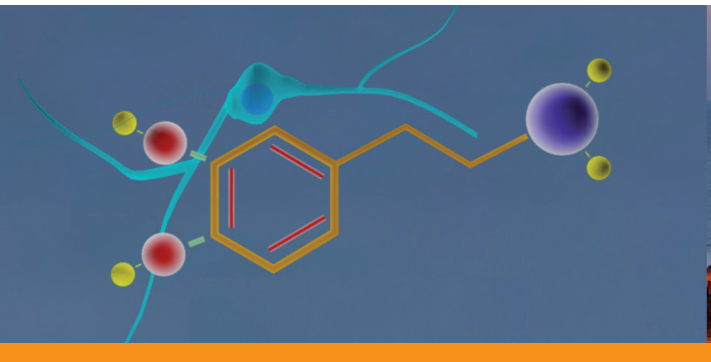
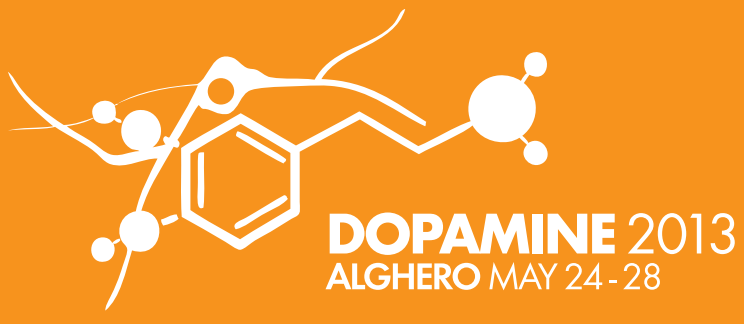
PROGRAM/ABSTRACTS



DOPAMINE 2013
ALGHERO MAY 24 - 28



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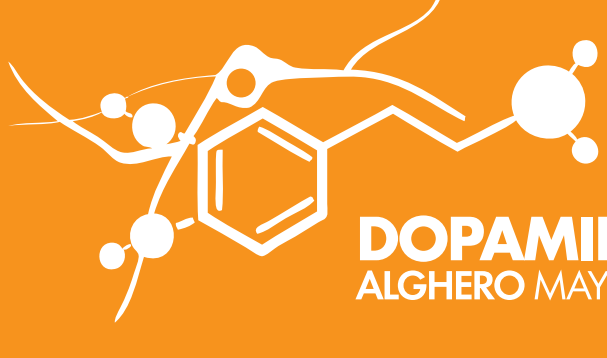

DOPAMINE 2013
ALGHERO MAY 24 - 28

Friday, MAY 24th

8:00 – 8:30 REGISTRATION

8:30 – 10:30 SESSION 1

Topic: *DOPAMINE AND REWARD*

Room: SAYÀL

ENDOCANNABINOID/DOPAMINE INTERACTIONS AND THE PURSUIT OF REWARD

Chair: E.L. Gardner (USA) - Co-Chair: J.F. Cheer (USA)
Organizer: J. F. Cheer (USA)
-**M. Melis (Italy)***Enhanced 2-arachidonoylglycerol signaling in dopamine neurons as a possible marker of heightened predisposition to addiction*
-**C. Lupica (USA)***Cocaine inhibition of synaptic GABA inputs to VTA DA neurons: Roles for serotonin and endocannabinoids*
-**L. Venance (France)***Endocannabinoid and dopamine interact to mediate spike-timing dependent potentiation and depression*
-**J.F. Cheer (USA)***Endocannabinoids modulate accumbal dopaminergic encoding during signaled shock avoidance*

Topic: *DOPAMINE AND COGNITION*

Room: QUARTÉ

DOPAMINE – RELATED LEARNING DYSFUNCTIONS IN ADDICTION

Chair: G. Hasler (Switzerland) - Co-Chair: P.N.Tobler (Switzerland)
Organizer: G. Hasler (Switzerland)
-**P.N. Tobler (Switzerland)***Role of dopamine in learning - findings from single cells and neuroimaging*
-**S. Flagel (USA)***Parsing the role of dopamine in stimulus-reward learning: Implications for Addiction*
-**Q. Huys (UK)***Learning processes in the development of addiction*
-**G. Hasler (Switzerland)** *Dopaminergic dysfunction and reward learning in bulimia nervosa*

Topic: *DOPAMINE AND AFFECTIVE DISORDERS*

Room: ARCHI

THE ROLE OF THE DOPAMINE TRANSPORTER IN HUMAN DISEASE

Chair: R.D. Blakely (USA) - Co-Chair: M. Kurian (UK)
Organizer: M. Kurian (UK)
-**M. Reith (USA)***The dopamine transporter: Structure, function and pharmacological considerations*
-**R.D. Blakely (USA)***The Role of the Dopamine Transporter in Attention Deficit/Hyperactivity Disorder*
-**A. Galli (USA)***De novo mutation in the dopamine transporter gene associates dopamine dysfunctionwith autism spectrum disorders*
-**M. Kurian (UK)***Dopamine Transporter Deficiency Syndrome: Characterisation of the Clinical and Molecular Features of this Novel "Dopamine Transportopathy*

Topic: *DOPAMINE RECEPTORS*

Room: LAZZARETTO

DOPAMINE RECEPTORS: NEW CELLULAR MECHANISMS RELEVANT FOR CNS DISORDERS

Chair: P. Sokoloff (France) - Co-Chair: B. Le Foll (Canada)
Organizer: B. Le Foll (Canada)
-**G. Collo (Italy)***Critical role of dopamine D3 receptor in structural plasticity generated by exposure to drugs of addiction in mesencephalic dopaminergic neurons*
-**B. Le Foll (Canada)***Neuronal circuitry underlying the impact of dopamine D3 receptors ligands on drug addiction*
-**E.A. Rabiner (UK)***Evaluation of the Dopamine D3 receptor in the living brain with [11C]-(+)-PHNO PET*
-**I. Boileau (Canada)** *Imaging D3 dopamine receptor levels across behavioral and drug addiction: positron emission tomography / [11C]-(+)-PHNO studies*
-**E. Merlo-Pich (Italy)***Pharmacological targeting of Dopamine D3 receptor: a review of therapeutic applications*

10:30 – 11:00 COFFEE BREAK

11:00 – 13:00 SESSION 2

Topic: *DOPAMINE NEURONS*

Room: LAZZARETTO IS OUR LIFE STYLE KILLING OUR DOPAMINE NEURONS?
Chair: M.J.Zigmond (USA) - Co-Chair: U. Spampinato (France)
Organizer: M.J.Zigmond (USA)
-**F. Cicchetti (Canada)***Impact of n-3 PUFA in Parkinson's Disease: evidence from animal models*
-**V.A. Russel (South Africa)***Interactions between early stress and exercise in a rat model of Parkinson's Disease*
-**J.L. Cameron (USA)***Physical activity protects the striatum against damage in nonhuman primates*
-**M.J. Zigmond (USA)** *Neurotrophic factors: a link between life style and the health of dopamine neurons?*

Topic: *DOPAMINE AND NEUROPLASTICITY*

Room: QUARTÉ NEW INSIGHTS INTO THE DEVELOPMENT AND PLASTICITY OF DOPAMINE CONNECTIVITY
Chair: C. Flores (Canada) - Co-Chair: P. Henny Vargas (Chile)
Organizer: C. Flores (Canada)
-**P. Henny Vargas (Chile)***Structural and synaptic substrates of activity in dopaminergic neurons*
-**J. Pasterkamp (Netherlands)***Molecular and genetic dissection of dopaminergic pathway development*
-**C. Bellone (Switzerland)***Drug-evoked synaptic plasticity in the VTA: a role for calcium-impermeable, GluN3A-containing NMDARs*
-**C. Flores (Canada)***Netrin-1 receptors organize mesocortical dopamine circuitry during adolescence*

Topic: *DOPAMINE AND REWARD*

Room: SAYÀL OPTOGENETIC PERTURBATION OF DOPAMINE NEURONAL CIRCUITRY
Chair: A. Bonci (USA) - Co-Chair: G. Stuber (USA)
Organizer: G. Stuber (USA)
-**L. Kravitz (USA)***Dissecting the striatal contributions to reinforcement and punishment*
-**N. Uchida (USA)***Dissecting computations in the dopamine reward circuit*
-**T. Kash (USA)***Genetic Modulation of Periaqueductal Grey Dopamine Neurons*
-**J. Britt (USA)***The organization and behavioral consequence of discrete glutamatergic input to the nucleus accumbens*
-**G. Stuber (USA)***Dissecting the neural circuitry that mediates reward and aversion*

13:00 – 15:00 LUNCH BREAK

15:00 – 17:00 SESSION 3

Topic: *DOPAMINE AND PARKINSON'S DISEASE*

Room: LAZZARETTO TRANSCRIPTION FACTORS AS MEDIATORS OF DOPAMINE NEURODEGENERATION IN PARKINSON'S DISEASE
Chair: A. Björklund (Sweden) - Co-Chair: A. Prochiantz (France)
Organizer: M. Decressac (Sweden)
-**T. Perlmann (Sweden)***Transcription factors for the development and maintenance of dopamine neurons*
-**A. Prochiantz (France)***Traveling homeoprotein transcription factors regulate mDA survival and physiology*
-**A. Björklund (Sweden)***Nurr1 as a regulator of survival and GDNF-induced neuroprotection in adult dopamine neurons*
-**M. Smidt (Netherlands)***Pitx3 is part of a homeodomain code for dopaminergic subset specification and survival*

Topic: *DOPAMINE AND REWARD*

Room: SAYÀL

NUCLEUS ACCUMBENS: CORE AND SHELL SPECIALIZATION IN REWARD FUNCTION?

Chair: R.A. Wise (USA) - Co-Chair: G. Di Chiara (Italy)
Organizer: R.A. Wise (USA)
-**S. Ikemoto (USA)***Drug and dopamine reward and the ventral striatum*
-**R.A. Wise (USA)***Cocaine reinforcement is a shell phenomenon; cocaine satiety involves the core*
-**B.J. Aragona (USA)***Regional specificity of real-time dopamine transmission: implications for reward and function*
-**J.A. Dani (USA)***Mechanistic Interactions between the Nicotinic Cholinergic and Dopaminergic Systems during Signaling and Synaptic Potentiation*

Topic: *ANATOMY AND PHYSIOLOGY OF DOPAMINE SYSTEMS*

Room: ARCHI

THALAMOSTRIATAL SYNAPSES - ANOTHER SUBSTRATE FOR DOPAMINE ACTION

Chair: J.P. Bolam (UK) - Co-Chair: G.W.Arbuthnott (Japan)
Organizer: G.W.Arbuthnott (Japan)
-**J.P. Bolam (UK)***The thalamostriatal projection: a major excitatory input to the basal ganglia*
-**G. Halliday (Australia)***Impact of thalamic changes in Parkinson's disease on the motor system*
-**J. Ding (USA)***Formation and Function of Thalamostriatal Synapses on Striatal Medium Spiny Neurons and Choliergic Interneurons*
-**K. Kobayashi (Japan)***Behavioral roles of thalamostriatal pathway in sensory discrimination learning*

Topic: *DOPAMINE AND REWARD*

Room: QUARTÉ

INDIVIDUAL DIFFERENCES IN DOPAMINE SIGNALING: ROLE IN LEARNING, RISK TAKING, AND IMPULSIVITY

Chair: A. Dagher (Canada) - Co-Chair: M. Frank (USA)
Organizer: A. Dagher (Canada)
-**M. Frank (USA)***Computational models of dopamine in learning and choice incentive*
-**A. Gjedde (Denmark)***Sensation-seeking propensity is directly proportional to dopamine storage and inversely proportional to dopamine turnover in human dorsal striatum in vivo*
-**R. Ebstein (Singapore)***Dopamine D4 Receptor (DRD4) Gene, Religious Affiliation and Spirituality Contribute to Dictator Game Altruism*
-**M. Leyton (Canada)***Vulnerability to Addictions: Dopamine Studies in Humans*

17:15 – 18:15 PLENARY LECTURE:

Room: SAYÀL

JAMES M. TEPPER (USA)
Striatal TH Interneurons: Physiology, Pharmacology and Transmitter Release
Host: M. Morelli (Italy)

18:30 OPENING RECEPTION

Saturday, MAY 25th

8:30 – 10:30 SESSION 1

Topic: *DOPAMINE AND NEUROPLASTICITY*

Room: SAYÀL

DOPAMINE REGULATION OF STRIATAL PLASTICITY
Chair: D.M. Lovinger (USA) - Co-Chair: M.S. Levine (USA)
Organizer: P. Belujon (USA)
-**P. Belujon (USA)***Altered hippocampal-accumbens synaptic plasticity in an animal model of depression*
-**R. Malenka (USA)***Role of distinct subtypes of dopamine neurons in reward and aversion*
-**A.A. Grace (USA)***Regulation of plasticity in the nucleus accumbens by afferent input: impact of stress*
-**J. Reynolds (New Zealand)***Timing dependent effects of substantia nigra dopamine on sensory and motor reinforcement*

Topic: *DOPAMINE AND SCHIZOPHRENIA*

Room: LAZZARETTO

DOPAMINE-GLUTAMATE INTERACTIONS IN BRAIN AND THE TREATMENT OF SCHIZOPHRENIA
Chair: T.H.Svensson (Sweden) - Co-Chair: B. Moghaddam (USA)
Organizer: P. Sokoloff (France)
-**K. Tseng (USA)***2B or not 2B modulated by dopamine: Input-specific late-adolescent emergence of NMDA-NR2B receptor-mediated synaptic transmission in the prefrontal cortex requires postsynaptic AKAP-PKA signaling*
-**T.H. Svensson (Sweden)***Presynaptic alpha2 adrenoceptor blockage in the treatment of schizophrenia*
-**P. Sokoloff (France)***Local and remote interactions of the dopamine D3 receptor with glutamate pathways: implications for the treatment of schizophrenia*
-**B. Moghaddam (USA)***Glutamate -dopamine interactions and prefrontal cortex function*

Topic: *DOPAMINE AND SIGNAL TRANSDUCTION*

Room: ARCHI

DOPAMINE SIGNALING IN HEALTH AND DISEASE: MECHANISMS AND INTERVENTIONS
Chair: J.Caboche (France) - Co-Chair: M.G. Caron (USA
Organizer: G. Fisone (Sweden)
-**P. Vanhoutte (France)***Dopamine-glutamate receptor interplay in the striatum modulates molecular, structural and behavioral responses to cocaine*
-**R. Moratalla (Spain)***Structural and synaptic plasticity associated with L-DOPA-induced dyskinesias in mouse models of Parkinson's disease*
-**G. Fisone (Sweden)***Identification of molecular targets for the treatment of Parkinson's disease and L-DOPA-induced dyskinesia*
-**R. Brambilla (Italy)***The Ras-ERK signalling pathway in the control of striatal function: from basic mechanisms to potential therapeutic approaches for hyperdopaminergic disorders*

Topic: *DOPAMINE AND PARKINSON'S DISEASE*

Room: QUARTÉ

NEURODEGENERATIVE MECHANISMS OF PARKINSON'S DISEASE AND POTENTIAL NEUROPROTECTIVE STRATEGIES
Chair: M. Morelli (Italy) - Co-Chair: M.A. Cenci (Sweden)
Organizer: L. Zecca (Italy)
-**M. Morelli (Italy)***Amphetamine-like drugs and degeneration of dopaminergic nigro-striatal neurons*
-**M. Pizzi (Italy)***Late-onset parkinsonism in NF-κB/c-Rel deficient mice*
-**D. L. Sulzer (USA)***Neuronal MHC-I display in T-cell mediated neurodegeneration*
-**L. Zecca (Italy)***Neuromelanin organelle pathways and neurodegeneration in Parkinson's Disease*

10:30 – 11:00 COFFEE BREAK

11:00 – 13:00 SESSION 2

Topic: *DOPAMINE NEURONS*

Room: QUARTÉ THE REGULATION OF DOPAMINE NEURON ACTIVITY STATES AND ITS RELEVANCE TO DOPAMINE SYSTEM FUNCTION

Chair: W. Schultz (UK) - Co-Chair: B. Hyland (New Zealand)
Organizer: A.A. Grace (USA)
-**A A. Grace (USA)***Tonic and phasic dopamine neuron activity and the regulation of information processing*
-**N. Mercuri (Italy)***Membrane properties and neurotransmitter-mediated responses of mesencephalic dopaminergic neurons*
-**J. Roeper (Germany)***Dopamine Diversity in the Substantia Nigra*
-**W. Schultz (UK)***Extension of dopamine reward responses by generalization and pseudoconditioning*

Topic: *DOPAMINE AND ADDICTION*

Room: LAZZARETTO STRUCTURE, REGULATION AND FUNCTION OF THE DOPAMINE TRANSPORTER

Chair: N.R. Zahniser (USA) - Co-Chair: G. Tanda (USA)
Organizer: A.H. Newman (USA)
-**H.H. Sitte (Austria)***The importance of kinases in the regulation of dopamine transporter-mediated efflux*
-**N.R. Zahniser (USA**) *Individual differences in acute low dose cocaine-induced locomotor activation in rats involve striatal dopamine transporters and predict cocaine addiction-like behaviors*
-**S. Jones (USA)***Cocaine and Methylphenidate Self-Administration Produce Opposite Changes in Dopamine Transporter Function*
-**K. Henry (USA)***Uncovering the cocaine binding site in the dopamine transporter using the cocaine-like photo affinity ligand RTI 82*
-**G. Tanda (USA)***Preference for Distinct Functional Conformations of the Dopamine Transporter alters the Relationship Between Subjective Effects of Cocaine and Stimulation of Mesolimbic Dopamine*

Topic: *DOPAMINE AND NEUROPLASTICITY*

Room: SAYÀL SYNAPTIC AND BEHAVIORAL PLASTICITY IN THE MESOCORTICOLIMBIC SYSTEM

Chair: C. Lüscher (Switzerland) - Co-Chair: A. Bonci (USA)
Organizer: C. Lüscher (Switzerland)
-**P. Janak (USA)***Illuminating the Role of Dopamine Neurons in Reward Learning*
-**C. Lüscher (Switzerland)***Drug-evoked synaptic plasticity of excitatory and inhibitory transmission*
-**M. Mameli (France)***Projection-specific synaptic plasticity in the lateral habenula*
-**R. Tonini (Italy)***Endocannabinoid signaling in action control*

13:00 – 15:00 LUNCH BREAK

15:00 – 17:00 SESSION 3

Topic: *DOPAMINE RECEPTORS*

Room: LAZZARETTO DOPAMINE RECEPTOR HETEROMERIC COMPLEXES AND THEIR FUNCTION

Chair: S.R. George(USA) - Co-chair: E. Borrelli (USA)
Organizer: S.R. George (CANADA)
-**S.R. George (USA)***The dopamine D1-D2 receptor heteromer: Novel signaling and function mediated by a unique subset of neurons in basal ganglia*
-**P.J. McCormick (Spain)***Diurnal Dopamine-Adrenergic heteromers can control melatonin production*
-**R. Franco (Spain)***Functional and pharmacological consequences of D2 receptor-containing heteromers. Relevance of heteromer quaternary structure and loss of heteromes in L-DOPA induced dyskinetic primates*
-**A. Kern (USA)***Growth Hormone Secretagogue Receptor (GHSR1a, aka ghrelin receptor) Antagonists as Selective Blockers of Dopamine Signaling in GHSR1a:DRD2 and GHSR1a:DRD1 Expressing Neurons*

Topic: *DOPAMINE AND ADDICTION*

Room: QUARTÉ

DIFFERENTIAL ROLE OF THE MESOLIMBIC SYSTEM IN PSYCHOSTIMULANT VERSUS OPIATE REWARD
Chair: A. Badiani (Italy) - Co-Chair: G. Di Chiara (Italy)
Organizer: A. Badiani (Italy)
-**D. Belin (France)** *Habitual drug seeking: from pathophysiology to treatment*
-**T.E. Robinson (USA)***Cocaine and opioid cues: incentive salience*
-**J. Bossert (USA)***Context-Induced Reinstatement of Heroin vs. Cocaine Seeking: Overlapping yet Distinctly Different Neuroanatomical Substrate Involvement*
-**A. Badiani (Italy)***Heroin and cocaine reward are different: clinical and preclinical evidence*

Topic: *DOPAMINE AND COGNITION*

Room: ARCHI

MAKING SENSE OF PREFRONTAL CORTICAL DOPAMINE FUNCTION
Chair: H.L.Fields (USA) - Co-Chair: P. O'Donnell (USA)
Organizer: P. O'Donnell (USA)
-**S. Floresco (USA)***Dissociable regulation of different executive functions by prefrontal D1 and D2 receptors*
-**P. O'Donnell (USA)***Adolescent Maturation of Prefrontal Cortical Function*
-**V.S.Sohal (USA)***Optogenetic stimulation of synaptic inputs reveals novel effects of dopamine D2 receptors in prefrontal cortex*
-**T. Weickert (Australia)***Prefrontal brain activity predicted by dopaminergic genes in healthy adults is modulated by antipsychotics in schizophrenia*

Topic: *DOPAMINE AND NEUROPLASTICITY*

Room: SAYÀL

REWARD LEARNING AND DOPAMINE
Chair: R.M. Wightman (USA) - Co-Chair: R.A. Wise (USA)
Organizer: R.M. Wightman (USA)
-**G. Meredith (USA)***The importance of circuit rewiring in amphetamine-associated contextual learning*
-**P.J. Kenny (USA)***Dopamine and overeating*
-**J.R. Wickens (Japan)***Methylphenidate specifically rescues deficient anticipatory dopamine release*
-**R.M. Wightman (USA)***In vivo measurements of dopamine release during behavior*

17:00 – 17:30 COFFEE BREAK

17:30 – 18:30 PLENARY LECTURE

Room: SAYÀL

REGINA M. CARELLI (USA)
Dynamics Of Mesolimbic Signaling In Learning, Decision Making And Addiction
Host: G. Di Chiara (Italy)

18:30 – 20:00 POSTER SESSION I

Topic: Dopamine and Parkinson's Disease

P001. A transcription factor-microRNA autoregulatory loop determines dopamine neuron numbers. A. Anderegg, H-P. Lin, G. Caronia-Brown, B. Yun, R. Johnson , B. Harfe, R. Awatramani*.
P002. Effects of antidepressant drugs in a rat model of depression in preclinical stages of Parkinson's disease. Klemencja Berghauzen-Maciejewska, Jadwiga Wardas, Katarzyna Kuter, Wacław Kolasiewicz, Urszula Głowacka, Krystyna Ossowska.
P003. Cognitive impairment and abnormal dentate gyrus plasticity in Parkinson's disease.Bonito-Oliva A., Pignatelli M., Spigolon G., Seiler S., Yoshitake T., Kehr J., Mercuri N., Nisticó R., Fisone G.
P003BIS. Transcriptional effects of LRRK2 kinase inhibition in LRRK2-expressing immune cells. Christensen KV*, Hentzer M, Daechsel JC, Herzig MC and Smith GP
P004. Longitudinal automated gait analysis of the MitoPark mouse, a model of progressive dopaminergic cell loss. M. Ronild, D.R. Andersson Clarke.
P005. Calpains inhibition increases striatal TH-immunoreactive neurons in hemiparkinsonian rats treated with L-DOPA. Christine Robitaille, Laure Chagniel, Geneviève Bureau and Michel Cyr.
P006. Nitric oxide modulates rotational behaviour: a comparison between selective D1, D2 receptor agonists and L-DOPA in a rat model of Parkinson's disease. A. Czarnecka*, T. Lenda, J. Konieczny, K. Kamińska, E. Lorenc Koci.
P007. Establishing a cell line model to study tyrosine hydroxylase deficiency. Héctor Díez*, Carlos Ortez, Noelia Fernández, Mercè Izquierdo, Pau Gorostiza, Bru Corman, Artur Llobet, Rafael Artuch, Àngels Garcia-Cazorla.
P008. MSK1 mediates L-DOPA-induced dyskinesia in an experimental Parkinsonian mouse model via expression of delta FosB. Michael Feyder, Emanuela Santini, Erik Södersten, Giada Spigolon, Klaus Hansen, Jocelyne Caboche, Vincent Vialou, Eric J Nestler, Gilberto Fisone.

P009. Antidyskinetic effect of memantine and amantadine in the rat Parkinson's disease model.C. Fidalgo, E. Tronci, C. Lisci, M. Collu, R. Stancampiano, M. Carta.
P010. Involvement of dopamine in generation of oxidative stress in a 6-hydroxy-dopamine-induced partial dopaminergic striatal lesion model in the rat. Y. Aluf, J. Vaya, S. Khatib, J.P.M. Finberg.
P011. Atypical dopamine transporter deficiency syndrome in an adult male: Molecular characterization of new transporter variants. Freja H. Henriksen, Tina Skjørringe, Saiqa Yasmeen, Natascha V. Arends, Thorvald Fauerschou Andreassen, Kevin Erreger, Aurelio Galli, Merete Karlsborg, Lena E. Hjermind, Lisbeth Birk Møller, Ulrik Gether.
P012. The stoichiometry of protomers in the complexes formed between tyrosine hydroxylase maximally phosphorylated at Ser19 and 14-3-3. Jorge-Finnigan Ana, Kleppe Run, Rosati Sara, Alvira Sara, Valpuesta José María, Haavik Jan, Heck Albert JR and Martinez Aurora.
P013. Behavioral and biochemical effects of combined treatment with L-DOPA and tricyclic antidepresants in 6-OHDA-lesioned rats. Kinga Kamińska, Tomasz Lenda, Anna Czarnecka, Jolanta Konieczny, Elżbieta Lorenc-Koci
P014. Mice with genetically evoked selective loss of noradrenergic system as a possible tool to study presymptomatic phase of Parkinson's Disease. Grzegorz Kreiner, Agnieszka Jurga, Marta Kot, Katarzyna Rafa-Zablocka, Monika Baginska, Rosanna Parlato, Guenther Schuetz, Wladyslawa A. Daniel, Irena Nalepa.
P015. Behavioral evidence of a hyperdopaminergic phenotype in LRRK2 G2019S mice.Longo F. & Morari M.
P016. Aberrant GABAergic tonic inhibition is present in the GP of parkinsonian rodents. Miguelez C, Morin S, Chazalon M, Ugedo L, Baufreton J.
P017. Enhanced vesicular storage to reduce dopamine toxicity. GW Miller, A Salahpour, A Bernstein, K Lohr, T Guillot, E Heath, K Stout, M Wang, Y Li
P018. The pathophysiology of Parkinson's disease: LRRK2, neurotransmission and synaptic maintenance. Beccano-Kelly D.A., Volta M., Co K., Chou P., Tatarnikov I., Cao L.P., Munsie L.N., Tapia L., Melrose H., Raymond L.A., Farrer M.J. & Milnerwood A.M.
P019. Direct generation of dopamine cells from fibroblasts and assessment of their therapeutic potential in animal models of Parkinson disease. Mus L., Caiazzo M., Leo D., Gainetdinov R.R.
P020. Elaboration of a rat model of depression present in preclinical stages of Parkinson's disease. Krystyna Ossowska1, Klemencja Berghauzen-Maciejewska, Katarzyna Kuter, Wacław Kolasiewicz, Anna Dziubina, Krystyna Gołembiowska, Jadwiga Wardas
P021. A new therapeutic strategy for Parkinson's disease.Pinna A., Costa G., Simola N., Tronci E., Carta M., Morelli M.
P022. Role of the transcription factor Pitx3 in modulating vulnerability of midbrain dopaminergic neurons to neurodegenerative stress. Abbas F Sadikot, Kelvin C Luk, Vladimir V Rymar, Pepijn van den Munckhof, Stefan Nicolau, Claude Steriade, Panojot Bifsha, Jacques Drouin
P023. Role of dopamine D3R/nAChR heterodimeric complex in the regulation of dopaminergic neurons function.P.Savoia*, C.Fiorentini, J.Grigoletto, A.Bellucci and C.Missale
P024. Down-regulation of the D1R/Shp-2/Erk1/2 pathway ameliorates L-DOPA-induced dyskinesia in the 6-Hydroxy-Dopamine rat model of Parkinson's disease. D. Savoldi*, P.Savoia, A. Prandini, C. Fiorentini, C. Missale
P025. Susceptibility of dopaminergic and GABAergic neurons in the rat substantia nigra to toxicity of the selective proteasome inhibitor lactacystin: in vivo study. T. Lenda*, A. Czarnecka, P. Nowak, K. Kamińska, E. Lorenc-Koci, J. Konieczny
P026. Effect of the serotonin precursor 5-hydroxytryptophan on L-DOPA-induced dyskinesia in parkinsonian rats.E. Tronci, C. Lisci, R. Stancampiano, P. Devoto, M. Collu, M. Carta.
P027. Dopamine-dependence of murine LRRK2 associated behaviors. M Volta; D. Beccano-Kelly; L. Tapia; C. Vilarino-Guell; K. Co; P. Chou; A. Huang; K. Yu; S. Bergeron; H. Melrose; M Farrer; A Milnerwood
P028. Impaired dopaminergic neurotransmission and vesicular recycling in human LRRK2 (R1441G) transgenic mice. SJ Choi, Y Huang, CJ Li and H Zhang*

Topic: Dopamine Co-Transmission

P 029. Contribution of TAAR1 receptors in effects of apomorphine. I. Sukhanov, S. Espinoza, D.S. Yakovlev, M.C. Hoener M, T.D. Sotnikova, R.R Gainetdinov

Topic: Anatomy and Physiology of Dopamine Systems

P030. Characterizing dopamine release and clearance in the striatum of c57/Bl6 mice using in vivo chronoamperometry. E. Arvidsson, Å. Mackenzie
P031. Role of Trace amine-associated receptor 1 (TAAR1) in the modulation of dopaminergic system and cortico-striatal signaling.S. Espinoza, I. Sukhanov, G. Lignani, L. Medrihan, D. Leo, S. Maggi, G. Giannotti, F. Fumagalli, F. Benfenati, V. Tucci, R. Gainetdinov
P032. Analysis of Glutamate, GABA, Noradrenaline, Dopamine, Serotonin and Metabolites using microbore UHPLC with electrochemical detection.M. Eysberg, V.Valentini, H.-J. Brouwer, N. J. Reinhoud, L. M. van Heerwaarden
P033. Spatial distribution of D1R- and D2R-expressing Medium-sized Spiny Neurons differs along the rostro-caudal axis of the Dorsal Striatum. G. Gangarossa, J. Espallergues, P. Mailly, A. De Kerchove D'Exaerde, D. Hervé, J. Antoine Girault, P. Krieger, E. Valjent.
P034. Reward responses of presumed dopamine neurons in the dorsal raphe nucleus. B. Hyland, Y. Li
P035. Taar1-mediated modulation of dopaminergic neurotransmission.D. Leo, S. Espinoza, M.C. Hoener, T. Sotnikova, R.R Gainetdinov.
P036. Immunocytochemical detection of PSD-95, TH and Golgi-Cox stained elements: visualization in the same slice. G. Mulas, S. Spiga, M. Diana
P037. Sex-specific effect of COMT on cortical anatomy.S. Sannino*, A. Gozzi, A. Cerasa, D. Scheggia, F. Managò, D. De Pietri Tonelli, A. Bifone, G. Spalletta, D. R. Weinberger, F. Papaleo
P038. Anatomical and functional characterization of the cortical input to D1 or D2 receptor-expressing striatal neurons. G. Spigolon, D. Fürth, A. Bonito-Oliva, Y. Xuan, I. Pollak Dorocic, E. Yoo, P. Krieger, M. Carlén, K. Meletis, G. Fisone
P039. Activity of VTA DA neurons induces a homeostatic plasticity of GIRK channels. K.R. Tan, A. Lalive, M. Munoz, P. Slesinger, C. Lüscher

Topic: Dopamine and Cognition

P040. The positive allosteric modulator of nicotinic alpha 7 cholinergic receptors, AVL-3288, stimulates cathecolamine release in the prefrontal cortex and nucleus accumbens shell of adolescent rats: potential utility in ADHD?. R. Cadeddu, M. Ibba, T. Johnstone, D. Hogenkamp, K.W. Gee and E. Carboni.
P041. Methylphenidate enhances cognitive stability at the expense of cognitive flexibility. Fallon.S.J., van der Schaaf, M., ter Huurne, N., Cools, R
P042. The Role of Midbrain Dopamine in Predictive Fear Learning. S. Li; G. P. McNally
P043. Impact of cortical alpha-synuclein overexpression on the performance in operant tasks of cortico-striatal function. Hanna S. Lindgren, Alex Klein,Andreas Heuerand Stephen B. Dunnett.
P044. Stuck in a loop: pre-training methamphetamine speeds up transition to habit dominated behavior. H. Pacitti, B. Balleine* & S. Killcross; Helena Pacitti
P045. Dysbindin-1 genetic disruption modulates cognitive flexibility in mice. D. Scheggia*, G. Kochlamazashvili, A. Dityatev, F. Papaleo

Topic: Dopamine and Neurodegeneration

P046. The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. Eleftheria K. Pissadaki and J. Paul Bolam
P047. Vulnerability to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity in adolescent mice chronically treated with 3,4-methylenedioxymethamphetamine (MDMA). Costa G., Frau L., Pinna A., Wardas J., Morelli M.
P048. SH-SY5Y neuroblastoma cells as a tool for studying designer drug toxicity - results on "bath salt" mephedrone. B. den Hollander, S. Rozov, I. Ojanperă, E. Kankuri, E. Korpi
P049. Effect of caffeine on the release of DA, 5-HT and production of hydroxyl radical induced by methamphetamine and 3,4-methylenedioxymethamphetamine in the mouse striatum. Krystyna Gołembiowska, Anna Maria Górska, Katarzyna Kamińska
P050. A translational study on Dopamine Transporter Deficiency Syndrome: development of new animal models. Placido Illiano, Jean-Martin Beaulieu, Claude Lamarre, Raul R. Gainetdinov

Topic: Imaging Dopamine

P051.Transgenic expression of GCaMP calcium indicator in dopaminergic neurons for optogenetic measurement of presynaptic calcium transients in striatum.C. Sgobio, G. Cui, Z. Li, D. M. Lovinger, H. Cai

Sunday, MAY 26th

8:30 – 10:30 SESSION 1

Topic: *DOPAMINE AND REWARD*

Room: LAZZARETTO

GENETIC AND FUNCTIONAL DISSECTION OF THE DOPAMINE D2 RECEPTOR IN MOTOR AND REWARD – MEDIATED BEHAVIORS

Chair: M. Rubinstein (Argentina) - Co-Chair: V.A. Alvarez (USA)

Organizer: M. Rubinstein (Argentina)

M. Rubinstein (Argentina) - Spatial and temporal genetic dissection of *Drd2* expression reveals an expanded functional repertoire of the dopamine D2 receptor

V.A. Alvarez (USA) - Low D2 receptors on dopaminergic neurons enhance the reinforcing properties of cocaine and reward-paired cues

J.T. Williams (USA) - Spontaneous D2-receptor dependent IPSCs in dopamine neurons

A. De Kerchove D'Exaerde (Belgium) - Roles of two efferent dopaminoceptive neuronal populations of the striatum in motor control and addiction: a transgenic approach

D.M. Lovinger - Dopamine-Cholinergic interactions in striatal synaptic plasticity

Topic: *DOPAMINE AND NEUROPLASTICITY*

Room: ARCHI

DOPAMINERGIC IMPACT ON NEUROPLASTICITY AND COGNITION IN ANIMALS AND HUMANS: A TRANSLATIONAL APPROACH

Chair: S. Otani (Japan) - Co-Chair: M.A.Nitsche (Germany)

Organizer: M. A. Nitsche (Germany)

-**Y. Goto (Japan)***Dopamine-dependent synaptic plasticity in cortico-limbic information processing*

-**S. Otani(Japan)***Functional and dysfunctional plasticity inductions in rodent prefrontal cortex*

-**M.A. Nitsche (Germany)***Dopaminergic impact on neuroplasticity in humans*

-**H. DenOuden (Netherlands)***Dissociable roles of dopamine and serotonin transporter polymorphisms in reversal learning*

Topic: *ANATOMY AND PHYSIOLOGY OF DOPAMINE SYSTEMS*

Room: QUARTÉ

LOCAL REGULATION OF STRIATAL DOPAMINE RELEASE

Chair: M.E. Rice (USA) - Co-Chair: P. Phillips (USA)

Organizer: M. E. Rice (USA)

-**S.J. Cragg (UK)** *Axonal Control of Striatal Dopamine Transmission by ACh*

-**J.C. Patel (USA)***Rapid inhibition of dopamine release by endogenous dynorphin and kappa opioid receptors in ventral but not dorsal striatum*

-**A.R. West (USA)***Striatal nitric oxide and dopamine interactions*

-**M.E. Rice (USA)***Hydrogen peroxide (H_2O_2) as a modulator of striatal dopamine release*

Topic: *DOPAMINE AND ADDICTION*

Room: SAYÀL

DOPAMINE-MEDIATED REWARD AND REINFORCEMENT IN PRECLINICAL DISEASE MODELS

Chair: L.J. Porrino (USA) - Co-Chair: K.M. Kantak (USA)

Organizer: K.M. Kantak (USA)

-**L.J. Porrino (USA)***Development of Dopamine Systems in Childhood and Adolescence*

-**E.M. Unterwald (USA)***Altered dopaminergic function and reward sensitivity in a rat model of Post-Traumatic Stress Disorder*

-**S. Izenwasser (USA)***Sexually dimorphic changes in reward and the dopamine system in a rodent model of diet-induced obesity*

-**K.M. Kantak (USA)***Cocaine Abuse Liability in a Rodent Genetic Model of ADHD: Comparison of Stimulant and Non-Stimulant Medication during Adolescence*

10:30 – 11:00 COFFEE BREAK

11:00 – 13:00 SESSION 2

Topic: *DOPAMINE AND PARKINSON'S DISEASE*

Room: QUARTÉ NEW MODELS OF NEURODEGENERATION FOR EXPERIMENTAL PARKINSON'S DISEASE

Chair: F. Blandini (Italy) - Co-Chair: G.U.Corsini (Italy)
Organizer: F. Blandini (Italy)
-**M.G. Spillantini (UK)***Alpha-synuclein dysfunction affects the dopaminergic system in a transgenic mouse model*
-**F. Blandini (Italy)***Central dopaminergic denervation affects splanchnic organ functions*
-**A.R. Carta (Italy)***The chronic MPTPp mouse model of progressive Parkinson disease*
-**M.T. Herrero (Spain)***The importance of the primate model of Parkinsonism*
-**Y. Wu (China)***Role of mTOR signaling pathway in dopamine D2 receptor-mediated suppression of neuroinflammation*

Topic: *DOPAMINE AND ADDICTION*

Room: SAYÀL ETHANOL-MECHANISMS ALONG THE MESOLIMBIC DOPAMINE SYSTEM

Chair: M. Ericson (Sweden) - Co-Chair: I. Diamond (USA)
Organizer: E.Jerlhag (Sweden)
-**M. Ericson (Sweden)***The role of gut-brain hormones in reward from addictive drugs*
-**P. Steensland (Sweden)***The monoamine stabilizer (-)-OSU6162 attenuates voluntary ethanol intake and ethanol-induced dopamine output in the nucleus accumbens*
-**M.S. Brodie (USA)***Actions of ethanol on protein kinase C function affecting dopaminergic ventral tegmental area neurons*
-**H. Morikawa (USA)***Dopamine Neuron Plasticity in Alcoholism*

Topic: *DOPAMINE AND SIGNAL TRANSDUCTION*

Room: ARCHI THE IMPACT OF STEROIDS ON DOPAMINE NEUROTRANSMISSION

Chair: M. Bortolato (USA) - Co-Chair: T. Di Paolo (Canada)
Organizer: M. Bortolato (USA)
-**T. Di Paolo (Canada)***Implication of the membrane estrogen receptor GPER1 in estrogenic neuroprotection and neuromodulation of brain dopamine*
-**C. Frye (USA)***Progestogens mediate mating behavior of rodents in part through dopamine signalling in the ventral tegmental area*
-**P.E.M. Phillips (USA)***Phasic dopamine transmission in the striatum during drug use*
-**M. Bortolato (USA)***Neurosteroids as dopaminergic modulators: neurobiological mechanisms and clinical implications*
-**C.S. Weickert (Australia)***Dopamine D2 Receptor Abnormalities in Schizophrenia: Possible Modulation by Sex Steroids and Genotype*

Topic: *DOPAMINE AND SCHIZOPHRENIA*

Room: LAZZARETTO DOPAMINERGIC FUNCTION IN MICE MUTANT FOR SCHIZOPHRENIA-RELATED GENES: CONVERGENCE OR DIVERGENCE?

Chair: J.L.Waddington (Ireland) - Co-Chair: T.H. Svensson (Sweden)
Organizer: J.L.Waddington (Ireland)
-**F. Papaleo (Italy)***Dysbindin-1 modulates cognitive deficits relevant to schizophrenia via dopamine pathways*
-**P. Moran (UK)***D-amphetamine and antipsychotic drug effects on Latent Inhibition in mice lacking dopamine D2 receptors*
-**H. Nawa (Japan)***Vulnerability of dopaminergic development to neuregulin-1 and EGF; Implication in schizophrenia-related behavioral deficits*
-**C.M. O'Tuathaigh (Ireland)***A mouse mutant model with disruption of two schizophrenia risk genes: dopaminergic modulation of schizophrenia-relevant phenotypes*

13:00 – 15:00 LUNCH BREAK

20:00 SOCIAL DINNER

Monday, MAY 27th

8:30 – 10:30 SESSION 1

Topic: *DOPAMINE AND PARKINSON'S DISEASE*

Room: QUARTÉ

PRE-SYNAPTIC AND POST-SYNAPTIC MECHANISMS IN L-DOPA-INDUCED DYSKINESIA

Chair: M. Carta (Italy) Co-Chair: G. Fisone (Sweden)
Organizer: M. Carta (Italy)
-**M.A. Cenci (Sweden)***Pre- and post-synaptic mechanisms in L-DOPA-induced dyskinesia*
-**P. Piccini (UK)***Pre-and post-synaptic mechanisms in L-DOPA-induced dyskinesia (LID): evidence from functional neuroimaging studies in PD*
-**E. Bezard (France)***Lentiviral-mediated silencing of PSD-95 diminishes L-DOPA-induced dyskinesia in experimental parkinonism*
-**M. Carta (Italy)***5-HT1 receptor agonists for the treatment of L-DOPA-induced dyskinesia: toward clinical investigation*

Topic:*DOPAMINE AND REWARD*

Room: LAZZARETTO

DOPAMINERGIC INVOLVEMENT IN EFFORT–RELATED ASPECTS OF MOTIVATION

Chair: M. Correa (Spain) Co-Chair: S. Ikemoto (USA)
Organizer: J.D. Salamone (USA)
-**X. Zhuang (USA)***Dopamine, economic decision making and energy balance*
-**W. Hauber (Germany)***Cost/benefit-related decisions in rats: role of dopamine and effects of dopaminergic drugs*
-**J.G. Hosking (Canada)***Cognitive versus physical effort: dopaminergic manipulation has dissociable effects on two rodent models of cost/benefit decision making*
-**J.D. Salamone (USA)***Development of animal models of the effort-related motivational symptoms of depression: Dopaminergic Mechanisms*
-**M. Treadway (USA)***Effort-related DA function and Motivational deficits in Psychopathology*

Topic:*DOPAMINE CO-TRANSMISSION*

Room: SAYÀL

THE MULTILINGUAL NATURE OF DOPAMINE NEURONS

Chair: D.L. Sulzer (USA) Co-Chair: J.M. Tepper (USA)
Organizer: L.E.Trudeau (Canada)
-**L.E. Trudeau (Canada)***Regulation and developmental role of the vesicular glutamate transporter VGLUT2 in dopamine neurons*
-**S. Rayport (USA)***The dopamine neuron functional connectome*
-**T. Hnasko (USA)** *Biophysical evidence for the vesicular co-packaging of glutamate with dopamine*
-**M. Morales (USA)***Subcellular Segregation of Dopaminergic and Glutamatergic Signaling by VTA Neurons*
-**A.W. Mackenzie (Sweden)***Vglut2-mediated glutamate release in the ventral midbrain*

Topic:*DOPAMINE NEURONS*

Room: ARCHI

SEROTONIN-DOPAMINE INTERACTION: NEW OPPORTUNITIES FOR IMPROVED TREATMENTS OF DOPAMINE-RELATED DISORDERS

Chair: K.A. Cunningham (USA) Co-Chair: U. Spampinato (France)
Organizer: K.A. Cunningham (USA)
-**K.A. Cunningham (USA)***Oppositional Control of Dopamine-Mediated Behaviors by Serotonin 5-HT2A and 5-HT2C Receptors: Implications for Pharmacotherapeutics in Addictions*
-**J. Neumaier (USA)***5-HT6 receptor oppose dopamine in striatum*
-**U. Spampinato (France)***5-HT2B receptors-DA interaction: control of DA ascending pathways and therapeutic implications*
-**A. Newman-Tancredi (France)***Biased agonism at pre or post-synaptic serotonin 5-HT1A receptors: therapeutic relevance for dopaminergic dysfunction*
-**V. Valentini (Italy)***5HT/DA system interactions in the reinforcing properties of psychostimulants: role of 5-HT6 receptors*

10:30 – 11:00 COFFEE BREAK

11:00 – 13:00 SESSION 2

Topic: *DOPAMINE AND SCHIZOPHRENIA*

Room: ARCHI

DA RECEPTORS AND THEIR INTERACTIONS WITH OTHER MONOAMINE RECEPTOR MECHANISMS REMAIN A TARGET FOR TREATMENT OF SCHIZOPHRENIA

Chair: P.F. Spano (Italy)Co-Chair: K. Fuxe (Sweden)
Organizer: K.Fuxe (Sweden)
-**K. Sahlholm (USA)***Reversibility of antagonism at the dopamine D2 receptor - how different are typical and atypical antipsychotics?*
-**T.H.Svensson (Sweden)***Dopamine, D1 receptors and noradrenergic mechanisms in the mode of action of antipsychotic drugs*
-**K. Fuxe (Sweden)***D2 receptor heteromers in the ventral striatum as targets for novel antipsychotic drugs*
-**S. Tanganelli (Italy)***Neurotensin receptor NT1 and dopamine receptor D2 heteromers as a target for antipsychotic drugs*

Topic:*DOPAMINE AND ADDICTION*

Room: SAYÀL

DA ROLE IN THE ADDICTED BRAIN: FROM SYNAPSES TO HUMAN BRAIN IMAGING

Chair: M. Diana (Italy) Co-Chair: D. Martinez (USA)
Organizer: A. Bonci (USA)
-**S. Russo (USA)***Mechanisms of synaptic plasticity in addiction and depression*
-**G.F. Koob (USA)***The role of dopamine in addiction: Neuroplasticity from the dark side*
-**N.D. Volkow (USA)** *The Role of Dopamine in the Addicted Human Brain*
-**A. Bonci (USA)***Optogenetic control of the prelimbic cortex: implications for cocaine craving*
-**C. Gipson (USA)***Prelimbic cortex and ventral tegmental area modulate nucleus accumbens core synaptic plasticity during cue- and cocaine-reinstated drug seeking*

Topic:*DOPAMINE NEURONS*

Room: QUARTÉ

BEATING AND BURSTING: THE JOYFUL NOISE OF DOPAMINE NEURONS

Chair: P. Shepard (USA) Co-Chair: J. Roeper (Germany)
Organizer: P. Shepard (USA)
-C.A. Paladini (USA)*Dopamine neurons require two inputs for bursting*
-P. Shepard (USA)*Ether-a`-go-go-related gene potassium channels in midbraindopamine neurons: Implications for a role in depolarization block*
-K. Herrik (Denmark)*Dopamine Beat Box*
-C. Canavier (USA)*Analysis of depolarization block and bursting in dopamineneurons*
-V. Seutin (Belgium)*Activity of dopaminergic neurons in freely moving rats: a new insight into the dynamics of the ventral tegmental area*

Topic:*DOPAMINE NEURONS*

Room: LAZZARETTO

NEUROENDOCRINE DOPAMINE NEURONS: PHYSIOLOGY, REGULATION AND CLINICAL ROLE

Chair: M. Zigmond (USA) Co-Chair: C. Broberger (Sweden)
Organizer: C. Broberger (Sweden)
-**D. Grattan (New Zealand)***Signal transduction pathways mediating prolactin-induced activation of tuberoinfundibular dopamine neurons*
-**C. Broberger (Sweden)***Electrophysiology of tuberoinfundibular dopamine (TIDA) neurons: network properties and modulation*
-**A. Martin (France)***TIDA neurons plasticity: The functional switch*
-**P. Velez (USA)***Electrophysiological Studies of TIDA Neurons From Female Rodents*

13:00 – 15:00 LUNCH BREAK

15:00 – 17:00 SESSION 3

Topic: *DOPAMINE AND NEURODEGENERATION*

Room: QUARTÉ A-SYNUCLEIN INDUCED TOXICITY: SYNAPTIC DYSFUNCTION AND NEURODEGENERATION IN DOPAMINE NEURONS
Chair: P.F. Spano (Italy) Co-Chair: R. Malenka (USA)
Organizer: A. Björklund (Sweden)
-**D. Rubinsztein (UK)***Autophagy and Neurodgeneration*
-**T. Outeiro (Portugal)***Extracellular alpha-synuclein induces neuronal dysfunction and toxicity*
-**P.F. Spano (Italy)***Alpha-synuclein regulates synapsin I and III in developing and mature dopaminergic neurons: implications for Parkinson's disease*
-**M. Decressac (Sweden)***Validation of therapeutic targets against alpha-synuclein toxicity in vivo*

Topic:*DOPAMINE RECEPTORS*

Room: ARCHI MOLECULAR DETERMINANTS OF D2 AND D3 RECEPTOR SELECTIVITY AND EFFICACY
Chair: D.R. Sibley (USA)Co-Chair: J.C. Schwartz (France)
Organizer: D.R. Sibley (USA)
-**D.R. Sibley (USA)***Identification of a novel dopaminergic ligand that is a biased agonist at the D2 dopamine receptor and an antagonist at the D3 receptor*
-**A.H. Newman (USA)***Molecular Determinants of Selectivity and Efficacy at the Dopamine D3 Receptor*
-**R. Lane (Australia)***Identification of a bitopic ligand that acts as a negative allosteric modulator across a dopamine D2 receptor dimer*
-**L. Shi (USA)***Identification of a second binding pocket for the subtype selectivity at dopamine D3 and D2 receptors*
-**J.C. Schwartz (France)***The D3R from discovery to clinical trials indrug abuse with BP1.4979, a partial agonist*

Topic:*DOPAMINE AND COGNITION*

Room: LAZZARETTO MOLECULAR IMAGING OF DOPAMINE NEUROTRANSMISSION DURING HUMAN COGNITIVE PROCESSING
Chair: R.D. Badgaiyan (USA) Co-Chair: A. Dagher (Canada)
Organizer: R.D. Badgaiyan (USA)
-**L. Backman (Sweden)***Cognitive and brain plasticity: The role of dopamine*
-**A. Strafella (Canada)***Imaging Cognitive Dysfunction in Parkinson's Disease*
-**G.R. Samanez-Larkin (USA)***Individual differences in dopaminergic neuromodulation of reward processing and cognition*
-**R.D. Badgaiyan (USA)***Molecular imaging of dopamine neurotransmission during human cognitive processing*

Topic: *DOPAMINE NEURONS*

Room: SAYÀL IMPACT OF NETWORK ACTIVITY ON THE INTEGRATIVE PROPERTIES OF DOPAMINE NEURONS IN RESPONSETO NATURAL REWARDS,DRUGS AND AVERSIVE EVENTS
Chair: F. Georges (France) Co-Chair: M. Ungless (UK)
Organizer: F. Georges (France)
-**F. Georges (France)***Modulation of dopamine neuron activity by afferents: Implications in reward and aversion*
-**M. Ungless (UK)***Functional diversity of dopamine neurons*
-**F. Chaouloff (France)***Cannabinoid type-1 receptors on ventral tegmental area GABAergic neurons control voluntary exercise performance in mice*
-**G.S. Aston-Jones (USA)***Linking context with reward*

17:00 – 17:30 COFFEE BREAK

17:30 – 18:30 PLENARY LECTURE

Room:SAYÀL **EMILIANA BORRELLI (USA)**
The Multiple Facets Of Dopamine D2 Receptor Signaling
Host: P.F. Spano (Italy)

18:30 – 19.30 TECHNOLOGY TRANSFER WORKSHOP

Room: SAYÀL VISUALIZING DA NETWORKS IN THE BRAIN AND THEIR RELATIONSHIPS TO THE NETWORKS THEY TARGET.

Chair: K. Fuxe (Sweden) Co-Chair: P.F. Spano (Italy)

-**S. Spiga/G. Mulas/M.Diana (Unica/Uniss, Italy)***Spine plasticity in the Nucleus Accumbens: confocal analysis.*

-**M. Wu (Neurodigitech, CA, USA)***High-throughput quantitation of 3D neuronal morphology*

-**J.P. Bolam (Oxford, UK)***Dopamine neurons: activity, chemistry and connections*

-**G.E. Meredith (USA)***Structural plasticity of medium spiny neurons: Dopamine-induced changes*

Discussants: **J.M. Tepper; H.L. Fields**

18:30 – 20:00 POSTER SESSION II

Topic:Dopamine and Addiction

P052. Striatal Dopamine Transporter Availability in Patients with Alcohol Dependence: Assessment by ^{123}I-FP-CIT SPECT. A Pilot Study. A. Ferrulli, G. Vassallo, M. Antonelli, A. Mirijello, C. D'Angelo,D. Di Giuda, A. Giordano, *M. Diana, G. Addolorato

P053. Striatal Modulation of BDNF Expression using MicroRNA124a-Expressing Lentiviral Vectors Impairs Ethanol- Induced Conditioned- Place Preference and Voluntary Alcohol Consumption. A. Bahi, J-L. Dreyer

P054. Changes of dopamine transmission in the nucleus accumbens shell and core during ethanol and sucrose self-administration.V. Bassareo, F. Cucca, R. Frau, G. Di Chiara

P055. Genotype dependent adaptive changes in mesolimbic dopamine transmission after adolescent nicotine exposure: possible underlying mechanisms in the gateway effect of nicotine. Cristina Cadoni, Elena Espa, Gaetano Di Chiara

P056. Vulnerability to Addictions: Dopamine Studies in Humans. K.F. Casey, E. Setiawan, I. Boileau, A. Fotros, S.P. Barrett, A. Dagher, C. Benkelfat, M. Leyton

P057. Individual contributions of drug and cues to striatal phasic dopamine signals during self-administration. L.M. Burgeno, I.Willuhn, P.EM. Phillips

P058. Altered response to stress in animals exposed to chronic cocaine treatment during adolescence. L. Caffino, G. Giannotti, G. Racagni, F. Fumagalli

P059. Cocaine self-administration results in neurochemical tolerance and dopamine transporter complex formation which is reversed by a single amphetamine bolus. E.S. Calipari, M.J. Ferris, J.H. Rose, D.CS. Roberts, S.R. Jones

P060. Acetaldehyde operant self-administration in rats: focus on D2-receptor activation. A. Brancato, R.A.M. Marino, F. Plescia, F.M. Sutera, C. Cannizzaro

P061. Ketamine produces structural plasticity of mouse mesencephalic dopaminergic neurons via activation of Akt-mTOR pathway: role of dopamine D3 receptor. L. Cavalleri, F. Bono, V. Tedesco, M. Di Chio, E. Merlo Pich, P.F. Spano, C. Missale, C. Chiamulera, G. Collo.

