is giving us unprecedented insight into characteristics of colonic motor patterns. Our objective was to explore this technique to gain insight into the motor patterns of the whole rat colon and the underlying mechanisms.
Methods: Video recording of the whole rat colon motility *In vitro* was analyzed using spatio temporal mapping. Pharmacological studies differentiated neurogenic and myogenic phenomena.
Results: In addition to recently published spontaneously occurring myogenic propulsive patterns, the ripples and rhythmic propulsive motor complexes (Frontiers in Neuroscience, 2011), we discovered a third propulsive motor pattern that invariably was initiated by fluid distention, but not balloon distention, of the proximal colon. These Long Distance Contractions (LDC's) involved progressing circular muscle ring contractions, slowly in the proximal colon, fast in the distal colon, that left the colon contracted at its wake for up to 12 s. When they approached the middle of the colon, a wave of relaxation started to precede the contraction. This relaxation abolished motor complexes (occurring at ~ 2 per min) that were happening in the distal colon. LDC's completely emptied the colon when unobstructed. An LDC was always followed by a period of elevated excitation in the proximal colon, which resulted in enhancement of weak ripples that propagated in oral direction. Ripples were orchestrated by ICC-SMP pacemaker cells and occurred at a frequency of 7–15 cycles min^{-1} with their force of contraction regulated by distention and the enteric nervous system. The LDC were inhibited but not abol-

ished by atropine indicating an important but not exclusive role for cholinergic motor neurons. In the presence of TTX, LDC's did not occur indicating their neurogenic origin. LDC's also occurred without proximal distention and then appeared rhythmic in nature with intervals of approximately 90 s. In atropine, TTX and lidocaine, rhythmic proximal contractions did occur suggesting that the pacemaker behind rhythmic spontaneous LDC's was myogenic.
Conclusion: Fluid distention of the proximal colon initiates a neurogenic LDC, characterized by a propagating and then sustained contraction preceded by a wave of relaxation. The pacemaker behind rhythmic spontaneous LDC's might be myogenic. This is the most effective propulsive motor pattern of the rat colon.



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Evidence for negative effects of Ethanol upon the enteric nervous system during pregnancy and in the regenerating postnatal gut
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Objective: Ethanol is a toxic agent which, as part of alcoholic beverages, is responsible for severe damage in different parts of the body. Whereas brain and liver are in the focus of alcohol research, little is known about its effects on gut motility and especially its intrinsic gastrointestinal innervation: the enteric nervous system (ENS). Especially the developing ENS can be affected and ethanol consumption during the pregnancy might lead to severe disturbances of the ENS, with subsequent functional disorders throughout the life.
Methods: The effect of various ethanol concentrations was investigated in various pre- and postnatal approaches. To investigate ethanol effects during development, embryonic gut from GFP-Nestin transgenic mice was exposed to different ethanol concentrations and the migration of neural stem cells evaluated. Additionally, individual experiments were performed in fertile chicken eggs, where ethanol was injected into the yalk sak. Neural crest derived stem cells were cultured under the influence of increasing ethanol concentrations. Postnatal myenteric plexus was isolatated, dissociated and cultured under the influence of ethanol. Here neurite length after 24 h, respectively the density of neurite networks after 5 days *In vitro* were evaluated.
Results: Ethanol changed both migration and neurite outgrowth in both pre- and postnatal ENS. The migration distances, as well as migration patterns were changed in both the chicken egg model, as well as in the embryonic mouse gut. Neural crest derived stem cells showed an increased proliferation rate at several ethanol concentrations. Neurite outgrowth, as well as neurite density were reduced with increasing ethanol concentrations.
Conclusion: Ethanol obviously interferes with the development of the ENS and might also alter postnatal regeneration rates of enteric neurons and networks.

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Age-relared change in cholinergic and nitrergic signaling in the mouse rectum
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Objective: Gastrointestinal disorders, including chronic constipation and faecal incontinence, increase with age leading to a reduction in the quality of life and a loss of independence. The rectum is known to play a key role in the storage of faecal matter and to initiate the defecation reflex, however little is known of the pharmacology of the rectum and how this changes with age.
Methods: Whole rectum from different aged C57BL/6 mice (3–4, 12–14, 18–20 and 24–26 months) were suspended in an organ bath in and longitudinal muscle contraction recorded following electrical field stimulation (EFS; 40 V; 1 ms pulse width; 0.1–25 Hz) and exogenous application of acetylcholine (ACh) and potassium chloride (KCl). Nitrergic responses were identified by their sensitivity to N?-Nitro-L-arginine (L-NNA) and cholinergic responses identified by their sensitivity to scopolamine. To examine the direct effects of nitric oxide on smooth muscle, dose-response curves to sodium nitroprusside (SNP) were performed in carbachol pre-contracted muscle.
Results: KCl induced a contraction of longitudinal muscle that significantly increased with age ($P < 0.01$). EFS evoked frequency-dependant responses in the rectum that were affected by age. A significant L-NNA-sensitive component was only recorded in 3 month animals ($P < 0.05$). In contrast, with increasing age a significant scopolamine-sensitive component was observed in 12, 18 and 24 month tissue ($P < 0.05$, $P < 0.001$ and $P < 0.01$, respectively). Responses of rectum to exogenous ACh did not change with age, however, responses to SNP were significantly reduced ($P < 0.05$).
Conclusion: We have shown an age-related decrease in nitrergic signalling and an increase in cholinergic signalling in the murine rectum. The rectal longitudinal muscle is important for driving appropriate defecation and an age-related change in the pharmacology of this tissue region may affect the defecation reflex.

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Fast (kHz) imaging with improved sensitivity and spatial resolution reveals stepwise Ca2+ increases in individual varicosities
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Objective: Calcium imaging is a powerful tool to record neuronal activity. Recording at kHz frame rates, extends the classical possibilities as individual $Ca2^+$ steps associated with action potential spikes can be resolved1. Unfortunately fast imaging goes at the expense of spatial resolution. 1Michel et al. J.Phys. 2011 Our aim was to record fast calcium events in individual varicosities in the mouse enteric nervous system using a 2 kHz CMOS camera with improved spatial resolution (512 × 512 pixels).
Methods: Mouse large intestines were dissected, loaded with Fluo4-AM and visualized under an upright microscope.
Results: First, we investigated whether improved spatial resolution would make it possible to assess signal propagation in neuronal fibers. We stimulated fiber tracts with a single electrical pulse, classically used to elicit fast excitatory postsynaptic potentials. By recording at 2kHz, we were able to continuously monitor the signal as it travelled through ganglia and connecting fibers and calculated propagation speeds of about 80 mm s^{-1} (7 ganglia, 4 mice). Secondly, we show that we have sufficient sensitivity and resolution in space and time to resolve calcium steps associated with a 20 Hz pulse train stimulus in individual varicosities (30 varicosities, 7 ganglia, 4 mice). The frequency was confirmed using Fourier transforms and varicosity localization using high resolution CCD camera recordings. Removal of extracellular $Ca2^+$ (0 m mol L^{-1} $Ca2^+$, 2 m mol L^{-1} EDTA) reduced the amplitude by 50% (4 ganglia, 2 mice) and TTX (10^{-6} mol L^{-1}) abolished the responses (4 ganglia, 3 mice), indicating the need for action potential firing. Finally, we also applied serotonin (5-HT) via a local spritz pipette to test whether, by analogy with nicotine1, 5-HT would increase intracellular calcium in a stepwise manner. We detected 2 to 3 steps of 50–200 ms duration at the onset of the $Ca2^+$ transients (4 ganglia, 4 mice), which were reduced down to 45.2% by ondansetron (10^{-5} mol L^{-1}, 4 ganglia, 2 mice).
Conclusion: Improved sensitivity and spatial resolution further adds to the possibilities of Ca2+ imaging in that signal propagation speed can be measured and stepwise Ca2+ increases associated with action potentials can be monitored in individual varicosities. This technique may be used to investigate the timing of pre- and postsynaptic signaling.

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Enteric glia mediate ion transport abnormalities in mouse colon during colitis
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Objective: Enteric glia are an important functional component of the enteric nervous system. Enteric glia play a role in cholinergic regulation of epithelial ion transport. However, the role of enteric glia in regulating ion transport during colitis is not well understood. Using a model of glial metabolic inhibition, we investigated the role of enteric glia in neurogenic regulation of ion transport in colitis.
Methods: Experiments were conducted in distal colon from wild-type CD1 mice. Colitis was induced using Dextran Sodium Sulfate (DSS; 5% w/v) dissolved in drinking water (5d DSS/2d water). Full-thickness colonic segments mounted in Ussing chambers were held under voltage-clamp conditions. The net electrogenic movement of ions across the epithelium was recorded as short-circuit current (ISC). Tissues were treated with the glial metabolic poison fluoroacetate (FA; 5 m mol L^{-1}; 120 min) and stimulated with electrical field stimulation (EFS; 50 V, 10 Hz, 5 s) in the presence or

absence of nitric oxide synthase (NOS) I & II inhibitors.
Results: Electrical field stimulation of colonic segments resulted in a monophasic increase in ISC (ΔISC, 44 ± 7 μA cm^{-2}, n = 12) that was unaffected by FA treatment (39 ± 6 μA cm^{-2}, n = 14), a NOS I inhibitor (Control: 35 ± 9 μA cm^{-2}, n = 4; FA: 38 ± 7 μA cm^{-2}, n = 4) or a NOS II inhibitor (Control: 30 ± 4 μA cm^{-2}, n = 6, P > 0.05; FA: 30 ± 4 μA cm^{-2}, n = 6, P > 0.05). In animals with colitis, there was no longer a response to EFS (3 ± 2 μA cm^{-2}, n = 14). FA treatment of inflamed colon reversed the inhibition of the EFS response (27 ± 5 μA cm^{-2}, n = 12; P < 0.05). The addition of a NOS II inhibitor (but not a NOS I inhibitor) also partially restored the response to EFS (24 ± 5 μA cm^{-2}, n = 8; P < 0.05). Together, FA and a NOS II inhibition normalized the response to EFS to control levels (39 ± 7 μA cm^{-2}, n = 6; P < 0.01).
Conclusion: Under physiological conditions, the activation of enteric neurons regulates ion transport in a glial-independent manner. During colitis, enteric glia inhibit ion transport, which is reversed through the metabolic inhibition of enteric glia and the inhibition of nitric oxide production. Our data show that enteric glia contribute to the dysregulation of ion transport during colitis.

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Effect of oxytocin on the chemical sensitivity of the mysenteric afferent fibers in rats *in vitro*
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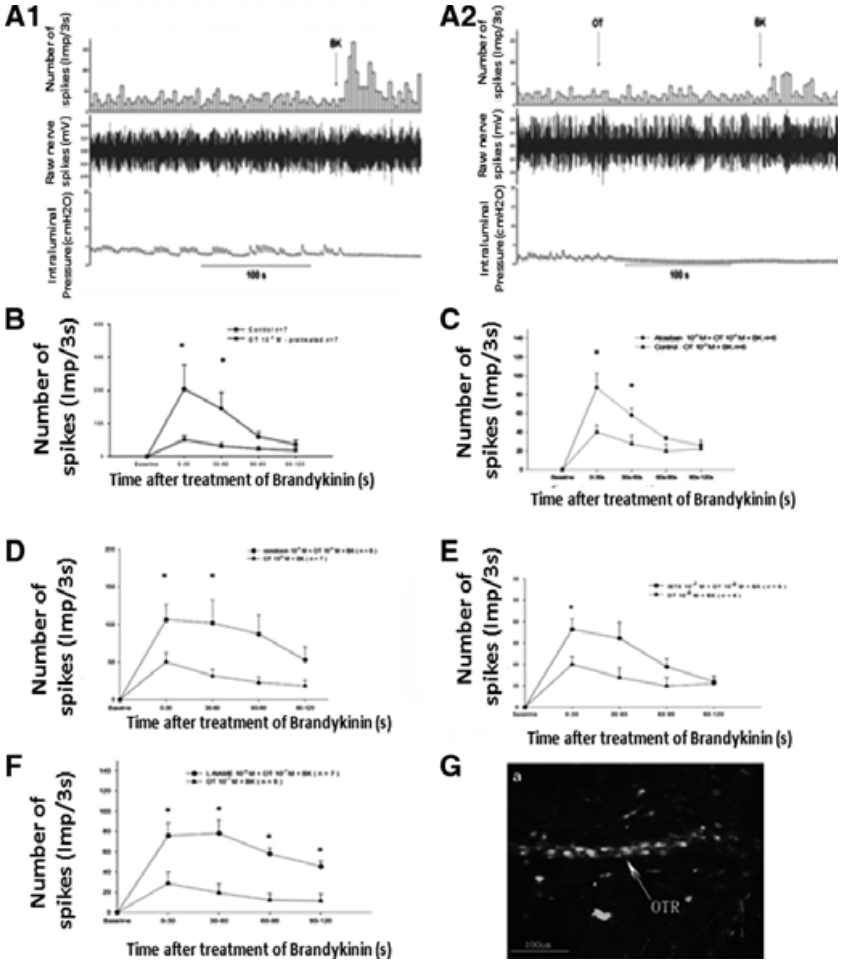
Objective: The purpose of this study was to investigate the effect of OT on the chemical sensitivity of afferent mesenteric nerve of the jejunum of the rat.
Methods: The mesenteric nerve was prepared from a 2-cm segment of jejunum in rat *In vitro* and the spikes were recorded by a polygraph.
Results: Both of 5-HT (250 μ mol L^{-1}) and bradykinin (BK, 0.5 μ mol L^{-1}) induced a increase of the afferent spikes. Administration OT (10^{-6}/10^{-7}/10^{-8} mol L^{-1}) did not influence the spontaneous afferent activity and afferent sensitivity to 5-HT but significantly decreased that to BK. With the pretreatment of OT (10^{-6} mol L^{-1}/10^{-7} mol L^{-1}) for 2 min, the maximal response of the afferent spikes to BK was decreased (59 ± 14 vs. 22 ± 7 spikes sec^{-1}; 75 ± 16 vs. 28 ± 10 spikes sec^{-1}; P < 0.05). The pretreatment of OT receptor antagonist atosiban, ω-conotoxin GVIA, iberiotoxin, NG-Nitro-L-arginine Methyl Ester (L-NAME) before OT (10^{-6} mol L^{-1}/10^{-7} mol L^{-1}) respectively partial reversed the maximal response of the afferent spikes to BK (P < 0.05). Oxytocin receptor (OTR) in duodenum enteric nervous system (ENS) was located by immunofluorescence staining.
Conclusion: Because BK excites the endings of the spinal afferent fibers which encode the noxious stimulation in the GI tract, we believed that OT might downregulate the visceral nociception through releasing NO.



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Expression of PrPC in glial cells of the cat intestinal tract
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Objective: Feline spongiform encephalopathy (FSE) is a fatal neurodegenerative disease affecting Felidae and belongs to transmissible spongiform encephalopathies (TSEs). FSE of domestic cats is thought to result from ingestion of bovine spongiform encephalopathy (BSE) contaminated food, whereas prion diseases of wild Felidae is due to ingestion of BSE-infected carcasses. TSEs are caused by pathological isoforms (PrPSc) of the constitutive prion protein (PrPC). A crucial factor for the initiation of infection is PrPC expression in the host cells. In acquired TSEs, the gastrointestinal tract is the main prion entry site. Thus, in order to better understand the early pathogenesis of FSE prion infections, this study was aimed to characterize the cell types which express PrPC in the cat gastrointestinal tract.
Methods: We performed an analysis of the distribution of PrPC-immunoreactivity (PrPC-IR) in cryosections of the cat duodenum, ileum and colon. Specifically, the co-localization of PrPC-IR with glial cells and neurons was tested by applying appropriate antibodies targeting glia and neurons. Single and double labeling experiments of indirect immunofluorescence were performed in gut segments as indicated above. PrPC was identified using the mouse anti PrPC monoclonal antibody (MAB1562, Millipore); enteric neurons were identified with the two pan-neuronal markers anti-protein gene product 9.5 (PGP 9.5) (AB176, Millipore) and anti-human neuronal protein (Hu) (20064, Invitrogen). Enteric glial cells were identified with antibodies anti-fibrillary acidic protein (GFAP) (AB5804, Millipore).
Results: PrPC-IR was mainly observed in enteric GFAP-IR glial cells. PrPC-IR glial cells surrounded myenteric and submucosal neurons lacking PrPC immunolabeling. PrPC-IR enteric glial cells were detected in ganglionated plexuses of the investigated gut segments.
Conclusion: The cat glial cells constitutively express PrPC. This finding indicates that glia in this species may be a selective target for infectious prions. Likely, enteric glial cells may be targeted by prions (PrPSc) not only via oral infection (i.e. first-step of neuroinvasion), but also as a result of a centrifugal spread of prions from central to the enteric nervous system in FSE.

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Edema-induced PAK activation negatively regulates MLC phosphorylation and inhibits intestinal motility

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Objective: Intestinal edema decreases intrinsic smooth muscle contractility via decreased myosin light chain (MLC) phosphorylation resulting in decreased motility that contributes to ileus in a trauma/resuscitation model. Intestinal edema also causes increased smooth muscle stress resulting in increased smooth muscle cell stretch. Activity of p21-activated kinase (PAK), a kinase that regulates myosin light chain (MLC) phosphorylation, was increased in edematous intestine and inhibition of PAK improved intestinal motility in edematous intestine. We hypothesize that increased intestinal smooth muscle stretch during edema development induces increased PAK activity resulting in decreased MLC phosphorylation. The objective of the current study was to investigate the effects of increased intestinal smooth muscle stretch on MLC phosphorylation and PAK activity.
Methods: We developed a cyclical stretch primary human intestinal smooth muscle cell (hISMC) model which mimics the *in vivo* intestinal edema model: control cyclical stretch (CCS) mimics the control, non-edematous conditions and edema cyclical stretch (ECS) mimics edematous conditions. We measured the effects of stretching on PAK activity and phosphorylation of myosin light chain (MLC) and the myosin targeting subunit of myosin light chain phosphatase (MYPT1). PAK constitutively active (ca) and kinase negative (kn) plasmids and specific PAK activators and inhibitors were utilized to determine the role of PAK in MLC phosphorylation.
Results: Subjecting hISMC to ECS significantly increased PAK activity and decreased both MLC and MYPT1 phosphorylation compared to CCS. Dose response curves using BPIPP, a PAK activator, and IPA-3, a PAK inhibitor, demonstrated a biphasic regulation of MLC phosphorylation by PAK. Transfection of hISMC with ca-PAK and kn-PAK before stretching in the CCS group resulted in increased MLC phosphorylation and decreased MLC phosphorylation, respectively. In contrast, transfection with kn-PAK prevented the decreased MLC phosphorylation induced by ECS but transfection with ca-PAK had no effect in the ECS group.
Conclusion: We conclude from these data that increased stretch of intestinal smooth muscle cells during edema development induces PAK activity which causes decreased MLC phosphorylation via decreased MYPT1 phosphorylation. Furthermore, we conclude that PAK regulation of MLC phosphorylation is biphasic with lower activity positively regulating and higher activity negatively regulating MLC phosphorylation.

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The influence amyloid-ß Protein upon Enteric Neurons - A possible diagnostic tool for early diagnosis in Alzheimer's disease

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Objective: Alzheimer's disease (AD) is characterized by fatal neuronal loss. Accumulation of amyloid-ß (Aβ) proteins between nerve fibers is a typical sign during development of AD, while the exact mechanisms underlying Aβ deposition remains unclear. While no curative treatment is developed until now an early diagnosis is fundamental to prevent further neurodegeneration. In case that the enteric nervous system (ENS) would also be affected by the amyloidosis, changes of the ENS could be used as a potential diagnostic tool.
Methods: Aß was generated with transgene neuroblastoma cell lines. Conditioned Aβ media of different compositions and concentrations was used for all experiments. Media with over-expressed Aß 42 protein, over-expressed Aß 40 protein, and control media were used. The jejunum of healthy adult rats was ligated and operatively removed with the superior mesenteric artery. In an organ bath approach the gut segment was perfused with the amyloid media through the mesenteric artery. Gastrointestinal motility changes were monitored and evaluated, as well as pressure changes within the gut segment. To investigate the toxic effect upon the ENS isolated rat myenteric plexus was cultured in the presence of the different Aβ conditioned media and analyzed concerning the density of neuronal fibers and surviving neurones.
Results: The perfusion of the gut through the mesenteric artery with Aβ conditioned media demonstrated a dramatically increase in the muscle tonus, combined with a decrease of the gut diameter, indicating an spasmodical contraction in comparison to control. The most significant effect was to be seen with Aβ 42 media where the diameter reduction was about 40%. In the cell culture experiment Aβ induced to a drastically decline in the density of neuronal fibers compared to neurons cultured in control media.
Conclusion: Aβ leads to alteration in the enteric nervous system as well as in gastrointestinal motility. This is a strong evidence for the involvement of the gastrointestinal tract in the course of AD. This might open up a new perspective for the early diagnosis of the disease using the ENS.

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Spatial analysis of the enteric nervous system of GFP-Nestin expressing mice

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Objective: The capability of the enteric nervous system to self-renew and using it as a neural stem cell source is an exciting matter discussed by several recent studies. We studied the enteric nervous system from different parts of the gastrointestinal tract of adult GFP-Nestin transgenic mice to estimate the neural stem cell potential in the individual segments of the gastrointestinal tract.
Methods: Whole mounts from adult GFP-Nestin transgenic mice were dissected from stomach, duodenum, terminal ileum, cecum and colon. The whole mounts were either used for myenteric plexus isolation or stained with either S100 or PGP 9.5 to be able to assess the nestin ratio To investigate the ENS *In vitro*, we have established a method to isolate the myenteric plexus from all parts of the gastrointestinal tract of adult mice without using any cell sorting techniques. The muscular layer was stripped from the submucosar layer, followed by enzymatic digestion with purified collagenase. Subsequently a final mechanical disruption step was performed. The MP-networks were used to generate neurospheres. The neurospheres were differentiated and the cellular composition was visualized by immunofluorescence staining again.
Results: Nestin positive cells are present in the muscle layer and the submucosal layer as well as the mucosal layer from the stomach to the distal part of the colon. We identified mainly nestin/S100b double positive glial cells throughout the gastrointestinal tract. Nestin PGP 9.5 double positive neurons occur in the duodenum, jejunum, ileum, caecum and colon. Neurospheres can be generated from all parts of the gastrointestinal tract and the spheres can be differentiated into glial and neuronal lineages. The myenteric plexus from the colon seems to be the most effective part of the GIT, concerning its neural stem cell potential, related to myenteric plexus yield per area.
Conclusion: Our results demonstrate that the ENS is a prospective neural stem cell source and it is possible to isolate the myenteric plexus selectively for transplantation experiments. The isolation of the myenteric plexus in an intact form is also a promising approach to analyze proteomic changes of the myenteric plexus during different intestinal conditions such as the influence of microflora or inflammatory bowl disease.

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Effects of oral administration of rotenone on the enteric nervous system

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Objective: The present study was designed to assess the effects of a chronic oral treatment by rotenone on enteric neurochemical phenotype and gastrointestinal (GI) motility.
Methods: Male C57BL6N mice received once daily oral rotenone administration for 28 days. GI motility was assessed by measuring gastric emptying, total transit time, fecal pellet output, and bead latency. Intestinal barrier permeability was both *in vivo* and ex vivo using Ussing chambers. The number of enteric neurons as well as the enteric neurochemical phenotype was analyzed by immunohistochemistry in the jejunum, ileum and proximal colon. Tyrosine-hydroxylase (TH) immunostaining of dopaminergic neurons of the substantia nigra was performed in a subset of animals.
Results: Mice treated orally with rotenone did not display any gross GI dysfunction. The only abnormality observed was a decrease in stool frequency that likely reflects a mild slowing in colon motility. The paracellu-

lar permeability of the IEB was not altered in mice treated orally with rotenone. A striking result of the present survey is the presence of neurodegeneration in CNS of mice treated with rotenone. Moreover the expression of alpha-synuclein in the jejunum was significantly lower in the jejunum of mice treated with rotenone.
Conclusion: Chronic oral treatment with rotenone induced only minor changes in the ENS and did not recapitulate the GI abnormalities seen in PD, while it replicated neurodegeneration of the substantia nigra.

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Differential regulation of 5-HT and neurotensin expression in rat colonic mucosal enteroendocrine cells by brain derived neurotrophic factor
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Objective: Brain Derived Neurotrophic Factor (BDNF) is present in enteric neurons and enteroendocrine cells (EEC) where it acts acutely to facilitate the release of serotonin (5-HT) from enterochromaffin cells and calcitonin gene-related peptide (CGRP) from enteric sensory neurons, thereby enhancing the peristaltic reflex. Little is known of the long-term role of BDNF in the gut. The Aim of this study was to determine the effect of reduced BDNF levels on the phenotype of key EEC in the rat colon.
Methods: Segments from the midcolon of BDNF heterozygous rats (BDNF+/−) and wild type (WT) rats were fixed and immunostained for Chromogranin A (Chr-A) (sc-1488; 1:100), 5-HT (sc7592; 1:200) and neurotensin (NT) (ImmunoStar 20079; 1:200). For Chr-A and 5-HT, the number of positive cells per crypt were counted; for NT, the number of crypts containing a positive cell were counted. In each section, 100 crypts were examined. Chr-A was chosen as a general EEC marker. 5-HT was chosen because of the acute interaction between BDNF and 5HT, and because we have previously shown that about 50% of 5-HT positive EEC co-express BDNF and about 30% of BDNF positive EEC co-express 5-HT. NT was chosen because of its role in motility of the distal gut and because NT and BDNF interact with a common receptor, the NT-3 or sortilin receptor, which is also present in colonic EEC.
Results: The architecture of colon was similar in wild type and BDNF+/− rats: there was no difference in the depth of mucosal crypts, or in length or thickness of muscularis mucosa. There was no significant difference in the number of Chr-A-positive EEC (WT: 5.8 ± 0.5 cells/crypt; BDNF+/−:6.5 ± 1.8 cells/crypt). In contrast, reduction in BDNF levels resulted in a significant decrease in 5-HT-positive EEC (WT:2.8 ± 0.2 cells/crypt; BDNF+/−:0.6 ± 0.1 cells/crypt; $P < 0.01$) and a significant increase in NT-positive cells (WT:20 ± 3% NT-positive crypts; BDNF+/−:50 ± 12% NT-positive crypts; $P < 0.05$).
Conclusion: We conclude that BDNF does not affect entry of mucosal stem cells into the EEC lineage because there is no difference in numbers of chromogranin-A positive cells. Rather, the changes in EEC phenotype in BDNF+/− rats suggests that BDNF promotes the expression of 5-HT and inhibits the expression of neurotensin.

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Evolution of the postnatal phenotypical maturation of glial cells in the enteric nervous system
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Objective: The postnatal period is crucial for the development of gastrointestinal (GI) functions. The enteric nervous system (ENS), composed of neurons and enteric glial cells (EGC), is a key regulator of GI functions. Increasing evidences demonstrate that (i) postnatal phenotypical maturation of enteric neurons controls the development of GI functions, and (ii) microbiota derived short chain fatty acids such as butyrate can be involved in this maturation. Although EGC are central regulators of GI functions, the postnatal evolution of their phenotype remains unknown. Therefore, we aimed to characterize in rat pups (i) the postnatal evolution of EGC phenotype in the colon (ii) the impact of butyrate upon their phenotype and (iii) transcriptional networks involved in postnatal or butyrate-induced EGC maturation.
Methods: The expression of glial markers (GFAP; S100b) and of transcription factors of interest such as Sox10 was analysed by RT-qPCR and immunohistochemistry in the colon of rat pups between 1 and 36 days after birth. *In vitro* analysis, using primary culture of ENS were performed to evaluate the impact of butyrate (500 μ mol L^{-1}) upon glial maturation.
Results: We showed that mRNA and protein expressions of the glial markers GFAP and S100b strongly increase within five weeks after birth. Moreover, major modifications in the cellular localization of these markers take place with expression becoming more pronounced in glial processes over time and the glial network appearing fully organized only 36 days after birth. Using a transcriptomic approach, we showed that mRNA expression of the glial-specific transcription factor Sox10 strongly increases during the postnatal period whereas expression of TFAP2a rapidly decreases over the same period. Analysis of TFAP2a expression in primary culture of ENS indicated that this transcription factor is specifically expressed in differentiated EGC. Moreover its expression is down-regulated after treatment with butyrate in primary culture of ENS while butyrate increased the expression of GFAP and S100b in this model.
Conclusion: These results demonstrate that the enteric glial network continues to set up after birth and suggest that TFAP2a might be involved in this maturation. The factors, in particular of nutritional origins, and the mechanisms responsible for enteric glial maturation *in vivo* remain to be identified.

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Glutathione promoves myenteric neuroprotection in the jejunum of diabetic rats
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Objective: Diabetic neuropathy is involved in changes of enteric neurons, primarily attributed to oxidative stress and reduction of endogenous antioxidants. The objective of this study was to investigate the effects of supplementation with 1% L-glutathione and 2% L-glutamine in the myenteric innervation of the jejunum of diabetic rats.
Methods: Forty-two male rats (Rattus norvegicus), 90 days old and 317.03 ± 16.29 g weight were used. They were randomly divided into six groups: normoglycemic (N), normoglycemic supplemented with L-glutamine (NG), normoglycemic supplemented with L-glutathione (NGT), diabetic (D), diabetic supplemented with L-glutamine (DG) and diabetic supplemented with L-glutathione (DGT). After 120 days, the jejunum were collected and processed for immunohistochemistry double staining of HuC/D protein, and neuronal nitric oxide synthase (nNOS) of myenteric plexus. Quantitative per unit area and the ganglion immunoreactive neurons HuC/D (HuC/D-IR) and nNOS (nNOS-IR) were measured.
Results: The disease was confirmed in animal D through elevated blood glucose levels and weight loss. The results of neuronal density are shown in Table 1.
Conclusion: 1% L-glutathione has been more effective compared to 2% L-glutamine, due greater neuroprotection observed in the total population of myenteric neurons of the jejunum in diabetic rats.

Table 1 - Density per unit area (cm²) and myenteric ganglion neurons HuC/D-IR and nNOS-IR normoglycemic (N), normoglycemic supplemented with L-glutamine (NG), normoglycemic supplemented with L-glutathione (NGT), diabetic (D), diabetic supplemented with L-glutamine (DG) and diabetic supplemented with L-glutathione (DGT).

Groups	Density per unit area (cm^2)		Density per ganglion	
(n=7)	HuC/D	nNOS	HuC/D	nNOS
N	13469.70±610.95^{a}	3455.63±186.66^{a}	36.62±0.80^{a}	9.76±0.25^{a}
NG	13858.23±296.75^{a}	3264.07±146.73^{a}	28.14±0.81^{b}	6.38±0.21^{b}
NGT	13680.73±264.16^{a}	3573.59±171.51^{a}	28.73±0.76^{b}	7.19±0.23^{b}
D	7931.82±469.32^{b}	2870.13±234.19^{a,c}	25.77±0.64^{c}	7.58±0.21^{c}
DG	9512.98±348.49^{b,c}	1719.70±93.75^{b,d}	23.00±0.65^{c,d}	4.87±0.16^{d}
DGT	11059.52±470.10^{c}	2349.57±162.52^{c,d}	29.27±0.68^{d}	6.19±0.17^{e}

Mean values followed by different letters in the same column are statistically different according to Tukey test ($p < 0.05$). Results were expressed as mean ± standard error.

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Myenteric neuron numbers are maintained in aging mouse distal colon

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Objective: The incidence and severity of conditions such as faecal incontinence, impaction and chronic constipation increase with age. Previous studies have shown that there is a loss of cholinergic myenteric neurons during aging in several different species. However, it is unclear whether all cholinergic neurons or a specific functional subgroup is susceptible to this age-related degeneration. Therefore, we wanted to analyse myenteric neuronal changes in mouse distal colon during aging in detail, specifically to determine if (i) one cholinergic subpopulation, the intrinsic sensory neurons, and (ii) nitrergic neurons are susceptible to age-related neuronal loss.
Methods: Antisera to calbindin, nNOS and Hu C/D were used to identify intrinsic sensory neurons, nitrergic neurons and total neurons respectively in whole-mounts from 3–4, 12–13, 18–19 and 24–25 month-old C57BL/6 mice. Neuronal numbers were counted manually in confocal Z stacks using new methods to ensure representative and unbiased sampling. Counts were corrected for both gut growth and changes in sample dimensions during processing.
Results: No statistically significant changes were detected in total neuronal numbers or numbers of either subgroup investigated with increasing age. The density of myenteric neurons decreased between 3–4 and 12–13 months however, because of gut growth. There was evidence of neuronal atrophy; swollen calbindin- and nNOS-immunoreactive (IR) nerve fibres were common in 18–19 and 24–25 month old animals. The density of nNOS-IR nerve fibres in the tertiary plexus increased significantly with age, up to 18–19 months ($P < 0.001$).
Conclusion: These results suggest that aging does not result in a loss of total, intrinsic sensory or nitrergic neurons in C57BL/6 mouse colon at the ages studied, although neuronal atrophy was observed. The increase in nNOS-IR nerve fibre density suggests that some generation of neurites occurs with age. It is however evident in these mice that some intrinsic colonic reflexes change during aging1. Therefore we speculate that the decline in gut function with age may be in part due to changes in the properties of neurons, rather than neuronal loss 1. Honey M.; Yeoman M. S.; et al. (2011) Neurogastroenterol Motil: 23(S1) 48.
Acknowledgements: This work was supported by an Open University Charter Studentship to P.P.K.M. Gamage, and by BBSRC 'Ageing bladder and bowel' grant numbers BB/G015988/1 to MJS and RR and BB/G015147/1 to MY.

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Changes in the enteric stem cell niche of children with Hirschsprung's disease

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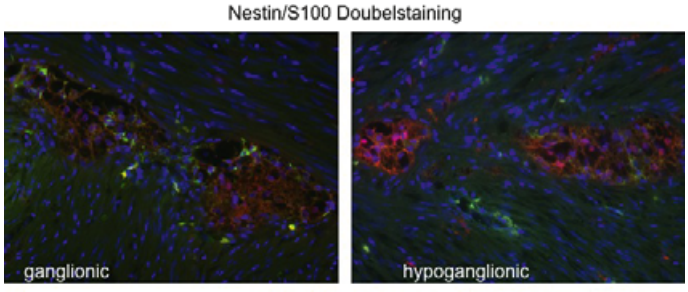
Objective: The transplantation of neural crest derived stem cells (NCSC) is a potent alternative for the treatment of Hirschsprung's disease (HSCR). Ideally, the cells transplanted are derived from progenitors within the gut. To gain further insight in possible stem cell niches in human colon from infants with Hirschsprung's disease was investigated with a panel of stem cell markers.
Methods: The tissue samples from ganglionic, aganglionic and transient segments were immuonstained either for S100/nestin, S100/p75 or GFAP/nestin. Altogether twenty-five tissue samples from infants with Hirschsprung's disease were investigated along the gut axis.
Results: In all samples investigated, nestin positive ganglia could be found, even in the distal parts were a severe hypo- or aganglionosis was verified. Beside nestin positive cells in all segments, there were also different expression pattern of glial markers within the ganglia, indicating that distinct phenotypes of glia cells could be found.
Conclusion: Neural and glial precursor cells are present in the ganglionic as well as in the hypoganglionic segments of Hirschsprung's colon, suggesting that these cells might be suitable for NCSC generation.



Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-19 Basic and Translational Session: Small Intestine, Colon and Aanorectum: Physiology and Pathophysiology

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Ischemia/ reperfusion damage in human intestine involves inflammation and changed expression of nitric oxide synthases

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Objective: Intestinal ischemia/reperfusion (I/R) injury is a serious clinical problem arising from transient disruption of blood supply due to disease, trauma or as a consequence of abdominal surgery and can lead to severe morbidities, even death. Clinical sequalae of I/R are increasingly recognised, as intestinal transplantation has become a treatment of choice for patients with intestinal failure and complications of parenteral nutrition. We have developed a unique protocol to investigate I/R damage in human intestine.
Methods: I/R was applied to a segment of jejunum in patients undergoing pancreatectomy (Whipple's procedure). During the surgery the blood supply to a 5–8 cm segment was occluded for 30 min while the adjacent segment provided control samples. After the occlusion, the clamp was removed and blood flow was restored for 2 h before control and I/R segments were removed and analysed.
Results: Histological examination revealed that segments subjected to I/R show significant neutrophil infiltration ($P < 0.05$) and minor damage to the epithelium and muscle. Concomitant with this observation, mRNA levels of major inflammatory cytokines show significant increase in I/R segments when compared to control segments including interleukin(IL)-1 ($P < 0.0001$), IL-6 ($P < 0.0001$) and IL-8 ($P < 0.001$). To assess the involvement of nitric oxide (NO) as a mediator of oxidative stress damage, the expression of genes involves in its production (nitric oxide synthases) and clearing was analysed. In segments subjected to I/R the expression of neuronal NOS decreased ($P < 0.05$), endothelial NOS increased ($P < 0.05$) and inducible NOS tended to increase. The analysis of anti-oxidant enzymes showed that in the external muscle layers SOD2 expression was doubled ($P < 0.001$) while GPX 7 and 8 were halved ($P < 0.001$).
Conclusion: This study shows clearly for the first time in human intestine tissue that following ischemia/reperfusion (i) an inflammatory response begins rapidly after the ischemic event; (ii) cytokines detected in the intestine tissue are produced locally in the early stages of the inflammatory cascade, but do not diffuse from the systemic circulation; and (iii) nitric oxide is

involved in the tissue reactions to I/R. These findings highlight the need to further characterise the extremely complex association between I/R injury, inflammation and NO-induced oxidative stress.

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Post-junctional cell types involved in inhibitory neuromuscular transmission in murine internal anal sphincter

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Objective: Inhibitory neuromuscular transmission (NMT) in the internal anal sphincter (IAS) includes purinergic and nitrergic neural components. Purinergic NMT persists in the W/Wv mouse IAS, but nitrergic hyperpolarization is reduced. The identity of the post-junctional cells mediating nitrergic and purinergic components of NMT is still uncertain. Intramuscular interstitial cells of Cajal (ICC-IM) are recognized as participants in nitrergic NMT. More recent studies suggest an additional role in inhibitory NMT for a second class of interstitial cells that express the receptor tyrosine kinase PDGFRα (PDGFRα+ cells). We characterized expression of post-junctional transduction and effector proteins involved in nitrergic and purinergic NMT in ICC and PDGFRα+ cells in the IAS of C57BL/6 and W/Wv mice.
Methods: Dual labeling immunohistochemical techniques and confocal microscopy were used to examine the distributions of two soluble guanylate cyclase isoforms (GCα/β), cGMP-dependent protein kinase I (PKGI), and small conductance Ca2+ activated K+ channels (SK3) in ICC and PDGFRα+ cells within the IAS.
Results: Intramuscular PDGFRα+ cells (PDGFRα+-IM) expressed GCα/β, but immunoreactivity for these proteins was not detected in myenteric, serosal or submucosal PDGFRα+ cells. Intense GCα/β labeling was also observed in vascular smooth muscle cells, whereas relatively faint uneven labeling was detected in IAS smooth muscle cells. GCα/β labeling was also observed in a subpopulation of ICC-IM. PKGI immunoreactivity was observed in myenteric ganglia and intramuscular nerve fibers but was not resolved in PDGFRα+-IM or ICC-IM. PDGFRα+-IM were SK3-positive whereas immunoreactivity for this protein was not detected in ICC-IM. Labeling differed in W/Wv mice in that ICC-IM were absent in these animals.
Conclusion: The persistence of both functional purinergic responses and SK3-expressing PDGFRα+ cells in W/Wv mice is compatible with a role for these cells in purinergic NMT. In contrast, whereas GCα/β were expressed in PDGFRα+-IM and ICC -IM, PKGI, normally associated with nitrergic NMT, was not detected in either of these cells. Thus the pathways leading to nitrergic NMT may be more complex with two classes of interstitial cells involved as well as possibly a PKGI-independent pathway. Grant support DK 078736.

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Analysis of changes in the cells of the mouse internal anal sphincter during aging

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Objective: Aging is associated with increased incidence of gastrointestinal dysfunction, including constipation, faecal incontinence and impaction. The causes of age-related dysfunction are complex and multifactorial, but are likely to include cellular changes in the gut during aging. The internal anal sphincter (IAS) has a critical role in regulation of defecation, but little is known of cellular age-associated changes in this part of the gut. The aim of this work is therefore to characterise the changes that occur in the cells of the mouse IAS during aging.
Methods: Samples from C57BL/6 mouse anal sphincter at 3–4, 12–13, 18–19 and 24–25 months of age were studied by conventional electron microscopic techniques and by immunofluorescence microscopy using antisera to neuronal nitric oxide synthase (nNOS), vasoactive intestinal peptide (VIP), substance P (SP) and PGP9.5 to identify subpopulations and total nerve fibres in tissue sections. Changes in Interstitial Cells of Cajal (ICC) were studied by confocal analysis of wholemount preparations of the IAS from 3–4 and 26–28 month mice immunolabelled with antiserum to c-kit.
Results: Semi-quantitative ultrastructural analysis of the smooth muscle layers and myenteric plexus indicate that degenerative changes occur in subpopulations of cells in IAS during ageing. Different types of inclusions are observed and present in increased numbers in samples from older animals. The density of nNOS- and SP- immunoreactive (IR) nerve fibres were significantly reduced in the circular muscle of the IAS of older animals, but no significant change in VIP-IR or total nerve fibre density was detected in this muscle. Mucosal VIP and SP-IR fibres were reduced in older animals. ICC volume was significantly reduced in the circular and longitudinal muscles and around the myenteric ganglia of old mice.
Conclusion: Ageing is associated with changes in the smooth muscle, ICC and innervation of the IAS. Reduction in the density of immunoreactive nerve fibres could result from reduction in the expression of the markers studied, or to neuronal degeneration. These results indicate that age-associated changes in the cells of the IAS are likely to contribute to changes in ano-rectal function in the elderly. Supported by BBSRC 'Ageing bladder and bowel' funding: BBSRC grant BB/G015988/1to MJS & RR and BB/G015147/1 to MY. PPMK Gamage is supported by an Open University Charter Studentship.

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The anal rectalfilling sensation receptor

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Objective: To evaluate the role of anal receptors in rectal filling sensation.
Methods: Anal electrosensitivity, anorectal manometry and rectal mano-volumetry were performed in 17 control subjects before and after superficial local anal anaesthesia, one subject before and after subepithelial injected local anesthesia and six control subjects before and after spinal anaesthesia.
Results: After superficial local anal anaesthesia, anal electrosensitivity decreased. All rectal filling sensation levels remained present. Two out of 17 subjects involuntarily lost the rectal balloon before reaching maximal tolerable sensation although they had normal rectal filling sensation prior to involuntary evacuation. superficial local anal anaesthesia reduced significantly ($P < 0.05$) anal pressure recorded in the distal anal canal during progressive rectal filling at all sensation levels. The rectal pressure to elicit rectal filling sensation did decrease at constant sensation, Urge and maximal tolerable sensation, but only significantly at maximal tolerable sensation ($P < 0.001$). A correlation was observed between proximal anal pressure and the pressure in the rectal balloon necessary to elicit rectal filling sensation at first ($P < 0.05$, $r = 0.6$) and constant sensation level ($P < 0.05$, $r = 0.5$). After subepithelial injected local anesthesia, although the anal pressure was decreased the pressure in the rectal balloon to elicit rectal filling sensation increased significantly. After spinal anaesthesia, no rectal filling sensation were felt anymore
Conclusion: These results support the hypothesis that anal rectalfilling sensation receptors are located in the proximal anal canal, with a spinal reflex pathway. These are stretch sensitive receptors located within the external sphincter into the subepithelium. Information from these receptors is not directly responsible for faecal continence.

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Age-related changes in nitrergic and purinergic signalling in the mouse internal anal sphincter

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Objective: Gastrointestinal disorders, including chronic constipation and faecal incontinence, increase with age leading to a reduction in the quality of life. The internal anal sphincter (IAS) is vital in maintaining continence and a range of neurochemicals have been shown to regulate its relaxation. This study examined the effects of increasing age on nitrergic and purinergic signalling in the mouse IAS.
Methods: Relaxations of isolated IAS smooth muscle strips from 3–4, 12–14 and 24–26 months C57BL/6 mice were recorded in an organ bath following nicotine or electrical field stimulation (EFS; 30 V; 1ms pulse

width; 0.5, 1 and-5 Hz). Nitrergic and purinergic responses were identified by their sensitivity to N-Nitro-L-arginine (L-NNA), and MRS2500, respectively. Dose-response curves to sodium nitroprusside were performed to determine the direct effects of nitric oxide on the IAS.
Results: IASs generated spontaneous basal tension that did not change significantly with age. Nicotine (10 *v* mol L^{-1}) induced a dose-dependent relaxation under control conditions, that was greater in 12 month compared to 3 and 24 month tissue ($P < 0.05$ for both). EFS-evoked relaxations at 1 Hz and 5 Hz were again greater in the 12 month compared to 3 and 24 month tissue ($P < 0.01$; $P < 0.05$, respectively). Increases in nitrergic signalling accounted for the increased relaxation in 12 month IAS in response to EFS ($P < 0.01$) with no significant change in the purinergic input. Age did not change the responsiveness of the IAS to SNP. The increased nicotine-evoked relaxation in 12 month tissue was due to increases in both nitrergic and purinergic components ($P < 0.05$ for both).
Conclusion: We have shown that both nicotine and EFS-evoked relaxations increase transiently in the 12 month group. Increases in EFS-evoked relaxations were due to increases in the release of NO. However, both the nitrergic and purinergic components were increased following nicotine stimulation in 12 month tissue. These data suggest that NO release is transiently increased in the 12 month tissue and that nicotinic stimulation can evoke the release of a purine from a non-neuronal source.

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Esophageal biomechanical properties as indicator of impaired gastrointestinal function in diabetes patients

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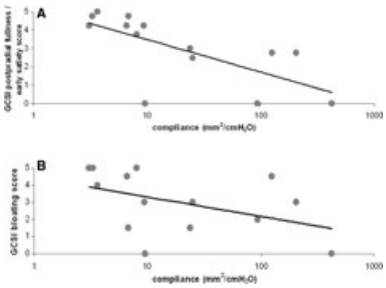
Objective: Gastrointestinal (GI) symptoms, such as nausea, vomiting, bloating, postprandial fullness and abdominal pain, are frequent in patients with diabetes mellitus (DM). The pathogenesis is complex and multi-factorial. In order to determine easy accessible and valid biomarkers for disordered GI function in DM patients, we aimed to study esophageal mechanical parameters and their relation to symptoms typically arising from the digestive tract.
Methods: Seventeen patients with longstanding DM and GI symptoms and 13 healthy controls were studied with ultrasound monitored esophageal distension. The sensory response was recorded and their symptoms registered. Biomechanical parameters such as compliance and stiffness were computed from luminal diameters during distension based on the ultrasound images and from pressure data. Biomechanical and sensory parameters were correlated with the clinical data.
Results: Diabetes patients had reduced esophageal sensitivity compared to controls ($P = 0.046$). The esophageal compliance was reduced ($P = 0.004$) and the esophageal stiffness was increased ($P = 0.004$) in the diabetes patients. Among patients, both postprandial fullness/early satiety and bloating correlated negatively to the esophageal compliance parameters (all $P < 0.05$).
Conclusion: Patients with long-standing DM and GI symptoms had reduced esophageal sensitivity together with reduced compliance and increased stiffness, which were correlated to the patients' GI symptoms. Biomechanical parameters obtained during distension may serve as valid independent biomarkers of the GI dysfunction. They may contribute to our understanding of the pathophysiology underlying GI dysfunction and symptoms in patients with longstanding DM.



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Principles of analysis of spatio-temporal mechanical events during neurogenic and myogenic activity in rabbit colon

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Objective: Motor activity of the intestine is determined by the contractions and relaxations of the smooth muscle. The state of the intestinal wall, described as tone, is synonymous with the mechanical stress generated in the muscle wall in response to myogenic or neurogenic mechanisms. Establishing where and when the intestinal muscle is actively contracting or relaxing or passively elongating or shortening is difficult. Here we propose a strategy to identify these mechanical states.
Methods: In the isolated rabbit distal colon (20–25 cm), we combined simultaneous video recording of changes in diameters (DMaps) and intraluminal pressure, recorded by fibre-optic, high-resolution intraluminal manometry (PMaps) to generate pressure/diameter spatio-temporal maps of colonic motility. Utilising software developed in Matlab we compared changes in diameter and pressure at any given point in the spatio-temporal map, enabling a number of unique theoretical relation associated with different states of the intestinal wall to be predicted. These include; (i) isotonic contractions of the circular muscle (reduction in diameter not associated with changes in pressure); (ii) isometric contractions (increase in pressure with maintained minimal diameter); (iii) common cavities (increase in pressure with maintained maximal diameter); (iv) active tonic contractions (parallel decrease in diameter and increase in pressure); (v) passive dilation (increasing diameter associated to increase in pressure).
Results: During myogenic activity there were phasic increases of active tone of the muscle due to either isotonic or isometric contractions. The neurally dependent propagating contractions (neural peristalsis) showed complex orbits of relation between pressure and diameter consistent with active dilation ahead of localized lumen occlusive isometric contractions, which propagated aborally generating passive dilation ahead of the advancing contraction.
Conclusion: Our method of portraying and analysis of the intestinal movements enables linking between myogenic and neurogenic mechanisms and the mechanical states of the intestinal wall and will permit quantitative analysis of different patterns of intestinal motor activity.

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Intestinal motility and nematode-induced protection against development of murine model of type 1 diabetes (T1D)

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Objective: Autoimmune T1D is associated with defects in intestinal motility and mucosal permeability. Non-obese diabetic (NOD) mice are a model for T1D that shares many characteristics of the human disease. Others showed that enteric nematode infection upregulation of Th2 cytokines prevented T1D in NOD mice. The objective of this study was to determine the contribution of altered intestinal function in the nematode-mediated protection from T1D.
Methods: NOD and WT mice were infected with the enteric nematode Heligmosomoides bakeri (Hb). Blood glucose was monitored weekly to determine the onset of diabetes. Segments of jejuna were suspended in organ baths to measure smooth muscle responses to acetylcholine and spontaneous contractions (SC). TTX was used to determine neural contribution. Transepithelial electric resistance (TEER) was measured in muscle-free jejuna. Cytokine and macrophage markers expression was determined using real time qPCR.
Results: Uninfected NOD developed T1D (84% incidence) by week 20. SC and responses to acetylcholine (10 μ mol L^{-1}) were lower in uninfected NOD mice (6.6 ± 0.8 vs 3.8 ± 1.1 N cm^{-2}) and less dependent on enteric nerves compared to WT (39% vs. 57% of responses -TTX). No differences were observed in smooth muscle contractility between diabetic and non-diabetic NODs. When compared to controls, TEER was constitutively lower in NODs. NODs had also constitutively lower levels of Th2 cytokines IL-13 and IL-4 (8.4% and 24% of WT) at 2 weeks post infection. Hb reduced the incidence of T1D in NODs (16%), with a transient increase in Th2 cytokines, that was 25% of that observed in WT, and increase in alternatively activated macrophages markers. NODs had significant worm burden at 20 weeks post infection whereas controls cleared by week 10.
Conclusion: NOD mice have ''leaky gut'', a characteristic of autoimmune diseases. We show here that these mice also have constitutively low smooth muscle contractility and, when compared to WT, an impaired Th2 response to infection that prolongs the chronicity of Hb infection. Recent studies in western populations indicate that nematode infections, which are generally treated as they are diagnosed, may not confer protection against autoimmune diseases. Our data suggest that the duration of exposure to nematodes is an important feature of the protective response.

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The anorectal defecation reflex

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Objective: To evaluate the mechanism controlling faecal defecation.
Methods: Anal electrosensitivity, anorectal manometry and rectal mano-volumetry were measured in 17 control subjects before and after superficial local anal anaesthesia, one control subject before and after (subepithelial) injected local anaesthesia and six control subjects before and after spinal anaesthesia.
Results: After superficial local anal anaesthesia anal electrosensitivity decreased. All rectal filling sensation levels remained present and the rectal volumes to reach the fillingsensation levels increased. The rectal compliance increased at all levels, but only significantly at constant sensation. A positive correlation was observed between proximal anal pressure and rectal compliance at first sensation and at constant sensation. After injected local anaesthesia, rectal compliance increased at all levels even more profound. After spinal anaesthesia, the anal canal became insensitive to electric stimulation, rectal filling sensation disappeared, while rectal compliance (calculated for the same rectal volumes as before spinal anaesthesia) decreased insignificantly at first sensation and constant sensation level but remained normal at feeling of urgency to defecate end maximal tolerable sensation level.
Conclusion: These results support the hypothesis that faecal defecation by contraction of the rectum is depending of an intestinal (intramural) defecation reflex without influence of the brain or the spinal cord (the anorectal defecation reflex). The afferent receptors of this reflex are located in the proximal anal canal and are contact receptors superficially subepithelial located. A not functioning anorectal defecation reflex results in chronic constipation.

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Release of β-NAD and ATP upon activation of myenteric 5-HT3 or nicotinic acetylcholine receptors in murine and primate colons

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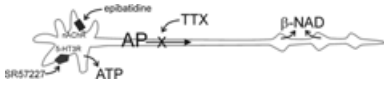
Objective: ATP has been assumed to be an inhibitory purinergic neurotransmitter in the gastrointestinal tract, however β-nicotinamide adenine dinucleotide (β-NAD) and its metabolite ADP-ribose mimic the purine neurotransmitter in murine, monkey and human colons better than ATP. We further investigated sources for purine release in murine and monkey colon muscles by measuring release of ATP and β-NAD and purine metabolites in response to stimulation of 5-hydroxytryptamine receptors (5-HT3R) or nicotinic acetylcholine receptors (nAChR) known to be expressed by enteric nerve cell bodies and processes.
Methods: The overflow of purine neurotransmitter candidates was measured by HPLC-FLD, and stimulation of muscles was performed in the presence of L-NNA (100 μ mol L^{-1}) and atropine (1 μ mol L^{-1}).
Results: The 5-HT3R agonist SR57227 (500 μ mol L^{-1}, 30 s) stimulated release of β-NAD, ATP and metabolites. Ondansetron (3–10 μ mol L^{-1}) reduced SR57227-evoked release of purines, confirming that release was mediated by 5-HT3R. Tetrodotoxin (TTX, 0.5 μ mol L^{-1}) inhibited the release of β-NAD (and its metabolites), suggesting that β-NAD release depended upon neural action potentials (AP). Release of ATP was not inhibited significantly by TTX, suggesting release of ATP might have come from intraganglionic sources (e.g. nerve cell bodies or glia) not requiring Na+ action potentials. Epibatidine (500 μ mol L^{-1}, 30 s), a potent nAChR agonist, also evoked release of β-NAD and ATP. Hexamethonium (500 μ mol L^{-1}) blocked epibatidine-evoked purine release. TTX reduced epibatidine-evoked release of β-NAD but did not affect release of ATP.
Conclusion: Our data demonstrate that the purine release occurs by stimulation of enteric neurons. Release of β-NAD (and its metabolite) requires activation of neuronal Na+ action potentials (Fig.), which is consistent with release of β-NAD at nerve varicosities. ATP release appears to be mainly from intraganglionic sources and not from varicosities of motor neurons. These results support previous findings from studies using electrical field stimulation to evoke neurotransmitter release, and suggest that β-NAD, not ATP, better fulfills the criteria for a purinergic enteric inhibitory neurotransmitter. Supported by DK41315.



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Properties of cholinergic and non-cholinergic submucosal neurons along the mouse colon

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Objective: Neurons in the submucous plexus are key regulators of normal gut secretion and are implicated in several pathologies including Vibrio cholera-induced hypersecretion. Submucosal neurons fall into two main classes: cholinergic (containing choline acetyltransferase, ChAT), or non-cholinergic (lacking ChAT). This segregation of submucosal neurons is conserved across several species. We investigated the properties of these two populations in the mouse colon.
Methods: ChAT-Rosa (yellow fluorescent protein, YFP) mice from a mixed background (progeny of ChAT-Cre X Rosa (YFP) reporter mice) express YFP in all neurons that have ever expressed ChAT. Submucosal preparations were taken from proximal, middle and distal colonic regions of ChAT-(YFP) and control (non-transgenic) C57Bl/6 mice. The proportion of submucosal neurons (marked by the pan-neuronal marker Hu) that expressed YFP, ChAT and/or vasoactive intestinal peptide (VIP) was studied immunohistochemically. Electrophysiological and morphological properties of submucosal neurons in the distal colon were examined using intracellular microelectrodes containing biocytin.
Results: The number of submucosal neurons per ganglion decreased (proximal: 12 ± 3; middle: 7 ± 1; distal: 5 ± 1 neurons/ganglion), and the proportion of Hu+ neurons expressing YFP increased (proximal: 2.0%; middle: 9.7%; distal: 25.2%) distally along the colon of ChAT-YFP mice. Most Hu+ neurons lacked YFP but contained VIP (middle: 86.0%, distal: 71.1%). The remaining neurons did not express YFP or VIP (middle: 2.7%, distal: 3.1%), or rarely expressed both YFP and VIP. The proportion of ChAT-immunoreactive submucosal neurons in control mice was similar to that of YFP+ neurons in ChAT-YFP mice. Intracellular biocytin injections showed that submucosal neurons in the distal colon were either uniaxonal (15/18) or had smooth cell bodies with 2 axons. Spontaneous and stimulus-evoked fast excitatory postsynaptic potentials (EPSPs) were seen in 24/25 submucosal neurons. Fast EPSPs were mainly mediated by nicotinic receptors and were seen together with inhibitory synaptic potentials and slow EPSPs.
Conclusion: The increase in the proportion of cholinergic neurons in distal colon suggests an increase in their role in this region.

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ß-NAD and not ATP acting via P2Y1 purinoreceptors is the purine that mediates inhibitory motor responses in the colon

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Objective: Inhibitory motor neurons participate in complex motor patterns of the gastrointestinal tract such as peristalsis, receptive relaxation, and sphincter opening. The neurotransmitters responsible for relaxation in the GI tract have been the subject of studies for many decades. Several substances have been proposed as neurotransmitters mediating inhibitory responses including ATP, however this purine does not fully mimic post-junctional responses. The objectives were therefore to determine the purine that may be released from inhibitory motor neurons and using mice lacking P2Y1 receptors (P2Y1R) evaluated the purinoreceptor responsible for inhibitory junction potentials in the colon. The role that P2Y1R play in colonic transit was also evaluated.
Methods: Intracellular microelectrode and isometric force measurements were performed on wildtype and P2Y1R null mice. Post-junctional inhibitory responses (IJPs) and membrane hyperpolarizations to picospritzed application of putative transmitters were studied. Spatio-temporal mapping was also performed to evaluate differences in colonic transit.
Results: Wildtype mice were characterized by biphasic IJPs and pharmacological dissection confirmed that the fast IJP (fIJP) was purinergic and the slow IJP (sIJP) was nitergic. The fIJP was completely absent in P2ry1-/- mice and the P2Y1 receptor antagonist MRS2500 had no effect on electrical activity or responses to electrical field stimulation of intrinsic nerves in these mice. Contractile experiments confirmed that purinergic responses were abolished in P2ry1-/- mice. Picospritzing of neurotransmitter candidates (ATP and its primary metabolite, ADP) and ß-NAD (and its primary metabolite, ADP-ribose, ADPR) caused transient hyperpolarization responses in wildtype colons, but responses to ß-NAD and ADPR were completely abolished in P2ry1-/- mice. Hyperpolarization and contractile responses to ATP and ADP were retained in colons of P2ry1-/- mice. Video imaging revealed that transit of fecal pellets was significantly delayed in colons from P2ry1-/- mice.
Conclusion: The results demonstrate the importance of purinergic neurotransmission in regulating colonic motility. The data reveals that ß-NAD rather than

ATP better fulfills the criteria as the inhibitory neurotransmitter in the murine colon and support pharmacological experiments suggesting that purinergic neurotransmission is mediated via P2Y1 receptors.

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Effect of acute ethanol exposure on the electrical and mechanical activities in the small intestine

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Objective: Ethanol ingestion causes a variety of gastrointestinal disorders including motility disturbances. Slow waves coordinate gastrointestinal motility and abnormal slow-wave activity is thought to contribute to motility disorders. To date however, little is known about the effect of acute ethanol on motility disturbances associated with slow wave activity.
Methods: Seven to ten centimetre duodenum, jejunum and ileal loops from adult male Wistar rats were isolated and mounted in a 300-ml organ bath superfused with a Tyrode solution (100 ml min^{-1}). The intestinal loops were connected to an inlet and an outlet and intra-luminally perfused with Tyrode (0.5 ml min^{-1}). 15–30 markers (soot) were applied onto the serosal surface of the segments. After a 15 min equilibration period, the loops were superfused with 1, 3 and 5% ethanol, corresponding to the consumption of different alcoholic beverages. Motility recordings were performed with a digital video camera and electrical activities were recorded using a single longitudinal row of 32 extracellular unipolar silver electrodes.
Results: In all duodenal, jejunal and ileal segments ($n = 6$), ethanol inhibits motility fully at 3% and 5%. Concomitantly, the action potentials (= spikes) had also disappeared from the electrical recordings. The slow wave activity however, was not altered at 1 and 3% ethanol but disappeared at 5% ethanol. All effects were reversible upon superfusing with normal Tyrode.
Conclusion: In conclusion, acute ethanol significantly inhibits small intestinal motility in a concentration dependent manner. Slow wave activity was only inhibited at the highest concentration. The ethanol induced mechanical and electrical disturbances were reversible. The concentration dependent differential in the inhibition of the electrical and the mechanical effects seems to suggest different mechanisms of action of ethanol.

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Mechano-sensory properties of hypertrophic intestine following chronic obstruction

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Objective: Partial obstruction of the intestine causes severe hypertrophy of smooth muscle cells, enteric neuronal plasticity, reducing number of interstitial cell of Cajal, motility disorders and remodeling of biomechanical properties. The present study aimed to study the discharge characteristics of intestinal afferents to mechanical stimuli and its relationship with biomechanical properties of the hypertrophic intestine.
Methods: Jejunal partial obstruction was surgically made by placement of a polyethylene ring for two weeks in 7 rats. Sham operation was made by suturing the polyethylene strip without placing a ring in 6 rats. Another 7 rats without surgery served as normal controls. Afferent nerve activity was recorded under various mechanical stimulations on the jejunal segments *In vitro*: ramp test with two different distension rates, a relaxation test (volume maintained constant from initial pressure of 20 or 40 mmHg) and a creep test (pressure maintained constant). Circumferential stress and strain, the spike rate increase ratio (SRIR) and increase firing rate in single unit were calculated for evaluation of afferent nerve activity during the mechanical stimulations.
Results: Jejunal hypertrophy was morphometrically and histologically evident proximal to the obstruction site in the obstructed group. The hypertrophic jejunum was stiffer in the circumferential direction than those from the sham operated and normal jejunums ($P < 0.05$). No significant difference was found in the SRIR- strain and SRIR –stress relationships among the three groups in all tests ($P > 0.05$). However, after-discharge (sustained afferent firing after cessation of the mechanical stimulation) was higher in the obstructed jejunum than in the sham operated and normal controls ($P < 0.05$). Single unit analysis showed absence of high threshold units, increased proportion of mechanosensitive units but reduced single unit activity in the obstructed jejunum.
Conclusion: Afferents from obstructed jejunum preserved their function in encoding ongoing mechanical stimulation in the intestinal wall. However, high after-discharge was characteristic for the obstructed jejunum. Furthermore, more percentage of the single units was activated but the activity in single units was lower in the obstructed intestine. The high after-discharge may mediate perception of pain in the obstructed patients.

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Decrease in serotonin-induced tachykinin signaling can account for age: Related reductions in the amplitude and the frequency of Colonic Migrating Motor Complexes (CMMCS) in the murine distal colon

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Objective: Altered motility is an important feature of age-related gastrointestinal disorders such as chronic constipation. Mucosal serotonin release can trigger motility via its actions on myenteric neurons. This study examined the effects of age on 5-HT-evoked tachykinin signaling and the properties of CMMCs.
Methods: Experiments were performed on colonic tissue segments from 3, 12, 18 and 24 month old C57BL/6J mice. Longitudinal muscle contractions were recorded from 1–2 cm lengths of distal colon to exogenously applied serotonin, neurokinin A (NKA) and electrical field stimulation (EFS). CMMC activity was recorded in the intact colon.
Results: Under conditions that isolated tachykinin signaling, 5-HT evoked dose-dependent contractions that were blocked by GR159897, a NK2 antagonist ($P < 0.001$). These responses were reduced in 18 and 24 month tissue compared to 3 and 12 month responses ($P < 0.05$ and $P < 0.001$, respectively). EFS-evoked tachykinin release was reduced in 24 month colon ($P < 0.05$). Age had no effect on colonic responses to exogenous NKA, a selective NK2 receptor agonist. Age decreased the frequency and amplitude of CMMCs ($P < 0.001$) and this was mimicked by application of GR159897 to the 3 month colon ($P < 0.001$ and $P < 0.01$, respectively). In addition, GR159897 increased the duration ($P < 0.05$) of CMMCs in 3 month tissue but was without effect in 24 month tissue.
Conclusion: We have shown an age-related decrease in the serotonin-evoked tackykinin mediated contraction in the mouse distal colon and a decrease in the amplitude and frequency of CMMCs. Application of the NK2 antagonist to 3 month tissue could account for the effects of ageing on CMMCs. These data suggest that the age-related decrease in the amplitude and frequency of the CMMCs is due to an impairment of 5-HT-evoked tachykinin signaling in the colon.

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Role of prostaglandins in the colonic motility in rat colon

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Objective: Role of prostaglandins (PGs) in the intestine is well known as a function to maintain the mucosal integrity. In the intestinal motility, it has been known that PGs have inhibitory and excitatory effects on circular and longitudinal muscles, respectively. However, the contribution of E prostaglandin (EP) receptors, which are currently identified in the molecular basis and subdivided into four subtypes, EP_1, EP_2, EP_3 and EP_4 receptors, to the intestinal motility, is unclear yet. Therefore the aim of the present study is to identify the role of EP receptors on the colonic motility distinct between circular muscle (CM) and longitudinal muscle (LM), and to reveal the distribution of several EP receptors in the colon.
Methods: Motilities of mucosa-free CM and LM strips in the rat middle colon were measured by utilizing isometric and isotonic transducers, respectively, in Magnus tube. In addition, localizations of EP receptors were analyzed by immunohistochemistry.
Results: Both CM and LM frequently induced phasic contractions called as ''giant contractions (GCs)''. In CM, neural blockade by TTX and COX inhibition by piroxicam enhanced the GCs, whereas in LM, TTX tended to enhance but piroxicam almost all abolished the GCs. Furthermore, an EP_3 receptor antagonist, ONO-AE3-240, concentration-dependently reduced the GCs in LM. On the other hand, EP_2 and EP_4 receptor agonists, ONO-AE1-259 and ONO-AE1-329, reduced the GCs of CM in concentration-dependent manners. These results have indicated that endogenous PGs partially inhibited the GCs in CM via EP_2 and EP_3 receptors, whereas they generates and enhances the GCs in LM via EP_3 receptors expressed in non-neuronal cells in the muscle layer. In immunohistochemical analysis, EP_1 and EP_3 receptor-immunoreactivities (IRs) were localized on smooth muscle cells in addition to the enteric neurons, whereas EP_2 receptor-IR was localized on glial cells, and EP_4 receptor-IR was localized on smooth muscle cells and enteric neurons.

Conclusion: The present results suggest that the endogenous PGs are necessary to generate the GCs in LM, and excitatory and inhibitory modulate the GCs in LM and CM, respectively, mediated via distinct EP receptors in the rat middle colon.