P062. Oxytocin decreases methamphetamine seeking in an animal model of relapse. B.M. Cox, R.E. See, C.M. Reichel

P063. Pharmacological characterization of JWH-018, a cannabinoid component of "spice" drugs. M.A. De Luca, P. Caboni, Z. Bimpisidis, V. Valentini, G. Margiani, G. Marsicano, M. Melis, M. Marti & G. Di Chiara

P064. Loss of control over food intake, observations from an animal model. J.W. de Jong, T.J.M. Roelofs, K.E. Meijboom, L.J.M.J. Vanderschuren, R.A.H. Adan

P065. JPC-077 interacts with VMAT2 to reduce the neurochemical and behavioral effects methamphetamine.
L.P Dwoskin, J.R. Nickell, J.S. Beckmann, E.D. Denehy, K.B. Siripurapu, J.P. Culver, P.A. Crooks, and M.T. Bardo

P066. The ethanol intake-reducing effect of acamprosate is associated with dopamine elevation. M. Ericson, P. Chau, B. Söderpalm

P067. Effects of the monoamine stabilizer (-) OSU6162 on dopamine output in the nucleus accumbens in rats after long-term voluntary ethanol drinking. K. Feltmann, B. Schilström, P. Steensland; Kristin Feltmann

P068. Dopamine D3 receptors and incubation of cocaine craving.E.L. Gardner, X. Li, J. Li, X-Q. Peng, R. Song, J. Gaal, Z-X. Xi

P069. Role of the Lateral Habenula and Tail of the Ventral Tegmental Area in Reinstatement of Cocaine Seeking. M.J. Gill, J. Kaufling, R.E. See RE

P070. Orexin signaling in the ventral tegmental area is critical for cue-induced reinstatement of cocaine-seeking but not natural reward-seeking behavior. M.H. James, E.M. Levi, J.W. Yeoh, D.W. Smith, C.V. Dayas

P071. Kappa-Opioid Receptors in the Nucleus Accumbens Shell are Important for Pair Bond-induced Attenuation of Amphetamine-induced Conditioned Place Preference. P.C. Keyes, S.L. Resendez, C.J. Austin, B.J. Aragona

P072. Delta9-THC and heroin exposure in adolescence differently affect heroin self-administration in adulthood D. Lecca, C. Cadoni, V. Valentini, G. Piras, A. Scifo, G. Di Chiara.

P073. Elevated activity of dopamine neurons during adolescence: implications for cocaine addiction. J.E. McCutcheon, W.C. Wong, M. Marinelli

P074. Dopamine involvement in Acetaldehyde drinking behaviour: role of Ropinirole on. R.A.M. Marino, A. Brancato, F. Plescia, C. Gagliardo, G. Gambino, C.Cannizzaro

P075. A Glucocorticoid Antagonist Shows Therapeutic Potential for Alcohol Dependence in a POC Human Laboratory Study. B.J. Mason

P076. Dopamine system adaptations in alcohol abstinence: Translational evidence from humans and rats for a hyperdopaminergic state. M. Meinhardt, N. Hirth, L. Broccoli, S. Perreau-Lenz, R.Rimondini, C. Harper, M. Heilig, R. Spanagel, W. Sommer, A. Hansson

P077. Characterization of input-specific innervation of the lateral habenula after exposure to drugs of abuse. F. Meye, M. Mameli

P078. Zinc modulates ethanol-induced dopamine output in the rat nucleus accumbens. J. Morud, L. Adermark, M. Ericson, B. Söderpalm

P079. D-TMS in cocaine addiction: preliminary findings. M. Pedetti, R. Panella, A.G. Frascella, M. Diana.

P080. Reversal of cocaine-evoked synaptic plasticity removes addiction related behaviors. V. Pascoli, J. Terrier, C. Lüscher

P081. Alpha-lipoic acid reduces ethanol self-administration in rats. A.T. Peana, G. Muggironi, A. Arru, M. Diana

P082. Differential effects of addictive drugs on dopamine release in shell and core of the Nucleus Accumbens in Hatano high and low-avoidance rats.G Piras, V Perra, R Frau, GP Serra, V Valentini, G Di Chiara.

P083. A novel BAC transgenic mouse strain expressing GFP under control of the dopamine transporter promoter. Sara Stilling, Jacob Eriksen, Gunnar Sørensen, Troels Rahbek-Clemmensen, Jan Pravsgaard Christensen, Ulrik Gether and Mattias Rickhag
P084. The role of rostromedial tegmental nucleus in the regulation of dopamine neurons in sardinian alcohol preferring rats. C. Sagheddu, M. De Felice, G. Colombo, M. Melis, M. Pistis
P085. Alterations in cached learning in alcoholism: pilot data. M. Sebold, M. Garbusow, N. Bernhard, C. Hägele, E. Friedel, C. Sommer, E. Jünger, A. Beck, A. Genauck, U. Zimmermann, M. Rapp, F. Schlagenhauf, M. Smolka, Q.J.M. Huys, A. Heinz
P086. Glutamatergic inputs to dopaminoceptive neurons affect novelty- seeking and sensitivity to alcohol. M. Sikora, M. Smutek, K. Lopata, J. Zajdel, R. Przewlocki, J.R. Parkitna
P087. The dopamine activating properties of ethanol - neurocircuitry involved. B. Söderpalm, S.Jonsson, R.Stomberg, L. Adermark, J. Morud, M. Ericson
P088. Contribution of ventral and dorsal striatal dopamine to the reinforcing properties of alcohol.M. Spoelder, H.M.B. Lesscher, L.J.M.J. Vanderschuren
P089. Implications for temporally precise optogenetic inhibition of reinstated cocaine seeking. M. Stefanik, P. Kalivas

Topic: Dopamine and Reward

P090. Dissociating the rewarding and motivational properties of social play behavior in adolescent rats: the role of dopamine, opioids and endocannabinoids. E.J.M. Achterberg, L.W.M. van Kerkhof, M. Servadio, M. van Swieten, V.. Trezza, L.J.M.J. Vanderschuren
P091. Regulation of ethanol-induced dopamine release by inhibitory receptors in striatal subregions.L Adermark*; R Clarke; B Söderpalm; M Ericson
P092. DREADD-regulated modulation of behaviour in TH-CRE rats by increased VTA dopamine neuron activity. L. Boekhoudt, G. van der Plasse, M.L. Luijendijk, R.A.H. Adan
P093. Anandamide interacts with the dopaminergic system to facilitate male rat sexual behaviour expression. A. Canseco-Alba, G. Rodríguez-Manzo
P094. Choosing between sucrose consumption or running on a wheel depends on dopamine in the motivational circuitry: Involvement of D2 and adenosine A2A receptors. M Correa*, M Pardo, L López-Cruz, P Bayarri, N SanMiguel, and JD Salamone
P095. Neurochemical and behavioural responsiveness during sucrose self-administration.F. Cucca, V. Bassareo, P. Musio, R. Frau, G. Di Chiara
P096. DNA methylation in the ventral tegmental area regulates associative reward learning. Jeremy J. Day, Daniel Childs, Mercy Kibe, Mikael Guzman-Karlsson, Jerome Moulden, Esther Song, & J. David Sweatt
P097. Working activity for palatable caloric or non caloric food in non food- deprived and food-deprived rats. De Montis MG,Scheggi S, Secci ME, Marchese G, Gambarana C
P098. Role of striatal indirect pathway dopamine D2 receptors in drug reward
L.K. Dobbs; V.A. Alvarez
P099.Conditioned saccharin avoidance induced by intra-accumbens shell amphetamine and intra-VTA morphine.Espa E., Fenu S., Cadoni C, Di Chiara G
P100. Spontaneous inhibitory synaptic currents mediated by D2 receptors. SC Gantz and JT Williams
P101. Mesolimbic dopamine transmission during choices involving cost-benefit tradeoffs. N.G. Hollon, M.M. Arnold, J.O. Gan, K.L. Reinelt, M.E. Walton, P.E.M. Phillips; N.G. Hollon
P102. The effect of LHb inactivation on Pavlovian versus Instrumental reversal learning. P. Jean-Richard dit Bressel & G.P. McNally; P. Jean-Richard dit Bressel
P103. Protein Kinase C β and the Dopamine Transporter Regulate Surface D2-Like Dopamine Receptor Localization. Kathryn Luderman1, Bipasha Guptaroy1, Prashant Donthamsetti2, Jonathan Javitch2, Paul R. Albert3, Margaret Gnegy1
P104. Role of nucleus accumbens dopamine in social play behavior in adolescent rats.Manduca, R. Damsteegt, P. Campolongo, V. Cuomo, L.J.M.J. Vanderschuren, V. Trezza
P105. Isoflavone administration reduces cocaine self-administration responses and cue-induced cocaine seeking behavior and relapse in mice. Miquel Martin, Roberto Cabrera, Rafael Maldonado, Marta Torrens, Rafael de la Torre, Magi Farré
P106. Inhibition of PKCbeta by enzastaurin attenuates amphetamine-stimulated efflux and behavior. SR Mikelman, OS Mabrouk, RT Kennedy, ME Gnegy
P107. Dopamine transmission and the role of D1- versus D2-like receptors in the nucleus accumbens during unexpected reward omission. Kirsten Porter-Stransky, Jillian Seiler, Omar Mabrouk, Robert Kennedy, Brandon Aragona
P108. Interactions between the Opioid and Dopamine System Regulate Pair Bond Formation and Maintenance in the Socially Monogamous Prairie Vole. Shanna L. Resendez, Caely Hambro, Morgan Kuehnmunch, Piper Keyes, Francis K. Maina, Tiffany A. Mathews, Brandon J. Aragona
P109. Real-time dopamine release to food-predictive Pavlovian cues in rats with a history of cocaine self-administration. Michael P Saddoris, Jonathan A Sugam, Regina M Carelli
P110. Effect of repeated administration of morphine and nicotine on 50-kHz ultrasonic vocalizations in male rats. Nicola Simola, Micaela Morelli
P111. Instrumental and Pavlovian conditioning in mice with inducible inactivation of NMDA receptors on cells expressing dopamine receptors D1. M. Smutek, M. Sikora, K. Tokarski, J. Zajdel, J. Rodriguez Parkitna, R. Przewlocki
P112. Optogenetic Modulation of Ventral Tegmental Area Dopaminergic and GABAergic Neurons Affects Cue-reward Seeking. Ruud van Zessen*, Geoffrey van der Plasse, Martin P. Smidt, Geert M. J. Ramakers, Garret D. Stuber, Roger A. H. Adan
P113. Neurosteroid agonist at GABAA receptor induces persistent neuroplasticity in VTA dopamine neurons. Elena Vashchinkina, Aino Manner, Teemu Aitta-aho, Olga Vekovischeva, Mikko Uusi-Oukari, Esa R Korpi

Tuesday, MAY 28th

8:30 – 10:30 SESSION 1

Topic:*DOPAMINE AND SCHIZOPHRENIA*

Room: LAZZARETTO HIPPOCAMPAL OVERDRIVE OF THE MESOSTRIATAL DOPAMINE SYSTEM AS A BASIS FOR PSYCHOSIS IN SCHIZOPHRENIA

Chair: A.A. Grace (USA) Co-Chair: F. Georges (France)
Organizer: A.A. Grace (USA)
-**D. Lodge (USA)***Aberrant hippocampal regulation of dopamine system function in the MAM model of schizophrenia*
-**O. Howes (UK)***Striatal dopamine & hippocampal glutamate in the development of schizophrenia*
-**A. Abi-Dargham (USA)***Mapping the cortical and striatal response of the dopaminergic system to amphetamine: relationships to symptoms*
-**T.W. Brown (Germany)***Functional imaging of the hippocampal-SN/VTA loop in humans*

Topic:*DOPAMINE AND PARKINSON'S DISEASE*

Room: ARCHI DOPAMINE AND RESTLESS LEGS SYNDROME

Chair: W. Paulus (Germany) Co-Chair: D. Rye (USA)
Organizer: W. Paulus (Germany)
-**C. Trenkwalder (Germany)***Treatment of the Restless Legs Syndrome (RLS)*
-**W. Paulus (Germany)***Dopaminergic Augmentation in RLS*
-**D. Rye (USA)***Functional Anatomy and Genetics Underlying Dopamine's Role in Restless Legs Syndrome*
-**I. Ghorayeb (France)** *The non-human primate A11 diencephalospinal pathway is notdopaminergic*

Topic:*DOPAMINE AND REWARD*

Room: QUARTÉ DUAL CONTROL OF DOPAMINE BY D2 AUTORECEPTORS AND DAT: REGULATION, EPISTASIS AND BEHAVIORAL IMPACT

Chair: M.E. Gnegy (USA)Co-Chair: E. Borrelli (USA)
Organizer: M.E. Gnegy (USA)
-**M. Benoit-Marand (France)***D2 autoreceptors stimulation enhances extracellular dopamine levels by acting synergistically on release and uptake mechanisms*
-**E.M. Gnegy (France)***Protein kinase Cβ modulates trafficking and function of the dopamine transporter and the dopamine D2 autoreceptor*
-**A. Bertolino (Italy)***Association of genetic and epigeneticvariation of dopamine genes with phenotypesrelated to risk for schizophrenia*
-**W. Sadee (USA)***Epistatic interactions between DRD2 and DAT genes: implications for clinical phenotypes*

Topic:*DOPAMINE AND ADDICTION*

Room: SAYÀL NEUROPLASTICITY OF CORTICOLIMBIC DOPAMINE AND GLUTAMATE PATHWAYS IN DRUG ADDICTION

Chair: R.E. See (USA) Co-Chair: R. Spanagel (Germany)
Organizer: R.E.See (USA)
-**L. Vanderschuren (Netherlands)***Striatal dopamine in compulsive drug use*
-**R. Spanagel (Germany)***mGluR2 loss in the corticoaccumbal neurocircuitry is a key pathophysiological mechanism mediating increased propensity to relapse*
-**E. Valjent (France)***Glutamatergic input from specific sources triggers psychostimulant-induced topographical and cell-type specific ERK activation in various nucleus accumbens shell subterritories*
-**F. Fumagalli (Italy)***Dynamic modulation of neuroplastic markers of dopaminergic and glutamatergic pathways following repeated exposure to cocaine during adolescence*
-**R.E. See (USA)***Methamphetamine-induced alterations in prefrontal cortex glutamate and dopamine function*

Topic:*DOPAMINE AND NEURODEGENERATION*

Room: RISTORANTE ALTERED ROLE OF DOPAMINE IN HUNTINGTON'S DISEASE
Chair: M.S. Levine (USA) Co-Chair: M. Parent (Canada)
Organizer: M.S. Levine (USA)
-**M. Parent (Canada)***Dopamine and serotonin innervation of the human striatum : A comparison between Huntington's chorea and Parkinson's disease*
-**K. Murphy (UK)***Altered Dopaminergic Function in the R6/1 Mouse Model of Huntington's Disease*
-**C. Cepeda (USA)***Time-dependent alterations in dopamine modulation of glutamate synaptic transmission in mouse models of Huntington's disease*
-**M. Johnson (USA)***Dopamine release and uptake alterations in Huntington's disease model rodents*

10:30 – 11:00 COFFEE BREAK

11:00 – 13:00 SESSION 2

Topic:*DOPAMINE AND PARKINSON'S DISEASE*

Room: LAZZARETTO IMPULSIVITY IN PARKINSON'S DISEASE
Chair: B. Averbeck (USA) Co-Chair: V. Voon (UK)
Oranizer: B. Averbeck (USA)
-**A. Djamshidian (UK)***Jumping to conclusions in Parkinson's disease*
-**V. Voon (UK)***Impulse control disorders in Parkinson's disease*
-**A. Lawrence (Canada)***Impulse Control Problems in Parkinson's Disease: An Incentive Sensitization Account*
-**J. Rowe (UK)***New approaches to treatment for impulsivity in Parkinson's Disease*

Topic:*DOPAMINE AND ADDICTION*

Room: SAYÀL MODULATION OF DOPAMINE SYSTEM FUNCTION BY NUCLEAR RECEPTORS: FOCUS ON ADDICTION
Chair: S.R. Goldberg (USA) Co-Chair: M. Melis (Italy)
Organizer: R. Ciccocioppo (Italy)
-**F. Tronche (France)***Glucocorticoid Receptors in dopaminoceptive neurons, key for the modulation of the dopamine system and stress-related behaviors*
-**S. R. Goldberg (USA)***Peroxisome Proliferator-activated nuclear receptor-alpha (PPAR-a): A novel taget for the development of anti-nicotine smoking Cessation Treatments*
-**M. Pistis (Italy)***Bidirectional control of nicotinic cholinergic function by PPAR-α in dopamine neurons*
-**R. Ciccocioppo (Italy)***Activation of PPARγ by pioglitazone reduces opioid reinforcement and opioid-induced activation of the mesolimbic dopamine system*

Topic:*DOPAMINE AND PARKINSON'S DISEASE*

Room: QUARTÉ PARKINSON'S DISEASE: FROM CAUSES TO NEUROPROTECTION
Chair: M.J. Zigmond (USA) Co-Chair: M.F. Chesselet (USA)
Organizer: M.J. Zigmond (USA)
-**A. Abeliovich (USA)***Altered regulation of αSynuclein expression as a key determinant of "sporadic" Parkinson's disease risk*
-**L. Stefanis (Greece)***Chaperone-Mediated Autophagy as a target for neuroprotection in Parkinson's Disease*
-**M. Tansey (USA)***Targeting inflammatory pathways to protect DA neurons in Parkinson's Disease*
-**M.F. Chesselet (USA)***Preclinical testing of neuroprotective strategies in Parkinson's disease*
-**S.A. Mandel (Israel)***In the rush for green gold: can green tea delay age-progressive brain neurodegeneration?*

Topic:*DOPAMINE AND AFFECTIVE DISORDERS*

Room: RISTORANTE ALTERING ADULT BRAIN FUNCTION: ENDURING EFFECTS OF EARLY-LIFE STIMULANT EXPOSURE
Chair: H. Steiner (USA) Co-Chair: G.R. Hanson (USA)
Organizer: H. Steiner (USA)
-**B. Kosofsky (USA)***Molecular Maladaptations Underlying Behavioral Deficits in Prenatal Cocaine Exposed Mice*
-**D. McCarthy (USA)***Morphological and molecular alterations associated with prenatal cocaine exposure in mice*
-**H. Steiner (USA)***Cocaine-like gene regulation by methylphenidate in the adolescent striatum: Potentiation by SSRI antidepressants*
-**C. Bolaños (USA)***Juvenile Administration of Concomitant Methylphenidate and Fluoxetine Alters Behavioral Reactivity to Reward- and Mood-related Stimuli and Disrupts Ventral Tegmental Area Gene Expression in Adulthood*

Topic:*DOPAMINE AND SCHIZOPHRENIA*

Room: ARCHI FUNCTIONAL CORRELATES OF DOPAMINE RECEPTOR SIGNALLING
Chair : M.G. Caron (USA) Co-Chair: C. Missale (Italy)
Organizer: C. Missale (Italy)
-**S. Nakanishi (Japan)***Dopamine-mediated neural plasticity in reward and aversive learning behaviors*
-**C. Missale (Italy)***D1 receptor signalling in basal ganglia function and dysfunctions*
-**J.A. Girault (France)***Signaling from dopamine receptors to the nucleus: striatal neurons epigenetics*
-**M.G. Caron (USA)***Consequences of Functionally Selective Signaling at the D2 Dopamine Receptor*

13:00 – 15:00 LUNCH BREAK

15:00 – 17:00 SESSION 3

Topic: *DOPAMINE AND PARKINSON'S DISEASE*

Room: RISTORANTE MRI AND PET INSIGHTS INTO PARKINSON'S DISEASE
Chair: P. Tuite (USA) Co-Chair: A. Dagher (Canada)
Organizer: P. Tuite (USA)
-**M.J. McKeown (Canada)***Resting State fMRI and other MR approaches to Parkinson's*
-**P. Tuite (USA)***Novel MRI and MRS methods in Parkinson's disease*
-**A. Dagher (Canada)***Functional brain imaging insights into cognitive and motivational disorders in Parkinson's Disease*
-**N. Bohnen (USA)***New PET imaging insights: Cholinopathy and gait and postural dysfunction in Parkinson Disease*

Topic: *DOPAMINE AND ADDICTION*

Room: SAYÀL THE DOPAMINE TRANSPORTER: NEW INSIGHTS INTO PSYCHOSTIMULANT ACTIONS AND THE RELATIONSHIP TO NEUROPSYCHIATRIC DISORDERS
Chair: A. Galli (USA) Co-Chair: G. Hasler (Switzerland)
Organizer: U. Gether (Denmark)
-**A. Yamamoto (USA)***Membrane microdomain localization of the dopamine transporter*
-**J. Sutcliffe (USA)** *De novo and inherited rare variants identified from exome sequecing impact the dopamine transporter and its regulatory network.*
-**S. Amara (USA)***Direct activation of intracellular signaling pathways by amphetamines: a mechanism for modulating neurotransmitter transporter function*
-**U. Gether (Denmark)***The dopamine transporter: new genetic mouse models and the relationship to psychostimulant addiction and neuropsychiatric disorders*

Topic: *IMAGING DOPAMINE*

Room: LAZZARETTO

IMAGING AND THE ROLE OF DOPAMINE ACROSS ADDICTIONS : DIFFERENCES AND COMMONALITIES

Chair: N.D. Volkow (USA) Co-Chair: M. Diana (Italy)

Organizer: D. Martinez (USA)

-**D. Martinez (USA)***Dopamine and Addiction: imaging and the neurobiology of substance abuse*

-**E. Forbes (USA)***Neural Response to Monetary Reward in Alcohol Dependence and Cannabis Use: Developmental Findings*

-**A. Beck (Germany)***Dopamine dysfunction in alcoholism*

-**R. Narendran (USA)***Imaging dopamine in prefrontal cortex*

Topic: *DOPAMINE NEURONS*

Room: QUARTÉ

REGULATION OF VTA NEURONS BY OREXIN/HYPOCRETIN: MECHANISMS, CIRCUITS AND BEHAVIORS.

Chair: G.S. Aston-Jones (USA) Co-chair: P.J. Kenny (USA)

Organizer: G.S. Aston-Jones (USA)

-**S. Borgland (Canada)***Hypocretin modulation of morphine-induced synaptic plasticity in the ventral tegmental area*

-**S. Mahler (USA)**Functions of VTA orexin, and orexin/glutamate interactions in reinstatement of cocaine seeking

-**C. Dayas (Australia)***Evidence for drug-induced modulation of the hypothalamic orexin to VTA dopamine neuron pathway and relevance for relapse-like behavior*

-**R. España (USA)***Hypocretin/orexin regulation of dopamine signaling: implications for motivated behavior*

-**G. McNally (Australia)***Orexin/dynorphin neuronal contributions to reinstatement and extinction of alcohol seeking*

Topic: *ANATOMY AND PHYSIOLOGY OF DOPAMINESYSTEMS*

Room: ARCHI

NEW MECHANISMS BY WHICH TRACE AMINE-ASSOCIATED RECEPTORS MODULATE DOPAMINE NEURON ACTIVITY

Chair: D.K. Grandy (USA) Co-Chair: M.C. Hoener (Switzerland)

Organizer: D.K. Grandy (USA)

-**D.K. Grandy (USA)***In Vivo Studies Reveal Trace Amine-Associated Receptor 1 Mediates a Novel Interaction Between Methamphetamine and Bupropion*

-**G.M. Miller (USA)***Trace Amine Associated Receptor 1 (TAAR1) Signaling Differentially Regulates Dopamine and Norepinephrine Transporter Internalization*

-**M.C. Hoener (Switzerland)***Discovery and characterization of selective TAAR1 agonists as potential therapeutic drugs in the field of mental illness*

-**R. Gainetdinov (Italy)***Neurochemical mechanisms involved in the modulation of dopamine transmission by Trace Amine Associated Receptor 1 (TAAR1)*

17:00 – 17:30 COFFEE BREAK

17:30 – 18:30 PLENARY LECTURE

Room: SAYÀL

HOWARD L. FIELDS (USA)

How Opioids Control Midbrain Dopamine Neurons and Contribute to Reward

Host: M. Diana (Italy)

18:30 – 20:00 POSTER SESSION III

Topic: Dopamine and Signal Transduction

P114. Combining DA application and time resolved FRET to investigate effects of physiological DA fluctuations on downstream signaling cascades in medium spiny neurons. Thorvald Andreassen, Kenneth Lindegaard Madsen, Ulrik Gether
P115. Growth Associated Protein-43 Regulates Dopamine Transporter Mediated Amphetamine-induced Reverse Transport. Bipasha Guptaroy, Aalisha Desai, Katharyn Luderman, Karina Meiri and Margaret E. Gnegy
P116. Erk1 map kinase regulates erk2 dependent signalling in the striatum. Marzia Indrigo, Daniel Orellana, Kerrie L. Thomas, Aura Frizzati, Raffaele d'Isa, Elena Marchisella, Riccardo Parra, Gianmichele Ratto, Stefania Fasano and Riccardo Brambilla
P117. SorCS2 is critical for dopaminergic firing pattern and the response to drugs of abuse. D. Olsen, S. Glerup, I. d. Jong, F. Sotty, J. Egebjerg, A. Nykjær
P118. Modulation of translational machinery in the striatum by d-amphetamine. Puighermanal E*, Biever A*, Gangarossa G, Valjent E
P119. Therapeutic approaches against Ras-ERK signaling for the treatment of L-DOPA induced dyskinesia and drug addiction. Nicola Solari, Francesca Marchisella, Livia Marrone, Alessandro Papale, Marzia Indrigo, Stefania Fasano and Riccardo Brambilla

Topic: Dopamine and Schizophrenia

P120. Dysfunction in metabolic mTORC2/Akt signaling disrupts brain D2R signaling and DA homeostasis. Olga Dadalko, Michael Siuta, Amanda Poe, Roxanne A. Vaughn, Kevin Niswender, Aurelio Galli
P121. Antipsychotic-like properties of antiandrogenic drugs: focus on dopaminergic system. Roberto Frau, Valentina Bini, Paola Devoto, Marco Bortolato
P122. Intranasal Oxytocin effects in mice. Huiping Huang, Caterina Michetti, Marta Busnelli, Luca Giancardo, Francesca Managò, Sara Sannino, Diego Scheggia, Diego Sona, Vittorio Murino, Bice Chini, MariaLuisa Scattoni, Francesco Papaleo
P123. D-cell hypothesis for mesolimbic dopamine hyperactivity of schizophrenia. Keiko Ikemoto
P124. Specific knockdown of the Drd2 gene in the NAcc reproduces the social novelty discrimination deficit induced by a neonatal treatment with phencyclidine. M. Ingallinesi, L. Le Bouil, N. Faucon Biguet, C. Mallet, C. Sauty, Ph. Ravassard, J. Mallet and R. Meloni
P125. Cariprazine preferentially induces c-fos mRNA in prefrontal cortical regions of rat brain: comparison with aripiprazole, SV-156 and SB-277011. B. Kiss, B. Pinter-Kubler, Sz. Ferenczi, K. Kovacs
P126. Cathecol-O-methyltransferase (COMT) modulates long-term memory in mice. Mereu M, Scheggia D, Papaleo F
P127. Arc involvement in Schizophrenia-related symptoms in mice. F. Managò; S. Sannino; R. Gainetdinov; K. Wang; F. Papaleo
P128. Dopamine and Other Monoamines Systems are Affected by Histamine(H3) Mechanisms: Combined Microdialysis and Electrophysiological Approaches. A.C. McCreary, E. Dremencov, G. Flik, J. H. A. Folgering, T. I. F. H. Cremers, B. H. C. Westerink.
P129. Midbrain dopamine neuron dysfunction in an infection-based rat model of schizophrenia: interaction with adolescent Cannabis exposure. L. Muntoni, A. Luchicchi, S. Lecca, M. De Felice, M. Melis, M. Scherma, P. Fadda, M. Pistis
P130. Pathophysiological alterations of midbrain dopaminergic neurons in their unit activity and channel properties in a schizophrenia model established by epidermal growth factor. Hisaaki Namba, Hiroyuki Nawa
P131. Modulation of dopaminergic signalling in the striatum by phosphodiesterase 10A (PDE10A) inhibitors. J. Nielsen, B. Steiniger-Brach
P132. Perinatal exposure to the cytokine EGF produces pallidal hyperinnervation of dopaminergic neurons and the indirect pathway dysfunction in the schizophrenia animal model. Hidekazu Sotoyama, Hisaaki Namba, Hiroyuki Nawa

Topic: Dopamine Receptors

P133. Testing drugs for structural plasticity in dopaminergic neurons: translation from mouse primary cell culture to human iPSC-derived neurons. Bono F., Cavalleri L., Gennarelli M., Merlo Pich E. , Rubin L., Spano PF., Missale, C., Collo G.
P134. Striatal D2 receptors in stress coping: genetic and environmental influences. Simona Cabib, Paolo Campus, Valentina Colelli
P135. Chronic alcohol disrupts dopamine receptor activity and the cognitive function of the medial prefrontal cortex. S.W. Centanni, H. Trantham-Davidson, J.T. Gass1, M.F. Lopez, P.J. Mulholland, S.B. Floresco and L.J. Chandler
P136. Distinct roles of dopamine D2 receptors in dorsal and ventral striatum on motor and drug-related behaviors. A. R. Kaplan, T. Doyle, E. Casey, R. B. Free, D. R. Sibley, M. Rubinstein, V. A. Alvarez
P137. Normalizing Dopamine D2 Receptor-Mediated Responses in D2-KO Mice by Virus-Mediated Restoration of D2 Receptors: Comparing D2L and D2S. K. Neve, D. Buck, C. Ford, J. Williams, R. Neve, T. Phillips
P138. Role of D1 and D3 dopamine receptor heterodimerization in the regulation of the receptor signaling.P. Tallarico*, R. Kumar, C. Busi, C. Fiorentini, PF. Spano and C. Missale

Topic: Dopamine and Affective Disorders

P139. Different classess of antidepressants increase dopamine and norepinephrine release in the bed nucleus of stria terminalis: an "in vivo" microdialysis study. Carboni Ezio, Ibba Marcello, Roberto Cadeddu
P140. The role of extended amygdala dopamine D2 receptor-expressing neurons in fear expression and fear generalization. De Bundel D, Espallergues J, Valjent E
P141. Molecular mechanisms behind inflammation-induced malaise and aversion. M. Fritz, A. Klawonn, D. Björk Wilhelms, M. Lazarus, U. Örtegren, M. Jaarola, J. R. Parkitna, C. B. Saper, A. Blomqvist, D. Engblom
P142. Initial pharmacotherapy by dopamine stabilizer, aripiprazole, for inpatients due to suicidal attempt. K Ikemoto, R Murao, R Ishiyama, K Sakamoto, M Hirai, I Fujimoto, K Iwai, A Koyama
P143. Tyrosine hydroxylase dysfunction and dopamine deficiency in phenylketonuria. Aurora Martinez, Tanja Scherer, Christineh N. Sarkissian, Ming Ying, Cary Harding and Beat Thöny
P144. Lateral Habenula Modulation Of Ventral Tegmental Area Dopamine Neurons in a Rodent Model of Depression. Jared L. Moreines and Anthony A. Grace
P145. The impact of co-treatment with antidepressant drugs and risperidone on the extracellular level of dopamine and its metabolites in rat frontal cortex. Zofia Rogóż, Katarzyna Kamińska, Krystyna Gołembiowska

Topic: Dopamine Neurons

P146. Dopamine neurons in opiate withdrawal: a computational perspective. Fabio Caboni, Michele Migliore , Giovanna Mulas, Francesca Piras, Saturnino Spiga, and Marco Diana
P147. Functional and topographical analysis of the brainstem cholinergic innervation of the ventral tegmental area. Daniel Dautan, Albert Souza, Ilana Witten, Karl Deisseroth, J. Paul Bolam, Todor Gerdjikov and Juan Mena-Segovia
P148. Neural bases for the excitatory control of VTA dopamine neurons by the ventral hippocampus. Christelle Glangetas, Delphine Girard, Giulia R.Fois, Marion Jalabert, Laurent Groc, François Georges
P149. SorLA Controls Neurotrophic Activity by Sorting of GDNF and its Receptors GFRa1 and RET. S. Glerup, M. Lume, D. Olsen, M. Saarma, A. Nykjaer, and C.M. Petersen
P150. High throughput single-cell expression analysis of midbrain dopamine neurons reveals Aldh1a1 as marker of vulnerability in a model of Parkinson's disease. JF Poulin, J Zou, J Drouin-Ouellet, F Cicchetti, RB Awatramani
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SYMPOSIA

FRIDAY, MAY 24TH

ENDOCANNABINOID/DOPAMINE INTERACTIONS AND THE PURSUIT OF REWARD

Organizer: J. F. Cheer (USA)

Endocannabinoids modulate accumbal dopaminergic encoding during signaled shock avoidance

Joseph F. **Cheer**

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The nucleus accumbens (NAc) is a neural substrate involved in integrating sensory and emotional information to initiate reward-directed behavior. We previously demonstrated that disrupting endocannabinoid signaling uniformly decreased NAc dopaminergic encoding of cue significance and reward directed behavior. Here, we investigate whether NAc neurons encode cues predicting the avoidance of aversive events and whether these responses are modulated by endocannabinoids during a signaled shock avoidance procedure. To assess for endocannabinoid modulation, we pre-treated rats with the CB1 receptor antagonist rimonabant, which dose-dependently weakened shock avoidance behavior, as a 1.3-5.6 mg/kg dose range shifted the behavioral outcome from avoidance to escape. This was accompanied by a significant inhibition of subsecond dopamine release at the presentation of the warning signal signaling the option to avoid. The behavioral effect was recapitulated by optogenetic inhibition of dopaminergic neurons specifically during presentation of the warning signal, suggesting a causal effect of mesolimbic dopamine signaling on conditioned stimuli driving behavioral responding. These data demonstrate that subsecond dopamine release encodes warning signals predicting the avoidance of punishment, similarly to what is observed during reward directed behavior, and that the endocannabinoid system modulates negative reinforcement, although greater disruption is required to reduce avoidance in comparison to primary reward.

Cocaine inhibition of synaptic GABA inputs to VTA DA neurons: Roles for serotonin and endocannabinoids

Huikun Wang and Carl R. **Lupica**

NIH/National Institute on Drug Abuse / Electrophysiology Research Section

Recent evidence suggests that endocannabinoids (eCBs) play a role in addiction-related behaviors associated with nicotine, alcohol, opiates or cocaine. Furthermore, cannabinoid CB1 receptors (CB1Rs) are activated by eCBs during drug intake, regulate cue-induced DA release in nucleus accumbens, and are involved in reinstatement of cocaine seeking. DA neurons of the ventral tegmental area (VTA) release eCBs that inhibit synaptic transmission and regulate long-term synaptic plasticity via retrograde signaling. However, the connections between abused drugs, the release of eCBs, and the activation of CB1Rs remains obscure. We used whole-cell recordings of DA neurons in VTA brain slices to measure electrically-evoked synaptic GABAB receptor-mediated currents to assess eCB function during cocaine exposure. We find that GABAB synaptic currents are tonically inhibited by 2-arachidonoylglycerol (2-AG), as revealed by a neutral CB1R antagonist, PIMSR1, and the diacylglycerol lipase inhibitor tetrahydrolipostatin (THL), and that depolarization of DA neurons can also cause phasic release of 2-AG. Single i.p. injections of cocaine to rats 2 hr prior to brain slice preparation resulted in the loss of phasic 2-AG function, but the tonic 2-AG effect was unchanged. In contrast, cocaine (10 µM) inhibited GABAB currents in naïve brain slices and these effects were partly blocked by CB1R antagonists. The remaining inhibition of synaptic GABAB currents produced by acute cocaine was blocked by the selective serotonin 5-HT1B receptor antagonist GR55562 (1-10 µM). Additional experiments revealed that the inhibition of GABAergic synaptic responses by 2-AG and 5-HT during cocaine exposure were additive, suggesting independent mechanisms for these actions on VTA DA neurons. Supported by the NIDA Intramural Research Program.

Enhanced 2-arachidonoylglycerol signaling in dopamine neurons as a possible marker of heightened predisposition to addiction

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Addiction is a psychiatric disorder, whose aetiology involves interaction of inherited predispositions and environmental factors. Addictive drugs share the properties of being self-administered by laboratory animals, and of activating the brain reward circuitry, which stems from the ventral tegmental area (VTA) where dopamine (DA) cells are located. Endocannabinoids serve as retrograde signaling molecules at many synapses in the brain, and regulate reward seeking by modulating DA signaling. We took advantage of significant sex differences in cannabinoid self-administration displayed by Lister Hooded (LH) female and male rats, and of one of the few pairs of lines of rats selectively bred for their voluntary alcohol preference, that is Sardinian alcohol-preferring (sP) rat line. Vulnerable phenotypes express different depolarization-induced suppression of inhibition (DSI), a form of endocannabinoid-mediated short term synaptic plasticity, at two discrete sets of inhibitory synapses onto VTA DA neurons. This phenomenon is selectively mediated by 2-arachidonoylglycerol (2-AG), which activates presynaptic CB1 receptors. However, the two discrete DSI do not depend upon differences in CB1 number and/or function, but rather on the rate 2-AG is degraded. Thus, 2-AG by differently depressing inhibitory synapses might indirectly alter DA neuron functional state, and enhance the responsiveness of the reward pathway to phasic DA. Given that both LH female rats and sP rats are vulnerable phenotypes, and that they share this DSI, our results suggest that differences in equipment of the endocannabinoid system machinery might control specific sources of vulnerability.

Endocannabinoid and dopamine interact to mediate spike-timing dependent potentiation and depression

Laurent **Venance**

Center for Interdisciplinary Research in Biology/College de France (Paris)

The extended capabilities of the brain for learning and memory admittedly rely mainly on synaptic plasticity. Yet, our knowledge of synaptic plasticity often depends on dedicated experimental protocols that imply a high number (hundreds) of stimulations to induce plasticity. However, it is still unknown if small numbers of paired stimulations can trigger spike-timing-dependent plasticity (STDP). Here, we investigated the frontiers of STDP and provide evidence that few (even 5 to 10) stimulations can trigger reliable and robust LTP at corticostriatal synapses. This LTP is NMDA receptor independent but endocannabinoid-mediated through activation of the type-1 cannabinoid receptor (CB1R) and the transient receptor potential vanilloid type-1 (TRPV1). In contrast to the widespread belief that endocannabinoids are only able to depress synaptic transmission, our data show that they in fact can also potentiate it. In addition, our study provides an exhaustive mapping of the molecular signaling pathways involved in this process using a sound combination of experimental and computational modeling approaches. We show it depends on the activation of metabotropic glutamate receptor type-5, dopaminergic receptors, voltage-sensitive calcium channels, phospholipase C, diacylglycerol lipase, CB1R, TRPV1 and presynaptic PKA. Finally, we demonstrate that this endocannabinoid-mediated LTP is a widespread process that we observed in the cortex and in the striatum, and occurs in juvenile adult rodents. Our results considerably enlarge the spectrum of action of endocannabinoids as (1) promoting not only depression but also potentiation, i.e. acting as a genuine bidirectional system and (2) supporting STDP at low numbers of paired stimulation.

DOPAMINE – RELATED LEARNING DYSFUNCTIONS IN ADDICTION

Organizer: G. Hasler (Switzerland)

Parsing the role of dopamine in stimulus-reward learning: Implications for Addiction

Shelly B. **Flagel**

University of Michigan / Psychiatry

In recent years, we have been using a rodent model to study individual differences in response to reward-related cues as a means to investigate the neurobiological mechanisms underlying motivational behavior. Using a Pavlovian conditioning paradigm, we have found that individuals vary in the degree to which cues can attain motivational value. For some rats, called "sign-trackers", a previously neutral stimulus that predicts reward acquires motivational properties and becomes an attractive and desirable stimulus. For others, called "goal-trackers", the reward cue merely serves as a predictor. We have shown that dopamine is required only for sign-tracking-a form of stimulus-reward learning in which incentive motivational value is assigned to reward cues. In individuals with a propensity for this form of learning, reward cues come to powerfully motivate and control behavior and can lead to maladaptive behavior. We have found that sign-trackers exhibit a number of addiction-related traits and are more susceptible to relapse. Thus, this work provides insight into the role of dopamine in a form of stimulus-reward learning that might confer increased susceptibility to addiction.

Dopaminergic dysfunction and reward learning in bulimia nervosa

Gregor **Hasler**

Psychiatry, University of Bern

Bulimia nervosa (BN) can be considered to be a complex food addiction. It is characterized by recurrent episodes of binge eating and inappropriate compensatory behavior to prevent weight gain. BN has been associated with behavioral and neural abnormalities in response to rewarding stimuli. Dopamine is involved in diverse aspects of reward processing, including the evaluation of rewarding proprieties of food, reinforcement learning, and in the development of addictions, which are likely associated with the pathogenesis of BN. We will present data from a dopamine depletion study, using the tyrosine hydroxylase inhibitior AMPT, in fully remitted subjects with bulimia nervosa (RBN). The purpose of this study was to uncover putative dopaminergic dysfunction in remitted subjects with BN who performed a reinforcement-learning task after AMPT. RBN subjects (but not healthy controls) showed marked blunted reward learning following AMPT that was associated with depletion-induced anhedonia, which is an important risk factor for binge eating episodes. These findings point to a trait-like dopamine-related reward processing deficit associated with risk of BN.

Learning processes in the development of addiction

Quentin JM **Huys**

Translational Neuromodeling Unit, ETH Zurich and Psychiatric University Hospital Zurich

Addictive substances have an intricate relationship with dopamine, which in turn has a well-described but complex role in affective learning. Dopamine has particularly been related to habitual aspects of learning, in that the phasic prediction error it correlates with is the key component of a so-called model-free estimate of expected values. However, addictive drug taking is yet more resistant to change than habits, sharing some aspects of compulsive behaviours. In this talk, I will first review the computational aspects of learning in multiple, parallel affective systems, focussing particularly on how computational accounts of the early phase of addiction may prove useful in assessing vulnerability. I will also discuss challenges faced by computational accounts of the transformation from early to established addictive processes.

Role of dopamine in learning - findings from single cells and neuroimaging

Philippe N. **Tobler**

Department of Economics / University of Zurich

Classical as well as more recent studies suggest that dopamine neurons encode errors in the prediction of reward. Such prediction errors play a central role in the form of teaching signals in theories of reinforcement learning. Thus, dopamine appears to play a role in reward learning. Converging evidence also comes from human neuroimaging studies on reward learning. The potential implications for drug addiction will be discussed briefly.

THE ROLE OF THE DOPAMINE TRANSPORTER IN HUMAN DISEASE

Organizer: M. Kurian (UK)

The Role of the Dopamine Transporter in Attention Deficit/Hyperactivity Disorder

Randy D. **Blakely**, Ph.D.

Vanderbilt University Medical Center / Pharmacology

Attention-Deficit/Hyperactivity Disorder (ADHD) is believe to affect 5-7% of school aged children and, in many individuals, can result in symptoms that persist into adulthood. A role for perturbed dopamine (DA) signaling in ADHD has been proposed for many years based on the known contribution of DA signaling in brain pathways subserving movement, arousal, reward and attention, as well as genetic and brain imaging studies. In 2008, we implicated ADHD as the first human disorder associated with coding variation in the DA transporter (DAT), and our studies since have uncovered other affected subjects bearing DAT coding variation. Our in vitro studies point to both functional and trafficking defects associated with these variants and have encouraged us to generate knock-in mouse models expressing these variants for a more in-depth analysis of biochemical, physiological and behavioral phenotypes in vivo. In my presentation, I will review the background and most recent findings pertinent to a further understanding of the role of DAT in ADHD and discuss the paths forward using our new models to improve our understanding of this prevalent disorder.

De novo mutation in the dopamine transporter gene associates dopamine dysfunctionwith autism spectrum disorders

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* P.J.H. and N.G.C. contributed equally to this work.

[#] H.J.M., J.S.S., and A.G. contributed equally to this work.

Mounting evidence suggests that *de novo*genetic variations represent an important class of risk factors for autism spectrum disorders (ASD). Recently, whole exome sequencing of ASD families has identified, for the first time, a novel *de novo* missense mutation in the human dopamine (DA) transporter (hDAT) gene, which results in a Thr to Met substitution at site 356 (hDAT T356M). The DAT is a presynaptic protein that regulates dopaminergic tone in the central nervous system by mediating the high-affinity re-uptake of synaptically released DA, making it a crucial regulator of DA homeostasis. Here, we report the first functional, structural, and behavioral characterization of an ASD associated *de novo* mutation in the hDAT. We demonstrate that the hDAT T356M displays anomalous function, identified as a persistent, reverse transport of DA (substrate efflux). Importantly, in the bacterial homolog leucine transporter, substitution of A289 (the homologous site to T356) with a Met, promotes,upon substrate binding, an outward-facing conformation. In the substrate-bound state, outward-facing is a required transporter conformation for substrate efflux. In *Drosophila melanogaster*, expression ofhDAT T356M in DA neurons lacking *Drosophila*DAT leads to hyperlocomotion, a trait associated with DA dysfunction and ASD. Taken together, our findings demonstrate that aberrant DAT function, associated with ASD, alters DA homeostasis and could confer risk for ASD.

Dopamine Transporter Deficiency Syndrome: Characterisation of the Clinical andMolecular Features of this Novel "Dopamine Transportopathy

M. **Kurian**

The dopamine transporter (DAT) is the major regulator of the duration and amplitude of dopaminergic transmission. To date, a number of neurological and neuropsychiatric disorders have been linked to variants in the dopamine transporter. In this presentation, we report the discovery of loss-of-function mutations in the gene encoding the dopamine transporter (*SLC6A3*) in a cohort of patients with autosomal recessive infantile parkinsonism-dystonia. Patients present with an early onset movement disorder characterised by early hyperkinesia with the evolution of dystonia and parkinsonism in early childhood, leading to rigidity, hypomimia and akinesia. Premature death is encountered in a number of patients. *In vivo* DATscan imaging shows a striking loss of dopamine transporter and *in vitro* studes suggest that mutant DAT has reduced dopamine recognition, binding affinity and impaired glycosylation. We report a new disease entity due to mutations in the dopamine transporter gene.

The dopamine transporter: Structure, function and pharmacological considerations

M.E.A. **Reith**

Psychiatry, and Biochemistry and Molecular Pharmacology

The dopamine transporter (DAT) is a target for psychostimulants, anti-attention deficit disorder (ADHD) drugs, and neurotoxins. The development of biochemical transport assays in the late sixties allowed pharmacological characterization of both substrates and blockers, leading to or enhancing our understanding of medications still used today such as amphetamine, methylphenidate, benztropine, or bupropion. Following the cloning of DAT in 1991, site-directed mutagenesis and chimera studies revealed interaction domains for DAT ligands and structural properties important for transport. The crystal structure of the bacterial leucine transporter LeuT, the closest homolog of DAT, became available in 2005, providing a crucial template regarding the permeation pathway for DA, and its extracellular and intracellular gate. DA binds to a primary binding site (S1) in the protein interior; the role of a secondary site (S2) above the extracellular gate is a matter of debate. Both substrates and blockers can bind to DAT pleiotropically in a conformation-specific manner akin to the functional selectivity principle for G protein-coupled receptors. The cloning of DAT also allowed elucidation of genetic coding variants occurring in humans, one of which is associated with ADHD, and a number of others with infantile Parkinsonism/dystonia, addressed in detail in this symposium in subsequent talks (Blakely and Kurian, respectively). Importantly, DAT is regulated by various signaling pathways, such as those utilizing MAPK (ERK1, 2) or Akt, playing a role in feeding and obesity (addressed in detail in the presentation by Galli).

DOPAMINE RECEPTORS:

NEW CELLULAR MECHANISMS RELEVANT FOR CNS DISORDERS

Organizer: B. Le Foll (Canada)

Title Imaging D3 dopamine receptor levels across behavioral and drug addiction: positron emission tomography / [11C]-(+)-PHNO studies

Doris Payer, Martin Zack, Arian Behzadi, Sylvain Houle, Alan Wilson, Stephen Kish

and Isabelle **Boileau.**

CAMH /

In contrast to consistent findings that D2-type dopamine receptor levels are low in addiction, evidence suggests that levels of the D3 receptor, a member of the D2 family, may in fact be higher than normal. The aim of the present study was to assess D3 receptor levels in humans in vivo, and to determine whether high D3 levels might be characteristic across addictions, using 3 cohorts: methamphetamine-dependent (MA), cocaine-dependent (COC), and pathological gamblers (PG). A total of 85 subjects participated underwent a PET scan following [11C]-(+)-PHNO. In the substantia nigra (SN), where 100% of the signal is attributable to D3 binding, [11C]-(+)-PHNO was higher in both MA and COC relative to controls but not in PG. Additionally, [11C]-(+)-PHNO binding in the SN correlated positively with self-reported drug-wanting (in the MA group), risk taking (game of dice task) in the COC and PG groups and self-reported impulsivity (in PG).The study is the first to show D3 receptor binding in human addiction in vivo, and suggests that D3 receptor involvement may be relevant to behavioral phenotypes of addiction (impulsiveness and drug-seeking), but that heightened expression levels may not be a feature of behavioral addiction. In addition, the findings support proposed therapeutic strategies targeting D3-selective antagonism in stimulant dependence.

Critical role of dopamine D3 receptor in structural plasticity generated by exposure to drugs of addiction in mesencephalic dopaminergic neurons

G. **Collo***, F. Bono, L. Cavalleri, L. Plebani, PF.Spano, C. Missale

Department of Molecular and Translational Medicine, Division of Pharmacology / University of Brescia

Dopamine plays a critical role in substance misuse and addiction. Recent view on the factors involved in the maintenance of drug taking indicates in structural plasticity of neuronal network controlling rewards and motivation one of the key cellular mechanisms. Addictive drugs generally increase the release of dopamine and it was suggested that dopamine itself could play a neurotrophic action on certain neurons, resulting in functionally relevant morphologic changes. Using an in vitro model of primary cultures of mesencephalic dopaminergic neurons prepared from the mouse embryo, we recently showed that cocaine, amphetamine, nicotine and ketamine increases dendritic arborisation and soma size, and that this effect depends upon the availability of functional dopamine D3 autoreceptor (D3R) and activation of ERK and Akt intracellular pathways. Interestingly, a strong activation of the mTORC1 pathway was also observed, indicating the engagement of a series of intracellular signalling involved in cell growth and survival. These effects were absent in mice carrying a null mutation for D3R and were pharmacologically blocked by D3R antagonists in a dose-dependent manner. Prenatal exposure to cocaine and nicotine in mice, resulted in increased soma size of dopaminergic neurons in the mesencephalon of the offspring, indicating that the structural changes observed in vitro are reflected in vivo. These data suggest a critical permissive role of D3R in drug-induced structural plasticity of mesencephalic dopaminergic neurons, suggesting that an increased dendritic harborization and capacity to receive synaptic inputs could play the role of common pathogenetic factor in the dysfunctional reward-related behaviour of drug addiction.

Neuronal circuitry underlying the impact of dopamine D3 receptors ligands on drug addiction

Bernard **Le Foll**

CAMH /

The dopamine D3 receptors (DRD3) are implicated in drug-seeking behaviors. Notably, we have previously shown that systemic administration of a selective DRD3 antagonist significantly reduces cue-induced reinstatement of nicotine-seeking in rats. However, the neural substrates mediating those effects are unclear. Among the areas exhibiting the highest levels of expression of the DRD3 in the rat brain is the nucleus accumbens (NAcc), the basolateral amygdala (BLA), lateral habenula (LHb) and ventral tegmental area. Results obtained in rats that were trained to lever press for intravenous infusions of nicotine, associated with illumination of a cue-light, under a fixed ratio (FR) schedule of reinforcement will be presented. Following extinction of the behaviour, where lever pressing had no consequences, reinstatement testing was performed by reintroduction of the cues after local administration of the DRD3 selective antagonist SB277011-A or vehicle, into discrete brain areas. SB277011-A (0.01-1µg/0.5µl/side), infused into the basolateral amygdala (BLA) or the lateral habenula (LHb), but not the nucleus accumbens (NAcc), significantly attenuated cue induced reinstatement of nicotine-seeking behaviour. Moreover, infusion of SB277011-A (1µg/0.5µl/side) into the BLA or LHb had no effect on food taking under a FR schedule. The current study supports an important role for the BLA in cue association processes and conditioned reinforcements. Interestingly, the current findings suggest an important role for the LHb DRD3 in cue-induced reinstatement of nicotine seeking. There is a strong potential for the use of DRD3 selective antagonists as therapeutic agents for the prevention of relapse to smoking in humans.