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Impacts of fungal alkaloids on intestinal motility in the isolated rat colon

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Objective: This study was motivated by reports that increased fecal moisture (or diarrhea) is more prevalent in sheep that graze pastures infected with an endophytic fungus, suggesting involvement of fungal metabolites. Two key fungal alkaloid classes are present, ergot alkaloids and indole-diterpenes, both of which increase smooth muscle contraction. We tested the hypothesis that key fungal alkaloids produced by endophyte act in concert to increase intestinal motility.
Methods: The effects of two types of endophyte compounds on motility were investigated using a rat model to determine effects on spontaneous contractile tension and frequency in the isolated distal colon. The compounds: ergotamine (1 μ mol L^{-1}) a 5-HT agonist and lolitrem B (0.1 μ mol L^{-1}) a BK channel inhibitor, were dissolved in 0.1% DMSO and applied separately or together. Differences in tension and frequency were measured relative to spontaneous activity in the presence of 0.1% DMSO (control =1.0) over 1 h.
Results: Ergotamine, an ergot alkaloid, produced an increase in contractile tension (1.5-fold) and a rapid increase in contraction frequency (1.6-fold) that decreased over one hour (1.3-fold). In contrast, lolitrem B, an indole-diterpene, increased contractile tension slowly over one hour (1.3-fold) but had no effect on contraction frequency. When applied together, the contractile tension was greater than that for either compound alone (2.2-fold, $P < 0.05$). The frequency of contractions was increased but was not significantly different from that for ergotamine alone.
Conclusion: The increase in contractile tension that occurred when both compounds were applied together indicates that ergotatmine and lolitrem B can act additively to increase motility. The finding that ergotamine increased the frequency of contractions whereas lolitrem B did not supports the idea that these compounds have functionally additive effects on motility. Since endophyte in grass pastures contain a mixture of these compounds and other structurally related alkaloids, our results suggest that ingestion of these fungal alkaloids could be a major contributing factor to the diarrhea that occurs in some animals grazing these pastures.

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The motility and biomechanical changes of rat small intestine after acute mesentery ischemia

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Objective: Acute mesentery ischemia (AMI) may affect intestinal biomechanical properties and motility. This study aimed to investigate the changes of intestinal motility and biomechanical properties after AMI.
Methods: AMI of jejunum was induced by ligating five mesenteric arterial branches to that segment in 40 Wistar male rats and lasted 15 30, 60, and 120 min (each group has 10 rats). Another 10 rats without ischemia served as normal controls. The 10-cm long ischemia and non-ischemia intestinal segments were excised and used for motility and passive mechanical tests. The motility test was done by flow test against different outlet pressures and by ramp distension test up to 12 cm H2O. The passive mechanical test was done by the same ramp distension protocol. The intestinal diameter and pressure changes were synchronously recorded. The amplitude and frequency of contractions were obtained by analysis of pressure and diameter curves. Circumferential stress and strain were computed from the diameter and pressure data and from the zero-stress state geometry. The opening angle, wall thickness, inner and outer circumferential length were obtained from the intestinal tissue rings at the no-load and zero-stress state. The residual strains in the inner and outer wall were computed from the morphometric data.
Results: The intestinal wall thickness and cross-sectional wall area were significantly decreased about 20% after AMI for 60 and 120 min ($P < 0.05$ and $P < 0.01$). The amplitude of contraction obtained from both flow and distension tests decreased after AMI for 30 minutes ($P < 0.05$). The opening angle and absolute value of residual strain decreased as function of the AMI duration ($P < 0.01$). Furthermore the stress-strain curves after AMI shifted to the left, indicating that the intestinal wall became stiffer. Significant difference was found after AMI for 60 and 120 min compared with normal control group ($P < 0.01$).
Conclusion: The morphometric and biomechanical properties of the intestinal wall changed after AMI. Furthermore, hypomotility was seen with AMI lasting more than 30 minutes. This may relate to morphological and biomechanical remodelling caused by AMI. Future studies should be directed towards studying the recovery of motility and biomechanical changes after the AMI has ceased.

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Age-related changes in colonic motility, faecal output and the properties of faecal pellets in the mouse

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Objective: Increasing age is a risk factor for a range of gastrointestinal disorders including constipation and faecal impaction. Aging studies to date have focused on alterations in neuronal numbers in the myenteric plexus and very few have investigated the functional changes observed during ageing. This study investigated how age affected faecal pellet output and geometry, the degree of compaction and colonic motility.
Methods: Faecal pellets from 3, 12, 18 and 24 month old C57BL/6 mice were collected over a 24 h period. Wet and dry weights of faecal pellets were determined. Photographic images of dry pellets were utilized to analyze the geometry of pellets. For colonic motility measurements the velocity of epoxy coated faecal pellets was determined in the isolated lower bowel (proximal colon to rectum) from 3 and 24 month old mice.
Results: Total faecal output was significantly decreased from 3 to 24 month old animals ($P < 0.01$, $n = 6$). There was a significant decrease in the number of faecal pellets from 3 to 12 month ($P < 0.001$, $n = 6$) which then remained constant in the 18 and 24 month old animals. The water content of the faecal pellets was significantly reduced between 3 and 24 month old animals ($P < 0.001$, $n = 6$). No differences in individual pellet width were observed, however faecal pellet length decreased between 3 and 24 months ($P < 0.05$, $n = 6$). There was a significant increase in the amount of bowel compaction in the 24 month animals compared to the 3 month animals ($P < 0.05$, $n = 5$), due to a reduction in faecal pellet motility between 3 and 24 month old animals ($P < 0.01$, $n = 6$). A more detailed analysis of the pellet velocity showed that proximal velocity was unaltered with increasing age and the changes in velocity could be attributed to alterations in distal colon function.
Conclusion: Overall we have found that with increasing age faecal pellet output and length are reduced. There is a decrease in colonic velocity and a consequential increase in impaction. These responses are suggestive of a model of constipation/faecal impaction, which is also observed in the elderly.

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Recto-anal manometry: Comparison between type 2 diabetics wit normal or high morning glycemia

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Objective: The influence of basal morning glycemia on the recto anal motility, in diabetic patients is not consensual. It is our aim to compare recto-anal manometric characteristics between diabetics according to the basal morning glycemia.
Methods: A recto-anal manometry was performed to 35 diabetics of both genders, without signs of autonomic neuropathy, with a mean age of 58.82 years. Of them, 12 had glycemia, ≤7 m mol L^{-1} with a mean age of 61.3 years and 23 had its value >7 m mol L^{-1} and a mean age of 60.0. The basal gycemia was similar in both genders, $P = 0.581$. Recto-anal pressures were recorded in mmHg. Student t Test was used and data are presented as mean ± standard deviation.
Results: The resting rectal pressure, resting pressure in the external and in the internal sphincter and in the anus were similar. The minimum rectal sensibility and the maximum tolerable capacity were also similar between groups. The percentage of relaxation during the recto-anal inhibitory reflex was not statistically different between them. During the squeeze (in diabetics with glycemia ≤7 m mol L^{-1} vs >7 m mol L^{-1}) the pressure increase was 57.66 ± 53.9 vs 100.0 ± 57.9; $P = 0.04$; average squeeze pressure was 33.41 ± 34.4 vs 65.91 ± 39.7; $P = 0.02$; amplitude under zero was 106.25 ± 59.7 vs 151.13 ± 66.6; $P = 0.05$ and area under curve 506.91 ± 232.6 vs 655.30 ± 285.3, $P = 0.1$. In the squeeze endurance the values were: pressure increase 70.0 ± 51.9 vs 114.8 ± 59.3, $P = 0.03$; average squeeze pressure was 41.1 ± 39.9 vs 69.13 ± 43.1, $P = 0.07$. The pressure after squeeze and the area under curve were similar.
Conclusion: The pressures augmentation were significantly higher in diabetics with glycemia >7mmol/l during the squeeze and the squeeze endurance. 2- The rectal and anal sphincter's resting pressure, the rectal sensibility thresholds and the recto anal inhibitory

reflex did not reveal significant differences. 3- The basal glycemia influenced the rectal manometric characteristics during the anal squeeze.

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Study of the biological effects of single-walled carbon nanotubes (SWNTs) on rabbit small intestine

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Objective: Single-walled carbon nanotubes (SWNTs) are structures of carbon of nanometric scale. Biomedical research is exploring possible application of nanotubes (NTs) as drug delivery carriers. Studies about the biological effects of NTs are necessary to ensure that they are not toxic when they are linked to a drug to be delivered.The aim of this study was to investigate the effects of SWNTs systemic administration on the motility, generation of free radicals and expression of inflammatory mediators in rabbit ileum.
Methods: Rabbits were injected intravenously with saline or SWNTs (0.02, 0.2 and 2 μg kg^{-1} body weight) for 24 h. Contractility studies were performed suspending pieces of ileum in an organ bath in the direction of longitudinal smooth muscle fibres. The ileum and plasma concentrations of malondialdehyde (MDA) + 4-hydroxyalkenals (4-HDA) and carbonyls were used as indexes of lipid and protein peroxidation respectively. The mRNA expression of iNOS and COX-2 in rabbit ileum was studied by RT-PCR.
Results: The frequency of the spontaneous contractions was slightly reduced in the ileum of rabbits treated with SWNTs 0.02, 0.2 and 2 μg kg^{-1}, while the amplitude of these contractions was increased only at the dose of 2 μg kg^{-1}. Serotonin 10–4 mol L^{-1}, ACh 10^{-4} mol L^{-1} and KCl 80 m mol L^{-1} induced contractile responses that were increased in ileum from rabbits treated with SWNTs 0.02, 0.2 and 2 μg kg^{-1}. The levels of MDA+4-HDA and carbonyls were increased in plasma from rabbits treated with SWNTs 0.2 and 2 μg kg^{-1}, while in ileum these levels were increased only at the dose of 2 μg kg^{-1}. The mRNA expression of iNOS and COX-2 in rabbit ileum was not modified after any of the treatments with SWNTs.
Conclusion: These results suggest that SWNTs 0.02 μg kg^{-1} does not modify the expression of inflammatory mediators and free radicals in ileum, although it has some slight effects on ileum motility. Thus, SWNTs 0.02 μg kg^{-1} could be use in future studies for drug delivery. Funding by Gobierno de Aragón (B61/2011).

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Recto-anal manometric characteristics of healthy individuals according to their age and gender

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Objective: Some investigators registered differences in recto-anal motor characteristics according age and gender. Others did not observe differences. Our aim is to compare rectal manometric characteristics in healthy individuals according to their age and gender.
Methods: A recto-anal manometry was performed to 34 healthy individuals, 14 women and 20 men, aged between 34 and 77 years old. Twelve had ≤60 years and 24 > 60 years. Pressure is in mmHg. Student *t* Test was used and data are mean ± standard deviation.
Results: According to the age, ≤60 *vs* >60 years, no significant differences were found. Comparing genders, male vs female, it was: resting external anal sphincter pressure 52.6 ± 27.4 *vs* 27.5 ± 17.1, $P = 0.06$; resting internal anal sphincter pressure 51.6 ± 27.4 vs 27.5 ± 17.1, $P < 0.004$; resting anal pressure 27.0 ± 12.8 vs 15.2 ± 10.4, $P = 0.008$; resting rectal pressure 14.1 ± 11.5 vs 7.1 ± 5.1, $P = 0.04$; recto anal inhibitory reflex 39.9 ± 26.5% vs 60.7 ± 21.5%, $P = 0.02$. The minimum rectal sensibility and maximum tolerable volume were similar. During the squeeze in the same groups, male vs female, pressure increase 146.7 ± 52.4 vs 95.5 ± 50.7, $P = 0.008$; average squeeze pressure 96.7 ± 36.3 vs 61.6 ± 36.8, $P = 0.01$; area under curve 916.1 ± 286.1 vs 666.5 ± 266.6, $P = 0.01$. In the endurance squeeze, pressure increase 158.2 ± 52.9 vs 103.5 ± 47.9, $P = 0.009$; average squeeze pressure 98.6 ± 44.8 vs 60.6 ± 30.1, $P = 0.01$; pressure after squeeze, 82.0 ± 56.0 vs 37.7 ± 25.7, $P = 0.004$; area under curve 2546.8 ± 1138.2 vs 1527.2 ± 782.3 mmHg, $P = 0.007$.
Conclusion: 1-The resting internal anal sphincter pressure, anal pressure and rectal pressure were higher in males. 2-The recto-anal reflex was significantly higher in female gender. 3-The pressures in anal squeeze were higher in males. 4-The rectal sensibility and capacity were similar. 5-Age did not influence the characteristics of recto-anal manometry.

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Intestinal contractility is not altered by guanylate cyclase C activation or by non cell-permeant cGMP

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Objective: The guanylate cyclase C (GC-C) receptor agonist, linaclotide, improved abdominal pain and bowel symptoms in patients with constipation-predominant irritable bowel syndrome, and abdominal and bowel symptoms in patients with chronic constipation. In preclinical models, linaclotide accelerated gastrointestinal (GI) transit and reduced visceral pain. Such pharmacological effects are thought to result from increased intracellular cGMP produced following GC-C activation, which initiates a signaling cascade resulting in the secretion of Cl- and HCO3-, promoting fluid secretion and accelerated GI transit. The effects on visceral pain are thought to be mediated by cGMP that is actively transported out of the epithelial cells where it modulates the activity of colonic afferent fibers. It is unclear whether the activation of GC-C receptors also results in a spasmolytic effect, thereby improving GI transit and abdominal pain symptoms. The objective of this study was to evaluate the effects of GC-C agonism and cGMP on the contractile activity of ileum and colon.
Methods: Rat ileum and colonic segments were used in a tissue bath assay using pharmacological and electrical field stimulation (EFS). An acetylcholine (ACh) concentration response curve or EFS was applied to tissues in the absence (baseline) and presence (test) of compound. Test response was expressed as a percentage of baseline. The GC-C agonist, Escherichia coli heat-stable enterotoxin (STc), cGMP, 5′GMP, guanosine and the cGMP cell-permeant analogue, 8-Br-cGMP, were used for these studies.
Results: ACh produced concentration-dependent increases in smooth muscle tension in both ileum and colon. STc up to 1 μ mol L^{-1} ($n = 6$) had no effect on ACh-induced contractions. Non cell-permeant cGMP (100 μ mol L^{-1}) and its metabolites 5′GMP (10 μ mol L^{-1}) and guanosine (1 m mol L^{-1}) also failed to alter ACh-induced contractions. EFS-induced contractions were not altered by STc, non cell-permeant cGMP, or 5′GMP. High concentrations of guanosine (3 m mol L^{-1}) significantly reduced EFS-induced contraction amplitude ($n = 6$, 65% inhibition in ileum; $n = 3$, 41% inhibition in colon). 8-Br-cGMP (100 μ mol L^{-1}) attenuated EFS-induced colonic contractions ($n = 6$, 58% inhibition in ileum, $n = 10$, 52% inhibition in colon).
Conclusion: This data suggests that neither STc nor extracellular cGMP affect intestinal smooth muscle contractility. Supported by Ironwood Pharmaceuticals Inc. & Forest Laboratories.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-20 Basic and Translational Session: Appetite Regulation, Satiety, Obesity and Nutrition

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Altered nutrient sensing in the transient receptor potential vanilloid 1 knockout mouse

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Objective: Enteral lipids have a potent effect on satiety and also attenuate inflammation triggered by various insults such as surgery, LPS and haemorrhagic shock via a cholecystokinin-mediated vagovagal reflex (Lubbers T, et al. 2010). Previous work has shown that lipid-rich nutrients causes augmented mechanosensitivity in afferents supplying the mouse jejunum (Lubbers T, et al. 2010). TRP channels are implicated in the response to CCK since treatment with ruthenium red attenuates CCK-evoked calcium entry into nodose neurones (Zhao and Simasko, 2010). However, the channel mediating this effect is unknown. Here we test the hypothesis that TRPV1 may contribute to nutrient sensing by comparing the mesenteric afferent sensitivity of 12 month old TRPV1-/- and wildtype (WT) littermates to lipid-rich nutrients.
Methods: Mesenteric afferent discharge was recorded *In vitro* from segments of mouse jejunum from TRPV1-/- mice and their wild type (WT) littermates. Responses to distension were characterized before, during and after luminal infusion with 1 ml of a lipid-rich emulsion (50.4%, 40.9 and 8.7 energy content derived from fat, carbohydrate and protein respectively). Whole nerve mesenteric discharge was quantified at baseline and during distension. Data were mean±SEM with $n \geq 6$. Statistical analysis was performed using one- and two way ANOVA as appropriate.
Results: Intraluminal infusion of the lipid-rich nutrient caused an elevation in baseline afferent firing. Surprisingly, the magnitude of this response was statistically augmented in the TRPV1-/- compared to the WT control ($P = 0.005$). Also surprising was the augmentation in mechanosensitivity in the TRPV1-/- animal that accompanied the lipid rich nutrient while in the WT animal this was not significantly different. This increased mechanosensitivity was particularly evident in the LT component of the response to distension ($P = 0.0003$). In contrast there was also a significant attenuation in the HT component ($P = 0.006$) in the TRPV1-/- mice resulting in a flattening in the pressure-response profile.
Conclusion: These data suggest that rather than a role for TRPV1 in mediating the response to intraluminal nutrient, TRPV1 may down-regulate nutrient signalling or that compensatory changes in excitability in the TRPV1-/- result in enhanced sensitivity to nutrient and CCK.

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Consequences of a diet-induced obesity on the plasticity of the Pancreatic Intrinsic Nervous System and the nervous control of pancreatic endocrine secretion in young mice

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Objective: Pancreatic Intrinsic Nervous System (PINS) is involved in the control of pulsatility and amplitude of insulin secretion (IS) both altered in obese and type 2 diabetic patients. However, little is known about the role of the PINS during obesity and in the genesis of pancreatic endocrine dysfunctions. The aim of our study was to determine the impact of obesity upon the PINS and its functional consequences upon IS in young mice.
Methods: C57BL/6J mice aged 4 weeks received either a normal diet (ND; $n = 20$) or a western (hyperlipidic hyperenergetic) diet (WD; $n = 20$) for 12 weeks. 4 weeks old mice ($n = 16$) were also used as initial controls (iC). After sacrifice, pancreases were removed and placed in short term organ culture for 1 h. The impact of PINS upon endocrine function was studied by adding to the culture the nicotinic receptors agonist dymethylphenylPiperazinium (DMPP) in presence or absence of hexamethonium (nicotinic antagonist) or Nω-nitro-L-arginine methyl ester (L-NAME, inhibitor of NO synthase NOS) or sodium nitroprusside, (SNP, NO donor). Supernatant of culture was collected and frozen for further analysis of insulin by ELISA. Immunohistological analyses were performed to determine nerve fibers density and to phenotype neurons with choline acetyltransferase (Chat) and NOS antibodies.
Results: PINS stimulation by DMPP induced a time dependent increase in IS which was significantly larger in ND as compared to WD mice ($P = 0.01$). Interestingly, the IS profile was identical in ND and iC mice ($P = 0.39$). This secretion was fully blocked in presence of hexamethonium. Addition of L-Name significantly inhibited insulin release in ND mice ($P < 0.01$) while SNP reduced IS at an intermediate level ($P = 0.06$). Neither L-Name nor SNP altered IS in WD mice. PINS density was less in ND compared to iC mice ($P < 0.05$), whereas there was no difference between WD and iC mice. Cholinergic innervation increased significantly with age in both WD and ND mice. Unlike ND mice in whom there was an increase of NOS-neurons with age ($P < 0.05$), WD mice had a significant decrease ($P < 0.05$).
Conclusion: Altogether, our study suggests that WD could induce neuroplastic changes in the PINS that could be involved in pancreatic dysfunctions observed during obesity.

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Glucose modulates glutamate release from vagal afferent terminals via a protein kinase C (PKC)-dependent pathway

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Objective: Alterations in blood glucose levels have profound effects upon gastrointesintal functions, including gastric motility and emptying. We have demonstrated previously that extracellular glucose levels modulate synaptic transmission from the central terminals of vagal afferent neurons via actions on tonically active 5-HT3 receptors. The aim of the present study was to investigate the role of protein kinase C (PKC) in the glucose-dependent regulation of 5-HT3 receptors.
Methods: Whole cell patch clamp recordings were made from NTS subnucleus centralis (NTSc) neurons in thin brainstem slices from rats 28–45 days of age. Neurons were superfused with PKC activators and inhibitors to investigate the modulation of glutamatergic synaptic currents.
Results: (i) As described previously, the frequency, but not the amplitude, of spontaneous excitatory glutamatergic potentials (sEPSCs) was increased by elevating extracellular glucose levels from 5 to 20 m mol L^{-1} in 4 of 6 neurons tested (to 170 ± 12% of control; $P < 0.05$). (ii) The protein kinase C (PKC) activator, phorbol myristate 12-acetate (PMA; 10 n mol L^{-1}) mimicked this increase in sEPSC frequency in 3 of 6 neurons tested (to 167 ± 23% of control; $P < 0.05$). (iii) In the presence of PMA, elevating glucose levels produced no further increase in sEPSC frequency (90 ± 9%; $P > 0.05$), i.e., PMA occluded the effects of glucose on sEPSCs. (iv) The non-selective PKC inhibitor, GF109203X (10 μ mol L^{-1}) blocked the ability of elevated glucose levels to increase sEPSC frequency (188 ± 25% in high glucose vs 94 ± 14% in high glucose + GF109203X; $P < 0.05$; $n = 4/5$ neurons). These actions were mimicked by the PKCbeta isoform blocker CGP55535 (4 μ mol L^{-1}) but not the PKCdelta isoform blocker rottlerin in two further neurons.
Conclusion: Previous studies have demonstrated that extracellular glucose levels regulates the number of 5-HT3 receptors present on the central terminals of vagal afferent neurons and that these tonically active receptors play a crucial role in regulating vagal afferent transmission to second order NTS neurons. The present data suggest that these actions of glucose to regulate 5-HT3 receptor translocation involves activation of a PKCβ-dependent pathway. Supported by NIH DK78364.

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MCH induced food intake in the NTS increases in obesity coinciding with increased MCH expression in vagal afferent neurons

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Objective: Vagal afferent neurons (VAN) convey information from the gastrointestinal tract important in the regulation of food intake (FI) to the nucleus tract solitarius (NTS) and play a major role in the regulation of meal size and duration. During fasting, VAN express the orexigenic neuropeptide transmitter melanin concentrating hormone (MCH); while in response to a meal MCH expression is downregulated and the anorexigenic neuropeptide transmitter cocaine and amphetamine regulated transcript (CART) is elevated. The aim of this study was to determine the physiological role of CART and MCH on feeding behavior in lean and obese animals at the level of the medial NTS (the site of vagal afferent nerve termination).

Methods: Male Wistar rats were fed chow (lean) or a high fat diet for 6 weeks (obese) and implanted with cannulas in the media NTS. Feeding tests were performed, in rats fed ad libitum or fasted 6 h, following intracranial injection of saline, MCHR1 antagonist (SNAP-94847), or CART antibody.
Results: In lean animals, inhibiting endogenous MCH within the NTS using SNAP-94847 (5 μ mol L^{-1}) significantly reduced 2 h FI in fasted rats compared to saline (6.78 ± 0.38 *vs* 10.00 ± 0.51 g, $P < 0.001$) but failed to have any effect in fed rats. Inhibiting endogenous CART in the NTS with CART antibodies (1 : 500 dilution) stimulated 2 h FI in fed rats compared to saline (5.91 ± 0.34 *vs* 2.58 ± 0.86 g, $P < 0.001$). In obese animals, SNAP-94847 inhibited 2 h FI in both fed (2.90 ± 0.52 *vs* 5.75 ± 0.63 g, $P < 0.001$) and fasted (6.54 ± 0.68 *vs* 9.20 ± 0.90 g, $P < 0.001$) conditions, while CART antibody failed to stimulate food intake. Immunohistochemical staining of obese VAN identified low CART abundance in both fed and fasted conditions; while MCH was constitutively abundant irrespective of the nutritional state of the animal.
Conclusion: CART induced inhibition of food intake in the NTS of fed rats is lost in obese rats, coinciding with constitutively low CART expression in VAN. Conversely, endogenous MCH in the NTS stimulated food intake of both fed and fasted obese animals, coinciding with constitutively high MCH expression in VAN. We propose that altered CART and MCH expression in VAN of obese rats accounts for the onset of hyperphagia.

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Glucagon-like Peptide-2 and mucosal changes induced by high fat diet in mouse small intestine

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Objective: Endogenous glucagon-like peptide 2 (GLP-2) is a key mediator of refeeding-induced- and resection-induced intestinal adaptative growth in rodents, but it is not known yet whether it may be implicated in the intestinal adaptive response induced by chronic high fat (HF) diet.
Methods: The small intestine adaptive response to HF diet was analyzed in mice following HF diet for 14 weeks and a possible involvement of endogenous GLP-2 was verified using GLP-2 (3-33) as GLP-2R antagonist. The relationship among plasma GLP-2 concentrations (measured by enzyme immunoassay), small intestinal GLP-2R expression levels (determined by real-time reverse-transcription polymerase chain reaction and western blot analysis) and intestinal morphometric parameters was determined.
Results: HF diet for 14 weeks induced hepatic steatosis and enhanced the body weight, but it did not increase small intestinal weight relative to body weight. The comparison with animals fed a standard diet (STD) showed that mice fed a HF diet exhibited an increase in crypt-villus mean height (duodenum 27.5 ± 3.00%; jejunum 36.5 ± 2.9%; $P < 0.01$) and in the thickness of the external muscularis layer (duodenum: 18.5 ± 3.53%; jejunum 13 ± 4.75%). No change in the height and width of the enterocytes or in the normalized number of goblet cells was observed. The chronic exposition to HF diet caused also a significant increase of GLP-2 plasmatic levels and of GLP-2R intestinal expression. Daily administration of GLP-2 (3–33) (30–60 ng) for four weeks reverted the increase in crypth-villus height and induced a reduction in the thickness of the muscular layer.
Conclusion: The results of the present study provide evidence for a role of endogenous GLP-2 in the intestinotrophic response to HF diet in obese mice and for a dysregulation of GLP-2/GLP-2R system after prolonged HF diet.

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Roux-en Y gastric bypass surgery-induced changes in enteroendocrine cell abundance and cytokine gene expression

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Objective: To characterize gut hypertrophic/hyperplasic and changes in gene expression in a rat model of Roux-en-Y gastric bypass surgery (RYGB).
Methods: Obese, high-fat-fed male Sprague-Dawley rats were subjected to Roux-en-Y gastric bypass or sham surgery and the gut wall was analyzed by immunohistochemistry and qPCR, 10 month after surgery.
Results: RYGB resulted in loss of all excess body weight and adiposity. Numbers of GLP-1/PYY producing L-cells as well as CCK, neurotensin, and 5-HT immunoreactive enteroendocrine cells were significantly increased about 2-fold in the hypertrophied Roux and common limbs but not in the biliopancreatic limb after RYGB, compared with the corresponding segments in sham-operated (obese) rats and unoperated lean, chow-fed rats. Expression of IL-1b was reduced and IL-10 was significantly elevated in RYGB rats compared to both other groups.
Conclusion: The results suggest general hyperplasia of the Roux and common limbs, with numbers of enteroendocrine cells increased proportionally to the increased cross-sectional areas but without increased densities of specific cell types, consistent with a GLP-2-mediated mechanism. Increased numbers of enteroendocrine cells are likely contributing to the increased basal and meal-stimulated circulating levels of GLP-1 and PYY and their combined actions on normalizing energy and glucose homeostasis.

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Weight regulating hormones correlate with parameters of the intestinal serotonergic system in obese individuals

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Objective: Recent studies have demonstrated an association between gastrointestinal hormone release and the regulation of appetite and body weight. However, most data derive from animal experiments, whereas human data investigating the regulation of gastrointestinal (GI) hormones are rare. Our study focuses on possible physiological interactions in obesity between weight-regulating hormones such as cholecystokinin (CCK), peptide YY (PYY), ghrelin, and the serotonergic system in the jejunum. In addition, we and others found that the serotonergic system, and in particular genetic variation of the serotonin transporter (SERT), is involved in the development of obesity.
Methods: Jejunum samples were collected from 102 obese patients (70 women; BMI (Mean ± SD): 43.1 ± 6.0 kg m^{-2}, Age: 41.5 ± 12.9 years) that underwent Roux-en-Y gastric bypass. Patients with diabetes ($n = 18$) or patients using psychotrophic drugs ($n = 14$) were not excluded since we could show that these factors had no significant influence on the parameters measured here. mRNA expression of CCK, PYY, nesfatin-1, ghrelin, ghrelin O-acyl transferase (GOAT), SERT and tryptophan hydroxylase 1 (TPH-1) was measured with qRT-PCR in jejunal tissue. Furthermore, ghrelin and GOAT positive cells were stained on jejunal cryostat sections (8 μm; $n = 10$) immunohistologically, quantified and correlated.
Results: We show a positive bivariate correlation of CCK with PYY ($r = 0.51$; $P < 0.001$), and with nesfatin-1 ($r = 0.57$; $P < 0.001$). In addition, CCK and PYY positively correlated with two components of the serotonergic system, SERT ($r = 0.46/0.49$; $P < 0.001/0.001$) and TPH-1 ($r = 0.26/0.56$; $P < 0.01/0.001$). The hunger hormone ghrelin, as expected, was positively correlated with GOAT ($r = 0.29$; $P < 0.005$). Surprisingly, the BMI showed no correlation with any measured weight regulating hormone. Ghrelin- and GOAT-positive cells show a positive but not significant correlation ($r = 0.53$; $P < 0.058$).
Conclusion: Our data show for the first time positive correlations between weight regulating GI hormones and the intestinal serotonergic system in obese subjects. Simulation of physiological levels of key GI signals using combination therapies may be a promising strategy in controlling meal size and subsequent weight gain.

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The study of GLP-1R expression in enteric nerve system of type 2 diabetic rat after duodenal-jejunal bypass surgery

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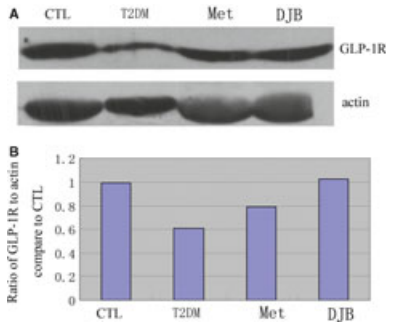
Objective: GLP-1 has multiple physiological functions beneficial in the treatment of diabetes. GLP-1R agonists and GLP-1 degradation enzyme inhibitors are proved for the treatment of T2DM. Enteric nerve system play an important role in regulation of GLP-1 secretion and also GLP-1 effect ENS functions, but the mechanism remains unclear. The purpose of this study was to determine whether GLP-1R expression in ENS was effected in T2DM and the gastric bypass could reverse the changes. In this study, duodenal-jejunal bypass (DJB) was used as a surgical model that induces glycemic control excluded the duodenal without significant weight loss.
Methods: Type 2 diabetes animal model was established by feeding intralipid and injecting low- dose STZ. These rats were randomly allocated into 4 groups: Wistar rats control group, T2DM control group, T2DM sham surgery group, DJB group. Fasting GLP-1 and blood glucose were measured before operation and 4 weeks after operation of different group rats. Animals were scarified at the end of 8 weeks, myenteric nerve plexuses layer were separated. Q-PCR was used to detect the GLP-1R expression in mRNA level; the

immunofluorescence and the western-blot were used to detect GLP-1R protein expression in the myenteic plexuses of ENS.

Results: The fasting blood glucose level of operation group decreased 4 weeks after DJB from 19.53 ± 3.45 m mol L^{-1} to 5.15 ± 0.67 m mol L^{-1} ($P < 0.01$). Immunofluorescence showed that GLP-1R expression increased significantly in the myenteric plexuses of the animals 8 weeks after DJB surgery compared to the animals of sham surgery. GLP-1R expression in MP of ENS detected by western-blot decreased to 61% in the T2DM rats compare to Wistar rats control (calculated by grey scan), and increased 103% compare to wistar group.

Conclusion: Our results showed that GLP-1R expression in ENS decreased significantly in the T2DM model rats, and DJB surgery can reverse the changes of GLP-1R expression. The results suggested that GLP-1R expression in ENS played an important role in the T2DM pathogenesis. Supported by: Supported by Scientific Research Foundation for Returned Scholars, Ministry of Education of China(2011, 43th); Shandong Province Natural Science Foundation, No. ZR2009DM024;No. ZR2011HM043; No. R2009CL047.



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The effect of motilin on gastric contraction using a newly developed diabetes mellitus model of the house musk shrew (Suncus murinus)

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Objective: We studied the functional mechanisms of motilin and ghrelin in the migrating motor complex by using the house musk shrew (Suncus murinus), a small animal model, and showed that Suncus has an almost identical gastrointestinal motility and motilin response as those found in humans and dogs. Motilin or erythromycin (motilin agonist) reportedly promotes delayed gastric emptying in diabetic human patients. However, the mechanisms of motilin in gastrointestinal motility are largely unknown. Therefore, we attempted to establish a long-survival diabetes mellitus (DM) model of Suncus, and investigated the motilin expression and the effect of motilin on gastric contraction.

Methods: To develop a DM Suncus model, streptozotocin (STZ) was intraperitoneally administered (50 mg kg^{-1}, 2–4 times). Blood glucose (BG) level, food intake, and water intake were measured. Three weeks after the BG level first exceeded 200 mg dL^{-1}, the motilin mRNA expression level was measured using quantitative PCR methods, the motilin-producing cells were immunostained, and organ bath studies were performed to investigate the effect of motilin on gastric contraction.

Results: STZ injection increased BG levels, and 73% of the DM Suncus models developed survived for more than 3 weeks. In addition, food and water intake were also significantly increased. Motilin mRNA expression in the duodenum of the DM Suncus model was 38.4% lower than that in the control. Moreover, the number of motilin-producing cells in the DM Suncus model were significantly lower (0.7 ± 0.16 cells mm^{-2}) than that in the control (2.9 ± 0.2 cells mm^{-2}). The gastric contraction following administration of motilin for the first time did not differ between the DM Suncus model and the control. However, the duration required to attain the half peak in motilin (10^{-9} mol L^{-1})-induced contraction was significantly delayed during the second administration of motilin in the DM Suncus model (control: 71.5 ± 6.1, DM Suncus model: 131.2 ± 18.7 s).

Conclusion: We established a method to develop a long-survival DM Suncus model using STZ, and observed that motilin-induced gastric contraction was delayed and motilin mRNA expression and production were decreased in the duodenum of DM Suncus. These results suggest that motilin may be involved in gastroparesis in DM.

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Effects of kaolinite ingestion on regulation by leptin of intrinsic nitrergic neurons activity in jejunum and proximal colon in rats

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Objective: Geophagy is the deliberate ingestion of soil by humans and animals and in particular of clay minerals such as kaolinite. In humans, geophagy has been observed in many parts of the world and is especially widespread in sub-Saharan Africa. Kaolinite ingestion has been previously shown to modulate gastric emptying and intestinal transit, and to interact with the intestinal mucosa in rats (Habold et al., Voinot et al.). As the inhibitory neurotransmitter nitric oxide is largely implied in gastrointestinal functions, the first aim of this work was to evaluate the impact of an imposed ingestion of kaolinite (5%) on enteric nitrergic innervation. Furthermore, kaolinite ingestion induced hyperphagia correlated to increase of plasmatic level of leptin. As leptin was shown to affect neurotransmission in the enteric nervous system, the second aim of this study was to determine whether the responses of enteric neurons in the presence of leptin were altered after kaolinite ingestion.

Methods: Male Wistar rats were fed with5% kaolinite in standard food pellets during 14 and 28 days. Ex vivo organ bath technique associated with pharmacological tools was conducted to evaluate smooth muscle contractility.

Results: The decrease in the nerve stimulation-induced relaxation due to L-NNA, a nitric oxide synthase inhibitor, was significantly reduced in the jejunum whereas the contraction was enhanced in the proximal colon at 14 days of kaolinite ingestion. Leptin inhibitory effects on relaxation in control animals were abolished in rats at 14 days of kaolinite ingestion. In the presence of L-NNA, leptin showed no effect on the relaxation in rats at 14 days of kaolinite ingestion compared to controls.

Conclusion: These data give evidence that kaolinite displays a reduction in the activation of intrinsic nitrergic neurons. This effect may explain the lack of leptin action in kaolinite rats. We hypothesized that kaolinite within the intestinal lumen stimulates intrinsic primary afferent neurons that project on these intrinsic nitrergic neurons. Changes in enteric nerves activities after kaolinite ingestion may also be due to non-beneficial effects such as iron deficiency and/or aluminum toxicity which are known to affect nitrergic neurotransmission.

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Palonosetron a serotonin-receptor-3-antagonist improves obesity-associated fatty liver disease in mice

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Objective: Obesity is a major cause for nonalcoholic fatty liver disease (NAFLD). Previous studies suggested that alterations in intestinal motility and permeability contribute to the development of NAFLD. Serotonin and serotonin receptor 3 are key factors in the regulation of intestinal motility and permeability. Therefore, we studied the effect of the specific 5-HT3R antagonist palonosetron on the development of NAFLD in leptin-deficient obese mice.

Methods: Four to six-week-old ob/ob mice and lean controls were treated for 6 weeks orally with palonosetron at 0.2 mg kg^{-1} per day. We determined physiological parameters, liver fat accumulation, portal endotoxin levels, and the tight junction protein occludin.

Results: Palonosetron treatment significantly reduced the liver to body ratio (15%), the liver triglyceride content (32%) and portal endotoxin (77%) in ob/ob mice. The beneficial effects of palonosetron were accompanied by a 30% decrease of tumor necrosis factor-alpha mRNA expression in the liver and a 130% increase of occludin in the duodenum.

Conclusion: In conclusion palonosetron is effective in attenuating NAFLD in a genetic mouse model of obesity.

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PS-21 Basic and Translational Session: Gastric Physiology, Pathophysiology

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Decreased vagal cholinergic pathway and altered gastric monoaminergic system contribute to the gastroparesis in the rats with 6-OHDA injection of substantia nigra

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Objective: Gastroparesis is a common non-motor system symptom of Parkinson's disease. However, the mechanism responsible for the gastric motor abnormality is not clear. We previously have reported the impaired gastric motility in the rats with microinjection of 6-hydroxydopamine (6-OHDA) in bilateral substantia nigra (SN) of rats (6-OHDA rats). In the present study, we explored neurochemical changes of vagal motoneurons and enteric nervous system (ENS) involved in the gastric dysmotility in 6-OHDA rats.
Methods: The strain gauge force transducer, digital X-ray imaging system, western blot, immunofluorecence and Radio Immunoassay were used in this study.
Results: Following 6-OHDA injected bilaterally into the SN, the number of tyrosine hydroxylase-immunoreactive (TH-IR) neurons and dopamine (DA) content in the lesioned SN were greatly reduced. In rats with 6-OHDA treatments, the expression of choline acetyltransferase (ChAT) ($P < 0.01$) and acetycholine (Ach) level ($P < 0.05$) were reduced in the dorsal motor nucleus of the vagus (DMV). To ascertain whether the decreased Ach could influence gastric monoamine neurotransmitters, we measured the expression of TH and serotonin transporter (SERT) and the content of DA, noradrenaline (NE) and serotonin (5-HT) in the gastric muscularis externa. SERT- and TH-IR neurons were existed in the gastric myenteric plexus. The protein levels of TH ($P < 0.05$) and SERT ($P < 0.05$) were significantly enhanced in the gastric muscular layer of 6-OHDA rats, where, correspondingly, the contents of DA ($P < 0.01$) and 5-HT ($P < 0.01$) were also markedly increased. But, no alteration of NE level was observed in the 6-OHDA rats. Next, we detected the receptor expressions of above monoamine in the gastric muscularis externa of 6-OHDA rats. The expression of cholinergic M1 receptor ($P < 0.001$) was extremely decreased, while the expressions of dopaminergic D2 receptor ($P < 0.01$) and both adrenergic β1 and β2 receptor ($P < 0.05$) were significantly increased. 5-HT4 receptor ($P < 0.05$) was found to be decreased. Furthermore, we still observed an impaired gastric motility and delayed gastric emptying in the 6-OHDA rats, which is same as our previous report.
Conclusion: Decreased excitatory Ach and M1 receptor, and enhanced inhibitory DA and D2 receptor in the gastric muscular layer may contribute to the gastric hypomotility in the 6-OHDA rats. The alterations of serotoninergic and adrenergic system in the gastric muscular layer of 6-OHDA rats might be also involved in the formation of the gastroparisis in the 6-OHDA rats. These results suggest that destruction of SN dopaminergic system caused DMV cholinergic neurons dysfunction may affect the monoaminergic system in the ENS and then lead to an impaired gastric motility.

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Insulin-like Growth Factor 1 (IGF1) encodes memory of past food intake via pleiotropic effects on the gastric neuromuscular apparatus

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Objective: Gastric dysfunction is common in protein-energy malnutritions and interferes with nutritional rehabilitation. Conversely, food intake is often reduced in gastroparesis. We found dystrophy of interstitial cells of Cajal (ICC) and enteric neurons in young female mice exposed to chronic dietary restriction. Patients with severe weight loss had similar deficits. Caloric restriction reduces IGF1, a growth/survival factor for smooth muscle cells (SMC) and, indirectly, for ICC. Here we studied whether weight gain after dietary restriction is limited by gastric neuromuscular dystrophy and whether it can be restored by IGF1.
Methods: Female BALB/c mice aged 4, 8 or 13 weeks ($n = 120$) were exposed for 170–224 days to dietary restriction that prevented natural weight gain. Subsets of mice were allowed to feed ad libitum starting at 0 (AL controls), 83, 96, or 152 days of limited feeding and/or were treated with R3LONG-rhIGF1 (150 μg kg^{-1} sc BID) starting with dietary restriction or refeeding. Solid gastric emptying was measured by 13C breath test. Estrous cyclicity was assessed by vaginal cytology. Blood glucose, ketones, serum malondialdehyde, insulin, IGF1 and corticosterone were measured by commercial kits. ICC-, SMC- and neuron-specific gene expression was studied by Western immunoblotting.
Results: Weight rose logarithmically in AL mice. Mice on restricted diet had lower ketones ($P = 0.002$) and IGF1 ($P = 0.016$) and 81% were in anestrus. Gastric emptying was delayed ($P = 0.016$), and Kit (ICC), Myh11 (SMC), Uchl1 and Nos1 (neurons) protein were significantly reduced. Weights of mice restricted from 13 weeks of age normalized in 18 days upon ad-libitum refeeding. In contrast, in mice first exposed to restricted diet at 4 or 8 weeks of age, weights only increased to 88 ± 2% and 86 ± 3% of AL weights, respectively ($P < 0.01$), and this difference was maintained for >60 days. In these cohorts, gastric emptying was further delayed ($P < 0.001$). IGF1 normalized gene expression and gastric emptying in both restricted and refed mice and also improved weight gain in refed mice.
Conclusion: Chronic dietary restriction reduced functional capacity of the gastrointestinal neuromuscular apparatus likely through the dystrophic effects of low circulating IGF1. In young mice these changes persisted after refeeding but could be minimized by IGF1 treatment. Grant support: NIH DK68055.

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Relaxation induced by guanosine in mouse stomach

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Objective: A number of evidence suggests that guanine-based purines can be considered part of the purinergic system, because they can be stored in synaptic vesicles and released from neurons following depolarization. They may act as neuromodulators, interfering with other neurotransmitters, including acetylcholine released by enteric neurons in mouse colon (Zizzo et al., 2011). The present study verified whether guanosine may affect gastric emptying and the mechanical tone of the isolated mouse stomach and examined the mechanism of action responsible for the effects observed *In vitro*.
Methods: The influence of guanosine on the gastric emptying rate was determined in mouse and its effects on spontaneous gastric mechanical activity, detected as changes of the intraluminal pressure, were analysed *In vitro* before and after different treatments.
Results: Oral guanosine (1.75–10 mg kg^{-1}) decreased the gastric emptying in a concentration-dependent manner. Guanosine (30 μ mol L^{-1}–1 m mol L^{-1}) induced a concentration-dependent relaxation of isolated stomach which persisted throughout the administration time and was reversible after washing out. At the maximal concentration tested, the effect reached about 60% of the relaxation induced by 1 μ mol L^{-1} isoproterenol. The inhibitory response was not affected by TTX (1 μ mol L^{-1}), a blocker of neuronal voltage-dependent Na^+ channels, but it was abolished by 6-[(4-Nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (NBTI) (10 μ mol L^{-1}), a nucleoside uptake inhibitor. Moreover, the guanosine-induced effects persisted in the presence of ODQ (10 μ mol L^{-1}), an inhibitor of nitric oxide-dependent guanylate cyclase or apamin (0.1 μ mol L^{-1}), a small conductance $Ca2^+$-dependent K^+ channels, but they were progressively reduced by increasing concentrations of DDA (10–30 μ mol L^{-1}), an adenylyl cyclase inhibitor.
Conclusion: The results of the present study provide the first experimental evidence that exogenous guanosine is able to inhibit gastric emptying and to induce gastric relaxation acting peripherally on the mouse stomach. The effect does not involve genesis and propagation of action potentials, nitric oxide release or opening of small conductance $Ca2^+$-dependent K^+ channels. Indeed, it appears to be dependent on guanosine cellular uptake and to involve adenylyl cyclase activation. References Zizzo et al.,. Eur J Pharmacol 2011; 650:350–5.

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P2Y1 purinoreceptors are essential forinhibitory neurotransmission in the murine gastric antrum

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Objective: Gastric inhibitory motor neurons regulate several motility patterns including receptive relaxation in the fore stomach, gastric peristaltic motor patterns and pyloric sphincter opening. The identity of the neurotransmitters involved in gastric motor patterns has been studied for decades, and numerous candidates have been suggested. Activation of enteric inhibitory motor neurons causes membrane hyperpolarization characterized by an inhibitory junction potential (IJP)

and muscle relaxation in mammalian stomachs. In the murine gastric antrum the inhibitory response consists of single fast hyperpolarization (fIJP) that is insensitive to the nitric oxide synthase inhibitor L-NNA and thus thought to be due to release of a purine neurotransmitter. However, the post-junctional receptor(s) that mediates the response to inhibitory purines in the antrum is not currently known. Recently, we have endeavored to identify the receptor(s) involved using mice with genetically deactivated P2y1 receptors.

Methods: Dual intracellular microelectrode and isometric force recordings were used in combination with electrical field stimulation (EFS) of enteric inhibitory nerves to examine post junctional inhibitory neural responses in the gastric antrum.

Results: Activation of inhibitory nerves by EFS produced a frequency-dependent fIJP that produced muscle relaxation. Stimulation of inhibitory nerves during a slow wave prematurely terminated the pacemaker event and associated contraction. In P2y1-/- mice the IJP and associated muscle relaxation either between or during a slow wave event was completely absent. MRS2500, a P2Y1R antagonist, inhibited the IJP and associated relaxation in wildtype controls but had no effect on the spontaneous electrical activity or responses to EFS in P2y1-/- mice. Picospritzing the purines, ATP and the newly identified ß-NAD resulted in differential responses in wildtype and P2y1-/- mutants. ATP produced membrane hyperpolarization in stomachs from both animals and this was not blocked by MRS2500 (1 μ mol L^{-1}). In contrast, the hyperpolarization produced by ß-NAD was inhibited by MRS2500 in wildtype animals but was absent in P2y1-/- mutants.

Conclusion: The results demonstrate that a purine inhibitory neurotransmitter via P2Y1R contributes to muscle relaxation in the gastric antrum. Since hyperpolarization responses to ß-NAD better mimicked the inhibitory post-junctional responses than ATP we conclude that ß-NAD is the more likely candidate to be the endogenous inhibitory neurotransmitter than ATP.

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Relaxation induced by menthol on mouse gastric tone: Analysis of action mechanism

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Objective: Menthol is the main active constituent of the essential oil of Mentha Piperita (peppermint oil), commonly used in alternative medical therapy in a variety of pathological gastrointestinal conditions, although available data on the mechanisms responsible for beneficial effects are poor and inconclusive. One of the main hypothesis concerns its interaction with the TRPM8 channels, a member of TRP superfamily. Since studies on menthol effects on the gastric motility are lacking, the aims of the study were to analyze the effects of menthol on the mechanical activity of mouse stomach and to clarify the mechanism responsible for the effects observed.

Methods: The mechanical activity was analyzed *In vitro* as changes of intraluminal pressure. The neurally-evoked cholinergic contraction, induced by electrical field stimulation (EFS: 0.5 ms duration, supramaximal voltage, in trains of 5 s, 8 Hz) was also analyzed in presence of increasing concentrations of menthol. TRPM8 expression was examined by reverse transcription-polymerase chain reaction (RT-PCR).

Results: Menthol (1 m mol L^{-1}–3 mol L^{-1}) produced a concentration-dependent relaxation of gastric tone. WS-12, a TRPM8 specific agonist, failed to affect the gastric tone. Moreover TRPM8 mRNA was not detected by RT-PCR analysis in whole organ suggesting the absence of these channels in mouse stomach. The inhibitory effects of the menthol were partly antagonized by tetrodoxin, a blocker of neuronal voltage-dependent Na+ channels, or ω-conotoxin GVIA, a blocker of N-type voltage-dependent $Ca2^+$ channels, but they were not affected by Nω-nitro-L-arginine methyl ester (L-NAME), a blocker of nitric oxide synthase, apamin, a blocker of small conductance $Ca2^+$-dependent potassium channels or [Lys1,Pro2,5,Arg3,4,-Tyr6]VIP7–28, a VIP receptor antagonist. Interestingly, atropine, a muscarinic cholinergic receptor blocker, significantly antagonized menthol effects. Moreover, menthol (1–100 μ mol L^{-1}) produced a concentration-dependent reduction of the EFS-evoked cholinergic contractions.

Conclusion: The present results suggest that menthol is able to induce gastric relaxation of mouse stomach in a TRPM8-independent manner. The inhibitory effect appears to be mediated, almost in part, by neural mechanism leading to a reduction of acetylcholine release from enteric nerves.

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The effects of progesterone on gastric motility in pregnant rats: A bradygastric response

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Objective: Several hormonal and physiological changes that occur during pregnancy seem to affect the gastrointestinal (GI) motility, however very little is known about relationship between sex hormones and mechanical activities of the stomach. The aim was to evaluate the relationship between female sex hormones (progesterone and estradiol) with the frequency of gastric contractions in fed pregnant rats using AC Biosusceptometry (ACB).

Methods: Seven female Wistar rats (weighting 250–350 g) were used and all procedures were approved by the local Animal Ethics Committee. Laparotomy was performed to implant a magnetic marker (ferrite with diameter of 3.5 mm and height of 3.0 mm) in distal stomach. Ten days after surgical recovery, mating occurred until pregnancy confirmation. Magnetic measurements were performed in anesthetized animals (pentobarbital, 30 mg kg^{-1}) during 30 min with an ACB sensor placed on the abdominal surface. This procedure was carried out in day-1, -7, -14 and -20 of pregnancy concomitant to blood collection. Magnetic signals were analyzed in MatLab to determine the frequency of gastric contractions and to compare these data with serum concentration of female sex hormones determined by means of ELISA methodology. Statistical analysis was performed by Student's *t*-test and Pearson Correlation test.

Results: The frequency of stomach contractions was 4.4 ± 0.2 cycles per minute (cpm), 4.0 ± 0.4 cpm, 3.5 ± 0.3 cpm ($P < 0.05$ vs day-1), 4.3 ± 0.3 cpm in day-1, -7, -14 and -20, respectively. The values of 44 ± 15 pg mL^{-1}, 31 ± 9 pg mL^{-1}, 25 ± 6 pg mL^{-1}, 22 ± 9 pg mL^{-1} in day-1, -7, -14 and -20, respectively, for 17ß- estradiol were not statistically different. Progesterone values were 13 ± 6 pg mL^{-1}, 54 ± 15 pg mL^{-1}, 127 ± 42 pg mL^{-1} ($P < 0.05$ vs day-1), 22 ± 13 pg mL^{-1} in day-1, -7, -14 and -20, respectively. The linear correlation coefficient between gastric contraction frequency and 17ß- estradiol was 2% ($P < 0.2$), whereas between gastric contraction frequency and progesterone was 81% ($P < 0.0001$).

Conclusion: Our data show a strong inverse relationship between gastric contraction frequency e progesterone serum levels throughout pregnancy in rats. To date, associations between hormones and motility changes have not been described in detail because, in part, of methodological constraints. Our biomagnetic technique offers new insights about hormone participation in bradygastria during pregnancy with accuracy and without interfere with normal physiology.

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A simple method for measuring gastric emptying in pre-weaned rats in natural free-feeding conditions

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Objective: Many techniques for assessing gastric emptying in small animals have been described in the literature. The majority, however, involve the use of chemical markers such as phenol red, performing surgery or sacrificing the animal to assess gastric emptying or involve the use of scintigraphy with a chemically specified meal after a long period of fasting. There are few non-invasive techniques reported in freely-feeding animals. It has previously been shown that gastric emptying increases toward the end of the lactation period in rat pups. The aim of this study was to investigate whether a non-invasive method of a water soluble contrast meal in a conscious freely suckling pup is a reliable and useful method of assessing gastric emptying in pre-weaned rats.

Methods: Pre-weaned freely-lactating Sprague Dawley rat pups were gavaged with 0.2 ml of Omnipaque (Iohexol 300 mg ml^{-1}) and were placed in an X-ray compatible red-tinted Perspex tube restrainer device and underwent time-lapsed imaging using an In-Vivo FX Pro Imaging System to assess gastric emptying. Contrast studies were performed on postnatal days 5, 7 and 19. Gastric emptying time was measured as the time when all contrast was seen to have entered the small bowel. Pups were marked and returned to the litter and were monitored and weighed regularly.

Results: Four Sprague Dawley rat pups (3 female, 1 male) were gavaged and imaged. Their weights ranged from 13–17 g at P5 to 42–43.8 g by P19. Gastric emptying time decreased as expected over the study period from a median of 120 min at P5 to 75 min at P19. The procedure was well tolerated by all pups and all were returned to their litter without incident and continued to gain weight. Some pups were noted to have brief perianal excoriation following passage of the contrast; however, this may have been due to over grooming by the mother.

Conclusion: Water soluble contrast studies are a simple, safe and reliable method of measuring gastric emptying in freely feeding pre-weaned rat pups without the need for scintigraphy or more invasive methods.

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The effect of the GLP-1 receptor agonist, exendin-4 on blood glucose levels, emesis and gastric myoelectric activity, in the ferret

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Objective: Glucagon-like peptide-1 receptor agonists, such as exendin-4, are being developed for the treatment of type 2 diabetes and obesity, but the treatment may be associated with nausea and emesis. In the present studies, we investigate the potential emetic mechanism of exendin-4 using the ferret.
Methods: Glucose tolerance test: Ferrets were anaesthetized and injected with the GLP-1 receptor antagonist, exendin (9–39) (300 n mol kg^{-1}, s.c.), or saline (0.5 ml kg^{-1}, s.c.). 15 min later, exendin-4 (30 n mol kg^{-1}, s.c.), or saline (0.5 ml kg^{-1}, s.c.) was injected, followed by a glucose load (1.5 g kg^{-1}, i.p.). Blood glucose was measured for 120 min (n = 3). Telemetric study: Radio telemetry transmitters (DSI) were used to measure GMA, blood pressure and body temperature. Under general anesthesia the two biopotential wires were implanted in the antrum and the pressure catheter was implanted in the abdominal aorta. Seven days later, telemetry baseline recordings were made 1 h before the treatment of exendin-4 (30 n mol kg^{-1}, s.c.), or saline (0.5 ml kg^{-1}, s.c.). Recordings were then continued for 24 h (n = 6). I.C.V. study: A guide cannula was implanted into the 3rd ventricle of the brain under general anesthesia. Seven days later, they were injected with exendin-4 (0.3–30 n mol, i.c.v.), or saline (15 μl, i.c.v.) and behavior was recorded for 1 h (n = 3–6).
Results: The basal blood glucose level was 5.2 ± 0.4 m mol L^{-1}. The glucose load progressively increased blood glucose in the saline-treated animals, which peaked at 30 min before decreasing gradually. Exendin-4 reduced the AUC0-120 by 36.3%. Exendin (9–39) antagonized the effect of exendin-4, but also increased AUC0-120 by 31.0% when used alone (P < 0.05). Subcutaneously administered exendin-4 (30 n mol kg^{-1}) failed to affect gastric myoelectric activity, heart rate, blood pressure or body temperature, but induced emesis in two out of six ferrets (P > 0.05). Exendin-4 at 30 n mol, i.c.v. induced 88.2 ± 48.7 retches and 11.6 ± 6.8 vomits, following a median latency of 14.9 min (P < 0.05). The threshold dose to induce emesis was 10 n mol (P > 0.05). Exendin-4 at 0.3–30 n mol, i.c.v. inhibited food (P < 0.05), but not water-intake.
Conclusion: These studies indicate that exendin-4 induces emesis in ferrets at doses that modulate glucose levels. The mechanism to induce emesis may have a central component.

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Dyspepsia and diet

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Objective: Although many patients recognize the impact of specific food in symptom occurrence, very few population-base studies evaluated the role of diet in dyspepsia. The aim of the study was to determine the prevalence of dyspepsia in general urban population and to evaluate the type of diet associated with dyspepsia.
Methods: A representative sample (n = 300) for the general population living in an urban area (18 000 habitants) was randomized from the family doctors patient lists and selected subjects were invited for interview in the family doctors office. An interview-based questionnaire was used to diagnose dyspepsia using Rome III criteria for functional dyspepsia and to evaluate eating habits and frequency of food intake. Socio-demographic factors and general medical history were also included in interview together with objective evaluation of overweight. Results from logistic regression were presented as odd ratio and 95% confidence intervals.
Results: 193 subjects (80 males, 113 women, mean age 50.8 ± 16.2, range 20–85 years) participated during January –April 2011. The prevalence of dyspepsia was 8.29% (4.62% for women and 13.75% for men, P < 0.05). The age distribution of dyspepsia subjects indicated increased prevalence of dyspepsia for subjects above the mean age of sample (87.5%, P < 0.05). Patients with dyspepsia had more common cardio-vascular diseases (50.0% vs. 25.4%, P < 0.05), including arterial hypertension (68.8% vs. 37.3%, P < 0.05). Smoking was not associated with dyspepsia: 31.3% of dyspeptic patients were smokers, vs. 26.0% non-dyspeptic subjects (P > 0.05). Dyspeptic patients perceived their well being status to be poor (15.4%, versus 8.1% in non-dyspepsia subjects, P > 0.05), acceptable (53.8% vs. 36.3%, P > 0.05), good (30.8% vs. 40.7%, P > 0.05). Just non-dyspepsia subject perceived a very good condition (14.8%). No subject perceived a very poor condition of well-being. In the sample studied, 49.3% were overweight and 20.8% obese. Presence of obesity was not different in dyspepsia (18.8%) and non-dyspepsia subjects (21.0%) (P > 0.05). Using median of food consumption frequency as cut-off point, dyspeptic patients consume significantly more frequent canned food (7.2, 1.6–32.8, P < 0.01) and less frequent grain cereals (0.2, 0.6–0.7, P < 0.05). There was not significantly different consumption for the following type of foods: milk, fish, eggs, fats, vegetables with 5% carbohydrate (lettuce, spinach, tomatoes and peppers), pulses, white bread, sugar and sweets, alcoholic beverages and coffee.
Conclusion: This study updated prevalence data and reveal association between food and dyspepsia.

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Morphological and functional aspects of experimental toxic gastritis induced by enteral impact of lignite charcoal

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Objective: The aim of this study was to evaluate the morphogenesis and development mechanisms of toxic gastritis induced by enterally administered lignite charcoal in experimental settings.
Methods: The experiment was carried out in 30 experimental male mice with a body weight between 25–28 g. Control group mice were fed a standard fodder, while experimental group mice received daily, along with the fodder, lignite charcoal particles of the 2B brand obtained at the Borodinskiy coal strip mine, Kansko-Achinskiy coal field. The charcoal dose was either 0.1 g or 0.2 g per animal. Animals were withdrawn from the experiment on 77th day, their stomachs were removed for histological examination and fixed in 10% formalin; afterwards, paraffin sections were prepared according to the conventional technique and stained with haematoxylin and eosin. Morphological examination of obtained material was performed using light microscopy. A number of parameters used in the morphological assessment of gastric mucous membrane structural abnormalities were also utilized to accumulate a database, which served for subsequent correlational and neural network analyses.
Results: Morphological examination revealed macroscopic signs of blood circulation disturbances, specifically microscopic haemorrhages.At microscopic level, pathomorphological alterations in mice of both study groups were characterized by dystrophy and focal atrophy in the integumentary epithelium (morphometry demonstrated the following mean integumentary epithelium heights:27.14 μm following administration of 0.1 g charcoal dose, 26.68 μm after 0.2 g dose, and 30.71 μm in controls, P 1, 2 < 0.05) accompanied by focal infiltration of mucosa lamina propria with leukocytes (lymphocytes) and macrophages, and, as a result, compression and deformation of gastric glands. All mice presented with pronounced dystrophy in the gastric glandular epithelium, and necrosis was observed as well in 60% cases. With the 0.2 g dose, the rate of integumentary epithelium atrophy increased by 30% compared with the 0.1 g dose, and the necrosis was more pronounced.
Conclusion: Enteral administration of lignite charcoal leads to development of toxic gastritis affecting all layers of the gastric mucous membrane. The most important morphological and functional alterations involve the integumentary epithelium and include focal atrophy and necrosis; the higher dose of the substance was shown to aggravate infiltration with lymphocytes, macrophages, and neutrophils of lamina propria in mucous membrane of stomach.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-22 Basic and Translational Session: Probiotics, Pharmacotherapy, Pharmacogenomics and Pharmacology

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Piperine improves swallow response of patients with neurogenic dysphagia

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Objective: To explore the expression of TRP (Transient Receptor Potential) V1 inthe areas innervated by the Trigeminal (V cranial nerve), the Glossopharyngeal (IX cranial nerve) and the Superior Laryngeal Nerve (branch of the X cranial nerve) in the human oropharynx and to characterize the effect of increasing sensorial input through the TRPV1 stimulation with the natural agonist piperine, on the swallow response of dysphagic patients.
Methods: (i) Real Time Polymerase Chain Reaction (qPCR): n = 10 surgical samples from the human tongue, pharynx and larynx were obtained. mRNA was extracted and retrotranscripted to cDNA. A qPCR was conducted to quantify the expression of TRPV1. (ii) Videofluoroscopy: 40 patients with neurogenic dysphagia (75.83 ± 2.03 year) were randomized in two groups. Patients were studied by videofluoroscopy during the deglutition of one series of nectar control boluses, and two series of nectar boluses supplemented with 150 μ mol L^{-1} piperine (Group 1) or 1 m mol L^{-1} piperine (Group 2). Prevalence of laryngeal vestibule penetrations and timing of the oropharyngeal swallow response were calculated.
Results: (i) qPCR: TRPV1 mRNA was detected in the human oropharynx and larynx. TRPV1 showed a maximal expression in the tongue and decreased towards the epiglottis. (ii) Videofluoroscopy: The addition of 150 μ mol L^{-1} piperine to the alimentary bolus reduced penetrations by 34.5% and 1 m mol L^{-1} piperine reduced them by 50.3%. Moreover, 150 μ mol L^{-1} piperine shortened the time to laryngeal vestibule closure from 0.366 ± 0.023 s to 0.270 ± 0.022 s (P < 0.001) and 1 m mol L^{-1} piperine from 0.380 ± 0.032 s to 0.306 ± 0.028 s (P < 0.01).
Conclusion: TRPV1 channels are extensively expressed in the human oropharynx and larynx. Piperine improves safety of swallow by shortening the time of airway closure. Increasing sensorial input through TRPV1 stimulation is a promising therapeutic strategy for patients with neurogenic dysphagia.

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Lactobacillus casei DG decreases LPS-induced nitric oxcide and enteroglial-S100B secretion in human intestinal biopsies

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Objective: Enteroglial derived S100B protein plays an active role in nitric oxide (NO)-dependent gut inflammation. Previous data show that pathogen bacteria are able to induce S100B release from enteroglial cells (EGCs). Whether probiotics are able to counteract these effects is not established yet. We aimed to study the effects of Lactobacillus Casei DG (LC-DG) on lipopolysaccharides (LPS)-induced iNOS protein expression, nitric oxide (NO) and S100B secretion from human intestinal biopsies.
Methods: Rectal biopsies from 3 healthy subjects undergoing screening for colorectal cancer were stimulated with LPS (10 μg mL^{-1}) and/or with LC-DG (108 bacteria mL^{-1}), as follows: experiment A) stimulation with LPS starts at 0 h and end at 5 h (0–5 h); B) LC-DG 0–5 h; C) LC-DG 0–5 h + LPS 2.5–5 h; D) LPS 0–5 h + LC-DG 2.5–5 h; E) LPS 0–5 h + LC-DG 0–5 h. To test EGCs stimulation, S100B release was evaluated by ELISA assay. Nitrite assay and Western Blot analysis were used to evaluate NO release and iNOS expression, respectively, in stimulated biopsies compared to unstimulated biopsies (control). Data are expressed as mean±SD of 6 independent experiments.
Results: In rectal biopsies, LPS (experiment A), but not LC-DG (experiment B), significantly increased S100B secretion (+1.8 ± 0.5 fold increase vs control; P < 0.05). With experiments C, D and E S100B secretion was not increased respect to control. In parallel, incubation with LPS (experiment A) led to a significant increase of iNOS protein expression (+2.0 ± 0.3 fold increase vs control; P < 0.05) and of NO secretion (+2.9 ± 0.7 fold increase vs control; P < 0.05), that was not observed with LC-DG (experiment B). With experiments C, D and E iNOS expression and NO secretion were similar to control.
Conclusion: We show that the probiotic LC-DG decreases LPS-induced NO secretion in human biopsies by inhibiting iNOS protein expression. This effect may be mediated by EGCs since LC-DG also decreases LPS-induced enteroglial-derived S100B protein secretion.

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Association between functional dyspepsia and neuronal nitric oxide synthase gene polymorphism

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Objective: Meal-induced satiety is under nitronergic control and impairment of this system can lead to functional dyspepsia (FD). Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) which exists in three isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible isoform (iNOS). NO production is known to be regulated by NOS polymorphisms. Our aims were to assess whether functional polymorphisms in eNOS, nNOS, or iNOS genes are involved in the susceptibility of FD and to investigate if genotype difference confers functional relevance in FD patients.
Methods: Genomic DNA from 89 patients with FD and 180 healthy subjects matched for age and gender were typed for nNOS (rs2682826), iNOS (rs2297518) and variable number tandem repeat in intron 4 of the eNOS gene. The subjects ingested 125 ml of liquid meal (Ensure liquid) at 5-min period intervals 4 times (total 500 ml). They scored the symptoms of fullness, discomfort, bloating and nausea using a visual analogue scale with 100-mm lines at 5, 10, 15, 20 and 50 min after starting the drink test.
Results: Genotype frequencies of the eNOS were not significantly different between FD patients and controls. In the iNOS, CC homozygotes were less frequent in FDs compared with controls, but the significance disappeared when the odds ratio of the iNOS CT and TT genotypes relative to the CC genotype was calculated after applying dominant model. Frequency of T allele in the nNOS was significantly higher in the FD patients compared to the controls (49% vs 16%; odds ratio, 5.01; 95% confidence interval, 2.83–9.01; P < 0.001). Patients with T allele in the nNOS polymorphism reported higher satiation score, as the sum of VAS during the drink test period, than those with CC genotype (median, 179 vs 117; P = 0.035).
Conclusion: nNOS gene polymorphism is linked to the susceptibility of FD and influences satiation in FD patients. Our data support the importance of NOS gene polymorphisms in the pathogenesis of FD.

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Effect of clomipramine on abdominal pain and brain activation in response to colorectal distention

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Objective: Brain-gut interaction is one of major pathophysiological features of irritable bowel syndrome (IBS). Although antidepressants are believed to be effective on IBS only after administration for a few weeks, physiological changes in brain-gut axis in humans by acute administration of antidepressants are quite obscure. Clomipramine is an injectable tricyclic antidepressant which mainly inhibits serotonin reuptake. Acute administration of clomipramine may modify visceral perception-related brain activation. The aim of this study is to test the hypothesis that acute administration of clomipramine reduces the specific regions of the brain activation during colorectal distention in healthy males.
Methods: Subjects were 18 normal healthy males aged 22 ± 1. Either clomipramine (250 μg kg^{-1}) (n = 9) or placebo (saline) (n = 9) was intravenously infused to them. A barostat bag was inserted into the descending colon or the rectum of each subject. Colorectum was distended with a computer-controlled barostat device with 0 mmHg, 20 mmHg, or 40 mmHg of the bag pressure at random order. During colorectal distention, regional cerebral blood flow with injection of H_2[15-O] was measured by positron emission tomography. After each scan, blood was sampled from the venous line for the analysis of plasma catecholamines, adrenocorticotropic hormone (ACTH) and cortisol. Visceral perception and emotion were assessed with ordinate scale.
Results: Intravenous infusion of clomipramine significantly suppressed colorectal distention-induced visceral perception compared with placebo (P = 0.034). Intravenous infusion of clomipramine significantly

more deactivated amygdala and posterior cingulate cortex in response to colorectal distention than that of placebo ($P < 0.005$). Compared with placebo, clomipramine significantly activated hippocampus and parahippocampal gyrus in response to colorectal distention ($P < 0.005$). By contrast, plasma ACTH and cortisol significantly increased after the administration of clomipramine ($P < 0.01$).