Pharmacological targeting of Dopamine D3 receptor: a review of therapeutic applications

Emilio **Merlo Pich**

Discovery & Biomarkers, Neuroscience DTA, Hoffman-La Roche, Basel CH

The last decade had witnessed the attempt to develop drugs selectively targeting dopamine D3 receptors (DRD3) with partial success. The first problem was the difficulties to achieve selectivity vs. the DRD2, a second was related to the difficult to obtain obtaining 'drug-like' physical properties. In the previous decade D2/D3 agonists with a moderate preferential DRD3 affinity were successfully developed for Parkinson's Disease indication. Recent data based on a better understanding of the mechanism-of-action suggest that D3R activation can be neurorestorative. Preferential D3R agonists, in particular partial agonists, have been also proposed for treatment of schizophrenia and drug addiction. It is unclear, in particular for the partial D3R agonists, if their effects are related to an overall antagonist-like effect occurring when the dopaminergic tone is high. Several antipsychotics also block DRD3 including clozapine, and compounds with mixed pharmacology that include DRD3 antagonism were proposed for negative symptoms and/or for cognitive enhancement. More recently, the synthesis of selective DRD3 antagonists allowed the exploration of the role of DRD3 in modulating the reward system. Extensive work conducted in preclinical species indicated reduction of drug-induced incentive motivation, attenuation of drug's rewarding effects and reduction in reinstatement of drug-seeking. These data and preliminary human studies suggesting a possible therapeutic use substance misuse, compulsive behavior and impulse control disorders. We propose that the improved knowledge of DRD3 biology, including genetic mutation, expression level regulation and PET ligand availability, will open to the possibility to use biomarker information to tailor personalized treatment with novel DRD3 therapeutics.

Evaluation of the Dopamine D3 receptor in the living brain with [11C]-(+)-PHNO PET

Eugenii A **Rabiner**[1,2]

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Dopamine D3 receptors (D3R) are autoreceptors affecting the tonic release of dopamine in the terminal fields, and have been implicated in the pathophysiology of neuropsychiatric disorders, however their examination in the living human brain has been technically challenging. D2R and D3R are expressed in similar brain regions, with D2R densities > D3R, while all D2/D3R ligands have similar affinity for the two receptors; hence the definition of a D3R specific signal has not been possible until recently. The D2/D3R agonist (+)-PHNO was labelled with 11C and characterised as a PET ligand. We quantified the regional contributions of D3R and D2R to the binding of [^{11}C]-(+)-PHNO in the brains of rodent, non-human primate and humans, enabling the first accurate quantification of D3 specific binding in the living brain. *In vivo* blockade with a variety of D2 and D3 selective compounds, and comparison of [^{11}C]-(+)-PHNO with the radioligands [^{11}C]raclopride and [^{11}C]fallypride (which have similar affinity for the D3R and D2R) enabled us to evaluate the regional densities of the D3R in comparison to the D2R. We found that while D2R comprises >90% of the combined D2R/D3R in the basal ganglia, extra-striatal regions such as thalamus, hypothalamus and the midbrain dopaminergic nuclei express proportionally more D3R, with the D3R fraction comprising >50% of the D2R/D3R population in the midbrain. Finally, we used [^{11}C]-(+)-PHNO PET in conjunction with relevant fMRI paradigms, to evaluate novel drugs in development for the treatment of addictive disorders, and demonstrated the relevance of D3R for human reward processing.

IS OUR LIFE STYLE KILLING OUR DOPAMINE NEURONS?

Organizer: M.J. Zigmond (USA)

Physical activity protects the striatum against damage in nonhuman primates

JL **Cameron**, AM Dettmer, BJ Lopresti, RK Leak, N Rockcastle, MJ Zigmond, CA Mathias, Z Zhang

University of Pittsburgh / Psychiatry

Exercise has been shown to be neuroprotective to dopamine (DA) neurons in the nigrostriatal pathway, such that exercising animals have less damage to DA neurons when exposed to a DA neurotoxin. However, it is unknown whether such protection required aerobic exercise. To examine this issue we monitored physical activity using accelerometers placed in a collar around monkey's necks in three groups of rhesus monkeys that were 1) sedentary, 2) running on a treadmill 1 hr/day 5 days/week at 60% maximal capacity, or 3) running at 80% maximal capacity (n=9 total; 3/group). Monkeys were studied for 3 months and then given 0.8 mg MPTP as a right-side intracarotid injection. Two months after MPTP PET scans of the VMAT2 tracer [11C]dihydrotetrabenazine (DTBZ) binding showed striatal binding was significantly predicted by level of physical activity ($r^2=0.636$, $p=0.032$), but not by exercise group. Striatal thyrosine hydroxylase (TH) concentration was also significantly predicted by level of physical activity ($r^2=0.767$, $p=0.01$). Our findings indicate that a more active lifestyle can protect DA neurons in the striatum from neurotoxic damage, and can ameliorate the motor deficits associated with such damage.

IMPACT OF N-3 PUFA IN PARKINSON'S DISEASE: EVIDENCE FROM ANIMAL MODELS

Cicchetti Francesca, Bousquet Mélanie, Calon Frédéric

CRCHUL/Université Laval / Neurosciences

In recent years and as part of a collaborative effort, our laboratories have undertaken a series of studies inves tigating the potential of nutraceutical approaches in the context of Parkinson's disease. We were more speci fically interested in studying the neuroprotective potential of n-3 PUFA as well as their mechanisms of action in various animal models of the disease. Conversely, we were also interested in the consequences of a high-fat intake on neuronal degeneration characteristic of Parkinson. The results of these studies have highlighted the beneficial and neuroprotective effects of n-3 PUFA against neurotoxins which can recreate various patho logical features of Parkinson's disease in rodents. We also found that the modulation of neurotrophic factors such as BDNF contributes, in part, to n-3 PUFA-induced neuroprotection observed in animal models. Importantly, we also found that high-fat intake can exacerbate the effects of these toxins used to provoke parkinsonian features. Taken together, the results hightlight the impact of dietary lipids on brain health.

In the rush for green gold: can green tea delay age-progressive brain neurodegeneration?

Mandel SA, Youdim MB.

Technion, Faculty of Medicine / Eve Topf Center

It is evident that brain aging engages changes in biological systems linked to synaptic function and cell metabolism and in the capacity to cope with different stresses that are either idiopathic in nature, or subject to environmental insults. In a substantial segment of the aging population there is a pathological transition to cognitive and behavioral dysfunction and thus, age constitutes the primary risk factor for Alzheimer's disease and other neurodegenerative disorders. To address the etiological complexity of aging and age-associated conditions, a new paradigm gaining increasing acceptance considers the use of multi-targeted ligands or combination of drugs to modulate several targets at once. During the past years intensive efforts are dedicated to the implementation of life style habits such as exercise and dietary compounds/supplements in combination with symptomatic treatment drugs to improve age-related cognitive decline and to attenuate motor and neurological dysfunction in neurodegenerative diseases. The catechin polyphenols constituents of green tea, which were for long time regarded merely as dietary antioxidants, have caught our and other scientist's attention because of their diverse pharmacological activities, which have been allied to a possible beneficial action on brain health. This review will elaborate on the impact of nutritional supplementation on brain function in general, and provide a compilation of the most updated literature on epidemiology, clinical and animal studies with green tea polyphenols in age-associated cognitive decline and in fighting neurodegenerative diseases. To conclude, a future perspective on the utility and assigned patents with green tea constituents will be presented.

Interactions between early stress and exercise in a rat model of Parkinson's Disease

Vivienne A **Russell**

University of Cape Town / Human Biology

It is generally accepted that exercise is beneficial to patients with Parkinson's disease but the timing, intensity, duration, and confounding effects of stress, are unknown. The purpose of this study was to address the critical question of how stress experienced during the early stages of development, alters brain function in ways that reduce the beneficial effects of exercise in adulthood. We showed that stress experienced early in life (maternal separation) increased the toxic effect of 6-hydroxydopamine in a rodent model of Parkinson's disease. Early stress also reduced the beneficial effects of exercise in the 6-hydroxydopamine-lesioned rat. Significantly, stress reduced not only the exercise-induced changes in behaviour but also the changes in brain neuroplasticity. We found that voluntary exercise stimulated the MAPK/ERK1/2 signaling pathway in the rodent hippocampus and that this stimulation was blocked in rats that had been subjected to early life stress (maternal separation). We have also shown that maternal separation down-regulates several proteins, including structural, energy-related and signaling proteins, in the prefrontal cortex of rats in adulthood, and that these changes are reversed by increased light exposure during adolescence. Identification of the brain areas that are affected by early life stress and the molecular mechanisms that are altered, will lead to a better understanding of the effect of stress on the risk of developing neurodegenerative disorders later in life.

NEW INSIGHTS INTO THE DEVELOPMENT AND PLASTICITY OF DOPAMINE CONNECTIVITY

Organizer: C. Flores (Canada)

Drug-evoked synaptic plasticity in the VTA: a role for calcium-impermeable, GluN3A-containing NMDARs.

Camilla **Bellone**

Department of Basic Neuroscience, University of Geneva

Drug-evoked synaptic plasticity of glutamatergic transmission in the mesolimbic dopamine (DA) system represents an early form of neuroadaptation that is permissive for circuit reorganization. In the VTA a single cocaine injection reorganizes the synaptic network driving the insertion of Ca2+ permeable AMPARs and changing the rules for the induction of synaptic plasticity. Whether cocaine changes the rules and the roles of NMDAR transmission in the DA system has not yet been fully investigated. Combining ex vivo electrophysiology, molecular biology and Ca2+ imaging, our data indicate that cocaine drives the insertion of Ca2+ impermeable, GluN3A-containing NMDARs. We propose the mGluR1 activation as the molecular mechanism controlling the removal of these non-conventional NMDARs and restoring the basal NMDAR transmission. Finally our data reveal an unexpected role for non-conventional NMDAR-containing GluN3A in the expression of cocaine-evoked synaptic plasticity in the VTA.

Netrin-1 receptors organize mesocortical dopamine circuitry during adolescence

C. **Flores**

McGill University / Psychiatry

Abnormalities in brain development and connectivity play an etiological role in certain psychiatric disorders leading to altered dopamine function and increased vulnerability to the effects of stimulant drugs in adulthood. The developmental guidance cue netrin-1 and its receptors are highly expressed by dopamine neurons in the rodent brain. The pattern of netrin-1 receptor expression by dopamine neurons, however, varies from embryonic life to adulthood, with a dramatic shift occurring during adolescence. We have found that netrin-1 receptor deficient mice have substantial structural and functional modifications in mesocortical dopamine circuitry. In turn, these mice display altered mesocorticolimbic dopamine function and related behaviors. These changes appear opposite to those observed in developmental animal models of schizophrenia or following chronic use of drugs of abuse. Significantly, the signs of this "protective" phenotype are not evident before adolescence (i.e., before the maturation of the mesocortical dopamine system). We propose that variations in netrin-1 receptor function in selective populations of dopamine neurons, and at specific critical periods during development, contribute to individual differences in susceptibility to psychopathology. Recently, we have identified an association between genetic variation in DCC and schizophrenia.

Structural and synaptic substrates of activity in dopaminergic neurons

Pablo **Henny Vargas**

Pontificia Universidad Catolica / Anatomia Normal

Subpopulations of midbrain dopaminergic neurons show different responses to aversive stimulation. We examined how differences in somato-dendritic architecture and afferent synaptic organization in individual dopaminergic neurons may account for differences in response. Substantia nigra neurons were recorded before, during and after nociceptive stimulation, and were subsequently labeled using the juxtacellular technique. Vector-based digital reconstructions of the same neurons showed that the relative extension of dendrites into the underlying pars reticulata predicted inhibition following stimulation. Quantitative analysis of synaptic inputs on the neurons revealed that GABAergic synapses were proportionally greater in number and denser on dendrites located in the pars reticulata than on those located in the pars compacta and overall, accounted for 40%-70% of all synapses received by individual neurons. In contrast, glutamatergic synapses were more numerous on dendrites located in pars compacta than on those located in the pars reticulata, and overall accounted for 30% of all synapses. The results thus reveal the existence of two synaptically distinct and region-specific subcellular domains, whose relative extension may underlie the observed differences in response to aversive stimulation.

Molecular and genetic dissection of dopaminergic pathway development

Ewoud R.E. Schmidt, Sara Brignani, and R. Jeroen **Pasterkamp**

Department of Neuroscience and Pharmacology, UMC Utrecht

Every aspect of mature brain function relies on a precisely sculptured neuronal network. This network forms during embryonic and postnatal development when neurons send out axons to their distant targets. Failure to establish appropriate neuronal connections or changes in existing networks lead to a variety of neural disorders. For example, abnormal wiring of the brain during development is believed to contribute to psychiatric disorders such as schizophrenia and autism, while neurodegenerative disorders including amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) are characterized by a marked loss of neuronal connections. To better understand and eventually treat these situations of perturbed neuronal connectivity, further insight is needed into the mechanisms that normally control nervous system wiring. Therefore the aim of our research is twofold: 1) to determine how neuronal circuits are formed during development at the molecular, cellular and systems level, and 2) to understand how and why neurons and their connections are changed or lost in neurodegenerative disorders. In this seminar I will discuss recent examples our work that reveal intriguing aspects of the development of dopaminergic axon projections that originate in the midbrain and are affected in schizophrenia and PD. We have identified novel molecular mechanisms essential for different aspects of dopaminergic pathway development (such as axon bundle polarity, axon targeting and axon-axon interactions) and are generating unique mouse genetics tools to for the first time simultaneously visualize different subsets of dopaminergic neurons and their projections.

OPTOGENETIC PERTURBATION OF DOPAMINE NEURONAL CIRCUITRY

Organizer: G. Stuber (USA)

The organization and behavioral consequence of discrete glutamatergic input to the nucleus accumbens

Jonathan **Britt**& Antonello Bonci

NIH / NIDA

The nucleus accumbens (NAc) plays a major role in the generation of motivated behaviors. It integrates dopaminergic reinforcement signals with glutamate-encoded environmental stimuli. Here, we examine the innervation patterns and synaptic properties of these glutamatergic inputs from the ventral hippocampus, basolateral amygdala, and medial prefrontal cortex. Bidirectional in vivo optogenetic manipulations were combined with brain slice electrophysiological recording techniques to identify the consequence of specific neural pathways during and following cocaine use. Challenging the idea that any of these inputs encode motivationally-neutral information, optical stimulations designed to offset the differential potency of each input proved that activation of each afferent pathway could reinforce instrumental behavior. Similar behavioral effects were observed following direct optical stimulation of dopamine D1 receptor-containing neurons in various regions throughout the striatum. This work characterizes some of the fundamental organizing principles of basal ganglia information processing.

Genetic Modulation of Periaqueductal Grey Dopamine Neurons

Thomas **Kash**, Chia Li

Department of Pharmacology, Curriculum in Neurobiology, Bowles Center for Alcohol Studies, UNC Chapel Hill School of Medicine

While a large number of studies have focused on the role of ventral tegmental area dopamine neurons in emotional behavior, to date none have evaluated the role of periaqueductal grey (PAG) dopamine neurons. These neurons are particularly interesting, as they appear to play a role in reward-related and pain-related behaviors. We used a combination of reporter mice, electrophysiology, optogenetic and chemical genetic approaches to determine the involvement PAG dopamine neuron in a range of behaviors and evaluate their sensitivity to opioid receptor ligands. Interestingly, similar to a subset of dopamine neurons in the ventral tegmental area, these PAG dopamine neurons appear to also express vGluT2, a finding that we functionally confirmed in target regions with optogenetic approaches. Ongoing studies are assessing how activation of these neurons can alter reward and pain related phenotypes.

Dissecting the striatal contributions to reinforcement and punishment

Kravitz AV1, Kreitzer AC2

1. National Institutes of Health / NIDDK 2. Gladstone Institutes of Neurological Disease

Many psychiatric diseases are characterized by alterations in reinforcement and punishment learning. For example, drug addiction is characterized by heightened reinforcement from drug-related stimuli, coupled with impaired punishment from the negative consequences of drug use. In the study presented here, we examined the hypothesis that reinforcement and punishment are mediated by separate cell types in the striatum, namely the classic direct and indirect pathway medium spiny neurons (dMSNs and iMSNs). We selectively targeted channelrhodopsin-2 to dMSNs or iMSNs using cre-dependent viral expression. Through implanted optical fibers, mice were allowed to self-stimulate their own dMSNs or iMSNs in both traditional operant and "real-time" place preference tasks. We also examined their behavior during extinction to characterize possible behavioral plasticity induced by the self-stimulation training. Direct pathway self-stimulation was highly reinforcing, and the reinforced behavior persisted during extinction, suggesting that direct pathway self-stimulation induced synaptic plasticity. Conversely, indirect pathway stimulation was punishing, but interestingly this punishment was not retained during extinction, suggesting a lack of plasticity. This work suggests that different symptoms of addiction may be localized to different cell groups. Specifically, the compulsion to use drugs may result from aberrant plasticity in dMSNs, while impaired punishment from negative consequences may reflect a lack of plasticity in iMSNs. Future therapies may selectively target these pathways to personalize the treatment of addiction, as well as other psychiatric disorders.

Dissecting the neural circuitry that mediates reward and aversion

Garret **Stuber**

University of North Carolina at Chapel Hill / Psychiatry

The Ventral Tegmental Area (VTA) is a heterogeneous brain structure containing neuronal populations that are essential for the expression of behaviors related to addiction and other neuropsychiatric illnesses. The VTA contains a mixture of dopaminergic (DAergic), GABAergic, and glutamatergic neurons, which are thought to act in concert to orchestrate and regulate behavior. The activity of VTA neurons is controlled by diverse anatomically specific excitatory and inhibitory inputs from regions such as the lateral habenula (LHb), bed nucleus of the stria terminalis (BNST). While neuroanatomical studies have demonstrated the presence of projections from these regions to the VTA few studies to date have selectively manipulated the activity of VTA afferents to determine 1) how distinct inputs are functionally connected to different types of postsynaptic VTA neurons and 2) how modulating the activity of these inputs affects behavior. Here, I will discuss our work to determine how pathway specific alterations in synaptic transmission within the VTA alters reward and aversive like behavior. Optical stimulation of glutamatergic inputs from either the LHb or BNST resulted in synaptic activation predominantly of non-DAergic neurons in the VTA. GABAergic inputs from the BNST also preferentially targeted non-DAergic VTA neurons. Direct in vivo activation of LHb or BNST excitatory inputs to VTA non-DAergic neurons results in aversive-related behaviors, while activation of inhibitory BNST inputs to these neurons produced reward-related behavior. These data demonstrate distinct VTA afferents can produce opposing behavioral responses.

Dissecting computations in the dopamine reward circuit

Naoshige **Uchida**

Harvard University / Department of Molecular and Cellular Biology

We make decisions based not only on current sensory inputs but also on the consequences of previous decisions. How do animals learn from the consequences of previous decisions? Psychological studies of animal learning have shown that temporal contiguity between two events (e.g. a sensory cue and reward) is not sufficient for establishing associations between them. Instead, the efficiency of learning critically depends on the discrepancy between predicted and actual outcomes (i.e. prediction errors) (Kamin, 1969; Rescorla and Wagner, 1972). Neurophysiological studies in non-human primates have shown that dopamine neurons in the midbrain signal discrepancies between expected and actual reward, i.e., they compute reward prediction error (Schultz et al., 1997). Because these firing patterns closely resemble a teaching signal used in machine learning theories (Sutton and Barto, 1998), this finding sparked great enthusiasm for understanding the function of dopamine neurons on a firm theoretical footing. Despite such interest, how dopamine neurons compute reward prediction error remains a mystery. To address how dopamine neurons compute reward prediction error, we have been taking a multidisciplinary approach using a mouse model amenable to emerging genetic and molecular techniques. In this talk, I will discuss our recent progress in dissecting neural circuits involved in reward prediction error calculations.

TRANSCRIPTION FACTORS AS MEDIATORS OF DOPAMINE NEURODEGENERATION IN PARKINSON'S DISEASE

Organizer: M. Decressac (Sweden)

Nurr1 as a regulator of survival and GDNF-induced neuroprotection in adult dopamine neurons

Anders **Björklund**

Lund University / Wallenberg Neuroscience Center

GDNF, and its close relative Neurturin, are currently explored in clinical trials for neuroprotection in patients with Parkinson´s disease (PD). In animal models, however, GDNF fails to protect nigral dopamine neurons against α-synuclein-induced neurodegeneration. Using viral vector delivery of wild-type human α-synuclein to nigral dopamine neurons in rats we show that the intracellular response to GDNF is blocked in α-synuclein overexpressing dopamine neurons. The blockade of GDNF signaling is accompanied by reduced expression of the transcription factor Nurr1 and its down-stream target, the GDNF receptor Ret. Similar reduction in Ret is observed also in nigral neurons in PD patients. Importantly, Nurr1 conditional knockout in mice results in reduced Ret expression and blockade of GDNF response. Conversely, we show that GDNF signaling is effectively restored by overexpression of Nurr1, and that this provides near-complete protection of nigral dopamine neurons against α-synuclein toxicity, also in the absence of exogenous GDNF. The results point to Nurr1 as a regulator of neurotrophic factor signaling and as a key player in the cellular defense against α- synuclein toxicity, and provide compelling evidence that Nurr1 is both necessary and sufficient to maintain GDNF signaling in neurons affected by toxic levels of α-synuclein.

Transcription factors for the development and maintenance of dopamine neurons

Thomas **Perlmann**

Cell and Molecular Biology

The presentation will cover our work on the transcription factor functions that are important for the development and maintenance of dopamine neurons. Increasing evidence support the hypothesis that disrupted transcription factor function may contribute to Parkinson's disease. In studies using conditional knockout animals we are investigating the consequences of temporally controlled knockout selectively in adult dopamine neurons in mice. We found that knockout of the transcription factors, including Nurr1 and Lmx1b, leads to dysfunction in the dopamine neurons that resemble early symptoms of Parkinson's disease, including axonal and neurite pathology, striatal dopamine loss and behavioral deficiencies. Moreover, specific roles in neurotrophic signaling and regulation of lysosomal function have also been revealed by these experiments. Together these studies indicate that ablation of key dopamine neuron-specific transcription factors recapitulates early features of Parkinson's disease.

Traveling homeoprotein transcription factors regulate mDA survival and physiology

Alain **Prochiantz**

Collège de France

Most homeoprotein transcription factors (HPs) contain two conserved regions allowing intercellular transfer. Several functions for HP signaling have been identified, including eye anlagen development, axon/cell guidance, Drososophila wing disk patterning, and the regulation of plasticity in the visual cortex. Striking findings are as follows. Internalized HPs regulate gene transcription and protein translation, HP signaling pathways interact with classical signaling pathways, the specific recognition of target cells by HPs involves complex sugars suggesting the existence of a "sugar code" for HP recognition and signaling. In addition HPs, thanks to their natural cell internalization domain, can be used as therapeutic proteins in animal models of human diseases. It was in particular found that Engrailed-1 infused into the midbrain is taken up by mDA cells and protects them in 3 mouse models of Parkinson disease. Internalized Engrailed regulates transcription and translation, in particular the translation of complex I mitochondrial mRNAs encoding Ndufs1 and Ndufs3 . Blocking the latter translation abolishes the protective effect of the transcription factor. In addition to protecting the cells, Engrailed-1 enhances DA synthesis, thus the amount of DA in the striatum. This effect is long lasting, suggesting epigenetic changes. Two references Sonnier et al. Progressive loss of Dopaminergic neurons in the ventral midbrain of adult mice heterozygote for Engraield1. J. Neurosci., 27, 1063-1071, 2007. Alvarez-Fisher et al. Engrailed protects mouse midbrain dopaminergic neurons against mitochondrial complex I insults. Nature Neurosci., 14, 1260-1268, 2011.

Pitx3 is part of a homeodomain code for dopaminergic subset specification and survival

M.P. **Smidt**

UvA / Molcular neuroscience

Mesodiencephalic dopaminergic (mdDA) neurons control locomotion and emotion and are affected in psychiatric and neurodegenerative diseases, including Parkinson's Disease (PD). Homeodomain transcription factor Pitx3 is pivotal in mdDA neuron development and loss of Pitx3 results in programming deficits in a rostrolateral subpopulation of mdDA destined to form the Substantia Nigra (SNc), reminiscent of the specific cell loss observed in PD. Critical mechanisms in dopaminergic subset-specification involve interplay between key regulators such as Pitx3, Nurr1, Lmx1b and retinoic acid signaling. Here, we reveal crosstalk between Pitx3 and a second homeoprotein, Engrailed-1 (En1) through genome wide expression analysis. Both in Pitx3 and En1 mutants dramatic loss of mdDA neurons and striatal innervation defects are observed. During development both En1 and Pitx3 are required to induce expression of mdDA genes in the rostrolateral subset destined to form the SNc. In contrast, Pitx3 and En1 reciprocally regulate a separate gene cluster, including Cck, demarcating a caudal mdDA subset. Whereas En1 is crucial to induce this caudal phenotype, Pitx3 antagonizes it rostrolaterally. These findings show that Pitx3 and En1, interplay in a homeodomain code for dopaminergic subset-specification, underlying specific molecular features, exemplified by specific vulnerability of the SNc as found in PD.

NUCLEUS ACCUMBENS: CORE AND SHELL SPECIALIZATION IN REWARD FUNCTION?

Organizer: R. A. Wise (USA)

Regional specificity of real-time dopamine transmission: implications for reward and function

Brandon J. **Aragona**

University of Michigan / Psychology

Across a variety of species and behavioral tasks, we have demonstrated that the nucleus accumbens (NAc) core and shell differentially mediate a variety of motivational behaviors. These behaviors are highly diverse, ranging from the formation and maintenance of social attachments, conditioned and unconditioned impacts associated with drugs of abuse, as well as both appetitive and aversive regulation of behavior. Measuring dopamine in real-time during behavioral tasks has revealed that dopamine transmission dynamics often vary greatly across the NAc core and shell but this is dependent on what behavior is being studied. Here, I will summarize our work and attempt to put it in a framework that attempts to understand dopamine regulation of reward and function. Briefly, our work (along with the work of many other labs) shows that an understanding of dopamine function requires that its communication be studied with temporal and regional contexts considered very carefully.

Mechanistic Interactions between the Nicotinic Cholinergic and Dopaminergic Systems during Signaling and Synaptic Potentiation

J.A. **Dani**, W.M. Doyon, Y. Dong, J. Tang, K. Yang; J.A.

Center on Addiction, Learning, Memory, Dept. of Neuroscience, and Menninger Dept. of Psychiatry & Behavioral Sciences / Baylor College of Medicine

Dopamine (DA) release varies between targets and within subregions and local environments suggesting that controls intrinsic and extrinsic to the DA fibers and terminals regulate release. While applying fast-scan cyclic voltammetry and using tonic and phasic stimulus trains, we investigated the regulation of DA release. The results indicate that intrinsic differences in the DA fibers combine with (at least) DA transporters, DA receptors, and nicotinic receptors (nAChRs) to regulate the frequency dependence of DA release. A combination of mechanisms provides specific local control of DA release that underlies pathway specific information associated with motor and reward related functions. Acting upon these mechanisms, nicotine at the concentration experienced by smokers also regulates DA release. While acting upon cellular and synaptic events in the midbrain and striatum, nicotinic mechanisms also influence synaptic plasticity and memory along the perforant path to the dentate gyrus of the hippocampus. In vivo recordings from freely moving mice indicate that both feedforward and feedback inhibition onto granules cells is diminished by nicotine. While local dentate inhibition is diminished, nicotine also induces a DA signal that enhances synaptic plasticity. These changes in local inhibition combine with catecholamine mechanisms contributing to nicotine induced synaptic potentiation and, likewise, to drug associated memories. Through this learning process environmental features become cues that motivate conditioned drug seeking and drug taking behaviors.

"Drug and dopamine reward and the ventral striatum "

Satoshi **Ikemoto**

DHHS/NIH/NIDA/IRP / BNRB/NMS

The ventral striatum (VStr) receives dense dopaminergic afferents and plays an important role in mediating rewarding effects of psychostimulant drugs. The nucleus accumbens can be divided into two parts, the core and shell, on the basis of peptide contents and connectivity. The striatal part of the olfactory tubercle (OT) may be considered as extension of the nucleus accumbens shell. We investigated the roles of the accumbens core and shell and OT in drug reward using intracranial self-administration procedures. Rats readily learn to self-administer dopaminergic drugs including D1/D2 receptor agonists, cocaine, and amphetamine into the medial shell and medial OT. In general, the core and lateral shell and lateral OT do not support self-administration of DAergic drugs except MDMA. MDMA is self-administered into the medial shell and core, not the OT. Although these data suggest the medial portion of the VStr particularly plays an important role in drug reward, they may not fully inform about the role of dopamine in reward and its action sites. Using an optogenetic procedure, we found that excitation of dopamine neurons in the substantia nigra pars compacta, which hardly projects to the medial VStr, is highly rewarding. Therefore, dopamine transmission in regions other than these may also play an important role in dopamine-mediated reward.

"Cocaine reinforcement is a shell phenomenon; cocaine satiety involves the core."

Roy A **Wise**

DHHS/NIH/NIDA/IRP / BNRB/BNS

When cocaine is injected into the core of nucleus accumbens it is rewarding (Slides 1-7). When it is infused by reverse dialysis into the core but not the shell, it makes the animal wait longer before responding for the next intravenous cocaine injection (Slides 8-20). What has been termed the "rewarding efficacy" of cocaine can thus be differentiated into two independent components.

THALAMOSTRIATAL SYNAPSES -

ANOTHER SUBSTRATE FOR DOPAMINE ACTION

Organizer: G.W.Arbuthnott (Japan)

The thalamostriatal projection: a major excitatory input to the basal ganglia

J.Paul **Bolam**

University of Oxford / Dept Pharmacology

Although the cortical input to striatum is often considered as the principal excitatory drive to the basal ganglia it is apparent that the thalamus (mainly intralaminar nuclei) also provides a major excitatory innervation of the striatum. In quantitative terms, the thalamostriatal pathway gives rise to a similar order of magnitude of synapses as does the corticostriatal pathway (Lacey et al 2005; Raju et al 2006) and equally innervates direct and indirect pathway medium spiny neurons (Doig et al 2010). Thalamostriatal synapses have the same spatial relationship with dopaminergic axons and terminals as do corticostriatal synapses and are thus likely to be similarly modulated by dopamine (Moss & Bolam 2008). However the thalamostriatal projection is highly heterogeneous. Combined electrophysiological and anatomical analyses (Lacey et al 2007) have demonstrated that the properties of thalamostriatal neurons in the rostral intralaminar thalamus (central lateral nucleus; CL) are markedly different from those in the caudal intralaminar thalamus (parafascicular; Pf). Furthermore using an optogenetic approach we have identified that CL and Pf synapses in the striatum have different functional properties (Ellender et al 2013). We conclude that the thalamostriatal projection possesses many characteristics in common with the corticostriatal projection but is highly heterogeneous. It is a major excitatory input to basal ganglia. Lacey et al (2005) Neuroscience 136:1083-1095.Lacey et al (2007) J. Neuroscience 27:4374-4384 Moss & Bolam (2008) J. Neuroscience. 28:11221-11230 Doig et al (2010) J. Neuroscience 30:14610-14618 Ellender et al (2013) J. Physiol. 591:257-272 Raju et al (2006 J Comp Neurol 499, 231-243.

Formation and Function of Thalamostriatal Synapses on Striatal Medium Spiny Neurons and Choliergic Interneurons

Jun **Ding**

Stanford University / Department of Neurology, SINTN

The two principal excitatory glutamatergic inputs to striatal medium spiny neurons (MSNs) arise from neurons in the cerebral cortex and thalamus. Using mouse brain slices that allowed each type of synapse to be selectively activated, we started to reveal key elements controlling thalamostriatal synapse formation and function. We find that Sema3e (encoding Sema3E) is highly expressed in thalamostriatal projection neurons, whereas in the striatum Plxnd1 (encoding Plexin-D1) is selectively expressed in direct-pathway medium spiny neurons (MSNs). Despite physical intermingling of the MSNs, genetic ablation of Plxnd1 or Sema3e results in functional and anatomical rearrangement of thalamostriatal synapses specifically in direct-pathway MSNs without effects on corticostriatal synapses. Thus, our results demonstrate that Sema3E/Plexin-D1 signaling is a critical determinant of synaptic specificity in cortico-thalamo-striatal circuits. In addition, thalamostriatal projection system exerts important function through activating striatal cholinergic interneurons. We found that activation of thalamostriatal axons in a way that mimicked the response to salient stimuli induced a burst of spikes in striatal cholinergic interneurons that was followed by a pause lasting more than half a second. This patterned interneuron activity triggered a transient, presynaptic suppression of cortical input to both major classes of principal MSNs that gave way to a prolonged enhancement of postsynaptic responsiveness in indirect pathway MSNs controlling motor suppression. This differential regulation of the corticostriatal circuitry provides a neural substrate for attentional shifts and cessation of ongoing motor activity with the appearance of salient environmental stimuli.

Impact of thalamic changes in Parkinson's disease on the motor system

Glenda M. **Halliday**

University of New South Wales and Neuroscience Research Australia / Ageing and Neurodegeneration

One of the most marked differences to be identified in Parkinson's disease is the change in activity of thalamic neurons in the motor circuits. Because dopamine replacement therapies largely alleviate these motor circuit abnormalities, it has been assumed that pathology in the basal ganglia is entirely responsible for the aberrant thalamic activity which then permeates the motor circuits. However, there is considerable evidence that pathology in the thalamus itself contributes to the abnormal neural activity characteristic of Parkinson's disease. In a series of studies examining the degree of degeneration in the thalamus, we have observed selective degeneration in the intralaminar thalamic nuclei in patients with levodopa-responsive Parkinson's disease. These nuclei provide important glutaminergic feedback from the thalamus to the putamen, as well as from the thalamus to the premotor cortices, pathways that are greatly enlarged in primates. There is 30-40% loss in this region of the thalamus in idiopathic Parkinson's disease, with non-parvalbumin-containing neurons degenerating the most (70% average loss), and preservation of nearby non-intralaminar nuclei. Our recent work suggests that the preservation of the intralaminar nuclei may contribute to dystonia in Parkinson's disease by allowing abnormal activation of both the thalamus and premotor cortices. In addition to showing additional focal pathology in patients with Parkinson's disease, these studies suggest that direct thalamic pathology contributes to their symptoms.

Behavioral roles of thalamostriatal pathway in sensory discrimination learning

Kazuto **Kobayashi**, Shigeki Kato, and Ryoji Fukabori

Fukushima Medical University / Department of Molecular Genetics

The dorsal striatum receives converging excitatory inputs from diverse brain regions including the cerebral cortex and the intralaminar/midline thalamic nuclei, and mediates learning processes contributing to instrumental motor actions. However, the behavioral roles of each striatal input pathway in these learning processes remain uncertain. We developed a strategy to target specific neural pathways and applied this strategy for studying the roles of the pathway originating from the parafascicular nucleus (PF) and projecting to the dorsolateral striatum (DLS). A highly efficient retrograde gene transfer (HiRet) vector encoding human interleukin-2 receptor α-subunit (IL-2Rα), which is the receptor for a recombinant immunotoxin anti-Tac(Fv)-PE38, was injected into the DLS in mice to express the human IL-2Rα in neurons innervating the striatum. Immunotoxin treatment into the PF of the injected animals caused a selective removal of neurons of the PF-derived thalamostriatal pathway. The removal of this pathway impaired the response selection accuracy and delayed the motor response in the acquisition of a visual cue-dependent discrimination task. When the pathway elimination was induced after learning acquisition, it disturbed the response accuracy in the task performance with no apparent change in the response time. These results indicate that thalamostriatal projection derived from the PF plays important roles in the acquisition and execution of discrimination learning in response to visual stimulus. The temporal difference in the pathway requirement for sensory discrimination suggests a stage-specific role of thalamostriatal projection in the regulation of response time of learned motor actions.

INDIVIDUAL DIFFERENCES IN DOPAMINE SIGNALING: ROLE IN LEARNING, RISK TAKING, AND IMPULSIVITY

Organizer: A. Dagher (Canada)

Dopamine D4 Receptor (DRD4) Gene, Religious Affiliation and Spirituality Contribute to Dictator Game Altruism

Richard P. **Ebstein** , Soo Hong Chew , Yushi Jiang

National University of Singapore / Psychology

Does altruism derive, as Aristotle believed from being "brought up in fine habits" or is this trait honed by evolution to enable us to act automatically and quickly guide decision-making? A molecular genetic strategy combined with behavioral economic paradigms can be leveraged to help solve the riddle of human altruism. To identify specific genes that contribute to generosity along with cultural factors that temper such hard wiring, we modeled altruism using the Andreoni & Miller's Dictator Game and genotyped 2288 Han Chinese undergraduates for the dopamine D4 receptor exon III VNTR (DRD4). DRD4 has been associated in previous smaller studies with altruistic traits. Subjects were also inventoried for religious affiliation (Christian, Buddhist/Tao & No Religion) and spirituality. Our analysis showed the importance of DRD4 genotype, religion and spiritual beliefs as well as their interactions specifically in determining the big decision to share or be selfish. For participants without self-declared religious affiliation, the exon III VNTR as well as the strength of spiritual belief are both significantly associated with likelihood of sharing. Moreover, there is an intriguing interaction between spiritual beliefs, genotype and religion. The emerging field of cultural neuroscience reveals the impact of culture on brain function mainly using functional imaging. Our report shows how dipping into the toolbox of molecular genetics can be leveraged to uncover how gene-culture interaction tips an individual's decision making towards either completely selfish or non-selfish choices.

Computational models of dopamine in learning and choice incentive

Michael J **Frank**

Brown University, Brown Institute for Brain Science

The striatum and dopaminergic systems have long been implicated in reward-based behavior, with debates focusing on the relative roles of this system in learning, motor performance, and reward-based decision making. I will present computational models (neural network and a novel algorithmic analysis) implicating the corticostriatal system at the intersection of all of these functions -- not independently, but interactively. Dopamine modulates the extent to which choice incentives are influenced by positive vs negative value during the decision process itself, during learning, with each of these effects reciprocally influencing the other. Imbalances of this system can lead to aberrant decision and learning processes in psychiatric conditions. These same principles can be extended to account for complex interactive dynamics among multiple corticostriatal circuits during higher level cognitive selection and learning.

Sensation-seeking propensity is directly proportional to dopamine storage and inversely proportional to dopamine turnover in human dorsal striatum in vivo

Albert **Gjedde**1,2, Arne Møller2, Doris Doudet2, Dirk Bender2, Jakob Linnet2, and Yoshitaka Kumakura1,2

1University of Copenhagen, Dept of Neuroscience & Pharmacology, and 2Aarhus University Hospital, Dept of Nuclear Medicine and PET Center.

Sensation-seeking is associated with increased dopamine and more numerous dopamine D2-3 receptors in ventral striatum, revealed by PET of [11C]raclopride (Gjedde et al. 2010). Previous studies indicated correlation of dopamine receptor numbers and synthesis capacity in psychosis (Reith et al. 1994, Cumming et al. 1997), measured with [18F]FDOPA (Gjedde et al. 1991, Gjedde et al. 1993, Cumming et al. 1995). We hypothesized that PET of [18F]FDOPA metabolism in human striatum would indicate positive correlation between dopamine synthesis capacity and sensation-seeking as cause or effect of increased dopamine turnover. We scored sensation-seeking of 18 male volunteers on the 40-point Zuckerman scale (ZS). Subjects had [18F]FDOPA PET with arterial sampling, analyzed by HPLC for BBB-penetrating metabolites (Kumakura et al., 2005, Kumakura et al., 2006). The analysis yielded estimates of net clearance (K), first-order rate constant of [18F]FDOPA efflux (kloss), and total tracer distribution volume (Vd). Voxel-based linear regression analysis for the correlation between Vd and the individual ZS scores identified a cluster of voxels with peak t-values in the dorsal part of the left caudate nucleus. Mean cluster estimates of Vd, K, and kloss were 4.2 ml/g, 0.010 ml/g/min, and 0.0034 /min, respectively. Individual estimates of Vd correlated positively with individual ZS scores (P=0.005), while individual estimates of kloss correlated negatively with the ZS scores (P=0.003), indicating decreased DA turnover. We conclude that sensation-seeking is inversely rather than directly proportional to dopamine turnover in left dorsal striatum.

Vulnerability to Addictions: Dopamine Studies in Humans.

K.F. Casey, E. Setiawan, I. Boileau, A. Fotros, E. Cawley, S.P. Barrett, A. Dagher, C. Benkelfat, M. **Leyton**

Dept. of Psychiatry, McGill University

Background: Animal studies suggest that dopamine neurotransmission influences responses to reward-related stimuli and susceptibility to drug-seeking behavior. The relevance of this work for humans, though, has been unclear. Methods: During the past 15 years, we have conducted a series of studies using positron emission tomography (PET) and acute phenylalanine/tyrosine depletion (APTD) to measure dopamine release and its behavioral significance. Results: The studies suggest that, in humans, compulsively abused drugs increase extracellular dopamine levels. With repeated drug administration, these responses can become progressively larger (sensitized) and conditioned to environmental cues. Diminishing the drug-induced dopamine response does not alter the substance's pleasurable effects, but does decrease the propensity to respond preferentially to rewards and the willingness to sustain effort to get them (alcohol, cigarettes, money). Finally, in people at risk for addictions, drug-induced dopamine responses are altered; strikingly, both increases and decreases have been observed, potentially related to the presence vs. absence of drug related cues. Discussion: Together, these studies raise the possibility that one biological vulnerability trait for addiction is susceptibility to labile dopaminergic and appetitive responses to cues.

SYMPOSIA

SATURDAY, MAY 25TH

DOPAMINE REGULATION OF STRIATAL PLASTICITY

Organizer: P. Belujon (USA)

Altered hippocampal-accumbens synaptic plasticity in an animal model of depression

Pauline **Belujon**

Dept of Neuroscience, University of Pittsburgh

Depression is a complex disorder that does not adequately respond to antidepressant treatment; therefore there is substantial unmet need. Recent studies show that depression is rapidly reversed by administering the NMDA antagonist ketamine, although the mechanism is unknown. In depression, patients show an abnormal focus on internal states, known as rumination. This could be described as inappropriate contextual focus, and the ventral subiculum of the hippocampus (vSub) plays a prominent role in context-dependent regulation of behavior via focused attention. We found that stressors will abnormally activate the vSub and increase its influence over the nucleus accumbens (NAc) via long-term potentiation. Therefore, a stress-induced hyper-augmentation of vSub-NAc contextual focus could drive an organism to a hyper-contextual state, with attention focused on the internal state to the exclusion of their surroundings. This presentation will present new data regarding the effects of ketamine on vSub-NAc synaptic plasticity in learned helplessness, a well established animal model of behavioral depression. Furthermore, the effects of ketamine on the dopaminergic system will be presented. The dopamine system is believed to have a role in depression due to its known involvement in anhedonia, a core symptom of major depressive disorder, and is a potent modulator of vSub-NAc synaptic plasticity. Studying the mechanisms underlying learned helplessness and the behavioral and electrophysiological effects of ketamine in this model of depression can help us determine which brain structures and neuronal networks may play a role in the pathophysiology and treatment of this condition.

Role of distinct subtypes of dopamine neurons in reward and aversion

Malenka R.C. (1), Lim B.K. (2), Huang K.W. (2), Ran C. (2), Tye K.M. (3), Deisseroth K. (3), Roeper J. (4) & Lammel S. (2)

Stanford University / Psychiatry and Behavioral Sciences

Midbrain dopamine (DA) neurons of the ventral tegmental area (VTA) are not homogeneous but differ in their molecular properties and responses to external stimuli. Evidence will be presented that the modulation of excitatory synapses on DA neurons by rewarding or aversive stimuli depends on the respective brain area to which these DA neurons project. In vivo administration of cocaine selectively modified excitatory synapses on DA cells projecting to nucleus accumbens (NAc) medial shell while an aversive stimulus selectively modified synapses on DA cells projecting to medial prefrontal cortex (mPFC). As an initial test of the hypothesis that the mesocorticolimbic DA system is comprised of anatomically distinct circuits each performing complementary functions, optogenetic manipulations of specific independent inputs to the VTA were performed. While activation of inputs from the laterodorsal tegmentum (LDT) elicited conditioned place preference, activation of inputs from the lateral habenula (LH) elicited conditioned place aversion. Electrophysiological recordings and viral tracing revealed that LTD neurons preferentially synapse on DA neurons projecting to NAc lateral shell while LH neurons synapse primarily on DA neurons projecting to mPFC as well as on GABAergic cells in the RMTg. These results suggest that specific VTA circuits involving distinct subpopulations of DA neurons contribute to the generation of reward and aversion.

Timing dependent effects of substantia nigra dopamine on sensory and motor reinforcement

John **Reynolds**, Simon Fisher and Yan Feng Zhang

University of Otago / Anatomy

Potentiation of cortical synapses onto spiny projection neurons in the striatum has been proposed to operate by a three-factor rule, requiring a conjunction of presynaptic activity, postsynaptic depolarisation and a dopamine reinforcement signal. Previously we demonstrated that phasic activation of the substantia nigra (SN) using behaviourally-reinforcing parameters induces dopamine-dependent corticostriatal potentiation. Recently we found that the relative timing between the activation of the SN and cortical inputs is critical to corticostriatal potentiation. Potentiation requires simultaneous dopamine cell activation and depolarisation to Up state, however the timing of this conjunction is most effective when it follows the cortical depolarisation by approximately one second. Thus, depolarisation originating from outside cortex must accompany the activation of dopamine cells for optimal induction of potentiation. We have shown that the thalamus can provide such a depolarising input simultaneous with a phasic dopamine signal, via pathways from the superior collliculus (SC) that innervate both the intralaminar nuclei and SN. To allow subcortical areas to 'see' the light in the anaesthetised rat, the deep layers of the SC were disinhibited using bicuculline. More recently we have found that the SC can begin to respond to light without bicuculline after pairing the light with behaviourally-rewarding SN stimulation at a similar timing relationship to corticostriatal plasticity. Thus, repeated presentation of a stimulus associated with the delivery of a reward reinforces inputs to primary sensory areas, and allows dopaminergic inputs and glutamatergic inputs from thalamus to access the striatum, to reinforce appropriately timed cortical motor inputs.

DOPAMINE-GLUTAMATE INTERACTIONS IN BRAIN AND THE TREATMENT OF SCHIZOPHRENIA

Organizer: P. Sokoloff (France)

Glutamate-dopamine interactions and prefrontal cortex function

Bita **Moghaddam**

University of Pittsburgh / Neuroscience

Background Several lines of evidence suggest that NMDA receptor hypofunction may lead to some symptoms of schizophrenia, especially cognitive deficits such as impairments in working memory and attention that are dependent on the functional integrity of the prefrontal cortex (PFC). Furthermore, postmortem studies indicate that schizophrenia may be associated with reduced cortical GABA function. We have hypothesized that NMDA receptor hypofunction results in disregulation of cortical ensembles during conditions of high cognitive demand and that this phenomenon may be relevant to the cognitive deficits of schizophrenia. Methods: Unit and LFP recordings in behaving animals were used to measure the impact of NMDA receptor antagonists and dopaminergic drugs on PFC activity at rest and during cognitive tasks. Results: NMDA hypofunction profoundly enhanced spontaneous activity of pyramidal cells at the same time that it inhibited the activity of putative inhibitory GABA interneurons. Local field potential recordings indicated that this treatment also produces transient disconnection of spike discharge from PFC network oscillations in the gamma range. Furthermore, we find that clozapine and positive modulators of metabotropic glutamate receptors 5 (mGlu5) reduce the impact of NMDA hypofunction on cortical neuronal activity and on behavior. Conclusions: These findings provide a better understanding of the dynamic coordination of neuronal processes that serve the behaviors that are disrupted in schizophrenia. Furthermore, they may provide physiological measures for testing the efficacy of targets for treating cognitive deficits of schizophrenia.

Local and remote interactions of the dopamine D3 receptor with glutamate pathways: implications for the treatment of schizophrenia

Pierre **Sokoloff**and Ludovic Leriche

Pierre Fabre Research Institute / Neurology-Psychiatry

The presentation describes direct and indirect interactions of the D3 receptor with NMDA receptor signalling and their functional consequences and therapeutic implications for schizophrenia. D3 receptor immunoreactivity at ultrastructural level with electron microscopy was identified at presumably glutamatergic, asymmetric synapses of the medium-sized spiny neurons of the nucleus accumbens. This finding supports the existence of a direct interaction of the D3 receptor with glutamate, in line with previously described interactions with NMDA signalling involving Ca2+/calmodulin-dependent protein kinase II at post-synaptic densities (Liu et al. Neuron 61:425-438). Indirect interactions of the D3 receptor with glutamate could involve a negative control exerted by the D3 receptor on mesocortical dopamine neurons and the complex regulation of the glutamatergic pyramidal cells by dopamine in the prefrontal cortex. This could be exemplified by the regulation of pyramidal cell activity in conditions of chronic NMDA receptor blockade with MK-801. BP897, a D3 receptor-selective partial agonist, reversed the dysregulation c-fos mRNA expression and pyramidal cell hyperexcitability, as measured by paired-pulse electrophysiology. At behavioral level, blockade of the D3 receptor, by known D3 receptor antagonists or the novel D3 receptor-selective antagonist F17141, produces antipsychotic-like effects in reversing hyperactivity and social interaction deficits induced by NMDA receptor blockade by MK-801 in mice. The glutamate-D3 receptor interactions described here offer a conceptual framework for developing new D3 receptor-selective drugs, which may appear as an original, efficacious and safe way to indirectly target glutamate in schizophrenia.

Presynaptic alpha2 adrenoceptor blockage in the treatment of schizophrenia

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Adjunct treatment with idazoxan (IDA), a selective alpha2-R antagonist, can markedly augment the effect of typical antipsychotic drugs (APD) in treatment-resistant schizophrenia on positive and negative symptoms, BPRS total score, thought disorder and withdrawal retardation, with an effect size similar to that of clozapine. Adding IDA to low doses of the typical APD raclopride (RAC), markedly enhances the RAC-induced suppression of conditioned avoidance response (CAR), a preclinical test of antipsychotic efficacy of high predictive validity, and also augments prefrontal dopamine (DA) outflow, an effect generated by most atypical, but not typical, APDs and thought to improve negative symptoms. Adjunct IDA was also found to augment the antipsychotic-like effect of both haloperidol and atypical APDs, such as olanzapine and risperidone (RISP), drugs with no or low alpha2-R affinity and, in parallel, to enhance prefrontal DA outflow. Taken as a group atypical, but not typical, APDs have been found to enhance NMDA-R mediated glutamatergic transmission in the medial prefrontal cortex (mPFC), which may contribute to cognitive enhancement. The combination of IDA with RAC or low, but not high, concentrations of RISP was also found to generate a large, clozapine-sized facilitation of prefrontal NMDA-R mediated transmission, an effect shown to be mediated by DA via D1-R activation. Moreover, in similarity with clozapine, the combination of idazoxan and raclopride completely reversed the working memory impairment in rats induced by the selective N-methyl-D-aspartate receptor antagonist MK-801. Several clinical studies confirm these experimental results and conclusions, as shown by a recent meta-analysis (Hecht and Landy 2012).

2B or not 2B modulated by dopamine: Input-specific late-adolescent emergence of NMDA-NR2B receptor-mediated synaptic transmission in the prefrontal cortex requires postsynaptic AKAP-PKA signaling

Kuei Y. **Tseng**

Cellular and Molecular Pharmacology/The Chicago Medical School at Rosalind Franklin University

Dopamine regulation of glutamate transmission in the prefrontal cortex is critical for sustaining working memory and decision-making, two cognitive processes that become enhanced late in adolescence. Adolescence is also a period of vulnerability for the onset of major psychiatric disorders including schizophrenia and drug abuse. However, we know very little about the neurodevelopmental processes that contribute to this vulnerability. This is due in part to our limited knowledge on the normative developmental regulation of dopamine-glutamate interaction during this developmental period. While it is evident that dopamine modulation of glutamate action increases in the prefrontal cortex during adolescence, the cellular mechanisms that contribute to this facilitation are incompletely understood. Here, I will present and discuss unpublished studies on how a protracted input-specific developmental facilitation of NMDA-NR2B function could enhance prefrontal plasticity and contribute to the maturation of dopamine action in the prefrontal cortex to improve working memory performance through late adolescence. Supported by Rosalind Franklin University (KYT) and National Institute of Health Grant MH086507 (KYT). Corresponding author: KY Tseng, kuei-yuan.tseng@rosalindfranklin.edu

DOPAMINE SIGNALING IN HEALTH AND DISEASE: MECHANISMS AND INTERVENTIONS

Organizer: G. Fisone (Sweden)

The Ras-ERK signalling pathway in the control of striatal function: from basic mechanisms to potential therapeutic approaches for hyperdopaminergic disorders

RIccardo **Brambilla**

San Raffaele Scientific Institute, Milan, Italy

Hyperdopaminergic disorders include addictive behaviour in response to psychostimulants and L-DOPA induced dyskinesia in Parkinsons's Disease patients. These disorders are characterised by abnormal cellular changes in the basal ganglia system and in particular in the striatum. Among the intracellular signalling cascades found altered in these two brain diseases, the Ras-ERK pathway seems to play a key role in their pathogenesis. Here it will be demonstrated that ERK and Ras-GRF1, as a striatal integrator of dopamine and glutamate signals to Ras, are not only essential for generating normal behavioural and electrophysiological responses in the striatum but also are implicated in both cocaine dependent alterations and aberrant motor symptoms associated to chronic L-DOPA treatment in a mouse model of Parkinson's Disease. Experimental evidence supporting combination therapies for these brain disorders targeting distinct components of Ras-ERK and related signal transduction pathways using small molecules, cell penetrating peptides and gene therapy approaches will be discussed.

Identification of molecular targets for the treatment of Parkinson's disease and L-DOPA-induced dyskinesia

Gilberto **Fisone**

Department of Neuroscience, Karolinska Institutet

In Parkinson's disease (PD), loss of dopamine in the basal ganglia is accompanied by profound modifications in the ability of striatal GABAergic medium spiny neurons (MSNs) to respond to dopaminergic drugs. Prominent among these changes is a remarkable enhancement in the ability of L-DOPA to activate cAMP-dependent protein kinase and phosphorylate the dopamine- and cAMP-regulated phosphoprotein of 32 kDa, DARPP-32. In addition, dopamine depletion confers to L-DOPA the ability to activate the extracellular signal regulated kinases (ERK), which control transcriptional activity. Abnormal ERK signaling has been implicated in the development of the serious motor side effects, or dyskinesia, which develop in response to prolonged administration of L-DOPA. L-DOPA-induced dyskinesia (LID) is accompanied by a large increase in the phosphorylation of the mitogen- and stress-activated protein kinase 1 (MSK1), a target of ERK involved in the regulation of gene expression. We found that, in dyskinetic mice, MSK1 is implicated in the accumulation of truncated splice variants (ΔFosB) of the early response transcription factor FosB. In transgenic mice, upregulation of ΔFosB exacerbates LID, whereas functional suppression of ΔFosB attenuates LID. A reduction of LID is also observed in MSK1-deficient mice. These results provide information on the mechanisms at the basis of the involvement of abnormal ERK signaling in dyskinesia and identify novel targets for therapeutic interventions.

Structural and synaptic plasticity associated with L-DOPA-induced dyskinesias in mouse models of Parkinson's disease.

L. M. Suarez, O. Solis, J. M. Solis, G. M. Murer, R. **Moratalla**

Instituto Cajal, CSIC, Madrid Spain and Fac de Medicina Univ Buenos Aires, Argentina

Treatment with L-DOPA is the most widely used and most effective non-invasive therapy for Parkinson's disease. However, the efficacy of this treatment decreases as the disease progresses and chronic L-DOPA administration causes abnormal involuntary movements (AIM) known as dyskinesias. We have shown that AIMs are associated with L-DOPA-induced changes in expression of ΔFosB and dynorphin and ERK activation in medium spiny neurons (MSN) of the direct pathway in completely denervated areas of the striatum and that these changes are D1R dependent. In the present work we studied whether AIMs correlate with structural and synaptic plasticity in MSN. The experiments were carried out in the classic 6-OHDA-lesioned mouse model. We found that dopamine denervation reduced dendritic length and spine density of striatal MSN and that L-DOPA treatment restored spine density and dendritic length. Electrophysiological studies in striatal slices indicated that SKF38393, a selective D1/D5 agonist, increased the number of action potentials in denervated animals in a dose-dependent manner. SKF38393 also induced a decrease in EPSP in denervated MSN of L-DOPA-treated dyskinetic animals, but not in parkinsonian saline-treated animals. These results indicate that L-DOPA treatment restores spine loss and changes the synaptic activity of denervated striatal MSN in either model. Funded by: The Spanish Ministries de Economia y Competitividad, grant BFU 2010-20664 and ISCIII, CIBERNED, grant CB06/05/0055, PNSD and Comunidad de Madrid ref # S2011/BMD-2336 to RM.

Dopamine-glutamate receptor interplay in the striatum modulates molecular, structural and behavioral responses to cocaine

Cahill Emma, Pascoli V, Trifilieff P, Besnard A, Lüscher C, Caboche J, **Vanhoutte** P

CNRS-UMR7224; INSERM-UMRS952; Université Pierre et Marie Curie-Paris VI

Activation of the extracellular-signal regulated kinase (ERK) pathway in the striatum is an essential signaling event for chromatin remodeling, gene expression and long-term behavioral adaptations induced by addictive drugs. A common feature of drugs of abuse is to increase extracellular DA in the striatum, especially in the nucleus accumbens, where it controls the efficacy of glutamatergic cortico-striatal synapses. The interaction between glutamate and DA signaling in striatal medium-sized spiny neurons (MSNs) has been proposed to be critical for long-term plasticity in the striatum and behavioral alterations induced by drugs of abuse. I will present data showing that the potentiation of NMDAR-dependent calcium influx by D1R is the triggering event for cocaine-induced ERK activation in the striatum and subsequent gene regulations and behavioral alterations. This interplay between D1R and NMDAR occurs at the level of signaling pathways (Pascoli et al. Biol. Psy 2011) but also involves direct physical interaction (oligomerization) of D1R and NMDAR. Finally I will also present data where showing that the phosphorylation of the transcription factor Elk-1 by activated ERK is crucial for cocaine-induced gene regulations, structural plasticity of medium spiny neurons and long-term behavioral alterations (Besnard et al. J. Neurosci 2011).

NEURODEGENERATIVE MECHANISMS OF PARKINSON'S DISEASE AND POTENTIAL NEUROPROTECTIVE STRATEGIES

Organizer: L. Zecca (Italy)

Amphetamine-like drugs and degeneration of dopaminergic nigro-striatal neurons.

Micaela **Morelli** [1, 2, 3], Giulia Costa [1], Antonio Plumitallo [4],Lucia Frau [1] .
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Several clinical reports have suggested that use of amphetamine-like drugs may be a risk factor to late development of Parkinson's disease (PD). Moreover preclinical studies in rodents have reported that 3,4 methylenedioxymethamphetamine (MDMA), also known as 'ecstasy', largely consumed by adolescent and young adults, has the ability to induce neuroinflammation and neurotoxicity towards dopaminergic (DA) nigro-striatal neurons. In order to evaluate whether adolescent mice may be more vulnerable than adults to MDMA-induced neurotoxicity toward DA neurons and neuroinflammation, we studied tyrosine hydroxylase (TH) and GFAP and CD11b immunoreactivity, as index of astroglia and microglia activation, in mice substantia nigra pars-compacta (SNc) and striatum. Acute-repeated administration of MDMA induced a similar decrease in the number and density of TH positive neurons in the SNc and striatum of adolescent and adults mice and a similar increase in astroglia and microglia in the same areas. However, when caffeine, which is contained in high concentration in energy drinks, was administered together with MDMA, we observed a potentiation in striatum and SNc of MDMA-induced DA neuron degeneration. In addition, a subchronic administration of MDMA during adolescence exacerbated DA neuron degeneration and neuroinflammation induced by MPTP in adulthood. The results indicate that adolescence is a critical period for MDMA-induced toxicity since administration of MDMA, in combination with caffeine during adolescence, increased MDMA-induced DA neuron degeneration and worsen neurodegeneration and neuroinflammation caused by MPTP in adulthood.

Late-onset parkinsonism in NF-κB/c-Rel deficient mice

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Activation of the NF-κB/c-Rel transcription factor can increase neuronal resilience to pathological noxae by regulating the expression of pro-survival managanese superoxide dismutase (MnSOD) and Bcl-xL genes. We have shown here that c-Rel deficient (c-rel-/-) mice developed a Parkinson's disease (PD)-like neuropathology with ageing. At eighteen months of age, c-rel-/- mice exhibited a significant loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), as assessed by tyrosine hydroxylase (TH)-immunoreactivity and Nissl staining. Nigral degeneration was accompanied by a significant loss of dopaminergic terminals and a significant reduction of dopamine (DA) and homovanillic acid (HVA) levels in the striatum. Mice deficient of the c-Rel factor exhibited a marked immunoreactivity for fibrillary α-synuclein in the SNc as well as increased expression of divalent metal transporter-1 (DMT-1) and iron staining in both the SNc and striatum. Aged c-rel-/- mouse brains was characterized by increased microglial reactivity in the basal ganglia, but no astrocytic reaction. In addition, c-rel-/- mice showed age-dependent deficits in locomotor and total activity and various gait-related deficits during a catwalk analysis that were reminiscent of bradykinesia and muscle rigidity. Both locomotor and gait- related deficits recovered in c-rel-/- mice treated with L-3,4-dihydroxyphenylalanine (L-DOPA). These data suggest that c-Rel may act as a regulator of SNc resilience to ageing and that aged c-rel-/- mice may be a suitable model of PD.

Neuronal MHC-I display in T-cell mediated neurodegeneration

C.Cebrian, F.A. Zucca, L. Zecca, J.D. Loike, D.L.**Sulzer**

Departments Neurology and Psychiatry, Physiology and Cellular Biophysics Columbia University, Institute of Biomedical Technologies, Italian National Research Council,

Parkinson's disease (PD) and other disorders feature the degeneration of ventral midbrain (VM) catecholamine neurons. Recent data suggest that neuroinflammatory mechanisms contribute to a cascade of events leading to chronic neuronal degeneration. In primary murine neuronal cultures, substantia nigra (SN) and locus coeruleus (LC) neurons are induced to express the major histocompatibility class I complex (MHC-I) by the proinflammatory cytokine, γ-interferon, L-DOPA, or conditioned medium from microglia exposed to α-synuclein or NM. SN DA neurons, moreover, process the foreign protein ovalbumin to an antigenic peptide that is presented by their MHC-I and triggers their specific destruction by CD8+ killer T-cells. In human postmortem samples, we find that neuromelanin (NM)-containing catecholamine SN and LC neurons in adult human control and PD brains express MHC-I, often in proximity to CD8+ T-cells. These data reveal a novel inflammatory T-cell mediated neurodegenerative processes that could underlie neuronal death.

NEUROMELANIN ORGANELLE PATHWAYS AND NEURODEGENERATION IN PARKINSON'S DISEASE

L. **Zecca**(1)*, C. Cebrián(2), R. Vanna(1), C. Bellei(1), A. De Palma(1), P. Mauri(1), L. Casella(3), F. A. Zucca(1), D. Sulzer(4)

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The investigation of aging mechanism is needed for understanding neurodegenerative diseases. In aging an accumulation of organelles containing neuromelanin occurs in all brain regions. This is particularly intense in substantia nigra(SN) and locus coeruleus(LC), the main targeted regions in Parkinson's disease(PD). Protein and lipid systems have been investigated in these organelles of human SN by LC-MS, western blot and immunoelectron microscopy to clarify the pathways of neuromelanin synthesis and their role in neurodegeneration of PD. Neuromelanin organelle appears as abnormal lysosome having a limited number of membrane and soluble proteins of typical lysosome. The organelle has reduced enzymatic activity and capability to fuse with lysosomes or autophagosomes. It has double membrane, is filled with neuromelanin and lipid bodies and has autophagy proteins like LC-3 in addition to storage proteins and engulfs precursors of neuromelanin and lipids from cytosol. Neuromelanin synthesis starts in cytosol from dopamine-protein adducts oxidized to form protein-melanin compounds which bind metals then taken into organelle. Here this complex is cleaved by proteases and reacts with dolichols to form neuromelanin. This organelle has low turnover, collects and immobilizes several non recycling molecules. We found in these organelles abundant MHC-I which can bind antigen derived from foreign proteins and present them on neuronal membrane. Then $CD8^+$ cytotoxic T-cells can target neurons presenting theseantigenic peptide and induce neuronal death. Infiltration of T cells occurs in SN and LC of PD subjects. The presence of MHC-I in neurons containing neuromelanin could explain their preferential vulnerability in PD.

THE REGULATION OF DOPAMINE NEURON ACTIVITY STATES AND ITS RELEVANCE TO DOPAMINE SYSTEM FUNCTION

Organizer: A. A. Grace (USA)

Tonic and phasic dopamine neuron activity and the regulation of information processing

Anthony A. **Grace**

Dept. Neuroscience, Psychiatry and Psychology, Univ. Pittsburgh

Dopamine neurons recorded in vivo exhibit several functionally different states of activity. In the basal state, nearly half are not firing due to GABAergic inhibition. This inhibition is driven by the ventral pallidum. In contrast, spontaneously firing dopamine neurons exhibit two firing patterns: a slow irregular tonic discharge pattern and phasic burst firing. These states are driven by different pathways, and have functionally unique actions within the ventral striatum. Activation of the context-related ventral hippocampus leads to a disinhibition of dopamine neurons, increasing the number firing tonically. We found that this leads to an increase in extracellular dopamine levels and an attenuation of prefrontal cortical input to the accumbens. In contrast, the pedunculopontine tegmentum will cause dopamine neurons to exhibit phasic burst firing, but only in neurons that are spontaneously firing. Burst firing is the behaviorally salient output of the dopamine system; however, since only firing neurons can burst, the amplitude of the phasic response is determined by hippocampal regulation of tonic dopamine neuron activity. Phasic bursts potentiate hippocampal drive of the accumbens, increasing the contextual focus on a task. Therefore, when the dopamine system is phasically active, as in a rewarding condition, there is increased hippocampal drive maintaining focus on the current activity. However, if the task fails to be rewarded, the drop in dopamine neuron activity removes inhibition of the prefrontal cortex, which increases flexible responding and task switching. This balance is essential for maintenance of optimal behavioral strategies.

Membrane properties and neurotransmitter-mediated responses of mesencephalic dopaminergic neurons

Nicola Berretta, Ada Le Donne, Dalila Mango, Ezia Guatteo, Mauro Federici, Paraskevi Krashia, Nicola B. **Mercuri**.

Dipartimento di Medicina dei Sistemi Tor Vergata University of Rome and IRCCS Fondazione Santa Lucia, Rome Italy.

The dopaminergic neurons of the ventral mesencephalon are able to fire spontaneously *in vitro* because of an intrinsic pace-maker current. This activity is under the influence of released neurotransmitters that transform the pacemaker activity in irregular- or burst-like in *in vivo* condition. In this presentation, common and different features of ventral tegmental area (VTA) and substantia nigra pars compacta neurons (SNpc) will be examined. Of paramount importance for the modality of firing and thus, the release of dopamine in the terminal fields, is the tone of dopamine itself that activates D2 autoreceptors at the somato-dendritic and terminal level of the dopaminergic cells. Therefore, different drugs by acting on the metabolism, release and uptake of this catecholamine affect the firing properties of the DAergic neurons. Moreover, the specific conductances and intracellular pathways activated by ionotropic and metabotropic receptors in these cells will be delineated.

We will also present data regarding possible differential responses to dihydropyridine calcium antagonists and dopamine within the VTAand SNpc.

A particular attention will be paid to the modalities of DA release in the striatum and the influence of abused drugs on catecholamine overflow .

To accomplish this we will use in vitro extracellular, intracellular and patch-clamp electrophysiological recordings plus voltammetric detections of DA release in mesencephalic and striatal slices obtained from wild and genetically modified rodents.

Dopamine Diversity in the Substantia Nigra

Jochen **Roeper**

Goethe University / Institute of Neurophysiology

While projection-specific functional diversity of dopamine (DA) midbrain neurons has been recognized, dopamine neurons in the neighboring substantia nigra (SN) is less clear. We provide evidence for differential regulation of intrinsic in vivo activity in medial versus lateral SN DA neurons. Only medial DA SN neurons depend on ATP-sensitive potassium channel activity for in vivo burst induction, which is necessary for novelty-induced exploratory behavior. In addition, we discovered differential changes of in vivo DA SN activity within the SN with aging and in response to proteasome inhibition or mutant alpha-synuclein expression. In summary, also DA SN neuron display functional diversity both in the intact brain and in response to pathophysiologically relevant stressors.

Extension of dopamine reward responses by generalization and pseudoconditioning

Wolfram **Schultz**

Department of Physiology, Development & Neuroscience, University of Cambridge, UK

Dopamine neurons signal the difference between obtained and expected reward. However, these neurons appear also to respond to stimuli without obvious appetitive consequences, such as loud sound, bright light, and painful pinch. To investigate the issue further, we studied the effects of reward and physically intense stimuli on single dopamine neurons. When we presented fruit juice or a large bright fractal picture in pseudorandomly interleaved trials, dopamine neurons showed equally large activations to all stimuli during the early response phase, although the responses began to distinguish the conditioned stimuli after the peak. We then tested dopamine neurons in a block design with several contextual cues; we introduced contextual pictures that stayed on the monitor indicating the presence or absence of reward, and put the juice spout in place only during the trial block in which juice was delivered. These contextual cues provided reward information even before stimulus onset. In this situation, the stimuli associated with intense visual stimuli evoked hardly any response, whereas the juice-predicting stimuli continued to evoke large responses. Closer analysis of these data suggest that dopamine responses are affected by generalisation to rewarding stimuli and by pseudoconditioning from rewarding contexts.

STRUCTURE, REGULATION AND FUNCTION OF THE DOPAMINE TRANSPORTER

Organizer: A. H. Newman (USA)

Uncovering the cocaine binding site in the dopamine transporter using the cocaine-like photo affinity ligand RTI 82

P. Bala, B. Sharma, R. Acharya, J. Foster, A. Newman, R. Vaughan, L K **Henry**

University of North Dakota / Pharmacology, Physiology and Therapeutics

The dopamine transporter (DAT) is the principal target for drugs of abuse such as cocaine and methamphetamine. However, the molecular interactions of these compounds with DAT are poorly understood. In this study, comparative models of rat DAT were constructed based on the bacterial transporter LeuT 'occluded' and 'open-to-out' crystal structures (PDBID: 2A65 and 3F3A, respectively) followed by iterative model refinement and energy minimization in the molecular modeling package Rosetta 3.1 in the absence of Na+ and Cl- ions. The twenty best DAT structures obtained (10 from both 2A65 and 3F3A) were carried forward for flexible ensemble docking of the cocaine-like photo affinity ligand, RTI-82 using RosettaLigand. In addition, Na+ and Cl- were placed in their putative binding sites in the 3F3A-based Rosetta 3.1 models and analyzed by induced fit docking (IFD). Here, analyses of the results obtained from the flexibly docked poses of RTI-82 revealed the orientation of the phenyl moiety of the aryl azido group of RTI-82 lies close to F319 on TM6 where its binding is stabilized by π-π interactions. In support of these findings, recent cross-linking data reveal that the RTI-82 aryl azido group adducts between residues F319 and L321 (F320 and L322 in hDAT). These data in addition to our previous analysis of the cocaine analog MFZ 2-48 reveal our models are highly predictive for these tropane-based DAT-inhibitor interactions.

Cocaine and Methylphenidate Self-Administration Produce Opposite Changes in Dopamine Transporter Function

Erin S. Calipari, Mark J. Ferris, David C. S. Roberts, Sara R. **Jones**

Wake Forest School of Medicine / Physiology and Pharmacology

Methylphenidate (MPH) is a commonly abused psychostimulant that is structurally similar to amphetamine, however, it has a mechanism of action similar to cocaine (COC) and is commonly characterized as a dopamine transporter (DAT) blocker. While there has been extensive work aimed at understanding dopamine (DA) nerve terminal plasticity following cocaine self-administration (SA), there is very little known about the effects of MPH SA on the DA system. We used fast scan cyclic voltammetry in brain slices from animals with a five-day SA history of 40 injections/day of behaviorally equivalent doses of MPH (0.56 mg/kg) and COC (1.5 mg/kg) to systematically explore alterations in baseline DA release and uptake kinetics as well as alterations in the interaction of each compound with the DAT. Although MPH and COC have similar acute effects, the consequences of SA on DA system kinetics were found to be opposite. MPH SA caused an increase in the maximal rate of DA uptake and the magnitude of stimulated DA release, while COC SA decreased both measures. Additionally, COC SA resulted in decreased COC potency at inhibiting DA uptake, with no change in MPH potency. On the other hand, MPH SA increased the ability of MPH to inhibit DA uptake while leaving COC unaffected. These data demonstrate that DA nerve terminal alterations following MPH SA are different from COC SA, and suggest that the factors governing DA system adaptations are more complicated than simple DA uptake blockade.

The importance of kinases in the regulation of dopamine transporter-mediated efflux.

Harald **Sitte**

Medical University Vienna / Center of Physiology and Pharmacology, Institute of Pharmacology

The dopamine transporter is a crucial regulator of dopaminergic neurotransmission. The dopamine transporter is also the primary target of psychostimulant drugs, such as cocaine and amphetamines. The action of amphetamines, which induce transport reversal, relies primarily on the ionic composition of the intra- and extracellular milieus. Recent findings suggest that dopamine transporter interacting proteins may also play a significant role in the modulation of reverse dopamine transport. Most importantly, the activity of several different kinases such as the serine/threonine kinase alpha CaMKII and protein kinase C support amphetamine-triggered dopamine transporter-mediated substrate efflux. Alpha CaMKII has also been shown to bind the dopamine transporter in vitro and is therefore believed to be an important player within the dopamine transporter interactome. A number of phosphorylation sites have been explored and mutagenesis approaches as well as the use of animal models supported their functional importance. Studies employing behavioural pharmacology examined part of the findings in vivo: the amphetamine-related phenotypes can be attributed to the kinase activity.

Preference for Distinct Functional Conformations of the Dopamine Transporter alters the Relationship Between Subjective Effects of Cocaine and Stimulation of Mesolimbic Dopamine

Gianluigi **Tanda**, Amy H. Newman, and Jonathan L. Katz

National Institute on Drug Abuse, IRP/NIH / DHHS

Binding to the dopamine (DA) transporter (DAT) in distinct conformations predicts differences in cocaine-like effects for structurally diverse DA uptake inhibitors (DUIs). We studied whether typical-DUIs suggested to bind and stabilize an outward-facing DAT conformation (cocaine, methylphenidate, WIN35,428) have a different relationship between stimulation of mesolimbic DA levels and their cocaine-like subjective effects compared to atypical-DUIs (the benztropine-analogs: AHN1-055, AHN2-005, JHW-007) which preferentially bind to an inward-facing DAT conformation. All drugs stimulated DA levels with different time-courses and maximal effects. The typical-DUIs, produced full cocaine-like subjective effects, which corresponded to about a 100-150 % increase over basal DA levels, regardless of dose and pretreatment time. However, atypical-DUIs produced inconsistent cocaine-like subjective effects with full cocaine-like effects, if at all, only at some pretreatment times at the highest doses. The atypical DUIs required much higher DA levels to produce cocaine-like subjective effects comparable to those produced by typical DUIs. Importantly, a linear, time-independent, relationship between cocaine-like subjective effects and stimulation of DA levels was obtained with typical- but not atypical-DUIs. The present results suggest a time-related desensitization process underlying the reduced cocaine-like subjective effects of atypical-DUIs that may be differentially induced by the binding modalities identified using molecular approaches. Since the DAT is the target of several drugs for treating neuropsychiatric disorders, such as drug abuse and ADHD, these results point to safe and effective DAT-based medications for these disorders that do not share the addictive liability of cocaine.

Individual differences in acute low dose cocaine-induced locomotor activation in rats involve striatal dopamine transporters and predict cocaine addiction-like behaviors.

Nancy R. **Zahniser**, Dorothy J. Yamamoto, Anna M. Nelson, Bruce H. Mandt, Richard M. Allen

University of Colorado Anschutz Medical Center / Pharmacology

Individual differences are a hallmark of drug addiction. To better understand their neurochemical underpinnings, we took advantage of the markedly different magnitudes of locomotor activation induced by acute, low dose cocaine in individual Sprague-Dawley rats. Rats with cocaine-induced activity below, or above, the group median are classified as low, or high, cocaine responders (LCRs or HCRs), respectively. Brain cocaine levels and novelty-induced activation are similar in LCRs and HCRs, but dopamine transporters (DATs) differ. Compared to HCRs, LCRs have higher numbers of striatal DATs, consistent with their insignificant cocaine inhibition of DAT-mediated DA clearance and cocaine-induced increases in extracellular DA levels. Overall, cocaine's behavioral activation and DAT-mediated effects are correlated. Interestingly, in HCRs, but not LCRs, maximal striatal DAT velocity is up-regulated shortly after cocaine exposure, suggesting greater compensatory DAT plasticity in HCRs. When exposed repeatedly to cocaine, LCRs more readily than HCRs develop cocaine locomotor sensitization and conditioned place preference; and LCRs and HCRs no longer differ in any measure of striatal DATs. Although LCRs and HCRs acquire cocaine self-administration similarly, under some conditions LCRs exhibit greater motivation than HCRs to self-administer cocaine. Thus, low initial responsiveness to cocaine-induced activation appears to predict a phenotype more sensitive to the consequences of repeated cocaine exposure. Together, our results emphasize the importance of striatal DATs in differential responsiveness of individuals to low dose cocaine and the predictive value of this initial drug response on subsequent expression of addiction-like behaviors.

SYNAPTIC AND BEHAVIORAL PLASTICITY IN THE MESOCORTICOLIMBIC SYSTEM

Organizer: C. Lüscher (Switzerland)

Illuminating the Role of Dopamine Neurons in Reward Learning

Patricia H. **Janak**

Ernest Gallo Clinic & Research Center/UCSF / Neurology

Dopamine is known to play a crucial role in reward learning, but exactly how it influences this process is an area of intense investigation and considerable debate. Dopamine neurons in the mammalian midbrain are strongly activated by unexpected rewards. This brief, phasic signal is proposed to support learning about cues that predict reward availability, featuring prominently in formal models of reinforcement learning as a reward prediction error signal. Despite strong correlational evidence, it is not known whether dopamine neuron activation during the consumption of primary rewards is causally related to learning about predictive cues. To address this question, we have leveraged the power of optogenetic tools to activate or inhibit DA neurons with precise temporal control in the ventral tegmental area of rodents during Pavlovian and instrumental conditioning. From associative blocking to extinction, we find a pattern of results that strongly supports a role for reward-related dopamine neuronal activation and conditioned responding. In addition, we find that inhibition of DA neuron activity inhibits initiation of reward-seeking actions, demonstrating a critical role for DA signaling outside the time of expected reward. The implications of these experimental findings for major hypotheses of DA function will be discussed.

Drug-evoked synaptic plasticity of excitatory and inhibitory transmission

C. **Lüscher**

University of Geneva /

Drug-evoked synaptic plasticity can be observed at several synapses of the mesolimbic circuitry and may represent a cellular correlate of drug-adaptive behaviour. We will present experiments where we characterise drug-evoked synaptic plasticity in neurons of the VTAex vivo, focussing at GABA transmission. We find that, cocaine exposure leads to a potentiation of transmission of inhibitory afferent onto VTA GABA neurons that occludes a from of presynaptic LTP. By this mechanism, cocaine increases tonic firing rate of dopamine neurons through disinhibition. We will discuss who cocaine-evoked potentation ofGABA transmission affects mesolimbic circuit function and drug-adaptive behaviour.

Projection-specific synaptic plasticity in the lateral habenula

Manuel **Mameli**

Inserm Institut du Fer a Moulin /

Addictive drugs increase dopamine (DA) levels and trigger synaptic adaptations in the mesocorticolimbic system. Neurons located in the lateral habenula (LHb) modulate the activity of DA neurons, DA release and adaptively tune goal-directed behaviors. We combined retrograde tracing and ex-vivo patch-clamp recordings to assess the influence of cocaine experience on excitatory transmission onto subsets of LHb neurons. We find that cocaine selectively strengthens glutamatergic synapses onto LHb neurons sending axons to the rostromedial tegmental nucleus, a GABAergic structure linking the LHb with DA neurons of the ventral tegmental area. Furthermore, cocaine-evoked synaptic strengthening, while inducing a postsynaptic accumulation of AMPA receptors, does not modify subunit composition or single-channel conductance. This synaptic potentiation unmasks a long-lasting AMPA receptor-dependent synaptic potentiation upon a stimulation protocol pairing presynaptic glutamate release with somatic hyperpolarization. This results unravels an early, projection-specific, cocaine-evoked synaptic potentiation in the LHb that may represent a key step for the functional reorganization of the mesolimbic system after drug exposure.

Endocannabinoid signaling in action control

Raffaella **Tonini**

Istituto Italiano di Tecnologia

Goal-directed control of actions is the ability to adapt the behavior to obtain specific outcomes and to efficiently respond in changing situations. With repetition, actions become more automatic and habitual and are controlled by sensorimotor associations. Neuronal projections from the limbic and associative cortical areas to the medial part of the dorsal striatum (cognitive control system) are involved in the goal-directed actions and behavioral flexibility, while projections from sensory motor cortices to the dorsolateral striatum (habit system) in motor planning and habit formation. Recent theories propose that the cognitive control system and the habit system interact to determine action control. The role of neuromodulatory pathways in regulating synaptic- and network plasticity to maintain a dynamic balance between these cortico-basal ganglia circuits is, however, still unclear. To address this question we investigated how dopamine and endocannabinoid (eCB) signals integrate to shape synaptic plasticity at discrete cortico-striatal circuits and how this is relevant for defined cognitive processes implicated in instrumental goal contingencies. We found that adaptations of the eCB pathway influence long-term synaptic depression preferentially at the cortical connections to striatopallidal neurons of the dorsolateral striatum. This is associated with a behavioral switch from goal-directed actions to inflexible habitual strategies. Our results thus reveal a direct relationship between synaptic eCB signaling in segregated dopaminergic circuits and control of instrumental behavior.

DOPAMINE RECEPTOR HETEROMERIC COMPLEXES AND THEIR FUNCTION

Organizer: S. R. George (CANADA)

Functional and pharmacological consequences of D_2 receptor-containing heteromers. Relevance of heteromer quaternary structure and loss of heteromes in L-DOPA induced dyskinetic primates.

Rafael **Franco**

Centro de Investigación Médica Aplicada. Univ. of Navarra

Adenosine-mediated regulation of dopaminergic neurotransmission was demonstrated in the late eighties. Careful studies in the striatum showed a high degree of segregation of dopamine D_1 and adenosine A_1 receptors in the direct pathway, and of D_2 (D_2R) and A_{2A} ($A_{2A}R$) in the indirect pathway. In the latter, D_2R activation by dopamine engages G_i proteins, while the presence of the neuromodulator adenosine activates $A_{2A}R$ and engages Gs proteins. Therefore, adenosine-dopamine interactions in the CNS were first explained by modulations at the cAMP second messenger level. However, the occurrence of $D_2R/A_{2A}R$ heteromers, which were among the first to be identified, has paved the path to understand a diversity of D_2R-mediated actions. Intramolecular cross-talk within the heteromers caused by activation of one of the receptors, or caused by calmodulin or by drugs such as cocaine, leads to a variation in ligand binding parameters that may be of pharmacological and drug discovery interest. The occurrence of heterotrimers of cannabinoid CB_1, dopamine D_2 and adenosine A_{2A}, first detected in transfected cells, was demonstrated in brain striatum. Interestingly, the hetometrimer links dopamine transmission to the MAPK signalling pathway. Mutation experiments indicate that the interactions of the intracellular domains of the CB_1 receptor with A_{2A} and D_2 receptors are fundamental for the correct formation of the quaternary structure needed for the specific function (MAPK signaling) of the $A_{2A}/CB_1/D_2$ receptor heteromer. These findings permitted to propose the first molecular model of the quaternary structure of a receptor heteromultimer. Studies in non-human primates show that $A_{2A}/CB_1/D_2$ receptor heteromers are present in naïve and parkinsonian animals but that their expression is markedly reduced in samples from L-DOPA-induced dyskinetic animals. Recent results showing the coupling to two different G proteins to a receptor heteromer will also be provided.

The dopamine D1-D2 receptor heteromer: Novel signaling and function mediated by a unique subset of neurons in basal ganglia

SR **George**, ML Perreault, A Hasbi, BF O'Dowd

Centre for Addiction and Mental Health, University of Toronto / Neuroscience

We identified a novel dopamine receptor signaling complex, the D1-D2 receptor heteromer. D1-D2 heteromer activation triggered intracellular calcium release through the Gq/PLC/IP3R signaling cascade, distinct from signaling activated by D1 or D2 receptors. D1-D2 heteromer activation led to CaMKII? phosphorylation, increased BDNF production, which enhanced neuronal differentiation and growth. D1-D2 heteromer is expressed exclusively in striatal MSNs that co-express dynorphin and enkephalin, and also contain GABA and glutamate with capability for dual regulation of these transmitters, unlike traditional MSNs, which are GABAergic and contain either dynorphin/D1 receptor or enkephalin/D2 receptor. D1-D2 heteromer in cultured striatal neurons and brain sections was demonstrated by confocal FRET between endogenously expressed D1 and D2 receptors, indicating proximity from each other under 100 A. The distribution of these neurons in basal ganglia revealed highest density in globus pallidus, followed by nucleus accumbens and with very low density in caudate nucleus. Activation of the D1-D2 heteromer with SKF 83959 resulted in increased grooming in rats, which was reproduced by injection of drug into nucleus accumbens bilaterally or into D5-/- mice. Amphetamine sensitization resulted in increased heteromer FRET signal, density and agonist affinity in striatum, providing the first measurable biochemical index of a sensitized state. In globus pallidus from schizophrenia brain, D1-D2 heteromer high affinity state was upregulated compared to globus pallidus from control brain. Thus this novel dopamine receptor signaling complex expressed within a unique subset of basal ganglia neurons has emerging neural functions and links to pathophysiologic processes that are being defined.

Diurnal Dopamine-Adrenergic heteromers can control melatonin production

Peter J. **McCormick**

Department of Biochemistry / University of Barcelona

The role of the pineal gland is to translate the rhythmic cycles of night and day encoded by the retina into hormonal signals that are transmitted to the rest of the neuronal system in the form of serotonin and melatonin synthesis and release. We found that the production of both melatonin and serotonin by the pineal gland is regulated by a circadian-related heteromerization of adrenergic and dopamine D4 receptors. Through α1B-D4 and β1-D4 receptor heteromers dopamine inhibits adrenergic receptor signaling and blocks the synthesis of melatonin induced by adrenergic receptor ligands. This inhibition was not observed at hours of the day when D4 was not expressed. These data provide a new perspective on dopamine function and constitutes the first example of a circadian-controlled receptor heteromer. The unanticipated heteromerization between adrenergic and dopamine D4 receptors provides a feedback mechanism for the neuronal hormone system in the form of dopamine to control circadian inputs.

Growth Hormone Secretagogue Receptor (GHSR1a, aka ghrelin receptor) Antagonists as Selective Blockers of Dopamine Signaling in GHSR1a:DRD2 and GHSR1a:DRD1 Expressing Neurons

Roy G. Smith, Celine Ullrich, and Andras **Kern**

Scripps Research Institute / Metabolism and Aging

GHSR1a was identified as an orphan receptor that mediates the action of a small molecule (MK-0677) which was designed to rejuvenate the growth hormone axis (Howard et al. Science 273:974, 1996). Subsequently, GHSR1a was deorphanized by discovery of an endogenous GHSR1a agonist (Kojima et al. Nature 402:656, 1999). GHSR1a is expressed in hypothalamus, hippocampus and mid brain; however, other than trace amounts in the hypothalamus, ghrelin is undetectable in the brain. Nevertheless, GHSR1a is expressed in subsets of neurons that also express either DRD2 or DRD1. We speculated GHSR1a could modify dopamine (DA) signaling in the absence of ghrelin. Indeed, we showed co-expression of GHSR1a with DRD2 or DRD1 alters canonical DA signaling resulting in mobilization of [Ca2+]i via formation of GHSR1a:DRD2 and GHSR1a:DRD1 heteromers. FRET confocal microscopy and Ca2+ imaging illustrated the presence of functional heteromers in mouse brain slices. Biological relevance was demonstrated by comparing DRD2 and DRD1 agonist-induced behavioral responses in WT and ghsr-/- mice, and in WT mice treated with a neutral GHSR1a antagonist (JMV2959). Both ghsr-/- mice and JMV2959 treated WT mice were resistant to DRD2 and DRD1 agonist-induced behaviors. Therefore, pharmacological intervention with a GHSR1a antagonist is a selective way to block DA signaling in neurons expressing GHSR1a heteromers without affecting signaling in neurons expressing DRD2 or DRD1 alone. These results show the potential of developing selective therapeutic agents that act by a new mechanism for treating psychiatric disorders involving abnormal DA signaling. Supported by NIH grant R01 AG19230 (RGS).

DIFFERENTIAL ROLE OF THE MESOLIMBIC SYSTEM IN PSYCHOSTIMULANT VERSUS OPIATE REWARD

Organizer: A. Badiani (Italy)

Heroin and cocaine reward are different: clinical and preclinical evidence

Aldo **Badiani**

Sapienza University of Rome / University of Sussex

Epidemiological studies have shown that the environment can exert a powerful modulatory influence on drug abuse and that this influence is substance-specific. We found that when human addicts are interviewed about the circumstances of drug taking, they indicate distinct settings for the two drugs: heroin being used preferentially at home and cocaine preferentially outside the home . Preliminary data from fMRI studies in heroin and cocaine co-abusers show that the two drugs produce distinct patterns of neuronal activation as a function of the context. Studies conducted in rats confirm the results obtained in humans. The setting of drug self-administration affects in a very different manner: 1) the intake on cocaine vs. heroin, 2) the preference for cocaine vs. heroin, 3) the vulnerability to cocaine- vs. heroin-induced reinstatement. The theoretical and therapeutic implications of these findings will be discussed.

Habitual drug seeking: from pathophysiology to treatment

David **Belin**

INSERM & University of Poitiers / U1084-LNEC

Drug addiction may be associated with a loss of executive control over maladaptive incentive habits. These incentive habits, operationalised in rats as well established cue-controlled drug seeking under second-order schedules of reinforcement, have been hypothesized to result from a pathological coupling of drug-influenced motivational states and a rigid stimulus-response habit system by which drug-associated stimuli through automatic processes elicit and maintain drug seeking. At the neurobiological level, incentive habits may depend upon an interaction between the basolateral amygdala and nucleus accumbens core, together with the progressive development of a ventral-to-dorsolateral striatum (DLS) functional coupling through the recruitment of striato-nigro-striatal dopamine-dependent loops. If the development of incentive habits for opiates seeking seems to develop faster than for stimulants, both have been shown to be sensitive to N-acetylcysteine, and its associated influence of glutamate homeostasis, thereby providing insights into potential common pathophysiological mechanisms between opiates and stimulant addictions.

Context-Induced Reinstatement of Heroin vs. Cocaine Seeking: Overlapping yet Distinctly Different Neuroanatomical Substrate Involvement

Jennifer M. **Bossert**

DHHS/NIH/NIDA/IRP / Behavioral Neuroscience

Data from animal models of drug self-administration and relapse indicate that there are distinct behavioral and neurobiological differences between opiates and psychostimulants. We and others have adapted an ABA renewal procedure to study the role of drug-associated contexts in drug-seeking in an animal model. Here, we will compare and contrast the neurobiological substrates involved in contact-induced reinstatement of cocaine and heroin seeking. While there is some overlap of brain area involvement between the two drugs in context-induced reinstatement (nucleus accumbens shell, ventral hippocampus), there are also distinctly differential roles of other brain areas in this behavior (medical prefrontal cortex subregions). Such neurobiological differences between opiates and psychostimulants may have implications for drug addiction treatment.

Cocaine and opioid cues: incentive salience

Terry E. **Robinson**

University of Michigan

Cues associated with rewards, such as food or drugs, can acquire considerable control over behavior, for example, instigating actions to obtain the reward. There are, however, large individual differences in the motivational properties of reward cues, and the extent to which they can be resisted. This presentation will address the implications of individual differences in cue-evoked motivational processes and in cognitive control in the development of impulse control disorders, including addiction, and possible neurobiological substrates.

MAKING SENSE OF PREFRONTAL CORTICAL DOPAMINE FUNCTION

Organizer: P. O'Donnell (USA)

Adolescent Maturation of Prefrontal Cortical Function

P **O'Donnell**, D Counotte, E Lewis, R Cardarelli

University of Maryland School of Medicine / Anatomy & Neurobiology

The balance between excitation and inhibition is a critical aspect of prefrontal cortical function tightly modulated by dopamine. The manner dopamine modulates pyramidal neuron and interneuron activity matures dramatically during adolescence. DA modulation of pyramidal neurons and fast-spiking interneurons is different in slices from adult vs. juvenile rats. Both the D1 potentiation of NMDA responses and the D2 activation of interneurons are stronger in the adult than in juveniles. In rodent models of schizophrenia, this maturation fails to occur, rendering cortical circuits in a disinhibited state in the presence of high levels of dopamine. Adult rats with a neonatal ventral hippocampal lesion (NVHL), rats with a neonatal immune activation, and mice with a truncated disrupted-in-schizophrenia 1 (DISC1) gene, exhibit a common deficit in this maturation. In all these models, cortical circuits lack the ability of D2 receptors to activate interneurons. The dopamine control of excitation-inhibition balance is critical for appropriate response selection and behavioral responses are altered in NVHL rats. It is therefore possible that the adolescent maturation of cortical circuits may be a factor in developmental disorders with symptoms that emerge during adolescence such as schizophrenia.

Optogenetic stimulation of synaptic inputs reveals novel effects of dopamine D2 receptors in prefrontal cortex

Vikaas S. **Sohal**

University of California, San Francisco / Psychiatry

I will review recent work from my laboratory on how dopamine can modulate specific subpopulations of pyramidal neurons in Layer 5 of the prefrontal cortex.

Dissociable regulation of different executive functions by prefrontal D1 and D2 receptors

Stan B. **Floresco**

University of British Columbia / Psychology

Mesocortical dopamine (DA) transmission is essential for modulating different executive functions governed by the prefrontal cortex (PFC). Much of our understanding of how DA regulates these functions comes from studies of working memory that have shown that blockade or excessive stimulation of PFC D1, but not D2 receptors induce deleterious effects on working memory functions (i.e., an "inverted-U" shaped function). Neurochemical studies have revealed that accurate retrieval of information from working memory is positively correlated with robust increases in PFC DA release, with lower levels of extracelluar DA efflux associated with poorer performance. However, recent pharmacological and microdialysis findings suggest that the principles of operation underlying PFC DA modulation of other executive functions are considerably different from those underlying working memory. Behavioral flexibility is dependent on both PFC D1 and D2 receptors, and supranormal stimulation of these receptors does not alter performance. Moreover, manipulations of PFC D1 and D2 receptors can exert opposing effects on cost/benefit decision making entailing judgments about uncertain rewards. Thus, PFC DA is an essential component of the neural circuitry that mediates a variety of executive functions, each of which engages distinct types of cognitive operations. Accordingly, the mechanisms through which DA exerts its effects are not unitary across these functions, but rather, each process relies on different patterns of activation of PFC DA receptors. Thus, the construct of an "inverted-U" shaped function underlying D1 receptor modulation of working memory does not necessarily to hold true for other executive functions mediated by the PFC.

Prefrontal brain activity predicted by dopaminergic genes in healthy adults is modulated by antipsychotics in schizophrenia

A. Vercammen, C.S. Weickert, A. Skilleter, R. Lenroot, P. Schofield, T.W. **Weickert**

University of New South Wales / School of Psychiatry

The dopamine system plays a crucial role in mediating cognitive and affective processes. Aberrant dopamine neurotransmission is also thought to underlie the symptoms of schizophrenia. Dopamine-dependent prefrontal response relies on the regulation of both dopamine availability and the relative balance of D1/D2 receptor mediated action. This study aimed to determine the extent to which the catechol-O-methyltransferase (COMT) val108/158met (rs4680) and dopamine D2 receptor (DRD2) G-T (rs2283265) polymorphisms combine additively to determine prefrontal brain activity during cognitive-affective processing in healthy people and in schizophrenia. Forty-three healthy adults and 27 people with schizophrenia received an fMRI scan while completing an emotional response inhibition test. Blood samples were collected and DNA was genotyped using Taqman SNP assays. The number of "risk" alleles was tallied in each participant to generate an oligogenic score which was used as a predictor of brain activation. We also tested whether task related brain activity was related to antipsychotic dosage in schizophrenia. People with schizophrenia showed a significantly reduced activity during inhibition of responses to negative words in left insula, left BA 10, right BA 10, right anterior cingulate, and right BA 9 compared to healthy adults. We detected a significant linear association between increasing prefrontal dopamine risk allele load and reduced activation in the left insula, bilateral BA10, and right BA9 in healthy adults and no such relationship in schizophrenia. We observed a negative association between daily chlorpromazine equivalent dose and dorsolateral prefrontal activation in schizophrenia. These results provide the first evidence that genetic variation controlling DRD2 receptor characteristics and synaptic dopaminergic availability combine to shape prefrontal cortex neural responses.

REWARD LEARNING AND DOPAMINE

Organizer: R.M. Wightman (USA)

Dopamine and overeating

Paul J. **Kenny**; Paul M. Johnson

The Scripps Research Institute / Molecular Therapeutics

There is a growing appreciation for the role of dopamine in overeating and in the development of compulsive patterns of food consumption. Recently, we reported that development of obesity is associated with the development of a progressively worsening deficit in brain reward function, measured as elevated intracranial self-stimulation thresholds in rats. Moreover, we observed compulsive-like patterns of palatable food consumption in obese but not lean rats, measured as palatable food consumption that was resistant to punishment-induced suppression of intake. Consistent with overweight human subjects, dopamine D2 receptors (D2Rs) in striatum were downregulated in obese rats. Moreover, virus-mediated knockdown of striatal D2Rs rapidly accelerated the development of reward deficits and compulsive-like food seeking in rats with access to palatable high-fat food. Currently, we are investigating which populations of dopaminociceptive neurons in the striatum are responsible for the emergence of addiction-like behaviors in obese rats.

The importance of circuit rewiring in amphetamine-associated contextual learning

Gloria E. **Meredith*** and David Rademacher

Pharmaceutical Science/Rosalind Franklin University

Drug seeking and the vulnerability to relapse occur when individuals are exposed to an environment with sensory cues where drug taking has occurred. Memory formation related to drug taking in the same environmental context may require plasticity in synaptic circuits. We used a conditioned place preference paradigm to understand the role of the basolateral amygdala (BLA) circuit in drug-induced associative learning. We used amphetamine (AMPH) as the unconditioned stimulus and conditioned rats with 1.0 mg/kg AMPH or saline. Rats were tested, drug free, 72 h after the last conditioning session. Controls included a saline-conditioned group and a home cage, delayed pairing AMPH injection group. Stereological analysis showed an increase in excitatory synapses following conditioning with AMPH but not after either saline or AMPH in the home cage. Excitatory synaptic activity in the BLA pyramidal neurons was measured using in vivo intracellular recordings in anesthetized rats and we found increased measures of synaptic drive that reflect the increase in excitatory synapse number. Thus, context-drug associations are accompanied by structural and functional plasticity in the BLA, findings that have important implications for drug-seeking behavior.

Methylphenidate specifically rescues deficient anticipatory dopamine release

Jeff **Wickens**, Yu-Ting Li, Yi-Ling Huang, Jia-Jin J. Chen, and Brian Hyland

Okinawa Institute of Science and Technology / 2Department of Biomedical Engineering, National Cheng Kung University/ University of Otago

Methylphenidate (Ritalin) is the most widely used and effective treatment for attention-deficit hyperactivity disorder (ADHD). Methylphenidate has a known cocaine-like pharmacological action on the dopamine transporter, but its therapeutic mechanism in ADHD is not completely understood. Many pieces of evidence indicate that there is altered processing of reward in ADHD, with increased sensitivity to delay of reward and a greater than normal preference for immediate over delayed rewards. Since dopamine is a key neurotransmitter in reward processing, modulation of reward processes by an action on dopamine is a possible mechanism for the effects of methylphenidate. Here we show that dopamine release in response to a classically conditioned cue that precedes reward is deficient in an ADHD model, and that methylphenidate specifically rescues this anticipatory dopamine release, at doses commonly used in treatment of ADHD. This specific action of methylphenidate provides a novel basis for its therapeutic action, in which facilitated release of dopamine by cues that predict reward is expected to bridge delays between actions and rewarding outcomes. We propose that by increasing the anticipatory release of dopamine, methylphenidate enhances the ability to stay on task when reward is delayed, which improves the performance of children with ADHD in classroom situations.

In vivo measurements of dopamine release during behavior

R, Mark **Wightman**, Catarina Ovesson-White, Anna Belle

University of North Carolina / Chemistry

Our recent work has shown that there is a surge of dopamine into the extracellular fluid in response to cues that predict reward. This dopamine release is a learned association that develops with repeated trials. The carbon-fiber electrode that is used to detect dopamine can also be used to monitor unit activity of adjacent neurons. This procedure enables chemical changes and their effect on unit activity of adjacent cells to be characterized. Furthermore, when coupled with controlled iontophoresis the types of receptors responsible for this activity can be probed. Using this approach we have examined the role of D2 receptors in the cue evoked response.

POSTER SESSION I

Saturday, MAY 25th

Dopamine and Parkinsons's disease

P001. A transcription factor-microRNA autoregulatory loop determines dopamine neuron numbers

A. Anderegg1, H-P. Lin1, G. Caronia-Brown1, B. Yun1, R. Johnson 2, B. Harfe 3, R. **Awatramani**1*

Neurology / Northwestern University

Coordinating the construction of the vertebrate brain requires the production of the correct numbers of neurons, at the proper place and time during embryogenesis. Imperative to this dynamic process is the appropriate activation and restriction of key transcription factors, which orchestrate the assembly of organizing centers, delineate progenitor cell domains along the neural tube, specify neuronal identity, and promote precise differentiation programs. Recently, microRNAs have been shown to play a significant role in post-transcriptional regulation of developmentally important transcription factors and provide a potent mechanism by which transcription factor expression can be titered. Accurate control of transcription factor stoichiometry could be important in determining neural progenitor allocation, and consequently the generation of the correct numbers of distinct neuron types towards normal physiologic function. Dopamine (DA) neurons are produced from a specific embryonic domain and during a defined window of time, suggesting that there must exist specific mechanisms governing the spatial specification as well as the time of neuron production. Here, using conditional gain and loss of function approaches in mice, we have identified a novel transcription factor-microRNA autoregulatory loop that determines the spatial size of the DA progenitor pool, as well as the temporal window of DA neurogenesis. This molecular pathway could be a key determinant of DA neuron numbers, and consequently, could impact our understanding of disorders involving the DA system.

P002. Effects of antidepressant drugs in a rat model of depression in preclinical stages of Parkinson's disease

Klemencja **Berghauzen-Maciejewska**, Jadwiga Wardas, Katarzyna Kuter, Wacław Kolasiewicz, Urszula Głowacka, Krystyna Ossowska

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Depression is a comorbid disturbance in the clinical and preclinical phases of Parkinson's disease (PD). Pramipexole, SSRIs and tricyclic antidepressants ameliorate depression in PD. Our studies have indicated that a moderate lesion of dopaminergic systems induced "depressive-like" behaviour in rats.The aim of the present study was to examine the influence of chosen antidepressants on the behaviour of rats with the moderate lesion of dopaminergic systems in the forced swimming (FS) test. Rats were injected bilaterally with 6-OHDA (15 µg/2,5 µl) into the ventral striatum (vSTR) and were treated with imipramine (10 mg/kg ip), fluoxetine (10 mg/kg ip) and pramipexole (1 mg/kg sc) for 14 days. Behavioural tests were performed on the 15th day after the surgery. The lesion extent was analysed by HPLC and immunohistochemically. Pramipexole shortened the immobility and prolonged the climbing time in the FS test in lesioned rats. Fluoxetine decreased immobility and increased swimming in both lesioned and control animals. Imipramine did not influence the immobility time, but increased climbing in lesioned rats. Moreover, imipramine reduced the exploratory activity in lesioned animals during the first 5 min of the test. HPLC analysis showed decreases in the dopamine level in the dorsal striatum, vSTR and frontal cortex (40-50%). The above findings support the view that a relatively moderate dopaminergic lesion may model depression of the preclinical phase of PD.
Acknowledgments Study supported by the Project DEMETER- POIG.01.01.02-12-004/09-00

P003. Cognitive impairment and abnormal dentate gyrus plasticity in Parkinson's disease.

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Parkinson's disease (PD) is characterized by the progressive degeneration of the nigrostriatal dopaminergic pathway and the emergence of rigidity, tremor and bradykinesia. Accumulating evidence indicates that PD is also accompanied by non-motor symptoms including cognitive deficits, often manifested as impaired spatial memory and object recognition abilities. We studied cognitive performance and synaptic plasticity in a mouse model of PD, characterized by partial lesion of the dopaminergic and noradrenergic inputs to striatum and hippocampus. Sham- and 6-OHDA- lesioned mice were subjected to the novel object recognition test and long-term potentiation was examined in the dentate gyrus and CA1 regions. Bilateral 6-OHDA lesion reduces long-term, but not short-term, novel object recognition and decreases long- term potentiation specifically in the dentate gyrus, a region of the hippocampus critically involved in memory function. These abnormalities depend on the loss of dopamine and are abolished by administration of the antiparkinsonian drug, L-DOPA, or by activation of dopamine D1-type receptors. In contrast, activation of dopamine D2-type receptors does not modify the effects produced by the lesion. These finding show that experimental parkinsonism leads to impairment of long-term recognition memory, associated with abnormal synaptic plasticity in the dentate gyrus. These pathological manifestations are independent of loss of noradrenergic innervation and are corrected by activation of dopamine D1, but not D2 receptors

P003BIS_Transcriptional effects of LRRK2 kinase inhibition in LRRK2-expressing immune cells

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H. Lundbeck / Neurodegeneration

Rare and common forms of LRRK2 are associated with familiar and sporadic Parkinson's Disease. Also, diseases of the immune system have been associated with the LRRK2 biology. In humans, expression of LRRK2 mRNA is highly enriched in spleen, lung, kidney, brain and immune cells. The role of LRRK2 in the immune system is not known; however, based on recent literature findings LRRK2 may be involved in host response to pathogens. As an example, LRRK2 is differentially expressed in the monocyte derived leukemia cell line THP-1 after PMA differentiation and IFN-? stimulation. To investigate the functional role of LRRK2 in immune cells a pharmaco-transcriptomic approach was performed on PMA differentiated and IFN-? stimulated THP-1 cells with or without LRRK2 inhibition. The transcripts of 755 genes had a 2-fold increased or decreased expression pattern after IFN-? stimulation. LRRK2 inhibition using either LRRK2- IN-1 or Cmpd A gave differential expression of 1065 and 23 genes, respectively. Between the two compounds the overlap was transcripts encoded by 13 genes. Of these, 10 genes were concentration- dependently affected by Cmpd A. Q-PCR showed that LRRK2-IN-1 but not Cmpd-A concentration- dependently inhibited differential expression of LRRK2, CD14 and FCGR3A. Further profiling of both compounds in enzymatic, phosphorylation and selectivity assays indicated that the dissimilar differential expression patterns of LRRK2-IN-1 and Cmpd A arises from differences in kinase selectivity. Further studies using more selective LRRK2 inhibitors will provide novel insight into the molecular mechanisms underlying LRRK2's role in diseases of the immune system and further to its role in Parkinson's disease.

P004. Longitudinal automated gait analysis of the MitoPark mouse, a model of progressive dopaminergic cell loss.

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The MitoPark mouse is a conditional knockout of mitochondrial transcription factor A (Tfam) specifically in dopamine (DA) neurons, and as such it displays a progressive dopaminergic cell loss and gradually develops deficits in motor performance-related assays, starting at around 10-12 weeks of age. While behavioural studies in these mice have previously been focused mainly on spontaneous locomotor activity (LMA), there is little information about more subtle and specific motor deficits. We have used the CatWalk gait analysis system (Noldus, Wageningen, Netherlands) to characterize gait-related parameters in MitoPark mice from an age of 6 weeks until 19 weeks, and as comparison we also studied LMA as well as forced motor behavior via the rotarod test. Our data suggest that MitoPark mice display deficits in several gait-related parameters - some of which appear earlier than deficits in LMA or rotarod performance. In addition, some of these deficits do not appear to be of a progressive nature, but rather display a fixed difference relative to control mice throughout the duration of the study, while other parameters do display a more progressive worsening as mice get older. Overall, the data suggests that automated gait analysis is a more sensitive way of investigating motor abnormalities and also that subtle differences in gait parameters precede deficits in gross motor behaviour, as measured by LMA and rotarod.