Conclusion: These results suggest that administration of clomipramine can suppress visceral nociception via inhibition of activities of amygdala and posterior cingulate cortex even during acute single infusion. Moreover, acute single administration of clomipramine and colorectal distention likely induces serotonin and cortisol secretion which may stimulate hippocampus.

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Effects of erythromycin on esophageal contractility in healthy volunteers: An advanced muscle contraction analysis

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Objective: Little is known of the basic physiology of the human esophageal contractility in response to motility enhancing drugs. Erythromycin has proved to increase contractility in the small bowl and been proposed to increase esophageal contractility too. The study aims were to examine Erythromycin induced changes in motility patterns of the human esophagus.

Methods: Sixteen healthy men and women with a mean age of 23 years (range 19–34, 6 females) participated in the study. An esophageal probe was fitted with a bag near the distal tip. The probe was positioned in the esophagus with a bag distanced 10 cm. from the esophago-gastric junction. The bag volume was increased step-wise from 5 to 25 ml before and after an IV Infusion of 250 mg erythromycin. Analysis included changes after erythromycin administration in: (i) esophageal baseline pressure (preload tension), (ii) swallowing force (primary esophageal peristalsis/ propulsive force), and (iii) esophageal luminal clearance (distension evoked secondary esophageal peristalsis). Furthermore, curve analysis was done of Pressure (P)-Cross sectional areas (CSA) (P-CSA) as well as wall tension-CSA areas.

Results: Erythromycin administration caused higher esophageal baseline pressure (2.45 ± 0.63 N m^{-1} *vs* 4.73 ± 0.60 N m^{-1}, $P = 0.049$), and swallowing force (7.41 ± 0.98 N m^{-1} *vs* 11.9 ± 0.92 N m^{-1}, $P = 0.0014$) at lower distension volumes. However, the drug did not cause significant changes in any parameters at the higher distension volumes (volumes >5 ml, $P > 0.1$)). During secondary peristalsis the area of tension-CSA curves increased (5388 ± 1864 μJ *vs* 16495 ± 1954 μJ at 25 ml, $F = 64.4$, $P < 0.001$) as well as the propulsive force (37.5 N. ±14.8 N m^{-1} *vs* 137.2 ± 15.5 N m^{-1} at 25 ml, $F = 108.1$, $P < 0.001$) compared to primary peristalsis. The peristalsis and following relaxation resulted in loop like P-CSA and wall tension-CSA curves.

Conclusion: The results do not support a role for Erythromycin in patients with dysphagia of solid foods and week esophageal peristalsis.

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Elucidation of the mechanisms underlying the potential usefulness of STW 5 in reflux esophagitis

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Objective: STW 5, a multi-component herbal preparation, was shown to relieve heartburn and concomitant reflux symptoms in patients with functional dyspepsia and to prevent inflammation in an acute model of reflux esophagitis (RE), without affecting the pH of the refluxate. The present study assesses the efficacy of STW 5 in a more chronic model of RE, and the underlying mechanisms both in-vivo and *in vitro*.

Methods: Rats were pretreated for 7 days with STW 5 or omeprazole (as reference drug) before surgical induction of esophagitis. RE was achieved by ligation of the fore-stomach and covering the duodenum near the pylorus with a piece of Nelaton catheter. After recovery, rats were treated for a further 10 days with the drugs and then sacrificed, their esophagi excised, weighed and evaluated macroscopically. Tissue homogenates were used for semi-quantitative determination of cytokines using a cytokine array. Since recent evidence indicates that the mucosal damage observed in GERD is due to release of inflammatory mediators from mucosal and sub-mucosal cells in response to bile salts and other substances in the gastric refluxate, the effects of STW 5 on cytokine release from the normal human esophageal cell-line HET-1A in response to stimulation with chenodeoxycholic acid (CDCA) was assessed.

Results: Both, STW 5 and omeprazole, improved body weight, tissue damage score and esophageal weight index to similar extents. However, STW 5 had a much more pronounced anti-inflammatory effect. As shown in the cytokine array (Fig. 1), STW 5 inhibited the majority of the measured pro-inflammatory cytokines induced by surgical reflux, suggesting a direct anti-inflammatory and/or mucosal protecting action. Furthermore, incubation of HET-1A cells with CDCA caused marked release of CD40-ligand, IFN-γ, IL-1ra, IL-6, IL-8 and IL-23, which were all inhibited by co-incubation with STW 5 (0.3–10 μl ml^{-1}) as assessed semi-quantitatively (cytokine array, Fig.2) and confirmed quantitatively (ELISA), without affecting cell viability. Similar effects were observed when cells were incubated with capsaicin as TRPV1 agonist (shown to be the main receptor involved in acid induced cytokine release from HET-1A cells), which evoked the release of CD40-ligand and IL-23.

Conclusion: The present findings suggest that multi-target anti-inflammatory drugs like STW 5 might present an alternative/additional treatment option for GERD patients not responding adequately to PPIs. Further elucidation of its exact mechanism of action might pave the way for a new class of anti-reflux agents.

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Terminal restriction fragment length polymorphism analysis of the diversity of fecal microbiota in patients with Irritable Bowel Syndrome (IBS)

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Objective: The pathophysiology of irritable bowel syndrome (IBS) might be related to abnormal host-microbial interactions. The aims of this study were to perform terminal restriction fragment length polymorphism (T-RFLP) analyses of fecal microbiota in IBS patients and to investigate the potential alterations in fecal bacterial communities.

Methods: A total of 85 subjects, including 36 patients with IBS and 49 healthy subjects (HS) were enrolled. DNA was extracted from their stool samples, and the 16S rRNA genes were amplified by real time polymerase chain reaction (RT-PCR). The PCR products were then digested with MspI and HinPlI restriction enzymes, and the length of the terminal restriction fragments (T-RFs) was determined. The sizes of T-RFs were rounded to the nearest number between samples to produce operational taxonomic units (OTUs). We used PRIMER v6 for statistical analysis. A one-way analysis of similarity (ANOSIM) was used to compare the microbial communities between the two groups. Hierarchical clustering and non-metric multi-dimensional scaling (nMDS) was performed to visualize the

Table. Operational taxonomic units contributing significant differences in fecal bacterial communities between the IBS patients and healthy subjects

Abundant OTUs in IBS Patients			Abundant OTUs in HS		
OTU	IBS/HS	Predicted bacteria	OTU	HS/IBS	Predicted bacteria
99	2.5	Prevotella, Bacteroides, uncultured bacterium	222	1.8	Uncultured bacterium
97	2.1	Bacteroidetes (Bacteroides, Prevotella, Porphyromonas), Epsilon proteobacterium (Helicobacter, Campylobacter)	300	1.6	Selenomonas, *Megasphaera elsdenii*, uncultured bacterium
216	2.1	Enterococcus, uncultured bacterium			
90	1.6	Bacteroidetes, Aeromonas, uncultured delta proteobacterium, uncultured gamma proteobacterium			

OTU, operational taxonomic unit; HS, healthy subjects; IBS, irritable bowel syndrome

degree of dissimilarity among each samples as dendrogram and multi-dimensional graphs. Analysis of similarity percentages (SIMPER) was done to determine the overall average dissimilarity and the OTUs significantly contributing the dissimilarity in microbial community compositions between the two groups. The bacterial species of the significantly different OTUs were predicted from the database that we developed (http://microbiology.cau.ac.kr) based on the silico PCR amplification and restriction of 16S rRNA sequences.
Results: The composition of the fecal bacterial communities in IBS significantly differed from that of HS ($P < 0.05$). Dendrogram and nMDS showed that the distribution of the fecal microbiota was clearly different between the two groups. Dissimilarity between the IBS patients and the HS was 78.6%. The OTUs that significantly contributed to the dissimilarities were shown in Table. Bacteriodetes (Bacteroides, Prevotella, Porphyromonas) and Epsilon proteobacterium (Helicobacter, Campylobacter) were significantly abundant in IBS. Selenomonas and Megasphaera elsdenii were significantly lack in IBS compared to HS.
Conclusion: The composition of fecal microbiota in IBS significantly differs from that of HS, and some bacterial species were significantly deceased in IBS. The lacking bacterial species in IBS may be some therapeutic targets.

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Mechanisms of action of Otilonium Bromide (OB) in human cultured smooth muscle cells and rat colonic strips

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Objective: Otilonium Bromide (OB) is a molecule with spasmolytic properties used to treat colonic motor disorders. In the present study we have investigated the mechanisms of action of OB.
Methods: We studied human cultured smooth muscle cells (HCSMC) using calcium imaging and rat colonic strips using both microelectrodes and muscle bath contractile experiments.
Results: OB concentration dependently inhibits 1- KCl (75 m mol L^{-1}) induced (nifedipine sensitive) calcium transients (Ratio=0.44 ± 0.04 at 1 μ mol L^{-1}, $n = 20$) and 2- Carbachol (1 μ mol L^{-1}) induced (atropine sensitive) calcium transients (Ratio=0.20 ± 0.05 at 1 μ mol L^{-1}, $n = 30$) in HCSMC. In colonic strips incubated in nominally calcium free Krebs, CaCl2 10 m mol L^{-1} induced a nifedipine sensitive contraction that was concentration dependently inhibited by OB (EC50 = 9.4 μ mol L^{-1}, $n = 6$). To avoid a possible interference with L-type calcium channels electrophysiological and mechanical experiments were performed in the presence of Nifedipine (1 μ mol L^{-1}). L-NNA (1 m mol L^{-1}) and MRS2500 (1 μ mol L^{-1}) were used to inhibit the nitregic and purinergic inhibitory junction potential (IJP). In these conditions, electrical field stimulation (EFS) caused 1- an atropine sensitive excitatory junction potential (EJP) that was concentration dependently inhibited by OB (EC50 = 8.7 μ mol L^{-1}, $n = 7$) and 2- an atropine sensitive contraction that was concentration dependently inhibited by OB (EC50 = 7.3 μ mol L^{-1}, $n = 6$). Carbachol 10 μ mol L^{-1} induced 1- a smooth muscle depolarization (10 mV) that was blocked by 100 μ mol L^{-1} OB ($n = 4$) and 2- a contraction that was concentration dependently inhibited by OB (EC50 = 13 μ mol L^{-1}, $n = 6$). Neurokinin A (1 μ mol L^{-1}) induced a contraction that was inhibited by OB 100 μ mol L^{-1} ($n = 4$).
Conclusion: In the present work we isolate each mechanism of action of OB: inhibition of L-type calcium channels, muscarinic and tachykinergic responses that when acting in synergy they explain the pharmacological properties of the compound to treat colonic motor disorders.

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Selective agonists of somatostatin receptor subtype 1 or 2 prevent visceral hyperalgesia induced by activation of peripheral CRF1 receptors with cortagine in mice

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Objective: To investigate whether selective peptide somatostatin receptor subtype 1 and 2 (sst1 and sst2) modulate visceral hyperalgesia induced by cortagine, a corticotropin-releasing factor receptor type 1 (CRF1) agonist, conscious mice.
Methods: Adult male C57Bl/6 mice (25–33 g, 2–4/cage) were monitored for visceromotor response (VMR) to phasic noxious colorectal distension (CRD) using a novel non-invasive manometric technique (Larauche et al., Stress 2010). The CRD protocol consisted of graded phasic distensions to constant pressures of 15, 30, 45, and 60 mm Hg (3 times each, 10-sec duration, 4-min interstimulus interval). The first CRD set served as a baseline and after a 1-h rest period, mice ($n = 5–8$/group) were injected intraperitoneally (IP) with saline or selective sst1 or sst2 agonist followed by IP cortagine (30 μg kg^{-1}) directly after sst1 or 15 min after sst2 agonist. The 2nd CRD was performed 15 min after cortagine administration. The mean value of VMR for each consecutive CRD set was expressed as percentage of the respective baseline. Comparison between groups was performed using 2-way ANOVA with Bonferroni post-test.
Results: The sst1 agonist compared with vehicle reduced the VMR to IP cortagine at CRD of 30, 45, and 60 mm Hg to 25.9 ± 6.6 vs 61.2 ± 11.2% ($P < 0.01$), 45.1 ± 9.1 vs 83.7 ± 4.2% ($P < 0.01$), and 49.7 ± 9.9 vs 110.5 ± 8.2% ($P < 0.001$), respectively. The sst2 agonist compared with saline reduced the VMR to IP cortagine at the highest CRD of 45, and 60 mm Hg: 72.2 ± 8.1 vs 106.1 ± 11.2% ($P < 0.05$), and 69.4 ± 7.9 vs 107.8 ± 9.5% ($P < 0.05$), respectively, but not at 30 mm Hg: 42.4 ± 4.2 vs 59.6 ± 4.1% ($P > 0.05$).
Conclusion: The selective agonists of sst1 or sst2 receptors injected peripherally prevents visceral hypersensitivity induced by the selective activation of peripheral CRF1 receptors. The sst1 agonist may provide a new venue to modulate visceral hyperalgesia induced by stress-related mechanisms and sensitization of mechanoreceptors and allow avoiding the widespread inhibitory effects of octreotide on the gut function. Supported by: NIH R01DK-57238, P30DK-041301 (YT), 1K01DK088937 (ML).

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Plecanatide, a superior analog of uroguanylin, as an oral drug candidate for treatment of gastrointestinal functional disorders and diseases

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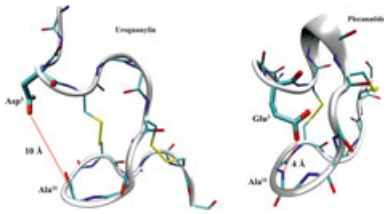
Objective: Agonists of guanylate cyclase-C (GC-C) are emerging as a new class of drug candidates for treatment of gastrointestinal (GI) disorders and diseases. Uroguanylin (UG) and guanylin (GN) are physiological agonists and natriuretic peptides that bind and activate GC-C receptors expressed on the epithelial cells lining the GI mucosa, leading to production of cyclic guanosine monophosphate and promoting electrolyte and water secretion needed for normal bowel movements. While GN and UG cooperatively regulate the GC-C in a pH-dependent physiological mechanism, the E. coli enterotoxin ST peptide activates GC-C in an uncontrolled and pH-independent fashion resulting in excessive fluid secretion to cause Traveler's diarrhea. Although UG seems an attractive choice as a drug candidate, the peptide assumes several topological isomers in solution, only one of which is biologically active. The objective of this study was to identify an analog of UG with superior properties for the treatment of chronic constipation (CC) and irritable bowel syndrome-constipation (IBS-c).
Methods: Based on bond energy calculations, 3-D structure modeling, structural activity relationships and molecular dynamics studies, we have identified plecanatide. Plecanatide's primary structure is similar to UG except that the Asp at the 3rd position in UG is substituted by glutamate.
Results: Plecanatide is a more stable peptide showing minimal interconversion between active and inactive topoisomers. Molecular dynamics studies demonstrate the increased stability of plecanatide is due to increased contact between the alpha-helical structure of the peptide and the interaction loop, minimizing interconversion between active and inactive forms. For example, in UG, the distance between the carboxylic acid side chain of Asp at the 3rd position and the amino group of Ala at the 11th position is longer (~10 Å *vs* 4 Å) and less rigid than for plecanatide (Fig 1).
Conclusion: Plecanatide is amenable to large-scale GMP manufacturing and is better suited for clinical development than UG. Plecanatide has successfully completed a Phase I trial, and a Phase IIa 14-day, ascending dose, placebo-controlled trial in CC patients. Plecanatide is currently in a Phase II/III clinical trial in CC patients.



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The cannabinoid agonist WIN 55,212-2 at a low, analgesic dose does not alter gastrointestinal motor function in type 2 diabetic rats

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Objective: To determine, using radiographic methods, the effect of an acute dose of the non-selective cannabinoid agonist WIN 55,212-2 with antineuropathic properties, on GI motor function of ZDF diabetic rats
Methods: Obese diabetic male ZDF rats were used. Controls were lean male rats. The effect of WIN (1 mg kg^{-1}) or its vehicle on mechanical allodynia (a sign of sensory neuropathy) was evaluated at 16–17 weeks of age using the von Frey filament test. In another group of rats, the effect of the cannabinoid or its vehicle was evaluated on GI motility at the same time point. Animals received an intragastric dose of contrast medium (barium sulphate) immediately after WIN/vehicle and serial X-rays were taken 0–8 h afterwards. Alterations in GI motility were quantitatively evaluated in each radiograph by assigning a compounded value (0–12) to each region of the GI tract (Cabezos et al, 2008). The size of the stomach was also evaluated in the different experimental groups. The cannabinoid tetrad was performed to control for possible central effects of WIN at the same dose tested.
Results: Neuropathy and altered gastrointestinal motor function were present in ZDF vs. lean animals. WIN reverted mechanical allodynia in ZDF rats. However, at the dose tested, GI motor function was not further modified in diabetic animals and no significant central effects were found in the cannabinoid tetrad.
Conclusion: Cannabinoids, at relatively low, non-psychotropic doses, could be useful to relieve neuropathic signs of type 2 diabetes without aggravating gastrointestinal diabetic dysmotility.

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Secretory action of individual extracts of the herbal medicine STW 5 in human intestine

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Objective: STW5 (Iberogast®) is a herbal combination from bitter candy tuft, chamomile, peppermint(P), caraway, liquorice, lemon balm(L), angelica(A), greater celandine and milk thistle, used for treatment of functional dyspepsia and irritable bowel syndrome (IBS). We reported that STW5 increased ion secretion in the human intestine and proposed that this prosecretory action may be involved in its clinical efficacy. Our aim was to investigate the secretory potential of the nine extracts on human gut epithelium in order to reveal target oriented herbal combinations.
Methods: We used the Ussing chamber voltage clamp method to measure the short circuit current (Isc) which reflects mucosal ion secretion. Studies were performed *In vitro* on 291 mucosa/submucosa preparations of human small or large intestinal specimens from 81 patients undergoing abdominal surgeries.
Results: We first confirmed that a combination of the individual extracts at concentrations present in 512 μg ml^{-1} of the mother compound STW5 evoked comparable prosecretory actions (medians [25/75 percentiles]: 17.7 [11.1/36] μA cm^{-2}; versus 25.8 [6.6/38.6] μA cm^{-2}). At concentrations that corresponded to their concentrations in 512 μg ml^{-1} STW5, only angelica evoked a secretion (18.6 [9.6/28.8] μA cm^{-2}). However, at 10x higher concentrations, which are still sub-therapeutic, additionally peppermint (7.7 [3.4/18] μA cm^{-2}) and lemon balm (7 [6/23.5] μA cm^{-2}) exhibited prosecretory potentials. A, P and L were equally potent in small and large intestine. Combined application of A+P+L as in 512 or 5120 μg ml^{-1} STW5 evoked an Isc increase of (14.2 [8.4/22] and 23.5 [18.34/32.6] μA cm^{-2}), respectively. This prosecretory action was significantly reduced by adenylate cyclase inhibitor MDL12, 330A, cAMP-activated Cl- channel blocker CFTRinh-172 or Ca^{++}-activated Cl^- channel blocker SITS by 76%, 71% and 88% respectively.
Conclusion: We showed that the prosecretory action of STW5 is mainly due to angelica, peppermint and lemon balm. Their effects involve activation of cAMP- and Ca^{++}-activated Cl^- channels. Our results suggest that STW5 or its prosecretory components may be used to normalize impaired intestinal secretion which may occur in C-IBS or chronic constipation.

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A model of *in vitro* perfused mouse intestine suitable for pharmacological testing of antiinflammatory drugs

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Objective: Inflammatory bowel diseases constitute a very important entity in gastrointestinal disorders. There are many different animal models available to mimic the disease. For the time being adequate *In vitro* models that can simulate the *in vivo* situation are not available yet. Different cell culture models for intestinal cells are very simple to use, but do not come close to the physiology of the small intestine as a whole. Therefore we established a mouse small intestine long time perfusion model primarily designed for pharmacological testing. The most important advantage of our model is its ability to imitate the tissue interaction in inflamed intestine more similar to the *in vivo* situation than cell culture approaches can do.
Methods: Before starting the surgical procedure, animals are anesthetized. Parts of the intestine that are not required for perfusion are ligated and removed. After resection of a 5–6 centimetre segment of the proximal small intestine with the adhering cannulated mesenterial root, *In vitro* longterm mesenterial perfusion is started immediately and the gut is perfused mesenterially in a custom designed perfusion chamber. This allows a luminal perfusion and serosal superfusion. Acute inflammation in the small intestine is induced by the application of LPS via the intestine's lumen and a cytomix through the mesenteric superior artery. Our perfusion model is used to evaluate the effects of novel anti-inflammatory drugs from fungi.
Results: Motility of the small intestine in response to perfusion and inflammatory stimuli is measured and interpreted by using optical recording and appropriate analysis software. Inflammation has been proven on the transcriptional level at different time points. Furthermore histological stainings were performed.
Conclusion: The model is especially valuable for the testing of new compounds intended for the treatment of inflammatory bowel disease. Antiinflammatory effects are evaluated in comparison to dexamethasone. Intestinal perfusion is a suitable alternative to various mouse *in vivo* inflammatory bowel disease models to assess the effects of new pharmacological treatment strategies. The assessment of drug effects in our *In vitro* model can help to accelerate drug development for the treatment of inflammatory conditions of the intestine.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-23 Basic and Translational Session: Neuroimmune Mechanisms

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Attenuation of colitis by acetylcholinesterase inhibitor is mediated via the vagus nerve and the dendritic cells

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Objective: Electrical stimulation of the vagus nerves reduces gut inflammation through a vagus-dependent cholinergic anti-inflammatory pathway. The vagus nerve regulation of peripheral functions is controlled by brain cholinergic neural network. Recently, we have shown that central activation of the vagus via galantamine (GAL, acetylcholinesterase inhibitor) treatment decreases colitis. Therefore, using GAL, we have examined whether the central increase of acetylcholine provide a tonic inhibitory influence in a model of experimental colitis through the vagus nerve.
Methods: C57Bl/6 mice received 5% dextran sulfate sodium (DSS) in drinking water for 5 days. Bilateral truncal sub-diaphragmatic vagotomy plus pyloroplasty (VXP) was performed and 10 days later GAL treatment was initiated one day before induction of colitis. Disease severity index was evaluated daily after induction of colitis. At sacrifice, inflammation was evaluated clinically, histologically and by myeloperoxidase activity (MPO) in colonic tissue. Cytokines levels were determined in colonic tissue and C-reactive protein (CRP) was determined in serum by ELISA. IL-12p40 level was evaluated from dendritic cell culture.
Results: GAL treatment reduced the onset of clinical disease as assessed by loose stools, weight loss and rectal bleeding. On day 5, macroscopic scores were 52% lower in GAL-DSS group compared to DSS group ($P < 0.05$). MPO and CRP activity decreased from 2.2 ± 0.1 to 0.6 ± 0.4 U mg $tissue^{-1}$ and from

39.4 ± 1.5 in DSS group to 27.8 ± 0.6 pg ml^{-1} in GAL-DSS group ($P < 0.05$) respectively. Colonic levels of IL-1b, IL-6 were reduced in GAL-DSS group ($P < 0.05$) and no effect was visible on IL-10 levels. When stimulated or not with LPS, CD11c+ DCs isolated from GAL-DSS group showed a significant decrease of IL-12p40 release when compared to DSS group. The attenuation of colitis and the decrease of IL-12p40 by GAL treatment were not evident in vagotomized mice.
Conclusion: These results support the hypothesis that pharmacological central stimulation of the vagus nerve modulates intestinal inflammation in a murine model of colitis through the vagus nerve and the DC population. Identification of the cellular mechanism underlying the protective role of parasympathetic nerves may lead to novel therapeutic targets in IBD.

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The influence of the immune environment and miRNA expression on colonic stem cell behavior in a parasitic infection

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Objective: Parasitic infection induction of colonic epithelial proliferation is required for clearance. The mechanisms involved are unclear, but microRNAs (miRs), a class of gene expression regulators, are implicated in control of intestinal stem and progenitor cell (ISPC) proliferation and differentiation. Aim: To elucidate the immune and non-immune factors involved in regulation of proliferation during colonic infection and potential regulation by microRNA (miR) expression.
Methods: BALB/c wild type (WT) mice and mice lacking the Th2 transcription factor STAT6 (STAT6-/-), were infected with the cecal-dwelling Trichuris muris and studied weekly ($n = 5$/group) for 4 weeks. Mice were given bromodeoxyuridine (BrdU) 2 h before sacrifice. BrdU-positive cells were counted and mucosal height measured in well-oriented crypts. Candidate miRs, cytokines and factors involved in proliferation and differentiation of ISPC were evaluated by qPCR.
Results: In WT T. muris infected mice, the maximal increase in IL4 and IL13, cytokines needed for clearance, occurred 14 days post infection (PI) and coincided with a modest upregulation of IFNγ and CXCL10 and a decrease in the absorptive enterocyte lineage marker HES-1. At this time there was also a transient increase in BrdU-positive cells per crypt and mucosal height preceding clearance. In contrast, at day 14 PI STAT6-/- mice failed to increase Th2 cytokines or epithelial proliferation despite the presence of worms. STAT6-/- mice developed a chronic infection with a marked proliferative response at day 28 coinciding with a Th1 immune response. The persistent epithelial proliferation in the STAT6-/- animals coincided with increased expression of the Wnt-target gene, CyclinD1, and downregulation of the secretory lineage differentiation factor Atoh1 and the goblet cell marker Muc2. Suppression of genes such as Mybl2 by miR-365 is needed for ISPC differentiation, however, miR-365 was downregulated significantly at day 28 in STAT6-/- mice.
Conclusion: The proliferative response of the colonic epithelium necessary for clearance of T. muris, is independent of a Th2 immune response. In addition, there is competition between proliferation of the ISPC and differentiation towards the secretory lineage. Finally, we implicated a role for miR-365 in the orchestration of the proliferative response.

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HSV-1 infection of enteric nervous system evokes inflammation-mediated myenteric plexus injury

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Objective: Enteric neuropathies are characterized by marked infiltration of neutrophils and cytotoxic lymphocytes into the myenteric plexus. We previously reported that Herpes simplex virus (HSV)-1 infection of the enteric nervous system (ENS) results in neuro-dysfunction and gastrointestinal dysmotility. Since the mechanisms of neuronal injury remain unclear, in the present study we investigated whether innate and/or acquired immune responses against HSV-1 harm neuronal functional integrity.
Methods: Adult C57Bl/6 mice were intranasally inoculated with HSV-1, after 4 weeks (W) intragastrically (IG), and sacrificed 1–8 W post IG. Molecular analysis was performed to ascertain viral transcripts in longitudinal muscle myenteric plexus (LMMP). Immune cells isolated from mucosa and LMMP were characterized using fluorescent activated flow cytometry (FACS). Muscular contractility and gut motility were evaluated by electric field stimulation (EFS) of isolated ileal segments and gastrointestinal transit, respectively. ENS integrity was assessed by immunofluorescence on LMMP whole mount preparations for peripherin expression.
Results: In the ENS of infected mice, HSV-1 latency-associated transcripts (LATs) correlated with the infiltration of activated macrophages (CD11b+CD49d+F4/80+ cells) and CD4+CD25+Foxp3+ regulatory T cells at 2 and 6 W post IG viral inoculum. Abortive viral replication was associated to HSV-1 reactive CD3+CD8+IFNgamma+ lymphocytes at 8 W post IG. EFS-evoked contractions were reduced at 2 W post infection but increased at 8 W. Intestinal transit time was reduced only at 8 W post IG infection as compared to sham infected animals. In myenteric plexus, peripherin expression augmented during the early time of infection but resulted highly fragmented at 8 W. Administration of anti-serum anti-CD8 at 6 W post IG infection ameliorated intestinal contractility in mice sacrificed at 8 W.
Conclusion: In the ENS, HSV-1 infection triggers innate and acquired immune responses in a time-dependent fashion. Thus, following neurotropic viral infection the recruitment of different immune-competent cellular populations into the myenteric plexus may account for the time-dependent neuronal damage and intestinal dismotility during enteric neuropathies.

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Modulation of the inflammatory response by mas-related gene receptor D during intestinal schistosomiasis

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Objective: Mas-related gene receptor (Mrg) family members have been suggested to play roles in nociception, in regulation of inflammatory responses to non-immunological activation of mast cells and in mast cell-sensory nerve communication. One member of this family, MrgD, is expressed in spinal nociceptive neurons, is encoded by a single copy MrgD having clearly defined rodent and human orthologs, is activated by beta-alanine, and is altogether an experimentally attractive therapeutic target. We aimed to investigate the putative role of MrgD in intestinal inflammation using a mouse model, i.e., murine intestinal schistosomiasis.
Methods: Immunohistochemical analyses were performed on the ileum and spinal ganglia of non-inflamed and Schistosoma mansoni-infected (inflamed) wild-type (WT) and MrgD-/- mice. Expression of MrgD mRNA was analysed on the afore-mentioned tissues by real-time RT-PCR.
Results: In WT mice, in the non-inflamed ileum, immunohistochemistry revealed no MrgD immunoreactivity (IR), whereas in the inflamed ileum, MrgD IR was observed in 5% of the myenteric neurons, which were intrinsic primary afferents. In addition, MrgD IR was detected in mucosal mast cells (MMCs) in the inflamed ileum. Furthermore, in both non-inflamed and inflamed WT mice, 15% of the spinal ileal afferent neurons showed MrgD IR. In MrgD-/- mice, no MrgD IR was detected, while a significantly increased calcitonin gene related peptide (CGRP) expression and MMC infiltration were observed in the inflamed ileum. Immunohistochemical results were corroborated by real-time RT-PCR, demonstrating the absence of MrgD mRNA in the non-inflamed ileum of WT mice and in MrgD-/- mice, no changes between MrgD mRNA levels in the spinal ganglia of non-inflamed and inflamed WT mice, and the presence and elevation of MrgD mRNA in the inflamed ileum of WT mice.
Conclusion: The de novo expression of MrgD in sensory neurons and MMCs in the inflamed ileum of WT mice, combined with the increased CGRP expression and MMC infiltration in the inflamed ileum of mice lacking MrgD, reflect a role for MrgD in the modulation of intrinsic sensory neuronal and mast cell-mediated inflammatory responses during intestinal schistosomiasis. These findings emphasize the need to further elucidate the precise functional mechanisms and pathways underlying MrgD-mediated responses during intestinal inflammation.

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Mucosal immune biological markers in patients with Irritable Bowel Syndrome (IBS)

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Objective: A biological marker (biomarker) is an indicator of a physiological or pathological state that can be objectively measured. At present a useful biomarker of irritable bowel syndrome (IBS) does not exist. In a recent randomized control trial of mesalazine against placebo, a significant reduction in mast cell number was demonstrated. This suggests that in mechanistic pharmacological studies, mast cell number could act as a biomarker both for entry into the study and as an endpoint. We assessed mucosal biopsies of patients with IBS, ulcerative colitis (UC) and healthy controls (HC) to evaluate if total immune cells and mast cells as well as their mediators may be useful IBS biomarkers.

Methods: One hundred forty-four patients with IBS, 32 patients with UC, and 68 HC were studied. All patients underwent colonoscopy and histological examination. Colonic immunocytes were identified with quantitative immunohistochemistry. Mast cell mediators released into the supernatant of cultured biopsies were assessed by immunoenzymatic assay. Median values of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA) were analyzed.

Results: We found the best cut-off range between HC and patients with IBS to be: (i) 20.2 to 20.3% (sensitivity, 86.3%; specificity, 87.5%; PPV, 95.8%; NPV, 65.6%; DA, 86.5%) regarding total immune cell count; (ii) 4.3 to 4.7% (sensitivity, 93.8%; specificity, 73.5%; PPV, 88.2%; NPV, 84.7%; DA, 87.3%) regarding mast cell count; (iii) 172.5 to 182.6 ng ml^{-1} mg^{-1} (sensitivity, 81.3%; specificity, 80.6%; PPV, 89.7%; NPV, 67.6%; DA, 81.1%) regarding histamine release; (iv) 0.91 to 0.95 ng ml^{-1} mg^{-1} (sensitivity, 91.4%; specificity, 72.7%; PPV, 85.5%; NPV, 82.8%; DA, 84.6%) regarding tryptase release. The best cut-off range between patients with IBS and UC was 33.8 to 34% (sensitivity, 87.5%; specificity, 94.4%; PPV, 87.5%; NPV, 94.4%; DA, 92.3%), regarding total immune cell count.

Conclusion: Our data suggest that mucosal total immune cells and mast cells as well as their mediators have good sensitivity and specificity in distinguishing patients with IBS from HC. Although these tests are difficult to perform and require cumbersome laboratory techniques, they may be of utility at least in clinical trials of anti-inflammatory drugs in IBS.

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Altered unfolded protein response in inflamed bio-breeding rats

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Objective: Unfolding protein accumulation, a.k.a.endoplasmic reticulum (ER) stress, is emerging as a pathological factor in several diseases, such as diabetes mellitus, motor neuron disease and inflammatory bowel disease. ER stress elicits a signaling cascade known as the unfolded protein response (UPR).The BioBreeding diabetes-prone (BBDP) rat has a mutation in the Gimap5(GTPase of the immunity-associated protein 5) which localizes to the ER. Increases in ER stress-associated chaperones in T cells from BBDP rats have been reported (Pino 2009).We reported myenteric ganglionitis, inflammation-induced nitrergic dysfunction and intestinal dysmotility preceded by increased intestinal permeability and mucosal inflammation in normoglycemic BBDP rats (Masaoka DDW2011, Vanormelingen UEGW2011). We designed this study to assess the pathophysiological role of the ER stress in the intestine of BBDP rats.

Methods: Normoglycemic BBDP rats (inflamed rats) and control rats (BioBreedingdiabetes-resistant) were sacrificed at 220 days. After harvesting jejunum, mucosal layer and longitudinal muscle myenteric plexus (LMMP) preparations were dissected. Jejunal inflammation, mRNA expression of ER stress chaperone GRP78, the UPR regulators (IRE1α, IRE1β, PERK, ATF6 and OASIS) and downstream UPR effectors CHOP were analyzed by MPO measurement and real-time PCR, respectively.

Results: Compared to control (n = 5), LMMP preparations from inflamed rats (n = 8) had higher MPO levels (0.0 ± 0.0 *vs* 5.4 ± 2.2 U mg^{-1}, $P < 0.01$), lower GRP78 mRNA expression (1.0 ± 0.1 *vs* 0.5 ± 0.1, $P < 0.01$) and PERK mRNA expression (1.0 ± 0.1 *vs* 0.4 ± 0.2, $P < 0.05$). A significant linear correlation was found between mucosal and LMMP expression of GRP78 mRNA expression ($P < 0.01$, $r = 0.72$). In LMMP, a significant linear correlation was also observed between MPO levels and GRP78 mRNA expression ($P < 0.05$, $r = -0.65$). Compared to control, inflamed rats had lower mucosal GRP78 mRNA expression (1.0 ± 0.1 *vs* 0.4 ± 0.1, $P < 0.001$), IREα mRNA expression (1.0 ± 0.2 *vs* 0.5 ± 0.1, $P = 0.07$), IREβ mRNA expression (1.0 ± 0.2 *vs* 0.3 ± 0.2, $P < 0.01$), PERK mRNA expression (1.0 ± 0.2 *vs* 0.3 ± 0.1, $P < P < 0.01$) and OASIS mRNA expression (1.0 ± 0.2 *vs* 0.4 ± 0.1, $P < 0.05$). Inflamed rats tended to have higher mucosal ATF6 mRNA expression (1.0 ± 0.2 *vs* 1.3 ± 0.1, $P = 0.16$) and CHOP mRNA expression (1.0 ± 0.3 *vs* 1.4 ± 0.1, $P = 0.16$).

Conclusion: The UPR was altered in the inflamed intestine in BBDP rats, both in mucosa and LMMP, which may contribute to the pathogenesis of increased permeability and intestinal dysmotility.

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Cyclooxygenase inhibitors modulate pro-fibrotic signalling mediated by transforming growth factor beta in experimental colitis

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Objective: In extra-gastrointestinal sites, cyclooxygenase isoforms (COX-1, COX-2) have been implicated in the development of fibrosis. In the setting of bowel inflammation, transforming growth factor beta (TGF-beta) has been identified as the main regulator of fibrotic remodelling. The present study evaluated the effects of cyclooxygenase inhibitors on pro-fibrotic signalling mediated by TGF-beta in experimental colitis

Methods: Colitis was induced in rats by intrarectal administration of 2, 4-dinitrobenzenesulfonic acid (DNBS, 30 mg/rat in 0.25 ml ethanol 50%). After 6 days, systemic [body and spleen weight] and tissue inflammatory parameters [macroscopic and microscopic damage] were assessed. Three days before colitis assessment, the animals were treated daily with indomethacin (IND, non-selective COX-1/COX-2 inhibitor, 2 mg kg^{-1}), SC-560 (SC, selective COX-1 inhibitor, 2.5 mg kg^{-1}), or celecoxib (CEL, selective COX-2 inhibitor, 1 mg kg^{-1}), by intragastric gavage. At the time of sacrifice, COX-1, COX-2, collagen I and III, fibronectin, matrix metalloproteinase(MMP)-2 and MMP-9, TGF-beta, RhoA, PCNA and phosphorylated p38, ERK1/2, Akt expression were analyzed by western blot. COX-2 was also examined by immunohistochemistry. Collagen fibers (Van Gieson) and elastic fibers (orcein) were detected by histochemistry.

Results: Western blot analysis of inflamed colon showed an increased expression of COX-2, collagen I/III, fibronectin, MMP-2, MMP-9, TGF-beta, PCNA, p-Akt, p-p38, RhoA. COX-1 was not affected, while p-ERK1/2 was reduced. The enhanced expression of COX-2 and TGF-beta was confirmed by immunohistochemistry. Histochemistry displayed an increased positivity for collagen fibers in concomitance with a dramatic decrease in elastic fibers. Based on western blot, IND, SC and CEL counteracted the increased expression of collagen I/III, fibronectin, TGF-beta, RhoA and p-p38. Histochemistry confirmed the inhibitory action of IND, SC and CEL on collagen deposition and showed their reverting effect on the loss of elastic fibers.

Conclusion: In the DNBS model of colitis, bowel fibrosis is characterized by enhanced collagen/fibronectin deposition, elastic fiber reduction, and TGF-beta induction. In this setting, the pharmacological blockade of COX-1 and COX-2 is able to down-regulate the fibrotic remodelling, and this action appears to depend on the modulation of TGF-beta and its signalling pathways.

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Immunomodulatory role of Nociceptin/Orphanin FQ – NOP receptor system in a rat model of experimental colitis

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Objective: Nociceptin/OrphaninFQ (N/OFQ) and Nociceptin Orphan Peptide (NOP) receptors represent an endogenous system modulating gastrointestinal functions and inflammation. The role of N/OFQergic system on some inflammatory variables in a rat model of colitis induced by intracolonic (IC) instillation of TNBS (2, 4, 6 trinitrobenzensulfonic acid) (60 mg kg^{-1}, IC) has been investigated.

Methods: Male Wistar rats received two intraperitoneal (IP) injections per day of N/OFQ (0.02–0.2–2–20 n mol kg^{-1}) or NOP receptor selective antagonist, UFP-

101 (1–3–10 n mol kg^{-1}), or saline for three days after induction of colitis. Four days after TNBS, animals were sacrificed and colonic histological damage, myeloperoxidase (MPO) activity, cytokine levels were evaluated. N/OFQ plasmatic concentration was assessed by radioimmunoassay.
Results: TNBS increased all the inflammatory variables considered in comparison with control animals. In colitic rats, N/OFQ at 0.02 and 0.2 n mol kg^{-1} improved microscopic damage and MPO activity, and decreased colonic IL-1β level in comparison with TNBS group, whereas at the highest dose (20 n mol kg^{-1}) worsened colitis. UFP-101 at a dose of 1 n mol kg^{-1}, without intrinsic activity, antagonized the protective effect of N/OFQ (0.2 n mol kg^{-1}) on colitis. At the doses of 3 and 10 n mol kg^{-1}, UFP-101 worsened colitis revealing an antiinflammatory role of the endogenous N/OFQergic system. No differences in N/OFQ plasmatic levels have been observed between controls and TNBS-treated rats. In contrast, treatment with UFP-101 at the doses (3 and 10 n mol kg^{-1}) that worsen colitis significantly reduced N/OFQ concentration in the plasma of colitic rats.
Conclusion: (i) the anti-inflammatory action of peripheral low doses of N/OFQ (1000–10000 fold lower than those that worsen colitis) is mediated by the activation of peripheral NOP receptors; (ii) the endogenous N/OFQergic system has a protective role in the control of inflammation in experimental colitis in rats, and it could be considered an interesting target for the treatment of pathological inflammatory conditions of the gastrointestinal tract.

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Mast cell and enterochromaffin cell distribution in the aged human bowel
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Objective: Intestinal motility disorders (constipation) are often associated with the ageing population. Number of the colonic enteric neurons declines with age (Bernard et al. 2009, Neurogastroenterolgy & Motility). Changes in sensory signaling may also be implicated in altered motility. Mediators released from the intestinal mast cells (MCs) and enterochromaffin cells (ECs) regulate visceral sensitivity (Jiang et al., 2000, Gastroenterology; Keating et al. 2008, J Physiol). Thus we aimed to examine whether the density and distribution of MCs and ECs was changed in the ageing human bowel.
Methods: Histologically normal segments of small (distal ileum) and large human bowel were obtained from patients (31-to-87-year-old) after surgical resection following patient consent and ethical approval. Specimens from patients >65-year-old were classified as the aged group; those from 20-to-65-year-old were the adult group. MCs were processed in resin-embedded cross-sections, labeled with 0.1% toluidine blue. ECs were processed in frozen cross-sections, labeled with primary anti-serotonin (AbD serotec) and Cy3 secondary antibody (Jackson ImmunoResearch). The density of MCs and ECs cells was calculated by the total number of specifically labeled cells within a defined area using an eye-piece graticule and ImageJ software.
Results: MCs were identified primarily in the mucosa, submucosa and serosa. ECs were labeled only in the epithelium. MCs in both mucosa and submucosa areas were predominantly located on either side of the muscularis mucosae. MCs density in the mucosa was significantly higher than the other two populations in both adult and aged large bowel (Mucosa vs. submucosa vs. serosa: 68.8 ± 10.7 *vs* 18.4 ± 2.1 *vs* 5.8 ± 0.9 cell mm^{-2}; n = 12, $P < 0.001$, One-Way-ANOVA). Highest density of MCs in the small gut was also revealed in the mucosa. The mucosal MCs were significantly augmented in the aged colon (51.5 ± 13.3 *vs* 94.5 ± 10.4 cell mm^{-2}, n = 5 each, $P < 0.05$, unpaired t-test). A similar trend was found in the aged ileum. In contrast, ECs population in the aged colon showed no difference from the adult group (adult versus aged: 36.3 ± 10.0, n = 3, vs. 37.4 ± 4.0, n = 6).
Conclusion: These data suggest that the mast cell but not enterochromaffin cells are increased with age. Such changes may be associated with the colonic motility disorders in the elderly. Supported by SHC and BDRF.

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Enteric nervous system activity inhibits TNFa but potentiates IL6 productions induced by LPS in a p38 and ERK independent manner
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Objective: Evidences pile up concerning the importance of the enteric nervous system (ENS) in controlling intestinal functions other than motility, such as intestinal epithelial barrier functions. Nevertheless, little is known about its direct participation in the inflammatory response profile observed during inflammatory bowel diseases or even obesity. In the present study, we have analysed if, in an inflammatory context, ENS could induce TNFa and IL6, and what were the mechanisms involved.
Methods: To analyse if, in an inflammatory context, ENS could induce TNFa and IL6 productions, we have treated ENS primary cultures with lipopolysaccharide (LPS) and have measured TNFa and IL6 mRNA (Q-PCR) and protein production (ELISA). To identify the mechanisms involved we have analysed p38/SAPK2 and ERK/MAPK signal transduction in ENS by western-blot and have used selective inhibitors of p38/SAPK2 (SB203580; 10 μ mol L^{-1}) or ERK/MAPK activators (U0126; 10 μ mol L^{-1}) as co-treatment to determine their respective implication. To determine the impact of ENS activity upon this TNFa and IL6 production, we have tested the effects of KCL (40 m mol L^{-1}), electrical field stimulation (EFS) or ATP (100 μ mol L^{-1}) co-treatments.
Results: Stimulation of ENS primary cultures with LPS induced an increase in TNFa and IL6 mRNA and protein production in a time (from 2 to 24 h) and dose (0.001 to 1 μg ml^{-1}) dependent manner. In another hand, LPS induced ERK/MAPK and p38/SAPK2 activation in primary ENS cultures. All two inhibitors almost completely prevented LPS-induced TNFa and IL6 production. In addition, EFS, KCl-induced depolarisation as well as ATP greatly reduced TNFa mRNA expression as well as protein production whereas they potentiated IL6 mRNA and protein expression. ERK/MAPK and p38/SAPK2 activity were modified by EFS but not correlated with the cytokine productions.
Conclusion: Our results show that LPS induces TNFa and IL6 production by ENS through the classical ERK/MAPK and p38/SAPK2 pathway. ENS activity could reduce TNFa production but increase IL6 production independently of these pathways. All together, this study demonstrates for the first time that ENS activation could modulate LPS-induced inflammatory cytokine production.

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Involvement of COX-2 derived from cerebral perivascular macrophages in the early gastrointestinal motor disturbances induced by Lipopolysaccharide (LPS) in sheep
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Objective: The aim of this study was to investigate the involvement of brain cyclooxygenase (COX) in the gastrointestinal motor alterations induced by LPS.
Methods: Myoelectric activity was recorded in conscious ewes with electrodes implanted in rumen, gastric antrum, duodenum and jejunum. Body temperature was continuously monitored with an intraperitoneal thermistor. In addition, groups of 3 ewes were treated intravenously (iv) with saline or LPS (0.1 μg kg^{-1}) from Escherichia coli and slaughtered 1 or 4 h later. Samples of hypothalamus and medulla oblongata were collected for immunohistochemistry studies.
Results: LPS reduced antral and intestinal myoelectric spiking activity, increased MMC frequency and induced hyperthermia. Intracerebroventricular pretreatment with the selective COX-2 inhibitor nimesulide (0.2 mg kg^{-1}) strongly reduced LPS-induced effects. In the hypothalamus and medulla oblongata of control animals, COX-1 and COX-2 were expressed in the vascular endothelial cells. Positive COX-1 immunostaining was also observed in scattered cells throughout the parenchyma and related with the blood vessels wall and in activated astrocytes of the brainstem. COX-2 was also expressed in some neurons, being in the hypothalamus particularly abundant in the preoptic area and in the paraventricular and ventromedial nuclei. In the cerebral tissues of control animals, positive immunostaining to the perivascular macrophage marker CD163 (ED2) was observed in scattered cells related with the blood vessels wall. LPS increased the number of these perivascular macrophages and induced their appearance throughout the parenchyma. All these cells coexpressed COX-2. In the hypothalamus, these macrophages were particularly abundant in the organum vasculosum of the lamina terminalis (OVLT) and in the medial preoptic nucleus. LPS also induced COX-2 expression in hypothalamic reactive microglia and increased the number of neurons positive to COX-2 in the paraventricular and ventromedial hypothalamic nuclei.
Conclusion: Our data suggest that gastrointestinal motor disturbances and fever induced by LPS in sheep are mediated through the increase in the expression of COX-2. Perivascular macrophages located in the medulla oblongata and hypothalamus would be

involved in these effects. Supported by DGI (AGL2006-04317/GAN), FEDER, Gobierno de Aragón (I-2011/017, B61/2010, B090/2009) and Universidad de Zaragoza (UZ2010-BIO-13).

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Modulation of inflammation in IBD: Alpha7nAChR expression and role of nicotine

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Objective: The divergent effects of nicotine on natural history of inflammatory bowel diseases (IBD) are well known: while it's beneficial in ulcerative colitis (UC) it increases risk of surgery and relapse in Crohn's disease (CD). It has been shown that in animal models the modulatory effect of nicotine in intestinal inflammation involves the alpha7 nicotinic acetylcholine receptor (α7nAChR), activating a cholinergic counterinflammatory mechanism. Thus, aims of the study were to evaluate α7nAChmRNA levels on macrophages from patients with MC, UC and controls (HV) and nicotine effects on LPS-induced TNFα and IL10 production.
Methods: Macrophages obtained from peripheral blood monocytes of UC and CD patients and HV were supplemented with M-CSF (7 days). α7nAchR-mRNA and protein levels were evaluated by qRT-PCR and FACS analysis using αBgt-FICS specific binding, respectively. Macrophages were pre-incubated with nicotine (1 mg ml^{-1} 30 min) and then stimulated with LPS (1 $\mu g\ ml^{-1}$ 24 h). TNFα and IL10 levels were measured on supernatant using ELISA.
Results: Macrophages from UC showed greater α7nAchR mRNA levels then cells from CD (0.00092 copies in UC Vs 0.000212 copies in CD, $P = 0{,}006$), while no differences were found with HV. FACS analysis confirmed greater α7AChR expression in UC patients (90.75 Gmean in UC Vs 15.62 Gmean in MC, P 0.031). Higher α7nAchR mRNA levels were observed in macrophages from IBD with colonic disease compared with small bowel involvement ($P = 0.007$). Nicotine significantly decreased LPS-stimulated TNFα release in macrophages from HV (from 13122.7 pg ml^{-1} to 9790.2 pg ml^{-1}; $P = 0.03$), CD (14291.7 pg ml^{-1} to 10613.2 pg ml^{-1}; $P = 0.003$) and UC (from 13723.9 pg ml^{-1} to 10238.5 pg ml^{-1}; $P = 0.000$). This effect was more pronounced in macrophages from UC patients (-22.9%) compared with CD (-6.1%) and in IBD with colonic disease (-17.4%) compared to CD with small bowel involvement (+3.8%), although it did not reach statistical significance. Pretreatment with nicotine inhibited LPS-stimulated IL10 production by macrophages in HV, UC and CD, but not differences were observed among groups.
Conclusion: Nicotine has a suppressive effect on TNFα and IL10 production by macrophages: disease-related or site-related differences in α7nAChR levels may justify the divergent effects of nicotine in IBD patients.

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Muscular and nerve-mediated responses associated with inflammation in human gallbladder

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Objective: Muscular and nerve-mediated responses associated with inflammation in human gallbladder are poorly characterized. We studied carbachol (CCh) and potassium chloride (KCl)-induced motor responses and electrically evoked contractions in human gallbladder from patients with cholelithiasis.
Methods: Gallbladder specimens were obtained from 21 patients (mean age 52 years, range 30–77 years;7M;14F) cholecystectomized for cholelithiasis with cholesterol calculi. Isometric tension were measured on longitudinal muscle strips (15 × 3 mm) exposed to CCh (10^{-5} mol L^{-1}). Moreover, cumulative concentration-response curves were obtained with CCh (10^{-7}–10^{-4} mol L^{-1}) and KCl (10–200 m mol L^{-1}). In addition, strips were exposed to electrical field stimulation (EFS), which was evoked every 2 min delivering 10-s trains of pulses (0.1–10 Hz, duration 0.3 ms, 20 V). Drugs tested: the association of atropine (2×10^{-6} mol L^{-1}) and guanethidine (5×10^{-6} mol L^{-1}) (30 min); the association of atropine, guanethidine and L-nitro arginine methyl ester (L-NAME) (2×10^{-4} mol L^{-1}) (45 min). Each gallbladder specimen was evaluated for the degree of inflammation, graded on the scale of Lennon et al. (1). Gallbladders were divided into three groups (G): mild chronic (6pts., G1); advanced chronic (10pts., G2) and acute cholecystitis (5pts., G3). Data were analyzed by analysis of variance to compare all pairs in the groups. $P < 0.05$ was considered to be statistically significant.
Results: The degree of inflammation was significantly ($P < 0.001$) different among groups: scores were 4.5+0.5 (G1), 7.80+1.40 (G2) and 13.0+0.93 (G3). The corresponding ED50 values to CCh (10^{-6} mol L^{-1}) were 1.236 (95% CI 0.75–2.05), 4.24 (95% CI 2.68–6.70), 5.48 (95% CI 3.49–8.61) respectively ($P < 0.001$), whereas those to KCl were not statistically significant. Electrically evoked contractions were linearly related to stimulation frequency in the 0.1–10 Hz, although contractions were bigger in G2 ($P < 0.05$, $P < 0.01$). Atropine and guanethidine per se inhibited electrically evoked contractions in three G ($P < 0.05$; $P < 0.01$). The association of L-NAME, atropine and guanethidine increased contractions at all frequencies in three G ($P < 0.05$; $P < 0.01$).
Conclusion: Chronic inflammation reduces sensitivity to CCh and enhances electrically evoked contractions. It may be important in impaired gallbladder motility and the formation of gallstones.

Reference

[1] Lennon F, Feeley TM, Clanachan AS et al. (1984). Gastroenterology 87: 257–62.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-24 Basic and Translational Session: Brain-gut-axis and Stress Mechanisms

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Acute psychological stress induces an immediate naloxone-independent visceral analgesia to colorectal distension in mice

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Objective: Mice repeatedly exposed to water avoidance stress (WAS) respond differentially to colorectal distension (CRD) depending on the method used to monitor the visceromotor response (VMR). WAS induces stress-induced visceral analgesia (SIVA) 24 h after the last exposure to stress in animals tested for visceral pain non invasively while it leads to visceral hyperalgesia in those equipped with the traditional method of EMG (Larauche et al., 2010). Here, we aimed to determine whether WAS in its acute form induces SIVA in male mice and to assess whether the analgesic response is opiate dependent.
Methods: Adult male C57Bl/6 mice (23–33 g, 1–4/cage) were monitored for VMR to CRD (15, 30, 45 and 60 mmHg, 3 times each, 10 s, 4 min interstimulus interval) using a novel non-invasive technique (Larauche et al., 2010). The 1st CRD (day 0) served as baseline VMR, then mice were exposed to 1 h WAS on day 1. Two groups were injected subcutaneously (sc) with naloxone (1 mg kg^{-1}) ($n = 6$) or saline (0.1 ml) ($n = 8$) 10 min before WAS, another control group ($n = 12$) received no injection. The 2nd CRD was monitored 45–50 min after the WAS. The VMR to CRD after WAS was expressed as percentage of the respective baseline. Defecation was monitored after each WAS session. VMR data were analyzed using two-way ANOVA and Bonferroni post-hoc test, defecation data by unpaired t test.
Results: Immediately after WAS, non-injected male mice exhibited a visceral analgesia at 60 mmHg (78.9 ± 7.2 *vs* 100.0 ± 0.0%, $P < 0.05$). Compared to non-injected mice, sc injections of saline or naloxone before WAS enhanced the visceral analgesia inducing an analgesic response at 30, 45 and 60 mmHg for saline and at 45 and 60 mmHg for naloxone (Table). Compared to non-injected mice, WAS-induced defecation was reduced by sc injection of saline (4.2 ± 1.3 vs 6.8 ± 0.5 pellets per hour per 20 g body weight, $P < 0.05$) but not naloxone (8.0 ± 2.7 vs 6.8 ± 0.5 pellets per hour per 20 g body weight $P > 0.05$).

Conclusion: Exposure to an acute psychological stress induces an immediate opiate-independent visceral analgesia in male mice. Subcutaneous injection potentiates the immediate analgesic response suggesting that the concomitant acute somatic noxious stimulus may exert heterotopic analgesic effects. Supported by NIH P50 DK-64539 (YT), 1K01DK088937 (ML), SNFGE (HD) and APHP (HD).

	CPD (mmHg)	30	45	60
Non-injected	Baseline	40.2 ±7.7	60.0 ±7.1	100.0 ±0.0
	Post WAS	37.5 ±5.1	66.7 ±6.8	78.9 ±7.2*
Saline SC	Baseline	52.6 ±11.1	81.5 ±8.8	100.0 ±0.0
	Post WAS	18.9 ±6.7**	55.9 ±7.9*	69.8 ±7.8*
Naloxone SC	Baseline	41.4 ±9.2	89.7 ±9.0	100.0 ±0.0
	Post WAS	22.3 ±4.8	43.7 ±9.3***	61.9 ±11.7**

Baseline and immediate post WAS VMR of mice non injected or injected sc with saline or naloxone (1 mg/kg) expressed in %. *p<0.05, ** p<0.01 and *** p<0.001 vs respective baseline.

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Strain differences in visceral nociception, anxiety and depression-like behaviour: Responses of 12 mouse strains

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Objective: Responses to painful stimuli differ between populations, ethnic groups, genders and even among individuals of a family. Furthermore, patients suffering from chronic pain disorders such as irritable bowel syndrome (IBS) also display co-morbid anxiety and depression. Previous studies in rodents have provided us with much needed data regarding strain differences using a vast array of somatic pain assays, but data regarding visceral nociception is still lacking. This study aimed to investigate strain differences in visceral nociception using manometric recordings of colorectal distension (CRD). Moreover, anxiety and depressive-like behaviours were investigated using the open field and the forced swim test respectively.

Methods: Adult male mice (>8 weeks) were sourced from Harlan, UK and Charles River, USA. The inbred strains used in this study were: CBA/JHsd, C3H/HeNHsd, BALB/c OlaHsd, C57 BL/6JOlaHsd, DBA/2J RccHsd, CAST/EiJ, SM/J, A/J OlaHsd, 129P2/OlaHsd, FVB/NHan Hsd, and the outbred strains: Hsd: ND4 (Swiss Webster), ICR (CD-1). CRD was performed as previously described by this group. The open field and the forced swim test were performed to assess anxiety and depressive-like behaviours respectively. All experiments were conducted in accordance with the European Community Council Directive (86/609/EEC) and approved by Animal Experimentation Ethics Committee of University College of Cork.

Results: A significant effect of distension pressure was observed for all animals thus demonstrating that all animals respond to the CRD paradigm. We also found a significant effect of strain and a significant interaction of distension pressure X strain. Post hoc analysis revealed that CBA/JHsd and C3H/HeNHsd strains displayed a significantly greater response to CRD. Furthermore, these two strains travelled significantly less in the inner zone of the open field and display increased immobility in the forced swim test.

Conclusion: This data demonstrate that strain differences occur in visceral nociception with both CBA/JHsd and C3H/HeNHsd strains displaying visceral hypersensitivity. Moreover, these two strains display anxiety and depressive-like behaviours, which are commonly co-morbid with chronic pain disorders. These findings reveal variations in basal nociception between mouse strains which may aid future work aimed at elucidating the mechanisms underlying visceral hypersensitivity in appropriate animal models.

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Changes in α- and β-adrenergic neurotransmission during postoperative ileus in rat

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Objective: α- and β-receptors participate in control of contractile activity and might participate in pathophysiology of postoperative ileus (POI). Our aim was to determine changes in neurotransmission via α- and β-receptor subtypes in rat jejunal circular muscle during POI.

Methods: Circular jejunal muscle strips (n = 8/rat) from male Sprague Dawley rats (n = 6/group) were studied in organ chambers. Groups: Naïve controls (NC), sham controls 3d after laparotomy (SC3d), and rats 3d after laparotomy and standardized small bowel manipulation (P3d) to induce POI. Dose-responses to exogenous α1- (α1a; phenylephrine; 10^{-8}–10^{-5} mol L^{-1}), α2- (α2a; clonidine; 10^{-8}–10^{-5} mol L^{-1}), β1- (β1a; xamoterol; 10^{-8}–3×10^{-6} mol L^{-1}), β2- (β2a; fenoterol; 3×10^{-9}–10^{-6} mol L^{-1}), and β3-agonists (β3a; BRL37344; 10^{-10}–3×10^{-8} mol L^{-1}) were studied -/+ tetrodotoxin (TTX; blocking enteric nerves; 10^{-6} mol L^{-1}). Intestinal transit was studied by charcoal gavage. Histology of jejunal whole mounts was performed for myeloperoxidase positive cells (MPO), macrophages, and mastcells (cells mm^{-2}). Data: mean±SEM.

Results: α- and β-agonists caused dose-dependent inhibition of contractile activity in all groups ($P < 0.05$). α2a, β1a, β2a, and β3a induced inhibition was increased in P3d ($P < 0.05$), whereas the effect of α1a was unchanged in P3d (P = NS). Inhibition by α1a, β1a, and β3a was also increased in SC3d ($P < 0.05$). In NC, TTX reduced α1a- and α2a-induced inhibition and enhanced β2a-inhibition ($P < 0.05$), while in P3d and SC3d, TTX reduced α1a-, β1a-, and β3a-inhibition ($P < 0.05$). Intestinal transit was delayed only in P3d (NC: 60 ± 8 vs P3d: 33 ± 4%; $P < 0.05$; SC3d: 53 ± 4%; P = NS). MPO positive cells (NC: 12 ± 2 vs P3d: 611 ± 59 mm^{-2}), mastcells (16 ± 4 vs 788 ± 51), and macrophages (376 ± 29 vs 1212 ± 97) were increased in P3d (all $P < 0.05$), and unchanged in SC3d (26 ± 10; 25 ± 1; 393 ± 6, respectively; P = NS).

Conclusion: Postoperatively, adrenergic inhibition is enhanced for all studied receptor subtypes, except for α1. Although this inhibition is predominantly mediated via receptors on muscle cells, α-adrenergic inhibition in NC is in part mediated via receptors on the enteric nervous system. In postoperative animals also inhibition via β1- and β3-receptor is partially mediated via the enteric nervous system. However, these changes appear not to be specific for POI as they were present also in SC3d, where no inhibition of gastrointestinal transit or inflammatory response, representing typical features of POI, was observed. DFG KA2329/5-1.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-25 Basic and Translational Session: Extrinsic Neural Pathways, Hypersensitivity and Pain

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Perivagal capsaicin alters the morphological, physiological and pharmacological characteristics of vagal motoneurons

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Objective: A supposedly selective degeneration of vagal afferent C-fibers by perivagal application of a 1% capsaicin (CAP) solution has often been used to ascribe a vagal mechanism of action to gastrointestinal (GI) peptides. As a result, the mechanism of action of many of these peptides have been attributed exclusively to a paracrine effect on vagal afferent fibers based on the dramatic reduction of their effects following perivagal CAP pretreatment. Circulating GI peptides can exert effects upon GI functions via actions at multiple sites, however, including at the level of the brainstem vagal nuclei. A reduction in the effects of circulating peptides following perivagal capsaicin could conceivably also be the result of actions of capsaicin to damage vagal efferent functions. The aim of the present study was to test the hypothesis that perivagal CAP treatment induces morphological and functional impairments of vagal efferent motoneurons in the rat.

Methods: Unilateral perivagal CAP treatment (0.1 and 1% solutions for 30 min) was carried out 7–10 days prior to monitoring: (i) the morphology and neurochemical phenotype of gastric-projecting DMV neurons; (ii) the gastric motility response induced by DMV microinjection of Thyrotropin Releasing Hormone (TRH); and (iii) the biophysical properties of DMV neuronal membranes.

Results: The effects of perivagal CAP was similar to those observed following posterior gastric branch vagotomy, viz., a reduction in ChAT-immunoreactivity, dendritic degeneration, and, possibly, death of a subpopulation of rat vagal preganglionic (DMV) neurons. Briefly, compared to control DMV microinjec-

tion, the the gastric motility response to TRH following perivagal capsaicin was significantly reduced. Furthermore, BK-type calcium-dependent potassium currents, a channel type previously reported to be associated with neuronal injury, were functionally expressed in DMV neurons ipsilateral, but not contralateral, to CAP application.
Conclusion: Our data show that perivagal CAP treatment induces functional impairment of DMV neurons. These results provide further indications that perivagal CAP treatment should not be used as an exclusive means to investigate the site of action of gastrointestinal peptides. Funds: NIDDK 55530.

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Reduced sensory neuron activity in the aged mouse
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Objective: Ageing is associated with changes in sensory neuron function that may correlate with decreased sensory perception. In this study we assess whether age-related changes in TRPV1 function occur in isolated mouse DRGs and assess the physiological relevance of this.
Methods: Young (3 months) and aged (24 months) mice were used in these experiments. Mouse DRGs (T9-L4) were isolated and cultured (for approximately 24 h) using standard protocols. Calcium mobilisation was assessed using Fura2-AM (4 μ mol L^{-1}), and the results were normalised to an ionomycin (5 μ mol L^{-1}) response. An in-vitro mouse colon preparation with attached lumbar colonic nerves, inferior mesenteric ganglion and lumbar splanchnic nerves was used to study chemosensory afferent function. In both experimental designs, test compounds were added to the bath or coverslips via the superfusion system. Data presented as mean ± SEM ($n \geq 4$). Data analysed by one or two way ANOVA or by Students *t*-test. $P < 0.05$ was taken as significant.
Results: 300 n mol L^{-1} and 1 μ mol L^{-1} capsaicin increased intracellular calcium mobilisation in DRGs isolated from 3 and 24 months animals. The response profile to 300 n mol L^{-1} capsaicin was moderately attenuated in 24 months DRGs compared to 3 months animals, although the maximum response was not altered. However the effect of 1 μ mol L^{-1} capsaicin was similar at both 3 and 24 months. Ageing had no effect on the number of DRGs which responded to capsaicin, although in similar experiments with 5-HT (10 μ mol L^{-1}) we found fewer DRGs responded to 5-HT in 24 months *vs* 3 months animals. Chemosensory function was also determined using an in-vitro mouse colon preparation. In these experiments, bath superfusion of 1.0 μ mol L^{-1} capsaicin produced significant increases in afferent discharge in 3 months and 24 months animals. The peak afferent response to capsaicin was similar in 24 months and 3 months animals, but the duration of the capsaicin-evoked afferent response was attenuated at 24 month ($P < 0.05$).
Conclusion: Ageing is associated with a decline in the number of DRGs responding to 5-HT, while the numbers of capsaicin responding cells are maintained in young and aged animals. However, the attenuated response to capsaicin in DRG and afferent discharge experiments suggest that ageing is associated with altered TRPV1 channel function. Funded by BBSRC.

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Developmental origins of functional dyspepsia like gastric hypersensitivity in rats
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Objective: Gastric hypersensitivity (GHS) contributes to epigastric pain in Functional Dyspepsia (FD) patients. The etiology or the cellular mechanisms of this dysfunction remain unknown. We tested the hypothesis that inflammatory insult to the colon in neonates induces GHS in adulthood.
Methods: We used cellular, molecular, and *in vivo* approaches to investigate the underlying mechanisms of GHS in adult rats subjected to neonatal colonic inflammation. Colonic inflammation was induced by intraluminal administration of 130 mg kg^{-1} trinitrobenzene sulfonic acid on postnatal (PND) day 10.
Results: Inflammatory insult to the colon on PND 10 significantly enhanced plasma corticosterone on PND 15 (180 ± 28%, $P < .05$) and induced GHS in adulthood (85 ± 24% increase at 80 mmHg, $P < 0.05$). We called these FD-like rats. The inhibition of glucocorticoid receptors with RU-486 following neonatal inflammatory insult blocked the induction of GHS in adulthood. The inopportune elevation of corticosterone during neonatal development significantly up regulated the expression of neurotrophic factor (NGF; 73 ± 10%, $P < 0.05$) in the muscularis externae of the gastric fundus; brain-derived neurotrophic factor (BDNF) in thoracic DRG (2.5 ± 0.5-fold, $P < 0.05$) and spinal cord (54 ± 8%, $P < 0.05$; and suppressed Kv1.1 mRNA (52 ± 10%, $P < 0.05$) in thoracic DRG without affecting Kv1.4, Nav1.8, TrpA1, TrpV1, and P2X3 in FD-like rats. Neonatal insult elevated the basal plasma levels of norepinephrine in adulthood (35 ± 6%, $P < 0.05$). The inhibition of adrenergic receptors with a cocktail of phentolamine (α1 and α2), propranolol (β1 and β2) and CL316243 (β3), or NGF neutralization with NGF antibody, or BDNF blockade with its receptor antagonist (trkB-Fc) suppressed the GHS in FD-like rats. Intrathecal administration of Kv1.1 siRNA increased GHS in naïve rats, compared to control siRNA treated rats (44 ± 15% increase at 100 mmHg, $P < 0.05$). These findings suggest that the up regulation of plasma NE, DRG/spinal cord NGF, BDNF, and concurrent suppression of Kv1.1 in DRG synergistically induce GHS in FD-like rats.
Conclusion: Our findings show that inflammatory insult to the colon in neonates is a major risk factor for the induction of GHS in remotely located stomach in adulthood. GHS results from altered expression of genes encoding neurotrophins, ion channels, and altered sympathetic nervous system activity.