P005. Calpains inhibition increases striatal TH-immunoreactive neurons in hemiparkinsonian rats treated with L-DOPA

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UQTR / Medical Biology

Tyrosine hydroxylase-immunoreactive (TH-ir) neurons have been identified in the striatum of dopamine-depleted animals and human parkinsonian patients. It is now well-accepted that these neurons are functionally active and distinct from classical neurons. On the other hand, little is known about the mechanism underlying their occurrence. Recently, it has been suggested that brain calpains could play critical roles in differentiation of embryonic stem cells into neural cells. The aim of this study was to investigate whether calpains inhibition can promote the emergence of striatal TH-ir neurons in 6-OHDA-lesioned rat treated with L-DOPA. According to previous studies, we observed an increased number of striatal TH-ir neurons in 6-OHDA rats treated with L-DOPA. Double immunofluorescence studies were performed to identify TH-ir cells expressing either glial fibrillary acidic protein (GFAP), neuronal nuclei (NeuN), calbindin, choline acetyltransferase (ChAT) or doublecortin (DCX). Virtually all TH-ir neurons were also immunoreactive for NeuN or calbindin. A chronic striatal infusion with the calpains inhibitor MDL28170, via a subcutaneous osmotic minipump, strongly increases the occurrence of TH-ir neurons within the striatum of 6-OHDA rats treated with L-DOPA. Phenotypic characterization revealed that 70% of TH-ir neurons were also immunoreactive for NeuN and 38% for DCX. These results suggested that calpains inhibition stimulated the emergence of striatal TH-ir neurons originating from migrating precursor cells. Promotion of these neurons may be beneficial in Parkinson's disease to compensate the loss in dopamine.

P006. Nitric oxide modulates rotational behaviour: a comparison between selective D1, D2 receptor agonists and L-DOPA in a rat model of Parkinson's disease

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Some strong evidence obtained from in vivo and ex-vivo studies suggests an interaction between dopaminergic and nitrergic systems. Our study was aimed at assessing the behavioral effects of selective D1 and D2 dopamine receptor agonists and the dopamine precursor L-DOPA, administered chronically alone or in combination with the nitric oxide donor, molsidomine to 6-OHDA-lesioned rats. Male Wistar rats were injected unilaterally with a 6-OHDA (8µg/4µl) into the left medial forebrain bundle. After two weeks only rats exhibiting a substantial loss of nigrostriatal dopamine neurons, evaluated by an apomorphine-induced rotation test, were treated chronically with molsidomine (2mg/kg ip) and the selective dopamine receptors agonists D1 - SKF38393 (3mg/kg sc), D2 - quinpirole (0,2mg/kg sc), or with L-DOPA (12.5 and 25mg/kg ip) once daily for 15 days. The intensity of rotations until their expiry was measured after the first and penultimate doses of the examined drugs. Both SKF38393 and quinpirole evoked contralateral rotations whose intensity increased during chronic administration but was much more relevant for the D2 agonist. Combined treatment with molsidomine and SKF38393 further increased contralateral rotations. No significant changes were observed after analogous treatment with molsidomine and quinpirole. In contrast to dopamine agonists, combined treatment with molsidomine and L-DOPA slightly decreased the number of contralateral turns. The obtained results indicate that exogenous nitric oxide differently modulate the rotational behaviour induced by selective dopamine D1 and D2 receptor agonists and dopamine derived from L-DOPA.

P007. Establishing a cell line model to study tyrosine hydroxylase deficiency

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Tyrosine hydroxylase deficiency is a rare metabolic disorder characterized by deficiencies in the enzyme responsible for converting the amino acid tyrosine to L-DOPA. This reaction is the rate limiting step in the production of catecholamines like dopamine, norepinephrine or epinephrine. These catecholamines are a group of neurotransmitters that play a crucial role in neurological processes like motor control, movement, sympathetic nervous system response, motivation, attention or learning. Tyrosine hydroxylase deficiency a recessively inherited disorder that is caused by mutations in tyrosine hydroxylase; these mutations can nullify tyrosine hydroxylase expression or codify a protein with low enzymatic activity which is unable to produce the L-DOPA levels necessary for correct organism function and development. Thus, patients with tyrosine hydroxylase deficiency can show a wide range of symptoms that go from mild locomotive alterations in the moderate forms to infantile parkinsonism in the severe ones. PC12 is a well characterized cathecolaminergic cell line that derives from albino rat pheochromocytoma. Using this system, we are developing a cell culture model to study the effects of human pathogenic variants of tyrosine hydroxylase in cathecolaminergic cells. In particular we aim to study the differences between mutations with different L-dopa response. Our work should provide a useful and manageable tool to study the tyrosine hydroxylase deficiency.

P008. MSK1 mediates L-DOPA-induced dyskinesia in an experimental Parkinsonian mouse model via expression of delta FosB

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The 6-OHDA mouse model of Parkinson's disease facilitates the dissection of signaling pathways responsible for the emergence of L-DOPA-induced dyskinesia (LID), a debilitating motor complication resulting from the repeated administration of the dopamine precursor L-DOPA to Parkinsonian patients. A causal role for the hyperactivity of the mitogen-activated protein kinase (MAPK) signaling pathway following L-DOPA administration has been reported for the development of LID. The mitogen-and stress-activated protein kinase 1 (MSK1), a nuclear kinase implicated in the nucleosomal response and immediate early gene expression, is situated to propagate cytoplasmic MAPK signaling into programmed transcriptional responses. Here, we report reduced dyskinetic behavior in MSK1 knockout mice, reduced phosphorylation of transcription factors and epigenetic states associated with active transcription, and reduced expression of the transcription factor delta FosB. Furthermore, using transgenic mouse lines in which delta FosB is over- or under-expressed, we demonstrate a causal role for this protein in the emergence of LID. We propose a model in which dyskinesia develops as a result, in part, of MSK1 activation, consequential phosphorylation of transcription factors and histones, and the expression of delta FosB.

P009. Antidyskinetic effect of memantine and amantadine in the rat Parkinson's disease model.

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Scienze biomediche

Although L-DOPA remains the most effective drug to treat motor symptoms in Parkinson's disease (PD), its long-term administration leads to development of dyskinesia. An increasing body of experimental evidence demonstrates that the glutamate system is involved in the appearance of dyskinesia. In fact, the NMDA receptor antagonist amantadine is the only antidyskinetic compound used in patients, albeit with limited efficacy and side effects. Here, we compared the effect of amantadine with memantine, another NMDA receptor antagonist, in clinical use for the treatment of dementia, in the rat model of L-DOPA-induced dyskinesia. 6-OHDA-lesioned rats were made dyskinetic by a chronic L-DOPA treatment, and then subjected to pharmacological challenges with memantine (5, 10, 15 and 20 mg/Kg) or amantadine (10, 20, 40 and 60 mg/Kg) in combination with L-DOPA (6 mg/kg). Abnormal involuntary movements (AIMs) were evaluated. Results showed that amantadine reduced AIMs by about 40% compared to the control group, when given at 60 mg/kg. However, side effect such as skin irritation and eye oedema were observed in the treated animals. Memantine produced about 50% reduction of AIMs at 20 mg/kg, and appeared to be well tolerated. To our knowledge, this is the first time memantine is reported to produce significant antidyskinetic effect in the rat PD model. In line with preliminary clinical reports, our study supports the use of memantine to control dyskinesia in PD patients.

P010. Involvement of dopamine in generation of oxidative stress in a 6-hydroxy-dopamine-induced partial dopaminergic striatal lesion model in the rat.

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In normal rat striatum in vivo, dopamine is thought to be preferentially metabolized by MAO-A, as shown by the profound reduction in DA metabolites via MAO when animals are pretreated with clorgyline (selective inhibitor of MAO-A) as opposed to rasagiline or selegiline (selective inhibitors of MAO-B). This situation may alter following dopaminergic nerve lesioning and resultant gliosis, since glial cells express mainly MAO-B and not MAO-A. In rats given a 50% dopaminergic lesion by injection of 6-hydroxydopamine intracerebro-ventricularly, extracellular oxidative stress was elevated as shown by microdialysis with a non-dialysable free radical trap, but intracellular oxidative stress was reduced as shown by measurement of GSSG/GSH ratio and oxidized cholesterol levels in tissue homogenate. In this situation, striatal content of glial cells and MAO-B was increased, but clorgyline still caused a much greater reduction in oxidized DA metabolites than rasagiline, but rasagiline was more potent in suppression of formation of oxidizing free radicals in the extracellular space. The reason for the greater antioxidant property of rasagiline than clorgyline appears to be that: a) clorgyline increases extracellular pro-oxidant DA levels, and b) rasagiline promotes increased expression of the antioxidant enzymes SOD-1 and catalase

P011. Atypical dopamine transporter deficiency syndrome in an adult male: Molecular characterization of new transporter variants

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Dopamine transporter (DAT) deficiency syndrome was recently described as an infantile neurological disease, causing movement disorders within months after birth. Previously disease-coursing mutants are characterized by misfolding and ER retention. We present an adult patient (45y) with early-onset parkinsonism and psychiatric problems, likely caused by missense mutations in the DAT encoding gene (SLC6A3). The patient had insidious onset of right-sided hand tremor at the age of 28, progressing to severe asymmetric PD with levodopa-response. SPECT scan showed bilateral reduction of DAT ligand binding. Sequencing of the SLC6A3 gene revealed two missense mutations on separate alleles, I312F (from the father) and a de novo mutation, D421N. Molecular characterization of the transporter variants, show markedly compromised function of both mutants. This is not a result of transporter misfolding and ER retention, as both western blotting, biotinylation experiments and confocal microscopy demonstrate, efficient targeting of the the mutant transporters to the plasma membrane. Furthermore, amperometry experiments revealed a dramatic reversal effect of amphetamine on the D421N DAT mutant, seen as a blockage of dopamine efflux by AMPH. Electrophysiological characterization likewise showed markedly altered characteristics of the D421N DAT mutant, evident as a pronounced sodium leak through the transporter. This patient provides important evidence on the contribution of DAT to parkinsonism as well as psychiatric disorders.

P012. The stoichiometry of protomers in the complexes formed between tyrosine hydroxylase maximally phosphorylated at Ser19 and 14-3-3

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Tyrosine hydroxylase phosphorylated on serine 19 (THpS19) interacts with the 14-3-3 proteins, but the molecular details about the complex are poorly understood. Recent evidence suggests a role for 14-3-3γ in regulating TH membrane association. Using gel filtration and native mass spectrometry (nativeMS) we confirmed the oligomeric state of purified 14-3-3γ and human TH, phosphorylated to full stoichiometry at S19 (THpS19,) to be >95% dimeric and tetrameric, respectively. Mixing the complex with excess 14-3-3γ rendered one dominating m/z series on nativeMS corresponding to a molecule (340.8 kDa) containing two dimers of 14-3-3 (2x58.4 kDa) and one tetramer of THpS19 (224 kDa). At lower 14-3-3γ to TH ratios, a complex containing only one 14-3-3 dimer (282.4 kDa) was also observed. By electron microscopy, the largest particles observed had estimated dimensions of 250 Å length and 200 Å wide, a size compatible with a complex consisting of one TH tetramer and two dimers of 14-3-3γ. The results from Blue Native Electrophoresis showed that the complex formed between one THpS19 tetramer and two 14-3-3γ dimers, can under our conditions, easily reorganize into a complex formed by dimeric THpS19 and two 14-3-3γ dimers. The different types of complexes identified, and in particular the TH dimer one, could have different functional roles, opening new ways to understand TH function in health and in diseases, such as Parkinson's.

P013. Behavioral and biochemical effects of combined treatment with L-DOPA and tricyclic antidepresants in 6-OHDA-lesioned rats

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The aim of the present study was to examine rotational behavior and monoamine metabolism in motor (striatum, substantia nigra) and limbic (prefrontal cortex, hippocampus) brain structures of 6-OHDA-lesioned rats, treated chronically with the antidepressant drugs amitriptyline or desipramine jointly with L-dopa. The experiment was performed on male Wistar rats injected unilaterally with 6-OHDA (16ug/4ul) into the medial forebrain bundle. Two weeks later, the animals were tested for the rotational behavior induced by apomorphine. Rats exhibiting more than 100 contralateral turns/1h received amitriptyline (10mg/kg) or desipramine (10mg/kg) and L-dopa (12mg/kg), alone or in combination, once daily for 21 consecutive days. Rotational behavior was recorded after the first and the penultimate doses of the examined drugs. The rats were sacrificed 1h after the last injection, and the examined brain structures were dissected. Noradrenaline, dopamine (DA), serotonin (5-HT) and their metabolites were determined using HPLC. Combined administration of amitriptyline and L-dopa, but not desipramine and L-dopa, resulted in an increase in the number of contralateral rotations compared to the L-dopa-treated group. Moreover, joint treatment with amitriptyline, but not desipramine, and L-dopa increased DA levels on the ipsilateral side of all the examined structures more visibly than did L-dopa alone. Either antidepressant differently affected the L-dopa-enhanced 5-HT turnover. The obtained data are discussed in the context of PD therapy. Study supported by the Project "DeMeTer" - POIG.01.01.02-12-004/09-00.

P014. Mice with genetically evoked selective loss of noradrenergic system as a possible tool to study presymptomatic phase of Parkinson's Disease

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Parkinson's Disease (PD) is associated not only with dopaminergic transmission but involves the noradrenergic system as well. Post-mortem examination of human brains revealed that neuronal loss associated with PD may proceed and is even greater in the region of locus ceruleus than substantia nigra (SN). The aim of this study was to determine whether selective loss of noradrenergic neurons may negatively influence the dopaminergic system. We applied the conditional inactivation of the gene encoding transcription factor TIFIA (rRNA synthesis regulator) by the Cre/loxP system to induce noradrenergic neurodegeneration by expressing Cre recombinase under dopamine beta-hydroxylase promoter. The selective loss of noradrenergic neurons in resulted TIFIADBHCre mice was confirmed by immunofluorescent staining with the anti-TH antibody. The number of TH+ cells was not changed in SN of 12-week mutant mice. Nevertheless, our data indicate that lack of the noradrenergic transmission in these mice may lead to enhanced expression of selected markers associated with neurodegeneration in dopaminergic system. We found 1.4 fold up-regulation of mRNA encoding for glial fibrillary acidic protein (GFAP) and higher level of oxidative stress shown by immunoblot detection of carbonyl groups in SN of mutant mice. Moreover, we have noticed 25% reduction of homovanillic acid (HVA) concentration in striatal tissue of mutant mice compared with the control group. If we provide additional evidences that selective noradrenergic degeneration impairs dopaminergic system functioning, TIFIADBHCre mice may became a valuable tool for study presymtomatic phase of PD. Acknowledgements: supported by grant NZ7/05949

P015. Behavioral evidence of a hyperdopaminergic phenotype in LRRK2 G2019S mice

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Mutations in leucine-rich repeat kinase 2 (LRRK2) are linked to late-onset autosomal dominant Parkinson's Disease (PD) [1]. Here, we describe the motor phenotype of transgenic mice expressing the G2019S mutation (knock-in mice, KI), which is most frequently found in PD patients. A battery of behavioral tests (the bar, drag and rotarod tests) were used to evaluate motor function in KI mice and wild-type littermates at different ages (3, 6, 9, 12 and 15 months).Wild-type mice developed motor impairment during aging, showing a 3-fold increase of immobility time in the bar test and a 70% reduction of stepping activity in the drag test at 15 months. Contrary to wild-type mice, no worsening of motor performance in the bar and drag test was observed in KI mice, which showed similar immobility time and stepping activity values at 3 and 15 months. Rotarod performance was similar between young and aged mice, both in KI and wild-type controls. To investigate whether such hyperkinetic phenotype could result from alterations of dopamine (DA) transmission, we analysed the responses to the D2/D3 receptor agonist pramipexole and the D2/D3 antagonist haloperidol. Pramipexole improved motor function in wild-type mice but worsened it in KI mice. Haloperidol was ineffective in wild-type mice but worsened motor function in KI mice. We conclude that the hyperkinesia observed in G2019S KI mice might be sustained by elevation of DA transmission, resulting in an increase of DA tone at postsynaptic D2 receptors and of (compensatory?)D2 autoreceptor sensitivity. Supported by Telethon grant n° GGP12237C. [1] Zimprich A et al., 2004,

P016. Aberrant GABAergic tonic inhibition is present in the GP of parkinsonian rodents

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The external globus pallidus (GP) is a GABAergic nucleus where GABA exerts dual functions by mediating phasic and tonic forms of inhibition. GABA released from synaptic vesicles activates GABA-A receptors located in the synaptic cleft responsible for phasic inhibition. Low concentrations of GABA can also spill from the synapse and result in a persistent tonic activation of extrasynaptic GABA-A receptors. So far, GABAergic tonic inhibition has been described under physiological conditions when extracellular GABA concentrations are artificially increasing by GABA transporter blockade in the GP. However, we still lack a description of what happens under parkinsonian conditions when dopamine is absent and GABA levels are abnormally increased in the GP. Using whole cell patch clamp recordings, immunohistochemical and behavioral approaches, we assessed the changes in the GABAergic tonic inhibition in the GP of 6-hydroxydopamine (6-OHDA) lesioned rodents and study the impact of its modulation on the excitability of GP cells and on motor performance. Under parkinsonian conditions, aberrant tonic inhibition was present in the GP. This tonic inhibition was independent of presynaptic neurotransmitter release, mediated by GABA-A receptor delta subunit and regulated, at least in part, by the activity of neuronal GABA transporters (GAT-1). The blockade of GAT-1 in 6-OHDA rodents suggested a dysfunction of this transporter in pathological conditions. In sum, tonic inhibition points to an important role in regulating GP neuron excitability and therefore, its pharmacological modulation may be an attractive therapeutic target for ameliorating motor disturbances associated with PD.

P017. Enhanced vesicular storage to reduce dopamine toxicity

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We have previously shown that the genetic reduction of the vesicular monoamine transporter (VMAT2) leads to progressive loss of dopamine neurons in the substantia nigra pars compacta as well as corresponding behavioral deficits. There is evidence of oxidative damage in dopamine systems, reduced locomotor activity, and shortened stride length. Given the negative outcomes of reduced vesicular storage of dopamine, we hypothesized that increasing VMAT2 may be beneficial. We have currently generated mice that overexpress VMAT 2 using bacterial artificial chromosomes (BAS). BAC-VMAT2 mice show increased VMAT2 protein expression on vesicles, which is accompanied by an increase in vesicular uptake of dopamine. Striatal dopamine turnover is also reduced. The mice exhibit increased locomotor activity, increased struggling in the forced swim test, and reduced marble burying which is indicative of reduced anxiety or perseveration. Overall, these data support the idea that enhanced storage of dopamine in vesicles may be the therapeutic benefit. Further, a recent report has suggested that the synaptic vesicle protein 2C (SV2C) may mediate vesicular dopamine function and represent a novel therapeutic target (the closely related 2V2A is a known drug target). To determine if SV2C expression alters vesicular dopamine release and uptake we have generated mice with a null mutation in SV2C. We will also present the initial characterization of these mice.

P018. The pathophysiology of Parkinson's disease: LRRK2, neurotransmission and synaptic maintenance

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Mutations in Leucine-Rich Repeat Kinase 2 (LRRK2) are the most common cause of familial and idiopathic PD. Despite many advances, understanding of LRRK2 biology is disappointingly inadequate. An improved understanding of LRRK2 is essential to development of therapeutic interventions to prevent onset and/or delay progression of PD. We, and others, have developed several transgenic mice to investigate LRRK2. Although models lack nigral degeneration, several exhibit key features including altered DA transmission, cognitive/motor dysfunction and tau pathology. To investigate LRRK2 we have conducted behavioural (Volta et al., this meeting) and electrophysiological characterization in LRRK2 overexpressing (OExp), knock-out (KO) and mutant G2019S knock-in (KI) mice and acute brain slices. We observe synaptic dysfunction in striatal medium spiny neurons (MSNs), prior to detection of hypodopaminergia in LRRK2 OExp mice. OExp mice exhibit decreased spontaneous EPSC frequencies, KO mice display the opposite phenomenon, suggesting a role for LRRK2 in synaptic function. Furthermore, striatal short-term plasticity is altered in OExp mice, and normalized by D2 antagonism. KI mice demonstrate similar alterations, suggesting the G2019S PD mutation confers a gain function upon LRRK2's role in the regulating synaptic transmission. We also performed acute manipulations of LRRK2 in primary neuronal cultures. Incubation of cortical cells with a LRRK2 kinase inhibitor or ASO knock-down (>80%) of LRRK2 produced marked alterations in miniature EPSC frequencies and synaptic markers by ICC. Our data demonstrate that LRRK2 is a central player in synaptic transmission. Futhermore, LRRK2 hyperfunction seems to be produced by PD

P019. Direct generation of dopamine cells from fibroblasts and assessment of their therapeutic potential in animal models of Parkinson disease

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FONDAZIONE ISTITUTO ITALIANO DI TECNOLOGIA / neuroscience and brain technologies

Latest publications have demonstrated the great success in the direct reprogramming of one somatic cell type to another through retroviral transduction of some transcription factors. It was shown that ectopic expression of Mash1, Nurr1 and Lmx1a factors cloned into lentiviral vector can induce direct conversion of fibroblast to DA neurons (induced DA neurons - iDA). The development of such polycistronic lentiviral vector opens the possibility of direct in vivo reprogramming of brain cells into iDA neurons in situ in the brain. This virus-based strategy can potentially become a powerful alternative to a cell replacement therapies for Parkinson disease since it negates many of the concerns of immunological rejection and cancerogenesis. In our study, the replication-deficient polycistronic lentivirus carrying Mash1, Nurr1 and Lmx1a transcription factors was constructed without tetracycline-controlled transcriptional activation system. The first aim of this study is to assess in vivo the therapeutic potential of iDA neuronal cells generated from mouse fibroblasts in vitro using ectopic expression of transcription factor upon their autologous transplantation into striatum of unilateral 6-OHDA lesion rodents. Furthermore, we are attempting to perform in situ reprogramming of other type of neurons to iDA via viral injection of transcription factors into striatum and/or substantia nigra of the lesioned animals. Results of these ongoing investigations will be presented.

P020. Elaboration of a rat model of depression present in preclinical stages of Parkinson's disease

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The clinical phase of Parkinson's disease (PD) is preceded by a preclinical period where depression is a frequent comorbid disturbance. Dysfunctions of monoaminergic systems may underlie depression in PD. The aim of the study was to examine whether a moderate lesion induced by bilateral injections of 6-OHDA (3.75-15 microg/2,5microl) into the ventral striatum (vSTR) would result in the 'depressive-like' behaviour of rats in the forced swimming (FS) test. The behaviour of rats was measured 2 and 4 weeks after the surgery. The lesion extent was analysed by HPLC and immunohistochemically. After 2 weeks an increased immobility time in FS test was observed in lesioned rats. Simultaneously no change of locomotor activity was noted. The levels of dopamine decreased moderately and dose-dependently in the dorsal striatum, substantia nigra and frontal cortex. In the vSTR losses of the dopamine level (40-60%) were similar after each dose of 6-OHDA. Dopaminergic neurons counted stereologically were slightly lowered in the substantia nigra and ventral tegmental area. Both the behaviour of rats in FS test and levels of dopamine in the vSTR were normalised between 2 and 4 weeks after the surgery. These results indicate that a moderate dopaminergic lesion which does not produce any motor disturbances, may induce "depressive-like" symptoms in rats and therefore may be regarded as a model of depression in preclinical stages of PD. Acknowledgments Study supported by the Project DEMETER - POIG.01.01.02-12-004/09-00

P021. A new therapeutic strategy for Parkinson's disease

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Preclinical and clinical studies showed that adenosine A2A receptor antagonists, like preladenant, significantly increase L-DOPA efficacy in Parkinson's disease (PD), without exacerbating dyskinetic-like behaviors. Recently, it has been reported that the mixed 5-HT1A/B receptor agonist, eltoprazine, produces a near to full suppression of dyskinetic-like behaviors; however, eltoprazine resulted in a partial reduction of therapeutic effect of L-DOPA. On this basis, we hypothesize that combination of eltoprazine with preladenant may produce suppression of L-DOPA-induced dyskinesia, without impairing the efficacy of L-DOPA in relieving motor symptoms. Thus, unilateral 6-hydroxydopamine-lesioned rats, rendered dyskinetic by repeated treatment with L-DOPA (6mg/kg), were administered with eltoprazine (0.3 or 0.6 mg/kg) and preladenant (0.3 or 1 mg/kg), singularly or in combination with L-DOPA (4 or 6 mg/kg) plus benserazide (6mg/kg), and turning behavior, as index of locomotor activity, and abnormal involuntary movements (AIMs) as index of dyskinesia, were evaluated. Results suggest that combined administration of L-DOPA (4mg/kg) plus eltoprazine (0.6mg/kg) plus preladenant (0.3mg/kg) significantly reduced dyskinetic-like behaviors, as revealed by AIMs- test without impairing the motor activity, as revealed by similar number of contralateral and ipsilateral turns, evaluated with turning test. Overall these data suggest the use of the combination of L-DOPA (4mg/kg) with eltoprazine (0.6mg/kg) and preladenant (0.3mg/kg) as new therapeutic strategy for treating motor symptoms and dyskinesia in PD. Ongoing experiments are meant to evaluate whether the positive effect of this drug combination can be maintained over a chronic administration.

P022. Role of the transcription factor Pitx3 in Modulating Vulnerability of Midbrain Dopaminergic Neurons to Neurodegenerative Stress

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The homeodomain transcription factor Pitx3 is critical for the survival of midbrain dopaminergic (mDA) neurons. Pitx3-deficient mice exhibit severe but selective developmental loss of mDA neurons, with accompanying locomotor deficits resembling those seen in Parkinson's disease (PD). We identify specific mDA cell subpopulations that are consistently spared in adult Pitx3-hypomorphic (aphakia) mice, demonstrating that Pitx3 is not indiscriminately required by all mDA neurons for their survival. In aphakia mice, virtually all surviving mDA neurons in the substantia nigra (SN) and the majority of neurons in the adjacent ventral tegmental area (VTA) also express calbindin-D-28k, a calcium binding protein previously associated with resistance to injury in PD and in animal models. Cell mapping studies in wildtype mice revealed that Pitx3 is primarily expressed in the ventral SN, a region particularly susceptible to 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) and other dopaminergic neurotoxins. Furthermore, Pitx3-expressing SN cells are preferentially lost following MPTP treatment. Finally, SN mDA neurons in Pitx3 hemizygous mice show increased sensitivity when exposed to MPTP. Thus, SN mDA neurons are represented by at least two distinct subpopulations including MPTP-resistant Pitx3-autonomous, calbindin-positive neurons, and calbindin-negative Pitx-3-dependent cells that display elevated vulnerability to toxic injury, and likely correspond to the subpopulation that degenerates in PD. Impairment of Pitx3 dependent pathways therefore increases vulnerability of mDA neurons to toxic injury. Together, this data suggests a novel link between Pitx3 function and dopaminergic vulnerability in PD.

P023. Role of dopamine D3R/nAChR heterodimeric complex in the regulation of dopaminergic neurons function

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Parkinson's disease (PD) is a neurological disorder characterized by the progressive degeneration of dopaminergic neurons. Dopaminergic neuron function is regulated by different receptors, including the dopamine D2-like receptors (D2R-like), such as the dopamine D3 receptor (D3R) and the acetylcholine nicotinic receptors (nAChR) containing the alpha4 and/or alpha6 subunits and the beta2 subunit. Increasing evidences suggest that D2R-like agonists may slow the progression of PD and that nicotine has neuroprotective effects on dopaminergic neurons. Moreover our recent data report that nicotine regulates dopaminergic neurons neuroplasticity by a mechanism that involve the D3R, suggesting a cross-talk between D3R and nAChR.

Therefore, the aim of this study was to investigate the mechanisms underlying the interaction between D3R and nAChR-mediated signals. By using co-immunoprecipitation, we found that in both the striatum and the substantia nigra of mice, D3R specifically interact with the alpha 4 nAChR subunit, suggesting that the D3R/nAChR interaction could be explained by the formation of an heteromeric complex. This result was strongly confirmed by using bioluminescence resonance energy transfer (BRET) carried out in HEK293T cells expressing D3R-GFP2 and alpha4-Rluc nAChR. Moreover, we found that in primary mesencephalic neurons, activation of the D3R/nAChR complex specifically inhibited alpha-synuclein aggregation induced by neurotoxic insults. Taken together these data suggest a crucial role of the D3R/nAChR complex in preventing pathological alterations that may lead to neurodegeneration of dopaminergic neurons. Moreover, D3R/nAChR complex may represent a novel target for compounds with neuroprotective effects for the PD therapy.Supported by Italian Institute of Technology, Genova, Italy.

P024. Down-regulation of the D1R/Shp-2/Erk1/2 pathway ameliorates L-DOPA-induced dyskinesia in the 6-Hydroxy-Dopamine rat model of Parkinson's disease.

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Striatal dopamine (DA) replacement with L-3,4-dihydroxyphenylalanine (L-DOPA) remains the most effective therapy, used in Parkinson' disease (PD). However, chronic L-DOPA administration leads to L-dopa-induced dyskinesia (LID). Numerous evidence showed that LID are associated to functional supersensitivity of the striatal dopamine D1 receptor (D1R), leading to abnormal Erk1/2 hyperphosphorylation. We have recently found that in hemiparkinsonian rats developing LID, striatal Erk activation mediated by the D1R/cAMP/PKA pathway specifically requires the tyrosine phosphatase Shp-2. In this study we evaluated whether striatal Shp-2 may be a new target for treatment of LID. To this aim, lentiviral particles (LV) carrying a specific shRNA was used to knockdown striatal Shp-2 in a rat model of PD. Unilateral 6-OHDA-lesion rats were stereotaxically injected into the striatum ipsilateal to the lesion with both non targeting LV and Shp-2 shRNA LV. Rats were then treated with L-DOPA (8 mg/kg) for 21 days and tested for development of LID. We found that in PD rats injected with non targeting LV, administration of L-DOPA induced the development of severe abnormal involuntary movements (AIMs); by contrast, striatal Shp-2 silencing deeply attenuated the severity of LID. Moreover, by using WB, we found that the severity of LID strongly correlated with both the striatal ERK activation and the levels of SHP-2. These results suggest that Shp-2, involved in the mechanism of D1R-mediated Erk activation in LID, could represent a potential

P025. Susceptibility of dopaminergic and GABAergic neurons in the rat substantia nigra to toxicity of the selective proteasome inhibitor lactacystin: in vivo study.

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Lactacystin is a selective proteasome inhibitor recently used to destroy dopaminergic neurons in animal models of Parkinson's disease. However, both in vitro and in vivo studies show discrepancies in terms of the sensitivity of non-dopaminergic neurons to lactacystin toxicity. Therefore our study was aimed at examining the toxic effect of lactacystin administration on dopaminergic and GABAergic neurons in the substantia nigra (SN) compared to the classic neurotoxin 6-OHDA. Male Wistar rats were unilaterally injected with lactacystin or 6-OHDA into the left SN pars compacta. The animals were sacrificed six weeks after the lesion. Dopamine levels were assayed in the striatum and SN, while GABA was measured in the SN using HPLC. Some brains were double-stained for tyrosine hydroxylase (TH) and cresyl violet in the SN. An unbiased stereological technique was applied for cell counting throughout the entire SN. Both toxins reduced by at least 95% dopamine levels in both the ipsilateral striatum and SN, but only lactacystin slightly decreased GABA levels in the SN. A stereological analysis showed that lactacystin reduced by 89% the density of nigral TH-ir neurons, while the density of cresyl violet-stained neurons (mostly GABAergic in the SN) was diminished by 24%. We also observed a positive correlation between the level of damage of GABAergic versus dopaminergic neurons in the SN. Our study demonstrates that lactacystin is not a fully selective toxin in relation to dopaminergic neurons, however, dopaminergic neurons are far more sensitive to lactacystin toxicity than GABAergic ones.

P026. Effect of the serotonin precursor 5-hydroxytryptophan on L-DOPA-induced dyskinesia in parkinsonian rats

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The serotonin system emerged as an important player in the appearance of dyskinesia in experimental models of Parkinson's disease (PD), as it provides an unregulated source of L-DOPA-derived dopamine release in the parkinsonian brain. Accordingly, toxin lesion or pharmacological silencing of the serotonin neuron suppressed L-DOPA-induced dyskinesia (LID) in the rat and monkey models of PD. However, 5-HT1 receptor agonists treatment was also found to partially compromise the therapeutic effect of L-DOPA. Here, we evaluated whether an increase of the serotonergic tone induced by the serotonin precursor 5-hydroxytryptophan (5-HTP) could affect induction and expression of LID, as well as the therapeutic effects of L-DOPA, in parkinsonian rats. Drug naïve and L-DOPA-primed 6-OHDA-lesioned rats were treated daily with L-DOPA (6 mg/kg) alone or in combination with 5-HTP (24-48 mg/kg) for two weeks. The abnormal involuntary movements test, as well as the stepping and the motor activity tests, were performed during the treatment. Results showed that 5-HTP, at both tested doses, significantly reduced LID. However, 5-HTP 24 mg/kg was more effective than 5-HTP 48 mg/kg in preserving the therapeutic effect of L-DOPA. 5-HTP 24 mg/kg also reduced expression of dyskinesia in L-DOPA-primed dyskinetic rats. These data suggest that 5-HTP is significantly effective in counteracting dyskinesia without compromising L-DOPA-induced therapeutic effects. Thus, these results may have interesting clinical application.

P027. Dopamine-dependence of murine LRRK2 associated behaviors

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Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most common cause of familial Parkinson's disease. The physiological role of LRRK2 is poorly characterized, despite several reports described involvement in varied neuronal processes. Behavioral motor and non-motor consequences of LRRK2 deletion, overexpression and mutations are also currently undercharacterized. We have compared young adult (3-6 months) non-transgenic (NT), knockout (KO) and BAC hWT-LRRK2 overexpressing mice in motor, cognitive and operant tests. Open field and cylinder testing have been performed to measure spontaneous horizontal and vertical locomotion. We also assayed physiologically-stimulated motor activity with "drag" test. Since PD clinical presentation is also characterized by non-motor symptoms, we expanded our behavioral characterization to non-motor paradigms. The tail suspension test was employed to study propensity to depressive-like states, while thigmotactic behavior in the open field gave indications on anxiety. Cognitive functions in these mice were investigated using novel object placement (spatial memory) and recognition (learning) and Y-maze (spatial memory, perseveration) tests. Short- and long-term working memory and functioning were investigated using the puzzle box (Abdallah et al., 2011). While LRRK2 KO mice behave normally, hWT-LRRK2 mice showed deficits in different parameters of spontaneous locomotor ability. Subsequently, the phenotypes observed have been characterized through pharmacological modulation. The activity of the nigrostriatal dopamine system has been studied through fast-scan cyclic voltammetry in acute brain slices and in vivo microdialysis. Finally, we measured dopamine neurone (SNc) and terminal (striatum) using immunohistochemistry.

P028. Impaired dopaminergic neurotransmission and vesicular recycling in human LRRK2 (R1441G) transgenic mice

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Emerging evidence suggest that synaptic dysfunction is an early event in the pathogenesis of Parkinson disease (PD) occurring prior to the onset of symptoms. In order to develop more effective therapeutic strategies, we need a better understanding of the underlying mechanisms of synaptic dysfunction of PD. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most prevalent causes of familial and sporadic PD, demonstrating an unprecedented significant role in PD pathogenesis. Recently a transgenic mouse model with over-expression of human LRRK2-R1441G has been shown to recapitulate robust motor behavioral, neurochemical and pathological features of PD (Li et al., 2009). In this study, we used a battery of tests (single pulse (1p), paired pulse, and train pulse stimulation) to characterize evoked dopamine (DA) release by fast cyclic voltammetry (CV) and amperometry in acute corticostriatal slices of 3, 5 and 10 month old transgenic R1441G-LRRK2 mice and their wild type littermates. We have found age-dependent deficits in DA release in the striatum in this model. Specifically, we found that vesicle trafficking and recycling in hLRRK2-R1441G TG mice is slower compared to that of WT and this deficit starts at 5 month old prior the onset of behavior deficits. While the data support the conclusion that LRRK2 plays a key role in regulating DA release and recycling, further investigations of the underlying mechanisms and how this dysfunction leads to axonal degeneration are ongoing (Supported by MJFF).

Dopamine co-transmission

P029. Contribution of TAAR1 receptors in effects of apomorphine

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G protein-coupled Trace Amine Associated Receptor 1 (TAAR1) is expressed in several regions of the brain and modulates dopaminergic activity partially via enhancing D2 dopamine receptor function. *In vitro*, nonselective dopamine agonist apomorphine can activate TAAR1. The present study was aimed to evaluate if activity of apomorphine at TAAR1 observed in *in vitro* studies contributes to behavioral effects of apomorphine. For this purpose, we compared behavioral effects of a wide range of apomorphine doses in wild type (WT) and TAAR1 knockout mice. Locomotor effects of apomorphine in doses 0.01-4.0 mg/kg were tested in locomotor activity boxes. For assessing effects of low doses (0.01 – 0.3 mg/kg), tests with drug started after 6 habituation sessions. Acute effects of apomorphine at higher doses (1.0 - 4.0 mg/kg) were analyzed in independent groups of mice. Stereotypic responses to 5 mg/kg of apomorphine were tested by ethological methods. Gnawing test was used to analyze effects of highest dose of apomorphine (10 mg/kg). No statistically significant differences were observed between KO and WT mice following inhibitory pre-synaptic low doses of apomorphine. At higher doses (2.0-5.0 mg/kg), apomorphine-induced climbing behavior was significantly reduced in TAAR1 mutants relative to WT controls. Also, lack of TAAR1 receptors decreased other types of stereotypies (total stereotypy score, licking) induced by 5.0 mg/kg of apomorphine. These data suggest that activity of apomorphine on TAAR1 may contribute to some particular behavioral manifestations following high doses of this drug.

Anatomy and physiology ofdopamine system

P030. Characterizing dopamine release and clearance in the striatum of c57/Bl6 mice using in vivo chronoamperometry.

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The c57/Bl6 mouse strain is one of the most commonly used strains in laboratories worldwide and it is also used for creation of various transgenic lines. Knowledge of dopamine (DA) release levels and clearance time could contribute to our understanding of how to interpret various behaviors and phenotypes observed. Both age and sex are parameters of importance when planning experiments and interpreting data, yet these parameters are often overlooked. The aim with this study is to characterize the DA release levels and the clearance rate in the striatum, the major DA-containing area in the brain, in c57/Bl6 mice, and to compare the levels observed in adult c57/Bl6 male and female mice with those in young c57/Bl6 male and female mice, respectively. For this, we use *in vivo*chronoamperometry and DA-selective microelectrodes that allows us to record DA release and clearance on a subsecond scale. We have applied a protocol of six consecutive pressure injections of KCl, spaced by two minutes apart and one additional injection after 15 minutes,into the dorsal striatum of urethane-anesthetized animals, This protocol makes it possible to study the basal capacity of the DA system as well asthe response to provocation and the ability for recovery. Results show that both adult and young females release more DA than adult and young males, respectively. When analyzing the clearance rate, there is no obvious difference between adult and young animals, however young females seem to have a slower clearance rate compared to young males. The findings suggest that both age and sex of the mice are important parameters of the functional DA system in mice.

P031. Role of Trace amine-associated receptor 1 (TAAR1) in the modulation of dopaminergic system and cortico-striatal signaling.

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Mammalian Trace Amine Associated Receptor 1 (TAAR1) is a GPCR that is mainly expressed in limbic regions and monoaminergic nuclei. There is evidence indicating that TAAR1 is involved in the modulation of dopaminergic system. In mice lacking TAAR1, amphetamine induces more pronounced locomotor stimulation and dopamine release. Moreover, it has been reported that D2 receptor function is altered in TAAR1-KO mice. In this study, our purpose was to describe how TAAR1 could influence dopamine system in vivo, in particular D2-related signaling in the striatum and its role in prefrontal cortex. We found that D2 but not D1 number was decreased in striatum of TAAR1-KO mice. Moreover, we analyzed D2 receptor-related signaling and we found a significant reduction in AKT and GSK-3β phosphorylation well as a reduction of beta-catenin. We also studied β-arrestin2 recruitment to D2R in vitro and its role in vivo in striatum of TAAR1-KO mice. While in cells it seems that TAAR1 does not modulate β-arrestin2 recruitment, in vivo we found an alteration of the AKT/PP2A complex. Since TAAR1 modulates dopaminergic neurons located in VTA we made investigations in the prefrontal cortex, particularly NMDA receptor functionality. By using patch clamp recordings we found that NMDA receptor located in layer V were less active. Finally, using a timing task, it was highlighted a deficit in the general performance, suggesting a deficit in cognition. These data indicates that TAAR1 is able to modulate dopamine system, in particular D2 receptor functions in striatum, and NMDA function in prefrontal cortex.

P032. Analysis of Glutamate, GABA, Noradrenaline, Dopamine, Serotonin and Metabolites using microbore UHPLC with electrochemical detection

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The applicability of microbore ultra-high performance liquid chromatography (UHPLC) with electrochemical detection for analysis of a number of well-known neurotransmitters in 2 - 10 µL microdialysis fractions is described. Two methods are presented: the analysis of monoamines and the analysis of amino acid neurotransmitters, which can both be run on the ALEXYS UHPLC system. Speed of analysis of noradrenaline (NA), dopamine (DA), serotonin (5-HT), and the metabolites homovanillic acid (HVA), 5-hydroxyindole acetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) is predominated by the retention behavior of NA, the non-ideal behavior of matrix components and the loss in signal of 5-HT. The method was optimized to meet the requirements for detection sensitivity and minimizing the size of collected fractions, which determines the temporal resolution in microdialysis.

The amino acid neurotransmitter glutamate (Glu) and gamma-aminobutyric acid (GABA) are analyzed after an automated derivatization procedure. Under optimized conditions Glu was well resolved from a number of early eluting system peaks, while the total runtime was decreased by applying a fourfold increase of the flow rate. Detection limit for both GABA and Glu is 10 nmole/L, and the monoamine neurotransmitters have a detection limit in the range of 20 - 90 pmol/L. Using UHPLC the analysis times vary from 15 min to less than 2 min depending on the complexity of the sample and the substances that need to be analyzed.

P033. Spatial distribution of D1R- and D2R-expressing Medium-sized Spiny Neurons differs along the rostro-caudal axis of the Dorsal Striatum.

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The current model of the anatomical organization of the striatum is built on the assumption that striatonigral and striatopallidal MSNs projection neurons, expressing preferentially dopamine receptor subtypes D1 (D1R) and D2 (D2R) respectively, are physically intermingled lacking therefore cytoarchitectural organization. Combining genetic, mathematical, tract-tracing and pharmacological approaches we demonstrate that, unlike in the dorsal part of the striatum, in the caudal striatal area, D1- and D2-expressing MSNs are not intermixed but instead physically segregated. This pattern was confirmed in Drd2-Cre and A2a-Cre mice crossed with several reporter lines. This striatal area, which receives specific cortical and thalamic inputs, lacks striatopallidal D2R/A2aR MSNs but is instead exclusively composed of D1R-containing MSNs. Moreover, we also provide evidence that this anatomical segregation is functionally relevant since dopaminergic signaling is differentially regulated. By identifying a clear anatomical and functional segregation between striatonigral and striatopallidal MSNs in the caudal striatum, this study establishes a new basis to reevaluate the anatomo-functional striatal circuitry in relationship to the D1R- and D2R-expressing MSNs.

P034. Reward responses of presumed dopamine neurons in the dorsal raphe nucleus.

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The dorsal raphé nucleus (DRN) is a neurochemically diverse structure that is implicated in a wide range of functions. It is a major locus of serotonin (5-hydroxytryptamine) neurons, but also contains inhibitory GABA interneurons, and small numbers of glutamatergic and dopamine (DA) neurons. Little is known of the functional properties of the raphe DA population. We performed extracellular recordings of single DRN neurons in rats performing a conditioned approach task in which an auditory cue either predicted or did not predict reward, depending on a global context cue (house light on or off) in a block design. We compared short latency responses to cue onset in the two contexts. Eight cells were tested with the DA D2 receptor agonist apomorphine, which inhibits ventral midbrain DA neurons. Three cells were strongly inhibited. All 3 of these neurons showed strong short latency responses to the cue in the reward context, which were lost (or converted to inhibitions) in the no-reward context. The remaining cells were either excited or not affected by apomorphine and showed distinctly different responses to the cue, being either more strongly or equally excited in the no- reward context. These data suggest that the DRN DA population selectively responds to reward signals in a similar manner to ventral midbrain neurons, consistent with them representing a caudal tail of the more rostral DA cell groups.

P035. Taar1-mediated modulation of dopaminergic neurotransmission.

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Trace Amine Associated Receptor 1 (TAAR1) is a GPCR hat is expressed in several brain areas in mammals. TAAR1 can be activated by endogenous biogenic amines called "trace amines" (TAs). TAs act as "false transmitters" by modulating efflux of other monoamines. The functional role of TAs is unknown, however discovery of their GPCR (TAARs), provided an opportunity to investigate their functions. To elucidate the role of TAAR1 in DA signaling we used a selective TAAR1 agonist (RO5166017) and a TAAR1 antagonist (EPPTB) in fast scan cyclic voltammetry experiments. We tested the ability of TAAR1 agonist and antagonist to modulate DA release in WT and TAAR1-KO brain slices. We have found that 10 uM of RO5166017 decreases DA release in WT mice, but not in TAAR1-KO animals. Application of EPTTB prevents the reduction in the evoked DA release induced by TAAR1 agonist in WT animals. Moreover, EPPTB is able to block the decrease in DA amount induced by TAAR1 agonist. We hypothesize that these effects could be mediated by an interaction between TAAR1 and D2. TAAR is, indeed, mainly expressed in D2 positive neurons and quinpirole effect on DA release is decreased in TAAR1 KO compared to control. Furthermore, short term plasticity induced by paired pulses is increased in TAAR1KO animals; this may indicate less inhibition of D2 autoreceptors. These observations confirm a closer interaction between TAAR1 and DA system and open new prospective for the modulation of dopaminergic system in a variety of neuropsychiatric disorders.

P036. Immunocytochemical detection of PSD-95, TH and Golgi-Cox stained elements: visualization in the same slice.

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The Golgi-Cox method has been usefully applied for qualitative analysis and quantitative evaluations (Lanciego & Wouterlood, 2011; Pinto et al., 2012), but it does not provide informations on the neurochemical nature of stained elements. On the other hand, immunohistochemical procedures provide detailed biochemical informations but poor morphological details . Although highly desired, their concomitant use has been classically considered as incompatible (Lee et al., 2006) in spite of the many attempts to combine them (Freund and Somogyi, 1983). These attempts have been only marginally successful, possibly for the high degree of complexity and low reproducibility of the procedures employed. We developed an innovative procedure that allows Golgi-Cox impregnation and immunofluorescence in the same section with a simplified and inexpensive method. It is based on three simple steps: 1) a paraformaldehyde perfusion followed by post-fixation to stabilize the immunofluorescence reaction; 2) Golgi-Cox impregnation and 3) immunofluorescence in previously impregnated material. This combination allows simultaneous visualization of structural details (Golgi-Cox impregnated neurons), antigens' characterization (PSD-95; TH; synapsin I), morphological-neurochemical interactions between discrete neuronal elements and reduction of animals required. The method allows unprecedented, simultaneous visualization of immunocitochemically-defined markers of dopamine transmission (TH; DAT), Glutamate signaling (PSD-95) and their close relationship with post- synaptic elements such as dendritic spines.

P037. Sex-specific effect of COMT on cortical anatomy.

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The catechol-O-methyltransferase (COMT) gene modulates dopamine availability in the prefrontal cortex (PFC) and it plays a role in cognitive performance in animal models as well as in humans. Interestingly, COMT enzymatic activity is sex dependent, with females having reduced dorsolateral-PFC COMT activity than males. Moreover effects of COMT genetic variants are sex dependent in cognitive abilities, emotional behavior and vulnerability to psychiatric disorders. In humans, polymorphisms in dopamine regulating genes such as dopamine transporter 1 (DAT1), COMT and dopamine receptor 4 (DRD4) impact brain morphology. Particularly, the functional Val-Met polymorphism in the COMT gene affects the volume and the thickness of the prefrontal areas. However, no study to date has addressed COMT*sex-dependent differences in brain morphology. In the present study, we examined whether COMT and sex interact to modulate the cortical thickness in healthy humans as well as in mice. We employed MRI for cortical thickness in healthy men and women. In parallel, thickness quantification was also performed in mice with MRI and with histological techniques. We demonstrated that genetic variations affecting COMT activity predict differences in the thickness of the PFC both in healthy men and in male mice. COMT genetic deletion in male but not female mice clearly leads to the thickening of the basal layers of the PFC, where we also observed an increase in neuronal density. Unraveling specific sex*genes interactions underlying brain dimorphism might be critical to understand sex-dependent differences in cognitive functions and vulnerability to psychiatric disorders.

P038. Anatomical and functional characterization of the cortical input to D1 or D2 receptor-expressing striatal neurons.

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The cerebral cortex gives rise to a substantial excitatory glutamatergic projection to the medium spiny neurons (MSNs) of the striatum, as part of the circuitry participating in motor control. MSNs are distinguished based on the expression of dopamine D1 (D1Rs) or D2 receptors (D2Rs). Activation of one or the other of these two groups of MSNs produces opposite motor responses. We combined the Cre/loxP gene expression system with rabies-virus-based retrograde tracing and optogenetics to study cortical inputs to either D1R- or D2R-expressing MSNs. A virus engineered to express the TVA receptor in a CRE-dependent manner was injected into the striatum of mice in which CRE expression was driven by the promoter for the D1R or D2R (D1- or D2-CRE). A second virus whose infection was dependent on TVA was then injected, resulting in pre-synaptic expression of EGFP or channelrhodospin-2 (ChR2). Barrel cortex analysis revealed that neurons projecting to D1R- expressing MSNs were predominantly localized in upper layer V, while neurons projecting to D2R- expressing MSNs were distributed throughout layer V. Morphological analysis showed smaller soma and thinner apical dendrites in D1-CRE animals when compared to D2-CRE animals. Functional analysis based on stimulation of ChR2 in layer V motor cortex revealed that corticostriatal neurons projecting to D1R- expressing MSNs induced contralateral rotation. No effect was seen after stimulation of neurons projecting to D2R-expressing MSNs. Our study supports the idea of anatomical and functional differences between corticostriatal neurons targeting D1R.

P039. Activity of VTA DA neurons induces a homeostatic plasticity of GIRK channels.

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In the ventral tegmental area (VTA), dopamine (DA) neurons represent the output cells that release DA in in target regions such as the nucleus accumbens and the frefrontal cortex to modulate specific behaviors. Any change in the excitability and eventually activity of DA neuron may therefore have a effect on behavior. For example, VTA GABA interneurons control DA neuron activity through activation of the fast inhibitory GABAA receptors. In addition, inhibitory synaptic transmission has also a slow component that is mediated by GABAB receptors, which are heteromeric metabotropic receptors that active G-protein inwardly rectifying potassium (GIRK) channels. While the role of GIRK channels in the control of the membrane potential is well established, it remains elusive whether they exhibit activity-dependent plasticity. In acute brain slices of the VTA, performing in vitro whole cell patch-clamp recordings in VTA DA neurons, we measured the amplitude of the slow inhibitory post synaptic currents (sIPSC). Phasic firing of VTA DA neurons (20Hz for 5 min) triggered a potentiation of the sIPSC. This potentiation required protein trafficking through specific GIRK subunit PDZ domain binding sites. In contrast, tonic firing (2Hz for 5 min) of VTA DA neurons led to a depression of the sIPSC. In summary our data reveal bidirectional plasticity of the sIPSC through GIRK channel trafficking in response to the activity of the DA neuron, suggesting a role in the homeostatic control of the excitability.

Dopamine and cognition

P040. The positive allosteric modulator of nicotinic alpha 7 cholinergic receptors, AVL-3288, stimulates cathecolamine release in the prefrontal cortex and nucleus accumbens shell of adolescent rats: potential utility in ADHD?

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The selective alpha7-nAChR positive allosteric modulator AVL-3288 (N-(4-chlorophenyl)-α-[[(4-chlorophenyl)amino]methylene]-3-methyl-5-isoxazolacet-amide), has been shown to evoke robust positive modulation of agonist-induced currents at alpha7-nAChRs. In order to evaluate its pharmacological potential in cognition disorders such as the attention deficit with hyperactivity disorder (ADHD), we assessed the effect of systemic AVL-3288 administration on noradrenaline (NA) and dopamine (DA) release in the medial prefrontal cortex (PFC) of adolescent spontaneous hypertensive (SHR) and Sprague Dawley (SD) rats, by using the microdialysis technique in freely moving animals. We also assessed the effect of AVL-3288 on NA and DA release in the nucleus accumbens (NAcc) shell, to gain insight into its potential motivational properties. Our results show that AVL-3288, upon acute systemic administration, stimulates NA and DA release in both areas of either SD or SHR rats, with a major effectiveness in the latter strain. The maximal effect on catecholamines release in the mPFC was obtained with the dose of 1 mg/kg. The maximal effect on NA output in the NAcc shell was obtained at 0.3 mg/kg, the maximal effect on DA output in the same area occurred at 1 mg/kg. Moreover, a previous systemic pretreatment with the selective alpha7-nAChR antagonist methyllycaconitine (3 mg/kg i.p.), completely blocked these effect. These data suggest that AVL-3288 has the potential of modulating catecholamine transmission in the PFC and in the NAcc shell, so it may possess cognitive and motivational properties, as shown by stimulant drugs currently used in ADHD therapy such as amphetamine and methylphenidate.

P041. Methylphenidate enhances cognitive stability at the expense of cognitive flexibility

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Methylphenidate (MPH) is commonly prescribed for the treatment of attention deficit-hyperactivity disorder (ADHD). However, the mechanisms by which MPH acts are unclear. Psychologically, it is unclear whether MPH improves cognitive function directly through augmenting cognitive resources, or indirectly through enhancing reward responsivity, which then boosts cognitive performance. Neurally, it is unclear whether MPH modulates activity in the prefrontal cortex (PFC) or striatum. Here, we present a pharmacological functional magnetic resonance imaging (fMRI) study designed to answer these questions. A delay match- to-sample task was modified so as to include two additional phases during the delay period between encoding and probe. Firstly, participants received an unexpected outcome (gain, neutral or loss) after playing a gamble. Next, novel intervening stimuli, which either had to be ignored or updated in working memory, were presented. MPH was found to improve the ability to ignore these intervening stimuli, but impair the ability to efficiently incorporate (update) novel information into working memory. This task- specific effect on performance was accompanied by task-specific effects on blood oxygenation level- dependent (BOLD) signal in the dorsolateral PFC. No task-specific effects were seen in the striatum. Furthermore, MPH did not modulate the effect of outcome on mnemonic performance or the BOLD response in the ventral striatum. These results support the idea that MPH acts directly on the PFC to improve the ability to retain information in the face of external distraction (enhance cognitive stability). However, this beneficial effect on cognitive stability comes at the cost of impaired flexible updating of working memory.

P042. The Role of Midbrain Dopamine in Predictive Fear Learning

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The firing of midbrain dopamine neurons during appetitive learning tasks conforms to the assumptions of associative learning theories. Some dopamine neurons also respond to aversive USs and CSs predictive of such USs. However, the role of dopamine in predictive fear learning, and its relationship to amygdala mechanisms for fear learning, remains unclear. Here we studied the role of dopamine in predictive fear learning using blocking designs and assessing fear via conditioned freezing and conditioned suppression. Blocking involved training rats to fear conditioned stimulus (CS) A in Stage I via pairings with shock. In Stage II, rats received pairings of CSA+CSB and shock. Blocking was shown by less fear to CSB than a control group that received Stage II, but not Stage I, training. Whereas microinjections of the D2 antagonist sulpiride into the VTA prior to Stage II conditioning prevented blocking using freezing as a measure of fear, they failed to do so when fear was assessed via conditioned suppression. Intra-VTA microinjections of the kappa opioid receptor antagonist nor-BNI also failed to prevent blocking as assessed via conditioned suppression. Dopamine manipulations at terminal regions in the amygdala (basolateral and central subregions) and the nucleus accumbens (core and shell) also did not affect blocking. These results contrast with previous studies showing dopamine receptor antagonism in the amygdala and nucleus accumbens prevents blocking when assessed via freezing. Further experiments studying the effects on blocking of glutamate and GABA receptor modulation in these regions will be reported.

P043. Impact of cortical alpha-synuclein overexpression on the performance in operant tasks of cortico-striatal function

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Although Parkinson's disease (PD) has traditionally been considered a pure motor disorder, impairmentsin cognition are increasingly recognized among the non-motor symptoms with significant clinical impact. More specifically, cognitive impairments typically fall within the 'executive' domain, affectingfunctions such as cognitive flexibility and rule learning.Cortical and subcortical pathological accumulation of intracellular alpha-synuclein aggregates are considered to be a key factor to this cognitive decline.

Rats were pre-trained on an operant version on the classical delayed alternation task before receiving an injection of adeno-associated viral vectors (AAV) encoding for alpha-synuclein into the medial prefrontal cortex. Another subset of animals received excitotoxic lesions of the same prefrontal region and all rats were re-tested on the delayed alternation task twelve weeks post-injection. To evaluate the impact of cortical alpha-synuclein accumulation on reversal and learning, the rats were also tested in the delayed-non-matching-to-position task and thereafter reversed to delayed-matching to position.

Excitotoxic lesions of the medial prefrontal cortex impaired performance in the delayed alternation and delayed-non-matching-to-sample task,showing a reduced choice accuracy at all delays, suggesting that the deficit is of the a frontal-type executive type. It had however no effect on the reversal to, or on the acquisition of the delayed-matching-to-position task. In contrast, rats with alpha-synuclein over-expression in the same cortical region showed impaired learning of this task. Post-mortem immunohistochemistry revealed widespread alpha-synuclein expression in several of the projection areas of the medial prefrontal cortex together with alpha-synuclein-positive axonal swellings.

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P044. Stuck in a loop: pre-training methamphetamine speeds up transition to habit dominated behaviour

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Chronic methamphetamine (mAMPH) use is associated with poor performance on cognitive control and response inhibition tasks. Moreover, mAMPH users engage in repetitive, involuntary behaviours. Although the nature of these behaviours is diverse across individuals, these "behavioural loops" share the common characteristic of being difficult to control, reminiscent of compulsions seen in OCD and habits more generally. In rats, mAMPH exposure increases synaptic density in brain areas involved in habits, whereas decreases are observed in areas involved in voluntary, goal-directed behaviour. It may be the case that these meth-dependent structural changes bias habitual over goal-directed behaviour. These studies used instrumental learning and outcome devaluation procedures to probe whether repeated exposure to mAMPH speeds up the transition to habit controlled behaviour in undertrained rats. At test, control rats display goal-directed performance as demonstrated by sensitivity to the current value of the outcome. In contrast, mAMPH animals were insensitive to the reduced value of the outcome at test, suggesting that behaviour was controlled by habits. This difference was not observed when mAMPH was administered post-training, indicating that exposure to mAMPH speeds up the transition from goal-directed behaviour to habits, but does not influence performance of established behaviours. These findings support the notion that dopamine is involved in the formation of habits.

P045. Dysbindin-1 genetic disruption modulates cognitive flexibility in mice

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Dysbindin-1 is located in the synaptic sites throughout human and mouse brain and is involved in intracellular protein trafficking and synaptic homeostasis. Genetic variation in dysbindin-1 are implicated in cognitive abnormalities associated with schizophrenia. The underlying mechanism is thought to be based in changes in dopaminergic and glutamatergic signaling in the prefrontal cortex (PFC) that is known to be critical for cognitive functions. Executive control plays a key role for PFC cognitive functions and flexibility, which are core impairments in schizophrenia. An effective measure of these functions in schizophrenia is the Wisconsin Card Sorting Test (WCST) or its more recent analogue ID/ED task of the CANTAB. We explored in dysbindin-1-mutant mice (dys-/- and dys+/-) (1) cognitive flexibility using a novel two-chamber "Operon" paradigm designed for rodents which is equivalent to WCST and ID/ED tasks commonly used in human, and (2) synaptic transmission and synaptic plasticity in the medial PFC that might be important for flexibility in cognitive control. Our results confirm that dysbindin-1 mutation in mice affects prefrontal-mediated cognition by modulating cognitive flexibility and regulates long-term potentiation in the medial PFC.

Dopamine and neurodegeneration

P046. The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease

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Dopamine neurons of the substantia nigra pars compacta (SNc) are uniquely sensitive to degeneration in Parkinson's disease (PD) and its models. Although a variety of molecular characteristics have been proposed to underlie this sensitivity, one possible contributory factor is their massive, unmyelinated, axonal arbor that is orders of magnitude larger than other neuronal types. We suggest that this puts them under such a high energy demand that any stressor that perturbs energy production leads to energy demand exceeding supply and subsequent cell death. One prediction of this hypothesis is that those dopamine neurons that are selectively vulnerable in PD will have a higher energy cost than those that are less vulnerable. Using a biology-based computational model of the axons of individual dopamine neurons, we examined the energetic impact imposed on SNc dopamine neurons by their axonal arbor and attempted to calculate the energy cost of action potential (AP) propagationthroughout the arbors. We show that the energy demand associated with AP conduction is related in a supra-linear manner to the axonal size and complexity. Futhermore, synaptic stimulation is necessary to ensure reliable propagation throughout larger axonal arbors and that calcium transients facilitate signal propagation. Thus SNc dopamine neurons, particularly in humans, whose axons we estimate to give rise to more than 1 million synapses and have a total length exceeding 4 metres (Bolam & Pissadaki 2012 Movement Disorders 12:1478-83), are at a distinct disadvantage with respect to energy balance which may be a factor in their selective vulnerability in PD.

P047. Vulnerability to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity in adolescent mice chronically treated with 3,4-methylenedioxymethamphetamine (MDMA)

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Amphetamine-like drugs induce neurotoxic and neuroinflammatory effects in experimental animals. Accordingly, several reports suggest that those drugs contribute to Parkinson's disease (PD)-associated neuronal loss. 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') is an amphetamine analogue consumed by adolescent and young adults, raising concern about its acute and long-term effects. In order to evaluate whether consumption of MDMA during adolescence might influence the neurotoxic effects of toxin known to induce PD, like MPTP, we evaluated the vulnerability to MPTP after a subchronic administration of MDMA during adolescence in mice. To this end we examined the activation of astroglial and microglial cells by GFAP and CD11b immunohistochemistry and the degeneration of dopaminergic neurons by tyrosine hydroxylase immunohistochemistry (TH) after administration of MPTP in male C57BL/6J mice subchronically treated for 9-weeks, from the late adolescence to the adult age, with MDMA (10 mg/kg i.p., two times x day, two days x week). Two weeks after the last administration of MDMA, mice were treated with MPTP (20 mg/kg, i.p, once a day for four days). In subchronic MDMA-treated mice, MPTP induced an increase in CD11b and GFAP-positive cells both in striatum and substantia nigra pars-compacta (SNc), as compared to vehicle, MDMA or MPTP alone. Inflammatory changes were paired with a decrease of TH immunoreactivity in SNc and striatum, as compared to the other groups. The results suggest that subchronic MDMA administered during late adolescence in mice exacerbates neurodegeneration and neuroinflammation caused by MPTP.

P048. SH-SY5Y neuroblastoma cells as a tool for studying designer drug toxicity - results on "bath salt" mephedrone.

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In recent years there has been a large increase in the abuse of the designer drugs mephedrone, commonly sold as "bath salts" via the Internet. Its chemical structure is very similar to the known recreational drug amphetamine (AMPH) which is known to be toxic to the dopamine system. Although mephedrone also affect the dopamine system, recent studies into the toxic effects have produced mixed results, with some studies reporting toxic effects of the drugs, whereas others find no such evidence. This warrants a closer investigation into the possible toxic effects of these drugs. AIMS Our aims are to investigate the effects of the designer drug mephedrone and AMPH in the SH-SY5Y neuroblastoma cell line. METHODS SH-SY5Y neuroblastoma cells were differentiated to a dopamine-like phenotype using retinoic acid (RA), 12-O- tetradecanoylphorbol-13-acetate (TPA) or a combination of RA and TPA. LDH-release and mitochondrial membrane potential was assessed after treatment with the drugs and cell dopamine content was assessed using HPLC. RESULTS Treatment with mephedrone (2 days, 1-2 mM) caused an increase in LDH-release in both RA- and TPA- differentiated SH-SY5Y cells. AMPH, on the other hand, affected LDH release only in TPA-differentiated cells. The increased LDH-release coincided with increased mitochondrial membrane potential. Furthermore, TPA- differentiated cells showed significantly higher intracellular dopamine content, compared to RA-differentiated cells. CONCLUSION Mephedrone appears to be toxic to cells across differentiation conditions. AMPH, conversely, conveys toxicity exclusively in TPA differentiated cells with higher dopamine content.

P049. Effect of caffeine on the release of DA, 5-HT and production of hydroxyl radical induced by methamphetamine and 3,4-methylenedioxymethamphetamine in the mouse striatum

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Methamphetamine (MTH) and 3,4-methylenedioxymethamphetamine (MDMA), psychostimulants with addictive potential are widely used as a recreational drugs. They increase release of DA and 5-HT into synaptic cleft by blocking their reuptake and cause dysfunction of vesicular monoamine transporters (VMAT2). The toxicity of MTH and MDMA directed against DA and 5-HT neurons is possibly related with generation of free radicals, as high cytosolic DA concentration leads to its autooxidation and production of highly toxic chinons. In addition, DA metabolism via MAO generates hydrogen peroxide, which in the presence of iron forms hydroxyl radical in Fenton reaction. Amphetamines are often used recreationally in combination with caffeine (CAF) to gain stronger stimulatory effect. CAF by blocking adenosine A1 and A2A receptors may affect neurotransmitter release and it is not known whether combination of amphetamine derivatives with CAF influence toxic effect of MTH and MDMA. In the present work we studied effect of combined application of MDMA or MTH and CAF on release of DA, 5-HT and production of hydroxyl radical in mouse striatum using microdialysis in freely moving animals. MDMA (20 mg/kg) and MTH (30 mg/kg) increased striatal release of DA, 5-HT and production of hydroxyl radical. CAF (10 mg/kg) inhibited generation of hydroxyl radical induced by MDMA and MTH, but potentiated effect of MDMA and MTH on DA and 5-HT release. These findings indicate the antioxidative and protective properties of CAF in its action against damage of DA and 5-HT terminals.

P050. A translational study on Dopamine Transporter Deficiency Syndrome: development of new animal models

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Dopamine Transporter Deficiency Syndrome (DTDS) is a newly recognized autosomal recessive disorder related to impaired Dopamine Transporter (DAT) function and the first identified parkinsonian disorder caused by genetic alterations of DAT in a cohort of 11 children. Movement disorders as parkinsonism, hyperkinesia and hypokinesia were described during infancy of these patients. In all cases homozygous or compound heterozygous SLC6A3 DAT gene mutations were detected. Humanized DAT (hDAT) knock-in mouse models featuring two particular mutations found in human SLC6A3 gene leading to DTDS phenotype are planned to be generated by means of microinjection of recombinant Embryonic Stem cells (ES) into C57BL/6 mouse blastocysts. Transfection of ES cells has been performed using plasmid vectors targeted with mouse DNA fragment containing site of mutation, either deletion of Guanine 399 in the DAT coding sequence (CDS) or single base mutation from Thymine to Cytosine at the residue 671 of DAT CDS. Selection markers were inserted in mouse DNA fragment. Standard diphtheria toxin/G418 double selection protocol was performed. Transient trasfection of ES cells with CRE recombinase expression construct allowed the removal of the LoxP flanked region, and Ganciclovir negative selection was carried out to select clones undergone to homologous recombination of the targeted DNA fragment. Development and characterization of these Knock-In mice will allow detailed investigation of the pathological molecular mechanisms involved in this disorder and generate experimental test systems for finding new treatments for DTDS and disorders related to DAT dysfunction in general.

Imaging dopamine

P051. Transgenic expression of GCaMP calcium indicator in dopaminergic neurons for optogenetic measurement of presynaptic calcium transients in striatum.

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Studies conducted over the last two decades have revealed a great deal about dopamine (DA) release from presynaptic terminals in brain regions such as the striatum. However, relatively little is known about signaling within dopaminergic axon terminals that regulates dopamine release. We expressed the genetically encoded calcium indicator protein "GCaMP3" in midbrain dopaminergic neurons. Using the tetracycline transactivator (tTA) system. After crossing a PITX3-IRES-tTA knock-in mouse with a tetO- GCaMP transgenic mouse, the resultant KI/transgenic Pitx3/GCaMP mice showed normal motor phenotype compared with wild type (WT) littermate, as well as normal striatal DA release and reuptake. Striatal slices were analyzed using a photomultipier tube-based system. Single-pulse (10 msec, 120µA) stimulation induced fluorescence presynaptic calcium transients that persisted for ~200 msec. These transients were totally blocked by application of cadmium, TTX, and by application of calcium-free aCSF. Transients were partially reduced by Quinpirole and N- and P/Q type calcium channel blockers omega-agatoxin IVA and omega-conotoxin GVIA (~-40%). When applied in combination, transient was completely blocked, indicating in N- and P/Q- type channels the main source of calcium in striatal DA terminals during electrical stimulation. L type calcium channel blocker Nifedipine did not affect calcium transient over time. Cholinergic modulation also affects calcium transient, in particular mAChR agonist (~-75%) and nAChR antagonist (~-50%) were able to strongly reduce calcium in DA terminals. In conclusion, this is the first report of a genetic expression of GCaMP conditioned for DA terminals, an useful tool for a better understanding about their calcium dynamic in physiological and pathological conditions.

SYMPOSIA

SUNDAY, MAY 26TH

GENETIC AND FUNCTIONAL DISSECTION OF THE DOPAMINE D2 RECEPTOR IN MOTOR AND REWARD – MEDIATED BEHAVIORS

Organizer: M. Rubinstein (Argentina)

D2 receptors on striatal and dopaminergic neurons play different roles in mediating cocaine reward and locomotor responses

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The work challenges the hypothesis that low D2R levels in the striatum predisposes for drug abuse and dependence. We use conditional mutant mice lacking D2Rs specifically on dopaminergic neurons or on striatal neurons. The experiments evaluate the acute and chronic behavioral responses to cocaine and the acquisition of intravenous cocaine self-administration behavior.

Roles of two efferent dopaminoceptive neuronal populations of the striatum in addiction and motor control: a transgenic approach

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One of the main targets of the dopaminergic neurones is the striatum. The dorsal striatum, divided into the dorsolateral striatum (DLS) (innervated by the sensorimotor cortex) and the dorsomedial striatum (DMS) (innervated by prefrontal and other associative cortices), is critically involved in motor behaviours, including regulation of motor activity, motor skill learning and motor response to psychostimulant and neuroleptic drugs, whereas the nucleus accumbens (NAc), is essential for motivation and drug reinforcement. To decipher the role of the two efferent dopaminoceptive neuronal populations of the striatum, D2R-striatopallidal and D1R-striatonigral neurons, we performed subregion- and cell population-selective ablation of striatal neurons thanks to new animals models combining BAC transgenesis, Cre/lox recombination and toxin receptor cell targeting. We demonstrated that the D2R-striatopallidal neurons in the NAc inhibit drug reward and drug memorisation. We found that the D2R-striatopallidal neurons in the DMS are responsible for the cataleptic effect of the antipsychotic drug haloperidol and the sensitization to psychostimulants such as amphetamine. We also demonstrated that the DMS exerts a population-specific control over locomotion and reactivity to novelty, D2R-striatopallidal and D1R-striatonigral neurons being inhibitors and stimulators of exploration, respectively. Further, DMS-D2R-striatopallidal neurons are involved in early motor learning whereas gradual motor skill acquisition depends on D1R-striatonigral neurons in the DLS. These results provide direct in vivo evidence for dissociations between neuronal subtypes and striatal subregions in the regulation of drug addiction, antipsychotic side effects, novelty-induced motor responses and motor learning.

Synaptic Plasticity in Direct and Indirect Pathway Striatal Projection Neurons

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Persistent synaptic efficacy changes at synapses onto striatal spiny projection neurons (SPNs) are thought to contribute to learning and memory for actions. The SPNs are organized into two output pathways; the D1 receptor-expressing direct pathway that projects to the substantia nigra and the D2 receptor-expressing indirect pathway projecting to globus pallidus. One prominent form of such plasticity is long-term synaptic depression (LTD) that occurs at glutamatergic and GABAergic synpapses on SPNs. At glutamatergic synpases, dopamine acting on D2 receptors facilitates LTD induction by stimulating production of endocannabinoids that serve as retrograde signals linking postsynaptic activation to presynaptic depression. However, LTD can be induced at both direct and indirect SPNs despite the fact that D2 receptors are not expressed by direct pathway neurons. Work is ongoing to determine the locus of D2 receptors that participate in LTD induction at synapses onto the two SPN subtypes. Endocannabinoid-dependent LTD is also expressed at GABAergic synapses onto SPNs. We have idenfified two forms of LTD, one ocurring when the postsynaptic neurons is at the resting potential "down-state" and another when the neuron is in the depolarized "up-state". These two forms of LTD involve different induction mechanisms and endocannabinoids, and affect synapses from different afferent targets. The LTD observed in the "down-state" is only expressed at synapses onto direct pathways MSNs. However, neither form of LTD at GABAergic synapses requires dopamine receptor activation. Our findings indicate that striatal synaptic plasticity is modulated by dopamine in a complex manner. It will be important to dtermine how dopamine-dependent and independent LTD participates in action learning and memory.

Spatial and temporal genetic dissection of Drd2 expression reveals an expanded functional repertoire of the dopamine D2 receptor

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Competition between adult males for limited resources such as food and receptive females is shaped by the male pattern of pituitary growth hormone (GH) secretion that determines body size and the production of urinary pheromones involved in male-to-male aggression. In the brain, dopamine (DA) provides incentive salience to stimuli that predict the availability of food and sexual partners. Although the importance of the GH axis and central DA neurotransmission in social dominance and fitness is clearly appreciated, the two systems have always been studied unconnectedly. Here we conducted a cell-specific genetic dissection study in conditional mutant mice that selectively lack DA D2 receptors (D2R) from pituitary lactotropes (lacDrd2KO) or neurons (neuroDrd2KO). Whereas lacDrd2KO mice developed a normal GH axis, neuroDrd2KO mice displayed fewer somatotropes, reduced hypothalamic Ghrh expression, pituitary GH content and serum IGF-I levels, and exhibited reduced body size and weight. As a consequence of a GH axis deficit, neuroDrd2KO adult males excreted low levels of major urinary proteins (MUP) and their urine failed to promote aggression and territorial behavior in control male challengers, in contrast to the urine taken from control adult males. These findings reveal that central D2Rs mediate a neuroendocrine-exocrine cascade that controls the maturation of the GH axis and downstream signals that are critical for fitness, social dominance and competition between adult males.

Dopamine-dependent transmission in the substantia nigra

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Oregon Health Sciences University / Vollum Institute

G protein-coupled receptors (GPCRs) are thought to alter physiological processes by modulation of both intrinsic membrane conductances and synaptic transmission. In this talk, spontaneous miniature inhibitory postsynaptic currents mediated by vesicular dopamine release acting locally on metabotropic D2 receptors leading to the activation of a G protein-coupled inwardly rectifying potassium conductance will be presented. These spontaneous IPSCs are not blocked by TTX or calcium channel blockers, are abolished by reserpine and are facilitated by L-DOPA and cocaine. The identification of individual exocytotic events resulting in spontaneous GPCR-mediated transmission places dopamine transmission in the substantia nigra in a position similar to synaptic activation by classical ligand-gated ion channels.

DOPAMINERGIC IMPACT ON NEUROPLASTICITY AND COGNITION IN ANIMALS AND HUMANS: A TRANSLATIONAL APPROACH

Organizer: M. A. Nitsche (Germany)

Dissociable roles of dopamine and serotonin transporter polymorphisms in reversal learning

H **den Ouden**, N Daw, G Fernandez, J Elshout, M. Rijpkema, M. Hoogman, B. Franke, R. Cools

Radboud University Nijmegen / Donders Institute for Brain, Cognition and Behaviour

Serotonin and dopamine are thought to subserve motivationally opponent functions, but this hypothesis has never directly been tested. We studied the role of these neurotransmitters in probabilistic reversal learning in nearly 700 individuals as a function of two polymorphisms in the genes encoding the serotonin and dopamine transporters (5HTTLPR plus rs25531; DAT1 3'UTR VNTR). A double dissociation was observed. The SERT polymorphism altered behavioral adaptation after losses (lose-shifting), while leaving unaffected perseveration after reversal. In contrast, the DAT1 polymorphism affected the effect of prior reinforcement on perseveration, while leaving unaltered lose-shifting. We fitted and compared different computational models to distinguish between different potential mechanisms that may underlie these observed differences in behavioural adaptation during reversal learning as a function of the dopamine and serotonin systems. Using this approach we showed that the effect of DAT1 on perseveration was best explained as a change in the integration of previous experience with new information. This study revealed a functional double dissociation between the effects of polymorphisms in regulatory regions of the SERT and DAT1 genes. Our results provide strong and direct evidence for functional dissociation for the serotonin and dopamine systems. In this dissociation, serotonin is involved in behavioral adaptation following losses, while dopamine plays a role in (reward-based) perseveration.

Dopamine-dependent synaptic plasticity in cortico-limbic information processing

Y **Goto**

Kyoto University / Primate Research Institute

Dopamine and its modulation on synaptic plasticity in the brain regions such as the prefrontal cortex and hippocampus are crucial neural substrates for cognitive and affective functions, and their deficits are thought to underlie several psychiatric disorders. Although molecular and cellular mechanisms of dopamine signaling and dopamine-dependent synaptic plasticity induction has been getting emerged, how these substrates are involved in cortico-limbic information processing mediating cognitive and affective functions at the system level are still unclear. In this talk, I present a model, mostly based on rodent studies, of cortico-limbic information processing with dopamine-dependent synaptic plasticity and its dysfunction in psychiatric disorders such as schizophrenia.

Dopaminergic impact on neuroplasticity in humans

Michael A. **Nitsche**

University Medical Center, Dept. Clinical Neurophysiology, Georg-August-University, Goettingen / Clinical Neurophysiology

As shown so far primarily in animal experiments, dopamine has a prominent impact on neuroplasticity, i.e. the enduring alteration of the strength of synaptic connections. Hereby, dopamine has a complex, and non- linear impact on neuroplastic alterations of cerebral activity, and excitability, which is determined by receptor type, dopamine concentration, and other factors. This complex action of dopamine might explain its non- linear effects on cognition. Non-invasive brain stimulation nowadays allows the induction of plasticity also in the human brain. Transcranial direct current stimulation (tDCS) induces non-focal plasticity of large cortical areas in the human motor cortex, while paired associative stimulation (PAS) generates focal plasticity of a limited set of synaptic connections. Both stimulation tools induce functional plasticity of the glutamatergic system. In a series of experiments, we explored the impact of dopamine, and subtypes of dopamine receptors, on motor cortex plasticity. It was demonstrated that dopamine has a non-linear dosage- dependent effect on plasticity, that the impact of dopaminergic receptor subtypes on plasticity differs, and that global dopaminergic activation, but not activation of receptor subtypes, results in a focusing effect on LTP-like plasticity. These results might be important for enhancing our understanding of the involvement of dopamine in cognitive and behavioural processes in healthy humans, as well as in patients suffering from neuropsychiatric diseases affecting the dopaminergic system.

Functional and dysfunctional plasticity inductions in rodent prefrontal cortex

Satoru **Otani**, Kevin Blot, Jing Bai

Ryotokuji University / General Education Center

Synaptic plasticity process in prefrontal cortex (prelimbic area in rodents) might play important roles for the functional regulation of this brain area, and maladaptive plasticity induction might underlie many cognitive disorders. Our previous efforts revealed that synaptic plasticity in rat prelimbic neurons in vitro is tightly controlled by dopamine, where the background (tonic) dopamine regulates synaptic potentiation induction through so-called "inverted U-shape" manner. This is an example for functional regulation of synaptic plasticity by dopamine. In the present work, we will present an example for dysfunctional dopaminergic regulation of synaptic plasticity through the blockade of dopamine transporter (DAT). DAT is a major target of psychoactive drugs such as cocaine in prefrontal cortex. Our results show that pharmacological block of DAT by the specific inhibitor GBR12909 (1-200 nM) blocks the induction of long-term depression (LTD) in rat prefrontal cortex. This blockade of LTD was through the over-stimulation of D1 receptors and over-activation of extracellular signal-regulated kinases (ERK1/2). A clinically useful drug CDPPB, the allosteric potentiator of subtype 1/5 metabotropic glutamate receptors (mGluR), cancelled LTD blockade and ERK1/2 over-activation by GBR12909. We suggest that cocaine-induced cognitive inflexibility might be related to the impairment of synaptic depression in prefrontal cortex by hyper-dopaminergia and that manipulation of mGluR might provide a way to control cognitive disturbances related to drug addiction.

LOCAL REGULATION

OF STRIATAL DOPAMINE RELEASE

Organizer: M. E. Rice (USA)

Axonal Control of Striatal Dopamine Transmission by ACh

Stephanie J. **Cragg**

University of Oxford

Activity in dopamine (DA) neurons has been assumed to be the principal driver of striatal DA release, and spans a broad range of tonic low frequencies to phasic bursts at higher frequencies. Phasic DA neuron activity encodes reward predictions, learning and/or salience. But how distinct modes of activity are reported to the striatum will depend on how DA axons and their presynaptic zones translate activity in to DA release. The extensive axonal arbours formed by DA neurons are endowed with a large variety of activity-dependent conductances and neuromodulatory receptors. We show how presynaptic nicotinic acetylcholine receptors (nAChRs) play a powerful role in gating DA transmission. Striatal nAChRs gate whether DA release reflects frequency of activity in DA neurons. Furthermore, using an optogenetic approach we have identified that single action potentials synchronized in a small population of cholinergic interneurons drives DA transmission directly by transiently activating presynaptic nAChRs, bypassing activity in DA neurons. These data revise several existing concepts. They suggest that presynaptic nAChRs can act to short circuit, or override, activity ascending from parent DA neurons, by generating ectopic excitability that evokes neurotransmission directly. They also suggest that inputs to cholinergic interneurons e.g. thalamostriatal inputs that can promote firing across the ChI network in response to salient unpredicted stimuli, might have previously unrecognized roles in driving DA transmission. These new findings shed new light on the function of presynaptic nAChRs, and suggest that the control of DA transmission by local mechanisms may be critical to DA function.

Rapid inhibition of dopamine release by endogenous dynorphin and kappa opioid receptors in ventral but not dorsal striatum

Jyoti **Patel**

NYU School of Medicine / Neurosurgery

Dynorphins (DYNs) form part of the opioid peptide family and are abundantly expressed throughout the striatal complex by a subset of medium spiny neurons. However, the dynamics of striatal dopamine (DA) release regulation by endogenous DYN acting at kappa opioid receptors (KORs) is not well established. Here fast-scan cyclic voltammetry was used to monitor DA release regulation by locally released DYN in coronal slices of guinea-pig striatum. Demonstrating that endogenously released DYN inhibits axonal DA release, the selective KOR antagonist nor-binaltorphimine (nor-BNI) markedly enhanced pulse-train (10 Hz) evoked [DA]o (by up to 300% of control) in the nucleus accumbens (NAc) shell, but not in the dorsal striatum. Moreover, DYN/KOR-dependent DA release modulation was fast: an increase in evoked [DA]o was seen within a few hundred ms of a 500 ms-train. Importantly, the lack of effect of nor-BNI in the dorsal striatum was not a result of a lack of functional DA release-regulating KORs in this region because the selective KOR agonist BRL-52537, decreased release evoked by a single pulse stimulation. This effect was markedly attenuated in nor-BNI, thereby confirming its action at KORs on DAergic axons. Instead, the ability of nor-BNI to enhance DA release in the NAc shell but not in the dorsal striatum is consistent with previous studies showing relatively higher basal expression of proDYN, the peptide precursor of DYN, in NAc versus dorsal striatum. These data demonstrate that endogenously released DYN acting at KORs provides a powerful, yet selective, sub-second suppression of mesolimbic DA signals.

Hydrogen peroxide (H_2O_2) as a modulator of striatal dopamine release

Margaret E. **Rice**

New York University School of Medicine / Dept Physiology and Neuroscience

The reactive oxygen species, hydrogen peroxide (H_2O_2), meets the criteria for classification as a neuromodulator through its effects on the local regulation of striatal dopamine (DA) release. This evidence was obtained using fast-scan cyclic voltammetry to detect evoked DA release in striatal slices, whole-cell recording, and fluorescence imaging to monitor H_2O_2 generation. The data show that: 1) exogenous H_2O_2 suppresses DA release in dorsal striatum and nucleus accumbens shell and the same effect is seen with elevation of endogenous H_2O_2 levels; 2) H_2O_2 is generated downstream from glutamatergic AMPA receptor activation in striatal medium spiny neurons (MSNs), but not DA axons; 3) generation of modulatory H_2O_2 is activity dependent; 4) H_2O_2 generated in MSNs diffuses from those neurons to cause a transient suppression of DA release by activating ATP-sensitive K^+ (K_{ATP}) channels on DA axons; and 5) the amplitude of H_2O_2-dependent inhibition of DA release is attenuated by enzymatic degradation of H_2O_2, but the subsecond time course is determined by diffusion H_2O_2 and/or K_{ATP}-channel kinetics. In dorsal striatum, neuromodulatory H_2O_2acts as a key intermediate in regulation of DA release by the classical neurotransmitters glutamate and GABA, as well as other striatal neuromodulators.

Striatal nitric oxide and dopamine interactions

Anthony R. **West**

Rosalind Franklin University / Neuroscience

Nitric oxide (NO) is a gaseous neuromodulator critically involved in the regulation of corticostriatal transmission and synaptic plasticity. Striatal NO-producing interneurons are activated by stimulation of glutamatergic NMDA receptors in a manner that requires coincidental dopamine (DA) D1/5 receptor activation. The most well studied signal transduction pathway for NO transmission in the striatum involves the activation of the soluble guanylyl cyclase (sGC) signaling cascade and subsequent production of the second messenger cGMP. The NO receptor sGC is highly expressed in striatal medium-sized spiny neurons, cholinergic interneurons, and various presynaptic elements. Thus, the striatal NO-sGC-cGMP signaling cascade is well positioned to modulate both presynaptic transmitter release and postsynaptic integration of excitatory and inhibitory transmission. Higher concentrations of NO can also facilitate the release of various neurotransmitters such as DA through cGMP dependent and independent mechanisms. The later mechanisms include nitrosylation of transporter proteins and synaptic release machinery. My presentation will review recent literature detailing how NO synthesis is regulated and how NO signaling impacts on striatal processing with a focus on DA transmission. I will also present new data addressing the impact of striatal dopamine depletion on NO signaling and describe the potential relevance of nitrergic dysregulation for the pathophysiology of Parkinson's disease.

DOPAMINE-MEDIATED REWARD AND REINFORCEMENT IN PRECLINICAL DISEASE MODELS

Organizer: K.M. Kantak (USA)

Cocaine Abuse Liability in a Rodent Genetic Model of ADHD: Comparison of Stimulant and Non-Stimulant Medication during Adolescence

K.M. **Kantak**

Boston University / Psychology

To examine comorbidity between ADHD and cocaine addiction, spontaneously hypertensive rats (SHR) exhibiting an ADHD phenotype were compared with Wistar-Kyoto (WKY) and Wistar (WIS) rats on attentional set-shifting while chronically treated with methylphenidate or atomoxetine during adolescence. After treatments were discontinued in adulthood, strain and treatment differences in cocaine abuse liability and dopamine transporter function were evaluated. Neither methylphenidate nor atomoxetine mitigated all deficits in SHR during the set-shifting task. Methylphenidate only improved formation of an initial attentional set and atomoxetine only improved performance during the extradimensional shift. After discontinuing vehicle treatment, SHR were more likely to self-administer cocaine than WKY or WIS, consistent with comorbidity between cocaine addiction and ADHD. SHR with previous methylphenidate treatment acquired cocaine self-administration fastest, identified cocaine as a highly efficacious reinforcer, and exhibited the highest progressive ratio breakpoints compared with vehicle-treated SHR and methylphenidate-treated WKY and WIS. In contrast, previous atomoxetine treatment did not further enhance these measures of cocaine abuse liability in SHR. An examination of DAT function revealed that previous methylphenidate treatment increased Vmax in mPFC of SHR, with no change in OFC or striatum, whereas previous atomoxetine treatment decreased Vmax in OFC and striatum of SHR, with no change in mPFC. These differential effects of methylphenidate and atomoxetine on DAT function, especially within PFC, may contribute to the increase in cocaine abuse liability after methylphenidate and the failure of atomoxetine to augment cocaine abuse liability further in rats with an ADHD phenotype.

Development of Dopamine Systems in Childhood and Adolescence

L. **Porrino**

Wake Forest School of Medicine / Physiology & Pharmacology

Dysfunction of the dopamine system has been shown to be associated with a multitude of neuropsychiatric disorders including schizophrenia, attention-deficit hyperactivity disorder, drug addiction, and more recently obesity. Adolescence, in particular, is a time of increased vulnerability to these disorders. Therefore, it is important to understand the factors that may lead to disruption of the development of these systems during childhood and adolescence. This presentation will provide an overview of the changes in dopamine during these critical periods. The developmental trajectory of dopamine innervation, dopamine receptors and transporters will be considered in both rodent and nonhuman primate models of development. We will focus on recent studies in juvenile nonhuman primates using positron emission tomography that have established a significant decline in dopamine D2 receptor availability throughout dorsal and ventral striatum. These levels in turn can be related to measures of physical and sexual maturation, as well as behavioral measure of cocaine-related behaviors assessed in adulthood. The potential environmental influences on the trajectory of the development of dopamine systems will also be discussed along with their implications for vulnerability to neuropsychiatric disorders.

Sexually dimorphic changes in reward and the dopamine system in a rodent model of diet-induced obesity

Sari **Izenwasser**

University of Miami / Psychiatry

Adolescence is a vulnerable period associated with a high incidence of drug abuse initiation and an increased risk for developing dependence and addiction. Drug use during this critical developmental period has been associated with higher incidence of cocaine abuse later during adulthood. Factors such as age and sex, as well as a variety of environmental variables (e.g. type of food available, social conditions, physical conditions) all influence drug reward during adolescence and these effects can persist into adulthood. Factors that modulate the effects of drugs of abuse during this critical period of development, and the underlying neurochemical changes mediating these effects, will be discussed. The purpose of these studies is to determine if psychostimulant effects depend upon diet, sex, age, and social or physical environment. The data show that social and environmental enrichment interact to differentially alter drug effects in male and female rats. Similarly, high fat diets produce different effects in males and females, both during adolescence and adulthood. For example, removal of a high fat diet greatly diminished cocaine reward in adult males, but made adult females more sensitive to the rewarding effects of cocaine. The data also show that basal and cocaine-mediated levels of proteins involved in dopaminergic transmission, as well as some inflammatory factors, are affected by cocaine as a function of sex, age and diet.

Altered dopaminergic function and reward sensitivity in a rat model of Post-Traumatic Stress Disorder

Ellen M. **Unterwald**, Nicole Enman, and Kayti Arthur

Temple University School of Medicine / Center for Substance Abuse Research

Exposure to severe stress can lead to the development of post-traumatic stress disorder (PTSD), which is characterized by symptoms including fear, anxiety, and depression. PTSD is highly comorbid with drug addiction, suggesting that PTSD may facilitate vulnerability to substance abuse. This study examined the effect of single-prolonged stress (SPS), a rodent model of PTSD, on behavioral activity, drug reward, and dopamine systems. Rats were exposed to a modified SPS paradigm consisting of 2 hours of restraint, 20 minutes of group swimming, isoflurane exposure, and 7 days of isolation. Following isolation or control handling, spontaneous and cocaine-induced activity was measured. Rats exposed to SPS exhibited significantly less spontaneous nocturnal activity than unstressed controls, however, SPS did not alter the locomotor-stimulant properties of cocaine. Conditioned place preference was used to determine the effect of SPS on the rewarding properties of cocaine. Control rats spent more time on the cocaine-paired side of the conditioning chamber than rats exposed SPS, suggesting a reduction in sensitivity to cocaine reward. Several components of the dopamine system were found to be altered by SPS. Tyrosine hydroxylase levels were lower in the amygdala and nucleus accumbens of SPS exposed rats. D2 receptor binding was significantly lower in the caudate putamen of rats exposed to SPS compared to controls, whereas dopamine transporter binding was significantly increased in the nucleus accumbens. These data suggest that SPS produces anhedonia-like effects and deficient reward function, which may be mediated by decreased dopaminergic neurotransmission in the striatum.

NEW MODELS OF NEURODEGENERATION FOR EXPERIMENTAL PARKINSON'S DISEASE

Organizer: F. Blandini (Italy)

CENTRAL DOPAMINERGIC DENERVATION AFFECTS SPLANCHNIC ORGAN FUNCTIONS

Fabio **Blandini** (1), Giovanna Levandis (1), Barbara Balestra (2), Ornella Pastoris (2), Maria Pia Vairetti (3), Marie-Therese Armentero (1)

(1) IRCCS National Neurological Institute C. Mondino, Pavia; (2) Dept. of Biology and Biotechnologies "Lazzaro Spallanzani", University of Pavia; (3) Department of Internal Medicine and Therapeutics, University of Pavia, Italy

Parkinson's disease (PD) is increasingly recognized as a complex entity. Typical PD motor symptoms are often combined with nonmotor symptoms; this association reflects, in most cases, the involvement of peripheral organs. A new view of PD, as a potentially systemic disease, is therefore being generated. Involvement of peripheral organs in PD animal models has been generally overlooked. Experimental studies have recently begun to address this issue by investigating, for example, the presence of gastrointestinal (GI) defects - a major nonmotor PD symptom - in various animal models of PD. Using a classic model of nigrostriatal lesion, obtained by stereotaxic injection of 6-hydroxydopamine in rats, we investigated the effects of central dopaminergic denervation on specific organs of the splanchnic area, including various segments of the GI tract and the liver. We observed that central dopaminergic denervation induces delayed, long-lasting alterations, at both levels. Neurochemical changes associated with modifications of GI motility are observed in 6-OHDA lesioned rats, particularly in the colon; as for the liver, rats bearing a nigrostriatal lesion show clear signs of mitochondrial dysfunctions, associated with reduced ATP production and increased formation of reactive oxygen species. The substrates of these phenomena are currently under investigation; it will be crucial, in particular, to clarify which circuitries are involved in linking the central dopaminergic denervation to the effects observed within the splanchnic district and whether specific signaling pathways and/or release of soluble mediators may play a role.

The chronic MPTPp mouse model of progressive Parkinson disease.

Anna R. **Carta**
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Biomedical Sciences

Parkinson's disease (PD) is characterized by a progressive degeneration of dopamine (DA) neurons and a chronic loss of motor function. The investigation of degenerative mechanisms and possible neuroprotective approaches for PD depends upon the development of an experimental animal model that reproduces the neuropathology observed in humans. Chronically treating mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in association with probenecid (MPTPp) for 5 weeks reproduces several key features of PD. Symptomatically, mice display pre-motor deficits such as olfactory dysfunction, followed by progressively worsening motor impairment. Histopathology reveals the progressive loss of tyrosine hydroxylase (TH) neurons in the substantia nigra (SN), that is associated with an increase of alpha-synuclein immunostaining. In the striatum, gradual drop of DA levels corresponds to post-synaptic changes typical of the parkinsonian striatum, such as an increase of enkephalin mRNA. Moreover, neuroimmune response is progressively altered along the MPTP treatment. Hence, gradual spreading of microgliosis in the SN is associated with an increase of pro-inflammatory cytokines and a transient increase of anti-inflammatory cytokines in damaged areas. Finally, analysis of synapsin-immunoreactivity as a marker of striatal synapses shows a gradual percent increase over TH-immunoreactivity, suggesting the formation of new synapses that may be related with the development of compensatory sprouting of dopaminergic terminals. Treatment with the anti-inflammatory compound PPAR-gamma agonist rosiglitazone arrested dopaminergic degeneration while counteracting the neuroinflammatory response. Therefore, the MPTPp mouse model provides a validated tool for the study of mechanisms contributing to the pathological dysfunction of PD at the cellular and whole animal level, and for testing neuroprotective strategies.

The importance of the primate model of Parkinsonism

MT **Herrero**

University Jaume I / Medicine

It is well known that MPTP-treated monkeys are the most closed experimental model to human PD probably because of the phylogenetic proximity between humans and non-human primates. It has been demonstrated that monkeys (like humans) have inter-animal and age differential MPTP susceptibility. The MPTP monkey model has been an invaluable tool to investigate and reveal the physiopathology of the basal ganglia but as well to study the molecular basis of the disease. In fact, neuropathological data indicate that acute MPTP-treatmentin monkeys causes almost identical damage to the nigrostriatal dopaminergic pathway to that seen in PD, but chronic MPTP intoxication results in damage to locus coeruleus noradrenergic neurons, to the serotoninergic system and to the cholinergic neurons in the pedunculopontine nucleus. Behaviourally, MPTP intoxication in monkeys reproduce motor symptoms (with the exception of resting tremor) and dyskinesias, the side effects of chronic L-dopa treatment. However, even if PD is characterized by both motor and cognitive decline, mostly experimental models of Parkinsonism fail to reproduce cognitive deficits in young animals with acute or sub-acute intoxication with MPTP. On the other hand, chronic intoxication in monkeys can reveal freezing of gait, sleep disorders (with an increase of sleepiness during the day), cognitive decline (impaired ability to sustain spatial attention or to focus attention, déficits in shifting attention sets or in motor readiness and planning among others) or psychotic-like behaviors after chronic L-dopa therapy. Additionally, there are many different regimens of MPTP intoxication which allows to analyse distinct aspects of Parkinsonism: compensatory mechanisms, motor symptoms, sleep disorders, cognitive impairment, disease progression and effects of potential (pharmacological and surgical) antiparkinsonian treatments as well as neuroprotective strategies.

Alpha-synuclein dysfunction affects the dopaminergic system in a transgenic mouse model.

Maria Grazia **Spillantini**

John van Geest Brain Repair Centre, Dept of Clinical Neurosciences, University of Cambridge

Parkinson's disease (PD) is the most common movement disorder clinically characterized by rigidity, resting tremor and bradikenisia which are associated with degeneration of neurones in the substantia nigra and other brain regions. Neuropathologically PD is characterized by the presence of intracellular protein aggregates known as Lewy bodies and Lewy neurites that are formed of filaments made of alpha-synuclein. The involvement of alpha-synuclein in PD pathogenesis is also supported by genetic findings showing that missense mutations and multiplications of the alpha-synuclein gene cause familial forms of PD. However, how the protein contributes to PD development, the mechanism leading to Lewy bodies formation and their role in the neurodegenerative process remain unclear. We have produced a transgenic mouse model that expresses truncated (1-120) human alpha-synuclein under the control of the tyrosine hydroxylase promoter specifically in dopaminergic neurones. In these transgenic mice, granular and filamentous alpha-synuclein are present. At 3 months of age these mice show dopamine reduction in the striatum associated with impaired LTP that is rescued by L-Dopa treatment. Alpha-synuclein synaptic aggregation can be detected from the age of 6 months and is associated with the redistribution of the SNARE complex and progressive reduction of dopamine release. The synaptic pathology is found in the absence of dopaminergic neuron death and it is also present in the striatum of PD patients with short duration of the disease. These results support an effect of alpha-synuclein on neurotransmitter release and indicate that PD is a dying back pathology starting at the synapse.

Role of mTOR signaling pathway in dopamine D2 receptor-mediated suppression of neuroinflammation

Yingying **Wu**, Hua Jiang and Jiawei Zhou

Institute of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences

Inflammatory attack plays a crucial role in the etiology of Parkinson disease (PD). Activated astrocyte is an important type of inflammatory cells in the brain. Our recent study shows that the astrocytic dopamine D2 receptors (DRD2) normally suppress neuroinflammation through αB-crystallin (CRYAB) (Shao, Zhang et al. Nature, 2013). However, the underlying molecular mechanisms have not been fully elucidated. Previous studies by others have demonstrated that the tuberous sclerosis complex-mammalian target of rapamycin (TSC-mTOR) pathway regulates inflammatory responses following bacterial stimulation in monocytes, macrophages, and primary dendritic cells. Rapamycin, an immunosuppressant drug preventing activation of T cells and B cells, protects against dopaminergic neuron death under MPTP toxic action. We thus hypothesized that DRD2 modulates innate immunity through mTOR signaling pathway. We found that p-P70s6k, which is directly activated by mTOR, was up-regulated after DRD2 deletion. Rapamycin appeared to be able to rescue dopaminergic neuron in MPTP-treated DRD2 KO mice much better compared with WT mice in MPTP model. We also examined neuroprotective activity of Rapamycin in MPTP-treated mice in the presence or absence of DRD2. We will present the data in the conference.

ETHANOL-MECHANISMS ALONG THE MESOLIMBIC DOPAMINE SYSTEM

Organizer: E. Jerlhag (Sweden)

Actions of ethanol on protein kinase C function affecting dopaminergic ventral tegmental area neurons

Mark S. **Brodie**

University of Illinois at Chicago / Physiology and Biophysics

Drugs of abuse increase dopamine (DA) outflow from the ventral tegmental area (VTA) to the extended amygdala, and this increased dopamine has been linked to reinforcement. Ethanol acts directly on DA VTA neurons to increase their firing rate. Other effects of ethanol in the VTA may affect the sensitivity of DA VTA neurons to that excitation. Extended exposure to dopamine desensitizes D2 dopamine autoreceptors on DA VTA neurons via dopamine inhibition reversal (DIR). DIR requires co-activation of D2 and D1-like dopamine receptors, and is mediated by Gq, phospholipase C, and a conventional isoform of protein kinase C (PKC). Activation of D2 receptors concomitant with activation of a Gq-linked receptor also results in D2 desensitization, but not D2 activation alone. Interestingly, ethanol blocks the development of DIR, and may act on protein kinase C to halt the development of DIR. In addition, ethanol may act on presynaptic elements within the VTA to alter the excitability of DA VTA neurons. In the presence of a phorbol ester, ethanol excitation of DA VTA neurons is blocked. This block of ethanol-induced excitation of DA VTA neurons by PMA is prevented by PKC antagonists. The net effect of alcohol on VTA dopamine neuron activity is the sum of the balance of influences in the VTA modulating the direct activation of VTA neurons by ethanol. Changes in those influences as a result of stress or chronic alcohol exposure may tip the balance in a direction that favors loss of control. Grant AA05846 and AA09125.

The role of gut-brain hormones in reward from addictive drugs

Elisabet **Jerlhag**1, Pia Steensland2, Ida Fredriksson2, Kristin Feltmann 2, Emil Egecioglu, Petra Suchankova1 Jörgen A. Engel1

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Alcohol dependence is a heterogeneous disorder where several signaling systems play important roles. By understanding the complex mechanisms underlying this disease new treatment strategies may be developed. While the gut-brain hormones ghrelin and glucagon like peptide-1 (GLP-1) were gaining status as food regulating peptides, the work pinpointing the midbrain dopamine system as a target for ghrelin and GLP-1, led us to hypothesize that these system may have a role that extends beyond regulation of food intake to include reward seeking behavior, such as reward induced by and intake of alcohol and other drugs of abuse. Indeed, we have shown that ghrelin receptor (GHS-R1A) antagonist reduces alcohol consumption in rats exposed to alcohol for a long period of time as well as prevents relapse drinking in rats using the alcohol deprivation model. Our recent human genetic studies show associations between polymorphisms in ghrelin signaling genes and addictive behaviors. Moreover, we have found that a GLP-1 analogue (exendin-4) suppresses the alcohol-induced locomotor stimulation, conditioned place preference and accumbal dopamine release in mice as well as reduces voluntary alcohol consumption and alcohol seeking behaviors in rats. Finally, our recent data show that exendin-4 attenuates the rewarding properties of cocaine as well as of amphetamine, as measured by locomotor stimulation, accumbal dopamine release and conditioned place preference. This implies that gut-brain hormones, ghrelin and GLP-1 may be involved in reward regulation. Collectively, our data indicate that the central ghrelin and GLP-1 signaling system may constitute a novel potential target for treatment of drug dependence.

Dopamine Neuron Plasticity in Alcoholism

H **Morikawa**

University of Texas at Austin / Waggoner Center of Alcohol and Addiction Research

During the course of development of alcoholism and other forms of addiction, the mesolimbic dopaminergic system increases its sensitivity to drug-associated environmental stimuli while at the same time there is a gradual reduction in baseline dopaminergic output. We have previously indentified two forms of neuroadaptive changes in ventral tegmental area (VTA) dopamine neurons after repeated ethanol experience. The first change is increased susceptibility to the induction of long-term potentiation (LTP) of NMDA receptor-mediated glutamatergic transmission. This results from an increase in the potency of inositol 1,4,5-trisphosphate (IP3) in producing action potential-induced Ca2+ signals, which is critical for LTP induction. The second change is an increase in dopamine-induced autoinhibition mediated by somatodendritic D2 autoreceptors, which results from attenuation of a Ca2+-dependent desensitization mechanism regulating D2 receptors. Enhanced synaptic plasticity of NMDA receptors may promote the formation of powerful memories of ethanol-associated stimuli by facilitating the development of NMDA receptor-dependent burst responses to those stimuli, while increased D2 autoinhibition will result in reduced tonic dopamine neuron activity during withdrawal and thus may drive excessive ethanol consumption to compensate for dopamine deficiency. I will further discuss our recent studies on the influences of social and dietary experiences on synaptic plasticity of NMDA receptors and D2 autoinhibition. We hypothesize that intensified phasic dopamine neuron burst responses to ethanol-related stimuli will work in concert with the deficit in tonic dopaminergic output to drive more and more ethanol consumption.

The monoamine stabilizer (-)-OSU6162 attenuates voluntary ethanol intake and ethanol-induced dopamine output in the nucleus accumbens

P. **Steensland**, I. Fredriksson, S. Holst, K. Feltmann, J. Franck, B. Schilström and A. Carlsson; P. Steensland

Karolinska Institutet / Clinical Neuroscience

This study evaluated the "monoamine stabilizers" (-)-OSU6162 as a novel treatment of alcohol use disorder (AUD). Long-term alcohol consumption leads to a dysregulated dopamine system. A novel approach to normalize these dysregulations may be treatment with "monoamine stabilizers", a novel class of compounds characterized by the ability to either suppress, stimulate or not influence dopamine activity depending on the prevailing dopaminergic tone. The effects of the monoamine stabilizer OSU6162 on voluntary ethanol intake, ethanol seeking behavior and cue-induced reinstatement paradigms were evaluated in rats voluntarily consuming ethanol for at least 3 months prior to testing. Furthermore, the interaction of OSU6162 with ethanol on dopamine output and metabolism were studied using microdialysis. OSU6162 attenuated several ethanol-mediated behaviors including voluntary ethanol consumption, ethanol withdrawal symptoms, operant ethanol self-administration under progressive ratio schedule, and cue-induced reinstatement of ethanol seeking in rats that had voluntarily consumed ethanol for at least three months before treatment. In addition, OSU6162 blunted ethanol-induced dopamine output in the NAcc of ethanol-naïve rats. These results highlight OSU6162's ability to stabilize dopamine activity depending on the prevailing dopaminergic tone and indicate that OSU6162 might decrease ethanol intake by attenuating the acute rewarding properties of ethanol. In addition, OSU6162 might have potential to prevent relapse triggered by alcohol craving, alcohol related cues and or an urge to relieve abstinence symptoms. The present study is to our knowledge the first indicating that OSU6162 may serve as a novel medication for AUD.