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Electrical vagus nerve stimulation reduces plasma cholesterol and triglycerides levels in rats
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Objective: There is growing evidence that vagus nerve stimulation (VNS) has a suppressive effect on both short- and long-term feeding in animal models. We previously showed that long-term VNS (102 days) with low-frequency electrical impulses (0.05 Hz) decreased food intake and body weight in rats. In the present study, we investigated the effect of high frequency (10 Hz) VNS on plasma lipids concentrations, feeding behavior and appetite in rats fed a high-fat diet; peptide secretion and other parameters were assessed as well.
Methods: Adult male Wistar rats were each implanted subcutaneously with a microstimulator (MS) and fed a high-fat diet throughout the entire study period (42 days). The left vagus nerve was stimulated by rectangular electrical pulses (10 ms, 200 mV, 10 Hz, 12 h a day) generated by the MS. Body weight and food intake were measured each morning. At the end of the experimental period, animals were euthanized and blood samples were taken. Total plasma cholesterol, triglycerides, low-density lipoproteins and high-density lipoproteins concentrations were measured. Serum levels of ghrelin, leptin and nesfatin-1 were assessed using radioimmunoassays. Adipose tissue content was evaluated by weighing epididymal fat pads, which were incised at the time of sacrifice. To determine whether VNS activated the food-related areas of the brain, neuronal c-Fos induction in the nuclei of the solitary tract (NTS) was assessed.
Results: Chronic vagus nerve stimulation significantly decreased food intake, body weight gain and epididymal fat pad weight in animals that received VNS compared with control animals. VNS also lowered total plasma cholesterol concentrations, triglycerides and LDL cholesterol levels. Significant neuronal responses in the NTS were observed following VNS. Finally, serum concentrations of ghrelin were increased, while serum levels of leptin were decreased. Although not significant, serum nesfatin-1 levels were also elevated.
Conclusion: These results support the theory that VNS leads to reductions in food intake, body weight gain and adipose tissue by increasing brain satiety signals conducted through the vagal afferents. VNS also evoked a feed-related hormonal response, including elevated blood concentrations of nesfatin-1.

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Isomalto-oligosaccharides reverts visceral hypersensitivity and intestinal epithelial damage induced by water avoidance stress in rats
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Objective: Prebiotics might improve the symptoms for patients with irritable bowel syndrome (IBS). This study aimed to evaluate the effects of isomalto-oligosaccharides (IMO), as a prebiotics, on intestinal function and ultra-structure in IBS modeling rats induced by water avoidance stress (WAS) and explore the potential mechanisms.
Methods: Male Wistar rats were initially divided into modeling group and control group ($n = 9$). After 10 days WAS, the modeling rats were divided to IMO treatment group (IMO group, $n = 9$) and water control group (water group, $n = 9$), in which 5% IMO 2 ml or distilled water were administrated twice a day by gastric gavage for 14 days. The body weight gain, Gastrointestinal(GI) transit rate, abdominal withdrawal reflex (AWR) score stimulated by colorectal distension(for first two lots of animals), isolation and culture for Lactobacillus, E. coli and Bacteroides with fresh feces, ultra-structure of intestinal epitheliums, serum cytokines concentration (IL-10, IL-12, TNF-α) were measured.
Results: WAS group had more fecal pellets per hour with a higher percentage of deformed feces than control group during modeling period. IBS modeling rats had slower body weight gain, higher AWR score for colorectal distension at pressure of 20 mmHg, and

lower pain thresholds than control group (data not shown). There was no obvious pathological changes on light microscopy in jejunum, ileum and colon of IBS modeling tats, WAS induced damages on intestinal epithelial ultrastructure which presented as secretary granules on gland pits, goblet cell shrinkage, E. coli or other bacteroide adherence on goblet cells, enlargement of epithelial space, partial loss of microvilli, uneven distribution of microvilli). After IMO administration (IMO group), body weight gain, GI transit rate in rats had no significant difference with water group; IMO intervention improved visceral hypersensitivity of IBS modeling rat(table 1) and reverted damages of intestinal epithelial ultrastructure, which were evaluated by independent observer under electron microscope. There were no significant differences among 3 groups in fecal flora or serum cytokines concentration.

Conclusion: Isomalto-oligosaccharides improved the visceral hypersensitivity of IBS modeling rats, which might be associated its reverse effect on ultrastructural damages of intestinal epitheliums induced with chronic stress.(Supported by grants of 2010AA023007, 2007BAI04B01).

Table 1 IMO intervention improved visceral hypersensitivity of IBS modeling rats

	Control group (n=6)	IMO group (n=7)	Water group (n=5)
AWR score(40mmHg)	2.23±0.53*	2.52±0.88*	3.52±0.36
pain threshold(mmHg)	51.00±2.76*	39.86±11.75	26.40±4.04

*vs water group, $P<0.05$

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Oxytocin hyperpolarizes cultured duodenum myenteric intrinsic primary afferent neurons by opening BKCa channels through IP3 pathway

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Objective: Oxytocin (OT) is clinically important in gut motility and constitutively reduces duodenum contractility. Intrinsic primary afferent neurons (IPANs), whose physiological classification is as AH cells, are the 1st neurons of the peristaltic reflex pathway. We set out to investigate if this inhibitory effect is mediated by IPANs and to identify the ion channel(s) and intracellular signal transduction pathway that are involved in this effect.

Methods: Myenteric neurons were isolated from the longitudinal muscle myenteric plexus (LMMP) preparation of rat duodenum and cultured for 16–24 h before electrophysiological recording in whole cell mode and AH cells identified by their electrophysiological characteristics. The cytoplasmic $Ca2^+$ concentration ($[Ca2^+]i$) of isolated neurons was measured using calcium imaging. The concentration of IP3 in the LMMP and the OT secreted from the LMMP were measured using ELISA. The oxytocin receptor (OTR) and large-conductance calcium-activated potassium (BKCa) channels, as well as the expression of OT and the IPAN marker calbindin 28 K, on the myenteric plexus neurons were localized using double-immunostaining techniques.

Results: We found that administration of OT dose dependently hyperpolarized the resting membrane potential and increased the total outward current. The OTR antagonist atosiban or the BKCa channel blocker iberiotoxin (IbTX) blocked the effects of OT suggesting that the increased outward current resulted from BKCa channel opening. OTR and the BKCa subunit were co-expressed on a subset of myenteric neurons at the LMMP. NS1619 increased the outward current similar to the effect of OT. OT administration also increased $[Ca2^+]i$ and the OT-evoked outward current was significantly attenuated by thapsigargin or CdCl2. The effect of OT on the BKCa current was also blocked by pre-treatment with 2-APB orU73122. OT also increased the IP3 concentration within the LMMP. Both of the spontaneous and KCl-induced secretion of OT was enhanced by atosiban. Most of OT-immunoreactive cells are also immunoreactive for calbindin 28 K.

Conclusion: In summary, we concluded that OT hyperpolarized myenteric IPANs by activating BKCa channels via the OTR-PLC-IP3-$Ca2^+$ signal pathway. OT might modulate IPANs mediated ENS reflex by an autocrine and negative feedback manner.

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Characterisation of a radio-induced colorectal chronic pain model

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Objective: Radiotherapy to treat abdominal or pelvic malignancies induces high incidence of undesirable acute and/or chronic gastrointestinal side effects. The growing number of cases declared each year and the specific complex symptoms have led some specialists to talk of a new disease termed ''pelvic-radiation disease''. Visceral chronic pain, one of these complications, is very intense and until now, no effective and well tolerate medical treatment has been found to diminish it. A better understanding of cellular and molecular mechanisms implicated in the radiopathology of visceral pain might open future therapeutic tracks. In this study, we develop and characterise an experimental new model of radiation-induced chronic visceral pain.

Methods: SD rats were exposed to locally (colorectal region) ionising radiation at a single dose of 27 Grays. At this dose, alterations observed in patients subjected to a pelvic bi-fractionated radiotherapy (total dose of 45 Grays) were similar. Visceral pain was tested in the irradiated rats versus the sham ones by the analysis of visceromotor response (VMR) to colorectal distension (CRD). Experiments were led two, four and eight weeks after irradiation. Immunohistochemical approach was used to evaluate CGRP (calcitonin gene-related peptide) +, SP (substance P) + fibbers and p-ERK (extracellular signal-regulated kinases) + neurons at the colon and spinal cord level respectively.

Results: Two, four and eight weeks after irradiation similar VMR to CRD were observed. Irradiation induced allodynia (for 15 mmHg distension pressure) and also reduced VMR in response to noxious CRD (45 and 60 mmHg). Four weeks after irradiation we showed colonic hyperplasia of the sensitive fibbers CGRP+ in the epithelium and SP+ in the muscle layer and enteric nervous system. Experiments performed at the L6-S1 spinal cord level demonstrated that colorectal irradiation increases neuronal p-ERK in the superficial layers of the dorsal horn ($P < 0.001$).

Conclusion: These results suggest that a single dose of colorectal irradiation (27 Grays) induced chronic visceral pain. Peripheral and spinal cord plasticity might participate to the development and maintain of chronic pain. We also reported a very intense radiation-induced visceral pain, which might in turn lead to inhibitory retro-controls activation.

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5HT2B receptor mRNA expression throughout the brain-gut axis is unaltered in the maternally separated rat

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Objective: Irritable bowel syndrome (IBS) is now seen as a disorder of brain-gut axis. Visceral pain is one of the more debilitating symptoms of IBS. Stress especially that in early life has been implicated in IBS making the maternally separated (MS) rat a valid model to study this disorder. There is significant evidence implicating the serotonergic (5-HT) system in the modulation of pain but the contribution of the 5-HT2B receptors remains unclear. Tegaserod, a 5-HT4 receptor agonist, which relieves IBS symptoms also acts at the 5-HT2B receptor. Moreover, we have shown that the 5-HT2B receptor antagonist, RS 127445, reduces visceral pain in animal models of IBS. It is likely thus that alterations in 5-HT2B receptor may contribute to the alterations in the brain-gut axis of rats subjected to MS.

Methods: Sprague dawley rats (SD) were subjected to MS (3h/day, post-natal days 2–12) and non-separated (NS) rats served as controls. At 11 weeks of age the animals were subjected to colorectal distension (CRD) to assess visceral sensitivity. The expression of the 5-HT2B receptor mRNA in naïve animals was analysed using in situ hybridization (ISH) and RT-qPCR.

Results: The MS group was viscerally hypersensitive compared to the NS group. The expression of the 5-HT2B receptor mRNA was demonstrated in the hippocampus and in the amygdala but there was no significant difference between the groups. In the other brain areas (dorsal raphe, periaqueductal gray, rostral ventral medulla, hypothalamus, pre-frontal cortex) and the spinal cord, the receptor levels were undetectable. Finally there was no difference in the receptor levels within the distal colon of the MS animals compared with NS animals.

Conclusion: Despite the clear relationship between IBS and psychiatric disease, the 5-HT2B receptors expression isn't affected by MS in either the hippocampus or the amygdala. The receptor mRNA levels were undetectable in brain areas more classically related to pain and in the spinal cord. The receptors were present in the colon yet there was no difference between our groups. Although no difference has been shown in the mRNA expression throughout the brain-gut axis, we can't exclude functional alterations of the 5HT2B receptor in this animal model.

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Effect of DA-9701, new prokinetic, on colonic function of spinal cord injured rat model

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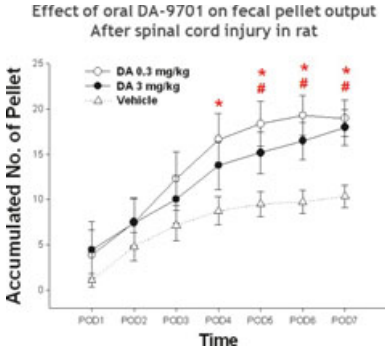
Objective: Defecatory disorder in patient with spinal cord injury (SCI) is an important symptom that decreases quality of life. The aim of this study was to investigate whether the DA-9701, newly developed prokinetic, could improve the defecatory dysfunction in SCI rat model.

Methods: S-D rats (250–300 g) were divided into a vehicle group, DA-9701 0.3 mg kg^{-1} group and DA-9701 3 mg kg^{-1} group (n = 7/group). A spinal cord transection was performed surgically at the T10 level. After operation, vehicle or DA-9701 was administrated per oral 2 times per day during 1 week. Number of fecal pellets and body weight were measured every morning. After 1 week, whole colon was removed under anesthesia and divided into proximal and distal colon. Each segment of colon was mounted with longitudinal or circular direction in an organ bath and spontaneous contraction was measured. The percent changes of contractility from the baseline value after ACh alone, ACh with M2 receptor blocker (AQ-RA 741), ACh with M3 receptor blocker (4-DAMP), and Ach with 5HT4 antagonist (GR113808) were compared between groups.

Results: There was no difference in body weight between groups after 1 week. The defecatory state was significantly improved in DA-9701 0.3 mg kg^{-1} or 3 mg kg^{-1} groups compared to vehicle group. From the 4th day, cumulative number of fecal pellet was significantly higher in DA-9701 groups than vehicle group ($P < 0.05$). In organ bath experiment, vehicle group showed increased colonic contractile response to ACh in distal colon in both direction compared to other groups ($P < 0.05$). This increased response was abolished by GR113808 pretreatment. In subgroup analysis, there was a significant difference in ACh response of proximal and distal longitudinal muscle direction in vehicle group between with and without GR113808. GR113808 did not show any effect in DA-9701 groups. Although 4-DAMP was more potent to decrease contractility, both of M2 and M3 antagonist pretreatment decreased contractility in all groups ($P > 0.05$ between groups).

Conclusion: DA-9707 could improve defecatory dysfunction after SCI in rat model. DA-9701 seemed to preserve normal 5-HT4 status which was abnormally enhanced after SCI and further study for this beneficial effect of DA-9701 was warranted.



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Changes in the modulation of spinal pain processing are related to severity in irritable bowel syndrome

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Objective: In irritable bowel syndrome (IBS) patients can be divided in two groups according to inhibition or facilitation of the RIII nociceptive spinal reflex induced by rectal distension. We further investigated the differences in pain processes in these two groups and their relationship to clinical symptoms.

Methods: This study included 10 female IBS-C patients with facilitation (Group F) and 10 patients with inhibition (Group I) of the RIII reflex recorded on the left lower limb during slow-ramp rectal distension, and 11 healthy females volunteers. Diffuse noxious inhibitory control (DNIC)-induced inhibition was assessed by measuring the effects of noxious cold stimulation of the right hand on the RIII reflex and the concomitant sensation of pain. Functional magnetic resonance imaging (fMRI) was performed to compare the changes in brain activity induced by painful and non painful rectal distension. IBS symptom severity, mood, anxiety and catastrophizing were also systematically assessed.

Results: Unlike the patients of Group I and healthy volunteers, Group F patients displayed no inhibition of the RIII reflex or of concomitant pain sensation during immersion of the hand in ice-cold water. The reduction of the inhibition induced by heterotopic noxious stimuli was directly correlated with the severity of IBS symptoms, but not with psychological symptoms. The fMRI study showed that non-painful and painful rectal distension induced similar changes in brain activity in the two groups of patients.

Conclusion: Alterations of the modulation of spinal pain processing in IBS correlates with symptom severity but not with psychological factors or brain activity.

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Analysis of CGRPergic, nitrergic, and VIPergic myenteric innervation after 2% L-glutamine supplementation in the ileum of diabetic rats

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Objective: Diabetes promotes changes in enteric innervations, likely via oxidative stress, which contributes to gastrointestinal dysfunction. Previous studies have shown that L-glutamine, a precursor of glutathione with antioxidant capacity, reduces diabetes-induced changes in neuronal density and reduces swelling of VIPergic varicosities in the submucosal plexus of the jejunum. The objective of this study was to investigate the effects of L-glutamine on CGRPergic, nitrergic, and VIPergic myenteric innervation in the ileum of diabetic rats.

Methods: Wistar rats were grouped as follows: normoglycemic (N); normoglycemic supplemented with L-glutamine (NG); diabetic (D); and diabetic supplemented with L-glutamine (DG). Diabetes was established in groups D and DG by streptozotocin (i.v.; 35 mg kg^{-1}). L-Glutamine (2%) was incorporated into the chow and fed to NG and DG animals for 120 days. Ileums were subjected to immunohistochemical techniques to localize myenteric neurons immunoreactive for HuC/D protein (HuC/D-IR) and neuronal nitric oxide synthase enzyme (nNOS-IR) and to analyze varicosities immunoreactive for vasoactive intestinal polypeptide (VIP-IR) and calcitonin gene-related peptide (CGRP-IR). Quantitative and morphometric analysis of micrographs were performed.

Results: L-glutamine in diabetic animals (DG group) (i) prevented the diabetes-induced increase in the cell body area of nNOS-IR neurons, (ii) prevented the diabetes-induced increase in the area of VIP-IR varicosities, (iii) did not prevent the diabetes-induced loss of HuC/D-IR and nNOS-IR myenteric neurons per ganglion, and (iv) reduced the size of CGRP-IR varicosities. L-glutamine in the NG group reduced (i) the number of HuC/D-IR and nNOS-IR neurons per ganglion, (ii) the cell body area of nNOS-IR neurons, and (iii) the size of VIP-IR and CGRP-IR varicosities.

Conclusion: L-glutamine reduced some aspects of experimental diabetic neuropathy in the myenteric plexus of the ileum. Newly observed in this study is the effect of L-glutamine to reduce neuronal density, cell areas and areas of VIP and CGRP varicosities of normoglycemic animals. Collectively, these results suggest there are additional actions of this amino acid beyond its antioxidant capacity and support the conclusion that there may exist a narrow therapeutic window for L-glutamine in the treatment of diabetic neuropathy.

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PS-26 Basic and Translational Session: Microbiota in Health and Disease

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Age-associated remodeling of the intestinal epithelial barrier

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Objective: Aging has profound effects on the gastrointestinal (GI) tract, which potentiates the susceptibility to GI disorders; however, there is a paucity of studies delineating the effects of aging that predispose individuals to these disorders. Most GI disorders are commonly characterized by enhanced intestinal permeability, which can involve degeneration of the tight junction proteins that adjoin the epithelial cells. Therefore, we hypothesized, that the normal aging process enhances permeability of the colon through a disruption of epithelial tight junction proteins. To test our hypothesis, we employed a nonhuman primate model with a lifespan closely resembling humans.
Methods: Colonic biopsies were excised from old (18+) and young (4–10 years) anaesthetized baboons (Papio anubis). Permeability was assessed by measuring basal transmucosal potential difference (PD) and diffusion of horseradish peroxidase (HRP) across the epithelial membrane in modified Ussing chambers. The tight junction proteins ZO-1, occludin, claudin-2, and JAM-A were quantified by immunofluorescence and Western blot. Regulatory modulators of tight junction proteins including the expression of miRNA, glutamine synthase (GLUL), and cytokines were assessed by qRT-PCR.
Results: Compared to young baboons, there was a decrease in PD (Old: -3.55 ± 0.45, n = 10; Young: -2.04 ± 0.46; n = 9; $P < 0.05$) and a greater HRP flux (Old: 0.31 ± 0.08, n = 10; Young: 0.72 ± 0.17; n = 9; $P < 0.05$) in colonic biopsies from old baboons, indicating enhanced permeability. Aging in the baboon colonic epithelial barrier was associated with a decrease in ZO-1, occludin, and JAM-A tight junction proteins, in contrast to a significant increase in claudin-2 expression (see table). There was a concomitant increase in miR-29a expression (2.35-fold; $P < 0.05$; n = 6/group), but no changes in GLUL expression ($P > 0.05$) in the aged colonic tissue. Additionally, in the absence of overt inflammation as indicated by lack of myeloperoxidase activity and histological examination, we found an increased expression of the cytokines INF-γ (17.62-fold; $P < 0.05$, n = 6/group), IL-6 (18.11-fold; $P < 0.05$ n = 6/group), and IL-1β (20.26-fold; $P < 0.05$; n = 6/group) in the colonic mucosa of old baboons in comparison to young.
Conclusion: Aging induces remodeling of the tight junction proteins in the intestinal epithelial barrier, potentially through an increase in miR-29a and/or inflammatory cytokine expression, resulting in enhanced intestinal permeability. Abnormal intestinal permeability exponentiates susceptibility to GI disorders, and therefore these mechanisms may be a critical contributing factor to the pathophysiology of age-associated GI disorders.

Tight Junction Protein	Young (n=4)	Old (n=4)
ZO-1	0.41±0.06	0.21±0.04*
Occludin	0.55±0.07	0.41±0.03*
Claudin-2	0.17±0.04	0.29±0.03*
JAM-A	0.25±0.03	0.17±0.02*

*$p < 0.05$

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The microbiome-gut-brain axis as a regulator of serotonergic neurotransmission and behaviour

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Objective: Dysregulation of the microbiome-gut-brain axis is implicated in functional disorders including irritable bowel syndrome (IBS) and previous studies have demonstrated CNS serotonergic disturbances following disruption of the intestinal microflora. It is unclear at present whether such alterations may be reversed by microbiome directed therapies later in life. Our specific objective was to compare germ-free animals with conventional and colonised germ-free animals for concentrations of serotonin (5-HT) and its main metabolite, 5-HIAA, in the hippocampus. The availability of plasma tryptophan, the amino acid precursor of serotonin, was also assessed along with anxiety-like behaviours.
Methods: Germ-free (GF), conventionally colonised (CC) and colonised GF Swiss Webster mice were compared. High performance liquid chromatography (HPLC) was used to assess the concentrations of 5-HT and 5-HIAA in the hippocampus and tryptophan concentrations in plasma. Anxiety-like behaviours were assessed in the light-dark box.
Results: There was a significant elevation in the hippocampal concentration of 5-HT (461.5 ± 24 vs 534.3 ± 11.30 ng g^{-1} tissue, $P < 0.05$) and 5-HIAA (289.7 ± 14.07 vs 380.7 ± 35.49 ng g^{-1}, $P < 0.05$) in GF animals compared to their CC counterparts. Moreover, plasma tryptophan concentrations were elevated in GF animals (15539 ± 1454 vs 21080 ± 2000 ng ml^{-1}, $P < 0.05$) who also displayed less anxiety-like behaviours than their CC counterparts as determined by an increased number of transitions in the light-dark box (33.60 ± 5.66 vs 56.56 ± 3.920, $P < 0.01$). Colonisation of the GF animals proved insufficient to reverse the hippocampal serotonergic alterations found but did restore both plasma tryptophan concentrations and anxiety-like behaviours to baseline values.
Conclusion: The results demonstrate that CNS neurotransmission can be profoundly disrupted by the absence of a normal gut microbiota. Moreover we have demonstrated that the neurochemical, but not the behavioural, consequences of growing up germ-free are resistant to interventions later in life aimed at restoring a normal gut flora. These findings should inform future studies seeking to exploit the therapeutic potential of microbiome manipulation.

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This abstract has been withdrawn.

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A novel mechanism for barrier dysfunction in mice on a high-fat diet

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Objective: Disruption of gut barrier function is associated with metabolic disease. Studies have reported a leaky barrier in obesity and in animals fed a high-fat diet. We have associated barrier dysfunction with a decreased proportion of fecal ursodeoxycholic acid (UDCA) and increased concentration of deoxycholic acid (DCA). We studied the effects of two bile acids, DCA and UDCA, on intestinal permeability ex vivo to address whether alterations in luminal bile acids may impact barrier function on a high-fat diet.
Methods: Tissue segments from healthy C57BL/6J mice were incubated in an Ussing chamber system with DCA, UDCA or both bile acids together, at various concentrations for 20 min. Barrier function was measured using 4 kDa FITC-labeled dextrans. The effects and interaction of the bile acids on tissue structure and tissue occludin were assayed *In vitro*.
Results: DCA disrupted epithelial integrity dose-dependently especially in colon at 1–3 m mol L^{-1} concentrations (1 m mol L^{-1}, 1.9-fold increase to control, $P < 0.01$; 3 m mol L^{-1}, 3.5-fold increase to control, $P < 0.01$; 3 m mol L^{-1} vs. 1 m mol L^{-1} $P < 0.05$). No effect was seen in lower concentrations. The concentrations 1–3 m mol L^{-1} for DCA are physiological in high-fat-fed mice, whereas feces from control mice contain only 0.3 m mol L^{-1} DCA. This lower concentration of DCA did not affect permeability. UDCA had no effect on permeability in colon, but decreased jejunal permeability at 0.3 m mol L^{-1} ($P < 0.05$). UDCA (0.6 m mol L^{-1}) protected from barrier dysfunction induced by 3 m mol L^{-1} deoxycholic acid ($P < 0.05$), which was reflected in decreased tissue disruption in H&E staining. Colon occludin content was unaffected by 3 m mol L^{-1} DCA.
Conclusion: DCA doubled intestinal permeability only at concentrations related to high-fat-feeding, but had no effect at concentrations found in the feces of healthy mice. The effect of DCA was ameliorated by UDCA. These results suggest a role for an altered luminal bile acid profile in the pathogenesis of barrier dysfunction on a high-fat diet in mice.

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Role of toll like receptors 2 and 4 in the serotonin-induced responses in mouse ileum and colon

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Objective: Gut mucosal enterochromaffin (EC) cells are regarded as key regulators of intestinal motility via release of serotonin (5-HT) to adjacent immune cells and nerve endings. Conditions associated with gut mucosal inflammation like inflammatory bowel disease (IBD) are accompanied by an increased EC cell number and hypersecretion of 5-HT. Toll-like receptors (TLRs), through interactions with microbe-associated molecular patterns, activate inflammatory gene expression in cells of the innate immune system. Recent knowledge has shown that secretory products from immune cells can activate EC cell secretion of 5-HT. However, the influence of TLR pathways in the motor responses induced by serotonin in the intestine is unknown.The aim of this study was to investigate the role of TLR2 and TLR4 in the 5-HT-induced responses in longitudinal muscle of mouse ileum and colon.

Methods: Segments of ileum and colon from male C57/BL6 wild-type (WT), TLR2 -/-, TLR4 -/- and TLR4/TLR2 -/- mice were suspended in an organ bath in the direction of longitudinal smooth muscle fibres. Concentration-response curves of 5-HT (10^{-7}–10^{-4} mol L^{-1}) were performed and 5-HT motor responses were measured.
Results: The frequency and amplitude of the spontaneous contractions were not modified in the ileum and colon of TLR2 -/-, TLR4 -/- and TLR4/TLR2 -/- respect to WT mice. 5-HT (10^{-7}–10^{-4} mol L^{-1}) induced a concentration-dependent contractile response in WT mice ileum and colon. The contractile response induced by 5-HT 10^{-4} mol L^{-1} in ileum was reduced in TLR2 -/-, TLR4 -/- and TLR4/TLR2 -/- respect to WT mice. In some ileum segments, 5-HT evoked a relaxing response before the contractile response. The relaxing response evoked by 5-HT 10^{-7} mol L^{-1} lasted less time in TLR2 -/-, TLR4 -/- and TLR4/TLR2 -/- respect to WT mice. There were no differences in the contractile responses evoked by 5-HT (10^{-7}–10^{-4} mol L^{-1}) in the colon of TLR2 -/-, TLR4 -/- and TLR4/TLR2 -/- respect to WT mice.
Conclusion: These results suggest that TLR2 and TLR4 are involved in the response induced by serotonin in mouse ileum. Funding by Gobierno de Aragón (B61/2011). E. Latorre has a personal grant (B105/11).

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-27 Basic and Translational Session: Signalling: Hormones, Neurotransmitters, Receptors, Channels, Secondary Messengers

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Endogenous H2S produced by CSE selectively modulates central cholinergic synaptic input in mouse superior mesenteric ganglion
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Objective: Hydrogen sulfide (H2S) is present in mammalian tissues including the CNS. Our previous studies have shown that cystathionine γ-lyase (CSE) is the predominant enzyme in mouse superior mesenteric ganglion (SMG) and that NaHS selectively potentiates fast cholinergic excitatory postsynaptic potentials (F-EPSPs) evoked by splanchnic nerve stimulation. The aim of this study was to determine if endogenously produced H2S selectively facilitates F-EPSPs evoked by splanchnic nerve stimulation.
Methods: The SMG together with attached splanchnic and colonic nerve trunks was dissected from adult SJL/J mice, CSE knockout (CSE-KO) mice and wild type (WT) litter mate controls. Sharp glass microelectrodes were used for intracellular recordings.
Results: First, the effect of inhibiting H2S breakdown on F-EPSPs was tested with stigmatellin, a specific sulfide quinone reductase inhibitor. Stigmatellin (1 μ mol L^{-1}) increased the amplitude and area of F-EPSPs evoked by splanchnic nerve stimulation (21.0 ± 4.8 mV and 441 ± 107 ms mV with stigmatellin vs. 18.4 ± 5.3 mV and 338 ± 108 ms mV before stigmatellin, $P < 0.05$, $n = 4$) but had no significant effect on F-EPSPs evoked by colonic nerve stimulation. Second, the effect of the CSE inhibitor dl-propargylglycine (PAG) was tested. PAG (1 m mol L^{-1}) decreased the amplitude and area of F-EPSPs evoked by splanchnic nerve stimulation (23.7 ± 3.9 mV and 824 ± 288 ms mV with PAG vs. 27.0 ± 4.1 mV and 1230 ± 414 ms mV before PAG; $P < 0.05$, $n = 6$) but it had no significant ($P > 0.05$, $n = 5$) effect on F-EPSPs evoked by colonic nerve stimulation. Third, CSE-KO mice and WT control mice were used. In WT control mice, F-EPSPs evoked by splanchnic nerve stimulation had an amplitude of 21.4 ± 3.7 mV and an area of 1200 ± 647 ms mV ($n = 6$), and F-EPSPs evoked by colonic nerve stimulation had an amplitude of 15.9 ± 2.5 mV and an area of 633 ± 146 ms mV ($n = 6$). In CSE-KO mice, the amplitude of F-EPSPs evoked by splanchnic nerve stimulation was significantly ($n = 5$, $P < 0.05$) reduced to 8.7 ± 2.2 mV and the area of F-EPSPs evoked by splanchnic nerve stimulation trended to be smaller (198 ± 57 ms mV, $n = 5$, $P = 0.06$). The amplitude and area of F-EPSPs evoked by colonic nerve stimulation in CSE-KO mice were unchanged.
Conclusion: Endogenously produced H2S selectively modulates central splanchnic cholinergic synaptic input in the mouse SMG. Supported by NIH DK17238.

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NO in the gastrointestinal tract: What it takes to relax
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Objective: The enteric nervous system regulates gastrointestinal (GI) motility via adrenergic, cholinergic, and nitrergic neurons. The release of nitric oxide (NO) as neurotransmitter leads to activation of NO-sensitive guanylyl cyclase (NO-GC) and production of the second messenger cGMP. In addition to smooth muscle cells (SMC), interstitial cells of Cajal (ICC) are involved in nitrergic relaxation. In this study, we intended to clarify the role of NO-GC regarding nitrergic relaxation of GI smooth muscle in mice.
Methods: Recently, our group has generated different mouse lines that lack NO-GC ubiquitously (total GCKO), specifically in SMC (SM-GCKO) or in ICC (ICC-GCKO) and in both SMC and ICC (SM/ICC-GCKO). Isometric force studies were performed to investigate the effects of NO on murine fundus. Using immunohistochemistry we evaluated the expression of NO-GC in the different GI cell types.
Results: NO-dependent relaxation of fundus smooth muscle was abolished in total GCKO mice. In SM-GCKO, NO still led to partial relaxation whereas ICC-KO showed a WT-like phenotype. Only in SM/ICC-GCKO we observed lack of nitrergic relaxation similar to that seen in total GCKO mice. Immunohistochemistry revealed NO-GC expression in SMC, ICC and, additionally, in a third cell type characterized as fibroblast-like cells (FLC).
Conclusion: To conclude, nitrergic GI relaxation is not evoked exclusively via NO-GC in SMC. Lack of NO-GC in both SMC and ICC, though, abolishes nitrergic signaling. In the fundus of SM/ICC-GCKO mice strong NO-GC expression was still detected in FLC. Therefore, generation of FLC-specific knock-out mice will give us the opportunity to investigate the function of NO-GC in this specific cell type.

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Mechanisms underlying motility in the aged gut: A role for altered serotonin signalling processes
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Objective: Ageing has a considerable effect upon gut function, but little is known about the physiological mechanisms underlying these changes. Serotonin signalling mechanisms are essential for normal peristaltic activity and as such age-related changes in gastrointestinal motility may reflect alterations in serotonin signalling pathways. In this current study we set out to explore this hypothesis.
Methods: Experiments were performed on young (3 m) and aged (18 and 24 m) mice. *In vitro* recordings of intraluminal pressure were used to monitor the presence of colonic peristaltic motor complexes (CPMCs) in colonic segments using a Trendelenburg-type preparation. Young (3 m) and aged (18 m) mouse colons were assessed for changes in CPMC activity as previously described (Keating et al., 2010). Compounds were tested for their effect on colonic motility using a cumulative dosing strategy. Real-time PCR was used to quantify gene expression of SERT, TPH1/2 and components of the serotonergic signalling pathways in young (3 m) and aged (24 m) animals. Statistical analysis was by one-way ANOVA or Students *t*-test on $n \geq 4$ experiments. $P < 0.05$ was taken as significant.
Results: CPMC frequency was moderately decreased in aged mice compared to young animals. Tropisetron (1 μ mol L^{-1}–10 μ mol L^{-1}) caused a concentration-dependent decrease in the frequency of CPMCs in both 3 m and 18 m animal groups although the effect was greater in the aged animal group. Fluoxetine had equipotent effects on both young and aged colon motility. Real-time PCR showed that, compared to young animals, expression of SERT and TPH-1 were significantly increased in aged animals, while expression of 5-HT4 receptors showed a large (approximately 20 fold), significant increase in aged animals. Expression of TPH-2, 5-HT2A-C, 5-HT3A and 5-HT3B were unchanged between aged and young animals.
Conclusion: Motility-related changes in the aged mouse colon are moderate, although the aged mouse colon appears more susceptible to 5-HT3 receptor blockade. Ageing is associated with increases in components of the serotonergic signalling pathways which are important in maintaining 5-HT bioavailability, motility and neuroprotection. As such, these results suggest that the ageing gut is capable of exhibiting a

degree of plasticity to maintain function. Funded by the Physiological Society and BBSRC

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Dynamics of purinergic fast inhibitory junction potentials in the human colon

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Objective: Purinergic and nitrergic neurotransmission is the dominant mechanism responsible for colonic smooth muscle relaxation. Previous data showed a ''rundown'' in purinergic neurotransmission elicited by single pulses.
Methods: Accordingly, in the present work we studied the dynamics of the purinergic inhibitory junction potential (IJP) during different protocols of electrical field stimulation (EFS) in human colon.
Results: Protocol 1. Single pulses (0.4 ms) at increasing voltages elicited a MRS2500 (1 μ mol L^{-1}) sensitive fast IJP with a progressive increase in amplitude which was fitted to a sigmoid voltage-response curve (V50 = 16 ± 0.8 V; Emax = -22.7 ± 1.1 mV; D.F. = 167 R^2 = 0.52, n = 17). No major changes were observed with L-NNA (1 m mol L^{-1}) confirming the purinergic nature of the response. Protocol 2. Stimulation at increasing frequencies (from 0.1 to 5 Hz) elicited consecutive fast IJP that ran down in a frequency dependent manner. Data (Frequency vs. IJP amplitude) were fitted to an exponential decay (D.F. = 170; R^2 = 0.94, n = 17). At high frequencies (>2 Hz), the amplitude of IJP ran down completely. The time constant was 2.01 ± 0.12 s and was independent of the amplitude of the IJP. Similar results were obtained in the presence of L-NNA. Protocol 3. Trains of 60 s were elicited at different frequencies (from 0.1 to 5 Hz). For each frequency, an IJP can be recorded after the first stimuli and the response remained constantly inhibited during the rest of the train. For example, purinergic neurotransmission at 1Hz elicited a first IJP followed by IJPs 80% inhibited in amplitude. The time course of recovery from junctional rundown was tested by eliciting a single stimulus every 30 s following each train of stimulation. Data (time vs. % of IJP recovery) were fitted to an exponential function (D.F. = 6, R^2 = 0.87, n = 3) that saturates (97 ± 11 s) at higher frequencies of stimulation.
Conclusion: In the human colon, purinergic neuromuscular transmission depends on the voltage and the frequency of stimulation. An increase in voltage increases post-junctional responses possibly due to an increase in neurotransmitter release. In contrast, the increase in frequency causes a rundown of the response that behaves like a low-pass filter. Prejunctional (i.e. prejunctional inhibition or transmitter depletion) or post-junctional (i.e. receptor desensitization) could account for the frequency dependent rundown. Recovering of inhibition of neurotransmsmission is time-dependent.

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Glucose malabsorption in critical illness: A molecular defect in intestinal sweet taste sensing?

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Objective: Provision of adequate nutrition is a major determinant of clinical outcome for critically ill patients. Effective delivery of enteral feeds, however, is often compromised by glucose malabsorption [1]. While no human data exists, levels of the major intestinal glucose transporter, SGLT1, are reduced in animal models of acute critical illness [2]. It is also established that the sweet taste receptors T1R2 and T1R3 are expressed in the proximal intestine of rodents and humans, and in rodents, act to increase SGLT1 levels in the presence of glucose or a sweetener [3, 4]. Here we determined if (i) the expression of intestinal SGLT1 and T1R2 and (ii) SGLT1 function was impaired in critically ill patients and in a mouse model of the disease.
Methods: Endoscopic biopsies were collected from fasted and ventilated critically ill patients and matched healthy subjects (n = 12) prior to, and after, 30 min duodenal infusion of glucose (4 kcal min^{-1}) plus the absorption marker 3-O-methylglucose (3OMG). Intestinal tissues were also collected from mice 4 days after cecal ligation and puncture (CLP) and after 30 min of duodenal infusion with glucose + 3OMG. Expression of SGLT1 and T1R2 in intestinal tissues was quantified by RT PCR; plasma 3OMG levels were determined by spectrometry.
Results: SGLT1 and T1R2 expression was lower in critically ill patients at baseline (50, 54% respectively, P < 0.05) and after glucose infusion (53, 61% P < 0.01), in association with reduced plasma 3OMG levels (55%, P < 0.05). Similar findings were apparent in CLP mice for SGLT1 and T1R2 transcript, and 3OMG levels (50 and 78, 91% reduced respectively P < 0.01).
Conclusion: Intestinal expression of T1R2 is markedly lower in human and modelled critical illness, in association with reduced expression and impaired absorptive function of SGLT1. Reduced T1R2 signalling may uncouple control of SGLT1 from luminal glucose, and represent the molecular basis of carbohydrate malabsorption in critical illness. Therapies targeting these molecules may have potential to improve clinical management in this setting.
References:
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Functional characterisation of human and mouse proximal colon as a site of nutrient sensing

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Objective: Nutrient sensing occurs via specific receptors that detect a variety of luminal stimuli throughout the gastrointestinal (GI) tract. Activation of these receptors leads directly to humoral and neural signals that modulate GI activity. The objective of this study was to elucidate the effect of nutrient exposure on enteroendocrine cells (EEC) specifically in human/mouse proximal colon which may be an important site of nutrient sensing/absorption.
Methods: Healthy human ascending colon samples were obtained from surgical resection specimens, while mouse proximal colon was obtained from 12 week aged female C57BL/6 mice. Nutrient exposure studies were performed on freshly dissected mucosa using an Ussing Chamber. Fluorescent immunohistochemistry was performed on sections cut from Zamboni's fixed tissue.
Results: In order to identify nutrients that would activate EEC, mucosa of mouse proximal colon was stimulated with 1 and 10 m mol L^{-1} phenylalanine (Phe)/tryptophan (Trp) (selective activators of CaSR), 12.5 and 25 m mol L^{-1} lauric acid (activates GPR84), 0.0375 and 0.057 g mL^{-1} protein hydrolysate (activates GPR93) and 10 m mol L^{-1} glutamic (Glu)/aspartic (Asp) acids (which activate T1R1 and CaSR). Using pERK as a marker for cell activation, more EEC were activated in all stimulated mucosa compared to buffer controls, however, an increase in the number of activated cells was not observed between low and high nutrient concentrations. Human mucosa of ascending colon was stimulated with 1 and 10 m mol L^{-1} Glu/Asp acids, and 25 and 50 m mol L^{-1} Phe/Trp. More EEC were pERK positive in Glu/Asp stimulated mucosa compared to buffer controls. However, in mucosa stimulated with Phe/Trp, pERK activation was not observed. Since recombinant CaSR activation is associated with pCAMKII activation (Rey et al., Cell Physiol 2010), we localised pCAMKII and found Phe/Trp stimulation markedly increased pCAMKII expression in EECs compared to buffer controls. Interestingly, double-staining studies revealed 5-HT and GLP-1 positive cells co-localise with pCAMKII.
Conclusion: Amino acid and medium chain fatty acid receptors are functionally active in the ascending colon. Furthermore, human colonic EEC respond to specific nutrient stimulation via distinct signalling pathways and activate gut hormone expression. Taken together, these results suggest that the colon is an important site for nutrient sensing and is therefore a potential target for appetite regulation.

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Expression of duodenal fatty acid receptors in humans is inversely related to body mass index

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Objective: Fatty acids (FAs) exert potent effects in the small intestine on gastrointestinal (GI) function, and trigger the release of GI hormones, including cholecystokinin (CCK) and glucagon like peptide-1 (GLP-1), which contribute to suppression of hunger and energy intake [1]. The recent identification of the novel G protein coupled FA receptors GPR120, GPR40 and GPR119 in the intestine has revealed new pathways of FA sensing. However, despite evidence that the effects of fats on GI function are attenuated in obesity [1], there is little known about the expression of intestinal FA receptors in humans and whether this is influenced by body weight.
Methods: 15 lean [age: 55 ± 5 year, body mass index (BMI): 24 ± 1 kg m^{-2}] and 5 obese subjects [age: 56 ± 12 year, BMI: 38 ± 5 kg m^{-2}] participated in this study. In fasted subjects, endoscopic biopsies were collected from the proximal duodenum and processed for quantitative RT-PCR assessment of GPR40, GPR120,

GPR119 transcript levels, and for immunolabelling of FA receptors, CCK and GLP-1.
Results: FA receptor transcripts were expressed in the human duodenum with relative abundance GPR40>GPR120>GPR119. In the group as a whole, expression of both GPR40 (r = -0.74, P < 0.001) and GPR120 (r = -0.53, P < 0.05) was inversely related to BMI (i.e. decreased as adiposity increased). Correspondingly, fewer cells were immunopositive for GPR40 and GPR120, and for CCK and GLP-1, in a subset of 2 obese compared to 2 lean subjects in early immunolabelling studies, while the density of GPR119-labelled cells was comparable.
Conclusion: This study provides the first evidence that the expression of FA receptor transcripts in the duodenum is inversely related to body mass index in healthy humans, and that the number of immunoreactive cells is reduced in obese, compared with lean humans. These changes, together with reduced numbers of duodenal cells equipped to release CCK or GLP-1 in obese humans, may underlie the attenuated GI hormone responses to fat, and the consequent increases in appetite and energy intake leading to obesity. Reference: (1) Little, Feinle-Bisset, Physiol Behav 104:613–20, 2011.

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Distinct effects of mechanical stretch on gene expression of PGE synthase and PGF synthase in lumen dilation
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Objective: Mechanical stretch in lumen dilation induces expression of cyclo-oxygenase-2 (COX-2) in gut smooth muscle cells (SMCs), which plays a critical role in motility dysfunction in obstruction. Prostaglandins PGE2 and PGF2α are products of a cascade of enzyme activities involving COX in common, and PGE synthase (PGES) and PGF synthase (PGFS), respectively. The aims of the study were to determine whether PGE2 and PGF2α were differentially mediated in bowel obstruction, and if so what are the molecular mechanisms.
Methods: Partial colon obstruction was induced with a silicon band implanted in the distal colon of rats. Static stretch (18% elongation) was mimicked *In vitro* in primary culture of rat colonic SMCs with a Flexercell System.
Results: Muscle bath experiments showed that colonic circular muscle contractility was inhibited by exogenous PGE2 (10^{-9}–10^{-6} mol L^{-1}), but enhanced by PGF2α (10^{-9}–10^{-6} mol L^{-1}) (n = 4). PGE2, but not PGF2α, was significantly increased in the colonic muscularis externae of the dilated segment oral to obstruction. The PGE2 level was 4577 ± 1249 pg mg^{-1} at day 3 of obstruction, compared to 1264 ± 259 in sham (P < 0.05). The expression of PGES mRNA was significantly up-regulated in obstruction on day 1 and day 3 (3.8 ± 1.1 and 1.8 ± 0.4 fold, respectively, P < 0.05), but not on day 7 (1.2 ± 0.5 fold). On the contrary, the expression of PGFS mRNA was down-regulated in obstruction to 0.6 ± 0.1, 0.7 ± 0.2, 0.5 ± 0.1-fold for 1, 3, and 7 days, respectively (n = 5, all P < 0.05). Direct stretch of rat colonic SMCs *In vitro* significantly up-regulated the PGES mRNA, but down-regulated that of PGFS (n = 5, P < 0.05). Inhibition of COX-2 activity with NS-398 (5 mg kg^{-1}, daily, n = 5) partially but significantly improved muscle contractility in obstruction. In the presence of NS-398, the increase of PGE2 level and PGES expression was blocked, but PGF2α level was significantly decreased with down-regulated PGFS mRNA.
Conclusion: Mechanical stretch up-regulates expression of PGES via activation of COX-2, but down-regulates PGFS expression directly. Distinct expression of PGES and PGFS leads to differential changes of PGE2 and PGF2α in lumen dilation, which contribute to the impaired motility function in obstruction.

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Mast cells are involved in post-inflammatory visceral hypersensitivity and exert their effects partially through histamine H1 receptors
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Objective: The location of mast cells in close vicinity to afferent nerves as well as the bidirectional communication between both, suggest mast cells play an important role in visceral hypersensitivity. Therefore we aimed to assess mast cell involvement by evaluating the effects of the mast cell stabilizer ketotifen and the histamine H1 receptor antagonist levocetirizine in our rat model of post-inflammatory visceral hypersensitivity.
Methods: Colitis was induced in male Sprague-Dawley rats by intrarectal administration of 15 mg trinitrobenzene sulphonic acid (TNBS) in 50% ethanol while controls received a saline instillation. Animals were monitored individually by colonoscopy: on day 3 to confirm the presence of colitis and from day 10 on every 4 days to monitor convalescence and specifically determine the time to complete colonic healing in each animal. Three days after endoscopic resolution of inflammation was achieved, visceral sensitivity was assessed by electromyographic (EMG) registration of the visceromotor responses (VMR) to colorectal distension (10–80 mmHg, 20 s, 4 min interval) and expressed as area under the curve of the corresponding EMG signal. VMR were recorded before and 30 min after intraperitoneal administration of ketotifen (25 mg kg^{-1}), levocetirizine (0.1–1 mg kg^{-1}) or vehicle (saline). Afterwards, colonic tissue was assessed by endoscopy, macroscopy, histology and myeloperoxidase activity (MPO) for remaining signs of inflammation.
Results: TNBS instillation resulted in mild colitis which resolved spontaneously after 18 days (range 10–26 days). In the post-inflammatory phase, VMR were significantly increased compared to controls (1199 ± 153 vs 2375 ± 207 μV, P < 0.001; n = 7/group). Post-inflammatory visceral hypersensitivity remained unaltered by administration of vehicle, but was significantly attenuated by ketotifen and the higher dose of levocetirizine (Table 1). In all animals, colonoscopic healing of inflammation was confirmed post-mortem and macroscopic and microscopic scores as well as MPO activity were comparable to those in control rats.
Conclusion: Heightened VMR after complete resolution of mild colitis were attenuated by ketotifen and levocetirizine, suggesting that mast cells are involved in TNBS-induced post-inflammatory visceral hypersensitivity and exert their effect, at least partially, through histamine H1 receptors.

Table 1

	Vehicle	Ketotifen 25 mg/kg	Levocetirizine 0,1 mg/kg	Levocetirizine 1 mg/kg
Pre-drug VMR	2550 ± 472	2957 ± 392	2337 ± 195	2658 ± 230
Post-drug VMR	2704 ± 496	1979 ± 270*	2014 ± 371	2113 ± 264*

VMR are expressed as area under the curve of the corresponding EMG signal (μV) and shown as mean ± SEM for n=6-10/group. * p<0.05, significantly different from pre-drug VMR, repeated measures anova.

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Adenosine modulates its own release through activation of A2A receptors on myenteric cholinergic nerve terminals of the rat ileum
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Objective: In the myenteric plexus, adenosine (ADO) released per se via equilibrative nucleoside transporters (ENT) may occur in parallel to the formation of the nucleoside from ATP hydrolysis (via the ectonucleotidase cascade) during prolonged periods of nerve stimulation (Duarte-Araújo et al., 2004; Correia-de-Sá et al., 2006). Endogenous ADO contributes significantly to maintain cholinergic neurotransmission at the myenteric plexus through the activation of facilitatory A2A receptors, which are localized predominantly on cholinergic nerve terminals (Vieira et al., 2011). It remains, however, to be elucidated whether ADO plays a role regulating its own release in the rat myenteric plexus.
Methods: Electrical stimulation (5 Hz, 3000 pulses) of longitudinal muscle-myenteric plexus (LM-MP) preparations of the rat ileum increased the outflow of ATP (306 ± 68%, n = 8) and ADO (74 ± 6%, n = 6) above baseline detected by bioluminescence and HPLC, respectively.
Results: Extracellular ATP accumulation was sensitive to blockade of nerve action potentials and of smooth muscle contraction, respectively with tetrodotoxin (1 μ mol L^{-1}, 54 ± 18%, n = 4) and nifedipine (5 μ mol L^{-1}, 112 ± 47%, n = 4). The nucleoside transport inhibitor, dipyridamole (0.5 μ mol L^{-1}), inhibited extracellular ADO accumulation by 59 ± 9% (n = 4), while the ecto-5′-nucleotidase inhibitor, concanavalin A (0.1 mg ml^{-1}) was less effective (-27 ± 1%, n = 5). Conversely, inhibition of intracellular ADO kinase with 5′-iodotubericidin (10 μ mol L^{-1}) and ABT-702 (100 n mol L^{-1}) facilitated stimulation-induced ADO outflow by 51 ± 6% (n = 8) and 109 ± 15% (n = 3), respectively. Activation of A2A receptors with CGS 21680C (3 n mol L^{-1}) increased stimulation-induced ADO outflow by 163 ± 33% (n = 8). The facilitatory effect of CGS 21680C (3 n mol L^{-1}) was prevented by the selective A2A antagonist, ZM241385 (50 n mol L^{-1}) and by dipyridamole (0.5 μ mol L^{-1}), but not by concanavalin A (0.1 mg ml^{-1}). The adenylate cyclase activator, forskolin (3 μ mol L^{-1}, 160 ± 44%, n = 4), the cAMP analogue, 8-Br-cAMP (1 m mol L^{-1}, 209 ± 38, n = 4) and the specific PKA activator, Sp-cAMPS (10 μ mol L^{-1}, 127 ± 27%, n = 4) mimicked the facilitatory effect of CGS 21680C (3 n mol L^{-1}). The facilitatory effect of forskolin (3 μ mol L^{-1}) was also attenuated in the presence of dipyridamole (0.5 μ mol L^{-1}).
Conclusion: Data suggest that myenteric neurons are an important source of purines (ATP and ADO) and that ADO mediates a positive feedback mechanism regulating its own transport via the activation of A2A receptors coupled to the adenylate cyclase/cyclic AMP pathway. Work supported by FCT (FEDER funding, PTDC/CVT/74462/2006 and UMIB-215/94).

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The CB1-selective cannabinoid antagonist AM251 partially prevents gastrointestinal dysmotility induced by the antitumoral drug vincristine in the rat

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Objective: 1- To characterise the effect of the antitumoral drug vincristine on the rat gastrointestinal (GI) motor function by radiographic means. 2- To determine whether the alterations induced by vincristine might be prevented by a CB1-selective cannabinoid antagonist.
Methods: Male Wistar rats received an intraperitoneal injection of vincristine (VC;0.1–0.5 mg kg^{-1}), and barium sulphate (2.5 ml; 2 g ml^{-1}) was intragastrically administered immediately, 24 or 48 h later. Serial X-rays were obtained 0, 1, 2, 4, 6 and 8 h after contrast and analysed as previously described (Cabezos et al, 2008). Second, AM251, a selective CB1 receptor antagonist was administered once (20 min before VC), twice (before and 24 h after VC), or 3 times (before, 12 and 24 h after VC); in these experiments, barium was administered 24 h after VC (0.5 mg kg^{-1}) and X-rays were obtained afterwards, as above.
Results: VC dose-dependently reduced GI motor function. This was particularly evident 24 h after administration. AM251 partially and significantly prevented VC effect when administered at least twice.
Conclusion: The fact that AM251 is capable of reducing the effect of VC suggests that, like in other experimental models of paralytic ileus, endocannabinoids are released and at least partially responsible for the alterations induced by the antitumoral drug on GI motor function. These results might have therapeutic implications.

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This abstract has been withdrawn.

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Endocrine and smooth muscle responses of the bitter agonist, denatonium benzoate, in the stomach

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Objective: The presence of bitter taste receptors, T2R, and the associated gustatory G-protein, alpha-gustducin, in gut endocrine cells similar to those on the tongue suggests that these receptors may function as nutrient sensors to regulate gastrointestinal responses to ingested food. *in vivo* studies in our lab showed that bitter agonists affect the release of ghrelin with functional effects on food intake and gastric emptying (PNAS 108: 2094, 2011). We want to elucidate the mechanisms by which the bitter tastant, denatonium benzoate (DB), influences ghrelin release and smooth muscle contractility.
Methods: Ghrelinoma cells, MGN3-1, were stimulated with DB for 3 h and the release of ghrelin in the cell culture medium was measured by radioimmunoassay. The effect on the release of $Ca2^+$ was measured by single cell $Ca2^+$-fluorescence imaging. *In vitro* contractility effects of DB were measured isometrically in fundic smooth muscle strips of wildtype (WT) and alpha-gustducin knockout (gust-/-) mice.
Results: Stimulating ghrelinoma cells with DB resulted in a dose-dependent release of octanoyl ghrelin (EC50: 84 μ mol L^{-1}), with a maximal increase (73 ± 18%) at 500 μ mol L^{-1}. This was accompanied by an increase in intracellular $Ca2^+$. The presence of the bitter taste receptor for DB, T2R108, was confirmed using RT-PCR. In fundic smooth muscle strips DB at 10^{-4} mol L^{-1} caused a TTX-insensitive contraction (11 ± 2% of ACh), followed by a relaxation (-14 ± 2%). The contraction was enhanced (38 ± 5% of ACh) by the $Ca2^+$ activated K^+-channel blocker, charybdotoxin, and blocked by thapsigargin, which depletes intracellular $Ca2^+$ stores, and by the L-type calcium channel blocker nifedipine. In gust-/- mice, the contraction to DB in the presence of charybdotoxin was reduced to 17 ± 3%. The following relaxation was unchanged.
Conclusion: The bitter agonist, DB, stimulates the release of ghrelin from endocrine cells by increasing intracellular $Ca2^+$. In fundic smooth muscle strips, DB activates a gustatory G-protein, alpha-gustducin, to induce the release of $Ca2^+$ from intracellular stores which induces capacitive $Ca2^+$ entry via a L-type $Ca2^+$ channel. The resulting contraction is masked by a relaxation mediated via activation of $Ca2^+$-activated K^+ channels. The relaxation at higher concentrations is mediated via another mechanism. Our studies indicate a functional role for bitter agonists in the gut both at the level of the endocrine and the smooth muscle cells.

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Selective gene expression by rat small and large bowel epithelia

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Objective: The gastrointestinal (GI) tract is separated into distinct anatomical as well as functional domains. We have previously described the transcriptome of purified epithelial cell suspensions from the gastric corpus mucosa by mRNA microarray analysis resulting in a distinct gene expression map of this region that is the most selective in the GI tract. Here we report the selective rat gene expression maps of the upper and lower small bowel as well as the colon.
Methods: Microarray hybridization of mucosal epithelial cells from the gastric corpus, the upper and lower small bowel and colon was used to evaluate gene expression of the small bowel and colon and identify genes that are individually expressed by these segments but not others.
Results: These data show not only expected distribution of known functions such as digestion and absorption but unexpected regulation of the immune system and fatty acid handling. Thus, the duodenum and jejunum are substantially enriched in messages for genes encoding proteins involved in digestion, fatty acid uptake and unique protection factors such as foveolin. The ileum selectively displays messages representing the distinct function of this segment and is most like the stomach expressing lipid uptake messages such as phospholipase B and fatty acid binding protein-6 and innate immune response genes such as matrilysin and defensins and ligands such as apelin and also the cholecystokinin-2-receptor. The colonic mucosa shows a number of specifically enriched genes, such as those coding for proteins such as carbonic anhydrase 1, lactoperoxidase and pentraxin and several Hox proteins.
Conclusion: This differential map of the bowel epithelium transcriptome will enable identification of previously unsuspected functions in successive regions of the intestine.

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Relationship between purinergic neurotransmission through P2Y1 receptors and the contractile effect of Orphanin FQ

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Objective: Although it has been described that Orphanin FQ (OFQ) stimulates colonic motor activity, little is known about its mechanism of action. *In vitro* studies suggest that the stimulatory effect of OFQ is due to the inhibition exerted by this neuropeptide on purinergic neurons. Our aim was to investigate the mechanisms involved in the effect of OFQ in the rat colon *in vivo*.
Methods: Motility recordings were obtained in anaesthetized Sprague-Dawley rats by means of a strain-gauge transducer sutured to the serosal surface of the proximal colon. Moreover, an electrode was placed in the lumen 1 cm distally to the strain-gauge to evaluate the response to the electrical mucosal stimulation (EMS). The response to EMS (30 V, 0.6 ms, 4 Hz) was recorded in the absence and presence of OFQ, UFP101 (a selective OFQ antagonist) and MRS2500 (a selective P2Y1 receptor antagonist). Response to intravenous administration of OFQ was evaluated in the absence and presence of UFP101 and MRS2500. Motor response was quantified measuring the area under the curve delimited by the tracing and results were analyzed using a paired-*t*-test.
Results: MRS2500 decreased EMS response (121 ± 30 g sec^{-1} vs 75 ± 23 g sec^{-1}, $P < 0.05$). As MRS2500 blocks P2Y1 receptors, and hence inhibits the relaxation induced by ATP released from purinergic neurons, this result suggests that the off-contraction that usually follows smooth muscle relaxation contributes to the contraction observed after EMS. UFP101 did not modify EMS response (85 ± 16 g sec^{-1} vs 103 ± 33 g sec^{-1}, $P > 0.1$) whereas, similarly to MRS2500, OFQ reduced it (110 ± 24 g sec^{-1} vs 67 ± 17 g sec^{-1}, $P < 0.05$), suggesting that OFQ inhibits ATP release from purinergic neurons. On the other hand, UFP101 reduced the motor activity increase induced by OFQ administration (340 ± 12 g sec^{-1} vs 99 ± 20 g sec^{-1}, $P < 0.01$). MRS2500 diminished, but not eliminated, the response to OFQ (534 ± 80 g sec^{-1} vs 294 ± 54 g sec^{-1}, $P < 0.05$), suggesting that other mechanisms besides the inhibition of ATP release are involved in its contractile effect.
Conclusion: The increase of colonic motor activity induced by OFQ is partly mediated by its inhibitory effect on purinergic neurons, preventing the relaxant effect of ATP interacting with P2Y1 receptors located on smooth muscle cells.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-28 Basic and Translational Session: Smooth Muscle and ICC in Health and Disease

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Voltage-gated sodium selective ion channel NaV1.5 mechanosensitivity is modulated by local anesthetics

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Objective: The voltage-gated sodium-selective ion channel NaV1.5 is found in circular smooth muscle cells and interstitial cells of Cajal of the human gastrointestinal tract. NaV1.5 is encoded by SCN5A. Mutations in SCN5A are associated with gastrointestinal motor and functional disorders. NaV1.5 is mechanosensitive – mechanical stimulation increases peak currents and hyperpolarizes half-points of activation and inactivation. A NaV1.5 mutation from a patient with irritable bowel syndrome resulted in loss of current and disrupted mechanosensitivity. The molecular mechanisms and pharmacology of NaV1.5 mechanosensitivity are poorly understood. Local anesthetics are known modulators of Na^+ channels and their effect on mechanosensitivity was the focus of this study.
Methods: Nav1.5 (hH1c3) was transiently expressed in HEK-293 cells. Mechanosensitivity was studied in voltage- and pressure-clamped membrane patches. Pressure was applied to patches at -30 mmHg. NaV1.5 mechanosensitivity was assessed by the shift of half-points of Boltzmann fits, ΔV=Vhalf, test – Vhalf, control. Lidocaine and QX-314 were used to modulate NaV1.5 mechanosensitivity.
Results: At resting tension, addition of lidocaine to the bath solution decreased peak Na^+ current (1 Hz depolarization to 0 mV) by 41 ± 22% (n = 4, P < 0.05) with an exponential time constant of 19.4 ± 12 sec (n = 4). Lidocaine did not affect voltage dependence of activation at rest (ΔV = 0.5 ± 6.3 mV, n = 4, P > 0.05). In the absence of lidocaine, 20 ms pressure steps to -30 mmHg resulted in ΔV of -9.2 ± 1.8 mV (n = 6, P < 0.05 compared to 0 mmHg). In the presence of 50 μ mol L^{-1} lidocaine, patch pressure resulted in a much smaller ΔV of -4.8 ± 3.1 (n = 5, P < 0.05). At physiologic pH lidocaine exists in charged and neutral forms, and neutral lidocaine partitions into the bilayer. The permanently charged lidocaine homolog QX-314 is membrane impermeable. QX-314 was used outside and inside to determine the need for bilayer partitioning for block of NaV1.5 mechanosensitivity. NaV1.5 mechanosensitivity was not affected by QX-314; ΔV was -12.0 ± 2.9 mV (n = 3) and -8.6 ± 3.3 mV (n = 4) for 500 μ mol L^{-1} outside and 50 μ mol L^{-1} inside (P > 0.05).
Conclusion: NaV1.5 mechanosensitivity is inhibited differentially by local anesthetics. Lidocaine but not QX-314 inhibits Nav1.5 mechanosensitivity, suggesting that membrane partitioning may be necessary for the effect of local anesthetics on mechanosensitivity.
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Restoring the density of interstitial cells of Cajal of the jejunum from diabetic rats after supplementation with quercetin

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Objective: Abnormalities in both Interstitial Cells of Cajal (ICC) number and function are associated with motility disorders. Oxidative stress has been implicated in diabetes-induced changes in ICC. Quercetin is an antioxidant that reduces diabetes-induced injury to enteric neurons. The aim of this work was to investigate the effect of quercetin on ICC in the jejunum of diabetic rats.
Methods: Thirty-two male Wistar rats, 90 days old, were divided into the following groups: normoglycemics (C), normoglycemics supplemented with quercetin (Q; 200 mg Kg^{-1}), diabetics (D), and quercetin-treated diabetics (DQ). Diabetes was induced in groups D and DQ by streptozotocin (i.v.; 35 mg kg^{-1}). After 120 days, the mean intensity of Ano1-immunoreactivity in 30 micrographs of whole mount preparations and the mean number of ICC ($Ano1^+/DAPI^+$) in 30 micrographs of thin sections were analyzed.
Results: Diabetes increased the intensity of Ano1 immunoreactivity and decreased the density of jejunal ICC-MY (P < 0.0001) when compared to group C. Conversely, the immunoreactivity and density of ICC in the deep muscular plexus (ICC-DMP) were similar in groups C and D (P > 0.05). Quercetin (group Q) did not change the intensity or density of ICC-MY with respect to group C (P > 0.05), or the density of ICC-DMP (P > 0.05), but the intensity of ICC-DMP was 27.8% higher in group Q than group C (P < 0.0001). In the DQ group staining intensity was 25.7% (ICC-MY) and 21.5% (ICC-DMP) higher compared with group D (P < 0.0001). The density of ICC-MY in the group DQ was 37.9% more than D (P < 0.01) but similar between groups for ICC-DMP.
Conclusion: Diabetes decreases the density of jejunal ICC-MY, and supplementation with quercetin significantly increases these measures supporting the concept that antioxidants protect ICC-MY during diabetes. Interestingly, ICC-DMP were not affected by diabetes, and yet quercetin enhanced Ano1 immunoreactivity in normoglycemics compared to controls, suggesting that antioxidants may promote ICC-DMP in the absence of disease.
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IL-10 reverses delayed gastric emptying, slow wave abnormalities and smooth muscle membrane potential gradient changes in diabetic NOD/ShiLtJ mice

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Objective: In non-obese diabetic (NOD/ShiLtJ) mice, development of delayed gastric emptying (GE) is associated with a loss of diabetes-induced up-regulated heme oxygenase-1 (HO1) in M2 macrophages, resulting in increased oxidative stress, loss of interstitial cells of Cajal (ICC) and abnormalities in slow wave frequency and regularity. IL-10 induces HO1 *in vivo* and therefore may be an option for the pharmacological treatment of gastroparesis. The aim of the study was to determine if IL-10 can reverse delayed GE, abnormal slow waves and smooth muscle resting membrane potential (RMP) gradient changes in diabetic NOD/ShiLtJ mice.
Methods: Mice were included in this study if they developed delayed GE (T½ >118 min) within 10 weeks of the start of hyperglycemia (glucose>250 mg dl^{-1}). GE was measured every week. Mice with delayed GE were treated with either vehicle (n = 4) or IL-10 (1 μg ip twice daily, n = 4). Smooth muscle membrane potential and electrical slow waves were recorded from the circular muscle layer of the stomach at 12 regions distributed evenly from the proximal body to distal antrum.
Results: Prior to treatment, the mean T½ value was180 ± 19 min (n = 8, all delayed). While GE remained delayed in mice treated with vehicle (T1/2 = 157 ± 11 min, n = 4 mice) GE returned to normal after 3.1 ± 0.9 weeks in IL-10-treated mice (T1/2 = 106 ± 4 min, n = 4). Peak amplitude (PA) and frequency of the slow wave were not different between vehicle- and IL-10-treated mice in the proximal body. In contrast, in the 3 sites recorded from in the distal antrum of IL-10-treated mice (see table), the slow waves frequencies were higher than vehicle controls (P < 0.05 indicated by *). Slow wave amplitude variability was assessed as variance in PA/amplitude (VPA), and showed significant difference between vehicle and IL-10 (table). The difference in RMP between the proximal body and distal antrum was greater in IL-10-treated mice compared to vehicle controls (–11.6 ± 3.2 mV vs -9.5 ± 3.3 mV).
Conclusion: These data suggest that the treatment of delayed GE with IL-10 normalizes delayed GE, slow wave abnormalities and smooth muscle membrane potential gradients.
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Distal Antrum	Posterior		Greater Curvature		Anterior	
Region	10		11		12	
Treatment	Veh	IL-10	Veh	IL-10	Veh	IL-10
Variance of PA (mV)	0.33±0.09	0.49±0.18	3.85±0.71	0.58±0.41*	0.27±0.06	0.29±0.17
RMP (mV)	-60.7±4	-63.3±6.8	-68.6±3.8	-64.9±2.6	-64.3±4.4	-57.9±2.2
Frequency (Hz)	0.03±0.01	0.06±0.02	0.04±0.01	0.07±0.02*	0.03±0.01	0.07±0.01*

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Intracellular Ca2+ release from Endoplasmic Reticulum (ER) regulates slow wave currents and pacemaker activity of interstitial cells of Cajal

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Objective: Interstitial cells of Cajal (ICC) generate electrical pacemaker activity in gastrointestinal smooth muscles. We sought to better understand the mechanism(s) controlling activation of spontaneous pacemaker currents in freshly dispersed ICC from murine small intestine.
Methods: We used patch clamp techniques to study spontaneous inward currents (STICs) in ICC isolated from transgenic mice with constitutive expression of copepod super green fluorescent protein (copGFP).
Results: Large amplitude STICs were generated spontaneously by ICC under voltage clamp. STICs corresponded to and are likely to be responsible for spontaneous transient depolarizations (STDs) recorded under current clamp. Previous studies have demonstrated that slow wave currents and STICs are due to activation of Ca 2+-activated Cl- channels (CaCC) encoded by Tmem16a in ICC. Periodic activation of CaCC explains the spontaneous electrical rhythmicity in these cells. Ryanodine, an inhibitor of caffeine-sensitive Ca2+ channels inhibited slow wave currents and the amplitude and frequency of STICs. Ryanodine also depolarized resting membrane potentials of ICC. After restoring depolarization caused by ryanodine by injection of current, STDs recovered and were blocked by 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), a CaCC blocker. The IP3 receptor inhibitor, 2-Aminoethoxydiphenyl borate (2-APB) also caused depolarization, decreased slow wave current, and reduced amplitude and frequency of STICs. Cyclocpiazonic acid (CPA) and thapsigargin, Ca2+-ATPase inhibitors in ICC. also decreased slow wave currents and STICs.
Conclusion: Our data suggest that Intracellular Ca2+ release from endoplasmic reticulum (ER) activates CaCC in ICC that is responsible for STICs and slow wave currents. It is possible that coupling between Ca2+ release channels (ryanodine and IP3 receptors) may be necessary to produce full amplitude slow wave currents and to maintain slow wave frequencies in GI muscles.