THE IMPACT OF STEROIDS ON DOPAMINE NEUROTRANSMISSION

Organizer: M. Bortolato (USA)

Neurosteroids as dopaminergic modulators: neurobiological mechanisms and clinical implications

Marco **Bortolato**

University of Kansas / Pharmacology

Over the last decade, converging lines of evidence have highlighted the role of neurosteroids in brain neurotransmission and behavioral regulation. The enzyme 5alpha-reductase (5AR) catalyzes the conversion of ketosteroid precursors - such as testosterone and progesterone - into their 5alpha-reduced neurosteroid metabolites. Capitalizing on these premises, we and other groups have investigated the role of 5AR in animal models of neuropsychiatric disorders, with particular focus on its effects on the modulation of dopamine signaling. Our preclinical and clinical findings suggest that 5AR inhibitors, such as finasteride, may elicit antidopaminergic effects in a number of disorders associated with dopaminergic hyperreactivity, including Tourette syndrome and schizophrenia. These effects are strikingly dissociated from extrapyramidal symptoms, and appear to be contributed by the negative modulation of D1 and D3 receptors in the nucleus accumbens, in coordination with other targets, such as sigma and beta-estrogen receptors. Notably, we identified that inhibitors of 17α-hydroxylase/17,20-lyase (CYP450-C17), the rate- limiting enzyme in testosterone synthesis, elicit behavioral effects akin to those produced by finasteride. Taken together, these data suggest that 5alpha-reduced androgens may play a critical role in the modulation of dopaminergic signaling in the nucleus accumbens, and offer a potential platform to account for the male predominance of Tourette syndrome and schizophrenia.

Implication of the membrane estrogen receptor GPER1 in estrogenic neuroprotection and neuromodulation of brain dopamine

M. Bourque, M. Morissette and T.**Di Paolo***

Laval University / Faculty of Pharmacy

The search for an estrogen analog mimicking the beneficial effects of 17β-estradiol against brain damage, but with minor effects in reproductive organs is of great interest. Agonists of the G protein-coupled estrogen receptor 1 (GPER1) could be promising compounds, GPER1 having a minor role in reproductive organs. In intact mice, the GPER1 agonist G1 reproduced the effect of 17β-estradiol in increasing striatal dopamine (DA) metabolites (homovanillic acid, HVA) concentrations and turnover of DA. GPER1 antagonist G15 blocked the effect of G1 on HVA/DA ratio and partially for 17β-estradiol. The 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) mice model of Parkinson disease treated with G15 were more susceptible to MPTP toxicity with a greater decrease in striatal DA concentration and DA transporter specific binding. G1 was as potent as 17β-estradiol in protecting striatum and substantia nigra of MPTP mice as measured with DA concentrations as well as DA and vesicular monoamine transporter specific binding. G15 antagonized completely the neuroprotective effects of G1 in the striatum and substantia nigra as well as protection by 17β-estradiol in the striatum but partially in the substantia nigra. Neuroprotection with the estrogen receptor alpha agonist PPT was also abolished by G15 suggesting collaboration between these two receptors. The selective estrogen receptor modulator raloxifene used to treat osteoporosis is a GPER1 agonist; its neuroprotective effect was also antagonized by G15. In conclusion, 17β-estradiol, PPT, G1 and raloxifene show an important role of GPER1 in DA neuroprotection.

Progestogens mediate mating behavior of rodents in part through dopamine signalling in the ventral tegmental area

Cheryl **Frye** and Sandra Petralia

University of Alaska Fairbanks / IAB

Research in my laboratory has focused on actions of progesterone and its metabolites (progestogens) in the ventral tegmental area (VTA) that facilitate lordosis of female rodents. In the VTA, there are few nuclear progestin receptors, through which progestogens can have their actions. In the VTA, progestogens can have agonist- and antagonist-like actions at GABAA and NMDA receptors, respectively. Actions of progestogens through membrane D1 receptors represent another possible steroid- and neurotransmitter-initiated pathway in the VTA. Notably, there seems to be extensive "cross-talk" between these receptors. P can alter second messengers that are part of the signal transduction pathway that lead to the phosphorylation of DARPP-32. Activation of GABAA can lead to DARPP-32 phosphorylation. Progestogens can alter GABAA, dopamine receptors, and second messengers, which effect DARPP-32 phosphorylation. Findings will be discussed which suggest that progestogens have actions in the VTA through dopamine receptors, second messengers, and phosphorylation of DARPP-32.

Phasic dopamine transmission in the striatum during drug use

Ingo Willuhn, Lauren M. Burgeno and Paul E. M. **Phillips**

University of Washington / Psychiatry & Behavioral Sciences

Dopamine release was measured with sub-second resolution in the core of the nucleus accumbens and the dorsolateral striatum of rats performing cocaine self-administration using fast-scan cyclic voltammetry at chronically implanted electrodes. In animals that had access to cocaine for one hour per day (short access) phasic dopamine release was observed following active responses to cocaine in both striatal regions. However, this signal emerged in the dorsal striatum in the second week of self-administration, whereas it was present in the nucleus accumbens core throughout the three-week testing period. In animals that had access to cocaine for six hours per day (long access), dopamine release was attenuated in both regions during escalation of drug intake. Phasic dopamine release was also diminished in the dorsolateral striatum of animals that underwent long-access self-administration but did not exhibit appreciable escalation of drug use. However, phasic dopamine signaling was spared in the nucleus accumbens of these animals. Therefore, to test whether the decline in dopamine release contributed to escalation of drug intake (akin to a dopamine antagonist), in a separate group of animals we administered L-DOPA (i.v.) prior to self-administration sessions. This treatment prevented escalation of drug taking in animals undergoing self-administration in long-access sessions, but did not change drug intake in animals self-administering cocaine in short-access session (which did not produce escalation). These findings suggest that a reduction in phasic dopamine signaling in the nucleus accumbens of animals performing long-access cocaine self-administration drives higher intake to escalated levels.

Dopamine D2 Receptor Abnormalities in Schizophrenia: Possible Modulation by Sex Steroids and Genotype

Cynthia Shannon **Weickert**, Debora Rothmond, Tertia Purves-Tyson, Samantha Owens, Xu-Feng Huang and Thomas Weickert

Neuroscience Research Australia, Schizophrenia Research Institute, University of New South Wales, University of Wollongong

Schizophrenia onset coincides with increased testosterone in males. The dopamine D2 receptor (DRD2) is a target for antipsychotics; however, the questions of how and why endogenous DRD2s may be changed in schizophrenia remain. We hypothesized that genetic factors (SNPs) and hormonal factors may contribute to putative changes in DRD2 transcription in schizophrenia. DRD2 SNPs determine the level of DRD2 short (D2S) splice variant, which encodes a truncated cytoplasmic loop protein. In a cohort of 74 humans, people with schizophrenia had significantly increased D2S (26%, p=0.01) and DRD2 long (D2L) (13%, p=0.04) mRNAs in the caudate. There was no correlation between antipsychotic dose and DRD2 mRNA, and in rodents given haloperidol, olanzapine or saline orally for 1-5 weeks, no significant effects of antipsychotics on DRD2 mRNA levels were found. However, 2 weeks of testosterone replacement to castrated adolescent male rats increased striatal D2S mRNA ($p<0.03$). In the PFC of people with schizophrenia, we found significant elevations in D2S and D2S**/**DRD2pan ratio mRNAs. We found that the DRD2 intron 5 SNP rs2283265 G/G genotype was associated with greater D2S mRNA. In a sample of 101 healthy controls, those with the G/G genotype performed significantly worse on attention and processing speed tasks than those with the other genotypes. Furthermore, we found that testosterone levels correlated with processing speed in people with schizophrenia (beta=0.49). Taken together, our results suggest that brain levels of D2S mRNAs are increased in schizophrenia and that D2S mRNAs may be influenced by genotype and modified by sex steroid levels.

DOPAMINERGIC FUNCTION IN MICE MUTANT FOR SCHIZOPHRENIA-RELATED GENES: CONVERGENCE OR DIVERGENCE?

Organizer: J.L. Waddington (Ireland)

D-amphetamine and antipsychotic drug effects on Latent Inhibition in mice lacking dopamine D2 receptors

Bay-Richter C, O'Callaghan MJ, Mathur N, Heery DM, Fone KC, O'Tuathaigh CMP, Waddington JL, **Moran** PM

School of Psychology/University of Nottingham, U.K.

Drugs that induce psychosis such as D-amphetamine (AMP) and those that alleviate it such as antipsychotics are suggested to exert behavioral effects via dopamine receptor D2 (D2). All antipsychotic drugs are D2 antagonists, but it is unknown whether D2 is necessary for their behavioral effects. Using D2 null mice (Drd2 -/-) we first investigated whether D2 is required for AMP (2.5mg/kg) disruption of latent inhibition (LI). LI is a process of learning to ignore irrelevant stimuli. In the LI procedure, one group of water restricted mice are pre-exposed to 60 85dB tones prior to two pairings with footshock (.38mA) ,while a second group are exposed to the same conditions without tone. Learning is measured 2 days later as suppression of drinking on presentation of the tone previously paired with shock. This suppression is reduced in pre-exposed relative to non-pre-exposed mice. Disruption of LI by AMP models impaired attention and abnormal salience allocation consequent to dysregulated dopamine relevant to schizophrenia. AMP disruption of LI was seen in both wild-type (WT) and Drd2 -/-. In contrast AMP-induced locomotor hyperactivity was reduced in Drd2 -/-. AMP disruption of LI was attenuated in mice lacking dopamine receptor D1 (Drd1-/-) suggesting that D1 may play a role in AMP disruption of LI. Further supporting this possibility the D1 antagonist SKF83566 (0.1mg/kg) attenuated AMP disruption of LI in WT. Remarkably, both haloperidol (0.1mg/kg) and clozapine (2.5mg/kg) attenuated AMP disruption of LI in Drd2 -/-. This demonstrates that these antipsychotic drugs can exert specific behavioural influence in the absence of Dopamine D2.

Vulnerability of dopaminergic development to neuregulin-1 and EGF; Implication in schizophrenia-related behavioral deficits

Hiroyuki Nawa, Hidekazu Sotoyama, Hisaaki Namba

Niigata University / Brain Research Insititue

Neuregulin-1 and epidermal growth factor (EGF) are both ErbB receptor ligands that are implicated in schizophrenia neuropathology and genetics. Neuregulin-1 receptor (ErbB4) and EGF receptor (ErbB1) are both enriched in midbrain dopaminergic neurons and regulate dopaminergic development through their mutual interactions. In vivo administration of neuregulin-1 or EGF up-regulated tyrosine hydroxylase (TH) and dopamine transporter (DAT) levels in distinct brain regions. In parallel, there were increases in the density of TH-positive dopaminergic varicosities as well as in local dopamine concentrations. These results confirm that these ErbB ligands have strong impact on phenotypic and functional development of postnatal midbrain dopaminergic neurons. EGF and neuregulin-1 are enriched in human amniotic fluid as well as in blood plasma and their expressions are regulated by their SNPs associating with schizophrenia risk. To evaluate the effects of prenatal and perinatal neuregulin-1/EGF hypersignaling on dopaminergic functions, these neurotrophic factors were subcutaneously administered to rodent pups. We found that these factors penetrated the immature blood-brain barrier and reach brain neurons and induced hyperdopaminergic states. The exposure to EGF and neuregulin-1 later resulted in various behavioral impairments associated with schizophrenia endophenotypes at the post-pubertal stage; acoustic prepulse inhibition (PPI), latent inhibition of learning, social interaction, and methamphetamine hypersensitivity in rodents. These findings suggest that dopaminergic development and functions are highly vulnerable to the neurotrophic factors of ErbB ligands (e.g. EGF and neuregulin-1) circulating in the pre- and peri-natal periphery.

A mouse mutant model with disruption of two schizophrenia risk genes: dopaminergic modulation of schizophrenia-relevant phenotypes

C.M.P. O **Tuathaigh**

University College Cork / Cork

Neuregulin-1 (NRG1) and Disrupted-in-Schizophrenia-1 (DISC1) are genetically associated with schizophrenia, a complex and highly heritable developmental disorder of unknown etiology that has been proposed to result from deficits in functional connectivity and synaptic plasticity. Both DISC1 and NRG1 signalling pathways play a role in dopaminergic and glutamatergic neurotransmission, which may underlie their relative contribution to the pathophysiology of the disorder. Recognising the polygenic basis of the disorder, clinical genetic analyses have studied gene × gene interactions in mediating risk for schizophrenia. Using a preclinical genetic approach, we have generated a mutant model with simultaneous disruption of DISC1 and NRG1, with a view to examining whether disruption of both genes is associated with a schizophrenia- and antipsychotic-relevant phenotypic profile which differs from that observed following disruption to either gene alone. Mice containing an ENU-generated mutation in exon 2 of mouse DISC1 [L100P] were intercrossed with mice with heterozygous deletion of NRG1. The presence of a schizophrenia-related phenotype was assessed at developmentally-relevant age points in mice with none, partial or complete disruption of either or both genes. This presentation will focus on behavioural, neurochemical, and molecular genetic data which addresses the role of dopamine in the expression of schizophrenia-related endophenotypes in this mutant model.

Dysbindin-1 modulates cognitive deficits relevant to schizophrenia via dopamine pathways

Papaleo Francesco

Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova - Italy

Cognitive abnormalities are core manifestations of schizophrenia that precede the onset of the diagnostic psychosis, dramatically contribute to poor functional outcomes in patients, and are currently not effectively treatable. Cognitive dysfunctions in schizophrenia have been linked to genetic susceptibility for the illness. Here we present findings showing in an animal model a nonlinear interaction between two schizophrenia-susceptibility genes - catechol-O-methyl transferase (COMT) and dysbindin (DTNBP1) - implicated through different mechanisms in cortical dopamine signaling and prefrontal cognitive function. In particular, we demonstrated as dysbindin-1 disruption alters dopamine/D2-related molecular, electrophysiological and behavioral changes. Moreover, using a well validated discrete paired-trial variable-delay T-maze task, we found that a single genetic mutation reducing expression of either COMT or DTNBP1 alone produces working memory advantages in baseline conditions, while, in dramatic contrast, challenging/stressful conditions or genetic reduction of both COMT and DTNBP1 in the same mouse produces working memory deficits. Similarly, using a new semi-automated attentional set-shifting "stuck-in-set" paradigm, we found that while single COMT knockout mice had a significant improvement in solving the extradimensional shift phase of the task, performance of double heterozygote COMT+/-dys+/- littermates was selectively impaired on the extradimensional shift stage. Working memory and extra-dimensional shifting abilities are among the cognitive functions mainly affected in schizophrenia and which depend on the prefrontal cortex. These results illustrate the importance of nonlinear relationships between genes and their cognitive effects.

SYMPOSIA

MONDAY, MAY 27TH

PRE-SYNAPTIC AND POST-SYNAPTIC MECHANISMS IN L-DOPA-INDUCED DYSKINESIA

Organizer: M. Carta (Italy)

Lentiviral-mediated silencing of PSD-95 diminishes L-DOPA-induced dyskinesia in experimental parkinsonism

E. **Bezard**

University of Bordeaux Victor Segalen / Institute of Neurodegenerative Diseases

L-dopa induced dyskinesia (LID), a detrimental consequence of dopamine (DA) replacement therapy in Parkinson's disease, is associated with an alteration in DA D1 (D1R) and glutamate receptors interactions. The synaptic scaffolding protein PSD-95 might play a pivotal role as it also interacts with D1R, regulates its trafficking and function and is overexpressed in LID. We here demonstrate in rat and macaque models that disrupting D1R and PSD-95 receptor interaction in the striatum reduces dyskinesia development and severity. Single quantum-dot imaging revealed that this benefit is achieved primarily by destabilizing D1R localization via increased lateral diffusion followed by increased internalization and thus diminished surface expression. These findings indicate that alterations in D1R trafficking via synapse-associated scaffolding proteins organization may be useful in the treatment of dyskinesia in Parkinson's patients.

5-HT1 receptor agonists for the treatment of L-DOPA-induced dyskinesia: toward clinical investigation

Manolo **Carta**, Elisabetta Tronci, Elsa Pioli, Qin Li, Gregory Porras, Anders Björklund, Erwan Bezard

Università di Cagliari / Scienze Biomediche, Sezione di Fisiologia

In the recent years, we have contributed to unveil the important role played by the serotonin system in the appearance of L-DOPA-induced dyskinesia (LID) in animal models of Parkinson´s disease (PD). In fact, dopamine released as false transmitter from serotonin neurons after L-DOPA administration appears to contribute to the pulsatile stimulation of dopamine receptors, leading to the appearance of LID. Indeed, drugs able to dampen the activity of serotonin neurons have been shown to suppress LID, and hold promise for the treatment of dyskinesia in PD patients. In the present study, we investigated the ability of the mixed 5-HT1A/1B receptor agonist eltoprazine to counteract LID in 6-OHDA-lesioned rats and MPTP-treated macaques. Our data demonstrate that eltoprazine is highly effective in suppressing dyskinesia in our experimental models; however, a partial worsening of the therapeutic effect of L-DOPA was observed after eltoprazine administration, and represents a concern, which needs to be taken into account also for the on-going clinical investigation. Our data also suggest that combination of low doses of eltoprazine with amantadine synergistically suppress LID in MPTP-treated macaques, without significant worsening of the L-DOPA therapeutic effect. Thus, combination of low doses of eltoprazine and amantadine may represent a valid strategy to increase the antidyskinetic efficacy and reduce the side effects of the treatment.

PRE- AND POST-SYNAPTIC MECHANISMS IN L-DOPA-INDUCED DYSKINESIA

M. Angela **Cenci**

Dept. Exp. Med. Science/Lund University

L-DOPA-induced dyskinesias (LID) are abnormal involuntary movements having hyperkinetic or dystonic features that complicate the pharmacotherapy of Parkinson´s disease (PD). In addition to being a clinically important problem, L-DOPA-induced dyskinesia (LID) represents an intriguing research topic to address the fundamental question of how the basal ganglia generate abnormal movements. Current pathophysiological models attribute LID to the combined effects of a severe nigrostriatal dopamine (DA) lesion and fluctuating presynaptic levels of DA, eliciting abnormal responses in striatal neurons via supersensitive DA receptors. An altered gating of cortically driven motor commands at the level of the striatum would then lead to a dyskinesiogenic neural output through basal ganglia-thalamocortical networks. While this general scheme fits well with a large number of observations, the relative importance of presynaptic versus post-synaptic factors in the generation of LID is a matter of ongoing debate. This talk will review preclinical experimental studies that have unraveled specific presynaptic or post-synaptic alterations at the basis of LID. Among the presynaptic alterations we shall focus on the role of the serotonin system as an aberrant source of DA release. Among the post-synaptic alterations we shall focus on the role of D1 receptor-dependent ERK1/2 activation in striatal neurons. We will show how these specific mechanisms are differentially targeted by pharmacological interventions that are now under clinical development. Understanding the relative impact of presynaptic or postsynaptic factors in LID will pave the way to individualized treatment interventions for PD patients.

Pre-and post-synaptic mechanisms in L-DOPA-induced dyskinesia (LID): evidence from functional neuroimaging studies in PD.

Paola **Piccini**

Imperial College London

Pre- and post-synaptic mechanisms have both been suggested to underlie the appearance of LIDs. Functional Imaging techniques, such as PET have been instrumental in assessing these mechanisms in vivo in patients with PD. 11C-raclopride (RAC) is a PET ligand with affinity for D2 receptors in the low nanomolecular range and therefore is subject to competitive displacement by endogenous DA. The acute administration of substances such as amphetamine or L-DOPA, which are known to increase the levels of DA, results in a reduction of striatal RAC binding and can give an estimate of the release of DA at the synaptic level. Using this method we have demonstrated that following single L-DOPA doses PD without LIDs show moderate synaptic DA levels that remain stable over several hours after L-DOPA, while in dyskinetic subjects the same dose of L-DOPA induces larger peaks and rapid swings in DA levels. Recently, we have also been able to demonstrate that striatal 5HT neurons play a role in LIDs by taking up L-DOPA and releasing DA as a false transmitter, therefore supporting previous studies in animal models of PD. 5HT agonists by dampening the excessive release of DA from 5HT terminals are able to improve LIDs. Post-synaptic mechanisms assessed so far in vivo with functional imaging include the role of D2/D3 receptors, glutamate NMDA receptors and Adenosine A2A receptors. The talk aims to give an overview of these mechanisms and to discuss their respective roles in PD patients.

DOPAMINERGIC INVOLVEMENT IN EFFORT–RELATED ASPECTS OF MOTIVATION

Organizer: J.D. Salamone (USA)

Cost/benefit-related decisions in rats: role of dopamine and effects of dopaminergic drugs

Wolfgang **Hauber**

University of Stuttgart / Institute of Biology

Rodent studies suggest that the anterior cingulate cortex (ACC), the basolateral amygdala (BLA), and the nucleus accumbens (NAc) are key components of an interconnected neural system that subserves effort-based decision making in T-maze or instrumental tasks. For instance, in rats not only inactivation of the ACC, BLA or NAc but also disconnection between the ACC and the BLA or between the ACC and the NAc impaired effort-based decision making indicating that an information transfer between these structures is essential to guide decisions requiring an assessment of costs and benefits. Furthermore, there is compelling evidence that brain dopamine (DA) systems, in particular in the NAc, regulate how much effort to invest for benefits such as food reward. For instance, relative to sham controls, rats with NAc DA depletion had a reduced preference for effortful but large-reward action. By contrast, stimulant drugs such amphetamine or methylphenidate, but not modafinil, can increase the preference for effortful large-reward action. Furthermore, motivational states can influence effort-based decision making, e.g., a shift in motivational state by means of satiety manipulations reduced the preference of rats for effortful large-reward action. Theoretical accounts suggest that NAc DA could mediate effects of motivational states on effort-based decision, however, in rats with NAc DA depletion effort-based decision making was still sensitive to motivational shifts. Thus, NAc DA seems not to mediate effects of motivational shifts on decision making policies.

Cognitive versus physical effort: dopaminergic manipulation has dissociable effects on two rodent models of cost/benefit decision making

J.G. **Hosking**, P.J. Cocker, C.A. Winstanley

University of British Columbia / Psychology

Individuals are constantly confronted with decisions involving both benefits and associated costs, the effort to obtain a reward being one such cost. A substantial body of research using animal models has revealed contributions of dopamine and cortico-limbic-striatal circuits involved in effort-based decision making. However, these measures have only involved tasks that differed in the level of physical investment. Here we present a rodent cognitive effort task (rCET) that offers animals the choice of easy trials, where visuospatial attentional demand is low but the potential sugar reward is small, or difficult trials on which both the attentional demand and available reward are higher. The effects of experimental manipulation often depended on whether animals chose high-effort/high-reward options more ("workers") or less ("slackers") than the average. Amphetamine and caffeine caused workers to "slack off", whereas amphetamine caused slackers to work harder. When animals were switched from the rCET to a well-established physical effort task, animals transiently retained their worker/slacker distinction. Dopamine D1- and D2-specific antagonists had no effect on choice for the rCET, whereas they caused animals to shift away from the high-effort option on the physical effort task. Finally, inactivation of the basolateral amygdala had opposing effects on workers versus slackers, whereas anterior cingulate cortex inactivation decreased willingness to work in all animals. Altogether, these data suggest overlapping yet distinct circuitry for cognitive versus physical tasks, suggesting that our understanding of effortful decision making requires expanding our definition beyond purely physical costs.

Development of animal models of the effort-related motivational symptoms of depression: Dopaminergic Mechanisms.

John D. **Salamone,** Eric J. Nunes, Patrick A. Randall, Laura López-Cruz, Merce Correa

University of Connecticut / Psychology; University of Jaume I/Psychobiology

Mesolimbic dopamine (DA) is a critical component of the brain circuitry regulating behavioral activation and effort-related processes. Accumbens DA depletions or DA antagonism produces a shift in effort-related choice behavior, biasing animals towards selection of low effort options in choice tasks. It has been suggested that studies of effort-based choice behavior in animals could be related to symptoms such as psychomotor retardation, anergia, and fatigue, which can be observed in individuals with depression and other disorders. These studies investigated the effects of the catecholamine depleting agent tetrabenazine (TBZ) on effort-related choice behavior using the multiple behavioral procedures. TBZ inhibits the vesicular monoamine transporter 2 (VMAT-2) and is used to treat Huntington's disease, but also produces depressive side effects in some patients, including motivational symptoms. TBZ (0.25-1.0 mg/kg IP) decreased extracellular DA and altered DARPP-32 signaling in nucleus accumbens, and produced alterations in effort-related choice behaviors in rats tested in multiple procedures (i.e., decreased selection of high effort task components and increased selection of low-effort components). These effects of TBZ on effort-related choice behavior were reversed by co-administration of the adenosine A2A antagonist MSX-3 as well as the widely used antidepressant bupropion. Taken together, these results demonstrate that administration of low doses of TBZ can alter effort-related choice behavior, biasing animals towards low effort alternatives. The present research may contribute to the development of novel treatments for effort-related motivational symptoms in humans.

Effort-related DA function and Motivational deficits in Psychopathology

Michael T. **Treadway**, Maribeth Memmer, Rob Tennyson, Justin W. Martin, Richard C. Shelton, David H. Zald

Harvard Medical School / Psychiatry

The term anhedonia has long been used in the clinical literature to describe reward-processing dysfunction in psychopathology, especially in the disorders of major depression and schizophrenia. While anhedonia literally describes a lack of pleasurable experiences in everyday life, recent advances suggest that reward processing symptoms in these disorders are much broader than hedonic responsiveness, possibly involving deficits in motivation and cost/benefit valuation. In this talk, I will summarize my recent work on the neural underpinnings of motivation and effort-based decision-making in healthy and psychiatric populations. This research highlights the potential of translational neuroscience to enhance diagnostic clarity by defining clinical symptoms in terms of systems-level pathophysiology.

Dopamine, economic decision making and energy balance

Xiaoxi **Zhuang**

The University of Chicago / Neurobiology

Phasic dopamine release has been hypothesized to mediate reward prediction learning, based on the correlation between dopamine neuron firing and appetitive Pavlovian learning. To access causality, we used a genetics approach. The adenylyl cyclase type V (AC5) is a major dopamine receptor signaling molecule. AC5 deficient mice have severely impaired corticostriatal LTD. They acquire instrumental responding but are unable to form appetitive Pavlovian learning, suggesting a critical role of dopamine signaling in stimulus-reward contingencies. Tonic dopamine has been shown to modulate performance without altering learning. We evaluated the role of tonic dopamine in the hyperdopaminergic DAT knockdown mice in a seminaturalistic foraging environment, and to study dopamine's overall role in adaptive behaviors. Mice earned all of their food by pressing either of two levers, but the relative cost for food on each lever shifted randomly. We fit the lever choice data using reinforcement learning models. Hyperdopaminergic mice displayed normal learning but showed a reduced coupling between choice and reward history. These data suggest that tonic dopamine modulates the degree to which prior learning biases action selection. Mice with lower tonic dopamine bias their economic decisions towards exploiting available resources, whereas mice with higher tonic dopamine bias their decisions towards exploring the unknown, they have high behavioral energy expenditure and impaired energetic thriftiness. Interestingly, we found similar phenomena in DAT deficient Drosophila using a simple foraging assay, and mutant flies cannot survive due to impaired thriftiness. These studies set the stage for a genetic screen in Drosophila for thrifty genes.

THE MULTILINGUAL NATURE OF DOPAMINE NEURONS

Organizer: L.E. Trudeau (Canada)

Biophysical evidence for the vesicular co-packaging of glutamate with dopamine

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Using optogenetics and electrophysiology, we and others have demonstrated that mature midbrain dopamine neurons have the capacity to co-release glutamate onto medium spiny neurons in the ventral striatum. Further, co-release depends on the expression of the vesicular glutamate transporter (VGLUT2) in VTA dopamine neurons, which mediates the vesicular uptake of glutamate into synaptic vesicles. But are dopamine and glutamate released from the same synapses or do they segregate? Why is this question important? Indeed, immunochemical data suggest that VGLUT2 and the vesicular monoamine transporter (VMAT2) localize to an overlapping population of synaptic vesicles. This observation is supported by functional biophysical data showing that in synaptic vesicles isolated from the ventral striatum, vesicular dopamine uptake is increased by glutamate. Because the co-entry of an anion is necessary to establish the large vesicular pH gradient (□pH) required for optimal dopamine filling, we propose that glutamate co-entry facilitates the vesicular loading of dopamine into synaptic vesicles by increasing the □pH. Moreover, the □pH produced by glutamate is larger and more stable than that produced by the classic counter-ion chloride, suggesting glutamate co-entry confers unique properties to the subset of vesicles that contain both VMAT2 and VGLUT2. We thus conclude that VGLUT2 serves two roles in dopamine neurons, 1) allowing them to signal postsynaptically through the activation of fast ionotropic glutamate receptors, and 2) increasing dopamine quantal size by making a larger and more stable □pH.

Subcellular Segregation of Dopaminergic and Glutamatergic Signaling by VTA Neurons

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NIDA/IRP

The mesocorticolimbic dopamine system plays a role in reward, motivation, learning and memory. This system arises from dopamine neurons from the ventral tegmental area (VTA) that innervate medial prefrontal cortex (mPFC) and nucleus accumbens (nAcc). VTA dopamine neurons, containing tyrosine hydroxylase (TH), are interspersed with GABA neurons and glutamatergic neurons expressing VGluT2 mRNA. Most of VGluT2-mRNA neurons lack either TH or GABA markers (VGluT2-only neurons) in the lateral VTA, but there is a small subpopulation that co-expresses TH (VGluT2-TH neurons) in the midline VTA. By tract tracing and in situ hybridization, we found that VGluT2-only and VGluT2-TH neurons targeted mPFC and nAcc. By ultrastructural analysis, we found that VGluT2-axon terminals from either VGluT2-TH or VGluT2-only neurons established asymmetric synapses with dendritic spines or dendrites. Activation of VGluT2-terminals from any of these cells elicited excitatory postsynaptic currents in nAcc or mPFC. At the ultrastructural level, we did not find coexistence of dopaminergic-markers and VGluT2 within the same vesicle or same terminal. Instead, we found that VGluT2 was confined to terminals making asymmetric synapses and dopaminergic markers occasionally were found in the contiguous axon segment. Lack of VGluT2 within dopaminergic vesicles was further confirmed by co-immunoprecipitation. We conclude that VGluT2-TH and VGluT2-only neurons excite neurons within mPFC or nAcc, and that VGluT2-TH neurons have unanticipated ultrastructural-domains segregated for dopamine- or glutamate-signaling. Our ultrastructural and biochemical findings do not support the notion of dopamine and glutamate co-release from the same pool of vesicles.

The dopamine neuron functional connectome

S. **Rayport**

Columbia University and NYS Psychiatric Institute

To define the DA neuron functional connectome, we used DAT-IRES-cre mice to restrict expression of a conditional ChR2-EYFP vector to DA neurons. We first mapped DA neuron axons by EFYP fluorescence and then colocalization with tyrosine hydroxylase; the striatum, nucleus accumbens (NAc) and olfactory tubercle received the densest DA neuron innervation, the amygdala and entorhinal cortex a moderately dense innervation, the neocortex a sparse innervation, and the hippocampus a negligible innervation. Then we recorded from identified principal neurons in target regions with strong ChR2-EYFP-fluorescence and did wide-field photostimulation of DA neuron varicosities impinging on the recorded cell to measure the strength of DA neuron connections. We found that DA neurons made the strongest glutamatergic connections in the NAc shell, followed by olfactory tubercle and NAc core, medium strength connections in the central amygdala and entorhinal cortex, and weak connections in cingulate cortex. Within the striatum, we examined DA neuron connections to interneurons. DA neurons made strong monosynaptic connections to cholinergic interneurons (ChIs) with both glutamatergic and dopaminergic components. These connections showed regional heterogeneity, driving a burst-pause firing sequence in the NAc medial shell, which was strong enough to synchronize ChI firing, and a pause in the dorsal striatum. Conditional deletion of VGLUT2 in DA neurons eliminated glutamatergic cotransmission and made DA neuron actions homogeneous across the striatum. Amphetamine strongly and differentially modulated DA neuron connections to ChIs, pointing to the crucial role of the connections in DA neuron control of motivated behavior.

Regulation and developmental role of the vesicular glutamate transporter VGLUT2 in dopamine neurons

Louis-Eric **Trudeau**

Universite de Montreal / Pharmacology

We discovered that expression of the vesicular glutamate transporter VGLUT2 underlies the ability of dopamine neurons to release glutamate as a second neurotransmitter. Evaluation of the expression of VGLUT2 in dopamine neurons using single-cell RT-PCR and immunocytochemistry revealed that expression of this gene in dopamine neurons can be regulated by cell contact. In addition, we found that the VGLUT2 gene is expressed early during the development of dopamine neurons, suggesting a possible developmental role. Using a conditional knockout strategy to inactivate the VGLUT2 gene from mouse dopamine neurons, we find that indeed, early inactivation of this gene leads to reduced neurite outgrowth from cultured dopamine neurons, reduced dopaminergic axon terminal density in the nucleus accumbens in vivo and a reduced number of tyrosine hydroxylase-positive dopamine neurons, both in vitro and in vivo. These findings provide strong evidence for a functional role of the glutamatergic co-phenotype in the development of mesencephalic dopamine neurons, complementing a postnatal role in fast synaptic transmission.

Vglut2-mediated glutamate release in the ventral midbrain

Åsa **Wallén-Mackenzie**, Emma Arvidsson, Ernesto Restrepo, Nadine Schweizer, Thomas Viereckel, Stefano Pupe Johann

Uppsala University /

The mesostriatal dopamine system contributes to several aspects of responses to rewarding substances and is thus heavily implicated in conditions such as substance dependence. In the present study, we addressed the functional significance of the recently described Vglut2 expression in DA neurons for reward-related behavior. Cocaine-induced locomotion and behaviors motivated by cocaine, as well as by drug-paired cues, were analyzed by the conditioned place preference (CPP) and operant self-administration paradigms in mice lacking VGLUT2 in DA neurons. Operant response to high- and low-sucrose food was also analyzed. Mice in which Vglut2 expression in DA neurons had been abrogated displayed normal CPP, but a strongly increased operant self-administration of both high-sucrose food and intravenous cocaine, yet their consumption of low- sucrose food was unaltered. Cocaine-seeking maintained by cocaine-paired cues was increased by 76%, indicating an effect on reward-dependent plasticity. This study shows that targeted loss of Vglut2 expression leads to alteration in reward consumption and reward-associated memory formation, features of relevance for substance dependence. In addition to Vglut2-mediated glutamate/dopamine corelease by the midbrain dopamine neurons, we are currently addressing possibilities for other neuronal cell types to use Vglut2- mediated aspects of corelease.

SEROTONIN-DOPAMINE INTERACTION: NEW OPPORTUNITIES FOR IMPROVED TREATMENTS OF DOPAMINE-RELATED DISORDERS

Organizer: K.A. Cunningham (USA)

Oppositional Control of Dopamine-Mediated Behaviors by Serotonin 5-HT2A and 5-HT2C Receptors: Implications for Pharmacotherapeutics in Addictions

K.A. **Cunningham**, M. Filip, T.H. Carbonaro, and N.C. Anastasio

University of Texas Medical Branch / Polish Academy of Sciences

The behavioral effects of cocaine are modulated by the serotonin 5-HT2A receptor (5-HT2AR) and the 5-HT2CR. Here, we assessed whether the behavioral effects of direct dopamine (DA) receptor agonists will be affected by 5-HT2R ligands. We analyzed the ability of 5-HT2R ligands to modify the behavioral effects of the D1-like receptor (D1R) agonist SKF 82958 or the D2-like receptor (D2R) agonist quinpirole in rats. We found that the 5-HT2R agonist DOI and the 5-HT2CR antagonist SDZ SER-082 enhanced while the 5-HT2AR antagonist SR 46349B and the 5-HT2CR agonist MK 212 blocked hyperactivity evoked by SKF 82958 (1 mg/kg). Hyperactivity induced by quinpirole (0.5 mg/kg) was unaffected by the 5-HT2R ligands. In rats trained to discriminate SKF 82958 (0.06 mg/kg) from saline, cocaine partially substituted, but quinpirole and 5-HT2R ligands did not substitute; MK 212 and SR 46349B produced rightward shifts for SKF 82958. In rats trained to discriminate quinpirole (0.1 mg/kg) from saline, cocaine, SKF 82958 or the 5-HT2R ligands did not substitute; some doses of MK 212 and SDZ SER-082 decreased quinipirole substuition. This oppositional 5-HT2AR/5-HT2CR influence over behaviors induced by a direct D1R, but not D2R, agonist supports the hypothesis that the 5-HT2R can influence the output generated by direct stimulation of a DA receptor. We are investigating the impact of DA ligands on the effects of a selective 5-HT2CR agonist to establish the reciprocal nature of this interaction. These findings will be discussed in the context of addiction therapeutics.

5-HT6 receptor oppose dopamine in striatum

John F **Neumaier**

University of Washington / Psychiatry

5-HT6 serotonin receptors are densely localized in dorsal and ventral striatum and have been implicated in reward motivated learning and habit behaviors. We have previously found that increased 5-HT6 expression using viral mediated gene transfer in rats inhibits reward motivated learning (in the dorsomedial striatum) but facilitates behavioral flexibility in compulsively responding animals (in the dorsolateral striatum). Since these receptors are localized in medium spiny neurons that contribute to both direct and indirect pathways, as compared to dopamine receptors that are segregated between these pathways, we developed the hypothesis that 5-HT6 signaling in striatum tends to oppose the effects of dopamine by reducing the differential activation and inhibition of direct and indirect pathways by dopamine, respectively. We tested this by developing viral vectors that target the direct or indirect pathway selectively by using dynorphin or encephalin promoters to drive gene expression. We dissected the role of these receptors in striatum by expressing them in either one pathway or the other, and discovered that increased 5-HT6 signaling in direct pathway neurons of dorsomedial striatum facilitated learning whereas increased 5-HT6 signaling in indirect pathway neurons impaired learning. Finally, increased expression of 5-HT6 receptors in indirect pathway neurons of dorsolateral striatum facilitated behavioral flexibility in the omission training model in compulsively responding rats, supporting the idea that the key to understanding these receptors in striatum involves considering the effects of these receptors in direct or indirect pathways, where they have generally opposite effects on behavior. Thus, balanced activation of dynorphin or encephalin expressing neurons via 5-HT6 receptors tends to oppose the effects of dopamine in striatum by reducing the differential activation of direct and indirect pathways.

Biased agonism at pre or post-synaptic serotonin 5-HT1A receptors: therapeutic relevance for dopaminergic dysfunction.

A. **Newman-Tancredi** / A.C. McCreary

NeuroAct Communication

Serotonin 5-HT1A receptor activation facilitates dopamine neurotransmission in the prefrontal cortex (PFC), an effect associated with improved cognitive function. In fact, 5-HT1A receptor activation underlies some of the actions of clozapine and aripiprazole, and the more-recently identified drugs, lurasidone and cariprazine. However, 5-HT1A receptors are also expressed as autoreceptors in mid-brain raphe, where serotonergic cell bodies are located. Activation of pre-synaptic 5-HT1A autoreceptors elicits a decrease in serotonin release and may disrupt cognitive flexibility potentially via dopaminergic mechanisms. Recent findings show that 5-HT1A agonists display distinct signaling properties, preferentially activating specific G-proteins and/or effectors. Such "biased agonism" translates to accentuated effects on pre- or post-synaptic brain regions that express the relevant signaling mechanisms. At a neurochemical level, biased agonists therefore preferentially influence dopamine release versus serotonin release, depending on their pre- or post-synaptic 5 HT1A receptor balance. Such considerations indicate that it is possible to identify 5-HT1A biased agonists that preferentially favor cortical dopaminergic transmission without a generalized inhibition of serotonin release. F15599 is a prototypical 5-HT1A biased agonist. It exhibits a distinctive signal transduction profile in vitro and stimulates PFC pyramidal neuronal activity and dopamine release at low doses not inhibiting raphe serotonergic neuronal activity or hippocampal 5-HT release. F15599 also preferentially stimulates cortical c-Fos expression and attenuates phencyclidine-induced cognitive impairments. Biased agonism at post-synaptic 5-HT1A receptors therefore represents a promising therapeutic strategy to titrate cortical dopamine neurotransmission.

5-HT2B receptors-DA interaction: control of DA ascending pathways and therapeutic implications

SpampinatoU., Devroye C., Cathala A., Piazza P.V.

Bordeaux 2 University / INSERM U 862

Several studies have recently suggested that the central serotonin2B receptor (5-HT2BR) may represent a new pharmacological target for pathological conditions depending upon mesolimbic DA dysfunction, such as drug addiction. However, the role of 5-HT2BRs in the control of DA ascending pathways remains weakly investigated to date. This study was therefore aimed at evaluating the influence of selective 5-HT2BR antagonists (LY 266097, RS 127445) on DA release, measured, using in vivo microdialysis, in the rat striatum, nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). LY 266097 (0.63 mg/kg, i.p.) or RS 127445 (0.16 mg/kg, i.p.) had no effect on striatal DA release, but significantly reduced and increased basal DA release in the NAc and the mPFC, respectively. LY 266097, reduced significantly the increase in accumbal DA outflow induced by haloperidol (0.01 mg/kg, s.c.) administration, but failed to alter haloperidol-induced DA outflow in the striatum. Conversely, the effect of haloperidol on mPFC DA release was significantly potentiated by RS 127445 administration. These findings demonstrate that 5-HT2B receptors exert a region-dependent modulation of DA ascending pathways, by providing, specifically, no effect in the striatum, and opposite facilitatory and inhibitory controls on NAc and mPFC DA release. Also, they highlight the therapeutic potential of 5-HT2BRs for pathological conditions requiring an independent modulation of DA pathways, such as schizophrenia or Parkinson's disease.

5HT/DA system interactions in the reinforcing properties of psychostimulants: role of 5-HT6 receptors

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5-HT6 receptors have a distribution limited to the brain and largely superimposable to that of dopamine. This distribution predicts a role in reward, motivation and extrapyramidal motor functions, and an interaction with DA. The putative 5-HT6 receptor agonist ST1936 has been shown to increase extracellular dopamine (DA) in the nucleus accumbens (NAc) shell and in the medial prefrontal cortex (PFCX). These observations suggest that 5-HT6 receptors modulate DA transmission in mesolimbic and mesocortical terminal DA areas. Consistent with its ability to stimulate DA release preferentially in the NAc shell, ST1936 is self-administered iv by rats at unitary dose of 0.5-1 mg/kg on a continuous reinforcement schedule (FR1) and on a progressive ratio schedule, with a breaking point of about 4. Pretreatment with the 5-HT6 antagonist SB271046 reduced by about 80% responding for ST1936. On the other hand, the 5-HT6 receptor antagonist reduced by about 60% the effect of a single dose of cocaine (10 mg/kg/ip) on DA release in the shell but not in the PFCX. Moreover, SB271046 impaired iv cocaine SA and reduced cocaine intake and the breaking point in a progressive ratio schedule. These observations indicate that ST1936 behaves as a weak reinforcer and suggest that 5-HT6 receptors play a permissive role in cocaine reinforcement via their interaction with DA projections to the NAc shell. This novel 5-HT/DA interaction might provide a novel target for a pharmacotherapy of cocaine addiction.

DA RECEPTORS AND THEIR INTERACTIONS WITH OTHER MONOAMINE RECEPTOR MECHANISMS REMAIN A TARGET FOR TREATMENT OF SCHIZOPHRENIA

Organizer: K. Fuxe (Sweden)

D2 receptor heteromers in the ventral striatum as targets for novel antipsychotic drugs

K.**Fuxe**, D.O. Borroto-Escuela, W. Romero-Fernandez, F. Ciruela, A.O. Tarakanov, L. Ferraro, S. Tanganelli, M. Perez-Alea, L.F. Agnati

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Our working hypothesis is that several of the potential D2R heteromers in the ventral striatum especially A2AR-D2R, D2R-5-HT2AR and D2R-OxytocinR (OTR) heteromers may be targets for typical and atypical antipsychotic drugs. The activation of the A2AR and 5-HT2AR protomers of the respective heteromers should result in anti-psychotic actions in view of their antagonistic allosteric receptor-receptor interactions with ventral striatal D2R protomers, especially in combination with low doses of D2R antagonists. Instead the blockade of D2Rs in D2R-OTR heteromers by antipsychotic drugs should result in side-effects in view of the role of these heteromers in social behavior. On the basis of the existence of the antagonistic A2AR-D2R interactions, A2AR agonists were proposed and demonstrated to be atypical antipsychotic drugs. A2AR agonists counteract the D2R induced reduction of the glutamate drive from the mediodorsal thalamic nucleus to the prefrontal cortex via their reduction of D2R signaling in the nucleus accumbens. Furthermore, we demonstrated that the D2R and the 5-HT2AR form stable and specific heteromers in mammalian cells and exist in ventral and dorsal striatum. Activation of the 5-HT2AR protomer by 5-HT within this D2R heteromer led to inhibition of the D2R functioning, thus suggesting the existence of a 5-HT2AR-mediated D2R trans-inhibition phenomenon. Certain D2R heteromers may be dysfunctional in schizophrenia leading to removal of the brake on D2R signaling in the striato-pallidal GABA circuits and are new targets for antipsychotic drugs. Receptor-receptor interactions between cortical NA, 5-HT2A and DA receptors in potential heteromers should be considered as targets for novel antipsychotics.

Reversibility of antagonism at the dopamine D2 receptor - how different are typical and atypical antipsychotics?

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Current antipsychotics are antagonists or weak partial agonists at the dopamine D2 receptor (D2R) and their use is frequently associated with extrapyramidal symptoms (EPS) that are particularly pronounced with typical antipsychotic drugs such as chlorpromazine and haloperidol. The widely considered "fast-off hypothesis" suggests that the lower EPS liability of atypical antipsychotics, including clozapine and amisulpride, is related to their faster rates of dissociation from D2R. Fast dissociation would produce rapid reversibility of antagonism, preserving the physiological dynamics of D2R signaling. While antipsychotic dissociation kinetics have been extensively characterized in radioligand experiments, the functional reversibility of D2R antagonism has received little attention. To examine the fast-off hypothesis in the context of signaling, we used an electrophysiology-based assay to compare the reversibilities of D2R antagonism by several clinically used or experimental antipsychotics. The assay is based on D2R-evoked activation of G protein-coupled potassium channel (GIRK) currents in Xenopus oocytes, and provides a greater temporal resolution than previous studies. The rates of recovery of D2R signaling from antagonism by ACR16, (-)-OSU6162, and remoxipride were four- to ten-fold faster than that of clozapine, whereas recovery from clozapine antagonism, unexpectedly, was less than two-fold faster than from chlorpromazine. Furthermore, the extent of response of recovery observed with different compounds correlated negatively with drug lipophilicity, suggesting that antipsychotics might differentially partition into cell membranes. The present data do not support the notion that the rate of reversibility of D2R antagonism is the distinguishing feature of atypical vs. typical antipsychotics.

Dopamine, D1 receptors and noradrenergic mechanisms in the mode of action of antipsychotic drugs

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Clinical data show a reduced cortical DA release in schizophrenia (SZ), contrasting the enhanced striatal DA release that correlates with psychosis. A reduced prefrontal DA release may impair cognition by insufficient D1-R signaling, as D1-R agonists reverse working memory (WM) deficits induced by the NMDA-R antagonist ketamine. Both clozapine (CLOZ) and quetiapine may improve WM in SZ. This work aimed to elucidate the role of noradrenergic mechanisms and D1-Rs in their modes of action. Since the clinical effects of quetiapine are partly mediated by its metabolite norquetiapine, which is not formed in rodents and is a potent NET inhibitor, we compared the behavioral and neurobiological effects of CLOZ and a combination of quetiapine and the selective NET inhibitor reboxetine (REB) with those of raclopride (RAC), a typical D2-R antagonist. In contrast to RAC, both CLOZ and the combination of quetiapine and REB effectively suppressed CAR at low D2 occupancy levels, enhanced DA outflow in the medial prefrontal cortex (mPFC) and, via D1-R activation, facilitated NMDA-R mediated transmission in this region. CLOZ also reversed the WM impairment induced by the NMDA-R antagonist MK-801. The effects of CLOZ were mimicked by a combination of RAC and idazoxan, an alpha2R antagonist, and those of quetiapine plus NET inhibition by a combination of RAC and REB. Our results implicate brain noradrenergic mechanisms in the modes of action ofCLOZ and quetiapine. A recent clinical meta-analysis (Hecht & Landy 2012) provides further support for he utility of noradrenergic targets, e.g. alpha2 antagonists, in antipsychotic therapy.

Neurotensin receptor NT1 and dopamine receptor D2 heteromers as a target for antipsychotic drugs

Sergio **Tanganelli**, Tiziana Antonelli, Maria C. Tomasini, Sarah Beggiato, Kjell Fuxe, Luigi F. Agnati, Luca Ferraro

Universita' Degli Studi Di Ferrara / Dipartimento Di Scienze Mediche

The tridecapeptide neurotensin (NT) acts in the mammalian brain as a primary neurotransmitter or neuromodulator of classical neurotransmitters. Morphological and functional in vitro and in vivo studies have demonstrated the existence of close interactions between NT and dopamine both in limbic and in striatal brain regions. Additionally, biochemical and neurochemical evidence indicates that in these brain regions NT plays also a crucial role in the regulation of the aminoacidergic signalling. It is suggested that in the nucleus accumbens the regulation of prejunctional dopaminergic transmission induced by NT may be primarily due to indirect mechanism(s) involving mediation via the aminoacidergic neuronal systems with increased glutamate release followed by increased GABA release in the nucleus accumbens rather than a direct action of the peptide on accumbens dopaminergic terminals. The neurochemical profile of action of NT in the control of the pattern of dopamine, glutamate and GABA release in the nucleus accumbens differs to a substantial degree from that shown by the peptide in the dorsal striatum. The neuromodulatory NT mechanisms in the regulation of the ventral striato-pallidal GABA pathways are discussed and their relevance for schizophrenia is underlined.

DA ROLE IN THE ADDICTED BRAIN: FROM SYNAPSES TO HUMAN BRAIN IMAGING

Organizer: A. Bonci (USA)

Optogenetic control of the prelimbic cortex: implications for cocaine craving

Antonello **Bonci** and Billy Chen

Intramural Research Program, NIDA/NIH

The dopaminergic neurons, originating in the ventral tegmental area (VTA) and projecting to forebrain areas, including the amygdala, prefrontal cortex, and nucleus accumbens (NAc) are essential for the manifestation of goal-directed behavior for both natural rewards as well as drugs of abuse, including ethanol. My laboratory has spent the past several years to elucidate the role of plasticity at excitatory synapses in the VTA in physiological and pathological behaviors, such as reward-related learning and excessive drug consumption. Recently, my laboratory has been using a multidisciplinary approach combining electrophysiology, optogenetic and behavioral procedures to better investigate the role of the dopaminergic signaling on ethanol as well as substance use disorders. During my presentation at the DA 2013 meeting, I will show our most recent optogenetic, electrophysiological and behavioral studies focused on the role of prelimbic neurons in modulating cocaine seeking.

Prelimbic cortex and ventral tegmental area modulate nucleus accumbens core synaptic plasticity during cue- and cocaine-reinstated drug seeking

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Medical University South Carolina / Neurosciences

Addiction to cocaine produces long-lasting, stable changes in brain synaptic physiology that might contribute to the vulnerability to relapse. However, it is not known if synaptic changes are initiated by and contribute to relapse. Cues associated with cocaine use and re-exposure to cocaine can precipitate relapse, and using a rat model of cue- and cocaine-induced relapse, we quantified two measures of synaptic plasticity in the nucleus accumbens core (NAcore) - morphological changes in dendritic spine head diameter and electrophysiological estimates of excitatory synaptic transmission (AMPA:NMDA ratio). Both re-exposure to cocaine and the presentation of cocaine-conditioned cues elicited rapid and reversible increases in spine head diameter and AMPA:NMDA ratio, although cues elicited this change within 15 min of reinstated cocaine-seeking, whereas cocaine prime elicited this change at 45 min following the priming injection. We then determined how the mesocorticolimbic afferents to the NAcore modulate synaptic plasticity during reinstated cocaine seeking. Inhibiting prelimbic (PL) cortex glutamatergic inputs to the NAcore prevented reinstatement in cue- and cocaine-primed reinstatement, but differentially inhibited and potentiated, respectively, NAcore synaptic strength and spine size. Inhibiting ventral tegmental area (VTA) inputs to the NAcore, however, abolished both cocaine-primed reinstatement and synaptic strength and spine head diameter. These results show that rapid synaptic potentiation in the NAcore may underpin relapse to cocaine use, and PL glutamatergic and VTA dopaminergic afferents to the NAcore may underlie synaptic adaptation induced by drug-seeking behavior.

The role of dopamine in addiction: Neuroplasticity from the dark side.

George F. **Koob**

CNAD, The Scripps Research Institute

Chronic administration of all drugs of abuse results in compromised reward system function and compulsive use is associated with dysregulated dopamine function. Current research in our laboratory has identified two neuroadaptive mechanisms from the brain stress systems that may contribute to longterm dopamine dysregulation: activation and recruitment of CRF neurons in the ventral tegmental area and activation of dynorphin-kappa opioid systems in the ventral striatum. Both CRF and kappa opioid antagonists administered systemically block the compulsive drug and alcohol seeking associated with extended access or exposure to the drugs. The sites for the actions of CRFantagonists include the central nucleus of the amygdala but also the ventral tegmental area. The sites for the actions of kappa antagonists include the ventral striatum. Together the results suggest that neuroplasticity related to CRF and dynorphin may drive within system neuroadaptations in the brain reward circuits in addition to between system neuroadaptations in the brain stress systems in the development of addiction.

Mechanisms of synaptic plasticity in addiction and depression

Scott **Russo**

Neuroscience/Mount Sinai School of Medicine

Depression and addiction are thought to involve lasting changes in synaptic structure and function of reward neurons in the mesolimbic dopamine system, although the mechanisms and behavioral relevance are unknown. Biochemical and transcriptional profiling of the nucleus accumbens (NAc) for RhoGTPases, known regulators of synaptic structure, following chronic social defeat stress or cocaine, revealed that both robustly down-regulate Rac1 activity or expression. Using targeted viral gene transfer and Rac1 conditional mice we show this adaptation is necessary and sufficient for depression- or addiction-like behavior and the formation of immature excitatory spines by redistributing synaptic cofilin, an actin severing protein downstream of Rac1. Our data identifies a converging role for Rac1 in regulating stress- and addiction-related behaviors and synaptic plasticity, which may explain the high degree of co-morbidity of these disorders.