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Inhibitory neurotransmission to circular smooth muscle in Crohn's ileitis

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Objective: Altered motility is believed to play a role in symptom generation in Inflammatory Bowel Disease (IBD) and abnormal neuromuscular function has been described in animal models of IBD. The current study assessed neuromuscular transmission to ileal circular smooth muscle (CSM) of patients with active Crohn's disease.
Methods: Fresh resected ileal tissue was obtained from patients with Crohn's disease as well as those undergoing right hemicolectomy for colon cancer. A 1 × 1 cm full thickness piece was excised, placed in oxygenated Kreb's solution and transported to the laboratory where the mucosa and submucosa were removed by sharp dissection, thereby exposing the underlying CSM. Following overnight storage at 4 degrees C, standard intracellular recordings were performed.
Results: Four diseased and 4 control ileums were studied. One diseased case was excluded from analysis as subsequent histological assessment revealed that the specimen showed a bland stricture (mural fibrosis) only with no inflammation. The other 3 specimens displayed acute and chronic transmural inflammation involving the muscularis propria. Baseline recordings were characterized by nifedipine-sensitive slow waves with superimposed spike potentials. Single pulse electrical field stimulation induced a fast inhibitory junction potential (fIJP) only, whereas a 1 s stimulus train (20 Hz, 0.5 ms pulse) evoked a biphasic IJP consisting of an initial fIJP followed by a slow IJP. The former was abolished by application of the P2Y1 antagonist MRS-2500 (1 μ mol L^{-1}) and the latter by application of L-NAME (200 μ mol L^{-1}). There was no statistically significant difference between control and diseased CSM with respect to resting membrane potential (-58.3 ± 4.1 mV *vs* -60.8 ± 6.9 mV), slow wave frequency (7.0 ± 1.2 min^{-1} vs 7.0 ± 1.0 min^{-1}), slow wave amplitude (8.4 ± 2.3 mV vs 16.8 ± 7.9 mV) or the magnitude of the IJPs (Table: shows data obtained with 1 s train stimuli only).
Conclusion: This novel preliminary study suggests that purinergic and nitrergic neurotransmission is intact in ileal CSM from Crohn's patients with active transmural inflammation. Given that acute and chronic animal models of intestinal inflammation reveal marked disruption of enteric nervous system (ENS) structure and function, our results suggest the presence of considerable ENS plasticity and compensatory ability in chronic IBD.

		fIJP Ampl (mV)	fIJP Dur (ms)	slJP Ampl (mV)	slJP Dur (ms)
Normal n = 4	control	14.9±5.9	2176±682	7.6±1.9	6041±833
	MRS-2500	13.9±9.6	1340±437	9.9±2.6	4653±319
	L-NAME	0±0	0±0	0±0	0±0
Ileitis n = 3	control	15.7±6.2	1163±298	4.3±3.0	4427±1905
	MRS-2500	3.7±3.1	1848±1486	10.0±5.0	4963±2828
	L-NAME	1.5±1.2	567±429	0±0	0±0

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Development of a biophysically based model of the anoctamin 1 calcium-activated chloride channel

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Objective: Anoctamin 1 (ANO1) is a calcium-activated chloride channel expressed in interstitial cells of Cajal (ICC) in mice and humans. ANO1 is activated synergistically by increased intracellular calcium concentration ([Ca2+]) and membrane depolarisation. ANO1 has been shown to be essential for ICC slow wave activity, but the specific role of ANO1 in generating slow waves is unclear. Ion channel electrical activity can be reproduced in biophysically-based mathematical models, enabling in silico investigation of ion channel behaviour in physiological conditions. This study aims to use mathematical modelling to predict how ANO1 may contribute to slow wave generation.
Methods: Patch clamp data from mouse ANO1 expressed in HEK 293 cells [1] were used to develop a novel Hodgkin-Huxley type model of coupled voltage- and calcium-dependent ANO1 activation. ANO1 activity in ICC was simulated using [Ca2+] predicted by the Means & Sneyd ICC model [2].
Results: The ANO1 model produced results comparable to published data. At 100 n mol L^{-1} resting [Ca2+] slow wave depolarisations did not activate ANO1 channels. At a typical ICC resting membrane potential (RMP) of -65 mV, steady-state ANO1 activation was half-maximal when [Ca2+] was 6.1 μ mol L^{-1}. At a peak potential of -10 mV, steady-state ANO1 activation was half-maximal when [Ca2+] was 2.2 μ mol L^{-1}. Preliminary simulations of ANO1 activation kinetics suggested the duration of [Ca2+] transients is also important. Localised [Ca2+] transients up to 7.3 μ mol L^{-1} activated ANO1 up to 17% at -65 mV RMP, with the highest levels of activation observed when [Ca2+] was maintained at micromolar concentrations for at least 50 ms.
Conclusion: In order for ANO1 channels to open at physiological membrane potentials in ICC they may need to be exposed to prolonged micromolar level [Ca2+] transients. ANO1 channels cannot be activated by slow wave depolarisations alone, but depolarisation can further increase ANO1 activation during [Ca2+] transients. To develop a better understanding of the role of ANO1 in ICC, this novel ANO1 model will be incorporated into a full ICC cell model to investigate how ANO1 interacts with the dynamic cellular system.
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Functional roles of interstitial cells of Cajal in aganglionic / hypoganglionic intestines

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Objective: Aganglionic / hypoganglionic segments of intestine in Hirschsprung's disease / hypoganglionosis do not show normal peristalses, resulting severe transit disturbances. However, we experienced these intestines can show ''to and fro'' pattern movements, possibly generated by interstitial cells of Cajal (ICC). Moreover, these ''abnormal'' intestines can work functionally after appropriate surgeries. An objective of this paper is to evaluate functional roles of ICC-derived gastrointestinal motility in aganglionic / hypogagnlionic intestines.
Methods: Subjects are a case of total intestinal aganglionosis and a case of hypogagnlionosis. Gastrointestinal motility was examined by a contrast study and an endoluminal manometry. The histological analyses were performed by hematoxyline-eosin staining for gangalion cells and anti-c-Kit immunohistochemical staining for ICC, respectively.
Results: Case 1 is an 1-year-old boy with total intestinal aganglionosis. Multi-points biopsies showed lack of ganglion cells in the jejunum 10cm from a ligament of Treitz and more distal points. A jejunostomy at 50cm from a ligament of Treitz has been working and a patient could eat meals. A contrast study showed ''to and fro'' pattern movements well. An endoluminal manometry revealed rhythmic contractions with various frequencies (4–19 times/min.) depending on locations of the intestines. Histological exam showed no ganglion cells in a resected small bowel, but apparent c-Kit immunopositivity between two muscle layers. Case 2 is a 15-year-old-boy with

hypoganglionosis who underwent small bowel transplantation. An ileal graft was anastomosed with native jejunum 25 cm from a ligament of Treitz. The patient could eat meals as much as he liked and no transit disturbance was seen. The movements of native jejunum seemed similar to that in case 1, showing ''to and fro'' pattern. Histological exam showed immature ganglion cells seldom but many c-Kit positive cells like in case 1.
Conclusion: The ''to and fro'' pattern movements were observed in aganglionic / hypogangionic intestines. It was interesting that even agangionic intestine contracted well and functionally transported endoluminal contents. Frequency of rhythmic contractions became fewer in distal location from the jejunum to the rectum. Although detailed underlying mechanisms should be addressed, rhythmic contractions and apparent expression of ICC suggested that functional transit in the aganglionic / hypoganglionic intestines likely owed to ICC-derived gastrointestinal movements.

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Interstitial cells of Cajal and their association with enteric nerves in human gastro-duodenal junction
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Objective: Interstitial cells of Cajal associated with the myenteric plexus (ICC-MP) are pacemaker cells in the human stomach and small intestine. Pacemaker activity by itself does not produce contraction but interactions between pacemaker ICC and neuromuscular system result in organized motor patterns. Although intramuscular ICC (ICC-IM) have been shown to form synapse-like junctions with nerve varicosities, this has not been observed with pacemaker ICC-MP. The aim of the present study was to provide a morphological basis for innervation of human pacemaker ICC.
Methods: Twenty surgical gastro-duodenal specimens were obtained from adult patients with pancreatic cancer. ICC distribution in distal antrum, pylorus, proximal duodenum and their relationship with enteric nerves were determined by immunohistochemistry and electron microscopy.
Results: There were more nerve cell bodies within the myenteric ganglia of human antrum and duodenum than pylorus. Myenteric ganglia had a sinuous contour, with different depth invaginations of the surrounding connective tissue capsule. On the section profile, they were displayed as multiple intersecting connective septa within the ganglia. ICC-MP were found either around ganglia or within the deep invaginations of the connective septa. They formed synapse-like connections with nerve varicosities within the myenteric ganglia and along nerve strands outside the ganglia. Direct contacts were detected between ICC within the connective septa of the ganglion and between ICC partially protruding into the ganglion and ICC outside the ganglia.
Conclusion: ICC-MP are embedded in the intersecting connective tissue septa of the myenteric ganglia creating a continuous dense network around the sinuous myenteric ganglia in human gastrointestinal tract. ICC more strictly border the ganglia than other interstitial cells, so there are more ICC located in deep invaginations of the ganglia thus having more chance to form direct contacts with enteric nerves. The connective tissue sheet around the ganglia is not continuous, so ICC can penetrate and form synapse like contacts with nerve varicosities. ICC-MP are directly innervated by myenteric nerves.

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Colonic interstitial cells of Cajal associated with the myenteric plexus harbour ion channels regulated by excitatory neurotransmitters
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Objective: Little is known about the colonic population of interstitial cells of Cajal (ICC) associated with the myenteric plexus (ICC-MP). Mounting evidence implicates ICC-MP as an inducible pacemaker responsible for generating slow waves associated with luminal content propulsion. This pacemaker is not constitutively active but may require activation by excitatory neurons. Slow wave generation requires ion channels which depolarize the ICC, but the identities and properties of these channels remain unknown. The objective of this study was to characterize ion channels involved in ICC-MP pacemaker response to excitatory neurotransmitters.
Methods: We recorded currents from colonic ICC-MP using patch clamp techniques with -80 to +80 mV voltage ramps. Cells were from primary cultures or exposed in situ by peeling longitudinal smooth muscle.
Results: Two different currents were observed. The first was inward between -20 to +20 mV and outward above +20 mV in physiological bath solution and KCl pipette solution (n = 22). It was insensitive to 5 m mol L^{-1} TEA (n = 4), but turned off after patch excision into the inside-out configuration (n = 11). It was similar to transient outward potassium currents from small intestinal ICC-MP. The second current was sporadically active and inwardly rectifying (n = 22). It was ''flickery'' and of high magnitude, ~200 pA at -80 mV. It was insensitive to 5 m mol L^{-1} TEA (n = 5) and did not inactivate upon patch excision. An outwardly rectifying current, similar to those from small intestinal ICC-MP, was also recorded from inside-out patches of colonic ICC-MP. Preliminary data suggests that 1 μ mol L^{-1} carbachol may be able to activate or increase activity of cell-attached outwardly rectifying currents.
Conclusion: Colonic ICC-MP harbour several channels that require further characterization. Two currents were active without pharmacological stimulation. One small conductance K+ channel may be involved in setting ICC-MP resting membrane potential. The other large conductance channel, possibly conducting chloride, may be modulated by muscarinic stimulation.
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'Real-time' high-resolution slow wave mapping using a novel analysis platform
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Objective: High-resolution (HR) mapping provides spatially detailed data on gastrointestinal slow wave propagation. Recent translational studies have revealed new patterns of normal and abnormal human gastric slow wave activation. An important limitation of HR mapping is that all analyses must currently be performed 'off-line', after recordings are completed. This delay to viewing data can compromise recording quality, limits opportunities for experimental interventions, and is a barrier to clinical translation. We developed a novel analysis platform to enable HR mapping in 'real time'.
Methods: Multi-electrode recordings were taken from the gastric serosa of six pigs in-vivo. Raw data was acquired using a commercial LabView acquisition package (BioSemi), and streamed to a novel software client via a 'socket'. The new software was coded in Python. Data was down-sampled (30 Hz) and filtered. Slow wave activation times (ATs) were identified and grouped into cycles using customized automated algorithms. A kurtosis estimate was introduced to identify channels not recording useful data (e.g. due to poor contact). A graphical user interface animates propagation patterns, displays isochronal maps, and plots selected electrograms. Validation was by comparison against existing off-line and manual analysis methods.
Results: Running on a standard laptop (Dell M1450), the novel analysis platform successfully mapped 256-channel data on-line, in near real-time (animation display delay approx. 18s). The software ran without system failures, on the acquisition computer or via network streaming. With kurtosis-estimation of bad channels, validation metrics demonstrated accurate performance against benchmarks: AT marking sensitivity = 0.92; specificity = 1.0; positive predictive value = 0.93 (range 0.86–0.96). 'On-line' animations and isochronal maps enabled the accurate identification of dysrhythmic initiation and conduction patterns, including conduction block, ectopic pacemaking, and colliding wavefronts.
Conclusion: This novel software platform enables automated and accurate HR mapping in 'real-time'. This software is an important advance because it allows on-line monitoring of recording quality, enables targeted experimental interventions (such as to dysrhythmia onset), and advances the clinical utility of HR mapping. The software will be available to academics without cost.

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A virtual model of human gastric slow wave dysrhythmias
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Objective: In the stomach, gastric slow waves set the pattern and the frequency of motility. The recent translation of high-resolution (HR) mapping has provided detailed information regarding the human gastric slow wave activity. The opportunity now exists to apply mathematical modeling techniques to analyze the effects of dysrhythmic gastric slow waves, in terms of visualizing the dynamic interactions between the dysrhythmic and normal activities, and testing the effects of the dysrhythmic activity on the electrical dipole, which is important for interpretation of electrogastrogram (EGG).
Methods: Recording: HR mapping was performed on 12 patients with diabetic or idiopathic gastroparesis, using flexible electrode arrays (256 electrodes; 36 cm^2 coverage). Three classes of dysrhythmias were identified for modeling: (i) an ectopic pacemaker in the antrum; (ii) slow conduction in the corpus; (iii) a partial conduction block in the corpus. Modeling: An anatomical model of the stomach was constructed. A

biophysical cell model was used to simulate slow wave activity. A validated whole-organ model was used to simulate normal gastric slow wave propagations in the human stomach. Every class of dysrhythmias was simulated over 120 s by perturbing the activation timing parameters in the region of the stomach that contained the dysrhythmias, based on the HR mapping results.
Results: The simulations demonstrated slow wave interactions between the normal slow waves (dipole: 467 mA mm^{-2}) and dysrhythmias: (i) For the ectopic pacemaker, the slow waves entrained by the ectopic pacemaker formed a retrograde propagation (dipole: 888 mA mm^{-2}); (ii) For the slow conduction, four waves stagnated in the corpus where only two waves existed in the normal case (dipole: 469 mA mm^{-2}); (iii) For the partial conduction block, slow waves propagated rapidly in the circumferential direction distal to the block site and quickly merged into a single wavefront in the corpus (dipole: 453 mA mm^{-2}).
Conclusion: This study used HR mapping and modeling to demonstrate how localized dysrhythmias may impart profound changes to gastric slow wave propagation, depending on their locality and form. This study promotes the hypothesis that gastric electrical dysrhythmias may generate specific electrical dipole profile that can be related to unique changes in the signal morphology of EGG.

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Slow wave conduction disturbances proximal and distal to ileal end-to-end anastomosis following ileocystoplasty

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Objective: End-to-end intestinal anastomosis is routinely performed in the course of managing several intestinal pathological conditions but there is no information regarding possible short- or long-term effects of such a procedure on the conduction of the electrical impulses in the vicinity of the anastomosis except for the fact that the slow wave will no longer propagate across the anastomosis. In the course of an ileocystoplasty project, we accidently discovered the presence of major conduction abnormalities of the slow wave in the region of the anastomosis following the surgery.
Methods: In a rat model (n = 12) of ileocystoplasty, an approximately 1 cm segment of terminal ileum was isolated (6–10 cm proximal to the cecum) and anastomosed to the bladder. The intestinal continuity was restored by an ileal end-to-end anastomosis and the animals were allowed to recover. After 1 day, 1 week or 1 month, the rats were again anaesthetized and the intestinal segment containing the anastomosis was isolated and positioned in a tissue bath where it was perfused with Tyrode. A 121-electrode array (11 × 11; 1 mm inter-electrode distance) was positioned at several locations proximal and distal to the anastomosis and recordings were performed from all 121 electrodes simultaneously. After the experiments, the signals were analyzed and propagation maps of the slow wave constructed.
Results: One day post-operatively, there was no slow wave propagation in the peri-anastomotic area (>5 cm proximal and distal to the anatomosis). After 1 week, the quiescent area was reduced, especially proximal to the anastomosis and had disappeared after 1 month. In the absence of the slow waves, multiple spikes were often seen at higher frequencies than normal. The distal segment still showed conduction disturbances 1 month after surgery.
Conclusion: End-to-end ileal anastomosis is followed by a relatively long period of absence of slow waves and the appearance of other electrical impulse propagation abnormalities which might explain the post-operative local paralytic ileus and the potential pseudo-obstruction related to it.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-29 Clinical Session: Esophagus: Clinical

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Kinetics of transient hiatus hernia during transient lower esophageal sphincter relaxations and swallows in healthy volunteers

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Objective: Proximal displacement of the gastro-oesophageal junction (GOJ) is present in hiatus hernia but also occurs transiently during transient lower oesophageal sphincter relaxations (TLOSRs) and swallows. Using a novel magnetic-based technique we have performed detailed examination of the GOJ movement during TLOSRs and swallows in healthy volunteers.
Methods: In twelve subjects, a magnet (2 × 1 mm) was endoscopically clipped to the GOJ and combined assembly of Hall-Effect locator probe and 36 channel high-resolution manometer (Sierra Scientific Inc., US) passed nasally. After a test meal the subjects were studied for 90 min.
Results: The median amplitude of proximal movement of the GOJ during TLOSRs was 4.3 cm (1.6–8.8 cm) and this was substantially greater than during swallowing at 1.2 cm (0.4–2.7 cm), P = 0.002. The median duration of GOJ migration during TLOSRs was 23.6 s (11.3–41.6 s) and this was greater than during swallowing at 6.9 s (2.7–14.3 s), P = 0.003. With both TLOSRs and swallows proximal GOJ movement coincided with LOS relaxation and return to its original position occurred 4s after return of LOS tone. Kinetic modeling of the movement of the GOJ during TLOSRs indicated 2 return phases with the initial return phase having the greater velocity (0.9 cm s^{-1}) and being strongly correlated with amplitude of proximal movement (r = 0.8, P < 0.001).
Conclusion: The marked proximal GOJ migration during TLOSRs represents very severe herniation of the GOJ. The rapid initial return of the GOJ following TLOSRs when the crural diaphragm is relaxed and its correlation with amplitude suggest it is due to elastic recoil of the phreno-oesophageal ligament. The marked stretching of the phreno-oesophageal ligament during TLOSRs may contribute to its weakening and development of established hiatus hernia.

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Postprandial changes in autonomic nervous system and frequency of transient lower oesophageal sphincter relaxation (TLOSR)

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Objective: Patients with gastroesophageal reflux disease show abnormalities in their autonomic nervous system (ANS), especially decreased cardiac vagal activity. Transient lower oesophageal sphincter relaxation (TLOSR) is a vagally-mediated reflex that occurs most frequently after a meal. Vagal activity, as measured by heart rate variability, decreases after a meal, and correlates with changes in gastric electrical activity (electrogastrography [EGG]). The ANS monitoring system Neuroscope provides beat-to-beat real time information on cardiac vagal activity. We aimed to characterize the time association between postprandial changes in cardiac vagal activity and the occurrence of TLOSRs.
Methods: Eight healthy volunteers (5 females, age 34.1 ± 3.7 years, body mass index 23.5 ± 1.1 kg m^{-2}) underwent simultaneous ANS (using Neuroscope), EGG, and lower oesophageal sphincter pressure (using high-resolution manometry) monitoring for 30 min in the fasting state, followed by a standard meal, and a further 4 h postprandially. Results are in Mean ± SEM.
Results: The number of TLOSRs increased after the meal, compared to baseline (0.6 ± 0.3), during the first (7.1 ± 1.1; P < 0.001), second (4.4 ± 0.7; P < 0.01), and third (3.3 ± 0.5; P < 0.05), hour. Cardiac Vagal Tone (CVT), a measure of efferent vagal activity, decreased after the meal, compared to baseline (10.3 ± 1.7), during the first (7.6 ± 1.4; P < 0.05) and second (7.0 ± 1.2; P < 0.01) hour. Cardiac Sensitivity to Baroreceptor reflex (CSB), a measure of afferent vagal activity, also showed a trend (P = 0.07) to decrease. Comparison between subjects showed that during the first postprandial hour there was a strong correlation between CVT and the number of TLOSRs (R2 = 0.56; P < 0.05). Heart rate increased (P < 0.0001), whilst mean arterial blood pressure decreased (P < 0.001), after the meal. There was also a decrease in the percentage of gastric arrhythmia (P < 0.05), and a trend for increase in the percentage of normogastria (P = 0.07) after the meal.
Conclusion: As expected, there was a dramatic increase in the number of TLOSRs after a meal. However, in contradiction to conventional wisdom, the highest number of TLOSRs occurred at a time of reduced cardiac vagal activity. On the other hand, during the first postprandial hour, those individuals with higher CVT also had higher numbers of TLOSRs. The relationship between cardiac vagal activity and TLOSR should be further explored in patients with reflux disease.

	Cortical damage (n 52)	Subcortical damage (n 25)	P
Tongue deficit	32	15	Ns
Soft palate deficit	7	4	Ns
Cheek deficit	2	2	Ns
Drop of bolus in cheek furrows	5	5	Ns
Repetitive swallowing	27	14	Ns
Delayed swallowing reflex	42	18	Ns
Diminished elevation of larynx/hyoid bone	7	5	Ns
Incomplete epiglottic tilt	9	6	Ns
Pharyngeal leakage	9	4	Ns
Retention of bolus in valleculae	16	9	Ns
Retention of bolus in pyriform sinuses	12	9	Ns
Retention of bolus on tongue	13	9	Ns
Penetration	8	9	Ns
Aspiration	16	5	Ns

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Swallowing alterations after Traumatic Brain Injury (TBI) without brainstem involvement

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Objective: After traumatic brain injuries (TBI) patients may present swallowing alterations that can be related to brainstem lesions. Little is known, instead, on the relationship between brain lesions not affecting the brainstem and swallowing disorders, if any. Aim of the present study was to evaluate the relationship between swallowing alterations and brain injury site(s), cortical or sub-cortical, in TBI patients with brain damage not involving the brainstem.
Methods: Seventy seven patients (62 M, age 26 years±8) with TBI (time from injury: 6–12 months) not affecting the brainstem as assessed at brain computerized tomography underwent a videofluoroscopic swallowing study (VFSS) with taperecording for slow motion analysis of the images. VFSS was performed in the antero-posterior and lateral upright position for the assessment of the oro-pharyngeal phase. During this study, videorecording of swallowing of a variety of boluses with different consistencies (semiliquid, semisolid of increasing volumes, liquid and solid) was performed for subsequent analysis. In each patient, we studied the presence of the following: motor alterations of tongue and soft palate, cheek deficit, drop of bolus in cheek furrows, repetitive swallowing, delayed swallowing reflex, diminished elevation of the larynx/hyoid bone, incomplete epiglottic tilt, pharyngeal leakage, retention of bolus in valleculae, pyriform sinuses, tongue, and penetration, aspiration in the airways.
Results: All patients presented one or more swallowing alterations that did not have any relationship with injury site(s). The table summarizes the results.
Conclusion: These observations demonstrate that traumatic brain injury causes a variety of swallowing alterations even in absence of brainstem lesions. Swallowing alterations are present regardless of cortical or sub-cortical localization of damage. This study suggests that a dynamic radiological swallowing study is indicated in every patient with TBI to detect possible alterations and it may be useful in the rehabilitation treatment.

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Esophageal high resolution manometry (HRM) in achalasia: Evaluation of the classification in a French multicentric cohort

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Objective: Achalasia is defined as impaired lower esophageal sphincter (LES) relaxation and absent peristalsis. Using HRM and the Chicago classification, achalasia is divided into 3 clinically relevant subtypes. The aim of this study was to apply this classification to a French cohort of achalasia and to compare clinical and manometric characteristics between the 3 subtypes.
Methods: Patients diagnosed with achalasia on HRM in 3 French university hospitals and without previous treatment were included. Symptoms (dysphagia, chest pain, regurgitation) were collected. HRM studies were retrospectively reviewed. Achalasia was classified as type 1 (no contractile activity, no pressurization), type 2 (no contractile activity, ≥20% of swallows with panesophageal pressurization) or type 3 (preserved fragments of distal peristalsis or premature contractions with ≥20% of swallows). End expiratory resting LES pressure, integrated relaxation pressure (IRP), and upper esophageal sphincter (UES) resting pressure were analyzed. Occurrences of double swallows and spontaneous UES relaxation were noticed. Data were expressed as mean ± sd and compared using Chi2 or Kruskall Wallis tests.
Results: From 2007 to August 2011, 169 patients (91 males; mean age 54 years, range 10–93) were diagnosed as achalasia. They were 24 type 1 achalasia (14%), 118 type 2 (70%) and 27 type 3 (16%). Type 3 patients were older than type 1 and 2 (62 years vs 52, $P = 0.03$). Ninety five percent of patients complained about dysphagia, 16% about chest pain (no difference between the 3 subtypes); 50% of type 1 patients presented regurgitations compared to 33% of type 2 and 22% of type 3 ($P = 0.10$). LES and UES pressures did not differ between the 3 groups. Double swallows were observed for 27 ± 29% of swallows and spontaneous UES relaxation for 13 ± 21%.
Conclusion: Our cohort was composed of a higher percentage of type 2 patients compared to the original cohort of Chicago (70% vs 54%). Patients with type 3 achalasia (spastic achalasia) were older than type 1 and 2 patients. This might suggest a different pathophysiology. Contrary to the original cohort we did not observe any clinical or sphincter pressure differences.

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Esophageal motility abnormalities in patients with laryngo-pharyngeal symptoms: Preliminary reports from a study conducted with high resolution manometry and oropharyngeal pH monitoring

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Objective: It is not known if patients with laryngopharyngeal symptoms (LPS) present specific esophageal motility abnormalities, especially in the upper esophagus. Aim of the study was to evaluate the relationship between pathological oropharyngeal pH-monitoring and esophageal motor abnormalities in patients with GERD-related laryngeal symptoms, assessed with high resolution manometry (HRM) and oropharyngeal pH-monitoring (Restech® probe).
Methods: Fifteen consecutive patients with chronic (> 6 months) LPS were prospectively enrolled from May to July 2011. A previous allergological and ear-nose-and-throat evaluation excluded other than GERD diagnosis. Reflux symptom index (RSI) score was recorded and only patients with a score >13 were considered eligible for the study. A solid state with 36 transducers HRM was performed before the pH probe positioning; lower esophageal sphincter (LES) pressure, integrated relaxation pressure 4 s (IRP-4s), upper esophageal sphincter pressure (UES), number of 5-ml wet swallows with a large transition zone (TZ) and motility characteristics (% peristaltic waves, distal contractile integral - DCI), of esophageal contraction were all recorded. Tracings were classified according to the Chicago Classification (Kahrilas PJ et al, J Clin Gastroenterol 2008). A≥2 cm defect in the 30 mmHg isobaric contour at the level of the TZ was considered abnormal and defined as large TZ. 24-h oropharyngeal pH-monitoring, as described by Ayazi S - J Gastrointest Surg 2009, was performed after having stopped antisecretive therapy for at least 14 days.
Results: Eight patients (53.3%) had a pathological oropharyngeal pH-monitoring study and 7 patients (46.7%) had a normal oropharyngeal acid exposure. These groups were comparable regarding to demographic aspects. Manometric findings in the two groups are presented in table 1, as well as the p-value. Values are presented as mean ± standard deviation. The only statistical significant manometric finding was the number of peristaltic contractions with a large

	Positive Restech® study	Negative Restech® study	p-value
LES pressure (mmHg)	35.1±20.6	27.3±5.1	0.31
IRP 4s	17.3±11.3	11.1±4.1	0.17
UES pressure (mmHg)	101±72.4	121±34.7	0.5
*DCI (cm*sec*mmHg)*	2361.3±1587.8	1739.8±1085.4	0.39
# peristaltic contractions with large TZ	38/67 (56.7%)	31/78 (40%)	**0.009**
# failed contraction	3	2	1

TZ, that resulted higher in patients with pathological oropharyngeal acid exposure.
Conclusion: Patients with LPS and a positive Restech® study showed a higher percentage of peristaltic contractions with a large TZ compared to patients with normal Restech® study.

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Assessment with the Functional Luminal Imaging Probe distinguishes oesophago-gastric junction distensibility in Barrett's oesophagus patients with hiatal hernias from healthy controls – A pilot study
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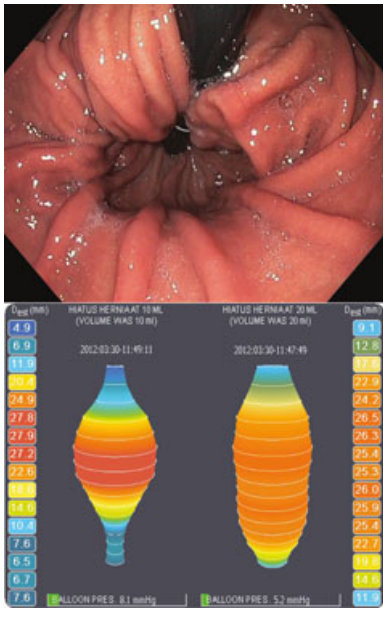
Objective: To use the Functional Luminal Imaging Probe (FLIP) to assess the distensibility of two hernia separated sphincter components; namely the true lower oesophageal sphincter (LOS) as well as the diaphragmatic crural component. Patients with gastro-oesophageal reflux disease have previously been shown to have increased distensibilty of the oesophago-gastric junction (OGJ). FLIP is a novel method to assess the distensibility as it relates to competency of sphincters in the gastrointestinal tract.
Methods: Seven Barrett's oesophagus patients with hiatal hernias were compared to six healthy, age-matched controls. First, all subjects underwent upper endoscopy. Next, a FLIP probe was placed straddling the OGJ. Spaced 5 mm apart, the probe has 16 electrodes that are capable of measuring cross-sectional area (CSA) during inflation of the probe balloon with saline. In patients, the diaphragmatic hiatus, the LOS and the hiatal hernia cavity were located and measured separately.
Results: In patients, the true LOS was more distensible and the balloon pressure was lower at all distension volumes. At maximum inflation volume (50 mL), the mean minimal CSA of the balloon was 327 mm^2 in patients vs. 193 mm^2 in healthy controls. The patients had a diaphragmatic sphincter component more distensible than their true LOS, mean minimal CSA 505 mm^2 vs. 327 mm^2. Due to the small number of subjects, none of the differences were statistically significant. At low distension volumes, the three-dimensional anatomy of a hiatal hernia could be assessed visually with both sphincter regions visible at each end of the hernia.
Conclusion: FLIP is a practicable method to assess the distensibility of the OGJ also in the presence of a hiatal hernia. The results indicate that the distensibility of the LOS in hiatal hernia patients is larger than in healthy controls and that the distensibility of the diaphragmatic hiatus is larger than that of the true LOS in hiatal hernia patients. This is a plausible explanation for the development of reflux disease in these patients. FLIP assessment may be used to better understand the function and competency of the OGJ and the roles that the different parts of the junction play.



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Can intraluminal multichannel pH-impedance monitoring be limited to 3h: Comparison between 24h ambulatory and 3h post-prandial recording
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Objective: Esophageal multichannel intraluminal pH-impedance (MII) recording is now a valid technique to determine the liquid, gas or mixed nature of gastro-esophageal reflux (GER), and to detect acid, weak or non acidic GER disease. However, recording analysis remains time consuming in the absence of validated automatic analysis. In addition, a few recording may stop prematurely due to technical reason or patient poor tolerance of the probe. Therefore, we questioned whether analysis of a recording limited to 3h post-prandially could predict results obtained in a 24-h ambulatory recording.
Methods: Fifty patients with either typical (n = 26) and/or atypical (n = 24) symptoms of GER were investigated. Esophageal MII was recorded in each patient 3h post-prandially (1h in sitting position followed by 1h supine and then 1h in sitting position) after a test meal (467 Kcal: 32.5% P; 23.2% G; 44.3% L). This was followed by a 24h ambulatory MII.). An increase of liquid/mixed reflux was defined by the occurrence of 75 or more liquid/mixed GER for 24h, and The association between symptoms and liquid reflux was considered positive if the symptoms association probability (SAP) reached 95%. Correlation between number of reflux in the 3 and 24-h recording were determined using the intra-class coefficient correlation.
Results: Correlation between the total number of liquid reflux in the 3 and 24-h recording was elevated (r = 0.71; P < 0.001), with a better correlation for acid (r = 0.80; P < 0.001) and weakly acid reflux (r = 0.56; P < 0.001) than non acid reflux (r = 0.44; P < 0.01). Sensitivity and specificity of a 3-h recording to detect elevated liquid reflux over 24h (id >75 reflux/24h) were 89 et 66%, respectively, for a number of 10 or more liquid reflux for 3h, and 78 et 88% respectively for a number of 15 or more liquid reflux for 3h. The predictive positive values were 38 and 56% for a number of 10 and 15 liquid reflux for 3h, respectively, while the predictive negative values were 96 and 95%. Lastly, Sensitivity and specificity of SAP calculated for 3h were 56 and 91%, respectively, with a predictive positive value of 88% and a predictive negative value VPN of 64%.
Conclusion: The occurrence of less than 15 liquid reflux for 3h may predict a normal total number of liquid reflux over 24-h of recording, while a positive SAP for 3-h may predict a positive SAP on a 24h ambulatory recording.

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Secretin level modulates the esophageal motor activity in type 2 diabetes mellitus
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Objective: Secretin influences the esophageal motility, and its level may be altered in type 2 diabetes. However, the effect of that alteration on esophageal motility in diabetics is not known. Our aim is to compare the esophageal manometric characteristics between diabetics with normal, higher or lower than normal plasmatic level of secretin.
Methods: The secretin plasmatic level was dosed to 31 diabetics of both genders, without signs of autonomic neuropathy, with a mean age of 60.25 years. Of them, 24 had a normal level of secretin (group I), 2 had an high level of secretin (group II) and 5 had the secretin level diminished (group III). An oesophageal manometry was performed to each of them. The characteristics of esophageal manometry were compared between the 3 groups. Student t Test was used and data are presented as mean ± standard deviation.
Results: In groups I, II and III, the percent of no transmitted waves were 6.78%, 45.5% and 4.66% respectively, P < 0.003. Wave amplitude (in mmHg) was higher in the group I, but the difference was not statistically significant. Similarly the maximum waves upstroke. However, the average upstroke (mm Hg/s) in the upper oesophagus was 23.79 ± 11.7 vs 56.5 ± 19 vs 17.4 ± 8.5; P = 0.001 and in the medium esophagus was 30.45 ± 10.6 vs 38.5 ± 0.7 vs 20.6 ± 10.4; P = 0.08. The wave duration and thevelocity were similar between groups.
Conclusion: No transmitted esophageal waves were significantly more frequent in diabetics with high level of secretin. 2: Average upstroke was significantly higher within the upper and slightly in the middle esophagus of diabetic with high level of secretin. 3: Wave amplitude was similar in both groups.

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Value of a rapid drink challenge test in the diagnosis of esophageal motility disorders
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Objective: Rapid multiple swallow has been proposed as a physiologic manoeuvre which could improve diagnosis in patients with suspected esophageal motility disorders. AIM: To compare the responses to a rapid drink challenge test in patients with suspected esophageal motility disorders but normal standard manometric results, and a control group of asymptomatic healthy volunteers, using high resolution manometry (HREM).

Methods: In 63 consecutive patients (43 female, 20 male; age range 21–81 years.) referred for suspected esophageal motility disorder (dysphagia 57%; chest pain 34%; pirosis 74%, regurgitation 50%) and normal results in standard HREM, and in17 healthy asymptomatic subjects (10 female, 7 male; age range 18–68 years), we tested the responses to rapid drinking of 200 ml of water immediately after a standard, 10 wet swallows protocol of HREM. We evaluated inhibition of esophageal contractions, lower esophageal sphincter (LES) relaxation, post-swalow LES contraction and post-swalow contractile wave.
Results: Healthy controls drank the 200 ml of water in 24 ± 2 s. All but one had a complete inhibition of esophageal contractility during rapid swallow, and all exhibited a normal LES relaxation (<15 mmHg). A normal peristaltic wave was observed post-swallow in 53% of the controls, and a non-transmitted or hypoperistaltic wave was observed in the remaining 47% of controls. Post-swalow LES contraction returned to pre-swalow values (0 ± 2 mmHg greater than pre-swalow; NS). By contrast, 31% of patients with normal manometry exhibited lack of inhibition of esophageal contractions during rapid swallow ($P < 0.05$ vs controls), and 47% produced a simultaneous post-swalow contraction which was never observed in healthy controls ($P < 0.001$ vs controls). No differences were observed in the time expended to drink, LES relaxation and post-swalow LES contraction vs controls (data not shown). Taking together the presence of any contractile activity during rapid swalow, and/or a post-swalow simultaneous contraction, 58% of patients with normal manometry, but only one healthy control, exhibited an abnormality during the rapid drink challenge test ($P < 0.001$).
Conclusion: Addition of a drink challenge test to the protocol of HREM, improves detection of motor abnormalities in patients with clinical suspition of esophageal motility disorder.

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Impact of bolus consistency and position on high-resolution manometric findings in patients

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Objective: Studies examining effects of posture and bolus consistency on manometric parameters report conflicting results. Recently we described that position and bolus consistency significantly affects contractile patterns seen with high-resolution esophageal manometry (HREM). The impact of experimental conditions is unclear. AIM: To determine whether sequence of swallows (wet supine vs. crackers in upright position) alter HREM parameters and final diagnosis.
Methods: Sixty-seven consecutive patients (25M, 43F, age 54.1 ± 1.9 (mean ± SEM)) underwent HREM using wet swallows when supine (WS) vs. crackers when upright (CU), and were randomized to which type of swallow was given first. A baseline followed ten swallows were performed with each bolus type.
Results: Wet swallows (WS) were performed first for 34 patients and with crackers when upright (CU) first for 33 patients. Dysphagia was the primary indication for 27 and GERD for 21 patients, equally distributed between groups. The mean LESP when supine was 21.7 ± 1.9 mmHg vs. 21.9 ± 1.7 mmHg when upright (NS). No differences in LES integrated relaxation pressure (IRP), mean contraction amplitude, wave duration and Distal Contractile integral were seen between positions and bolus type. When individual contractions were examined, the distribution of types of contractions differed following WS versus CU irrespective of order, with 53% of WS followed by normal contractions vs. 40% of CU when WS were given first and 40% of WS followed by normal contractions vs. 25% with CU when CU were given first ($P < 0.001$). The percent normal contractions after swallowing crackers was less if crackers rather than WS were given first, $P < 0.001$. The final diagnosis was different based on WS compared to CU but only when WS were given first. 13/33 were diagnosed as normal based on WS study vs. 7/33 when studies used CU ($P < 0.05$). When crackers were given first, 4/34 patients were normal based on studies with CU vs. 8/34 based on studies with WS (NS).
Conclusion: Position and bolus consistency do not significantly affect the mean LES pressure and IRP, or the mean amplitude of contractions, but affect the distribution of contraction type and diagnosis. The clinical significance and impact of consistency vs. position needs further study.

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Multiple rapid swallows using high resolution esophageal manometry for motor disorders of the esophagus

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Objective: Alteration in inhibitory neural pathways has been implicated in achalasia. The aim of this study was to assess inhibitory neural pathways using deglutitive inhibition during multiple rapid swallows (MRS) on high resolution esophageal manometry (HRM) in patients with esophageal motor disorders.
Methods: Consecutive patients with achalasia [type I($n = 5$), II($n = 8$) & III($n = 6$)], scleroderma with aperistalsis($n = 5$), diffuse esophageal spasm(DES)($n = 5$) and nutcracker esophagus($n = 5$) based on Chicago classification and 10 healthy volunteers were included for the study. HRM protocol included 5 minutes of baseline recording, followed by 12 supine wet swallows and 1 set of MRS. MRS constituted of 5 rapid swallows (2 cc of water with each swallow) 1 second apart. Esophageal body inhibition (EBI) and esophago-gastric junction (EGJ) relaxation were assessed during MRS and esophageal contractions and rebound EGJ contraction were assessed after MRS.
Results: Normal response of MRS in volunteers was EBI (90% of volunteers) with EGJ relaxation (100% of volunteers) during MRS followed by a peristaltic esophageal contraction(90% of volunteers) and rebound EGJ contraction(60% of volunteers). There was no EBI in type II and III achalasia and 60% of the patients with DES during MRS. EGJ relaxation was incomplete(>15 mmHg) in type I(80% of patients) and type II(87.5% of patients) achalasia during MRS. Median EGJ relaxation pressure was 18.9 mmHg and 24.2 mmHg in type I and II achalasia respectively. Esophageal contractions after MRS were absent in scleroderma(80% of patients) and DES(60% of patients). There were rebound EGJ contractions after MRS in DES(60% of patients), type III achalasia(83.3% of patients) and nutcracker(100% of patients).
Conclusion: Normal response during MRS is EBI and EGJ relaxation followed by esophageal peristaltic contraction. No EBI was seen in type II and III achalasia and many patients with DES, suggesting alteration in deglutitive inhibition to esophageal smooth muscle not only in achalasia but also in DES. There was predominant rebound EGJ contraction after MRS in type III achalasia and nutcracker esophagus suggesting alterations in excitatory pathways. Thus, MRS may be helpful to understand the pathophysiology of patients with esophageal motor disorders.

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Esophageal motor disorders and gastroesophageal reflux disease in lung transplanted patients

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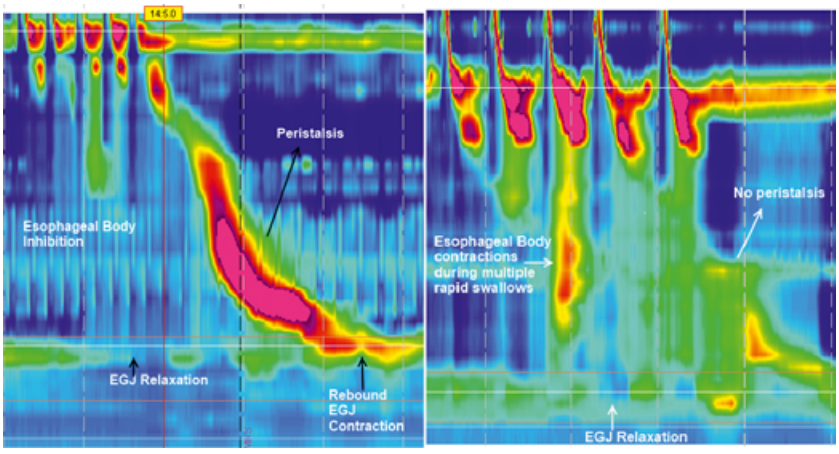
Objective: In lung transplanted patients a high prevalence of gastroesophageal reflux disease (GERD) was shown (D'Ovidio F 2005). This condition is considered the main non-alloimmune cause of chronic reject, due to bronchiolitis obliterans syndrome (Castor 2010). On the contrary, few data are available on esophageal



motility. The aim of this study was therefore to evaluate the prevalence of esophageal motility disorders, besides gastroesophageal reflux in a cohort of lung transplanted (LTx) patients.
Methods: We enrolled twenty-nine consecutive LTx patients and 19 NERD patients with typical symptoms on PPI as a control group. All patients underwent symptom severity evaluation by VAL, stationary esophageal manometry (Polygram, MedTronic) and 24-hr impedance-ph monitoring (Sleuth ZepHr, Sundhill Sc.) on therapy.
Results: As expected, twenty-four out of 29 patients were symptomatic for typical and/or atypical GERD symptoms. Compared to NERD patients, LTx patients showed a higher acid exposure of distal esophagus: upright 1.7%±3.2% vs 5%±8% and supine 0.96%±3.2% vs 4.8%±7.3%, $P < 0.05$. The number of acid refluxes was significantly higher in LTx than in NERD (16.5 ± 12.7 vs 10 ± 9.6); no significant difference was observed in the number of weakly acid refluxes (29.3 ± 34.4 vs 46.6 ± 28.4, P=NS). Weakly acid refluxes showed a proximal extent more frequently in NERD patients than in LTx ones. In LTx patients peristalsis was normal in 6 (22%) patients, 7 (25%) showed aspecific abnormalities, in 3 (6%) ineffective esophageal motility was present; nutcracker esophagus was evident in 7 (25%); diffuse esophageal spasm in 6 (22%). Hypotonic LES (<10 mmHg) was evident in 7% of subjects, hypertonic LES in 34%. No significant difference in LES tone was found between the two groups.
Conclusion: In lung transplanted patients, GERD is highly prevalent and PPI treatment shows a low efficacy in the control of esophageal acid exposure. Both esophageal body peristalsis and LES tone alterations are frequent. Further studies are needed to analyze whether regional alterations of esophageal motility may be responsible for the intraluminal persistence of refluxate that could facilitate aspiration.

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Lack of seasonal variation in the incidence of eosinophilic esophagitis

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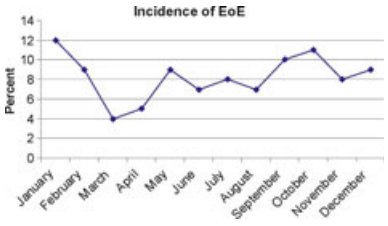
Objective: Eosinophilic esophagitis (EoE) has been associated with allergic disorders as well as aeroallergens. The current literature has shown a possible association between seasonal variation, mainly in the spring, and the incidence of EoE. However, this data was based on small population studies. AIM: The primary aim of this study was to determine if there is a seasonal variation associated with the diagnosis of EoE in our population.
Methods: Esophageal biopsies were obtained from a cohort of patients who presented with symptoms of dysphagia, odynophagia, globus sensation, and heartburn during a 10-year period. Patients that had biopsies from the mid and distal esophagus with greater than 20 eosinophils per high power field were diagnosed as having EoE. The monthly incidence and seasonal incidence were determined. (Winter: January- March, Spring: April-June, Summer: July- September; Fall: October- December).
Results: A cohort of 19,172 patients with esophageal biopsies was evaluated. A total of 167 patients (M/F:113/54, median age=27 years) had biopsy proven EoE (≥ 20 eosinophils/HPF). Incidence per month as follows: January 12%, February 9%, March 4%, April 5%, May 9%, June 7%, July 8%, August 7%, September 10%, October 11%, November 8%, and December 9%. Seasonal variation showed: Winter 25%, Spring 21%, Summer 25%, and Fall 28%.
Conclusion: The incidence of EoE was consistent across all 12 months as well as during the four seasons. Our data does not support a seasonal variation in relation to the incidence of EoE.



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Can the 3-dimensional animation plot be a 3rd diagnostic tool in high-resolution manometry?: Comparison to conventional linear and spatiotemporal plot

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Objective: The diagnostic accuracy of spatiotemporal plot (STP) in esophageal manometry compared to the linear plot (LP) has been reported to be increased, especially higher diagnostic yield in novice than advanced learners. Three-dimensional animation plot (3-DP) (Sandhill™, Highland Ranch, CO) can aggregate the information from the STP profiles and regenerate the esophageal motion as three-dimensional model with moving pictures. However, the clinical usefulness and diagnostic yield of 3-DP have not been fully validated. We aimed to investigate the additional diagnostic yield and ease of interpretation of 3-DP compared to STP and LP.
Methods: After standardized electronic tutorials, 20 medical students, 10 nurses, and 6 GI fellows, all of whom were never spoiled from the conventional manometry or HRM previously, were asked to answer to the questionnaire regarding diagnosis to the classified 30 examples of esophageal motility findings in LP, STP, and 3-DP formats in random order ((normalcy ($n = 15$), type I achalasia ($n = 6$), type II or III achalasia ($n = 7$), ineffective esophageal motility (IEM) ($n = 2$)), and asked about subjective preference and availability of interpretation among 3 formats. The diagnostic accuracy was compared among the 3 different display formats, as well as a subjective rating of preference in the diagnosis.
Results: The diagnostic accuracy was significantly higher in fellow group (OR=1.62, 95%CI: 1.12 to 2.35, $P = 0.01$), especially in case of normalcy (OR=2.42, 95% CI: 1.42 to 4.11, $P < 0.01$) and IEM (OR=9.42, 95% CI: 3.00 to 29.62, $P < 0.01$). There was no significant difference in diagnostic accuracy among the 3 different display formats. However, 3-DP gained significantly higher diagnostic yield in type I achalasia (OR=1.72, 95%CI: 1.08 to 2.76, $P = 0.02$) than other findings, but lower diagnostic yield in ineffective esophageal motility (OR=0.39, 95%CI: 0.22 to 0.69, $P < 0.01$) than other findings. Students and GI fellows favored STP (58.3%) over 3-DP (36.1%) and LP (5.6%) due to intuitively rapid interpretation. However, nurses preferred 3-DP (80%) over STP (20%) or LP (0%) due to easy understanding.
Conclusion: The newly developed 3-DP did not provide more diagnostic yield than LP or STP. However, in type I achalasia, 3-DP showed significantly additional diagnostic yield than other esophageal findings.

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Oesophagogastric junction relaxation in chagasic achalasia

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Objective: To evaluate oesophagogastric junction (OGJ) relaxation in Chagasic achalasia employing high resolution manometry (HRM), and comparing the results with those seen in idiopathic achalasia and healthy volunteers.
Methods: HRM was performed on 21 healthy volunteers (8 males, 20–54 years) and 19 subjects undergoing a work up for a presumptive diagnosis of achalasia using a solid state 36 sensor catheter system and dedicated display software (Sandhill Instruments). The protocol comprised a baseline recording and 10 5 mL saline swallows supine. HRM recordings were analysed blindly; end expiratory OGJ pressure (eeOGJP) and integrated relaxation pressure (IRP) were obtained using the Insight 3.0 analysis software (Sandhill Instruments). Achalasia patients were classified as either Chagasic achalasia ($n = 12$; 8 males, 35–73 years) or idiopathic achalasia ($n = 7$; 3 males, 26–54 years) according the results of a serological complement fixation test for Chagas' disease.
Results: eeOGJwas similar in Chagasic achalasia patients(16.4 ± 6.8 mmHg) and healthy volunteers (14.2 ± 7.5 mmHg) but was higher ($P < 0.05$) in idiopathic achalasia patients (33.6 ± 9.8 mmHg) than in both Chagasic achalasia and healthy volunteers. IRP in Chagasic achalasia patients (24.9 ± 11.7 mmHg) was higher ($P < 0.05$) than in healthy volunteers (13.9 ± 5.1 mmHg), and tended to be lower ($0.05 < P < 0.10$) than in idiopathic achalasia patients (34.9 ± 6.9 mm Hg). The upper 95% CI of IRP mean in healthy volunteers (16.3 mm Hg) was lower than the lower 95% CI of IRP mean in both Chagasic achalasia (17.5 mmHg) and idiopathic achalasia (24.3 mmHg).
Conclusion: Although eeOGJP is normal in Chagasic achalasia, OGJ relaxation is as incomplete in Chagasic achalasia as in idiopathic achalasia. IRP might be useful for distinguishing Chagasic achalasia from healthy volunteers.

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The role of high gastroesophageal reflux monitoring in diagnosing of ear, nose, and throat (ENT) manifestations of GERD

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Objective: The 24-hours pH-monitoring is the "gold standard" to verify the presence of pathologic reflux and confirmation of GERD presence. But there is lack

of data whether it is helpful to verify the link between concomitant ENT pathology with GERD.
Aim: To evaluate the utility of dual probe 24-hours pH monitoring for diagnosing the ENT manifestations of GERD.
Methods: One hundred GERD patients (53 men, 47 women, age (M±m) 46 ± 16.3 year old) were enrolled to the study. Among them 68 had signs of concomitant ENT pathology. Confirmed allergic, infectious and toxic etiology of ENT diseases were exclusion criteria. Dual-probe 24-hours pH studies (Gastroscan, Istok-systema, Russia) were performed to all of the patients. Distal probe was placed 5 cm above esophago-gastric junction, the proximal one – in the upper 1/3 of esophagus over upper esophageal sphincter. The presence of high gastroesophageal reflux (HGR) was diagnosed when at least one episode of drop of pH<4 for 20 s occurred at proximal probe during the study. The evaluation of sensitivity and specificity of the method was calculated. The results of pH studies were supposed as true positive when there were signs of HGR in patients with concomitant ENT pathology; false positive – when HGR occurred in patients without ENT patology, true negative – when no HGRs were present in patients without ENT pathology and false negative – when no HGRs were present in patients with ENT pathology.
Results: The age and sex distribution were equal in both groups. Presence of HGR was found in 54 patients of ENT group and in 14 of controls in group free of ENT signs.
Conclusion: Detection of high gastroesophageal reflux with placing the distal probe in the upper esophagus may be helpful for the diagnosing of extraesophageal manifestations of GERD.

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Swallow induced acid and non-acid Gastroesophageal Refluxes (GERs) in patients who were suspected of GERD: The role of hiatal hernia

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Objective: To investigate the role of swallow-induced acid and non-acid GERs in patients who had GERD symptoms with and without hiatal hernia(HH).
Methods: Thirty-eight patients(24 F, age 46 ± 14 years) with clinically suspected of GERD underwent 24 h esophageal pH-impedance monitoring following high resolution esophageal manometry (HRM) during off therapy. All patients had no or LA grade A reflux esophagitis. GER events recorded by the pH-impedance monitoring were analyzed visually regarding swallowing association, reflux composition, proximal extension, bolus contact time and time relation to meal ingestion. Reflux events during meal ingestion were excluded. Swallow-induced GERs were defined as the reflux events which the point of impedance dropped to 40% from baseline at most distal segment occurring within 12 seconds after the swallowing onset. The HH was identified using the HRM.
Results: 13, 10, 12 and 3 patients had reflux-like dyspepsia, typical reflux symptoms, chronic idiopathic ENT problems and noncardiac chest pain as their main symptoms, respectively. Three of 17 patients with HH had positive pH monitoring which was similar to patients without HH (4/21). Total reflux number and hourly swallowing rate were similar between patients with and without HH (22.3 ± 10.5vs.27.1 ± 14.2 times and 47.5 ± 9.5vs.40.2 ± 23.9 swallows/hr, respectively; $P > 0.05$). However, proportion of non-acid refluxes/total GERs in patients with HH was significantly greater than patients without HH (0.67 ± 0.32vs.0.34 ± 0.21, $P < 0.05$). In addition, the proportion of swallow-induced refluxes/total GERs was significantly greater in patients with HH than patients without HH (0.34 ± 0.23vs.0.15 ± 0.10, $P < 0.005$). Swallow induced GER was associated with a greater chance of being non-acid reflux (0.64 ± 0.36) compared to non-swallow induced GERs (0.46 ± 0.36, $P < 0.01$). Proportion of swallow induced refluxes/total refluxes during fasting period and 0–3 hour postprandial period was similar (0.24 ± 0.24vs.0.27 ± 0.29, $P > 0.05$). Bolus contact time and proximal reflux extension were similar between patients with and without HH, $P > 0.05$. Prevalence of esophageal dysmotility, which was hypotensive peristalsis in all patients, was similar between patients with and without HH (8/17vs.7/21, $P > 0.05$).
Conclusion: Patients with hiatal hernia had significantly higher chance to develop swallow-induced GERs than patients without hiatal hernia. In addition, swallow-induced GERs associated with non-acid refluxes more often than non-swallow induced GERs.

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Use of impedance monitoring for the diagnosis of secondary oesophageal peristalsis impairment in patients with gastroesophageal reflux disease

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Objective: To evaluate the opportunities of impedance monitoring in the detection of secondary oesophageal peristalsis impairments in patients with gastroesophageal reflux disease(GERD).
Methods: 145 patients with GERD were evaluated. Age of study subjects was 37 ± 7 years, men-to-women ratio was 4.3:1. Control group consisting of 22 virtually healthy subjects was recruited as well. Diagnosis of GERD was based on characteristic clinical data and endoscopic signs of reflux oesophagitis. Oesophageal motility was studied in all study subjects by means of intracavitary impedance monitoring with a Rheogastrograph RGG9-01 device(Saint Petersburg) working at a frequency of 10Hz, in dynamic mode. An impedance monitoring probe connected in advance to a polyvinylchloride tube(diameter, 1mm) was introduced into the oesophagus during this study. We evaluated oesophageal motility at baseline, in the fasting state, and then during a functional acid test. For this test, we used tube to inject 5mL of 0.1n hydrochloric acid solution pre-heated to 37C into the lower one-third of the oesophagus. The probe was placed so that the distal orifice of tube was 4–5cm above the lower oesophageal sphincter.
Results: Intracavitary impedance monitoring with functional acid testing revealed an increase in the frequency and amplitude of propulsive oesophageal movements after introduction of acid into the oesophagus in 68.2% of healthy individuals, statistically significantly more frequently($P < 0.001$)than in patients with GERD(40.0%). This was manifested by increased amplitude and frequency of impedance waves,as compared with baseline. Patients with GERD and preserved secondary peristalsis presented with minimal reflux-induced involvement of oesophageal mucous membrane on endoscopy. The most severe type of oesophagitis was observed in GERD patients with impairment of secondary oesophageal peristalsis. These patients presented significantly more frequently($P < 0.05$) with reflux oesophagitis grades C(24%) and D(8.0%), as well as with the complication Barrett's oesophagus (12.0%). Mild reflux oesophagitis, gradeA, as well as endoscopy-negative disease, were more commonly seen in patients with preserved secondary oesophageal peristalsis (50.0% and 10.9%, respectively).
Conclusion: Intracavitary oesophageal impedance monitoring with functional acid testing allows assessment of secondary oesophageal peristalsis in patients with gastroesophageal reflux disease.

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Preoperative esophageal manometry as a predictor of patient outcome following adjustable gastric banding

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Objective: There is a high prevalence of postoperative adverse symptoms in patients with laparoscopic adjustable gastric banding (LAGB). We previously demonstrated a high prevalence of esophageal dymotilities in obese patients. The aim of this study is to evaluate if the result of preoperative manometry was correlated with patients outcome after LAGB.
Methods: Prospective study from January 2009 to December 2011 at the University of Montreal Hospital Research Center (CRCHUM). All patients undergoing bariatric surgery were included and had a preoperatory standardized esophageal manometry and gastrointestinal symptoms survey. Patients included were subsequently divided into two groups according to their manometry results (normal vs abnormal). File reviews were performed to assess these postoperative criteria: upper digestive tract symptoms, LAGB revision and weight loss.
Results: Ninety-one patients were included (average age: 44 ± 9 years old, average BMI: 47 ± 10). Esophageal dysmotility was found in 54% of patients ($n = 49$). Patients were similar between the two groups (normal vs abnormal manometry) in terms of age, sex, BMI and comorbidities. There was no association found between preoperative oesophageal tract function and postoperative adverse upper digestive tract symptoms (57% vs 47%; statistically non significant (NS)) and LAGB revision rates (59% vs 63%; NS). Mean postoperative weight loss between 1 and 3 months was similar between our two groups (11.4% vs 9.3%; NS). Nonetheless, patients with a normal manometry presented with a more significant weight loss between 1 and 3 months (10–25% weight loss from weight at surgery: 43% vs 22%).
Conclusion: There is no association found in our study between preoperative oesophageal tract function and patients outcome after gastric banding.

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Esophageal disorders and psychological disadaptation in patients with globus sensation

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Objective: To evaluate the role of esophageal dysmotility and psychological disadaptation etiology in globus sensation.
Methods: The study population consisted of 27 consecutive patients with globus sensation referred to the Central Research Institute of Gastroenterology. In all patients there was no abnormal otorhinolaryngological status. Upper GI Endoscopy, 24-h pH monitoring, high resolution esophageal manometry. The State-Trait Anxiety Inventory (STAI) was used in clinical settings to diagnose anxiety and to distinguish it from depressive syndromes. The Beck Depression Inventory (BDI) is a series of questions developed to measure the intensity, severity, and depth of depression.
Results: Nonerosive reflux esophagitis was found in 8/27 (29.6%) of the patients. 18/27 (66.7%) demonstrated abnormalities in esophageal manometry, the most frequent finding being a nonspecific esophageal motility disorder (47.4%) and segmental esophagospasm (Nutcracker esophagus) (36.8%), pH monitoring was normal in 21/27 of patients (77.8%). Psychological disadaptation 22/27 (81.5%) were diagnosed following (of which 41.0% had depressive disorder in combination with anxiety's reactions and 59.0% had an anxiety disorder). The most of patients connected the beginning of disease with severe life events. It was noted, that many of the patients had some symptoms of autonomic nervous system disorders such as tachycardia (17/27 – 62.9%), disposition to perspire (16/27- 59.2%), lowering of capacity for work (20/27-74.7%), problems with slumber (22/27- 81.5%) and easy fatigability (16/27- 59.2%).
Conclusion: Globus sensation has a multiple etiology. The local reasons are rare, but they should be first ruled out. Abnormalities in esophageal motility are commonly found. Esophageal manometry should be included in the evaluation of a globus's patient. The indications of psychological disadaptation in combination with symptoms of autonomic nervous system disorders are common in these patients. A presence of globus sensation can aggravate patient's anxiety. An elucidation of the reason of globus sensation is very important for effective treatment.

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Effects of high-resolution esophageal manometry on oxygen saturation and hemodynamicfunction

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Objective: To evaluate the hemodynamic changes and oxigen desaturation induced by high-resolution esophageal manometry.
Methods: Prospective study of consecutive patients referred for high-resolution esophageal manometry. Demographic data, history of heart and respiratory diseases and smoking habit were collected in all patients. The difficulty of intubation; patient tolerance and duration of manometry were evaluated. The study variables were oxygen saturation (pulse oximetry), heart rate and blood pressure. Oxygen saturation and heart rate were measured at baseline, during intubation, during the test and 5 min after manometry. Blood pressure was measured before, during and after manometry.

	Oxigen saturation	Heart rate	Systolic blood pressure	Diastolic blood pressure
Before manometry	97 (2)	76 (13)	148 (24)	81 (14)
During intubation	96 (3)*	96 (20)*	------	------
During manometry	97 (2)	80 (13)	135 (21)	73 (14)
After manometry	97 (2)	77 (12)	130 (20)	71 (13)

Mean (SD) *p<0.05 compared with basal values

Results: One hundred and fifteen patients (54% women) with a mean age of 56 (SD 16) years were included. Thirty-one (27%) were obese and 38 (33%) had overweight. Thirteen (11.3%) patients had a history of respiratory disease; 12 (10.5%), had heart disease; 18 (15.7%), and 18 (15.7%), were smokers. Intubation was easy in 73%, difficult or very difficult in 27%. Exploration tolerance was good in 81%, poor or very poor in 19%. The average duration of the test was 12 (SD: 3) minutes. Values of oxygen saturation, heart rate and blood pressure are expressed in the table. Thirty-two (28%) and 25 (22%) patients had oxygen desaturation under 96% during intubation and during manometry respectively. One patient had saturation under 90% but his basal value was similar. Forty-five (39%) patients had tachycardia during intubation and 6 (5%) during manometry. No patient had clinically significant increase of blood pressure during manometry. Reduction of oxygen saturation during intubation was associated with higher age. Reduction of saturation during manometry was associated with higher age and overweight or obesity. Tachycardia during intubation was not predicted by any variable.
Conclusion: High-resolution esophageal manometry produces a reduction in oxygen saturation and tachycardia; however, these changes are not clinically significant. A higher age and a higher body weight predict a higher reduction of oxygen saturation.

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The influence of positional changes on upper esophageal sphincter pressure using high resolution esophageal manometry

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Objective: Esophageal manometry is used in evaluating patients with esophageal motility disorders. The standard protocol for esophageal manometry involves placing the patient in the supine position with head turned to left while evaluating liquid bolus swallows. It has been shown that UES pressure varies in relation to head rotation, but liquid swallows were not evaluated. Another study had demonstrated that the UES response to esophageal distension is affected by combined effects of posture physical properties, and volume of refluxate. We hypothesized that different bodily positions could affect UES resting pressures. The data on this topic is limited.
Aim: To compare esophageal pressure morphologies and function during swallowing in SHL and SHR using HREPT.
Methods: Healthy adult volunteers were screened using a GERD symptom assessment scale (GSAS) questionnaire. Those eligible underwent an HREPT. The probe (36 sensors, 2.75 mm in diameter, 1 cm apart) was positioned from the nasopharynx to stomach. The volunteers were placed in the following positions: supine head left(SHL), supine head right(SHR), seated at a 90° angle with head facing forward, and standing with head facing forward. Ten liquid swallows were performed in each of the supine positions, and three swallows were performed in the seated and standing position. The following parameters were assessed: resting lower esophageal sphincter (LES) pressure, integrated relaxation pressure (IRP), resting upper esophageal sphincter (UES) pressure, distal contractile integral (DCI), contractile front velocity (CFV), and intra bolus pressure (IBP). Data was compared using repeated test ANOVA corrected for multiple comparison using Tukey.
Results: A total of 38 healthy adult volunteers (M/F:22/16, mean age=27 years, mean BMI=25.2) were evaluated. Compared to SHL, the resting UES pressure was significantly lower in SHR, seated, and standing.
Conclusion: When compared to SHL, the UES resting pressure was significantly lower in all of the positions. These findings could have a diagnostic implication on HREPT protocols, and treatment in patients suffering from GERD and dysphagia.

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Factors associated with dysphagia symptoms in patients who were suspected of GERD: A high resolution esophageal manometry study

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Objective: To determine the factors which associated with dysphagia symptom in patients who were suspected of GERD.
Methods: Seventy-eight consecutive patients with typical or atypical GERD symptoms for >3 months, each underwent a high resolution esophageal manome-

Position	*Supine Head Left*	Supine Head Right	Sitting	Standing
LES, mean ± SD (mmHg)	***22.9 ± 9.6***	25.1 ± 9.1	20.9 ± 9.8	18.2 ± 9.1
IRP, mean ± SD (mmHg)	***3.4 ± 3.2***	3.4 ± 3	4.1 ± 12	2.2 ± 2.6
UES, mean ± SD (mmHg)	***78 ± 35***	**34.8 ± 19.9***	**56.7 ± 50.7***	**55.9 ± 27.2***
DCI, mean ± SD (mmHg/sec cm)	***1626.5 ± 806.4***	1655.5 ± 789	1610.1 ± 1541.6	1227.3 ± 725.7
CFV, mean ± SD (cm/sec)	***3.1 ± 0.7***	3.2 ± 0.7	2.9 ± 1.1	3.5 ± 1.2

try (HRM) and a 24 h pH test during off treatment. All patients had a negative upper GI endoscopy except for hiatal hernia or LA grade A esophagitis. Gastrointestinal symptoms profiles were evaluated using a symptom questionnaire. The dysphagia symptom was defined as the present of disturbing difficulty swallowing symptom >1/week. Esophageal motor functions were determined using the HRM technique. Achalasia patients were excluded. The HRM parameters including peristalsis contraction amplitude, upper and lower esophageal sphincter pressure, residual LES relaxation pressure, proximal contractile integral (CI), distal CI, proximal contractile front velocity(CFV), distal CFV, spasm length, distal latency (DL), intrabolus pressure (IBP), normal peristalsis length at distal esophagus, total esophageal length and the diagnosis from the HERM result were determined as described by the Chicago classification.

Results: Thirty and 17 patients had dysphagia symptom and positive pH tests, respectively. The patients' age, spasm length, normal peristalsis length at distal esophagus, prevalence of abnormal LES relaxation and ratio of normal peristalsis length/total esophageal length were significantly different comparing between patients with dysphagia and without dysphagia (54.9 ± 13.1vs47.9 ± 13.4 yrs, 2.3 ± 2.7vs0.9 ± 1.4 cm, 6.4 ± 4.8vs11.2 ± 12.4 cm, 15.3 ± 27.1%vs.3.9 ± 15.7% and 0.3 ± 0.2 vs 0.5 ± 0.5, respectively; $P < 0.05$). Binary logistic regression analysis demonstrated that age ≥50 years, esophageal spasm length ≥2 cm, and normal peristalsis length < 4 cm at the distal esophagus was associated with greater risk of dysphagia symptom [adjusted odd ratio (95% CI) was 7.5(2.1-27.7), 7.9(2.1-30.3), and 9(2.1-39.3), respectively]. The adjusted odd ratio (95% CI) for the risk of dysphagia for abnormally high residual LES relaxation pressure, abnormal distal latency and abnormal intrabolus pressure was 0.9(0.8-1.1), 1(0.5-2), and 1(0.9-1.2), respectively). The gender, BMI, pH test results, and the other HRM parameters were not significantly associated with dysphagia symptoms.

Conclusion: This study suggests that old age, esophageal spasm length, and normal peristalsis length at distal esophagus were independently associated with dysphagia symptom in patients who were suspected of GERD.

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Is the reflux characteristic to provoke heartburn, chest pain and cough different? The study using multichannel impedance-pH monitoring in patient with positive symptom index

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Objective: It is not clearly understood why esophageal exposure to gastric contents causes heartburn in some patients and chest pain or cough in others.To investigate the reflux characteristics that provoke heartburn, chest pain and cough in patients with gastroesophageal reflux disease (GERD), with a positive symptom index using multichannel intraluminal impedance-pH monitoring (MII-pH).

Methods: Thirty-eight patients with GERD based on symptom Index over 50 based on MII-pH results, were recruited. Patients were divided into three groups according to presenting symptoms; that is heartburn, chest pain or cough. Ninety-three reflux episodes which are considered to provoke each symptom, were analyzed retrospectively. The characteristics of reflux episodes among the three groups were compared.

Results: The delta pH, mean pH and pH-maximum were higher in the cough group ($P < 0.050$, $P < 0.010$, 0.001, respectively). The acid and bolus clearance time showed a tendency to increase in the chest pain group ($P = 0.069$, 0.073, respectively). No statistically significant differences were found for other various parameters, including acidity, bolus contents, distance of proximal extent and time interval from reflux event to the development of symptoms, among the three groups.

Conclusion: In GERD patients, reflux events that provoked cough showed different characteristics, as assessed by MII-pH monitoring, compared to patients with heartburn or chest pain. Specifically, delta pH, mean pH and pH-maximum were higher in the cough group. Cough may be a response to a change in pH sensitively. In reflux events that provoke chest pain, acid and bolus clearance time tended to be longer than in other events. We assumed that decreased acid clearance in the esophagus and large volume of refluxate may be related to the development of chest pain.

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Diagnostic thresholds of proximal pH monitoring for ENT manifestations of GERD

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Objective: GERD is associated with a number of extraesophageal manifestations (EEM) such as pulmonary, ENT etc. pH monitoring in distal esophagus is widely used to confirm the acid reflux but there are no optimal cut-off values for its parameters in the upper esophagus for detection the relationship between extraesophageal pathology and GERD. Aim: To establish the optimal cut-off values for mean pH, % time pH<4 a day and number of high gastroesophageal refluxes (HGR) in regard to presence of extraesophageal manifestations of GERD.

Methods: One hundred GERD patients (53 men, 47 women, 46 ± 16.3 year old) were examined using dual-probe 24-hours pH monitoring. The proximal probe was placed in the upper 1/3 part of esophagus over the upper esophageal sphincter. To confirm the presence of ENT manifestations of GERD all the patients were examined by qualified otolaryngologist; special ENT tests (laryngoscopy, pharyngoscopy with cytology and bacteriology) were performed. Toxic, allergic and infectious etiologies of ENT pathology were exclusion criteria. ROC curve analysis was used to find optimal cut-off values of pH-studies.

Results: ENT pathology was found in 68 patients. HGR was found in 79.41% of patients with ENT and 43.75% of group without ENT diseases, $P = 0.0011$. Optimal cut-off values of number of GER for proximal pH-probe with good sensitivity (DSn)/ specificity (DSp) ratio were 2 (DSn 72.1%, DSp 68.7%) or 3 (DSn=63.2%, DSp=71.9%), for mean pH – optimal values were 6.35 (DSn=75%, DSp=51.5) or 6.45 (DSn=68.8%, DSp=58.8%); duration of time pH<4 - 35 (DSn=72.1%, DSp=68.7%) to 45 (DSn=67.6%, DSp=71.9%) sec.

Conclusion: Proximal pH monitoring may be useful in diagnosis of ENT manifestation of GERD. Optimal cut-off values for number of high GER are 2 to 3, mean pH 6.35-6.45 and duration of time pH<4 35-45 sec.

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Esophageal contractile activity might re-appear after myotomy for achalasia

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Objective: Myotomy is a therapeutic option for achalasia. This treatment alleviates functional esophagogastric junction (EGJ) obstruction. The effect of myotomy on the esophageal body is not known. This study aimed to determine the effect of myotomy on esophageal contractile activity and pressurization characterized with esophageal pressure topography studies.

Methods: Patients who underwent esophageal high resolution manometry (HRM) before and after laparoscopic or endoscopic myotomy in two tertiary centers (Chicago and Lyon) were included. Before myotomy, achalasia was classified into three types according to the Chicago classification. Pre and post-operative HRM were characterized with resting EGJ pressure, integrated relaxation pressure (IRP), distal esophageal contraction, and pan-esophageal pressurization.

Results: Twenty-six patients (16 males; mean age 40 years, range 17-77) were included: 7 type 1 (27%), 15 type 2 (58%) and 4 type 3 (15%). Twenty-one patients underwent a surgical myotomy and 5 a peroral endoscopic myotomy. EGJ basal pressure and IRP decreased in all but two patients after myotomy. The median (interquartile range) decrease was 65% (47-77%) and 60% (32-67%) for EGJ basal pressure and IRP respectively. Whereas distal esophageal contraction was evident only in patients with type 3 achalasia before treatment, it was encountered in 86% of type 1, 40% of type 2 and 100% of type 3 after myotomy. When contractile activity was present, the median (IQR) of swallows with contractile activity was 85% (58-100%). Among the 6 patients with post myotomy IR $P > 15$ mmHg, 5 had an EGJ outflow obstruction pattern and one a type 1 achalasia pattern. Among patients with post myotomy IR $P < 15$ mmHg, esophageal contractile patterns were absent (9 patients), weak (7), frequent failed peristalsis (3) and spasm (1). Pan-esophageal pressurization disappeared after myotomy in 14 of 17 patients and was never encountered after myotomy if not noticed before. No difference of EGJ pressure, IRP, distal contractile activity or pressurization was observed between treatment modalities or centers.

Conclusion: Distal esophageal contractile activity frequently reappears after reduction in EGJ pressure by effective myotomy to treat achalasia. This suggests that EGJ outflow obstruction plays a role in causing absent peristalsis in achalasia.

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Transition zone defect as a negative prognosticator of the response to proton pump inhibitor treatment in patients with globus sensation

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Objective: Although gastroesophageal reflux disease (GERD) is associated with globus sensation, the etiology of this disorder remains unclear. This study was to evaluate the use of manometric parameters in proximal esophageal contraction as measured by topographical plots of high resolution manometry (HRM) as predictive factors of the response to PPI treatment in patients with globus sensation.
Methods: Forty-one patients with globus sensation were treated with rabeprazole 20 mg daily for 4 weeks and classified as positive and negative responders based on symptom intensity score. HRM topographical plots were analyzed for manometric parameters of upper esophageal sphincter (UES), proximal esophageal contraction, and transition zone (TZ).
Results: Nineteen patients (46%) were clinically diagnosed as GERD. Based on the pH testing and endoscopy, the prevalence of GERD was 20% (8 of 41). Three patients (7%) had complete resolution and 15 (37%) had more than a 50% improvement in the globus symptom score. There was no difference of mean basal pressure, relaxation time to nadir, or relaxation duration with regard to UES function between groups classified by the symptomatic response. About the proximal contraction segment, contractile integral (647 ± 165 mmHg cm s vs. 597 ± 157 mmHg cm s, $P = 0.342$) and contractile velocity (3.1 ± 0.5 cm s^{-1} vs. 3.3 ± 0.7 cm s^{-1}, $P = 0.332$) in the positive responders was similar to those of negative responders. In patients with GERD, spatial dimension of TZ was greater in the negative responders than in the positive responders (2.35 ± 1.24 cm vs. 1.15 ± 0.56 cm, $P = 0.031$). In similar, an increased temporal dimension of TZ was observed in the negative responders compared to the positive responders (1.58 ± 0.70 s vs 0.87 ± 0.36 s, $P = 0.026$). By ROC curve analysis, 2.1 cm and 1.1 s were found to be the spatial and temporal TZ dimensions that best differentiated positive and negative responders.
Conclusion: Transition zone defect, associated with striated-to-smooth muscle coordination, was a significant prognosticator for response to PPI treatment in patients with GERD-related globus sensation.

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Different effects of esomeprazol on gastric and gastro-oesophageal junction PH

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Objective: Gastro-oesophageal reflux (GOR) typically occurs postprandially and is more pronounced in the distal oesophagus. Previous studies have shown the presence of a pocket of highly acidic gastric juice at the gastro-oesophageal junction (GOJ) postprandially, and this has been implicated in the pathogenesis of postprandial GORD symptoms. The effect of proton pump inhibitor (PPI) therapy on the acid pocket has not been studied.
Methods: Seven PPI-responsive GORD patients (3 men and 4 women, mean age 32 (range 24–55)) were studied twice; and/or off PPI therapy and/or after 2 weeks of esomeprazole 40 mg daily. They underwent 24 h pH impedance monitoring followed by stepwise GOJ pull-through of a pH probe (1cm/min, lower oesophageal sphincter (LOS) -10 to +5 cm) before and 10, 30 and 50 min after a standard liquid meal (200 ml Nutridrink®, 300 Kcal). Simultaniously, a high resolution manometry was performed, mainly to determine the LOS localisation.
Results: Twenty-four hour pH monitoring confirmed efficacy of acid suppression. Mean gastric pH was significantly increased by PPI therapy, both pre- (3.42 ± 0.44 vs. 5.96 ± 0.66, $P < 0.005$) and postprandially (e.g. at 10 min 4.55 ± 0.63 vs. 6.62 ± 0.2, $P < 0.01$). Off therapy, the nadir pH of the acid pocket straddling the GOJ region, was 3.47 ± 0.52, 4.28 ± 0.42 and 3.77 ± 0.36 at respectively 10, 30 and 50 min postprandially. During esomeprazole therapy, these values were significantly higher (6.45 ± 0.14, 6.10 ± 0.37 and 5.95 ± 0.45 at respectively 10, 30 and 50 min, all $P < 0.05$). The drop in pH at the GOJ at 10 min tended to be suppressed by esomeprazole therapy (1.09 ± 0.47 vs. 0.17 ± 0.08, $P = 0.08$).
Conclusion: PPIs increases intragastric pH as well as pH at the GOJ, thereby suppressing the postprandial acid pocket at the junction.

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Optimization of treatment efficacy for patients with various forms of gastroesophageal reflux disease

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Objective: To evaluate the efficacy of different treatment regimens in patients with gastroesophageal reflux disease (GERD) by obtaining clinical-endoscopic and immunohistochemical characteristics of oesophageal mucous membrane(OMM).
Methods: A total of 150 patients with GERD were evaluated. The first group consisted of 30 patients with non-erosive GERD (NEGERD) who received treatment with omeprazole 20 mg/day; the second group was composed of 30 patients with NEGERD given omeprazole and Dibicor (500 mg twice daily); the third group included 30 patients with erosive GERD (EGERD) who were given omeprazole; the fourth group united 30 patients with EGERD who were given omeprazole and Dibicor. Numbers of OMM epithelial cells immunopositive to NO synthase, melatonin, and p53 molecule were determined. The clinical condition was evaluated daily using the Likert scale. Endoscopy was carried out at 2 and 4 weeks in patients with NEGERD and at 2, 4, 6, 8 weeks in patients with EGERD. Immunohistochemical characteristics were obtained prior to treatment and during a GERD remission. Control group was composed of 30 virtually healthy individuals.
Results: NEGERD was associated with an increase of number of gastric epithelial cells immunopositive to NO synthase, which was accompanied by a proportionately increased p53 expression and decreased function of melatonin-producing cells. Patients with EGERD were found to have significantly increased, as compared with NEGERD, p53 expression, hyperplasia and increased activity of NO synthase-immunopositive cells, as well as pronounced reduction in functional activity of melatonin-producing cells. After 3 days antisecretory therapy, patients in the GERD groups administered Dibicor-containing regimen had symptom scores on the Likert scale significantly below than respective scores of omeprazole-treated patients. Patients on Dibicor-containing regimen proceeded to better recovery of functional morphology of oesophageal diffuse endocrine system(DES) and to better reparation of OMM epithelial cells, as compared with omeprazole-treated patients, while remission time was reduced by a mean of 5, 3 days.
Conclusion: Including of Dibicor in the treatment of GERD improves the quality of GERD remission, which can be explained by property of Dibicor to potentiate antisecretory effect and improve NO metabolism and cellular homeostasis in epithelial cells of upper gastrointestinal tract.

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The Gerd-Q is a useful tool for evaluating the patients' satisfaction and identifying the patients with "unmet medical needs"

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Objective: Gastroesophageal reflux disease (GERD) is often encountered in daily clinical practice. Primary care physicians must make an accurate diagnosis of GERD and manage it effectively. Therefore, easy-to-use questionnaires that can validate the diagnosis and treatment of GERD are attracting attention. The GerdQ is a patient-centered self-assessment GERD questionnaire only with 6 questions (Aliment Pharmacol Ther. 30:1030-8, 2009). The present study was designed to evaluate the usefulness of GerdQ compared with the classical Carlsson–Dent questionnaire (CDQ) and the relationship among the GerdQ score, the treatment with prescribed agents, and patients' satisfaction with the medication.
Methods: The Japanese versions of the GerdQ and the CDQ, demographic characteristic questionnaires including the history of the treatment for GERD, and the 5-step scales of the patients' satisfaction with the treatment were posted to 840 subjects (20–59 years old). All subjects underwent EGD at Keio University Hospital between December 2010 and May 2011 and had no systematic diseases or findings of malignancies or ulcers. In addition, 1630 subjects with heartburn and/or regurgitation answered the same questionnaires on the web.
Results: 303 (168 men; 135 women) in the mail survey and 1024 (479 men; 545 women) in the web survey provided informed consent and answered the questionnaires. Although the GerdQ and CDQ scores were not associated with the patients' satisfaction in the mail survey, there was a negative correlation between the GerdQ score and the satisfaction score in the web survey ($P < 0.05$). In the medication group of the mail survey, 14/127 (11.0%) had taken OTC medications (GerdQ-Q.6 positive), and their total GerdQ scores was statistically higher than those of subjects who did not take OTC medications ($P < 0.001$), unlike the CDQ scores. Besides, more subjects who were prescribed for GERD symptoms took OTC medications in the web survey (45.0%) than the mail survey (11.0%).
Conclusion: The GerdQ, but not the CDQ, was useful to evaluate the efficacy of the treatment, and was able to detect patients with inadequate response to GERD treatment as indicated by the need for OTC medication. Furthermore, indirect approaches, such as web questionnaires, are more useful to find out dissatisfaction of patients than direct questions.

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Otorhinolaryngological manifestations of gastroesophageal reflux disease and efficacy of their treatment

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Objective: To evaluate the prevalence, clinical variants, and treatment efficacy in patients with the otorhinolaryngological GERD form.
Methods: The study sample consisted of 82 patients with chronic ear, nose, and throat conditions hardly treated by conventional conservative therapy, aged from 18 to 64 years and complaining of voice hoarsening, pain in the throat, chronic cough, nasal discharge,stuffed-up ears.Patients were followed up at the Department Otorhinolaryngology of SBFEHC City Clinical Hospital for Emergency Care named after G.A.Zakharin and Penza polyclinics.At screening, all patients were asked to answer the questions of the Reflux Symptoms Index clinical questionnaire(J.A.Koufman,2005).
Results: The clinical work-up produced a diagnosis of GERD in 62.2% of study subjects(n = 51) with ear,nose, and throat conditions;54.9% of them had laryngeal abnormalities:chronic hyperplastic laryngitis in 51% and Reinke's oedema 3.9%.Pharyngeal disorders were diagnosed in 52.9%: various forms of hypertrophic pharyngitis 41.2%, chronic tonsillitis 11.8%.Among patients with GERD,9.8% had disorders of the nose or paranasal sinuses (recurrent rhinosinusitis 3.9%, hypertrophic posterior parts of the inferior nasal conchae 5.9%); while 7.8% of subjects presented with auditory tube disorders.All study subjects diagnosed with GERD were administered itopride hydrochloride at a dose of 50 mg three times per day. After 4 weeks of itopride hydrochloride therapy, 89% of patients felt better, as they made no complaints of voice hoarsening, impaired nasal breathing and smelling,or nasal discharge,and had less pain and obstruction in the throat and less cough. A follow-up quality of life assessment demonstrated statistically significant improvements on the Role-Physical(RP),Bodily Pain(BP), General Health(GH), Vitality(VT), Social Functioning (SF), Role-Emotional(RE), and Mental Health(MHn) scales.
Conclusion: Patients with chronic ear, nose, and throat conditions hardly treated by conventional conservative therapy can be diagnosed with GERD in 62.2% of cases, their otorhinolaryngological symptoms are viewed as extra-oesophageal manifestations of GERD and can be reduced by itopride hydrochloride therapy in 4 weeks 89% of patients. Patients with GERD have decreased quality of life, useing of the prokinetic itopride hydrochloride results in significant improvements of the physical and mental health of these patients.

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What factor associates with health-related quality of life in patients with gastroesophageal reflux disease under treatment with PPI?

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Objective: Benefits on symptoms management of proton pump inhibitor (PPI) therapy are widely recognized in gastroesophageal reflux disease (GERD). However, not a few GERD patients experience persistent and troublesome symptoms despite PPI treatment. In addition, the factors for affecting health-related quality of life (HRQoL) have not been investigated well. The aim of this study was to prospectively evaluate variables associated with HRQoL measured by 8-items Short Form Health Survey (SF-8) in Japanese patients with GERD who were treated with PPI.
Methods: Ninety four patients taking PPI diagnosed GERD filled in SF-8 and four treatment response questionnaires of heartburn, regurgitation, sleep disturbance, additional medication defined in a validated questionnaire, GerdQ. Partial responder to PPI was defined that there were 2–3 day of symptoms at least 1 item of GerdQ. As variables associated with summary score of SF-8, treatment response, age, body mass index (BMI), gender, smoking, alcohol, non-steroidal anti-inflammatory drugs (NSAIDs), PPI daily dose, concomitant gastrointestinal drug other than PPI, esophagitis grade by Los Angeles classification were evaluated.
Results: Mean (s.d.) age and BMI (s.d.) were 64 (14) years and 22.5(3.5) kg m^{-2}, respectively. Fifty patients were partial responders to PPI therapy. These patients reported significantly lower scores in the General health (GH), Bodily pain (BP), Vitality (VT), Mental health (MH) and physical health component summary (PCS) than responder to PPI (P < 0.05). A multiple regression analysis presented that taking NSAIDs and partial response to PPI therapy were significant factors that affected PCS score (P < 0.05).
Conclusion: Partial responder to PPI therapy and NSAIDs use could be factors associated with decreased HRQoL in patients with GERD.

SF-8 domains		Partial responder to PPI (n=50) mean (s.d.)	Responder to PPI (n=44) mean (s.d.)	p-value
General health	GH	44.4 (6.9)	48.5 (6.5)	0.005 *
Physical functioning	PF	45.7 (8.9)	47.9 (6.5)	0.173
Role-physical	RP	44.9 (10.2)	48.3 (6.8)	0.066
Bodily pain	BP	45.3 (8.5)	52.0 (10.2)	0.001 *
Vitality	VT	46.7 (8.4)	50.8 (5.7)	0.009 *
Social functioning	SF	46.4 (7.7)	48.3 (8.4)	0.262
Mental health	MH	46.3 (9.4)	50.0 (6.1)	0.015 *
Role-emotional	RE	46.3 (9.4)	49.4 (6.1)	0.073
Physical health component summary	PCS	43.7 (7.8)	47.8 (6.5)	0.008 *
Mental health component summary	MCS	46.6 (8.6)	49.1 (6.5)	0.125

Univariate analysis * p<0.05

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Correlation between the new oropharyngeal pH-monitoring with the Restech® probe for laryngo-pharyngeal reflux and responsiveness to anti-secretive therapy

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Objective: To assess the ability of the oropharyngeal pH-monitoring in predicting the response to a 3-month double dose PPI (proton pump inhibitor) therapy in patients with GERD-related supra-esophageal symptoms.
Methods: Twenty-five consecutive patients with chronic (>6 months) LPS were prospectively enrolled from May to September 2011. A previous allergological and ear-nose-and-throat evaluation excluded other than GERD diagnosis. Reflux symptom index (RSI) score was recorded and only patients with a score >13 were considered eligible for the study. 24-h oropharyngeal pH-monitoring was performed after having stopped antisecretive therapy for at least 14 days. All the patients were given a 3 months therapy with pantoprazole 40 mg bid and then repeated the RSI score. Patients were considered as responders if a 5-point decrease in RSI score was recorded.
Results: 15/25 patients (60%) responded to therapy; Groups of responder and non responder patients were comparable regarding to demographic aspects. 10 patients (40%) had a pathological oropharyngeal pH-monitoring study. All the patients with a positive Restech® study (n = 10; 40%) resulted responsive to PPI; 5 patients (20%) with a positive Restech® study resulted responsive too and 10 patients (40%) with a negative Restech® study were non responsive to PPI; with this difference being statistically significant (P = 0.002). Responsive patients resulted also in a higher rate of oropharyngeal acid exposure in the orthostatic position, expressed as Ryan Score, compared to non responsive patients (49.8 ± 58.1 vs 2.12 ± 0.0, P = 0.002). Considering responsiveness to medical therapy as the gold standard for the diagnosis of LPR, the oropharyngeal pH-monitoring with the Restech® probe showed a global sensitivity of 67% with a specificity of 100%.
Conclusion: All the patients with a pathological oropharyngeal acid exposure assessed with the Restech® probe clinically responded to a 3 months course of double dose PPI therapy. The oropharyngeal pH-monitoring resulted in a very high positive predictive value (PPV), however, further data are warranted before the Restech® evaluation could be routinely proposed for patients with LPS.

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Treatment of halitosis: Results of itopride hydrochloride therapy

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Objective: To evaluate the efficacy of itopride hydrochloride in the treatment of halitosis in patients with reflux disease.
Methods: A total of 53 patients with halitosis combined with reflux disease were evaluated (36 women and 17 men aged from 23 to 65 years); oral cavity diseases and ear, nose, and throat inflammatory abnormalities were first ruled out in them. The diagnosis of reflux disease was verified by oesophagogastroduodenoscopy. To evaluate upper gastrointestinal motility and evacuation, all patients underwent ultrasonography of the stomach and duodenum.

Results: Apart from unpleasant breath odours, study subjects had other complains, such as heartburn (42 subjects), a bitter taste (36 subjects - 67.9%), a sour taste in the mouth (39 subjects - 73.6%), eructation (32 subjects - 60.4%), and nausea (28 individuals - 52.8%). Four people cited halitosis as their only complaint. The following conditions were diagnosed in study subjects: endoscopy-negative gastroesophageal reflux disease in 32.1% of cases (17 individuals), catarrhal oesophagitis - 64.1% (34 subjects), erosive oesophagitis - 3.8% (2 patients). Duodenogastric reflux was observed in 12 individuals. All study subjects took Nolpaza 20 mg twice daily (half an hour before breakfast and before dinner) plus Gaviscon 15.0 mL three times per day for 2 weeks, after which clinical changes in the symptoms were evaluated. Halitosis remained at the same level in 5 patients, while 14 subjects had it improved. Duodenogastric reflux ($P < 0.05$), as well as low-motility stomach dyskinesis ($P < 0.05$), was observed statistically significantly more frequently in this subgroup ($n = 19$). We therefore added itopride hydrochloride 50 mg three times per day (half an hour before a meal) to ongoing treatment in this patient subgroup. Treatment outcomes were assessed 3 weeks later: halitosis was eliminated in 17 out of 19 patients, 2 subjects had it significantly improved, which was confirmed by ultrasonography data, as improved gastric motility and a duodenogastric reflux relief were seen in 84.2% and 73.7% of cases, respectively.
Conclusion: The development of halitosis in patients with reflux disease was considerably affected by the duodenogastric reflux and low-motility stomach dyskinesis. Itopride hydrochloride showed a high effectiveness in the treatment of halitosis in this patient category.

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On the choice of prokinetic in the complex treatment of gastroesophageal reflux disease

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Objective: To investigate the influence of different generations of prokinetics on oesophageal lower oesophageal sphincter (LOS) motility in patients with gastroesophageal reflux disease (GERD).
Methods: The study included 78 patients with a diagnosis of gastroesophageal reflux disease verified by clinical and instrumental evaluations. Oesophageal and LOS motility was evaluated using a Polygraf ID multichannel monitoring system (Denmark). Obtained data were analyzed with the Polygram NET application package (Medtronic A/S).
Results: Subjects were divided into three groups comparable in gender, age, and severity of oesophageal inflammation. All patients received a proton pump blocker as basic therapy, once daily in the morning, before a meal. In accordance with the study objective, Group 1 patients ($n = 24$) were also administered metoclopramide; domperidone was given in Group 2 ($n = 28$) and itopride hydrochloride in Group 3 ($n = 26$). After the end of treatment (6 - 8 weeks), 74 patients were evaluated (94.9%). All of them presented with improved LOS tone and average peristaltic wave amplitude in the oesophageal body. The mean LOS pressure values were 17.8 ± 3.1 mm Hg, 16.8 ± 3.6 mm Hg, and 18.1 ± 2.7 mm Hg for Groups 1, 2, and 3, respectively. The mean oesophageal body contraction amplitude values had increased to 83.6 ± 22.3 mm Hg, 78.7 ± 22.6 mm Hg, and 77.5 ± 21.9 mm Hg, respectively. However, metoclopramide-treated patients presented with no statistically significant changes in oesophageal body conduction rate or contraction duration, while their post-swallow LOS relaxation degree had decreased considerably, to a mean of 82.3 ± 9.1% of baseline, leading to symptoms of oesophageal dysphagia in some of the patients. Group 2 patients retained adequate LOS relaxation during swallowing (more than 90% of baseline); they too, like in Group 1, had no statistically significant changes in oesophageal body conduction rate or peristaltic wave duration, and retained their biphasic pattern of distal oesophageal contraction. Group 3 patients were observed to have, along with still adequate LOS relaxation upon swallowing, an increased peristaltic wave conduction rate in the oesophageal body, to a mean of 0.09 ± 0.01 m/sec, and a reduced duration of distal contraction, to a mean of 2.5 ± 0.5 sec, along with a normalized contraction pattern (biphasic to monophasic).
Conclusion: The inclusion of itopride hydrochloride in the complex treatment of gastroesophageal reflux disease was thus shown not just to increase LOS pressure and oesophageal contraction amplitude but also to normalize the contraction pattern without causing LOS relaxation.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-30 Clinical Session: Stomach: Clinical

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Development and validation of a mixed liquid / solid test meal for scintigraphic assessment of gastric function in clinical practice

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Objective: Dyspeptic symptoms after meals (postprandial distress) are common in the community; however current investigations, including scintigraphic measurement of gastric emptying (GE) does not explain the causes of symptoms (Pasricha CGH11). This may be related to use of small, solid test meals that stimulate little response from gastric function or symptoms. Pilot studies using a large liquid meal have shown potentially diagnostic abnormalities in functional dyspepsia (Tucker DDW12); however there are many causes of dyspepsia and assessment of solid emptying may provide additional information in other conditions (e.g. diabetic gastroparesis). Here, we describe the development and validation of a mixed liquid / solid test meal for use in clinical practice.
Methods: Optimal volume and composition of the liquid component for assessment of gastric function was established and GE assessed in 60 age and sex stratified healthy subjects and 8 patients with functional dyspepsia (FD). Optimal physical characteristics were assessed for the non-nutrient solid component (agar balls with known breaking strength) by both scintigraphy and MRI. Pilot values for scintigraphy using a double labelled solid / liquid meal were assessed together with reproducibility of liquid GE alone ($n = 9$) and with non-nutrient, solid component ($n = 11$) healthy subjects.
Results: All healthy volunteers and 92% FD patients tolerated 400ml (0.75kcal/ml) liquid nutrient taken at 40ml/min. Compared to age and sex matched healthy subjects, FD patients had rapid early (gastric content@t = 0 after ingestion 345(333–358) v. 325(310–350)ml; $P = 0.052$) followed by slow late GE rate (3.5(3.0–4.2) v. 2.7(2.1–3.1)ml/min; $P = 0.012$); as a result GE half time was not different (t50 48(39–56) v. 52(44–54)min; $P = 0.710$). Good reproducibility for liquid GE was demonstrated (t50 Bland-Altman bias 5(SD±11)min). All participants could swallow 12x12mm agar balls. Optimal breaking strength to assess solid GE was 0.8N/m2. In healthy subjects at 60min and 120min a median 8(5-10) and 3(1-5) agar balls were present in the stomach on MRI and gastric retention of labelled solid was 80%(74–87%) and 64%(45–81%) on GS respectively. Liquid GE was not altered by co-ingestion of the agar beads (t50 Bland-Altman bias -9 (SD±19)min with solid compared to liquid alone).
Conclusion: The mixed liquid / solid test meal presented provides an objective assessment of (1) liquid GE sensitive to gastric dysfunction in FD and (2) solid GE with objective assessment of work done breaking down solids. The presence of non-nutrient agar balls had no systematic effects on liquid GE and, thus, liquid and solid GE can be assessed independently.

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Performance characteristics of scintigraphic measurement of gastric emptying of solids in health

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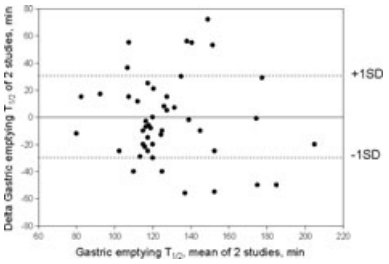
Objective: Gastric emptying of solids (GE) is measured in pharmacodynamic and diagnostic studies. There is limited information about the performance characteristics of scintigraphic GE. Aims: To assess inter- and intra-subject coefficients of variation (COV) of scintigraphic GE measurements in healthy subjects, and associations of GE with gender and BMI.

Methods: Data from healthy participants with scintigraphic measurements of GE of solids were analyzed. Primary endpoints were gastric emptying T1/2 (GE T1/2) and GE at 1, 2, 3 and 4 h.
Results: The patient cohort consisted of 105 males and 214 females; at least 2 studies were performed in 47 subjects [16 males (M), 32 females (F)]. Inter-subject COV (COVinter) for GE T1/2 were similar in M and F: overall 24.5% (M 26.0%, F 22.5%); COV are predictably lowest for GE at 4h (COVinter 9.6%). COVintra for T1/2 and GE4h were overall 23.8% and 12.6%, respectively, and were similar to COVinter values. Gender (but not BMI) was significantly associated with GE T1/2 [$P < 0.001$, F 127.6+/- 28.7 (SD) min; M 109.9+/- 28.6 min] and with GE at 1h and 2h. Repeat GE T1/2 values in 47 participants were significantly correlated ($r = 0.459$, $P < 0.001$) with median difference of -6 min (mean -1.6, range -56 to 72 min). Bland-Altman plots showed Δ GE T1/2 similarly distributed across mean GE T1/2 100 to 155 minutes, and across studies conducted 90 to 600 days apart.
Conclusion: Inter-subject variations in scintigraphic GE results are only slightly higher than the intra-subject measurements; intra-subject estimates are reproducible over time in healthy volunteers. Gender, but not BMI, is significantly associated with GE results.



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Gastroparesis in the joint hypermobility syndrome
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Objective: The Joint Hypermobility Syndrome (JHS) is a non-inflammatory connective tissue disorder characterised by hyperflexibility of the joints and skin. Affected patients have a high prevalence of gastrointestinal (GI), especially postprandial, symptoms. GI dysmotility is common in symptomatic JHS patients. Therefore our aim was to determine the prevalence of gastroparesis in JHS patients with postprandial nausea, vomiting or satiety and to compare this with a non-hypermobile control group that required gastric emptying studies for presumed gastroparesis.
Methods: Over a 3 year period we evaluated 72 consecutive patients (both JHS and non JHS) who were referred from our hospital for gastric emptying studies: age 13–72 years, 54 female. Hypermobility status was assessed using the Brighton classification. Demographic data, GI symptom profile, presence of diabetes and medication history were obtained through medical interview. Gastric emptying was assessed using 13C-labelled octanoic acid breath testing. Diagnosis was based on T 1/2: Normal: <135 min, delayed: 135–200 min, severely delayed: >200 min.
Results: Of the 72 patients, 29 had JHS, 37 had no JHS and 6 were undetermined, and therefore excluded. JHS patients were significantly younger (28 ± 10 vs 39 ± 13, p:0.00), and more likely be female, though this was not significant (83% vs 65%, p:0.1). Gastroparesis was present in 66% of JHS patients and 62% non JHS patients, p:0.78. JHS patients were significantly more likely to have SEVERELY delayed gastric emptying (35% vs 11%, p:0.016)., which was not accounted for by coexistent diabetes, or medication which could cause gastroparesis. The prevalence of GI symptoms was similar in both groups except for higher postprandial satiety as a primary complaint in the JHS patients (21% vs 0%, p:0.039).
Conclusion: There is a high incidence of gastroparesis in JHS patients with post prandial symptoms and this is more likely to be severe and associated with post prandial satiety in comparison to those without JHS. Possible mechanisms in JHS include gastric connective tissue abnormalities, as in scleroderma, or coexistent autonomic dysfunction, which is often associated with JHS. As there is an association between JHS and unexplained GI symptoms, the diagnosis of JHS should be considered in patients with idiopathic gastroparesis.

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Prevalence and outcomes of gastroparesis among hospitalized patients in US
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Objective: Gastroparesis is a common complication of diabetes (DM). Little information is available in regards to the health care burden due to gastroparesis due to diabetes or due to other causes. The aim of our study was to comprehensively describe the frequency, impact and outcomes all causes of gastroparesis among hospitalized patients.
Methods: We used the Nationwide Inpatient Sample database (NIS-The largest all-payer inpatient database of United States) for year 2006 in the age group 18–90 years for our study. We identified patients who were admitted with gastroparesis as the principal diagnosis identified by International Classification of Diseases 9 Clinical Modification (ICD9 CM) diagnosis code for gastroparesis (536.3). To identify patients admitted due to diabetic gastroparesis an additional secondary diagnosis of DM (ICD 9 CM - 250) along with a primary diagnosis of gastroparesis was used. Similarly, cardiovascular complications of DM hospitalizations were identified based on an ICD 9 CM 390 - 448, retinal complications ICD 9 CM-362, renal complications ICD 9 CM-585 as principal diagnosis respectively along with any listed secondary diagnosis of DM. Outcomes including in-hospital mortality, Length of Stay (LOS), total hospital charges were obtained from the database. All statistical analyses was done using STATA MP 10.0 (College Station, TX) using the appropriate survey commands to adjust for the complex sampling design of the NIS.
Results: DM gastroparesis is a common non cardiac complication of DM which accounted for 3488 hospital admissions in 2006. However, DM gastroparesis is the most common diabetic complication for hospitalization among younger age groups. It comprises of 74.5% of the total hospital admissions among 18–35 year old group and 58.1% among 36–50 year old age group. Interestingly, though DM gastroparesis (28.8%) is a significant cause of hospitalizations due to gastroparesis, it was not the most common cause of gastroparesis which comprised of a 12104 total admissions. Also, the mean LOS due to DM gastroparesis was 4.9 days which is slightly lower than that due to non diabetic causes of gastroparesis(5.74).The mean difference in the cost of hospitalization due to non DM and DM causes of gastroparesis was $2293. The total mean economic burden of DM gastroparesis was $76 million and a non-DM cause is $289 million.
Conclusion: Gastroparesis is a significant cause of morbidity and has a significant economic burden in the US. DM gastroparesis is a significant cause of hospitalizations among people of younger age groups. However, non-DM causes of gastroparesis are the most common cause of gastroparesis in general.

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Gallbladder and gastric motility in obese newborns, preadolescents and adults
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Objective: To comparatively study gallbladder and gastric motility in 3 age groups of lean and obese subjects: newborns, preadolescents and adults.
Methods: Lean and obese subjects from 3 different age groups were studied noninvasively: 50 newborns (1–12 months, 6 obese), 18 preadolescents (7–8 years, 7 obese), and 99 adults (22–80 years, 32 obese) classified according to standard normal tables and body mass index. Changes of fasting/postprandial gallbladder and gastric motility were assessed simultaneously by functional ultrasonography in response to milk (newborns and preadolescents) and to a liquid test meal (adults).
Results: In newborns, fasting and postprandial gallbladder volumes and gastric emptying were similar between obese and lean subjects. In preadolescents, obese subjects had a larger fasting gallbladder volume, with slower postprandial gastric emptying than lean subjects. In obese adults, the most evident dysfunction emerged, with larger fasting and postprandial residual gallbladder volume, and slower postprandial gastric emptying than lean subjects.
Conclusion: Obesity is associated with abnormal gallbladder and gastric motility patterns which appear in preadolescents and deteriorate in adults. Such abnormalities are absent in obese newborns. Functional ultrasonography can detect altered cholecysto-gastric motility at the earliest stage. Our findings suggest an age-related decline of motility, probably secondary to excessive fat and insulin-resistance.

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Functional dyspepsia syndrome is extremely frequent across the eating disorders
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Objective: There is a complex relationship between Eating Disorders (ED) and gastrointestinal symptoms. It is well known that a large part of ED patients seek attention from gastroenterologists for GI symptoms. Aim: to study the prevalence of Functional Dyspepsia (FD), according to the Rome III criteria, across the ED

diagnoses: anorexia nervosa (AN), bulimia nervosa (BN) and eating disorder not otherwise specified (EDNOS) (classified according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, DSM-IV) and to compare their prevalence to Constitutional thinness (CT), obese (OB) patients and healthy volunteers (HV).
Methods: 20 AN, 6 BN, 10 EDNOS patients were recruited from an outpatient clinics devoted to ED. Their data were compared to that of 9 CT subjects, 32 OB patients and 22 HV. All participants underwent a careful interview to diagnose FD (Rome III criteria) and a standardized questionnaire for the intensity-frequency score of early satiety, epigastric fullness, pain and burning (0–6). Chi-square, ANOVA and post-hoc Sheffè were used for comparisons.
Results: The study's groups were similar for age and gender distribution. The prevalence of FD was 90% in AN, 100% in BN, 89% in EDNOS, 56% in CT, 18% in HV and 0% in OB patients (χ-square, $P < 0{,}0001$). All participants, who received a diagnosis of FD, fulfilled criteria for Post Prandial Distress Syndrome (PPDS), except 1 BN patient who fulfilled Epigastric Pain Syndrome (EPS) criteria. AN, BN and EDNOS patients showed a significantly higher postprandial fullness intensity-frequency scores compared to CT, OB patients and HV (Scheffè, $P < 0.05$, respectively). Early satiety intensity-frequency scores were significantly higher in AN, EBNOS and CT compared to OB and HV group (Scheffè, $P < 0.05$, respectively).
Conclusion: There is a very high prevalence of FD, in particular PPDS, in ED. Is it mandatory in an outpatient gastroenterology clinic to investigate about ED when patients meet criteria for PPDS?

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Comparison of Continuous Breath Test (CBT) versus Gastric Scintigraphy (GS) for Gastric Emptying Rate (GER) measurement in healthy and dyspeptic subjects

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Objective: Gastric emptying rate (GER) measurement by continuous breath test (CBT) may offer an alternative to gastric scintigraphy (GS). However, there is still little information about normative values of the CBT. The objectives of this study were to determine local normative data for GS and CBT and to compare an automated, real-time, non-radioactive, CBT method versus GS for GER measurement in healthy controls and dyspeptic patients.
Methods: A standard meal of 250 Kcal, double labeled both with 1 mCi of Tc-99 m colloid and 100 μg of non-radioactive C-13 octanoic acid was administered to 22 patients with functional dyspepsia and to 20 healthy controls. Simultaneous, 4 h GER measurement by sequential GS and CBT (BreathID, Exalenz Bioscience, Israel), was performed on all subjects. Gastric half-emptying time (T½) obtained by GS and CBT were compared by linear regression analysis. GS and CBT were compared by kappa test of agreement for normal/abnormal results.
Results: Mean age, M/F ratio and mean BMI (kg m^{-2}) were 51.5 ± 15.12 years., 20/22 and 25, respectively. The upper limit of normal gastric T½ was 100 min by GS and of 140 min by CBT respectively. Linear correlations (R-values) for CBT and GS were 0.63 for gastric T½. The kappa test for agreement of CBT and GS (normal/abnormal) gastric T½ was 0.73.
Conclusion: The novel CBT may be suitable as an alternative method for on-line GER evaluation, in a simple, office based and non-radioactive setting.

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Upper functional gastrointestinal disorders in young adults

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Objective: Functional Gastrointestinal disorders (FGID) are common disorders in gastroenterology which are common in young adults.The aim of this study is evaluating the prevalence of upper FGID in iranian young adults.
Methods: This was a cross-sectional study which was on 995 persons who were going to marry. A ROME III based questionnaire was used to determine the frequency of upper GI Syndromes among the sample population.
Results: Our results determined 74 subjects had functional dyspepsia (36 subjects diagnosed as postprandial distress syndrome patient and Epigastric pain syndrome was seen in 38 subjects). Functional heartburn was diagnosed in 52 participants. Globus was seen in 35 subjects and 41 had unspecified excessive belching.
Conclusion: Many epidemiologic studies were done all around the world but there are different reports about prevalence and incidence of FGIDs. Our results were agreed with reported prevalence of FGIDs in Iran in adults. And our findings were agreed with some other Asian studies.

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Upper gastrointestinal symptoms after laparoscopic sleeve gastrectomy for morbid obesity

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Objective: In the last years, Laparoscopic Sleeve Gastrectomy (LSG) has increased in popularity as both a definitive and staged procedure for morbid obesity. The hypothesized anatomic and physiologic modifications produced by LSG lead to a balance between exacerbating and protective factors for gastro-esophageal reflux disease (GERD). However some of these factors, such as alterations in gastric emptying or compliance, can also contribute to the genesis of dyspeptic symptoms. In addition the relationship between upper gastrointestinal symptoms and GERD is controversial and may have a different origin. Aim of this study is to evaluate upper gastrointestinal symptoms associated to LSG and to investigate PPI efficacy.
Methods: Validated symptom questionnaires for dyspepsia and GERD, following Rome III and Montreal criteria respectively, have been filled in by 67patients (F = 51 and M = 16; median age 45 years, range 27–67) after LSG for morbid obesity (median BMI pre-surgery 46 Kg m^{-2}, range 31.3–64.6). Symptoms were considered newly developed after LSG when appearing for the first time or the pre-surgical symptoms worsen after surgery, and present at least once a week. After performing an overall analysis of symptoms, the influences of the time interval from LSG, weight loss and symptomatic response to PPI treatment have been evaluated.
Results: After LSG, the most prevalent symptoms are postprandial fullness and early satiation configuring a PDS-like dyspepsia rather than GERD. PDS-like is present in 61.2% (41/67) whereas GERD in 23.8% (16/67). No epigastric pain syndrome-like dyspepsia is observed. In dyspeptic patients, nausea is present in 29.3% (12/41) and vomiting in 24.4% (10/41). In GERD patients, atypical symptoms are present: chest pain in 31.2% (5/16), extraesophageal symptoms (hoarseness and cough) in 31.2% (14/67) and dysphagia in 25% (4/16) of patients. This last symptom results also to be associated to PDS-like in 24.4% (10/41). GERD symptoms disappear or improve in 20.9% (14/67) of patients after LSG. Finally, time interval, and weight loss from surgery, do not influence symptoms presentation and the response to concomitant PPI therapy.
Conclusion: After LSG the prevalence of PDS-like dyspepsia is higher than GERD. In addition typical GERD symptoms responded poorly to PPI therapy.

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Comparison of a breath test versus gastric scintigraphy for the measurement of gastric retention in healthy subjects

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Objective: Gastric emptying rate (GER) measurement by breath test (BT) may offer an alternative to gastric scintigraphy (GS). There is no data about normative gastric retention (GR) values of BT. The aims of the study were to determine local normative data for GS and BT and to compare the BT method versus GS for GR measurement in healthy volunteers.
Methods: A standard meal of 250 Kcal, double labeled both with 1 mCi of Tc-99m colloid and 100μg of non-radioactive C-13 octanoic acid was administered to 12 healthy volunteers. Simultaneous, 4 h GER measurement by sequential GS and BT was performed on all subjects. Gastric emptying halftime (T½) and the 4-hour gastric retention (GR) percents obtained by GS and BT were compared by linear regression analysis. GS and BT were compared by kappa test of agreement for normal/abnormal results.
Results: Mean age, M/F ratio and mean BMI were 51.1 ± 14.12 years, 7/5 and 26 kg m^{-2}, respectively. The upper limit of normal gastric T½ was 100 min by GS and of 150 min by BT respectively. Linear correlation (R-value) between BT and GS for gastric T½ was 0.628, $P = 0.028$. The kappa reliability test between GS and BT (normal/abnormal) gastric T½ was 0.625. When GS and BT were assessed for agreement on normal/abnormal results of 4-hour gastric retention (GR) percents, in 12 out of 12 healthy volunteers GS and CBT concurred (100% overall agreement).

Conclusion: The BT method may be suitable as an alternative method for the measurement of gastric T½ and 4-hour gastric retention (GR) percents.

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Chronic transcutaneous electroacupuncture ameliorates dyspeptic symptoms in patients with diabetic gastroparesis: A placebo-controlled multicenter clinical trial using a newly developed microstimulator

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Objective: Acupuncture is performed by a doctor/acupuncturist. Infrequent treatment (2–3 times/week), noncompliance of patients and lack of controlled studies are major contributing factors to inconsistent results in literature. The aims of this project were 1) to develop a novel method of transcutaneous electroacupuncture (TEA) using a custom-made wireless microstimulator and to assess the efficacy of chronic TEA in treating dyspeptic symptoms in diabetic gastroparesis patients.
Methods: Twenty six pts with diabetic gastroparesis were enrolled and 18 of them (50.4 ± 10.8 years, 5M, 13F) completed the study. In a crossover design, each pt was blindly treated at home with 4-week TEA and 4-week sham-TEA in a randomized order. TEA was performed via surface ECG electrodes placed at acupoints PC6 and ST36 using pulse trains: train on of 2 s and off of 3 s, 0.5 ms, 25 Hz and 2–6 mA (appropriate and well tolerated). Sham-TEA was performed using same parameters via non-acupoints. The pts were asked to self-apply TEA/sham-TEA for 2 hrs after lunch/dinner. The electrogastrogram (EGG) and ECG were recorded during 4 visits (beginning and end of each 4-week treatment). Gastroparesis cardinal symptom index (9 questions) and Quality of Life (SF36) were assessed weekly.
Results: (i) Watch-size stimulators were developed, tested and used in the study without any problems. It allowed the pt to resume daily activity during the treatment; (ii) Good compliance was noted: the actual usage of the therapy was 300.3 ± 82.5 min per day (requested usage: 300 min per day). (iii) 4-wk TEA not sham-TEA significantly improved 5 of 9 gastroparesis symptoms: nausea by 29.7% (P = 0.005 vs. baseline), vomiting by 39.3% (P = 0.055), abdominal fullness by 21.4% (P = 0.0047), bloating by 20.6% (P = 0.006), and retching by 31.1% (P = 0.006). A significant improvement in body pain was also noted with TEA. (iv) 4-week TEA not sham-TEA increased % of normal slow waves in both fasting (72.8 ± 14.1% vs. 79.9 ± 13.5%, P = 0.05) and fed (69.5 ± 12.1% vs. 77.4 ± 16.5%, P = 0.04) states. (v) 4-week TEA not sham-TEA resulted in a trend of increase in vagal activity in the fed state (P = 0.08).
Conclusion: The home-based TEA therapy is feasible and effective in treating gastroparesis symptoms and mechanisms involving central, gastric and autonomic functions require further elucidation.

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Transcutaneous electrical stimulation (TES) therapy improves gastric emptying in children with slow-transit constipation – Implication for non-invasive treatment for delayed gastric emptying or gastroparesis

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Objective: Transcutaneous electrical stimulation (TES) therapy improves colonic transit in children with slow-transit constipation (STC). Gastric emptying (GE) in children with STC was recorded as part of a standardised nuclear transit scintigraphy (NTS) protocol. We hypothesized that TES may also affect gastric emptying in these children. We aimed to assess the effect of TES on GE in STC children using NTS.
Methods: STC children (n = 62) treated by TES (2–6 months) were reviewed retrospectively from a NTS database (1999–2011, 344 with STC in 667 children; Ethics30059A&30116A). TES was applied at the level of the umbilicus using quadripolar stimulation. The gastrointestinal nuclear transit study measured GE, small bowel transit and colonic transit. GE was measured by t½ (rate of tracer emptied to 50% of its original activity from stomach, normal <50 mins). Colonic transit was measured by geometric centre based on region of interest at 6, 24, 30 and 48 hours.
Results: Paired NTS of 62 STC children (27 female, age 3–17 years, mean 9 years) pre- and post-TES were available. Twenty-one children had delayed gastric emptying (DGE). STC children who were treated medically (n = 29, 15 female, age 3–17 years, mean 9 years, 9 with DGE) showed no change in GE and colonic transit in NTS assessment repeated after more than 6 months (Table 1). Forty-one STC children with normal gastric emptying (NGE) had TES with no effect on GE. However, in 21 STC children with DGE, TES improved GE significantly.
Conclusion: In STC children, TES improved GE if GE was delayed. Similar effects have been reported for direct gastric electrical stimulation via implanted electrodes in adult diabetic patients with gastroparesis and in children with delayed gastric emptying. TES could be investigated as a non-invasive treatment for patients with gastroparesis or DGE.

Table 1. Effects of medical treatment and TES on GE.

Treatment group	Gastric emptying, t ½ (mins) – Mean ± SD		p-value	n (number of patient)
	Pre	Post		
Medical + NGE	31±10	42±15	<0.01	20
Medical + DGE	77±19	64±19	0.19	9
TES + NGE	33±10	38±18	0.08	41
TES + DGE	67±20	43±19	**<0.001**	21

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Effects of the amino acid, glutamine, on gastric emptying and the glycaemic response to oral glucose in healthy young subjects

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Objective: The rate of gastric emptying is a significant determinant of postprandial glycaemia. Slowing gastric emptying is associated with a reduction in postprandial blood glucose in both healthy subjects and patients with type 2 diabetes. Recent studies suggest that the amino acid, glutamine, may slow gastric emptying and improve the glycaemic response to carbohydrate-containing meals. Accordingly, glutamine may prove useful in the management of type 2 diabetes. The aim of this study was to determine the effects of glutamine on gastric emptying and the blood glucose response to oral glucose in healthy young subjects.
Methods: Eight males (age: 21.6 ± 0.7 years, body mass index: 22.9 ± 0.7 kg m^{-2}) were studied on two separate occasions after an overnight fast. Gastric emptying (2D ultrasound) and blood glucose (glucose meter) were measured following ingestion of 75 g glucose monohydrate (255 kcal) with and without glutamine (30 g) in water (total volume 300 ml) between t = 0–180 min.
Results: The magnitude of the rise in blood glucose from baseline at t = 60 minutes was inversely related to the T50 of the drink after both glutamine (r = -0.74, P = 0.03) and glucose alone (r = -0.77, P = 0.02). Glutamine reduced (P = 0.05) the area under the blood glucose curve between t = 0–30 minutes when compared to glucose alone and there was a trend for gastric emptying of glucose to be slowed by glutamine (T50: glucose 88 ± 9 minutes vs glucose with glutamine 107 ± 11 minutes; P = 0.12).
Conclusion: In a dose of 30 g, glutamine attenuates the blood glucose response to an oral glucose load which is likely to be attributable to changes in gastric emptying.

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This abstract has been withdrawn.

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Identification of novel domperidone metabolites in gastroparesis patients' plasma and urine

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Objective: Domperidone is a dopamine type 2 receptor antagonist that is used as a prokinetic agent to treat gastroparesis. After oral absorption, it is extensively metabolized in the liver. Previous studies reported oxidative metabolites of domperidone, detected by radiometric-HPLC or single quadrupole mass spectrometric (LC-MS) techniques. Our aim was to identify domperi-

done 'phase I' (oxidation/reduction) and 'phase II' (conjugation) metabolites with liquid chromatography (LC) and tandem mass spectrometry (MS/MS).
Methods: Gastroparesis patients (n = 11, 18–65 years old) currently being treated with domperidone at a dose of 10 or20 mg 3–4 times/day were recruited. Subjects were non-smokers and were not on other medications known to be substrates, inducers or inhibitors of cytochrome P450 (CYP) 3A4. At the start of the study, an initial urine sample was collected. Patients were then administered their usual domperidone dose. A blood sample was collected one hour after the dose. Urine was collected (entire volume collection) for 4 h post-dose. All blood and urine samples were stored immediately upon collection at -20 °C until further analysis. *In vitro* incubations were performed in human liver microsomes (HLM) and recombinant purified CYP3A4 to characterize metabolism mediated by CYPs and UD-Pglucuronosyltransferases and in human liver cytosol for metabolism via sulfotransferases. Metabolite identification studies were carried out using LC- MS/MS.
Results: Seven metabolites were detected in human plasma and urine samples. Domperidone was metabolized to two mono-hydroxylated metabolites (M1, M2), a de-alkylated metabolite (M5), and a di-hydroxylated metabolite (M7). M1 was further glucuronidated and sulfated to M8 and M11 respectively. To the best of our knowledge, M7, M8, and M11 have not been reported previously. Six additional metabolites were identified *In vitro* in human subcellular fractions which comprise two additional mono-hydroxylated metabolites (M3, M4), an alcohol metabolite (M6) possibly formed from an aldehyde intermediate, and other conjugative metabolites (M9, M10 and M12).
Conclusion: In total, 12 domperidone metabolites including 6 new metabolites have been identified. Furthermore, M1 to M7 were identified in CYP3A4 incubations indicating that it contributes to domperidone metabolism. These results will ultimately allow a better understanding of domperidone disposition in-vivo in humans.

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Improvement in the quality of life related to reflux symptoms after Helicobacter pylori eradication therapy
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Objective: The relationship between *Helicobacter pylori* (*H. pylori*) eradication therapy and the risk of developing gastroesophageal reflux disease (GERD) is controversial. We investigated the influence of *H. pylori* eradication on the risk of GERD by focusing on the quality of life (QOL) and evaluating reflux symptoms.
Methods: Patients with *H. pylori* infection were administered triple therapy for *H. pylori* eradication. At 3 months and 1 year after the eradication therapy, surveys were conducted to determine the health-related QOL (quality of life in reflux and dyspepsia-Japanese version, (QOLRAD-J)) and the severity of GERD symptoms (Carlsson-Dent questionnaire, [CDQ]).
Results: Forty patients were included in the analysis. Both the scores on CDQ and QOLRAD-J were significantly improved at one year after the eradication as compared with the scores recorded before the eradication (P < 0.05, P < 0.05), although no significant changes were found at three months after the eradication. In regard to the CDQ score being worse than 4 points, which is the recommended clinical cutoff value, significant improvement of the CDQ score was observed even at three months after the eradication (P < 0.01). In particular, 29.4% of patients with worse scores than 4 became totally asymptomatic within three months. The proportions of patients in whom no worsening of the CDQ and QOLRAD-J scores was observed were 71.0% and 82.0%, respectively, at three months after the eradication, and 85.7% and 88.2%, respectively, at one year after the eradication. Moreover, the number of patients who needed to take antisecretory agents was decreased, and the CDQ and QOLRAD-J scores were improved except in patients taking antisecretory agents (P < 0.05, P = 0.055). Furthermore, the PG I/II ratio was significantly increased even at three months after the eradication (P < 0.001).
Conclusion: Improvement in GERD-related QOL and reflux symptoms was observed at one year after *H. pylori* eradication therapy. In addition, the degree of improvement was even more pronounced in cases with severe symptoms. These data suggested that *H. pylori* eradication therapy may be a valid therapeutic option for improving the GERD-related QOL and reflux symptoms.

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Alterations of body weight and hormones regulating food intake during a 1-year weight reduction program in obese outpatients
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Objective: In Germany, 70% of men and 50% of women are overweight (body mass index, BMI >25 kg m^{-2}) or obese (BMI >30 kg m^{-2}). The prevalence is increasing and effective treatment strategies urgently needed. A sustained weight loss of 10% already leads to a marked improvement of the patient's metabolic condition. Aims: To establish an outpatient weight reduction program and investigate effects on body weight and food intake regulatory hormones.
Methods: Eighty-seven obese patients (BMI >30 kg m^{-2}, 66 women, 17 men) were enrolled. The program consisted of increasing physical activity, reducing calories (-500 kcal per day), patient education, behavioral therapy and relaxation. Examinations (interview, physical examination, assessment of body weight and blood withdrawal) were performed before (T0) and at 3, 6, 9 and 12 months. Plasma levels of the anorexigenic hormones leptin and nesfatin-1 and the orexigenic ghrelin were assessed by ELISA.
Results: At the start of the program age matched men and women did not differ in BMI (43.4 ± 3.2 vs. 41.6 ± 1.7 kg m^{-2}, P > 0.05). The BMI decreased at all time points (e.g. T3: -4.9 ± 0.4%, T12: -8.3 ± 1.5%) compared to T0 (P < 0.001) corresponding to a sustained average weight loss of 12.2 kg at 12 months. In line with the decrease of body weight, leptin plasma levels were decreased at all time points (e.g. T12 vs.T0: 4.3 ± 0.3 vs. 6.2 ± 0.4 ng ml^{-1}, P < 0.01) also reflected in a positive correlation of the change of BMI with the change of leptin levels (r = 0.57, P < 0.01). Plasma nesfatin-1/NUCB2 levels were decreased at T6 (0.14 ± 0.02 vs. 0.29 ± 0.07 ng ml^{-1}, P < 0.05) and afterwards increased again. In contrast, ghrelin levels increased at T3, T6 and T9 (e.g. T6: 12.3 ± 1.1 vs. 9.9 ± 0.5 ng ml^{-1}, P < 0.05) and decreased again at T12.
Conclusion: We established an effective program for obese patients leading to sustained weight loss of 12 kg (BMI -8%) after 12 months that was accompanied by decreased leptin plasma levels. Based on the colocalization of ghrelin and nesfatin-1 in the same gastric cell in rats, a finding recently confirmed in humans, this cell could play a pivotal role in the adaptation mechanisms altered under the present conditions of weight loss. The alterations of ghrelin and nesfatin-1 at 12 months irrespective of the sustained decreased body weight may facilitate maintenance of body weight loss after one year.

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This abstract has been withdrawn.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-31 Clinical Session: Small Bowel: Clinical

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Gastrointestinal motility involvement of systemic sclerosis in Chilean patients
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Objective: To characterize the gastrointestinal motility involvement in Chilean patients fulfilling ARA diagnostic criteria for systemic sclerosis (SSc).
Methods: An esophageal manometry, surface electrogastrography, intestinal manometry, and lactulose H2 breath test were performed to 30 SSc patients, mean age 52.7 year old (21–73 year old), 28 female, mean onset 5.8 ± 6.6 year, mean Rodnan score 12.3 ± 8.7. Nine patients presented diffuse SSc (dSSc) and 21 had limited SSc (lSSc). Predominant symptoms (dysphagia, nausea, vomiting, abdominal pain and distension, diarrhea, and constipation) were registered using a VAS score.
Results: Impaired esophageal motility was found in 80% of patients, mostly with hypotensive peristalsis and reduced lower esophageal sphincter pressure (11.9 ± 1.3 mmHg). Only 30% of patients had gastric involvement, mainly bradigastria and low power ratio. The intestinal motility was impaired in 80% of SSc patients, 54% of them with myopathic involvement, 37.5% with mixed pattern (neuromyopathic), and only

8.3% with neuropathic intestinal motility. Orocecal transit time assessed by lactulose breath test was delayed in 30% of SSc patients and 53.3% had small intestinal bacterial overgrowth. The most frequent symptoms in these patients were heartburn (76%), abdominal distension (70%), and dysphagia (63.3%). Comparing dSSc with lSSc patients, we found that dSSc had lower distal esophageal body pressure (25.1 ± 13.6 mmHg versus 48.6 ± 12.1 mmHg*), and a more impaired intestinal motility (with lower amplitude and frequency of MMC's phase II and III*). Orocecal transit time was more delayed in dSSc compared to lSSc (112.5 ± 27.1 min. versus 89.5 ± 20.4 min.*), also dSSc patients reported more severe dysphagia and vomiting*. There were no differences in electrogastrography measures, small intestinal bacterial overgrowth, and the rest of symptoms between these two clinical presentations of SSc. (*$P < 0.05$).

Conclusion: Gastrointestinal motility involvement of systemic sclerosis in Chilean patients is very common, being more severe in diffuse SSc and mostly impairing esophagus and small intestine. This highlights the role of early diagnosis in these patients in order to protect as much as possible, the gastrointestinal tract of microvasculopathy and fibrosis.

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Duodenal mucosal lymphocyte subgroups in patients with persisting abdominal symptoms after Giardia lamblia infection

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Objective: Persisting abdominal symptoms may develop after acute bacterial or viral gastroenteritis and immune activation has been proposed to play an important role. We investigated duodenal mucosal changes in patients after an acute Giardia outbreak in Bergen, Norway, in 2004.

Methods: Ninety-nine consecutive patients with persisting abdominal symptoms after confirmed Giardia infection 3–14 months previously treated with metronidazole, were divided into: (1) patients with treatment refractory chronic giardiasis ($n = 39$), and (2) patients who had no current infection, and diagnosed with post-infectious functional gastrointestinal disorder (PI-FGID) ($n = 60$). Controls were: Recovered controls ($n = 19$), who rapidly recovered after metronidazole treatment; and healthy controls of Giardia unexposed volunteers ($n = 18$). All subjects underwent gastroduodenoscopy with duodenal biopsies. Immunohistochemical staining for CD3, CD4, CD8 and CD20 was performed in intraepithelial lymphocytes (IEL), lamina propria villus (Lpv) and lamina propria crypt (Lpc).

Results: IEL CD4 lymphocytes were significantly increased in chronic giardiasis compared to PI-FGID group ($P < 0.0001$) and healthy controls ($P < 0.0001$), while recovered controls had more IEL CD4 than PI-FGID ($P = 0.008$) and healthy controls ($P = 0.003$). All Giardia exposed subjects had lower IEL CD8 counts compared to healthy controls. CD4 Lpc were decreased in chronic giardiasis ($P < 0.0001$) as well as in PI-FGID ($P < 0.0001$) compared to healthy controls. Recovered controls had more CD4 Lpc cells than both chronic giardiasis and PI-FGID patients. CD8 Lpc lymphocytes was decreased in the chronic giardiasis group compared to PI-FGID ($P = 0.03$), recovered controls ($P = 0.04$), and healthy controls ($P = 0.005$). CD20 Lpc B-cell counts were increased in chronic giardiasis ($P = 0.002$), PI-FGID group ($P = 0.004$) as well as in recovered controls ($P = 0.002$) compared to healthy controls.

Conclusion: We found significant alterations in lymphocyte distribution in duodenal biopsies after Giardia lamblia infection. Postinfectious FGID after Giardia infection as well as chronic giardiasis had decreased CD4 T-cell numbers in lamina propria, suggesting that these patients may have a mucosal immune dysfunction. B-cells (as stained by CD20) remained elevated in both chronic giardiasis and PI-FGID as well as in recovered controls, indicating long-term mucosal immune alterations after Giardia infection.

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Impact of gastric electrical stimulation on duodenal motility

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Objective: Gastric electrical stimulation is currently used to treat medically refractory nausea and vomiting. Most of the studies found no a little association between efficacy of GES and gastric motility or gastric emptying, while duodenal motility has never been investigated to date in implanted patients.

Methods: A 16h-antro-duodenal manometry was performed in 8 patients before and at least 6 months after implantation for GES. Duodenal motility was analyzed 4 h before a test meal (750 Kcal) and then for 12 h. Frequency, amplitude, and duration of duodenal contractions and phase III of the motor migrating complex, as well as the duodenal motility index (MI) and area under the curve (AUC), were calculated pre and post-prandially.

Results: Pre-prandial motility parameters were not different between pre- and post-implantation periods. The 4h post-prandial MI (+11%; $P = 0.04$) and AUC (+43%; $P = 0.01$) were increased after implantation compared to the 4h post-prandial MI and AUC before implantation. This was in part due to the increase in the mean amplitude (+21%; $P = 0.04$) and frequency (+43%; $P = 0.05$) of the 4h post-prandial duodenal contractions. The number and frequency of the phase III of the motor migrating complex was not different between recording made before and after implantation (5.3/16h vs 7.9/16h; $P = 0.08$). However, the amplitude but not the duration of phase III contractions was enhanced after implantation (+36% $P = 0.04$). Lastly, the time to return of phase III after the meal was not different before and after GES (-27%; $P = 0.32$).

Conclusion: An overall increase in the amplitude of the post-prandial duodenal contractions as well as the phase III was observed after GES. Whether this effect is related to a direct action of GES or to an improvement of the nutritional status remains to be assessed.

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Use of ambulatory simultaneous measurements of small bowel intra-luminal impedance and pressure to predict the efficacy of migration motor complex phase III

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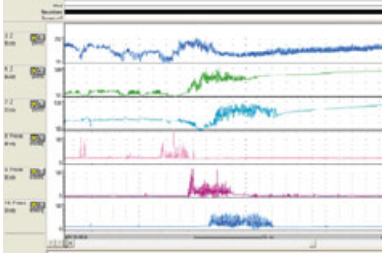
Objective: Standard investigations for patients with suspected enteric neuropathy are small bowel manometry and fluoroscopic assessment. Depending on these results a decision to perform a full-thickness biopsy for histopathological evaluation can be made. Small bowel manometry gives us information of neuromuscular integration by evaluating migrating motor complex (MMC). Transport of intra-luminal contents in the small bowel can be measured by multi-channel intra-luminal impedance. MMC phase III (pIII) is thought to be a house keeping phenomena by clearing intestinal contents. We hypothesised that impedance could be used to assess the efficacy of MMC pIII for antegrade transport of luminal contents, by comparing changes in impedance before and after passage of the pIII.

Methods: Twenty-four patients with suspected enteric neuropathy underwent 24h small bowel manometry+impedance measurements. A three-channel manometry catheter (15 cm apart) was combined with a 3ch impedance electrodes. The catheter was positioned in the proximal small bowel, so that the most proximal pressure+impedance channel was located in D1. Data were recorded on a portable data-logger (Sleuth system, Sandhill Scientific, USA). Visual analysis was performed, on a computer screen (BioView, Sandhill Scientific, USA), to identify MMC pIIIs. The pIIIs were classified as normal or abnormal (simultaneous or very rapid propagation). The mean baseline impedance values in the most distal channel were measured before and after MMC pIIIs.

Results: A total of 67 MMC pIIIs were recorded by pressure, and all of them were identifiable by impedance channels with distinct patterns, consisting of initial drop in impedance coincided with arrival of the pIII front and rapid oscillation of impedance values during phase III contractions followed by a plateau period with increased baseline impedance corresponding to phase I (Fig 1). With normal MMC pIIIs, changes in the baseline impedance before and after the pIII were significantly larger (median 246 Ω, range 139–452, in 44 pIIIs) than those with abnormal pIIIs (median 49 Ω, range -92–224, in 23 pIIIs) ($P < 0.0001$).

Conclusion: Propagated MMC pIIIs were associated with increased baseline impedance after the passage of the pIIIs, suggestive of complete clearance of the intestinal segment. Small or no impedance changes suggest incomplete clearance. The efficacy of pIII clearance measured with impedance can be used to evaluate MMC in patient with suspected enteric neuropathy.



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Double dissociation between severe CIPO, mild neurological, but severe neuroradiological findings: Presentation of 6 cases of Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE)

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Objective: MNGIE is a rare autosomal recessive disorder caused by loss of function mutations in the nuclear gene encoding tymidine phosphorylase that led to mitochondrial DNA defects. Typical findings are severe gastrointestinal dysmotility, cachexia, ptosis, ophthalmoparesis, peripheral neuropathy, and white matter (WM) changes on brain MRI often diffuse despite the absence of mental retardation or deterioration. The aim of this study was to characterize in six MNGIE patients the leucoencephalopathy combining advanced MR techniques such as MR spectroscopy (MRS), and diffusion tensor imaging (DTI).
Methods: Brain MR studies were performed using a 1.5T GE scanner. Six patients with a molecular diagnosis of MNGIE and 14 healthy controls were studied. Conventional T1 and T2-weighetd MR images were acquired. 1H- MRS was used to assess in brain WM concentrations of N-acetyl-aspartate (NAA), marker of neuronal and axonal integrity; creatine-phosphocreatine (Cr); choline (Cho), component of membranes phospholipids. Diffusion tensor imaging (DTI) was acquired to quantify diffusion parameters of water molecules, such as mean diffusivity (MD). Group differences were calculated using the Student T-test. Correlations were performed between 1H-MRS, DTI and clinical parameters, using the Pearson test 1-tailed (statistical significance: $P < 0.05$).
Results: In all six patients a diffuse confluent cerebral and/or cerebellar leucoencephalopathy was present. In patients WM [NAA], [Cr], and [Cho] were lower ($P < 0.05$) and WM MD higher ($P < 0.05$) than in controls. WM MD values were negatively correlated with WM [NAA] and positively correlated with the age of patients ($P < 0.05$). The reduction in all 1H -MRS metabolites in the brain WM of MINGIE patients can be explained by a dilution effect due to increased brain water content demonstrated by the increased WM MD values. This pattern is not reported among other leukoencephalopathies or mitochondriopathies.
Conclusion: Our results are consistent with the functional alteration of the blood brain barrier and the absence of detectable brain axonal/neuronal loss, demyelination, and gliosis demonstrated in a previous post-mortem study of two MNGIE patients (Szigeti K. et al., Ann Neurol 2004). The correlations between MRS, DTI changes and the age of patients suggest that these MR parameters are robust bio-markers of disease, usable for monitoring therapeutic trials.

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Assessment of cine-MRI as a novel diagnostic modality for chronic intestinal pseudo-obstruction (CIPO)

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Objective: Chronic intestinal pseudo-obstruction (CIPO) is an intractable disease in which clinical symptoms of intestinal obstruction appear without mechanical cause. Diagnosis of CIPO is based on radiological evidence of distended bowel loops with air-fluid levels, and exclusion of organic obstruction. Until recently, no single established non-invasive method could have detected the motility disorder of the entire small bowel. Cine-MRI is an emerging technique for video imaging, and has a potential to evaluate small bowel motility function. We assessed the lower gastrointestinal motility in patients with CIPO by cine-MRI in order to evaluate its utility as a novel diagnostic method for CIPO.
Methods: Sequential MRI, using balanced turbo field echo (b-TFE) sequence, was performed in ten healthy volunteers and ten CIPO patients. Using the cine-mode display, luminal diameters of three different points of the small bowel were measured on each image, and temporal changes were plotted on a graph. Luminal diameter, contraction cycle and contraction ratio were compared between healthy volunteers and CIPO patients, and were statistically analyzed by unpaired *t* test.
Results: Mean values with standard deviations of luminal diameter were significantly larger in CIPO patients than in healthy volunteers (44.1 ± 17.1 mm and 10.8 ± 4.0 mm respectively, $P < 0.001$), and those of contraction ratio were significantly lower in CIPO patients than in healthy volunteers (18.7 ± 20.9% and 70.6 ± 11.4% respectively, $P < 0.001$). On the other hand, no significant differences were observed between these groups in contraction cycle (7.54 ± 2.63 s and 7.35 ± 1.41 s respectively, $P = 0.75$). Graphs of temporal changes of luminal diameter showed several abnormal patterns of bowel contraction in CIPO patient.
Conclusion: Cine-MRI can be performed quickly without pain, and therefore is an easier procedure compared to conventional methods. Abnormalities of small bowel movement can be detected clearly because of its high temporal, spatial, and contrast resolution. Measurement of bowel caliber allows both quantitative and qualitative assessment of small bowel motility function. Cine-MRI is a useful and novel modality for diagnosis of CIPO.

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Small bowel motility disorders in vagotomized patients

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Objective: The role of the vagus nerve in initiation and coordination of intestinal motility remains controversial. After a vagotomy, is described a lower and more disorganized activity of the migrating motor complex (MMC). Aim: To evaluate the presence of abnormal intestinal motility in vagotomized patients.
Methods: We studied the small intestinal motility of 22 symptomatic vagotomized patients, mean age 48 years (range 28–76); 17 female, compared to 20 healthy subjects, mean age 38 years (range 22–58); 12 female. Intestinal motility was assessed by perfused catheters and external transducers with standardized technique during 309 ± 47 min and 279 ± 49 min in patients and healthy subjects, respectively. The cyclic activity (CA), duration of MMC, duration of MMC's phases, amplitude and frequency of contractions in phase II and III, intestinal motility score (IMS) and clustered contractions were evaluated. The referral for consultation was chronic diarrhea in 10 patients, constipation in 4, abdominal pain in 3, gastroesophageal reflux in 3, intestinal pseudoobstruction in 3 and 2 patients with gastric retention syndrome. History of total or partial gastrectomy plus vagotomy was found in 12 patients. Statistical analysis was assessed using t-test and Kruskal-Wallis.
Results: From the patients group, 9 did not showed a phase III during the entire study, whereas all from the control group had cyclical activity. The presence of clustered contractions was pathological (>4 per hour) in 4 patients and none in the control group (0.036). There were no differences between gastrectomized and non gastromized patients.
Conclusion: Vagotomized patients with digestive symptoms show severe small intestinal motor disorders. The most frequent symptom after vagotomy was chronic diarrhea. The presence of gastrectomy did not increase the severity of the intestinal motor disorder. The absence of cyclical activity found in almost one third of the patients and the differences in intestinal motility, suggest a neuropathic pattern. However, studies with a greater number of patients are needed.

	N° patients	CA	N° Phase III per hour	Frequency of Phase II (cpm)	Amplitude of Phase II (mmHg)	IMS	Amplitude of Phase III (mmHg)	MMC (min)	Clustered contractions
Vagotomy	22	13	0.31 ±0.4*	0.59 ±0.4*	21.4 ±10.3*	13.4*	22.7 ±5.9*	91.4 ±50	1.68 ±2.3*
without gastrectomy	10	5	0.36 ±0.5*	0.6 ±0.4*	19.1 ±6.8*	12.4*	21.2 ±6.3*	67.5 ±31.8*	1.3 ±2.2*
with gastrectomy	12	8	0.27 ±0.2*	0.5 ±0.5*	23.3 ±12.4*	14.2*	23.5 ±5.8*	101 ±54.2	1.8 ±2.5*
Control	20	20	0.7 ±0.3	1.2 ±0.6	30.5 ±6.7	35.8	37.9 ±11.7	115 ±43	0.2 ±0.5

*p<0,05 compared to control group

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Natural course of adult patients with chronic intestinal pseudo-obstruction exhibiting transition zones in Korea

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Objective: Chronic intestinal pseudo-obstruction (CIPO) is a rare and severe disabling disorder, characterized by chronic and/or recurrent symptoms suggest-

ing bowel obstruction in the absence of fixed occluding lesion. Our objectives in this study were to describe the clinical course of CIPO patients exhibiting transition zones, and to analyze the risk factors for poor outcome.
Methods: In total, 46 adult CIPO patients showing transition zones in radiological exams (20 men/26 women, median age at symptom occurrence 37 [range, 5-73] years) were included, who were followed-up between 1989 and 2010. Clinical characteristics were described and ordinal logistic regression was used to analyze the risk factors for increased number of surgery.
Results: At the time of diagnosis, the patients' median age was 46 years (interquartile range [IQR], 29-63), body mass index 21.2 kg/m2 (IQR, 19-23.4), and prior symptom duration 2.5 (IQR, 1-5). Small bowel dilatation was present in six patients (13%), and transition zone was most frequently located at descending colon (n = 25 [54.4%]). Median number of admissions per patient was 2 (range, 0-22). Cumulative incidence of surgery was 0.59, and median number of surgery among the operated patients was 1 (range, 1-5). There were two patients who showed poor clinical outcome (4.3%). Patients with small bowel dilatation (2/6 [33%] vs. 0/40 [0%], P = 0.014) or myopathy (2/2 vs. 0/44, P = 0.001) showed significantly higher incidence of poor outcome. Multivariable ordinal logistic regression showed that small bowel dilatation was associated with increased number of surgery (odds ratio, 22.6; 95% confidence interval, 1.9-265.8; P = 0.013).
Conclusion: Compared to previous reports, CIPO patients exhibiting transition zones showed considerably milder clinical course, where only 2/46 patients (4.3%) showed poor outcome. Small bowel dilatation seems to be a risk factor for poor outcome and increased number of surgery. Being a single center study, the given results need to be validated in a larger sample of patients.

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Prevalence and clinicoepidemiological features of chronic intestinal pseudo-obstruction in Japan: Findings from a nationwide epidemiological survey

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Objective: The study consisted of 2 quesionnarie surveys, which were distributed to randomly selected departments of gastroenterology, internal medicine, surgery and clinical psychiatry in hospitals throughout Japan. The first survey inquired about the number of the patients treated in 2010, and the second requested additional detailed clinicoepidemiological information about each patient identified in the first survey.
Methods: The study consisted of 2 quesionnarie surveys, which were distributed to randomly selected departments of gastroenterology, internal medicine, surgery and clinical psychiatry in hospitals throughout Japan. The first survey inquired about the number of the patients treated in 2010, and the second requested additional detailed clinicoepidemiological information about each patient identified in the first survey.
Results: In 2010, total number of patients treated in Japan was estimated at 1240 (95% confidence interval, 627 to 1853). This number is lower than that of Crohn's disease that is 30563 in 2008 in Japan. And This number is nearly equal to that of Primary Sclerosing Cholangitis (PSC) in 2007 in Japan. Sex ratio (women to men) of the patients was 1.1. For men, the peak of chronic intestinal pseudo-obstruction was observed in patients aged 70 to 79 years and for women aged 40 to 49 years. The majority (91.0%) were treated as outpatients.
Conclusion: This was the first study to estimate an annual number of patients with chronic intestinal pseudo-obstruction in Japan and to describe the clinicoepidemiological features of the disease.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-32 Clinical Session: Large Bowel: Clinical

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Automated cross-correlation analysis og pan-colonic manometry data clearly differentiates healthy controls from patients with constipation

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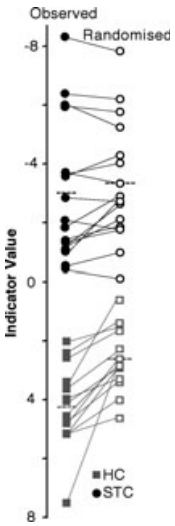
Objective: Colonic manometry traces are generally analysed manually with the majority of studies focusing upon the frequency of propagating pressure waves. Utilising such methods, differences have been noted between patients and controls. However, these findings are not consistent enough to provide a biomarker of disease that will differentiate all patients from healthy controls. Accordingly, we have utilised an automated cross-correlation technique in an attempt to separate the motor patterns of patients with slow transit constipation (STC) from those recorded in health controls (HC).
Methods: Colonic manometry (caecum - rectum) was recorded from 17 patients with STC and 14 HC. The catheter contained 16 recording sites at 7.5 cm intervals. Analysis was performed on the first 4 h from each 24 h recording. The automated analysis involved the calculation of an indicator value, after removing any baseline drift and synchronous anomalies. The data was split into 50% overlapping 2 min blocks. Cross-correlations were calculated between adjacent recording sites for each block, then modified by subtracting negative from positive lags, and squared. The log of the average of the resulting data was used as the indicator value. Automated analysis was conducted on blinded data sets. To determine the likelihood of positive and negative indicator values occurring by chance, the channel numbers within each individual data set were randomised and then re-analysed.
Results: In controls the observed indicator value (4.2 ± 1.4) was significantly greater than that predicted by chance (random distribution) (2.6 ± 1.0; P < 0.0001). In patients the observed indictor value (-3.0 ± 2.4) did not differ from chance (-3.3 ± 2.0; P = 0.1). The average indicator value for the controls differed significantly from the patients (HC, 4.2 ± 1.4 vs STC, -3.0 ± 2.4; P < 0.0001), with no overlap between groups.
Conclusion: Automated analysis of colonic manometry data using cross correlation clearly separated the HC from STC. The indicator value suggests that motility in HC consists of coordinated higher amplitude propagating pressure waves in comparison to patients which display a high frequency of poorly co-ordinated lower amplitude pressure waves. The indicator values may represent a means for defining subtypes of constipation (Supported by NHMRC & FMC Clinicians Special Purpose Trust Fund).



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24 hour assessment of pancolonic motor activity in health and disease using solid-state technology: Compatision with traditional water-perfused catheters

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Objective: Study of colonic motor activity is limited by available technology. To date, assessment of pancolonic motility has only been effectively achieved with the use of water-perfused catheters (WP). However, these do not allow subject ambulation and involve infusion of water during the study period. Only few colonic manometric studies performed using solid-state (SS) catheter with very limited sensors. Longer SS catheter able to span the whole length of the colon are now available, and have the potential to overcome many limitations, which may aid our understanding of the mechanisms regulating colonic contractility in health and disease. This study aims to compare quali-

tative and quantitative differences (if any) of 24 h pancolonic motor activity recorded with a WP and SS catheter on two different occasions in the same subjects.
Methods: Four healthy volunteers (all F, median age 47, range 31 - 55) underwent colonoscopic placement of custom-made pancolonic WP and SS catheters, which were clipped to the caecum on two separate occasions. Both catheters recorded pressures at 16 sites, spaced 7.5 cm apart. Recording commenced 24 hr following intubation and continued for 24 h. Standard meals were provided throughout the study period.
Results: SS catheter recordings showed a significant increase in the overall frequency of antegrade ($P = 0.0005$) and retrograde ($P = 0.04$) propagating sequences (PSs); the frequency of high amplitude PSs was similar between both studies ($P < 0.05$). The colonic motor response to meals and morning waking was normal in recordings obtained from both catheters. However, the well-recognised nocturnal suppression of colonic contractility was practically absent in the SS catheter recordings. Spatiotemporal organisation (regional linkage), pre-defaecatory stereotypical patterning of PSs and amplitude of PSs was similar between both catheters. Interestingly, only one defaecation episode was recorded during the 4 SS studies, compared to 11 episodes during the WP studies ($P = 0.003$).
Conclusion: Detection of overall frequency of colonic propagating contractile activities is markedly influenced by the recording technique. SS technology appears to be more sensitive than WP catheters, and nocturnal suppression of motor activity was not evident, challenging traditional concepts. These data highlight the fundamental importance of recording technique when comparing studies performed within different institutions.

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An evaluation of neuropsychological performance in Irritable Bowel Syndrome (IBS): Relationship between altered visuo-spatial memory function, salivary cortisol levels and tryptophan metabolism along the kynurenine pathway

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Objective: Irritable bowel syndrome (IBS), is a poorly understood stress-related disorder of the brain-gut-microbiome axis. Recently, a cognitive neurobiological model of IBS has been proposed (Kennedy et al., 2012), based on some of the key pathophysiological features of IBS, such as stress-related changes in hypothalamic pituitary adrenal (HPA)-axis functioning, and the immune-mediated degradation of tryptophan along the kynurenine pathway. However, a comprehensive neuropsychological assessment across a broad number of cognitive domains is lacking. Moreover, the influence of mechanistically relevant biological indices on neuropsychological performance in IBS is unknown. This study sought to redress these deficits by assessing if IBS patients display altered cognitive performance when compared to healthy controls (HC) across the domains of executive function,visuo-spatial memory, working memory and attention.
Methods: Thirty-eight IBS patients (6M/32F; mean age 28 years; IQ: 105.9; BMI: 23.9), and 38 matched HC (11M/27F; mean age 29 years; IQ: 108.6; BMI: 23.6) were assessed using a selection of cogntive tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) and Stroop test. A venous blood sample was acquired for tryptophan analysis by HPLC.
Results: No significant differences in performance were found between IBS patients and HC on the Intra-Extra Dimensional Set Shift (IED; executive functioning); total errors (25.2 ± 3.3 vs 25.3 ± 3.4, $P > 0.05$), Spatial working memory (SWM); total errors (22.8 ± 2.6 vs 19 ± 3.1, $P > 0.05$), or Stroop response time (219.9 ± 35.7 vs 192.7 ± 39.1, $P > 0.05$). IBS patients made significantly more errors than HC on the Paired associated learning (PAL; visuo-spatial memory) 6 pattern stage (3.3 ± 0.7 vs 1.5 ± 0.4, $P < 0.05$). There was a trend towards an inverse correllation between cortisol concentrations and errors on the PAL 6 pattern stage in IBS patients ($r = -0.32$, $P = 0.06$). Moreover, there was a positive correlation between PAL 6 errors and the kynurenine: tryptophan ratio in IBS patients ($r = 0.37$, $P < 0.05$).
Conclusion: These data demonstrate for the first time that IBS patients display a visuo-spatial memory deficit. Furthermore, these deficits may be related to mechanistically-orientated biomarker determinations. This lends support to the cognitive neurobiological model of IBS and may inform the future management of this debilitating disorder.

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Investigation of the efferent spinofugal axis by trans-lumbar and trans-sacral magnetic stimulation in Irritable Bowel Syndrome (IBS) and Interstitial Cystitis (IC)

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Objective: We investigated the trans-lumbar and trans-sacral MEPs induced by magnetic stimulation in patients with IC and IBS.
Methods: We measured latency onset (ms) and amplitude (μV) of the rectal and anal MEP following trans-lumbar and trans-sacral magnetic stimulation (figure) bilaterally (efferent spinal-anorectal axis), in 16 IBS patients, 7 IC patients and compared the data with 7 healthy controls (HC).
Results: Median latencies for the onset of MEPs responses in the anal and rectal regions following similar intensity magnetic stimulations of the spine at the lumbar and sacral regions were shorter ($P < 0.05$) on both sides in IC and IBS patients compared to healthy controls (table) indicating more rapid conduction and hyperexcitability of lumbar and sacral root nerves that innervate the anorectum. MEP amplitudes were not significantly different ($P > 0.05$). There were no differences between IBS and IC patients.
Conclusion: The efferent spinofugal-anorectum neurobiologic axis is significantly deranged and shows hyperexcitability in both IBS and IC patients. This rapid conduction across lumbar and sacral pathways and at both, the rectum and anus regions suggest that the peripheral neuroenteric system is more easily excitable and reactive for the same intensity of stimulation and may explain why these patients experience stool and urinary urgency. This study was funded by MAPP grant (NIH-1U01DK082344-01).

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The relationship between colonic breath methane excretion and IBS symptoms: The role of colonic methane production

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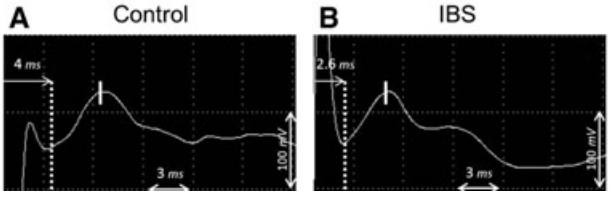
Objective: It was previously suggested that In IBS patients breath methane (CH4) excretion correlates with clinical presentation and colonic transit (Kunkel D 2011, Chatterjee S 2007), but the pathophysiological role of CH4 in functional disorders needs further investigation. Very few data on colonic production of CH4 are available; therefore, the aim of this study was to evaluate in IBS patients the relationship between intestinal production and breath excretion of CH4 and correlate CH4 production with presence and severity of symptoms.
Methods: A group of 225 IBS patients (Rome III criteria) was enrolled. In all the patients, presence and severity of symptoms by VAL and gastrointestinal transit with radio-opaque markers (Metcalf AM 1987) were evaluated. All patients underwent breath H2/CH4 measurement for 7 hours after lactulose, 12g/250cc water (Strocchi A 1993); in a subgroup ($n = 68$), H2 and CH4 were also measured in rectal samples, obtained through a catether positioned at the rectosigmoid junction.
Results: Eighty-three (37%) patients were breath H2 and CH4 excretors, 142 (63%) excreted only H2. Symptom severity and gastrointestinal transit in CH4 excre-

	Age	Translumbar MEP (ms)				Trans-sacral MEP (ms)			
		Left		Right		Left		Right	
	mean (±SD)	Rectal	Anal	Rectal	Anal	Rectal	Anal	Rectal	Anal
HC	39 ± 4.4	3 (2.9-3.5)	3.5 (3.1-3.8)	3.5 (2.8-4)	3.5 (3.1-3.8)	3.6 (3.2-4)	3.5 (3.2-3.8)	3.4 (3-4.2)	3.3 (2.8-3.8)
IBS	33 ± 2.4	2.6 (2.4-2.9)*	2.3 (2.1-2.6)*	2.3 (2.2-2.5)*	2.5 (2-2.7)*	2.3 (2.2-2.9)*	2.3 (2.2-3.4)*	2.3 (2.2-2.5)*	2.5 (2.3-2.8)*
IC	47 ± 6.4	2.3 (2.2-2.5)§	2.3 (2.1-2.4)§	2.3 (2.5-2.9)§	2.4 (1.8-2.8)§	2.4 (2.3-2.5)§	2.3 (2.2-2.5)§	2.3 (2.2-2.4)§	2.4 (1.4-2.6)§

* HV vs IBS; § HV vs IC; p< 0.05 *ms = median, 25-75%*



tors were not different than in non excretors. Cumulative breath CH4 and H2 excretion were not significantly correlated with severity of symptoms. Moreover, IBS subtype prevalence was not significantly different between CH4 excretors and non excretors. In 26 out of 68 tested patients (38%) CH4 was detected in rectal samples. Among them, 14 subjects (54%) excreted CH4 in breath, and the remaining 12 (46%) did not. Mean CH4 colonic production in breath excretors was 169054 ± 162925 ppm x min, significantly higher than in non excretors (6508 ± 8101 ppm x min), but breath CH4 excretion was not correlated with colonic production. Colonic CH4 production did not correlate with severity of symptoms.
Conclusion: Our data show that breath CH4 excretion is not associated with clinical presentation in IBS patients and does not correlate with colonic production, which, in turn, is not correlated with symptom severity. Clinical inferences based on breath CH4 excretion should undergo an in-dept revision.

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Lack of any correlation among colonic transit time, bowel motions frequency and stool form in patients with Spinal Cord Injury

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Objective: Bowel dysfunction has a significant impact on the Quality of Life of Spinal Cord injured (SCI) patients, and moreover morbidity and even death may follow it. In healthy and disease, hard as well as fluid stools are predictive for different intestinal transit patterns. Delayed Colonic Transit Time (CTT) is found in patients with SCI, independently by level and completeness of the lesion. We investigated what relationship among transit time, evacuation frequency and stool consistency characterize bowel dysfunction in SCI patients
Methods: A single abdominal X-ray film was obtained after daily ingestion of 10 radioopaque pellets for six days, during which 65 patients (17 males, 15 tetraplegic and 50 paraplegic, mean age 37 ± 11 years) scheduled defecation with the usual modalities adopted for assisting evacuation. Evacuation number and types of stool according to the Bristol Stool Form Scale (BSFS), were recorded in the same study week. Total Intestinal Transit Time (TITT) was calculated according to the Bouchoucha's method [DCR 1992; 35: 773]
Results: The average CTT was 98 ± 62 hours (range 31 – 155 h), resulting a delay (normal value <60 h) in the 86% of patients. No differences were found between tetraplegic and paraplegic (95 ± 43 h vs 101 ± 81 respectively) patients. By contrast, bowel motion frequency was in the normal range (4.2 ± 2.5 in the 7 days). Patients reported 268 bowel motions: hard stools (BSFS types 1, 2 and 3) characterized the 30% of evacuations and fluid/watery stools (types 6 and 7) the 22%. Regular and soft stools (types 4 and 5) occurred in the remaining 48%. No inverse correlation between CTT and evacuation frequency was shown (Pearson's correlation: r = -0.15, P = 0.3). Moreover, no relationship was found also between BSFS score and CTT for each patient (Pearson's correlation: r = 0.23, P = 0.2).
Conclusion: This study confirms that in a very high percentage of patients with SCI colonic transit is delayed, and no relationship exists with level of the lesion. In these patients slow transit occurs also if the bowel motion frequency is normal and, moreover, stool form and consistency are not predictive for the pattern of colonic transport, suggesting that evacuation is incomplete and fecal impact can occur even if defecation features are normal.

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Functional diarrhoea is the most frequent diagnosis in patients with chronic diarrhoea

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Objective: Chronic diarrhoea is common and a key symptom in many different conditions. Diseases causing diarrhoea can be organic, such as inflammatory bowel disease (IBD), microscopic colitis and idiopathic bile acid malabsorption (IBAM), as well as functional. The aim of this study was to investigate the proportion of subjects with functional diarrhoea versus organic GI diseases in patients submitted for investigation of chronic diarrhoea.
Methods: Patients referred to our outpatient clinic because of chronic diarrhoea were included. Subjects with a previously known disorder causing chronic diarrhoea were excluded. All subjects had a standardised investigation plan including laboratory testing, stool cultures, upper endoscopy and colonoscopy with biopsies as well as SeHCAT test for bile acid malabsorption. All patients were asked to complete a questionnaire assessing stool frequency and consistency, as well as other GI symptoms (pain, bloating and gas). A commonly used criteria for chronic diarrhoea were then applied, i.e. ≥3 defecations/day for at least four weeks (faecal weight was not measured).
Results: We included 207 subjects (mean age 45 (17 – 84) years, 121 females). Sixty-eight of these were excluded - six because of a previously known cause of the diarrhoea, 31 withdrew their consent and 31 did not complete the questionnaires - leaving 139 subjects for evaluation. Forty-eight patients had functional diarrhoea and 91 had an organic cause: IBD (n = 8), IBAM (n = 48), microscopic colitis (n = 24), and other (n = 11). 53/139 had ≥3 defecations/day (10/53 functional disorder, 43/53 organic disease), whereas 86/139 had <3 defecations/day (38/86 functional disorder, 48/86 organic disease) (P = 0.004). GI symptom scores did not differ significantly between the groups.
Conclusion: Functional diarrhoea is the most common diagnosis in patients referred for investigation of chronic diarrhoea. The most commonly used criteria for chronic diarrhoea is not a clinically useful tool to differ between functional and organic disorders.

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Impact of functional dyspepsia, GERD, depression and anxiety on patients with Irritable Bowel Syndrome (IBS)

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Objective: The patients with irritable bowel syndrome (IBS) frequently accompany with functional dyspepsia (FD), gastroesophageal reflux disease (GERD), depression (DP) or anxiety (AX). The aim of this study was to analyze the impact of FD, GERD, depression and anxiety on patients with IBS, using Likert score of IBS symptoms, IBS-specific quality of life (IBS-QOL), and Short Form (SF)-36 representing Health related-QOL (HR-QOL).
Methods: A total of 175 IBS patients fulfilled the Rome-III criteria were enrolled. One hundred forty eight patients completed self-administered questionnaires on sociodemographics, symptoms for IBS, FD and GERD, Zigmond's Hospital Anxiety-Depression Scale, IBS-QOL and SF-36. The remained 27 patients completed the questionnaires except the symptoms for FD and GERD.
Results: The 148 patients who completed the questionnaires were divided into two major groups (A and B) and each group into three subgroups (A-1, A-2, A-3, B-1, B-2, and B-3), and the remained 27 patients were included only for group B analysis as followings; IBS with either FD or GERD (A-1, n = 39), IBS with both FD and GERD (A-2, n = 72), IBS without FD and GERD (A-3, n = 37), IBS with either DP or AX (B-1, n = 30), IBS with both DP and AX (B-2, n = 18), and IBS without DP and AX (B-3, n = 127). There was no difference between A-1 and A-3 on IBS-QOL. The IBS-QOL scores tended to be lower in A-2 than in A-3. The scores of dysphoria, social reaction and relationship domains were significantly lower in A-2 than those in A-3 (Table). The IBS-QOL scores of B1 or B2 were significantly lower than those in B-3 (Table). The results of SF-36 had the same patterns with those in IBS-QOL. The IBS symptoms were more severe in B-1 and B-2 than those in B-3.

Table. The comparisons of IBS-QOL between the patient groups

Domains of IBS-QOL	A-2	A-3	*P-value*	B-1 and B-2*	B-3	*P-value*
Dysphoria	62.3±25.6	73.5±22.3	0.04	59.1±22.1	70.0±24.0	<0.001
Interference with activity	64.6±22.5	70.7±23.8	0.28	59.8±21.4	70.6±22.7	<0.001
Body image	71.9±24.9	76.9±21.6	0.22	64.7±23.4	77.4±21.4	<0.001
Health worry	58.3±24.0	63.4±23.7	0.21	54.3±21.7	62.5±23.5	0.03
Food avoidance	56.8±28.9	64.1±28.9	0.06	52.9±25.3	60.3±29.1	0.12
Social reaction	68.7±22.3	78.8±21.3	0.03	65.8±22.0	77.4±21.4	<0.001
Sexual	85.2±22.4	85.6±20.4	0.41	77.3±23.3	86.9±20.3	<0.001
Relationship	72.1±24.5	83.9±20.4	0.03	69.2±21.9	79.1±22.4	0.01
Overall	65.3±21.4	74.7±20.5	0.06	61.3±18.7	71.1±20.7	<0.001

IBS, irritable bowel syndrome; QOL, quality of life.

A-2 group, IBS with both FD and GERD; A-3, IBS without FD and GERD; B-1, IBS with either depression or anxiety; B-2, IBS with both depression and anxiety; B-3, IBS without depression or anxiety.

* The combined scores of B-1 and B-2 groups

Data are shown as mean ± SD.

Conclusion: The QOL of IBS patients accompanied with FD and GERD at the same time tended to be worse than those of IBS without FD and GERD. The IBS patients with either depression or anxiety showed worse QOL and symptoms than those without depression and anxiety.

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Use of antibiotics during infancy and childhood and risk of recurrent abdominal pain

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Objective: To explore the relationship between antibiotic (Ab) use during infancy and childhood and the occurrence of recurrent abdominal pain (RAP) at 1, 2 and 12 years.
Methods: A Swedish population based birth cohort (BAMSE) of 4089 newborns was followed for 12 years. Parental questionnaires were used to collect information on Ab use and of RAP in infancy and childhood. Our main outcome at 12 years was RAP self-reported by the children. Logistic regression was used to calculate Odds Ratios (OR) and 95% Confidence Intervals (CI) for RAP at 1, 2 and 12 years as a function of Ab use in earlier ages and adjusted for potential confounders.
Results: Questionnaires with complete data were available for 2,663 children. The proportion of children who had received Ab treatment at different ages was: 42% during the first year of life, 61% during the second year of life, 72% between 2–4 years and 88% between 0–8 years. The prevalence of RAP at different ages was 4% (1 year), 3% (2 years) and 9% (12 years). Children with parent-reported RAP at 1 year had more often received Ab in the first year of life (OR 1.65, CI 1.13-2.42; $P = 0.01$). RAP at 12 years was not associated with use of Ab during the first year of life (OR 1.20, CI 0.92-1.57, $P = 0.18$), the second year of life (OR 1.13, CI 0.86-1.49, $P = 0.39$) or between 2–4 years (OR 0.88, CI 0.66-1.17, $P = 0.38$). Children who had received Ab between 0-8 years were less likely to report RAP at 12 years (OR 0.68, CI 0.47-0.98, $P = 0.04$), suggesting that Ab use between 5–8 years decreases risk of RAP at 12 years. Risk estimates did not differ by indication for Ab treatment (data not shown).
Conclusion: Use of Ab during the first year of life is associated with an increased occurrence of RAP at 1 year, but reverse causality cannot be excluded. Use of Ab between 0–4 years was not associated with occurrence of RAP at 12 years, whereas use of antibiotics between 5–8 years was associated with a decreased occurrence of RAP at 12 years. The mechanisms involved are unclear but we speculate that antibiotics interfere with the gut microbiota early in life and thus may modulate immune activity in the gut mucosa and pain sensitivity to visceral stimuli.

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Nutritional profile and fiber intake in children with functional gastrointestinal disorders related to constipation

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Objective: Few studies in pediatric population concerning fiber intake due to nutritional status in patients with functional gastrointestinal disorders related to constipation (FGDRC) exist. Objective: To describe nutritional profile of children with FGDRC and associate with fiber intake.
Methods: Sample; 49 children aged 5 to 17 years diagnosed with FGDRC according to Rome III Criteria, establishing subgroups: Functional Constipation (FC) and Irritable Bowel Syndrome with Constipation (IBS-C). Study period: 01-01-11 to 31-12-11. Z scores were estimated for weight, height and body mass index (BMI) according to WHO references adapted by the Argentine Ministry of Health. Nutritional status (NS) was established according to Z score percentile for BMI: Normal (No), (Pc 10 to 85), Underweight (Un) (Pc <10) and Overweight/Obesity (Ov/Ob) (Pc >85). Fiber intake was estimated by the formula: age + 5 and adecquacy was established according to initial consumption by 24 hour dietary recall.
Results: 44.9% female, 55.1% male, mean age 9 years 5 months ± 3 years 1 month. Diagnosis: a)FC 75.5%, IBS-C 24.4%, no significant differences by gender (Fisher $P = 0.507$) or between children aged 10 and under (Fisher $P = 1.00$), b) NS: Normal 73.4%, (95% CI 61-85), Ov/Ob 21.6% among children with IBS-C and 41.6% among those with FC without enough evidence to differ significantly (Proportion p Test=0.17), with mean height Z score -0.37 ± 1.1, and mean BMI Z score 0.66 ± 1.58. Total fiber intake was inadequate for 98% of the sample with a mean of 6.7 ± 1.58 (95%CI 6.1-7.3) without differences between the two groups (Welch Test $P = 0.60$).
Conclusion: The sample of children with FGDRC showed an overall 26.5% Ov/Ob (95% CI 14-39) with an inadequacy of fiber intake for almost all of the population study (98%) regardless of FGD diagnosis.

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Postinfectious irritable bowel syndrome after traveller's diarrhoea: Incidence and risk factors

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Objective: Infectious gastroenteritis is known as a risk factor for developing irritable bowel syndrome (IBS). There are only few studies concerning postinfectious IBS after traveller's diarrhoea (TD) and reported incidences range between 3% and nearly 15%.
Methods: This study aims to establish the incidence and risk factors of postinfectious IBS after TD. For this purpose outpatients of the Institute of Tropical Medicine in 2009 and 2010 with microbiologically confirmed and/or clinical highly suspected TD were contacted ($n = 475$) and invited to fill out a questionnaire. The questionnaire consisted of items concerning IBS according to the Rome III criteria, the Hospital Anxiety and Depression Scale (HADS) and the somatization module of the Patient Health Questionnaire (PHQ). Additional information concerning clinical features of TD and sociodemographic data were collected.
Results: 175 patients agreed to take part, 135 completed questionnaires were sent back (77.1%). Just under half (41.5%) were male, the mean age was 36.6 ± 14.6 years. Nearly all participants (97.8%) lived in Germany. Excluding patients with pre-existing IBS the incidence of postinfectious IBS after TD was 8.9%. Subjects with postinfectious IBS were predominantly female (11/12; $P = .015$) and had significantly higher anxiety (t = 2.98; $P = .003$) and somatization scores (t = 3.77; $P < .001$). There were no associations found between the development of postinfectious IBS and weight loss, use of antibiotics or the causing pathogen.
Conclusion: The found incidence of 8.9% underlines the importance of infectious gastroenteritis in the development of IBS. Female sex, a predisposition to anxiety and somatization are associated with the development of postinfectious IBS. (supported by a grant from fortuene - No. 2049-0-0, Tuebingen).

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Prevalence of irritable bowel syndrome during pregnancy trimesters

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Objective: Irritable bowel syndrome is one of the most common functional gastrointestinal disorders which is presented by bowel habit change and abdominal pain without any structural disease. Prevalence of IBS in western countries is more than eastern countries. Prevalence of IBS in Iran is reported between 2.6-18%. The etiology of IBS is still unknown but many factors such as motility disorders, genetics, nutrition and inflammation, behavioral disorders and abdominopelvic intervention may cause IBS or exacerbation of symptoms. Pregnancy is a status in which anxiety will increase. Gastrointestinal symptoms are common in pregnancy. Nausea and vomiting is seen in half of pregnant women with an unknown etiology. According to the relation between IBS symptoms and abdominopelvic intervention, anxiety and depression this study was designed to evaluate the IBS symptoms changes during pregnancy.
Methods: This was a cross-sectional study which was done in Shariaaty hospital in Isfahan on pregnant women in three trimesters of pregnancy to evaluate IBS symptoms changes and comparing them with control group. Patients were included to the study after full-filling the questionnaire.
Results: 101 patients in first trimester, 110 in second and 112 in third trimester and 98 as control were included. IBS was seen in 17 (17%) of first trimester group, 21 (19%) of second trimester group, 38 (33.9%) of third trimester group and 13(13.3%) patients in control group. There was a significant deference between groups ($P < 0.05$). IBS-C had a significant increase in third trimester comparing with first and second trimester ($P < 0.05$).IBS-D had a significant increase in second and third trimester comparing with control group ($P = 0.042$).IBS-M had a significant increase in third trimester comparing with control group ($P = 0.008$).
Conclusion: In this study prevalence of IBS was higher than control group and this could be because of hormonal changes and psychological factors which are changing during pregnancy.

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Intestinal symptoms and psychological status determined the quality of life for patients with Irritable Bowel Syndrome with Diarrhea (IBS-D)

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Objective: The quality of life (QOL) in patients with irritable bowel syndrome (IBS) is poor. Both IBS symptoms and psychological status might impair patient's QOL; it is unclear which plays the determinant role. We aimed to explore the relation between IBS symptoms and psychological status, and to reveal determinant factor for QOL in IBS with diarrhea (IBS-D).
Methods: IBS-D was diagnosed with Rome III criteria and all patients had biochemical and colonic examinations to exclude with organic and metabolic diseases. Gastrointestinal symptoms were collected by well trained investigators, psychological status was evaluated with Hamilton Rating Scale for Depression/Anxiety (HAMD/HAMA), IBS-specific quality of life (IBS-QOL) was used to evaluate patient's QOL.
Results: A total of 155 IBS-D patients were consecutively enrolled. The overall score of IBS-QOL was 71.61 ± 19.22, patients with persistent IBS symptoms had lower QOL than those with episode symptoms (65.62 ± 22.47 vs 80.26 ± 12.63, $P = 0.003$). Among eight domains of QOL, score of food avoidance was lowest (53.23 ± 26.82), body image domain was with highest score (85.28 ± 16.75). There was no association between global bowel symptoms score and HAMD/HAMA score ($r1 = 0.103$, $P = 0.203$; $r2 = 0.104$, $P = 0.200$), but comorbid with moderate ~severe depression and anxiety did worsen patients' feeling to subjective bowel symptoms, such as abdominal pain, urgency. There were negative correlations between global bowel symptoms score, HAMD score, HAMA score and IBS-QOL ($r1 = -0.387$, $P1 < 0.001$; $r2 = -0.268$, $P2 = 0.001$; $r3 = -0.262$, $P3 = 0.001$, respectively). Frequency of abdominal pain and/or discomfort with defecation and passing mucus, intensity of urgency to defecation were the independent predictors for lower QOL. The score of ''retardation'' factor in HAMD scale and ''somatic anxiety'' factor in HAMA scale decreased IBS-QOL score significantly ($P1 = 0.007$, $P2 = 0.027$). The increase of global bowel symptoms score diminished the QOL score more intensively than the comparable change of HAMD/HAMA score ($P < 0.001$).
Conclusion: While IBS-D patients had persistent onset, the global bowel symptoms did play a determinant role for their quality of life, comorbid with depression and anxiety worsened patients' feeling to bowel symptoms and quality of life.(Supported by grants of 2007BAI04B01, 2010AA023007).

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Design of studies with combined pharmacodynamic and clinical endpoints for colonic motility disorders: Clinical and statistical considerations

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Objective: Colonic transit by scintigraphy has well characterized intra- and inter-subject coefficients of variation (COV), responsiveness to therapy, and prediction of efficacy of experimental medications in phase IIB or III trials in patients with lower functional gastrointestinal disorders (FGID) (Clin Pharmacol Ther 2010;87:748–753). Aim: To appraise the COVs of bowel function endpoints in patients with lower FGID, and compare with those of the validated pharmacodynamic (PD) endpoint, colonic geometric center at 24 h.
Methods: We evaluated data from the placebo arm of 5 previously published phase IIA, parallel-group, placebo-controlled clinical trials of the PD effects of drugs (linaclotide, dexloxiglumide, renzapride, elobixibat, and chenodeoxycholate) in patients with lower FGID. Patients filled out daily diaries for at least 7 days documenting each bowel evacuation, the stool consistency based on 7 point Bristol stool form scale, and ease of passage on a 7 point scale ranging from manual disimpaction to incontinence. Eleven patients had received placebo in two separate studies, allowing assessment of intra-patient COVs for the GC and bowel functions. We sought to identify the sample size required to demonstrate a 30% effect size (with 80% power) in colonic transit, stool frequency per day, stool consistency and ease of passage.
Results: Data from 52 patients and COVinter are shown in Table 1A. Data from 11 patients who had 2 sets of data are shown in Table 1B.
Conclusion: The COVs for PD endpoints are lower than those for clinical endpoints; however, clinically relevant effects can be identified with modest (~50%) increases in the sample size in parallel-group design studies. The total number of studies in a crossover design would be somewhat lower for most endpoints than for parallel-group design studies, although not sufficiently so to mitigate the potential pitfalls associated with crossover studies such as order effect, loss to follow-up, and lack of comparability of disease states at entry to each arm of study, despite washout period.

Table 1A

Endpoint	Mean ± SD	COV_{inter}, %	#/group to show 30% effect* (number of units)
Transit GC24	2.05 ± 0.80	38.9	19/group (0.75)
Transit GC48	3.09 ± 1.02	33.3	27/group (0.80)
Stool #/day	1.14 ± 0.67	59.0	27/group (0.53)
Stool consistency	2.70 ± 1.10	40.6	23/group (0.95)
Stool ease of passage	3.44 ± 0.81	23.6	14/group (0.89)

COV_{inter} was calculated by SD/mean *Difference as a percentage of overall mean

Table 1B

Endpoint	Mean ± SD	SD of Deltas	COV_{intra} %	Total # to show 30% effect*
Mean transit GC24	2.04 ± 0.55	0.86	42.2	13
Mean transit GC48	3.12 ± 0.70	1.18	37.8	19
Mean stool #/day	1.31 ± 0.66	0.83	63.4	22
Mean stool consistency	2.47 ± 0.99	1.58	64.1	24
Mean stool ease of passage	3.65 ± 0.55	0.92	25.2	11

COV_{intra} was calculated by SD of deltas/overall mean * Number of subjects in a paired study to detect 30% effect sizes (number of units in Table 1A)

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Effect of chronic chili ingestion on postprandial gastrointestinal symptoms and rectal sensation in diarrhea predominant Irritable Bowel Syndrome (IBS): A randomized double-blinded crossover study

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Objective: To investigate the effects of chronic chili ingestion on postprandial gastrointestinal (GI) symptoms and rectal sensation in IBS-D patients.
Methods: Sixteen patients (11 F, age 46 ± 3 year) who fulfilled the Rome III criteria of IBS-D received placebo or chili powder in capsules orally before meals, 3 times/day (2.1 gm/day of chili with 2.5 mg/day of capsaicin) for 6 weeks in a randomized double-blinded crossover fashion with a 4-week washout period. GI symptoms in respond to a standard meal (a cup of noodle soup with 2 gm chili) were evaluated at before and at the end of treatment. Severity of each postprandial GI symptom was assessed every 15 min for 2 h using 10-cm long visual analog scales. All patients underwent rectal barostat study to evaluate rectal sensation at the end of each treatment.
Results: All patients completed the studies. All GI symptom scores in response to the standard meal were similar at baseline. At the end of treatment, maximum abdominal burning symptom score in response to the standard meal were significantly decreased after chili treatment compared to placebo (0.30 ± 0.16vs.0.91 ± 0.36, $P < 0.05$). However, abdominal pain symptom (1.42 ± 0.60 vs. 1.05 ± 0.51) and other GI symptom scores in response to the standard meal were not significantly different between chili and placebo treatment ($P > 0.05$). At the end of treatment, chronic chili ingestion significantly increased sensory threshold for first rectal sensation [16(12-16)vs.8(8-16) mmHg; median (interquartile range), $P < 0.05$)] with a trend of increase threshold for first [20(16-20)vs.16(13-23)], moderate [24(17-27)vs.16(16-31)] and severe urgency [32(29-43)vs.24 (24-40)], respectively, without significant effect on rectal compliance (7.3 ± 1.0vs.7.1 ± 1.8 ml/mmHg, $P > 0.05$). Daily abdominal burning and postprandial fecal urgency symptom scores of the patients trended to be lower by chili in relative to placebo at the end of treatment (1.07 ± 0.39 vs.1.49 ± 0.56 and 1.96 ± 0.67vs.3.22 ± 0.83) but did not reach statistical significance ($P > 0.05$).
Conclusion: In IBS-D patients, chili ingestion for 6 weeks can increase rectal sensory threshold without significant effect on rectal compliance and reduce postprandial abdominal burning symptom in response to the standard meal. These results suggest the desensitization effect on capsaicin receptors in the proximal gut and rectum by chronic chili ingestion.

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Psychometric evaluation of patient-reported outcome measures for assessing IBS-C symptom severity and change: Results from two randomized, double-blind, placebo-controlled phase 3 trials of linaclotide

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Objective: Linaclotide, a minimally absorbed peptide agonist of the intestinal guanylate cyclase type-C receptor, is an investigational treatment for irritable bowel syndrome with constipation (IBS-C) and chronic constipation (CC). Analyses of two Phase 3 clinical trials of linaclotide were conducted to provide evidence of the psychometric properties of patient-reported outcome (PRO) measures assessing changes in the severity of abdominal and bowel symptoms of IBS-C.
Methods: 1602 adult patients with IBS-C in the pooled ITT population who participated in two Phase 3 multicenter, double-blind, placebo-controlled, clinical trials of linaclotide were randomized to 290 μg or placebo administered orally once daily. Nine daily PRO measures addressing abdominal symptoms (pain, discomfort, bloating, fullness, cramping) and bowel symptoms (spontaneous bowel movement [SBM]/complete SBM [CSBM] frequency, stool consistency, straining) and four weekly PRO measures (constipation severity, IBS symptom severity, degree of relief, adequate relief) were administered using interactive voice response system technology. Test-retest reliability (intra-class correlation coefficients), construct validity (Pearson correlations), discriminating ability (known-groups F-tests), and responsiveness (Guyatt's statistics) were evaluated.
Results: The IBS-C PRO items showed highly satisfactory test-retest reliability, ranging from a minimum of 0.75 for Stool Consistency and Adequate Relief to a maximum of 0.95 for Abdominal Fullness. Correlation analyses supported the construct validity of the IBS-C PRO items (see Table). All hypothesis tests based on a variety of responder status groups were statistically significant and in the predicted direction, substantiating the discriminating ability of the IBS-C PRO items. With few exceptions, responsiveness statistics were highly satisfactory, demonstrating that the items were easily capable of detecting change.
Conclusion: In two large Phase 3 trials of IBS-C patients, linaclotide significantly improved patient-reported measures of abdominal symptoms, bowel symptoms, and global assessments. The psychometric analyses strongly support the reliability, validity, responsiveness, and usefulness of these IBS-C PRO items.

Table. Validity Correlations Between the IBS-C PRO Items and Available Measures

IBS-C PRO Item	Change in Degree of Relief	Change in PRCQ	IBS-SSS (Last 4 Weeks)	IBS-QOL Overall	Dysphoria	Interference with Activity	Body Image	Health Worry	Food Avoidance	Social Reaction	Sexual	Relationships
CSBM Frequency	-0.54*	-0.51*	-0.38*	0.21*	0.20*	0.11*	0.28*	0.24*	0.19*	0.17*	0.16*	0.16*
SBM Frequency	-0.48*	-0.45*	-0.26*	0.13*	0.12*	-0.00	0.19*	0.19*	0.12*	0.11*	0.10*	0.10*
Stool Consistency	-0.39*	-0.35*	-0.25*	0.11*	0.08*	0.01	0.17*	0.14*	0.14*	0.09*	0.09*	0.11*
Straining	0.49*	0.54*	0.48*	-0.32*	-0.28*	-0.22*	-0.36*	-0.32*	-0.29*	-0.27*	-0.22*	-0.23*
Abdominal Pain	0.61*	0.60*	0.68*	-0.35*	-0.32*	-0.29*	-0.35*	-0.32*	-0.28*	-0.28*	-0.25*	-0.26*
Abdominal Discomfort	0.63*	0.63*	0.71*	-0.38*	-0.36*	-0.30*	-0.41*	-0.36*	-0.32*	-0.30*	-0.27*	-0.28*
Abdominal Bloating	0.63*	0.61*	0.68*	-0.37*	-0.33*	-0.26*	-0.47*	-0.35*	-0.31*	-0.29*	-0.25*	-0.25*
Abdominal Cramping	0.59*	0.59*	0.62*	-0.31*	-0.27*	-0.27*	-0.32*	-0.30*	-0.25*	-0.25*	-0.22*	-0.25*
Abdominal Fullness	0.64*	0.62*	0.70*	-0.37*	-0.34*	-0.27*	-0.45*	-0.38*	-0.31*	-0.29*	-0.25*	-0.25*
Constipation Severity	0.71*	---	0.66*	-0.45*	-0.43*	-0.29*	-0.50*	-0.46*	-0.39*	-0.34*	-0.32*	-0.30*
IBS Symptom Severity	0.70*	---	0.70*	-0.46*	-0.44*	-0.33*	-0.50*	-0.47*	-0.38*	-0.36*	-0.32*	-0.32*
Degree of Relief	---	---	0.68*	-0.40*	-0.39*	-0.27*	-0.45*	-0.41*	-0.34*	-0.29*	-0.29*	-0.28*
Adequate Relief	---	---	–0.57*	0.32*	0.32*	0.20*	0.37*	0.32*	0.27*	0.23*	0.23*	0.22*

* $P < 0.05$
Note: Pearson correlations are presented.
CSBM = complete spontaneous bowel movement; SBM = spontaneous bowel movement; IBS = irritable bowel syndrome; PRCQ = patient rating of change question; IBS-SSS = Irritable Bowel Syndrome Symptom Severity Scale; IBS-QOL = Irritable Bowel Syndrome Quality of Life questionnaire.

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Effect of a synbiotic preparation on stool consistency, intestinal transit time and gut microbiota in patients with severe functional constipation: A double blind controlled trial

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Objective: Aim was to evaluate if administration of a symbiotic product (Psyllogel Megafermenti®, Nathura Srl, Montecchio RE - Italy) was able to normalize stool consistency and to decrease Intestinal Transit Time (ITT) in a group of patients with severe Functional Constipation, in relationship with its capacity to modify concentration in resident microflora.
Methods: A double blind, randomized, controlled trial was carried out in patients fulfilling to ROME III Criteria. Patients reporting previous abdominal surgery and abdominal pain were excluded. Moreover exclusion followed evidences for anterior rectocele >4 cm and/or rectal prolapse at defecography, Dyssinergic Defecation during straining at anorectal manometry, as well as pathologic score for more than 50% of items for the psychometric tests CES-D; SCL-90-R and IBQ. During a 2 week run-in period and the 8 weeks of study period evacuation diary was maintained so that evacuation frequency and stool consistency according to Bristol Stool Form Scale (BSFS) were recorded. ITT, measured by 10 radiopaque markers ingested daily for 6 days and abdominal Xray at 7th day, and PCR- DGGE and Quantitative real-time PCR analysis on fecal specimen were carried-out during run-in and last week of study period. Control Group (GroupC) assumed 1 sachet with 2.8 g of maltodestrine b.i.d., while in Active Group (GroupA) each sachet contained 2 g of psyllium fiber and 24 billions of Lactobacillus and Bifidobacteria.
Results: Thirty-one patients were randomized, but 17 completed the study in GroupA (15 F, 39 ± 14 years) and 12 in GroupC (12 F, 38 ± 12 years). Bowel motions with type 4 and 5 stools increased from 35%±33 to 53%±35% of total in GroupC and from 29%±31 to 54%±34* in GroupA, * $P = 0.001$. Even ITT decreased significantly only in GroupA: from 82 ± 43 to 58 ± 37* hours, $P = 0.022$, versus from 90 ± 42 to 82 ± 36 hours in GroupC. Patients who encountered all the efficacy end-points (25%) were only in the GroupA and in these all the probiotic species contained in the synbiotic harboured after treatment.
Conclusion: Synbiotic Psyllogel Megafermenti supply is related to an increase in bowel motions with normal stools and to colonic transit quickening in a group of very homogeneous patients with severe functional constipation. These effects were associated to changes in intestinal microbiota composition.

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Effects of sinusoidal modulated current on large intestina motility

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Objective: To specify the effects of different operating modes of sinusoidal modulated currents on large intestinal motility.
Methods: Ten healthy volunteers (5 men and 5 women) with a mean age of 19.75 ± 0.53 years and no gastrointestinal complaints, were evaluated. The SMC impact was done using an Amplipulse-4 device: the anode was applied on the mesogastric region and the cathode on the L1 - L2 region. Stimulation was alternating, with OM IV, III (frequency 100 Hz, modulation depth 50%), and II (70 Hz and 75%, respectively), with an impact duration of 2 to 3 seconds, in rectified mode, 5 minutes for each OM. Intestinal motility was examined using an EGS-4M electrogastrograph set for the frequency range of 0.01 - 0.03 Hz, which corresponds to the electrical activity of the large intestine. The frequency and amplitude of the obtained curve were evaluated. Study results were analyzed by means of variational statistics.
Results: The mean frequency and amplitude of electrocolonograms in study subjects corresponded to normal values, being 1.15 ± 0.1 per minute and 0.74 ± 0.07 mV, respectively. These means were 3.15 ± 1.38 per minute ($P < 0.05$) and 0.9 ± 0.03 mV after the OM II SMC impact, 0.74 ± 0.13 per minute ($P < 0.05$) and 0.81 ± 0.06 mV after OM III SMC; 1.05 ± 0.21 per minute and 0.76 ± 0.08 mV after OM IV SMC, respectively.

Conclusion: OM II produced a stimulating effect of the greatest magnitude and can thus be recommended for large intestinal hypokinesis (in patients with obstipation syndrome). OM III had the greatest effect on large intestinal contraction amplitude and can be employed for decreased motility. OM IV has no marked stimulating effect on the frequency or amplitude of large intestinal smooth muscle contractility and can thus be utilized in patients with excessive bowel motility (i. e. those with diarrhoea).

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Psychometric evaluation of patient-reported outcome measures for assessing chronic constipation symptom severity and change: Results from Phase 2b and Phase 3 trials of Linaclotide

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Objective: Linaclotide is an investigational minimally absorbed guanylate cyclase-C receptor agonist (GCCA). Psychometric analyses using data from one Phase 2b study and two Phase 3 trials of linaclotide for the treatment of chronic constipation (CC) were conducted to document the measurement properties of patient-reported outcome (PRO) measures assessing changes in the severity of symptoms.
Methods: Each study had a multicenter, randomized, double-blind, placebo-controlled, parallel-group design, comparing placebo to four doses of oral linaclotide taken once daily for 4 weeks in the Phase 2b dose-ranging study (n = 307 ITT) and to two doses of linaclotide taken once daily for 12 weeks in the Phase 3 trials (n = 1,271 ITT). Seven PRO measures addressing abdominal symptoms (pain, discomfort, bloating) and bowel function (spontaneous bowel movement [SBM]/complete SBM [CSBM] frequency, stool consistency, straining) were assessed daily using interactive voice response system technology. Patients completed the Patient Assessment of Constipation Quality of Life Questionnaire (PAC-QOL) at the Randomization and End-of-treatment Visits. Intra-class correlation coefficients (ICCs), Pearson correlations, F-tests, and effect sizes were computed to evaluate the reliability, construct validity, discriminating ability, and responsiveness of the PRO measures in a clinical trial context.
Results: The CC PRO measures showed satisfactory test-retest reliability; ICCs ranged from 0.68 for straining in Phase 2b to 0.91 for abdominal pain in Phase 3. Convergent and divergent validity correlations between the CC PRO measures and PAC-QOL provided evidence of the validity of the PRO measures (see Table). Known-groups F-tests were statistically significant and in the expected direction, substantiating the discriminating ability of the CC PRO items. Responsiveness statistics for bowel symptom PRO measures were large (0.86-3.29 in Phase 2b and 0.79-2.68 in Phase 3), and for abdominal symptom PRO measures, moderate to large (0.54-1.03 in Phase 2b and 1.02-1.21 in Phase 3). Results were consistent across the Phase 2b and 3 studies.
Conclusion: In large Phase 2b and Phase 3 studies of CC patients, linaclotide significantly improved PRO measures of abdominal and bowel symptoms. The psychometric analyses strongly support the reliability, validity, discriminating ability, responsiveness, and usefulness of the PRO measures assessing CC symptom severity and change evaluated in these clinical studies.

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Efficacy of combination therapy with osmotic laxatives and bifid triple viable for the elderly persons suffering non-orgnic constipation

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Objective: To evaluate the efficacy of combination therapy with osmotic laxatives and bifid triple viable for the elderly persons suffering non-orgnic constipation.
Methods: Inpatients and outpatients aged 60 and over 60 were randomly divided into five groups, patients in group A treated with glycol 4000, group B treated with lactulose, group C treated with bifid triple viable, patients in group D treated with combination of glycol 4000 and bifid triple viable, patients in group E treated with combination of lactulose and bifid triple viable.
Results: In the primary 2 weeks, the effective rates of groups of A, B, C, D and E were 50.9%, 50.9%, 43.7%, 70.5% and 73.2% respectively. Logistic regression showed there was no difference on therapeutic effect among groups of A, B,and C, but effective rate of group D or E was higher than groups of A, B and C. Furthermore, there was no significantly difference between group D and group E. In 6 months, ineffectiveness and recurrence ratios of groups of A, B, C, D and E were 68.4%, 66.0%, 72.9%, 57.4% and 35.7% respectively. Kaplan-Meier survival analysis showed the ineffectiveness and recurrence rate of group E was the most lowest compared with the other four groups.
Conclusion: Bifid triple viable can improve the therapeutic efficacy of osmotic laxatives for the elderly persons with non-orgnic constipation. Combination of glycol 4000 and bifid triple viable can improve short-term therapeutic efficacy,while combination of lactulose and bifid triple viable can enhance both short-term and long-term therapeutic efficacy.

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Mediation analysis suggests that linaclotide has a direct effect on relief of abdominal pain independent of constipation improvement

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Objective: To estimate the direct effect of linaclotide (LIN) on improving abdominal pain by controlling for a concurrent increase in CSBMs (bowel movements associated with a sensation of complete evacuation occurring in the absence of rescue medication).
Methods: Patients meeting Rome II criteria for irritable bowel syndrome with constipation (IBS-C) were randomized to oral LIN or placebo (PBO) for 26 weeks. Patients reported abdominal and bowel symptoms, and rescue medication use daily. A multilevel mediation analysis (for weeks 13–26 of a Phase 3 trial) was performed on percent improvement from baseline in abdominal pain scores to estimate the proportion of the LIN treatment effect attributable to increased CSBM rate. CSBMs occurring on the day of the reported abdominal pain score and also on the previous 6 days were considered as mediation variables. An additional analysis summarizing patient improvement in abdominal pain on a particular day was also performed using a 2-way CSBM stratification: the number of days since a patient's last CSBM (0, 1, 2, and ≥3 days) and the number of CSBMs a patient had through the previous 3 days (0, 1, 2, and ≥3 CSBMs).
Results: The 20% effect of LIN on abdominal pain above the PBO effect was a combination of direct effect (18%) on abdominal pain and an indirect effect (2%) mediated by increasing a patient's CSBM rate. Spontaneous bowel movements (SBMs) that were not CSBMs and BMs associated with rescue medication use did not qualitatively increase the amount of mediated LIN pain effect. The results of the 2-way CSBM stratifica-

Table. Validity Correlations Between Change in CC PRO Measures and Available Measures

CC Measures	CSBM Frequency	SBM Frequency	Stool Consistency	Straining	Abdominal Pain	Abdominal Discomfort	Abdominal Bloating
PAC-QOL Overall							
Phase 2b	–0.47*	–0.37*	–0.44*	0.54*	0.50*	0.56*	0.53*
Phase 3	–0.43*	–0.40*	–0.33*	0.46*	0.53*	0.57*	0.57*
PAC-QOL Physical Discomfort							
Phase 2b	–0.46*	–0.36*	–0.48*	0.59*	0.47*	0.56*	0.56*
Phase 3	–0.42*	–0.37*	–0.32*	0.45*	0.56*	0.61*	0.63*
PAC-QOL Psychosocial Discomfort							
Phase 2b	–0.29*	–0.21*	–0.24*	0.34*	0.41*	0.42*	0.36*
Phase 3	–0.26*	–0.23*	–0.18*	0.31*	0.40*	0.42*	0.42*
PAC-QOL Satisfaction							
Phase 2b	–0.52*	–0.48*	–0.48*	0.47*	0.37*	0.44*	0.47*
Phase 3	–0.48*	–0.44*	–0.40*	0.44*	0.39*	0.44*	0.47*
PAC-QOL Worries and Concerns							
Phase 2b	–0.35*	–0.25*	–0.32*	0.46*	0.45*	0.50*	0.44*
Phase 3	–0.34*	–0.33*	–0.25*	0.39*	0.46*	0.50*	0.48*

* $P < 0.05$

Note: Pearson correlations are presented.

CC = chronic constipation; PRO = patient-reported outcome; CSBM = complete spontaneous bowel movement; SBM = spontaneous bowel movement; PAC-QOL = Patient Assessment of Constipation Quality of Life Questionnaire.

tion analysis (Table) indicated that improvement in abdominal pain was influenced by the time since last CSBM and the number of recent CSBMs. However, consistent with the predominant direct effect of LIN on abdominal pain shown in the mediation analysis, in each cell of the Table, the LIN-treated patients had greater abdominal pain relief than PBO-treated patients when controlling for these CSBM factors.

Conclusion: These findings suggest that the LIN effect on abdominal pain (over PBO) was predominantly a direct effect and, to a lesser extent, a mediated effect of increasing CSBM frequency.

Table. Percent Improvement in Abdominal Pain Stratified by Number of Recent CSBMs and Time Since Last CSBM by Treatment Group (LIN/PBO [LIN-PBO difference])

Total Number of CSBMs Through the Previous 3 Days	Number of Days Since Last CSBM			
	0 (CSBM that Day)	1	2	3+
0				45/25 [20]
1	58/43 [16]	61/37 [24]	60/40 [20]	51/38 [14]
2	74/56 [19]	72/54 [17]	65/56 [9]	57/39 [18]
3+	76/67 [9]	76/60 [16]	76/68 [8]	74/48 [25]

ITT Population, Weeks 13-26, median values presented. For patients with multiple values in a particular cell, the patient's median value was used.

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Factor Analyses of Chronic Constipation Patient-Reported Outcome Measures: Results from Phase 2b and Phase 3 Trials of Linaclotide

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Objective: Factor analyses of patient-reported outcome (PRO) data from one Phase 2b and two Phase 3 clinical trials of linaclotide for the treatment of chronic constipation (CC) were conducted to examine the patterns of correlations among the PRO measures and provide evidence of their construct validity.

Methods: 307 patients with CC were analyzed in a Phase 2b multicenter, randomized, double-blind, placebo-controlled, dose-range-finding, parallel-group clinical study comparing placebo and four doses of oral linaclotide taken once daily for 4 weeks. Seven daily PRO measures addressing abdominal symptoms (pain, discomfort, bloating) and bowel symptoms (spontaneous bowel movement [SBM]/complete SBM [CSBM] frequency, stool consistency, straining) and one weekly measure (constipation severity) were administered using interactive voice response system technology. Using these data, principal component analyses (PCA) and exploratory factor analyses (EFA) explored the structure of the CC PRO symptoms. Using confirmatory factor analysis (CFA), the EFA results were evaluated with data (n = 1,272) collected in two Phase 3 multicenter, randomized, double-blind, placebo-controlled, parallel-group 12-week clinical trials (145 or 290 μg oral linaclotide, or placebo).

Results: The PCA of data averaged over the treatment period indicated two dimensions accounting for 70.4% of the total variance. The two-factor EFA produced one factor consisting of abdominal symptoms (pain, discomfort, and bloating) and a second factor consisting of bowel symptoms (SBM frequency, CSBM frequency, stool consistency, straining, and constipation severity). When the two-factor CFA model was applied to the correlation matrix with select correlated error variances, the fit of the model to the data was very satisfactory, as were the factor loadings (Table).

Conclusion: The factor analyses enhance the construct validity of the PRO measures by establishing the composition of two factors that explain the pattern of correlations among the seven CC PRO items. One factor explained the correlations among the bowel symptoms, and a second factor explained the correlations among the abdominal symptoms. The weekly rating of constipation severity was more strongly related to the bowel symptoms factor than the abdominal symptoms factor. Additionally, the two factors, abdominal symptoms and bowel symptoms, were highly related.

Table. Factor Loadings (Standard Errors) and Model Goodness of Fit Statistics - CC PRO Measures

	Exploratory Factor Analysis		Confirmatory Factor Analysis	
CC PRO Measures	**Factor 1**	**Factor 2**	**Factor 1**	**Factor 2**
CSBM Frequency[b]	0.704 (0.05)	−0.132 (0.05)	0.631 (0.02)	—
SBM Frequency[b]	0.703 (0.05)	0.065 (0.05)	0.545 (0.02)	—
Stool Consistency[c]	0.706 (0.04)	0.260 (0.04)	0.521 (0.02)	—
Straining[a]	−0.531 (0.06)	0.224 (0.06)	−0.754 (0.02)	—
Constipation Severity[a]	−0.648 (0.05)	0.285 (0.05)	−0.939 (0.01)	—
Abdominal Pain[a]	0.064 (0.03)	0.847 (0.03)	—	0.868 (0.01)
Abdominal Discomfort[a]	−0.001 (0.02)	0.984 (0.02)	—	0.753 (0.02)
Abdominal Bloating[a]	−0.176 (0.04)	0.752 (0.03)	—	0.939 (0.01)
Goodness of Fit				
χ^2 (all $P < 0.05$)	75.86 df = 13		274.309 df = 16	
Comparative Fit Index	0.953		0.965	
Tucker-Lewis Index	0.899		0.939	
RMSEA	0.126		0.113	
SRMR	0.038		0.056	

CC = chronic constipation; CSBM = complete SBM; df = degrees of freedom; PRO = patient-reported outcome; SBM = spontaneous bowel movement; RMSEA = root mean square error of approximation; SRMR = standardized root mean square residual.
Note: The confirmatory factor analysis solution includes correlated residuals between the following: CSBM Frequency and SBM Frequency; Straining and Stool Consistency; Abdominal Pain and Abdominal Discomfort.
[a] 5-point ordinal scale.
[b] These were derived from daily PRO items about bowel symptoms: number of BMs, use of rescue medication, and completeness of evacuation.
[c] 7-point Bristol Stool Form Scale (BSFS).

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What is a clinically significant rectocele in functional constipation?: Relationship between rectal sensitivity, compliance, post-defecatory residue and rectocele size

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Objective: Rectocele is reported in more than 50% of patients with functional constipation (FC) [1] and, in a retrospective study, rectoceles >4 cm were associated with altered rectal sensitivity and compliance [2]. However rectal sensitivity can be decreased and rectal compliance increased in FC even in absence of rectocele and no previous study investigated the relationship between the rectocele size and rectal functional alteration. This prospective study assessed the relationship between the size of the rectocele, rectal sensitivity, rectal compliance, and post-defecatory residue in FC to identify the clinically significant rectoceles.

Methods: Eighty female patients (age 50 ± 15 years) with FC according to Rome III criteria underwent anorectal manometry and cinedefecography. At cinedefecography radiographs were performed at rest, during contraction, straining and evacuation. Maximal depth of the rectocele was assessed during defecatory effort. Contrast rectal residue was assessed by a semi-quantitative evaluation (small, moderate, abundant). During standard recto-anal manometry a balloon distended intermittently the rectum with progressively increasing air volumes from 10 to 200 ml. The threshold of rectal sensation was defined as the minimum volume of distension perceived by the patients as stimulus to evacuate; rectal compliance was calculated as ratio between 100 ml air volume and intra-rectal pressure. For statistical analysis we used the Mann-Whitney and Spearman correlation tests.

Results: The rectocele was <35 mm in 25 patients (31%), between 35 and 50 mm in 44 patients (55%) and > 50 mm in 11 patients (14%). No correlation was found between sensitivity (r = 0.3) and rectal compliance (r = 0.2) and rectocele size less than 50 mm. In the group with rectocele >50mm, the size correlated with threshold of rectal sensitivity (r = 0.6) and rectal

compliance (r= -0.8). Patients with moderate/abundant residue had a higher threshold of rectal sensitivity (58 ± 27 vs 90 ± 57 ml air, $P = 0.006$) than, and a not different compliance from, those with small residue.
Conclusion: Rectoceles with size >50 mm correlate with altered rectal sensitivity and altered rectal compliance. Rectocele >50 mm associated with altered rectal sensitivity identifies a condition that may play a clinically relevant role in the defecatory dysfunction of functional constipation.
References: [1] Pescatori M, Spyrou M, Pulvirenti d'Urso A. Colorectal Disease. 2007; 9: 452–456. [2] Rotholz NA, Efron JE, Weiss EG et al. Tech Coloproctol. 2002; 6: 73–77.

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How accurate is computerized analysis versus an expert analysis for evaluating anorectal function using 3-D high definition anorectal manometry

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Objective: Our aim was to analyze and compare the topographic measurements of anorectal manometric parameters, as assessed by a commercial software and by an expert.
Methods: We used a 10-mm diameter probe containing 256 circumferentially arrayed sensors and 2 additional rectal sensors in a standardized rectal balloon (Given Imaging, Yoqneam, Israel) in 24 healthy subjects (13:11) age (42 yrs 95% CI 35–49).The probe was held in place by operator throughout the study. We evaluated anal and rectal topographic pressure changes during squeeze, when blowing up a party balloon and during bearing down maneuvers. The data were analyzed by an expert investigator (>25 years experience) who was blinded to the automatic software analysis (Manoview Analysis, Given Imaging, Yoqneam, Israel) for the same variables. An independent investigator blinded to expert analysis performed the analysis using commercial software. Statistical analyses were performed with SPSS v. 20 (Chicago, IL).
Results: No differences were observed for any of the key manometric parameters ($P > 0.05$) such as anal resting pressure, rectal and anal pressures during party balloon maneuvers and during bearing down maneuver with and without rectal balloon inflation. Correlations between expert and software analysis of data were excellent ($r2 = 0.82–0.99$).
Conclusion: The software analysis program appears to provide reliable data that is sufficient routine for clinical purposes. Although there is some learning curve, when used properly, the software can provide accurate information.

	Comparison			Correlations	
	Expert	Software	p	r^2	p
Rest Pressure Mean, (95%, CI)	75 (67-82)	78 (71-85)	0.5	0.99	0.0001
Max Squeeze, Mean (95%, CI)	219 (192-247)	217 (188-247)	0.9	0.99	0.0001
Rectal Pressure Party Balloon Mean (95%, CI)	52 (43-61)	50 (38-63)	0.8	0.97	0.0001
Anal Pressure Party Balloon, Mean (95%, CI)	139 (122-156)	134 (118-151)	0.7	0.99	0.0001
Rectal Pressure Bear-Down (95%, CI)	49 (38-59)	49 (38-59)	0.9	0.93	0.0001
Anal Pressure Bear-Down (95%, CI)	57 (45-70)	61 (48-74)	0.5	0.98	0.0001
Rectal 60cc Pressure Bear-Down (95%, CI)	75 (65-89)	77 (65-88)	0.9	0.93	0.0001
Anal 60cc Pressure Bear-Down (95%, CI)	48 (36-61)	55 (43-66)	0.4	0.86	0.0001
Defecation Index (95%, CI)	1.1 (0.7-1.5)	0.7(0.5-0.9)	0.08	0.97	0.0001
Defecation Index 60cc (95%, CI)	1.9 (1.5-2.3)	1.6(1.3-2)	0.4	0.81	0.0001

p value, Student t-test; 95% CI, Confidence Interval

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Fecal incontinence in patients with IBS

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Objective: The Irritable Bowel Syndrome (IBS) is a common disorder with a prevalence between 2.1–22%. Fecal incontinence (FI) can be found in 8% of the general population; however the prevalence of FI in IBS is mainly unknown. The aims of this study were to determine the presence of FI in IBS, its relationship with symptoms, quality of life and colorectal sensorimotor function.
Methods: We included 320 IBS patients fulfilling the ROME II criteria (mean age 40 years; 229 women), consecutively referred to our centre. The presence of FI was determined using the Rome II modular Questionnaire. The patients also completed the Gastrointestinal Symptom Rating Scale (GSRS), Short Form 36 (SF-36) questionnaires and underwent a rectal barostat study before and after a standardized meal.
Results: 64/320 patients (20%) reported accidentally leaking or passing stool more than once a month. Of these 19 (30%) had gross incontinence, leaking the amount of two teaspoons or more. Patients with FI were older than those without (48 ± 19 vs. 39 ± 12 years (mean ± sd); $P < 0.0001$), and FI was more common in D-IBS, 27/115 (24%) and A-IBS 33/126 (26%) than in C-IBS, 4/79 (5%) ($P < 0.001$). The perceived severity of diarrhea was higher in patients with FI – (GSRS diarrhea: 4.3 ± 1.7 vs. 3.0 ± 1.6; $P < 0.0001$), including more frequent stools (4.1 ± 1.8 vs. 2.9 ± 1.8; $P < 0.01$), looser stools (4.2 ± 1.7 vs. 3 ± 1.8; $P < 0.001$) and urgency (4.5 ± 1.9) vs. 3.2 ± 1.8; $P < 0.001$). FI was also associated with reduced physical quality of life (SF36 Physical Component Summary: 41 ± 11 vs. 45 ± 8; $P < 0.05$). Rectal balloon volumes were similar in patients with versus without FI in the fasting state (84 ± 25 vs. 84 ± 37 ml; NS), but after meal intake IBS patients with FI had lower balloon volumes (44 ± 40 vs. 61 ± 41 ml; $P < 0.05$), indicating a more pronounced rectal tone response. No differences in rectal sensitivity were noted.
Conclusion: The prevalence of FI in patients with IBS can be as high as 20%, being more common in A- and D-IBS. These conditions should be included as risk factors for FI especially in older patients with more severe diarrhea. The exaggerated rectal tone response after meal intake warrants further investigations.

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High resolution anorectal manometry in the diagnosis of pelvic floor dyssynergia

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Objective: Pelvic floor dyssynergia is a disorder of defecation and is characterized by dysfunction of coordination of anorectal muscles and pelvic floor muscles during defecation. Objective: To evaluate patients with constipation and defecatory disorder caused by pelvic floor dysfunction with high resolution anorectal manometry (HRARM).
Methods: Twelve patients, 10 females and 2 males, average age 54,7 years (range 24–87) with chronic constipation and symptoms of pelvic floor dyssynergia were recruited after exclusion of obstructive or metabolic cause. HRARM quantifies IAS and EAS function, rectal sensation, rectoanal reflexes, rectal compliance, and attempted defecation. HRARM involves a solid-state manometric assembly with 12 circumferential sensors spaced at 1-cm intervals (4.2 mm outer diameter) (Sierra Scientific Instruments, Los Angeles, CA). This device uses proprietary pressure transduction technology (TactArray) that allows each pressure sensing element to detect pressure over a length of 2.5 mm in each of 12 radially dispersed sectors. Rao's types classification of dyssynergia. has been used: in type I dyssynergia, the subject can generate an adequate propulsive force (increase in intrarectal pressure ≥45 mm Hg) along with a paradoxic increase in anal sphincter pressure; in type II dyssynergia, the subject is unable to generate an adequate propulsive force together with paradoxic anal contraction; in type III dyssynergia, the subject can generate an adequate propulsive force along with an absent relaxation (a flat line) or incomplete (≤20%) relaxation of resting anal sphincter pressure; in type IV dyssynergia, the subject is unable to generate an adequate propulsive force together with absent or incomplete relaxation of anal sphincter pressure.
Results: In the classification of attempted defecation, classified by HRARM Rao' types of dyssynergia Type I (4 patients) Type II (1 patient) Type III (6 patients) Type IV (1 patient).
Conclusion: HRARM was well tolerated, a reliable method to evaluate defecatory disorder of pelvic floor dysfunction and identified the dyssynergic defecation's mechanisms.

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Simplified saline continence test for evaluation of anorectal capacity: Technique and normative data

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Objective: Saline continence test (SCT) is a comprehensive test of rectal and anal sphincter function in retention of liquid stool. By using automatic peristaltic pump, constant rate of infusion is achieved. However, the pump is expensive and sophisticated. We described a simple and cheap technique.
Methods: Simplified SCT set is comprised of an 8-Fr feeding tube, an infusion set (20 drops ml^{-1}) connected to a bottle containing 750 ml of 0.9%NaCl and a stopwatch. Healthy subjects were enrolled. With the feeding tube inserted 10 cm up into the subject's rectum, they were asked to sit on the commode. The infusion set connected to a saline bottle was then joined to the feeding tube. The infusion, at an estimated rate of

Table Characters of saline retention

	Male (n=15)	Female (n=31)	Total (n=46)	Correlation coefficient (r)
Age (years)	45.1 ± 0.0	47.4 ± 4.4	46.5 ± 3.7	0.41*
Body weight (kg)	61.0 ± 5.9	56.3 ± 3.7	59.8 ± 3.3	0.36*
Height (cm)	158.9 ± 4.7	154.5 ± 2.6	158.1 ± 2.5	0.23
Body surface area (m^2)	1.55 ± 0.06	1.74 ± 0.07	1.61 ± 0.05	0.34*
Total volume retained (ml)	655.8 ± 72.6	633.2 ± 56.7	638.0 ± 46.0	
%volume retained (ml)	90.8 ± 9.3	88.3 ± 7.4	90.0 ± 5.9	
Volume at first leak (ml)	313.0 ± 76.4	263.6 ± 52.3	283.1 ± 43.5	

*p <0.05. r<0-no correlation; 0.1≤ r ≤0.3 - weak correlation; 0.3 ≤ r ≤ 0.5 - moderate correlation; 0.5 ≤ r ≤ 1; high correlation

60 ml min^{-1} (750 ml in 12 min) was started. The subject was asked to retain fluid as long as possible. Fluid leakage was collected in a graduated jar. Volume of saline infused at the onset of first leak, total volume retained (Vretained) and percentage of saline retained (= Vretained / Vinfused x 100) were recorded. Student's *t*-test was used to compare differences between genders. Correlation coefficients (r) between total volume retained versus age, body weight, height and body surface area were analyzed.

Results: Forty-six subjects (M:F 15:31, mean age 46.5 ± 3.7 years) without bowel symptoms and no previous pelvic/abdominal surgery other than simple appendectomy were enrolled. All subjects cooperated well without complication. Thirteen subjects (28.3%) had leakage. Data is shown in table (mean ± 95%confidence interval). Total retained volumes, % volume retained and volume at first leak were not different between genders ($P > 0.55$). Moderate but significant ($P < 0.05$) correlations was found between total volume retained and age (0.41), body weight (0.36) and body surface area (0.34). However, the correlation with height was small (0.23).

Conclusion: Simplified saline continence test is an easy-to perform and well-tolerate test to assess anorectal function. These normative data will guide diagnosis and treatment of subjects with anorectal disorders.

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Randomized trial of biofeedback or medical treatment for fecal incontinence

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Objective: Both biofeedback and medical treatment have been advocated as first-line therapies for mild to moderate fecal incontinence. There is limited data comparing and combining these two treatments. Therefore, we aimed to evaluate the effect of standardized biofeedback therapy and medical treatment, separately and in combination, in a randomized fashion.

Methods: Sixty-five consecutive female patients, median age 57 (range 27–78) referred to a tertial center for fecal incontinence were included. Median number of deliveries were 2 (range 1–5). Fifty-three patients had findings of a sphincter injury on anal ultrasound. Median duration of symtoms were 5 (range 1–22) years. Gastrointestinal malignancy or inflammatory bowel disease were excluded. The patients were randomized to start with either biofeedback (4–6 months) or medical treatment with loperamide and bulking agents (2 months). Then both groups continued with a combination of both treatments, i.e. medical treatment was added to biofeedback and vice versa. The total duration of the study was 8 months. A two-week prospective bowel symtom diary and anorectal physiology were evaluated at base-line, after singel- and both treatments. Disease related quality of live (QoL) was evaluated with the disease specific short health scale questionnaire (SHS). Non-parametric statistics was used (paired sign test and Whitney-U test).

Results: Fifty-seven patients completed the study. Median number of leakage episodes per week decreased from 3.5 to 3.0 after single treatment ($P < 0.05$) and to 1.2 ($P < 0.0001$) at completion of the study with both treatments. The combination treatment was superior to both single treatments in terms of symptoms and functions. There was no significant difference between the two treatment groups at any time-point. From start to completion of the study patients demonstrated a significant (1) increase in the proportion of normal stool consistency from 52 to 65%; (2) decrease of proportion of stools with urgency ($P = 0.02$); decrease of rectal sensory thresholds, both for maximum tolerable rectal pressure and first sensation, from 50 to 40 cmH2O ($P = 0.004$) and 20 to 10 cmH2O ($P = 0.03$), respectively; (3) improvement of QoL in SHS dimensions of function ($P = 0.04$) and disease related worry ($P = 0.02$).

Conclusion: The combination therapy with biofeedback and medical treatment, but not each treatment alone, is successful for symptom relief in fecal incontinence. This symptom improvement was associated with improved fecal consistency, reduced urgency and increased rectal sensory thresholds.

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Surgical correction does not improve dyssynergic defecation in patients with rectal prolapse

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Objective: The patients with rectal prolapse suffer from not only a prolapse rectum but also associated dysfunction. The purpose of this study was to investigate the functional and physiological results after surgical correction in patients with rectal prolapse.

Methods: Seventeen patients with rectal prolapse (15 females, mean age 66.8 ± 10.6 years) underwent anorectal manometry before and after Delorme's procedure. The results of anorectal manometry and clinical symptoms were analyzed preoperatively and postperatively.

Results: The mean period of follow-up was 20.2 months (range 8–46 months). The mean age of the study group was 66.8 ± 10.6 years. Thirteen patients (76.5%) had rectal mucosal prolapse and 4 patients (23.5%) had complete rectal prolapse. The two most prevalent symptoms before operation were rectal tenesmus (14/17, 82.3%) and excessive straining (13/17, 76.5%). The two most prevalent symptoms after operation were rectal tenesmus (10/17, 58.8%) and excessive straining (12/17, 70.6%. Eight patients (47.1%) were able to expel the balloon before surgery and 10 patients (58.8%) were able to expel the balloon after operation. There was no significant improvement of ability to expel the balloon after surgery. No significant differences in resting anal pressure, squeezing anal pressure, defecation index, and rectal sense were found postoperatively. However, vector asymmetry index was higher in postoperative patients than in preoperative patients (34.8 vs 38.2, $P = 0.049$). Ten patients (58.8%) had type I dyssynergic defecation before surgery. No improvement of dyssynergic pattern occurred after surgery.

Conclusion: Surgical correction of rectal prolapse does not correct dyssynergic defecation in patients with rectal prolapse despite of reduction of the prolapse. So, potential role of combination treatment with biofeedback therapy in these subgroups requires further studies.

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Transcutaneous tibial nerve stimulation in the treatment of faecal incontinence: A randomised trial

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Objective: While transcutaneous tibial nerve stimulation (TENS) is being increasingly used to treat faecal incontinence (FI), its efficacy has never been proved using controlled trials.

Methods: In this randomised, double-blind, sham-controlled trial, 144 patients aged 30–82 years from nine centres were randomly assigned to receive either active or sham stimulations for 3 months. The primary endpoint was the response to treatment based on the number of incontinence and urgency episodes. Secondary endpoints were severity scores, quality of life scores, delay to postpone defecation, patient self-assessment of treatment efficacy, physician assessment of TENS efficacy, anorectal manometry, adverse events.

Results: No statistically significant difference was seen between active and sham TENS in terms of an improvement in the median number of FI/urgency episodes per week. Thirty-four patients (47%) who received the active TENS treatment exhibited a ≥ 30% decrease in the FI severity score compared with 19 patients (27%) who received the sham treatment (odds

ratio 2.4, 95% CI 1.1-5.1, $P = 0.02$). No differences in delay to postpone defecation, patient self-assessment of treatment efficacy, or anorectal manometry were seen between the two groups. The evaluating physicians rated the active stimulations as more effective than the sham stimulations ($P = 0.01$). One minor therapy-related adverse event was observed (1.5%).
Conclusion: We failed to demonstrate any benefit of TENS on our primary end-point.

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Does deep brain stimulation modify ano-rectal motility?: Result of a randomized cross-over study

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Objective: Anorectal motility impairment is often observed during Parkinson's disease (PD), generating symptoms as constipation and/or incontinence. In most case, this is ascribed to dyssynergic anorectal contraction during defecation or to impaired squeezing contraction of the anal sphincter. Deep brain stimulation (DBS) is an effective treatment for advanced levodopa-responsive form of PD, but data on the impact of DBS on gastro-intestinal motility are lacking. We therefore hypothesized that DBS could either normalize anorectale dyssynergia and/or anal squeezing contraction in PD patients.
Methods: Seventeen patients (age: 62+/- 9 year old) with chronic bilateral high frequency stimulation of the subthalamic nucleus for at least 6 months were randomized with stimulator either turned OFF (2h) then ON (2h), or turned ON (2h) and OFF (2h) thereafter. An ano-rectal manometry was performed at the end of each period using perfused catheters.
Results: No groups effect (ON-OFF vs OFF-ON) was observed for the different parameters analyzed. A similar rate of ano-rectal dyssynergia was observed during the two periods (7/17 during the ON period vs 8/17during the OFF period). No difference was observed during the two periods for anal resting pressure (OFF: 72.5+/- 8.6 cm H_2O vs ON: 64.4+/- 7.2 cm H_2O; $P = 0.24$). However, during the ON period, an increase of the amplitude (108+/- 21 cm H_2O) of anal squeezing pressure was observed compared to the OFF period (amplitude: 85+/- 14 cm H_2O; $P = 0.02$ vs ON), while the duration was not different (OFF: 26.2+/- 3.6 s vs ON: 33.1+/-3.1 s; $P = 0.10$). Lastly, rectal distension induced recto-anal inhibitor reflexes with similar amplitude and duration between the ON and the OFF periods. Likewise, maximal tolerable volume was comparable in the two periods (OFF: 231+/- 24 ml vs ON: 241+/- 26 ml; $P = 0.68$).
Conclusion: DBS does not modify ano-rectal dyssynergia in PD patients, while it increases anal squeezing pressure. This interventional study thus shades a new light on the voluntary control of ano-rectal motility by the dopaminergic nigro-striatal loop targeted by DBS.

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Effect of biofeedback therapy in irritable bowel syndrome patients with intractable constipation

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Objective: Many patients with irritable bowel syndrome with constipation do not respond to conventional medical treatments. This study evaluated the efficacy of biofeedback therapy in irritable bowel syndrome patients with intractable constipation; identified factors that influence treatment outcome.
Methods: Of the 99 patients with irritable bowel syndrome (IBS) with constipation as defined by Rome II and III criteria, 25 patients with intractable constipation were selected (22 female, mean age 37.0 ± 13.3 years). In all patients the Anorectal manometry was carried out with the help of Multichannel recording system PC Polygraf (Synectics Medical). Anorectal physiological studies were compared with those of 23 healthy volunteers (22 female, mean age 37.5 ± 10.5 years). Twenty five IBS patients with intractable constipation received 6.3 ± 0.3 sessions of biofeedback therapy (BFT). IBS symptoms were evaluated immediately after the completion of biofeedback therapy and during the follow-up period of about 6.2 ± 0.5 months. Patients with slow transit constipation were not included.
Results: Twenty-four per cent of IBS patients with intractable constipation exhibited paradoxical contraction of the anal sphincter on straining. The biofeedback therapy was successful in 18 patients through the follow-up periods of 6.2 ± 0.5 months: in 73.7% of IBS patients without dyssynergia and in 66.7% of IBS patients with dyssynergic defecation. Treatment reduced IBS patients with need to strain (22 vs. 4, before BFT vs. after BFT, $P < 0.001$), sense of incomplete evacuation (22 vs. 5, $P < 0.001$), abdominal pain (25 vs. 5, $P < 0.001$), bloating (19 vs. 5, $P < 0.001$), and laxative, enemas or suppositories use (10 vs.3, $P = 0.009$). Spontaneous bowel frequency was significantly improved by treatment (1.3 ± 0.2 vs. 5.1 ± 0.4 bowel movements per week, before BFT vs. after BFT, $P < 0.001$) in 72% IBS patients. High defecation index was associated with success of BFT (OR 10.8; 95% CI, 1.02–114.2).
Conclusion: Biofeedback is an effective long term treatment for the majority IBS patients with constipation unresponsive to traditional treatments. High defecation index at baseline was a strong predictor of successful biofeedback therapy.

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Top down medical treatment for dis-impaction of patients in aged-care setting

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Objective: Literature in recent years, discusses the idea of high-dose, top-down medical treatments. This study investigated the success of colonic disimpaction using high levels of stimulant laxatives in combination with stool softeners at a suburban aged care facility. Current practice within the facility was daily suppository and high dose lactulose.
Methods: Ten residents (75–92 years) with faecal impaction were randomly selected from ward list with ≥day 5 post faecal output. Patients were initially identified with faecal impaction from bowel charts the facility maintained by nursing staff. Residents unable to ambulate for independent toileting were excluded. Residents were given an initial dis-impaction regimen of Movicol (polyethylene glycol) 8 sachets per day plus 15-25 Dulcolax drops, for 2 consecutive days. Defecation volume & consistency, activity, food and water intake were monitored within bowel chart. Residents were encouraged to be participants and in control of their own treatment.
Results: Residents began to defecate within 10–12 h. They continued to defecate daily on a maintenance regimen of Movicol and Dulcolax medications. Maintenance medication was maintained to continue producing 1 defecation/day. There were no reported episodes of soiling following dis-impaction or during maintenance. There was enthusiastic compliance with all residents and staff following the intervention due to significant increase in residents' quality of life. In the absence of further treatment alteration, their colons gradually filled up again, with impaction after 1–2months.
Conclusion: Use of high doses of Movicol and Dulcolax is effective to dis-impact elderly people with chronic constipation. Residents avoided invasive procedures to manage impaction including rectal interventions. Time commitment from nursing staff was minimal. Success and compliance was high.

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Home electric stimulation for women with fecal incontinence: Effectiveness and predictors of outcome

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Objective: Home electric stimulation (ES) is an effective treatment for fecal incontinence (FI). Available data on effectiveness and predictors of outcome for ES in Israel are scarce. The aim of the study was to evaluate effectiveness and predictors of outcome in women suffering from FI that were treated with home ES.
Methods: Home ES was offered for a period of 6 weeks. Patients were evaluated by a questionnaire regarding their demographics, medical and childbearing history. Subjective measures of outcome included the Fecal Incontinence Grading Scale (FIGS), quality of life questionnaire (the SF-36) and a 10 point Likert scale for the assessment of satisfaction. Objective measure of outcome included the pelvic floor muscle (PEM) contraction.
Results: A total of 19 women with FI were included. Mean age: 66.6 ± 9.8 years, mean BMI (kg/m^2): 26.8 ± 4.7, mean disease duration: 3.6 ± 3.1 years and mean number of births: 3.6. Obstetric trauma was reported by 21% of patients. Significant improvement of subjective measures was reported at the end of treatment for both FIGS (12.5 vs. 8.6, $P = 0.013$) and at the Likert rating (7.61 vs. 4.9, $P = 0.021$). A significant improvement of PEM contraction was achieved in 86% of patients. Mean number of FI episodes dropped below 50% by 62.5% of patients. Older age in general or at first episode of FI, and higher BMI significantly deteriorated FIGS ($r = -0.555$, $P = 0.021$) and the capability to reduce FI episodes/week ($r = -0.728$, $P = 0.001$), respectively.
Conclusion: Home electric stimulation is efficient in FI. Factors affecting treatment failure include older age and higher BMI.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-34 Clinical Session: Miscellaneous

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Transcutaneous electrical acupuncture therapy for severe gastroparesis and constipation in a diabetic patient

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Objective: A 51 years old diabetic F with severe drug refractory gastroparesis (GP) was implanted with gastric electrical stimulation (GES) system in 2000. More than 85% symptoms improvement was observed over the next 10 years. In March of 2011 patient noticed a gradual return of her GP symptoms. The GES battery was depleted, requiring replacement of the implantable pulse generator (IPG).
Methods: Clinical evaluation revealed GP symptoms score 25 points (max 28) and severe, debilitating constipation. Patient reported bowel movements (BM) every 3–4 weeks with passing a little liquid stools with gas and painful straining. Cytotec, Go-Lightly, Amitiza, Colchicine, herbs and all laxatives were introduced without success. While waiting for GES surgery, Transcutaneous Electrical Acupuncture (TEA) therapy was initiated. Two pairs of electrodes were connected to the micro- stimulators which were programmed based on patient's preference and tolerance. One pair was positioned on the arm- PC6 acupoint, and the second was placed on her leg at acupuncture point ST36 location. Trains of TEA pulses were programmed with current 2 mA (arm) and 4 mA (leg). Other parameters were: ON-time 2 s, OFF-time 3 s; frequency 25Hz. Patient was instructed to use TEA for 30 min before and 2 h after each meal.
Results: During the first TEA session nausea was completely resolved within 15 min, and 6 h later patient had a normal, ''healthy'' BM. Similar BM responses were observed in the next few days until TEA therapy was discontinued for surgical IPG replacement. In the next 10 weeks of GES, patient was doing well with her GP symptoms (score 6 points) but she did not have any ''normal'' BM. GES was then turned OFF and TEA was re-introduced. Patient was using TEA 2–3 times / day in the next 2 months and she continued to have sustained control of GP symptoms and had daily BMs. She gained 4 lbs and improved her glucose control.
Conclusion: TEA therapy is an innovative approach which was efficacious in controlling severe symptoms of DMGP, as well as in stimulating bowel movements in a setting of colonic inertia attributed to gut neuropathy.

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Translation and validation of the ROME-III questionnares for the diagnosis of functional gastrointestinal disorders in Korea

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Objective: A self-report questionnaire is frequently used to measure symptoms reliably and distinguish patients with functional gastrointestinal disorders(FGIDs) from other conditions. This study was aimed to produce and validate a cross-cultural adaptation of Rome III Questionnaires for screening and diagnosis of FGIDs in Korea.
Methods: The Korean version of the Rome III questionnaire had been developed through translation, assessment of comprehensibility, back translation, and appraisal of applicability. Subsequently, reliability was measured by a test-retest procedure in 151 persons. Following completion of the questionnaire by 196 outpatients, gastroenterologists who were blinded the patient's self-reported responses interviewed them. And concurrent validity was evaluated by comparing self-reported questionnaire data to the following doctor's completion of questionnaire based on the interview.
Results: Most of items (30/34) had an acceptable consistency with median value of Cronbach's α 0.830(range 0.706–0.971). The questions to judge IBS, GERD, FD, functional constipation, functional diarrhea and functional bloating had median value of 0.720(0.514–0.866), 0.830(0.824–0.836), 0.828(0.598–0.860), 0.790(0.514–0.863), 0.757(0.514–0.832) and 0.805(0.514–0.889), respectively. The Korean ROME III had excellent sensitivities (97.2%, 95.4%, 94.4%, 100%, 75.0%, 97.5%) and specificities (98.7%, 98.0%, 97.7%, 100%, 100%, 99.3%) to diagnose the FGIDs, respectively. The percent agreement of IBS subtype was 94.4%(34/36) and that of FD was 100%(17/17).
Conclusion: The Korean version of ROME III questionnaire is reliable and valid for diagnosing FGIDs in Korean people.

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Probiotics for prevention of radiation induced bowel disease: New findings and updated meta-analysis

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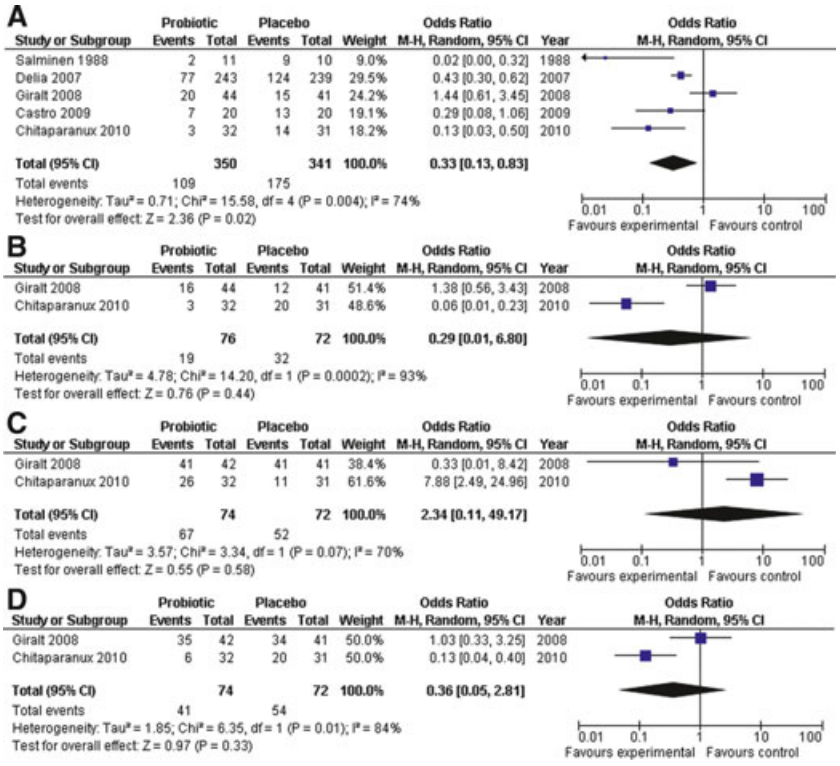
Objective: Our aim is to perform a meta-analysis to estimate the efficacy of probiotics to prevent radiation-induced bowel damage. Previous attempts have failed to fully address and take into account the complete outcomes measured in clinical trials.
Methods: We searched studies indexed in Medline, EMBASE, Cochrane Library, and on-line clinical trials registers. There was no language or time limit in our literature search. Each study was evaluated for meth-



odological quality and outcomes. We identified four outcomes to perform meta-analysis: incidence of diarrhoea, loperamide use, watery and soft stools (Bristol's Stool Chart). We used odds ratio (OR) to compare efficacy and estimated the pooled OR, using random effects (RE) or fixed effects (FE) models for certain outcomes; heterogeneity was assessed with Cochran's Q and Higgins I2 test. Analyses were performed with Review Manager 5.
Results: Six randomized controlled trials were included (n = 897). Quality assessment showed an unclear risk due to low reports in incomplete outcome data and performance of ITT analysis, while blinding and randomization issues were present in certain studies. The pooled OR for the incidence of diarrhoea was synthesized from 5 studies and favoured the use of probiotics significantly against control (RE: OR=0.33, 95%CI 0.13–0.83). Probiotics seem to decrease loperamide use, although the RE model produced a non-significant OR (RE:OR=0.29, 95%CI 0.01–6.80; FE:OR=0.45, 95%CI 0.24- 0.88); the incidence of watery stools also decreased (RE:OR=0.36, 95%CI 0.05–2.81; FE:OR=0.36, 95%CI 0.17–0.77), but not that of soft stools (RE:OR=2.34, 95%CI 0.11–49.17; FE:OR=4.77, 95%CI 1.76-12.98). Pooled results showed heterogeneity (Cochran's Q: P < 0.05; I2: high); publication bias wasn't assessed due to the low number of studies.
Conclusion: Probiotic supplementation showed beneficial effect in the prevention and treatment of radiation-induced diarrhoea.

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High frequency of overlap between functional dyspepsia, irritable bowel syndrome and overactive bladder
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Objective: Overactive bladder syndrome (OAB) is defined as a symptom complex comprising urgency, with or without urge incontinence, and usually frequency and nocturia. The association between irritable bowel syndrome (IBS) and bladder symptoms has been reported. On the other hand, we recently reported that functional dyspepsia (FD) patients with concomitant constipation or diarrhea show different characteristics from those without bowel symptoms, suggesting a possible high overlap frequency between FD and IBS (Neurogastroenterol. Motil. 24(4):325-e164, 2012). This study is designed to investigate whether FD, like IBS, is associated with OAB.
Methods: A web surveys containing questions about OAB, FD, IBS, and demographics were completed by 5,494 public individuals (2,302 men and 3,192 women) who have no history of severe illness. The prevalence and overlap of OAB, FD, and IBS were examined.
Results: Among participants with FD, 20.5% could also be diagnosed with OAB (odds ratio [OR]: 2.85; 95% confidence interval [CI]: 2.21–3.67). Although concomitant FD and IBS were more strongly associated with OAB (OR: 4.34; 95% CI: 2.81–6.73), OAB was also highly prevalent among participants with FD but without IBS (OR: 3.09; 95% CI: 2.29–4.18). Among participants with FD, an overlapping OAB condition was more prevalent in those with both postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) (OR: 3.75; 95% CI: 2.48–5.67) than in those with PDS or EPS alone. Among participants with OAB, the severity of bladder symptoms was greater in participants with dyspeptic symptoms than without them.
Conclusion: OAB is common among FD patients, even without overlap of IBS. In order to improve FD patients' QOL, we should pay attention to the overlapping of OAB.

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Image guided radiation therapy (IGRT) reduces gastrointestinal (GI) sequelae compared with external beam radiation therapy (EBRT) in patients with carcinoma of the prostate (CaP)
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Objective: EBRT for CaP delivered without real time imaging to guide delivery of treatment is associated with acute and chronic GI sequelae which impair quality of life of these patients(1). Although IGRT reduces exposure to normal tissues by allowing on-line corrections to the mobile prostate target of irradiation, it is not known if this has any impact on GI radiation sequelae of the patients. We have performed a study comparing the two treatment techniques.
Methods: Thirty-two patients (72 (56-83) years) with localised CaP treated by either EBRT (n = 16) or equivalent dose IGRT (n = 16, including 7 patients who also had image guided brachytherapy boost) were matched by risk disease category according to the National Cancer Clinical Network criteria. Each patient was evaluated for (i) GI symptoms (questionnaire), (ii) anorectal motor and sensory function (manometry using sleeve senor and graded balloon distension) and (iii) anal sphincter morphology (endoanal ultrasound) before RT and at one month and one year after treatment. Two way ANOVA was used to test for changes with time after RT in each of the 2 groups and unpaired t and $\chi 2$ tests were used to test for differences between the treatment techniques. A $P \leq 0.05$ was considered significant in all analyses.
Results: Overall, faecal urgency scores increased (0(0-3) vs 1(0-5), P < 0.001) and rectal compliance reduced (6.5 ± 0.5 ml/mmHg vs 4.8 ± 0.5ml/mmHg, P < 0.001) at 1 year compared to pre treatment. However, when the measurements were compared between the treatment techniques at 1 year, EBRT patients had an increased prevalence of faecal incontinence compared with the IGRT patients (13% vs 8%, P < 0.05, respectively). Lower basal anal pressures (34 ± 3mmHg vs 51 ± 6mmHg, P < 0.05) and squeeze pressures (80 ± 5mmHg vs 110 ± 6mmHg P < 0.01) were also recorded in the EBRT patient group at this time.
Conclusion: At 1 year IGRT not only reduced the prevalence of faecal incontinence, but was also associated with quantitatively less anorectal dysfunction compared with EBRT. The findings represent the first hard evidence of reduced GI sequelae associated with IGRT for CaP. (1)Yeoh et al, 2006, Int J Radiat Oncol Biol Phys 66(4):1072.

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Level of Acetylcholine and serotonin in patients with chronic pancreatitis and pain after eating
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Objective: To establish changes in the concentration of acetylcholine (Ach) and serotonin (5-HT) in the blood of patients with chronic pancreatitis (CP).
Methods: The study involved 15 healthy (control) and 20 CP patients, including 10 patients without complications and 10 patients with a complicated disease. The content of 5-HT and Ach in the blood.
Results: In the blood serum of fasting patients with CP showed elevated levels of 5-HT 0.39 ± 0.05 mg ml^{-1} (P < 0.01) and Ach 1.8 mg ml^{-1} (P < 0.05), reducing holinesteraznoy activity (Che) 0.1 m mol ml^{-1}.30 min (P < 0.01); healthy 0.19 ± 0.02 mg ml^{-1} and 0.8 ± 0.9 mg ml^{-1}.30 min. After a meal in the control group (healthy people) a tendency to increase 5-HT from 0.19 ± 0.02 to 0.23 ± 0.019 pg ml^{-1} (P > 0.05). Ach levels are increased from 0.8 ± 0.06 to 1.0 ± 0.05 m mol l^{-1} (P < 0.05). Increase Ach was carried out by reducing the activity of Che 0.9 ± 0.1 to 0.5 m mol l^{-1}.30 min (P < 0.01). In patients with CP the reaction of biologically active substances on food intake was different: the concentration of 5-HT increased from 0.39 ± 0.05 to 0.6 ± 0.07 mg ml^{-1} (P < 0.05), and Ach decreased from 1.8 ± 0.4 to 1.6 ± 0.3 m mol l^{-1} (P > 0.05). Che is low and the activity did not change before and after the meal (0.1 ± 0.02 and 0.1 ± 0.01 m mol l^{-1}.30 min). The original high level of Ach in CP does not imply an additional increase it after food stimulation. In the control group, the content of neurotransmitters in the blood serum before and after load changes of the same type of food and has a strong correlation in the test series. In patients with CP to meal installed a high correlation, which decreases after a meal, stimulating formation of new functional and regulatory context. Through the analysis of morphological, instrumental, biochemical data in patients with CP, and using food as a stress test for the detection of violations of regulatory mechanisms can be assumed that an increase in the content of Ach and 5-HT is a compensatory response to reduced exocrine activity.
Conclusion: Stimulation of pancreatic secretory activity moves to an autonomous type of regulation that is a poor prognostic factor and 5-HT is involved in the formation of pain after eating.

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Correlation between gastrointestinal symptoms(GI) with clinical factors and the treatment with Metformin in Diabetis Type 2 (type2DM) patients
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Objective: To identify which clinical factors are associated with Gastrointestinal symptoms, including metformin use, in patients with Type2DM.
Methods: We performed a study with 116 outpatients with Type2DM recruited at Antonio Pedro Universitary Hospital, using a validate questionnaire to measure GI symptoms. The questionnaire was divided in

two GI Symptoms categories (upper and lower GI symptoms), glicemic control (glycolsilated hemoglobin HbA1c), diabetic complications (renal, neuropathy, retinopaty), and metformin treatment. 81 consecutive subjects with age and gender matched control were recruited for this study.
Results: Among the 116 Type2DM 75(64.6%),55 female (55%),mean age 59.6 years old – (Group I GI) presented GI symptoms. Forty one 41 (GroupII GII) Type2DM, 23(56%), mean age 58.5 years old not presented digestive complaints. Thirty 30(37.5%) of the control group(GIII),58 female(62.5%), mean age 52.5 years old had GI symptoms respectively($P < 0.05$). In GI group 51(7 4.6%) and GII 35 (83.3%) patients were in treatment with metformin ($P > 0.05$).The GI had mean values of HbA1c 10.3g% and 69(92%) of these patients presented diabetic complications. In the GII mean values of HbA1c were 7.87g% and diabetic complications were associated to 21 (48.7%) of them.($P < 0.05$).
Conclusion: Gastrointestinal symptoms (GI) occur frequently in patients with DM and have a negative impact on the quality of life in diabetis. These symptoms may reflect poor glycemic control or autonomic nervous system dysfunction.In our study, Type2 DM was associated with increased prevalence of GI symptoms, and these symptoms appeared to be independently linked to poor glicemic control and diabetic complications. Digestive disorders represent the most common metformin side effects (around 30%) with the first-line drug treatment for type2DM.These symptoms may cause frequently to discontinue metformin treatment. In present study the prevalence of gastrointestinal symptoms were similar in patients regardless of metformin administration. These results suggest that many of GI symptoms can be attributed to the diabetic functional gastrointestinal disorders and the metformin treatment discontinuation could be not the therapeutic solution in all clinical situations.

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Does a westernised diet predispose to the development of FGIDs?

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Objective: The reported prevalence of Functional Gastrointestinal Diseases (FGIDs) in particular irritable bowel syndrome (IBS) and functional dyspepsia (FD) in Asia has been rising. We postulate this is associated with the increasing adoption of Western dietary patterns. Our aim is to explore the contribution of a westernised diet to the development of FGIDs.
Methods: A proportional stratified sample of adults over 21 years old (according to housing types) was obtained from the Singapore Department of Statistics. We conducted face-to-face interviews using a structured questionnaire comprising a battery of prompted questions designed to gather data on demographics, socio-economic, environmental, lifestyle, socio-cultural and dietary factors. Anthropometric measurements and saliva for Helicobacter pylori were also collected. FGIDs were defined according to ROME III criteria. Western diet is defined as a diet that is high in saturated-fat and red meats but low in fruits and vegetables.
Results: There were 297 subjects (F:M 179:118). 31.3% fulfilled FGID (20.9% IBS, 24.2% FD, 9.1% IBS/FD overlap). Woman (F 36.9% v M 22.9%) and younger subjects had more FGID ($P < 0.05$). There was no difference in BMI between FGIDs and normal subjects but FGIDs subjects reported more weight gain (32.3% v 13.2%, $P = 0.001$). There was no significant difference in education, smoking and Helicobacter pylori status. FGID subjects took more Western meals compared to normal subjects ($P < 0.001$). (Table 1). FGIDs also had higher consumption of cereals but lower intake of bread and coffee. There was a trend of lower consumption of chili among FGIDs but the result was not significant ($P = 0.099$).In terms of dietary habit, we found that FGIDs subjects tend to omit breakfast (17.2% vs 8.8%, $P = 0.048$).
Conclusion: Westernisation of Asian diet may be associated with FGID in Asia. This may explain the rise in the prevalence of FGID in Asia. Cultural and traditional Asian beliefs influenced the choice of dietary avoidance. Coffee and chili are important dietary factors that deserve more studies to investigate their effects on FGIDs.

Table 1: FGID and Food Culprit

	FGID N (%)	Non-FGID N (%)	P value (two-sided)
Total	93 (31.3)	204 (68.7)	
At least one standard portion a week			
Western meals	51 (54.8)	66 (32.4)	0.000
Cereals	41 (44.1)	65 (31.9)	0.050
At least one standard portion a day			
Coffee	38 (40.9)	114 (55.9)	0.018
Bread	28 (30.1)	88 (43.1)	0.040
Chili	32 (34.4)	92 (45.1)	0.099
Oily fried food	20 (21.5)	28 (13.7)	0.125

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Gastrointestinal contraction patterns and segmental transit times evaluated with ambulatory capsule tracking

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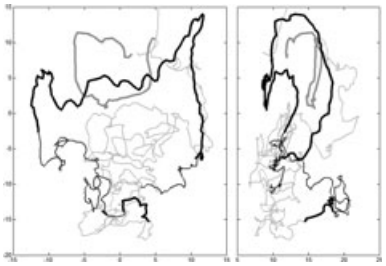
Objective: To test the possibility of ambulatory assessment of gastrointestinal movement patterns and segmental transit times by tracking of ingested electronic capsules.
Methods: Motilis-3D-transit is a novel ambulatory system whereby one or more electronic capsules (diameter 8 mm) emitting magnetic fields are tracked by external sensors carried in a belt. In an ongoing study four healthy subjects ingested an electronic capsule in the morning and the evening on day 1 and again in the morning on day 2. The three capsules were tracked simultaneously while located in various parts of the gastrointestinal tract. The subjects were allowed to keep up most aspects of daily life including travelling by public transport.
Results: In the ambulatory setting simultaneous location and tracking of three capsules located in various segments of the gastrointestinal tract was possible. Continuous recordings were obtained from ingestion until exit of the capsules or for up to 60 h. Characteristic basic contraction frequencies of 3 min^{-1} in the stomach, 8–11 min^{-1} in the small intestine, and 3–6 min^{-1} in the colon were clearly visible. Gastric emptying, duodenal passage (thick grey line in figure), small intestinal transit (thin grey line), ileocecal passage (thick black line) and segmental colorectal transit times were determined. Distance covered during colonic mass movement (thick black line) was identified.
Conclusion: Ambulatory and continuous tracking of three electronic capsules throughout the gastrointestinal tract was realized by using Motilis-3D-transit. This approach allows minimally invasive estimation of gastrointestinal contraction patterns, gastric emptying, small intestinal transit, and segmental colonic transit times.



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Biliary tract motility in patients with chronic hepatitis C

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Objective: Evaluation of biliary tract motility and evacuation in patients with chronic hepatitis C.
Methods: Forty-eight patients aged 22–64 years and diagnosed with chronic hepatitis C(CHC). Control group 35 healthy individuals was recruited. All patients had blood biochemistry and serological tests, virological evaluation of pathogen, duodenal intubation with fractional chromatic bile sampling procedure, ultrasonography, oesophagogastroduodenoscopy,liver morphology examination. Every patient signed Informed Consent Form study participation. Differences between compared values were considered statistically significant $P \leq 0.05$.
Results: The duodenal intubation test produced following results: Sample A volume in CHC patients was twice as high as that in control group, and bile flow rate (mL/min) of this sample differed with statistical significance between patients with CHC and controls, which was an indirect indication of duodenal hypertension in study subjects. Considerable differences, at high statistical significance ($P < 0.001$), were obtained for sphincter Oddi (SO) function, as

CHC patients were shown to have SO hypertension. We observed hypermotor gallbladder dysfunction with accelerated evacuation ($P < 0.05$) and increased bile flow ($P < 0.05$). A follow-up ultrasonography performed to assess gallbladder contractility yielded significantly more rapid gallbladder evacuation ($P < 0.05$) in CHC group,36% quicker at 40 minutes compared with controls. It should be mentioned that 32 patients had gallbladder contraction rate that was over 50% higher than that in controls, thus indicating pronounced hypermotor gallbladder dysfunction in these patients.

Conclusion: Our diagnostic data obtained in patients with CHC suggest marked biliary tract dysfunction: hypertonic sphincter of Oddi, biliary type (type 3), and hypermotor gallbladder dysfunction, which is an apparent indication of significant quality of life deterioration in study subjects. Abnormalities in patients with this condition are not limited to biliary tract motility impairment. All these findings suggest an undisputable, close link between all elements of the hepatic-biliary-duodenal zone. Patients with CHC have impaired bile-producing and bile-secreting hepatic functions, along with biliary tract motility impairment, abnormal physico-colloidal properties of the bile, increased formation of microliths, and development of biliary insufficiency in more than 80% of patients, which definitely must be taken into account when administering treatment. Treatment of these disturbances must include mebeverine (Duspatalin) 200 mg twice daily and ursodeoxycholic acid 10–12 mg kg^{-1} body weight.

500

Cortical brain activation in response to somatic pain in symptomatic diverticular disease

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Objective: Increased somatisation and anxiety are associated with increased reporting of pain in diverticular disease however the underlying mechanisms are unclear. Our aims were to identify differences in pain processing between Asymptomatic (ADD) and symptomatic (SDD) groups.

Methods: Forteen patients with ADD, 14 patients with SDD and a Patient Health Questionnaire 12 (PHQ12) score ≤6 (Low SDD, LSDD) and 14 patients with SDD and a PHQ12 score ≥7 (High SDD, HSDD) participated. A Medoc Pathway system was used to deliver thermal stimuli to the back of the left foot. The temperature at which participants consistently rated pain at 6–7 on a visual analogue scale (Pain Threshold, PT) was identified. Participants underwent fMRI in a 3T Philips Achieva Scanner. A total of 10, 5 s duration VAS temperature stimuli were applied to the back of the left foot, interspersed with 25–30 s rest periods. Differences between the ADD and SDD groups (2 sample t-test, Uncorrected $P < 0.05$, Voxel threshold 5) were analysed using SPM8.

Results: ADD and LSDD subjects were significantly older than the HSDD group (Median yrs [IQR]: 61.5 [59.5–67.3], 62.0 [55.8-67.3], 54.5 [50.3-59.5] $P < 0.05$) and had <% female (% Female; 42.5%, 57.1% & 78.6% respectively $P > 0.05$) The LSDDs had a greater reported incidence of diverticulitis compared to HSDDs (50% VS 35.7% $P > 0.05$). ADD and LSDDs also had lower scores on the hospital anxiety and depression score (Median [IQR]: 5.5 [3.0–7.0], 5.5 [3.0-7.3], 8.5 [5.5-12.8], $P < 0.05$). There was no significant difference in the temperature PT between groups. Significant differences between groups in brain areas related to emotional pain processing (insula, anterior cingluate cortex and amygdala) were observed (Table 1).

Conclusion: This the first brain imaging study of pain processing in DD patients shows greater activation of affective pain processing areas in the SDD groups compared to ADD. This is similar to changes previously reported in irritable bowel syndrome suggesting central active treatments may be beneficial in SDD.

Title: CORTICAL BRAIN ACTIVATION IN RESPONSE TO SOMATIC PAIN IN SYMPTOMATIC DIVERTICULAR DISEASE

Table 1

	Region	MNI Coordinates x, y, z	T score	P value (uncorrected)
ADD>LSDD				
	R pINS	34,-28,6	2.22	0.013
LSDD>ADD				
	R aINS	32,0, -2	3.26	0.001
	L aINS	-40,20,2	1.91	0.028
	R ACC	4,28,22	2.09	0.019
	R MCC	4,-22,40	2.24	0.013
	R Amy	32,0,-2	3.26	0.001
	L Amy	-20,0,-16	2.07	0.019
ADD>HSDD				
	L MCC	0,-22,34	2.53	0.006
HSDD>ADD				
	R aINS	36,18,-2	2.29	0.011
	R ACC	6,32,20	1.85	0.032
	L ACC	-6,2,30	1.82	0.034
	R MCC	16,16,36	1.8	0.036
	L Amy	-32,12,-20	2.21	0.013

R Right; L Left; A Activations; ACC Anterior cingulate cortex; aINS Anterior insula; Amy, Amygdala; MCC Midcingulate cortex; pIns Posterior insula.

Saturday, 8 September 2012, 12.30 – 14.30, Foyer Sala Magenta
PS-35 Late Breaking Abstracts

001

Expression and hierarchic role of serotonin receptors in the control of the guinea pig distal colon peristalsis

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Objective: The main source of serotonin (5-HT) in the body are enterochromaffin cells (EC) of the intestinal mucosa. EC cells when stimulated by mechanical/chemical stimuli release 5-HT in the lumen. Released 5-HT participate to the regulation of intestinal motility by activating at least five receptor types, 5-HT1, 2, 3, 4, 7. Aims of this work were to establish the presence of 5-HT3, 5-HT4 and 5-HT7 receptors and define their role in peristalsis.

Methods: The presence and relative expression of 5-HT3, 5-HT4 and 5-HT7 receptors was evaluated by quantitative real time RT-PCR, and their hierachic role in the activation and propagation of peristaltic reflex in isolated guinea pig colonic segments was defined by using selective antagonists.

Results: Real time RT-PCR showed that 5-HT3 and 5-HT7 receptor mRNAs were found in comparable amounts, but lower in density compared to the 5-HT4 mRNA. In functional studies, peristaltic activity was reduced by 65%, 85% and 40% by 5-HT3, 5-HT4 and 5-HT7 receptor blockade, respectively. Peristaltic activity was reduced by the administration of the antagonists given in pairs by 16% (5-HT3+5-HT4), 70% (5-HT4+5-HT7), 87% (5-HT4+5-HT7), and invariably blocked by the simultaneous administration of the three antagonists. At submaximal distension (about 75% of control response), peristaltic activity was blocked by the administration of 5-HT3, 5-HT4 or 5-HT7 antagonists while at maximal distension was reduced by 75, 60, and 65% respectively.

Conclusion: Endogenous 5-HT plays a key role in the modulation of peristalsis since simultaneous blockade of the 5-HT3, 5-HT4 and 5-HT7 receptors suppresses peristaltic activity. Among receptor subtypes, 5-HT4 are the most functionally important for peristalsis, followed by 5-HT3 and 5-HT7. Our findings bear implications for the development of novel therapeutic targets for patients with functional bowel disorders.

002

Impact of late anorectal dysfunction on quality of life after radiotherapy for prostate cancer

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Objective: Anorectal dysfunctions including decreased sphincter pressures and rectal capacity, are common after pelvic radiotherapy. This study aims to explore the relation of subjective and objective anorectal func-

tion with quality of life (QoL) and their relative impact on QoL in patients irradiated for prostate cancer.
Methods: Patients underwent anal manometry, rectal barostat measurement and they completed validated questionnaires, at least 1 year after prostate radiotherapy (range 1–7 years). QoL was measured by the Fecal Incontinence Quality of Life scale (FIQL) and the Expanded Prostate Cancer Index Composite Bowel domain (EPICB)-bother subscale. Severity of symptoms was rated by the EPICB-function subscale.
Results: Anorectal function was evaluated in 85 men (mean age 72 years; range 53–84 years). Sixty-three percent suffered from one or more anorectal symptoms. Correlations of individual symptoms ranged from $r = 0.23$ to $r = 0.53$ with FIQL domains and from $r = 0.36$ to $r = 0.73$ with EPICB-bother scores. They were strongest for fecal incontinence and urgency. Correlations of anal sphincter pressures, rectal capacity and sensory thresholds ranged from $r = 0.00$ to $r = 0.42$ with FIQL domains and from $r = 0.15$ to $r = 0.31$ with EPICB-bother scores. Anal resting pressure correlated most strongly. Standardized regression coefficients for QoL outcomes were largest for incontinence, urgency and anal resting pressure. Regression models with subjective parameters explained a larger amount (range 26–92%) of variation in QoL outcome than models with objective parameters (range 10–22%).
Conclusion: Fecal incontinence and rectal urgency are the symptoms with the largest influence on QoL. Impaired anal resting pressure is the objective function parameter with the largest influence. Therefore, sparing the structures responsible for an adequate fecal continence is important in radiotherapy planning.

003

A new rat model for enteric neurodegeneration

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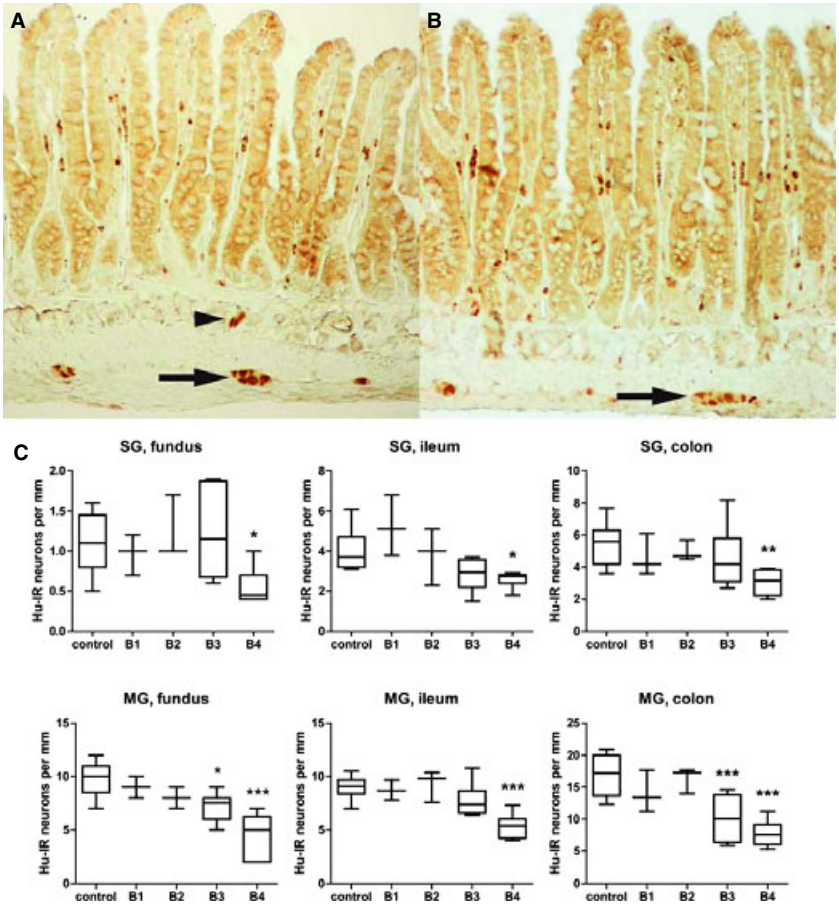
Objective: The gonadotropin-releasing hormone (GnRH) analog buserelin is given to women undergoing In vitro fertilization (IVF). Development of chronic intestinal pseudo-obstruction (CIPO) when treated with buserelin has been described. The purpose of this study was to investigate possible effects on enteric neurons in response to repeated administration of buserelin in rat.
Methods: Rats were treated with subcutaneous injections of buserelin or saline daily for 5 days followed by three weeks recovery, representing one session, and were treated one to four sessions.
Results: Rats treated four sessions with buserelin displayed a markedly reduced numbers of submucous and myenteric neurons in fundus, ileum and colon compared to saline-treated controls. The neuronal loss may be explained by increased apoptosis, reflected by increased immunoreactivity of activated caspase-3. No GnRH or GnRH receptors were identified in rat gastrointestinal tract, but numerous IR for LH receptors was seen. The relative number of neurons immunoreactive for LH receptors decreased after buserelin treatment. No increase in inflammation in the gastrointestinal tract or increases in interleukins/cytokines in the circulation were noted.
Conclusion: Repeated administration of buserelin induces gastrointestinal neurodegeneration in rat. The mechanisms probably involve activation of LH receptors since numerous submucous and myenteric neurons along the gastrointestinal tract posses LH receptor immunoreactivity.



004

Irritable bowel syndrome impairs psychological well-being and gastrointestinal symptoms in patients with microscopic colitis

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Objective: The primary purpose of this study was to compare gastrointestinal symptoms and subjective well-being in microscopic colitis (MC) female outpatients, with or without co-existing irritable bowel syndrome (IBS)-like symptoms. Moreover, autoantibodies against gonadotropin-releasing hormone (GnRH) have recently been detected in patients with IBS. The second purpose was to examine the prevalence of autoantibodies against GnRH in patients with MC.
Methods: Women with biopsy-verified MC, collagenous and lymphocytic colitis (CC and LC, respectively), at any outpatient clinic of the Departments of Gastroenterology, Skåne, between 2002 and 2010 were invited to participate in the study. Questionnaires about gastrointestinal symptoms (Gastrointestinal Symptom Rating Scale, GSRS), quality of life (Psychological General Well-being Index, PGWB), life style, medical history and the Rome III questionnaire were answered within two weeks prior to blood sampling. Autoantibodies against GnRH and GnRH- receptor (GnRH-R) were determined by an enzyme-linked immunosorbent assay (ELISA) and blood donors served as controls.
Results: Altogether, 159 (66.2%) of 240 invited patients with MC were recruited. Of these, 134 (55.8%) also accepted to provide blood samples for analysis of antibodies against GnRH. Patients with IBS-like symptoms (55%) experienced much more symptoms and worse psychological well-being in all dimensions in GSRS and PGWB compared to patients without IBS symptoms. Anti-GnRH antibodies were detected in 11/133 MC patient and 7/98 in the control group. The prevalence of anti-GnRH-R were 11/133 and 6/98 in MC patients and control group, respectively. There were no significant differences between the groups.
Conclusion: MC patients fulfilling Rome III criteria for IBS experience more gastrointestinal symptoms and worse psychological well-being in all dimensions in GSRS and PGWB. Furthermore, no elevated prevalence of autoantibodies against GnRH or GnRH-R was observed.

005

Use of a more specific inhibitor, T16Ainh-A, confirms a key role for Ano1 in the regulation of ICC proliferation

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Objective: In the muscle layers of the gastrointestinal tract Ano1 is selectively expressed in ICC and appears to be required for normal gastrointestinal function. Ano1 is expressed in all classes of ICC, including those that do not contribute to generation of the slow wave, suggesting that it may have additional functions. One proposed role is in the control of cellular proliferation. General Cl- channels blockers resulted in a decrease in ICC proliferation. However, these compounds can also act at a number of other targets. Recently, a high throughput screening identified a novel small molecule, T16AinhA01, as a more specific inhibitor of Ano1. We used T16AinhA01 to specifically target Ano1 to study its role as a regulator of ICC proliferation.
Methods: Primary cultures of ICC and smooth muscle strips were obtained by enzymatic dissociation or dissection of the small intestine of 3 day old mice. CFPAC1 cell line, endogenously expressing Ano1, was also used. Cells and tissues were treated with T16AinhA01 at a final concentration of 10 μ mol L^{-1} for 24 h and then fixed for immunofluorescence staining. Antibodies against Kit were used to stain ICC. Proliferating cells were identified by immunoreactivity for Ki67 or EdU.
Results: Proliferation of ICC was significantly reduced in primary cultures from mice following treatment with T16AinhA01, as assessed by counting the number of Ki67positive ICC per field. Similar results were obtained also with the use of CFPAC1 cells. Proliferation of ICC was also reduced in organotypic cultures of smooth muscle strips. Moreover, the total number of proliferating cells/confocal stack was also assessed and no significant difference was observed, suggesting that the inhibitory effect was specific for ICC.
Conclusion: The novel compound T16AinhA01 reduced the number of proliferating ICC in culture confirming our previous observations that Cl- entry through Ano1 is important for optimal ICC proliferation. Ano1 appears therefore to have multiple roles in the muscle layers of the gastrointestinal tract. Funded by DK57061.

006

Epidemiology of Chronic Constipation in Germany in 2012 (GECCO)

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Objective: Prevalence of chronic constipation (CC) in Germany has been reported to be substantially lower than in other European countries and worldwide, but reliable data are unavailable.
Methods We ran a data sampling frame using a RDD approach (Random Digit Dialing), and conducted both a telephone interview (CATI; Computer assisted telephone interview) and a mailing of a written questionnaire to those that acknowledged the presence of constipation symptoms during the preceding 12 months.
Results: We contacted 1005 individuals age 18 or older who agreed to participate in CATI. Of these, 175 (17.4%) (52.3 ± 17.8 years, 26.3% males) acknowledged having experienced constipation during the last 12 months, 59 (5.9%) reported constipation during the last 4 weeks, and 20 (2.0%) having current constipation symptoms. Of all respondents 36 (3.6%) had consulted a doctor for constipation, and 50 (5.0%) had taken medication for constipation. Of all 175 individuals, 73 (43.4%) were willing to provide their postal address and were sent the written questionnaire that asked for health-related quality of life, the presence of Rome-III defined symptoms of functional CC and the irritable bowel syndrome, and the presence of other diagnoses. A total of 52 questionnaires were returned (71.2%). Based on these data, we estimated that contacting 15.000 persons in a second phase of GECCO by the same strategy will allow collecting these data from 500 to 1000 individuals with constipation. During this phase we will obtain more precise information on medication, health care utilization and absence from work during the last 12 months.
Conclusion: This preliminary study shows that this population-based method is appropriate to recruit individuals to participate in surveys related to constipation. Up to 17% of the general population may report symptoms of constipation, but the majority of them are probably not of clinical relevance. (Supported by research funds from Shire-Movetis NV, Turnhout, Belgium).

007

Effect of hypothermia on human colonic smooth muscle contractility

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Objective: Paralytic ileus is a frequent complication of major abdominal surgery, but the exact mechanism is unknown. The aim of the study was to assess the effect of thermal cooling on human colonic circular muscle strip (HCCMS) contractility.
Methods: HCCMSs were obtained from disease-free margins of resected segments for cancer. After removing the mucosa and serosa layers, strips were mounted in separate chambers. After 30 min spontaneous contractions gradually developed. Isometric contractions were measured using force displacement transducers connected with a computer. Temperature was decreased every 45 min (from 37 °C to 36 °C and to 35 °C). Temperature values were established on the basis of intra-operative colonic temperature measurements during surgery. The effect of cooling was analyzed on mean contractile amplitude and on contraction to Acetylcholine (Ach, 10^{-5} mol L^{-1}).
Results: At 37 °C HCCMSs developed a stable phasic contraction with a significant Ach-elicited contractile response (39% compared to baseline). Lowering thermal bath temperature higher mean contractile amplitude was observed, being 20 and 19% increased at 36 °C and 35 °C respectively, compared to 37 °C mean percentage of contraction. By decreasing temperature, response to Ach slightly increased, being 40% at 36 °C and 46% at 35 °C. By setting the temperature at the initial value (37 °C), mean percentage of contraction and Ach response remained higher during the entire 30-min period of observation. A preliminary theoretical model has shown that thermal cooling leads the transmembrane potential to change its period as well as its duration and amplitude.
Conclusion: Acute thermal cooling affects colonic neuromuscular function by increasing basal tone and response to cholinergic stimulus. These effects are not reversible after short-term observation. We hypothesize that temperature gradients, by changing the ion dynamics, could influence the electrochemical behaviour of intestinal muscle, with a memory effect which should require long time to be deleted. Further studies are needed to establish the exact involved mechanisms in order to better understand clinical consequences of hypothermia during abdominal surgery.

008

Exposure of human colonic mucosa to Lipopolysaccharide induces impairment of muscle cell contractility: Role of oxidative stress and mucosal translocation

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Objective: Impairment of gastrointestinal (GI) motility is frequently observed in humans during severe infection and in animal models of sepsis. The study was aimed to assess whether exposure of human colonic mucosa to pathogenic LPS may affect SMC contractility and to examine possible involved mechanisms.
Methods: Human colonic mucosa and submucosa were sealed between two chambers, with the luminal side of mucosa facing upward and covered with 5 mL of Krebs solution with or without LPS 0111:B4 and the submucosal side faced downward into 25 mL of Krebs. After 30 min, the Krebs solution on the submucosal side, in absence (N-Undernatant) or presence of LPS (LPS-Undernatant), were collected to measure LPS and H2O2 levels. Enzymatically isolated SMCs were exposed for 30 min to N-Undernatant or to LPS-Undernatant in presence of catalase, indomethacin or MG132. At the end of pre-incubation periods the cells were contracted with acetylcholine (ACh). Some SMCs were directly exposed to LPS.
Results: LPS level was higher in LPS-Undernatant (2.7 ± 0.7 ng mL^{-1}) than in N-Undernatant (0.45 ± 0.06 ng mL^{-1}) ($P < 0.001$). H2O2 level was significantly higher in LPS-Undernatant (133.75 ± 15.9 vs 82 ± 7.5 n mol L^{-1}). SMCs incubated with N-undernatant had a maximal contraction of 32 ± 5% that was reduced of 62.9 ± 12% in SMCs exposed to LPS-Undernatant. Inhibition of maximal contraction was reversed by pre-incubation with catalase, indomethacin or MG132. Inhibition of contraction was also observed after direct LPS exposure (57 ± 15%).
Conclusion: Acute exposure of colonic mucosa to pathogenic LPS impairs muscle cell contractility. It's reasonable that this effect is due either to LPS translocation throughout the mucosal and submucosal layers, and to production of free radicals and prostaglandins which leads to suppression of muscle cell contractility via nuclear factor-kappaB (NF-kB) activation.