The Role of Dopamine in the Addicted Human Brain

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Addiction affects multiple neurobiological substrates in the brain. We have used neuroimaging technology (PET and fMRI) paired with preclinical studies and optical imaging to investigate the role of dopamine in the brain functional changes that occur in addiction. Although large and rapid increases in dopamine have been linked with the rewarding properties of drugs, the addicted state, in striking contrast, is marked by significant decreases in striatal dopamine D2 receptor (D2R) mediated signaling both at rest and during drug intoxication. At rest, striatal D2R decreases are associated with reduced glucose metabolism (marker of activity) in prefrontal regions including orbitofrontal cortex, cingulate gyrus and dorsolateral prefrontal cortex, which could underlie the associated phenotype of impulsivity and compulsive drug intake. Upon challenge with a stimulant drug (methylphenidate or MP) the increases in DA are markedly attenuated in addicted individuals and in alcoholics. Moreover in cocaine abusers MP-induced DA stimulation of striatal D2 receptors (measured as their occupancy by DA) was markedly attenuated even when MP was given with concomitant exposure to cocaine-cues. In contrast MP significantly decreased resting functional striato-cortical connectivity in cocaine abusers. In parallel optical imaging studies in mice that express GFP in D2R neurons we corroborated that repeated cocaine decreases the sensitivity of D2R-expressing neurons to cocaine. Inasmuch as D2 receptor signaling attenuates cocaine's rewarding effects this could contribute to cocaine's enhanced incentive value in cocaine abusers whereas the associated decreases in striatal-frontal connectivity during stimulant intoxication could facilitate their compulsive drug use.

BEATING AND BURSTING:

THE JOYFUL NOISE OF DOPAMINE NEURONS

Organizer: P. Shepard (USA)

Analysis of depolarization block and bursting in dopamine neurons.

Carmen **Canavier**, Kun Qian, Na Yu, Kristal Tucker, Ed Levitan

LSUHSC / Cell Biology and Anatomy

Depolarization block limits the maximum steady firing rate of dopamine neurons in vitro to about 10 Hz. We confirm experimentally our previous modeling predictions that decreasing the sodium conductance pharmacologically causes dopamine neurons to go into depolarization block with lower maximal frequencies at lower values of applied current, whereas augmenting this conductance using the dynamic clamp has the opposite effect. Using a reduced model that faithfully reproduces the sodium current measured in these neurons, we show that adding an additional slow component of sodium channel inactivation, recently observed in these neurons, changes the mechanism by which the model enters depolarization block and imposes a firing rate limitation consistent with the data. We demonstrated a switch between these two mechanisms in actual dopamine neurons by selectively removing the slow component of inactivation using the Dynamic Clamp. We next show that in a more biophysically detailed model of a dopamine neuron, the plateau potentials observed in the presence of SK channel blockers also depend upon the slow component of sodium channel inactivation. Furthermore, blocking the ERG K+ channel accelerates entry into and retards recovery from depolarization block in the model due to the loss of the hyperpolarizing current that flows through these channels after an action potential, removing sodium channel inactivation. Our modeling results suggest that activation of NMDA receptors may contribute to circumventing the firing rate limitation during behaviorally relevant, high frequency bursts in vivo.

Dopamine Beat Box

Kjartan **Herrik**

Lundbeck

The 'sound' of a dopamine neuron is a hallmark feature when identifying dopamine neurons electrophysiologically in the laboratory. But the dopamine beat is more than just a characteristic of this specific cell type. Changes in the beat from regular to bursty appear to encode the salience of incoming stimuli and have profound downstream effects in terminal areas. Dopamine neurons in the SNc release dopamine in the striatum in an activity dependent way. Increases in firing rate lead to increased release from dopamine terminals. Changing their firing pattern towards more bursting induces temporal variations in local [DA], which may differentially affect dopamine receptors with low and high affinity. Burst firing favors the lower affinity D1 dopamine receptors; whereas tonic firing favors the higher affinity D2 dopamine receptors. Small conductance Ca2+ activated potassium channels play a central role in shaping dopamine neuron action potentials as well as their firing pattern. SK channels are voltage insensitive and activate when the intracellular Ca2+ concentration increases. Thereby, SK channels function to provide negative feedback to increased cellular activity. SK channels may be regulated through phosphorylation, internalization or pharmacological intervention. Inhibiting SK channel conductance with pore blockers or negative allosteric modulation induces irregular or burst firing activity, whereas positive modulation of SK channels increases the amplitude and duration of afterhyperpolarizations leading to increased firing regularity or even silencing of neuronal activity. This talk will exemplify how SK channels in dopamine neurons shape firing rate and pattern and how SK channel modulation may affect release and modulate behavior. The scope is to think of the dopamine neuron as a beat box instead of a black box.

Dopamine neurons require two inputs for bursting

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Dopaminergic cells integrate complex environmental stimuli from a variety of different afferents and switch from a tonic firing state to firing a discrete burst of action potentials. We report that specific activation of glutamatergic afferents originating from either subthalamic nucleus (STN) or pedunculopontine nucleus (PPN) fails to produce bursting, but concurrent STN and PPN activation reliably drives bursting. The two inputs provide mechanistically distinct types of glutamatergic drive to dopaminergic neurons: STN input is mediated solely by GluR2-lacking AMPA receptors, while PPN input is mediated by both NMDA receptors and GluR2- containing AMPA receptors. Injection of parametrically-controlled, whole-cell currents with dynamic clamp reveals that coincident STN and PPN activation results in prolonged NMDA receptor-mediated current decay kinetics and the capacity for a temporal summation of inputs that underlies bursting. Our results illustrate a mechanism for functionally parsing glutamatergic drive arriving from distinct nuclei, and support a role for dopaminergic neurons as AND operators that integrate glutamatergic inputs to produce phasic bursts in firing.

Activity of dopaminergic neurons in freely moving rats: a new insight into the dynamics of the ventral tegmental area.

Vincent **Seutin**

Giga-Neurosciences, University of Liège, Belgium

Although much is known about dopamine neuron physiology in slices and anaesthetized rodents, data about their activity in behaviorally relevant conditions is scarce. Using telemetric recordings, we have recently found that their reactivity to passive cocaine administration is drastically different in awake vs anaesthetized rats. We also observe that the second to second variability in their firing rate and pattern is extremely large in awake animals and correlates to some extent with some frequencies of local field potentials that can also be observed in the region (see Fujisawa and Buczaki, 2011). Among other projects, we are currently trying to quantify relationships between single-cell firing and these local field potentials.

Ether-a`-go-go-related gene potassium channels in midbrain dopamine neurons: Implications for a role in depolarization block

H Ji; KR Tucker; I Putzier; MA Huertas; JP Horn; CC Canavier; ES Levitan and PD **Shepard**

Dept Psychiatry, University of Maryland School of Medicine; Depts Pharmacology, Chemical Biology and Neurology University of Pittsburgh and Department of Cell Biology and Anatomy, LSU Health Sciences Center

Antipsychotic drugs exert their therapeutic effects by inhibiting dopamine receptors, but are also known to produce side effects in the heart by inhibiting cardiac ether-a-go-go related gene (ERG) K+ channels. Recently, it has been discovered that these channels are also present in the brain, where they are expressed in midbrain dopamine neurons. Here, we report that selective ERG channel blockade increases DA cell excitability and accelerates onset of depolarization inactivation during bursts induced by blockade of Kca2.3 channels or elicited by virtual NMDA receptors. In vivo, somatic ERG blockade was associated with an increase in spontaneous bursting activity that was attributed to a reduction in doublet firing. These results indicate that ERG K+ channels in dopamine cells play a prominent role in limiting neuronal excitability and in minimizing depolarization inactivation. As the therapeutic actions of antipsychotic drugs are associated with depolarization inactivation of dopamine neurons and blockade of cardiac ERG channels is a prominent side effect of these drugs, ERG channels in the central nervous system may represent a novel target for antipsychotic drug development.

NEUROENDOCRINE DOPAMINE NEURONS: PHYSIOLOGY, REGULATION AND CLINICAL ROLE

Organizer: C. Broberger (Sweden)

ELECTROPHYSIOLOGY OF TUBEROINFUNDIBULAR DOPAMINE (TIDA) NEURONS: NETWORK PROPERTIES AND MODULATION

David Lyons, Virginie Briffaud, Stephanos Stagourakis, Arash Hellysaz, Christian **Broberger**

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Pituitary release of the hormone, prolactin, is controlled by tonic inhibition from hypothalamic neuroendocrine tuberoinfundibular dopamine (TIDA) neurons. We recently demonstrated that TIDA neurons discharge in a synchronized gap junction-dependent oscillation. This finding begs the question: are the network properties of TIDA neurons a target for neuromodulation in prolactin release, and if so, through what mechanisms? Whole-cell patch clamp recordings were performed on slice preparations of the male rat mediobasal hypothalamus, and ion conductances were identified by pharmacological manipulation and ion substitution experiments. Thyrotropin-releasing hormone, a stimulator of prolactin release, was found to depolarize TIDA cells, shifting phasic to tonic firing, by activating a TRP-like conductance. Administration of prolactin itself caused a similar TRP-dependent depolarization, but in addition also spike broadening through inhibition of a Ca2+-dependent K+ conductance. Thus, negative prolactin feedback may involve causing TIDA cells to discharge not only more, but also more efficient action potentials, leading to higher dopamine release. Finally, local application of serotonin, another transmitter that stimulates prolactin release, caused TIDA neurons to hyperpolarize, abolishing the oscillation and all action potential discharge. These actions were the result of the opening of G-protein coupled inwardly rectifying K+ channels. These studies have identified a novel circuit-based control of prolactin that suggests novel therapeutic targets for hyperprolactinaemia and related reproductive disorders. By changes in the discharge pattern and action potential waveform of TIDA neurons, pituitary concentration of dopamine may be tuned to adjust serum prolactin levels to reproductive demands.

Signal transduction pathways mediating prolactin-induced activation of tuberoinfundibular dopamine neurons

DR **Grattan**, SJ Bunn, SH Yip

Anatomy/University of Otago

The tuberoinfundibular dopamine (TIDA) neurons, located in the arcuate nucleus of the hypothalamus, represent the major system controlling prolactin secretion from the anterior pituitary gland. Dopamine is released from nerve terminals located in the median eminence, and is transported by a portal blood system to the pituitary, inhibiting prolactin secretion. In a classical negative feedback system, prolactin acts directly on the TIDA neurons to increase the synthesis and release of dopamine, thereby inhibiting its own secretion. Prolactin action on these neurons is mediated through the prolactin receptor, a member of the cytokine receptor family. As such, the predominant mode of signalling is through the JAK/STAT pathway, particularly involving STAT5b. This is a transcriptional regulatory pathway, and appears to be essential for the regulation of TIDA neurons by prolactin, likely through the regulation of tyrosine hydroxylase gene expression. In addition to this transcriptional regulation, however, prolactin also induces very rapid actions on the spontaneous firing rate of these neurons, mediated through a less well-defined pathway. These signaling pathways exhibit remarkable plasticity during late pregnancy and lactation. The prolactin negative feedback system is altered, and while the TIDA neurons remain responsive to prolactin, they no longer produce dopamine and begin to synthesize enkephalin. The appears to involve a change in the balance of signalling through the JAK/STAT and through a MAP kinase pathway involving Erk1/2. This change allows a prolonged increase in prolactin secretion that is critical for milk production and maternal behaviour.

TIDA neurons plasticity : The functional switch.

Nicola Romano, Anne Guillou, Xavier Bonnefont, Patrice Mollard, Agnès O.**Martin**,

Institut de génomique fonctionnelle

TIDA neurons are the central regulators of prolactin (PRL) secretion. Their extensive functional plasticity allows a change from low PRL secretion in the non-pregnant state to the condition of hyperprolactinemia that characterizes lactation. To allow this rise in PRL, TIDA neurons are thought to become unresponsive to PRL at lactation and functionally silenced. We demonstrated that TIDA neurons displayed network organization of their electrical activity that was unchanged during lactation. Furthermore, the neurons remained electrically responsive to a PRL stimulus. PRL-induced secretion of dopamine (DA) at the median eminence, however, was strongly blunted during lactation. This suggest that lactation, rather than involving electrical silencing of TIDA neurons, represents a condition of decoupling between electrical activity at the cell body and DA secretion at the median eminence. This functional switch is indispensable for successfull lactation to be achieved. This unique physiological plasticity takes place in context where the whole organism translates and enter into a new physiology.

Electrophysiological Studies of TIDA Neurons From Female Rodents

P**. Velez**/ C. V. Helena/ A. M. Stathopoulos/ P. Fletcher/ J. Tabak/ P. Q. Trombley/ R**.** Bertram

Neuroscience/Florida State University

We use the loose patch technique to record the electrical activity of tuberoinfundibular (TIDA) neurons from the arcuate nucleus of female rodents. These neurons are known to play a key role in the regulation of prolactin secretion from pituitary lactotrophs, and to be stimulated by prolactin at fast, intermediate, and slow time scales. Recent intracellular recordings from the Broberger lab have focused on male rats. Our focus is on the female rodent, in which prolactin secretion patterns, and presumably regulation by TIDA neurons, is very different. We discuss our recent data on the spontaneous activity of these neurons and how this is modulated by endogenous agents.

A-SYNUCLEIN INDUCED TOXICITY: SYNAPTIC DYSFUNCTION AND NEURODEGENERATION IN DOPAMINE NEURONS

Organizer: A. Björklund (Sweden)

Validation of therapeutic targets against alpha-synuclein toxicity in vivo

Mickael **Decressac**

Neurobiology, Lund University, Sweden

The lack of a relevant model of the Parkinson's disease impedes the development of effective neuroprotective or rescuing therapies. Alpha-synuclein is known to play a central role in the PD pathogenesis. Using viral vector delivery of the disease-causing protein, we recently characterized a novel rat model alpha-synuclein-induced neurodegeneration that replicates the progressive nature of the human condition, allowing to study of stage-dependent pathological mechanisms and to test disease-modifying strategies in a context more relevant to the human pathology. Using this model, we identified important pathways affected by alpha-synuclein toxicity, which were interestingly also impaired in PD patients. Notably, processes involved in the clearance of the disease-causing protein are altered and drive the affected neurons into a vicious circle leading to their death. We identified therapeutic targets to counteract alpha-synuclein toxicity and afforded recovery. This pre-clinical model provides a useful tool for a better understanding of mechanisms underlying alpha-synuclein-induced neurodegeneration and for the development of disease-modifying strategy potentially successful in clinic.

Extracellular alpha-synuclein induces neuronal dysfunction and toxicity

Tiago Fleming **Outeiro**

University Medical Center Goettingen / Neurodegeneration and Restorative Research

Parkinson's disease is the most common representative of a group of disorders known as synucleinopathies, in which misfolding and aggregation of alpha-synuclein (a-syn) in various brain regions is the major pathological hallmark. Here, we conducted an RNAi-based genetic screen to identify novel modifiers of a-syn oligomerization in living cells, a step that precedes the formation of larger Lewy-body like inclusions. We found that genes involved in trafficking and in signalling pathways are potent modulators of a-syn oligomerization. In addition, we investigated the impact of different a-syn species (monomers, oligomers, and fibrils) on readouts of synaptic transmission and plasticity. We found that a-syn oligomeric species, but not monomers or fibrils, caused an enhancement of synaptic transmission and impairment of long-term potentiation in Schaffer-collaterals/CA1 connections through a mechanism dependent on NMDA receptor activation. Moreover, a-syn oligomers caused an increase in synaptic calcium-permeable AMPA receptor expression. Since excitotoxicity is implicated in neurodegeneration, our findings shed light into the mechanisms through which specific forms of a-syn may trigger neuronal dysfunction and therefore enable the development of novel strategies for therapeutic intervention.

Autophagy and Neurodgeneration

David C. **Rubinsztein**

Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0XY, U.K.

Intracellular protein misfolding/aggregation are features of many currently incurable late-onset neurodegenerative diseases, like Alzheimer's disease, Parkinson's disease and polyglutamine expansion diseases like Huntington's disease (HD) and various spinocerebellar ataxias. The mutations causing many of these diseases confer novel toxic functions on the target proteins. We showed that the autophagy inducer, rapamycin, reduced the levels of mutant huntingtin and attenuated its toxicity in cells, and in Drosophila and mouse HD models. We have extended the range of intracellular proteinopathy substrates that are cleared by autophagy to other related neurodegenerative disease targets including forms of alpha- synuclein and have provided proof-of-principle in cells, Drosophila and mice. In order to induce autophagy long-term, we have been striving to identify safer alternatives to the mTOR inhibitor, rapamycin. To this end, we have been trying to discover novel components of the autophagy machinery and new signalling pathways and drugs that impact on autophagy. I will discuss our drug screens that have yielded new insights into mTOR-independent autophagy pathways and how we have validated hits from such screens in cell-based, Drosophila, zebrafish, and mouse models of disease. While autophagy induction is protective in models of various neurodegenerative diseases, certain other conditions, including alpha- synuclein excess, are associated with compromised autophagy. I will review these data and then describe how impaired autophagy compromises cellular processes, including the ubiquitin-proteasome system.

Alpha-synuclein regulates synapsin I and III in developing and mature dopaminergic neurons: implications for Parkinson's disease

A. Bellucci [1], C. Missale [1] and P.F. **Spano** [1,2]

[1] *Division of Pharmacology, Department of Molecular and Translational Medicine and National Institute of Neuroscience, University of Brescia, Brescia, Italy;* [2] *IRCCS S. Camillo Hospital, Venice, Italy*

Emerging evidence indicates that alpha-synuclein (a-Syn) shares numerous biochemical and functional similarities with synapsins. Likewise a-Syn, synapsin I is a member of dopamine transporter (DAT) interactome (Maiya et al., 2007), and synapsin III negatively regulates striatal DA release (Kile et al., 2010). We thus investigated whether the lack of a-Syn may coincide with specific changes of synapsin I and III expression and distribution in developing and mature DA terminals.

We found that the expression and distribution of DAT, synapsin I and III were changed in developing and mature striatal dopaminergic terminals lacking a-Syn. The distribution of these proteins in neuronal cells lacking a-Syn was similar to that observed in primary dopaminergic mesencephalic neurons exposed to a-Syn pro-aggregating insults. The lack of a-Syn coincided with changes in synaptic vesicle pool arrangement. Consistently, a-Syn null mice, when compared to control mice, showed significant differences in DA release, as observed by microdialysis, and age-related divergences in the locomotor responses to the administration of cocaine, a DAT blocker which is able to increase DA release by mobilitating synapsin-dependent reserve pool.

Our findings indicate that a-Syn controls dopaminergic synapse arrangement by regulating the expression and distribution of synapsins both during embryonic and postnatal development and adulthood. Furthermore, a-Syn overexpression and aggregation can alter synapsin III expression and distribution thus suggesting that specific alterations of this protein may occur in the early stages of PD when AS aggregation likely coincides with a loss of function of the protein.

MOLECULAR DETERMINANTS OF D2 AND D3 RECEPTOR SELECTIVITY AND EFFICACY

Organizer: D.R. Sibley (USA)

Identification of a bitopic ligand that acts allosterically across a dopamine D2 receptor dimer

J.Robert **Lane**, Prashant Donthamsetti, Jeremy Shonberg, Samuel Dentry, Mayako Michino, Lei Shi, Laura López, Peter J. Scammells, Ben Capuano, Patrick M. Sexton, Jonathan A. Javitch, Arthur Christopoulos

Monash Institute of Pharmaceutical Sciences

SB269652 is the first drug-like allosteric modulator of the dopamine D2 receptor (D2R), a prototypical Family A G protein-coupled receptor (GPCR). However, this small molecule contains a number of structural features associated with orthosteric D2R antagonists. To understand its mechanism of action, we synthesized truncated derivatives of SB269652 and characterized their interaction with the D2R using both functional and radioligand binding assays. By combining this chemical biological approach with analytical methods for quantifying drug action, we identified two key components of SB269652, a purely orthosteric pharmacophore and a purely allosteric pharmacophore, indicating that the parent molecule represents a hitherto-unappreciated bitopic (dual orthosteric/allosteric) ligand. We subsequently used a novel functional D2R complementation system to control the identity of the individual protomers comprising a dimeric D2R signalling unit. Mutational impairment of ligand binding to one protomer converted the interaction between the bitopic molecule and dopamine into simple competition. We infer that SB269652 exerts its cooperative effect by binding in a bitopic mode within one protomer but allosterically modulating the other. Demonstration of allosteric modulation of dopamine function by SB269652 in rat striatum is consistent with the presence of D2R dimers in this tissue and highlights that this approach can be used to chemically differentiate GPCR oligomerization status in native tissue.

Molecular Determinants of Selectivity and Efficacy at the Dopamine D3 Receptor

Amy Hauck **Newman**, Ashwini K. Banala, Prashant Donthamsetti, Mayako Michino, Robert R. Luedtke, Jonathan A. Javitch, Lei Shi

NIDA-IRP, NIH / Molecular Targets and Medications Discovery Branch, Center for Molecular Recognition and Departments of Psychiatry and Pharmacology, Columbia University College of Physicians and Surgeons, New York, New York, USA, Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, Texas, USA, Department of Physiology and Biophysics and Institute for Computational Biomedicine, Weill Medical College of Cornell University, New York, NY, USA.

The dopamine D3 receptor (D3R), a member of the dopamine D2 receptor family, has been investigated as a potential target for medication development to treat substance use disorders (SUDs) with a particular focus on cocaine and methamphetamine. The primary distribution of D3R in limbic regions of the brain, especially the nucleus accumbens has fortified interest in the D3R as a potential target for drug discovery. However, the high degree of sequence identity within the transmembrane (TM) segments of the D2-like receptors and the near-identity of the residues that form the orthosteric binding site (OBS) in these receptors have made it challenging to create subtype-selective agents that possess physicochemical properties suitable for in vivo characterization of their physiological roles. Nevertheless, D3R selective compounds such as SB 277011A, NGB 2904, BP 897 and PG01037 have provided both critical tools for further characterization of D3R in addiction and pharmacophoric templates for the evolution of subsequent generations of D3R-selective agents. Structure-activity relationships (SAR) have been derived through extensive medicinal chemistry efforts, resulting in highly potent and D3R-selective agents with varying intrinsic activities. We have recently taken the approach of deconstructing several D3R-selective substituted-4-phenylpiperazine antagonists and partial agonists into pharmacophoric elements. Using computational simulations based on the D3R crystal structure, and binding and functional assays, we have begun to dissect the structural bases for D3R selectivity and efficacy. Our findings reveal structural features of the receptor that are critical to selectivity and efficacy that can be used to design highly D3R-selective ligands with targeted efficacies.

The D3R from discovery to clinical trials indrug abuse with BP1.4979, a partial agonist

Jean-Charles **Schwartz**

BIOPROJET / Univ. René Descartes

From its discovery, the striking localisation of the D3R, restricted to reward brain areas, suggested the therapeutic interest of ligands in drug abuse. This hypothesis was confirmed with the design of BP897, a partial agonist with an acceptable D3R/D2R selectivity ratio, and the demonstration of its activity in a rat model of cue-induced cocaine self-administration (Pilla et al Nature, 1999, 400, 374). Our development of BP897 development in smoking cessation was interrupted for safety reasons but we continued the search for a successor, synthesizing and testing over thousand compounds, finally succeeding with the identification of BP14979. BP1.4979 is a D3R partial agonist (i.a. 0.3) with subnanomolar potency and over 200-fold D3/D2 selectivity. It is orally active, brain penetrating and inhibits the expression of cocaine or nicotine place preference in rats at 0.01-0.1 mg/kg doses and cocaine self-administration in monkeys at 0.3 mg/kg. BP1.4979 showed good tolerance in regulatory toxicity and pharmacological safety studies, and in Phase I trials; its half-life indicates a single oral administration per day. Proof of concept was obtained in heavy smokers (?20 cigarettes/day) at 10 mg/day and Phase II trials are progressing in smoking and cocaine cessation.

Identification of a second binding pocket for the subtype selectivity at dopamine D3 and D2 receptors

Lei **Shi**

Weill Medical College of Cornell University / Physiology and Biophysics

Antipsychotic drugs block dopamine D2-like receptors (D2R, D3R, and D4R) non-selectively, but it is generally thought that antagonism of D2R is essential for therapeutic efficacy. On the other hand, antagonists of the cognate D3R have been proposed as potential medications for drug abuse. However, side effects can be a serious issue for the drugs targeting D2-like receptors due to non-selective blockade of the receptors and thereby their downstream effectors. In addition, the full therapeutic potential of these targets has been difficult to explore rigorously due to the lack of selective drugs with appropriate physicochemical properties and pharmacokinetics for in vivo selectivity. The crystal structure of D3R reveals an orthosteric binding site (OBS) located in the upper-half of the transmembrane domain containing the nonselective antagonist eticlopride. The high degree of sequence identity in the OBS exposes the challenge in creating selective agents with drug-like properties. Our recent analysis of a series of D3R-selective bitopic 4-phenylpiperazines has revealed important leads towards exploiting the structural basis of D3R over D2R selectivity. A key element is the identification of a divergent second binding pocket (SBP) that accommodates the arylamide moiety of the compounds. We found that pharmacological efficacy depends on the binding mode of the 4-phenylpiperazine in the OBS, which is modulated by phenyl ring substitutions and linker length. By using computational modeling and simulations, and exchanging the divergent segments in the SBP, we have also identified the segment specifically responsible for the selectivity.

Identification of a novel dopaminergic ligand that is a biased agonist at the D_2 dopamine receptor and an antagonist at the D_3 receptor.

David R. **Sibley**, R. Benjamin Free, Jennie Conroy, Rebecca A. Roof, Trevor Doyle, Noel Southall, Hoon Shin, Mayako Michino, Prashant Donthamsetti, Kyle A. Emmitte, Yang Han, Marc Ferrer, Craig Lindsley, Veronica Alvarez, Lei Shi, Jonathan A. Javitch

NINDS/National Institutes of Health / Molecular Neuropharmacology

In order to develop novel small-molecule scaffolds for the D_2 receptor (D_2R), we used high-throughput screening to identify ligands with unique functional characteristics among dopamine receptor (DAR) subtypes. Using a beta-arrestin recruitment assay to compare activity at all DARs, we identified a ligand (compound 3508) that selectively activates the D_2R, but not other DAR subtypes. Compound 3508 is an antagonist at the D_3R for beta-arrestin recruitment and no activity at the D_4R or D1-like DARs (D_1R and D_5R). Compound 3508 exhibits full agonist activity in three different functional assays for the D_2R: beta-arrestin recruitment, Ca^{2+} mobilization, and inhibition of cAMP accumulation. Using a Go BRET assay, we found that 3508 is a full agonist at the D_2R, but displays weak partial (<15%) agonist activity at the D_3R. Interestingly, 3508 is a full antagonist with no agonist activity on D_2R- or D_3R-coupled GIRK channel activation, indicating that it is a biased agonist. Consistent with our studies in heterologous cells, application of 3508 elicited a minimal response in D_2R-activated, whole cell GIRK-mediated currents measured in dopaminergic neurons in mouse midbrain slices, while it effectively blocked the response elicited by the full agonist quinpirole. Molecular modeling studies suggest subtle differences in 3508 binding poses to the D_2R and D_3R that may underlie its functional properties. In summary, 3508 is a full and selective agonist at G-protein-linked and beta-arrestin-mediated D_2R signaling pathways; however, it is an antagonist for D_2R GIRK activation, indicating biased agonism. In contrast, 3508 generally functions as a D_3R antagonist.

MOLECULAR IMAGING OF DOPAMINE NEUROTRANSMISSION DURING HUMAN COGNITIVE PROCESSING

Organizer: R.D. Badgaiyan (USA)

Cognitive and brain plasticity: The role of dopamine

Lars **Bäckman**

Karolinska Institutet

I describe a program of research that examines the trainability of a key intellectual function critical to most everyday cognitive activities, namely working memory (WM). A recurrent observation in this research is that the dopamine is critically implicated not only in general WM functioning, but also for the ability to benefit from training procedures that seek to improve WM performance. Work using functional and molecular imaging, as well as research that examines the role of specific genetic polymorphisms in WM plasticity is reviewed. Several avenues for future research along these lines are discussed.

Molecular imaging of dopamine neurotransmission during human cognitive processing

Badgaiyan, RD

SUNY at Buffalo / Psychiatry

In recent years a number of investigators have used dynamic molecular imaging for detection and mapping of dopamine neurotransmission during human cognitive processing. Since dopamine neurotransmission is directly 'visualized' in these experiments, the results provide novel insight on dopaminergic processing of human cognition. Not surprisingly, the findings have begun to reshape our concept of dopaminergic control of human cognition. The symposium will focus on findings of these experiments and will highlight how these findings are advancing our understanding of dopaminergic control of human cognition. It will also discuss methodological constraints and latest development in dynamic molecular imaging technique. This discussion is important because it is a novel and evolving technique. Since it will be the first international symposium focused specifically on imaging of dopamine neurotransmission in the human brain, it will advance and expand research in this evolving area. Dopamine researchers will find the symposium interesting and thought provoking because of the novelty of technique and data.

Individual differences in dopaminergic neuromodulation of reward processing and cognition

Gregory R. **Samanez-Larkin**, David H. Zald

Vanderbilt University / Psychology

Over the past several years our research group has used positron emission tomography (PET) imaging to measure individual differences in dopamine functioning in humans. This approach has included studies of task- induced dopamine release during reward related decision-making measured with [11C]raclopride PET as well as multimodal imaging combining PET measures with active measures of broader neural system function using fMRI. These studies examine the relationship between individual differences in dopamine functioning, personality traits, and cognitive ability. We will summarize a set of recent studies that have identified relations between individual differences in D2 receptor availability, amphetamine-induced dopamine release and traits associated with reward seeking and impulsivity. We have recently begun to extend these studies by examining how these measures additionally relate to activations within neural circuits involved in inhibition and self-control. Together, these studies allow increasing precision in understanding the role of individual differences in dopamine function in personality and cognitive ability.

Imaging Cognitive Dysfunction in Parkinson's Disease

Antonio P. **Strafella**

University of Toronto

In the last few years, non-motor symptoms like cognitive dysfunction are increasingly being reported in Parkinson's disease (PD) patients. Given the social implications, these disorders represent a cause of significant distress not only for the patients but also their families. To date, the mechanisms underlying cognitive abnormalities in PD are poorly understood. Proposed mechanisms include abnormal functioning of nigrostriatal and mesocorticolimbic systems resulting in dysregulation of dopamine.

IMPACT OF NETWORK ACTIVITY ON THE INTEGRATIVE PROPERTIES OF DOPAMINE NEURONS IN RESPONSE TO NATURAL REWARDS, DRUGS AND AVERSIVE EVENTS

Organizer: F. Georges (France)

Linking context with reward

Gary S.**Aston-Jones**, PhD

Medical University of SC / Neurosciences

Reward-motivated behavior is strongly influenced by the learned significance of contextual stimuli in the environment. However, the neural pathways that mediate context-reward relations are not well understood. We have identified a circuit from area CA3 of dorsal hippocampus to ventral tegmental area (VTA) that uses lateral septum (LS) as a relay. Theta frequency stimulation of CA3 excited VTA dopamine (DA) neurons and inhibited non-DA neurons. DA neuron excitation was likely mediated by disinhibition because local antagonism of GABA acid receptors blocked responses to CA3 stimulation. Inactivating components of the CA3-LS-VTA pathway blocked evoked responses in VTA and also reinstatement of cocaine-seeking by contextual stimuli. This transsynaptic link between hippocampus and VTA appears to be an important substrate by which environmental context regulates goal-directed behavior.

Cannabinoid type-1 receptors on ventral tegmental area GABAergic neurons control voluntary exercise performance in mice

F. **Chaouloff**

NeuroCentre INSERM U862, Bordeaux, France

We have previously shown by means of cannabinoid type-1 (CB1) receptor knock-out mice that the endogenous stimulation of CB1 receptors is a prerequisite for voluntary wheel-running in mice (Dubreucq et al., 2010). However, the precise mechanisms through which the endocannabinoid system exerts a tonic control on running performance remain unknown. Herein, we have analyzed the respective impacts of cell type-specific CB1 receptor mutations and of CB1 receptor blockade on wheel-running performance. Conditional deletion of CB1 receptors from brain GABA neurons, but not from principal neurons, glutamatergic neurons, serotonergic neurons, D1 receptor-expressing neurons or from astrocytes, decreased wheel-running performance in mice. CB1 receptor antagonists provided systemically or directly into the ventral tegmental area (VTA) diminished wheel-running behavior, these effects being abolished in GABA-CB1-/- mice. Moreover, in vivo electrophysiology experiments revealed that the absence of CB1 receptors from GABAergic neurons led to a tonic inhibition of VTA DA neuronal activity (number of spontaneously active cells, firing rate and bursting activity) after either acute or repeated wheel-running. This study provides evidence that CB1 receptors on VTA GABAergic terminals exert a permissive control on rodent voluntary running performance, possibly through a disinhibition of reward-based motivation processes. Furthermore, our results indicate that CB1 receptors located on GABAergic neurons impede negative consequences of voluntary exercise on VTA DA neuronal activity. These results position the VTA endocannabinoid control of inhibitory transmission as a prerequisite for wheel-running performance in mice.

Modulation of dopamine neuron activity by afferents: Implications in reward and aversion

François **GEORGES**

IINS CNRS UMR 5297

The hippocampal formation including the ventral subiculum and the ventral CA1 area (vSUB/CA1) is a brain region that is involved in context-dependent processes, and also is considered as a regulator of emotion. The ventral tegmental area (VTA) plays a role in the acquisition of learned appetitive behaviors and in the development of drug addiction. It is now well accepted that the vSUB/CA1 activates the dopamine system, however, the mechanism by which the vSUB/CA1 regulates the activity of the VTA DA neurons is still unclear. The tail of the ventral tegmental area (tVTA) or rostromedial tegmental nucleus (RMTg) is a recently described brain region which is a major inhibitory control centre for dopaminergic systems. A proper balance between the excitatory and inhibitory neurotransmitters is essential for dopamine neurons excitability. The focus of this presentation will be to understand how GABAergic and glutamatergic terminals innervate and regulate the activity states of DA neurons. We combined tract-tracing and in vivo electrophysiological approaches to dissect the polysynaptic projection from vSUB/CA1 and tVTA/RMTg to VTA dopamine neurons. The main objective of this work is to highlights the functional inhibitory and excitatory impact of tVTA/RMTg and vSUB/CA1 on in vivo synaptic adaptation of dopamine neurons.

Functional diversity of dopamine neurons

Mark **Ungless**

MRC Clinical Sciences Centre, Imperial College London

Although dopamine neurons are often viewed as functionally homogeneous, several emerging lines of research indicate that anatomically distinct subgroups of dopamine neurons may exhibit distinct information coding properties and distinct synaptic adaptations in response to rewards, aversive events, and drugs of abuse. To illustrate this, I will present evidence concerning synaptic adaptations in dorsal raphe/ventro-lateral periaqueductal grey dopamine neurons in response to drugs of abuse and social isolation.

POSTER SESSION II

Monday,MAY27th

Dopamine and addiction

P052. Striatal Dopamine Transporter Availability in Patients with Alcohol Dependence: Assessment by ^{123}I-FP-CIT SPECT. A Pilot Study.

A. Ferrulli, G. Vassallo, M. Antonelli, A. Mirijello, C. D'Angelo,D. Di Giuda, A. Giordano, *M. Diana, G. **Addolorato**

Dept. of Internal Medicine, and Nuclear medicine, Catholic University of Rome, Rome, Italy.

*Dept. Of Chemistry of Pharmacy, Univ. Of Sassari, Italy

Background: The mesolimbic dopaminergic system is hypofunctional in alcohol dependence. Preclinical and clinical evidence suggests an association between alcoholism and the dopamine transporter (DAT), although the nature of this association is unclear.
Aim: To assess striatal dopamine transporter (DAT) availability using ^{123}I-FP-CIT SPECT in long-term alcoholic patients.Materials & Methods: We enrolled 8 right-handed untreated patients (7 M, mean age: 48 ± 9 yrs) with a DSM-IV diagnosis of alcohol dependence and without a major psychiatric disorder. Specific to non-specific ^{123}I-FP-CIT binding ratios in the entire striatum and striatal subregions were bilaterally calculated, based on ROI analysis. The control group consisted of 16 healthy subjects (12 M, mean age: 45 ± 10 yrs). Results: Alcoholic patients showed significantly lower ^{123}I-FP-CIT binding ratios in bilateral striatum and putamen (right striatum: -16%, left striatum: -17%; right putamen: -23%; left putamen: -22%; Mann-Whitney U test, $p<0.05$). DAT availability in striatal regions inversely correlated with the duration of alcohol dependence and the intake of alcohol per day (Spearman's rank correlation analysis, $p<0.05$). The number of days of abstinence before SPECT imaging, as assessed by TLFB, positively correlated with DAT availability in bilateral caudate (Spearman's rank correlation analysis, $p<0.05$). Conclusion: DAT availability is reduced in alcoholic patients, supporting the hypothesis that alcohol-dependent subjects may have a hypofunctioning dopaminergic system (Melis et al., 2005; Diana, 2011). These findings suggest that dopaminergic neurons could represent a target for potential useful treatments of alcohol dependence. Results of ^{123}I-FP-CIT SPECT after repeated TMS stimulations will be discussed.

P053. Striatal Modulation of BDNF Expression using MicroRNA124a-Expressing Lentiviral Vectors Impairs Ethanol- Induced Conditioned- Place Preference and Voluntary Alcohol Consumption.

A. **Bahi**, J-L. Dreyer

Dept. of Anatomy, College of Medicine & Health Sciences, United Arab Emirates University, Al Ain, UAE

Alcohol abuse is a major health, economic and social concern in modern societies but the exact molecular mechanisms underlying ethanol addiction remain elusive. Recent findings show that small non-coding microRNA (miRNA") signaling contributes to complex behavioral disorders including drug addiction. However, the role of miRNAs in ethanol-induced conditioned place preference (CPP) and voluntary alcohol consumption has not yet been directly addressed. Here, we assessed the expression profile of miR124a in the dorsal striatum of rats upon ethanol intake. The results have shownthat miR124a was down regulated in the dorso-lateral striatum (DLS) following alcohol drinking. Then, we identified BDNF as a direct target of miR124a. In fact, BDNF mRNA was up-regulated following ethanol drinking. We used lentiviral vector (LV) gene transfer technology to further address the role of miR124a and its direct target BDNF in ethanol-induced CPP and alcohol consumption. Results revealed that stereotaxic injection of LV-miR124a in the DLS enhanced ethanol- induced CPP as well as voluntary alcohol consumption and preference in the two bottle choice drinking paradigm. Moreover, miR124a- silencer (LV-siR124a) as well as LV-BDNF infusion in the DLS attenuatedethanol-induced CPP as well as voluntary alcohol consumption and preference. Importantly, LV-miR124a, LV-siR124a and LV-BDNF hadno effect on saccharin and quinine intake. Our findings indicate that striatal miR124a and BDNF signaling have crucial roles in alcohol consumption and ethanol conditioned reward.

P054.Changes of dopamine transmission in the nucleus accumbens shell and core during ethanol and sucrose self-administration

V. **Bassareo**, F. Cucca, R. Frau, G. Di Chiara

Dept. of Biomedical Sciences, University of Cagliari, Cagliari, Italy

Ethanol is a psychoactive compound of several beverage abused by humans and it is well known that, as well as other drugs of abuse, increases dopamine (DA) transmission preferentially in the nucleus accumbens (NAc) shell. The aim of our study was to investigate by microdialysis the role of the NAc shell and core DA in the response to ethanol and to ethanol-conditioned stimuli (ethanol-CS) using an instrumental conditioning paradigm with fixed-ratio 1 (FR1). Rats were trained to acquire sucrose and ethanol oral self-administration under a FR 1 paradigm (1 nose poke corresponds to 0.25 ml administration of 20% sucrose or 10% ethanol in 20% sucrose solutions). We found that oral ethanol, either self-administered or given passively, produces an increase of DA transmission in the shell and in the core, strengthened during the self-administration session, while ethanol-CS increased DA preferentially in the NAc shell. Sucrose oral self-administration and its conditioned cues affects DA exclusively in the shell, but the passive administration increases DA in the shell and in the core. These data suggest that the two compartments of the NAc are differently implicated in the responsiveness to natural and to pharmacological rewards. While DA transmission in the NAc shell seems to play a key role in the operant responding for both sucrose and ethanol, the DA core appears to be more involved in the responsiveness to ethanol.

P055. Genotype dependent adaptive changes in mesolimbic dopamine transmission after adolescent nicotine exposure: possible underlying mechanisms in the gateway effect of nicotine.

Cristina **Cadoni**[1,3,4], Elena Espa[2], Gaetano Di Chiara[1,2,3,4]

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Epidemiological studies have shown that smoking during adolescence may increase the likelihood to develop a nicotine dependence and lead to abuse other illicit drugs such as heroin and cocaine. In spite of greater efforts to understand this progression and the underlying neurobiological mechanisms, less attention has been paid to the role of genetic factors. To elucidate the neurobiological mechanisms in the gateway effect of nicotine we investigated, in a genetic animal model, differences in the long-term effects of adolescent nicotine exposure on mesolimbic dopamine (DA) transmission responsiveness to nicotine, heroin and cocaine at adulthood. Fischer344 (F344) and Lewis (LEW) male rats of 6 weeks of age (38-42 PND) were exposed once a day for 5 days to nicotine (0.4 mg/kg s.c.). At adulthood (10-12 weeks) animals were implanted with two microdialysis probes in the nucleus accumbens (NAc) shell and core. The following day challenge with nicotine (0.4 mg/kg. s.c.) showed that adolescent nicotine exposure potentiated DA response to nicotine in the NAc core of both strains, while DA responsiveness in the NAc shell was increased only in the vulnerable strain (LEW). Adolescent nicotine exposure potentiated moreover DA increase induced by heroin and cocaine in the NAc shell of LEW but not F344 rats, leaving unchanged DA response in the NAc core of both strains. Given the role attributed to DA in the NAc shell in the rewarding properties of drugs of abuse these results would suggest that adolescent nicotine exposure might increase the rewarding properties of nicotine, heroin and cocaine in genetically vulnerable individuals, thus functioning as a gateway to abuse nicotine but also other illicit drugs.

P056. Vulnerability to Addictions: Dopamine Studies in Humans.

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Background: Animal studies suggest that dopamine neurotransmission influences responses to reward-related stimuli and susceptibility to drug-seeking behavior. The relevance of this work for humans, though, has been unclear. Methods: During the past 15 years, we have conducted a series of studies using positron emission tomography)PET) and acute phenylalanine/tyrosine depletion (APTD) to measure dopamine release and its behavioral significance. Results: The studies suggest that, in humans, compulsively abused drugs increase extracellular dopamine levels. With repeated drug administration, these responses can become progressively larger (sensitized) and conditioned to environmental cues. Diminishing the drug-induced dopamine response does not alter the substance's pleasurable effects, but does decrease the propensity to respond preferentially to rewards and the willingness to sustain effort to get them (alcohol, cigarettes, money). Finally, in people at risk for addictions, drug-induced dopamine responses are altered, and both increases and decreases have been observed, potentially related to the presence vs. absence of drug related cues. Discussion: Together, these studies might identify more closely the role of dopamine in drug-seeking behaviors in humans. Moreover, they raise the possibility that one biological vulnerability trait for addiction is susceptibility to labile dopaminergic and appetitive responses to cues.

P057. Individual contributions of drug and cues to striatal phasic dopamine signals during self-administration.

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Addiction is a neuropsychiatric disorder defined as the endpoint of the series of stages beginning with recreational drug use and culminating in habitual and compulsive drug use. Dopamine signaling in the ventral striatum is strongly implicated in mediating the acute reinforcing effects of drugs of abuse. Recent work from our lab demonstrates that phasic dopamine signaling in the dorsal striatum is engaged during later stages of drug use as behavior becomes more habitual, while this signaling is not observed earlier in self-administration. Drug-associated cues play a major role in the control of such drug-seeking behavior and relapse. Here we study the evolution of drug-associated and cue-elicited phasic dopamine release in the dorsal and ventral striatum throughout cocaine self-administration in order to better understand how these signals control drug-taking behaviors. During self-administration sessions, drug and cue presentations overlap in time, thus, to determine the independent contributions of drug and drug-associated cues to the signals observed during self-administration we measured phasic dopamine signals elicited by non-contingent delivery of drug and cues independently using fast-scan cyclic voltammetry (FSCV) in both ventral and dorsal striatum repeatedly over the course of three weeks. These measurements were performed both before and after 1-hour access cocaine self-administration sessions in male Wistar rats. Preliminary findings suggest that ventral striatal dopamine signals to both cue and drug become larger as self-administration progresses. We have not observed any phasic dorsal striatal signals to drug-associatedcuepresentations, and onlyobservedsignals to drug delivery during the very first exposure to drug.

P058. Altered response to stress in animals exposed to chronic cocaine treatment during adolescence.

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The interaction between drug abuse and stress is a critical component of drug addiction, but the underlying molecular mechanisms remain elusive. Evidence exists that both the dopamine and glutamate neurotransmission play a role in cocaine abuse; however, their interaction in the modulation of the stress response after chronic exposure to cocaine is still obscure. We investigated whether the rapid coping response of both dopaminergic and glutamatergic synapse to stress was influenced by previous cocaine history during the adolescence. We exposed adolescent male rats to cocaine (20mg/kg/day) from PND 28 to PND 42; on PND 45, rats were subjected to swim stress (5 min) and sacrificed 15 min later. We focused on the medial prefrontal cortex (mPFC) that is still developing during adolescence and might be more vulnerable to stress. The interaction between cocaine and stress altered the glutamatergic synapse by increasing the vesicular glutamate transporter, reducing glial glutamate transporters and increasing the activation of the NMDA receptor. The dopamine system seems to be more sensitive to the effects of cocaine alone and not to the combination of cocaine and stress: in fact, the expression of dopamine D2 receptors is increased primarily by cocaine rather than by the combination. Our data suggest that the interaction between glutamate and dopamine may play an important role in the response to stress in animals exposed to cocaine during adolescence, although further data need to be collected to draw a precise picture of the underlying mechanisms.

P059. Cocaine self-administration results in neurochemical tolerance and dopamine transporter complex formation which is reversed by a single amphetamine bolus.

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Agonist therapies, especially amphetamine, have been greatly studied as pharmacotherapies for cocaine addiction. Because the dopamine transporter (DAT) is the main site of action of cocaine, and the dopamine elevating effects are critical for the reinforcing and rewarding properties of the compound, much work has focused on the dopamine system. As such, we wanted to assess the ability of cocaine to inhibit the DAT following cocaine self-administration (SA), and explore the ability of an amphetamine bolus to modulate this effect. We found that binge cocaine SA results in reduced cocaine-induced DAT inhibition as measured by in vitro voltammetry. Further, reduced cocaine potency could be reversed with a single bolus of amphetamine administered intravenously one hour prior to cocaine potency assessment. We propose that the reversal of the effects of cocaine SA by amphetamine is due to DAT recycling as a single amphetamine injection resulted in a robust increase in baseline uptake rates in animals with a history of cocaine SA. To investigate this further we used western blot hybridization to assay DAT levels and complex formation. We found that cocaine SA resulted in the formation of higher order DAT complexes that were dissociated following amphetamine bolus. Taken together, we proposed that cocaine SA results in the formation of DAT complexes, which are responsible for reduced uptake function as well as reduced cocaine-induced uptake inhibition. In addition, amphetamine administration reverses complex formation, reverses the reduced uptake rates and reverses the reduced cocaine uptake inhibition caused by cocaine SA.

P060. Acetaldehyde operant self-administration in rats: focus on D2-receptor activation.

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Acetaldehyde(ACD), ethanol first metabolite, is rewarding in rodents and humans; it induces "place preference", is self-administer directly in the VTA, orally in an operant/conflict paradigm and increases DA neurons' firing. This research aims at investigating DA2-receptor role in the reinstatement of acetaldehyde operant-drinking behaviour, following induction, maintenance and abstinence in the rat. Male Wistar rats are trained to orally self-administer ACD solution (3.2% v/v) or water, in an operant chamber under a FR1. Afterwards animals undergo cyclic periods of deprivation and relapse to ACD. The effect ofD2-receptor activation by quinpirole (0.03mg/kg,i.p.) on operant ACD self-administration is tested during relapse sessions. Rats show a peak-and-drop drinking pattern that reaches regular and higher values in the last training days. Quinpirole administration produces lever press reduction in ACD group when compared to basal intake($p<0.001$) and to vehicle ($p<0.05$;$p<0.001$), while when treatment is suspended, rats reinstate lever presses for ACD. ACD incentive properties involve dopamine neurotransmission: D2-receptor activation is able to reduce reinstatement of operant drinking behaviour for ACD, following periods of abstinence, probably acting at a pre-synaptic level, thus reducing DA release in mesolimbic areas. These findings further support ACD pivotal role in ethanol central effects.

P061. Ketamine produces structural plasticity of mouse mesencephalic dopaminergic neurons via activation of Akt-mTOR pathway: role of dopamine D3 receptor.

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Ketamine is an anaesthetic and drug of abuse. Its effects on glutamate and GABA transmission have been well documented but little is known about its effects on the dopamine (DA) system. Several studies have demonstrated that a single sub-anaesthetic dose of ketamine rapidly increased DA release in the prefrontal cortex of rats indicating its involvement in DA system. Chronic exposure to other addictive drugs such as cocaine or amphetamine produces morphological changes in neurons of the mesolimbic DA system in rodents. Using mouse primary cultures of mesencephalic DA neurons we recently showed that cocaine increased dendritic arborisation and these effects were blocked by D3R antagonists. In this work we have extended our observations to ketamine. Experiments were performed in vitro on primary mesencephalic DA neurons from wild-type and D3KO mice at 12.5 embryonic day. After pharmacological testing the cultures were immunostained using an anti-tyrosine hydroxylase (TH) antibody to detect DA neurons. Morphometric assessments showed that ketamine produced time- and dose-dependent increases of dendrite arborisation and soma area of DA neurons in wild-type cultures. By contrast, ketamine was ineffective in mesencephalic neurons from D3KO mice. Using confocal microscopy and dual immunocytochemistry we showed a D3R-dependent activation of the Akt-mTOR pathway, a critical signalling involved in neuronal plasticity. Our observations suggest that dopamine D3Rs play a central role in the neuroadaptative effects of ketamine on mesencephalic dopaminergic neurons. In vivo experiments on wild-type and D3KO mice are on progress and will be presented at the meeting.

P062. Oxytocin decreases methamphetamine seeking in an animal model of relapse.

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Oxytocin receptors are distributed throughout the brain, including regions of the mesocorticolimbic dopamine system, and dopamine neurons in the ventral tegmental area receive oxytocin inputs. Additionally, hypothalamic oxytocin-containing cells express dopamine receptors. While oxytocin has been best characterized for its interactions with the dopamine system in pair bonding, data also suggests that oxytocin plays a regulatory role in various reward related behaviors, including drugs of abuse. However, the role of oxytocin in drug seeking behavior has not been well studied. To examine whether oxytocin would modulate drug taking and drug seeking, we used a methamphetamine (meth) self-administration and relapse model in male and female rats. Rats self-administered meth along a fixed ratio 5 schedule of reinforcement and were subsequently tested on a progressive ratio schedule of reinforcement following systemic oxytocin or vehicle treatment. Additionally, we assessed the effects of oxytocin on cue-, prime-, and pharmacological stress-induced reinstatement of meth seeking. Oxytocin reduced motivation to seek meth on the progressive ratio test and during cue-induced reinstatement in females, but not males. In both sexes, oxytocin attenuated meth-primed and stress-induced reinstatement of meth seeking. The results suggest that oxytocin may have greater efficacy as a treatment of meth addiction in females. Such differences may arise from sexually dimorphic neural interactions of oxytocin and dopamine during meth seeking.

P063. Pharmacological characterization of JWH-018, a cannabinoid component of "spice" drugs

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'Spice' is a smokable herbal mixture marketed under many brands as herbal alternatives to Cannabis. Spice is comprised of shredded plant material laced with a variety of synthetic cannabinoid agonists, specifically the compound 1-pentyl-3-(1-naphthoyl)-indole (JWH-018) has been detected in about 140 Spice samples. JWH-018 is a more potent CB1 and CB2 agonist than 9-THC, and this likely contributes to the greater incidence of dependence associated with Spice use. We characterized the rewarding properties of JWH-018 and the neurochemical mechanisms that produce these effects. We found that Sprague-Dawley rats consistently acquired operant behavior (nose-poking) that resulted in JWH-018 infusion (20 µg/kg/inf iv, FR3). Nose-poking behavior diminished when JWH-018 was replaced with vehicle (*extinction*), it was rapidly re-established when JWH-018 was again made available (*reinstatement*) and was blocked by pretreatment with Rimonabant. Using *in vivo* microdialysis we found that JWH-018 (0.25 mg/kg ip) preferentially increased DA release in the NAc shell vs. NAc core or mPFCX and this effect was blocked by CB1 antagonists (Rimonabant, AM 251) and lacking in CB1KO mice. Electrophysiological recordings from DA neurons of the VTA revealed that JWH-018 was able to decrease GABAA-mediated post-synaptic currents in a dose-dependent fashion. Finally, by using the "tetrad" paradigm for screening cannabinoid-like effects (hypothermia, analgesia, catalepsy, hypomotility) we found that JWH-018 produces CB1 receptor-dependent behavioral effects in mice and rats. These findings not only link JWH-018 with other cannabinoids with known abuse potential, but also to other classes of abused drugs that increase DA signaling in the NAc shell.

P064. Loss of control over food intake, observations from an animal model.

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To test the hypothesis that chronic overeating is a form of addiction we designed an animal model based on a prominent model from the addiction field. In this model rats are tested for three criteria to assess if they express 'addiction-like behavior'. The criteria are: 1. Extremely high motivation to obtain the reward. 2. Difficulty in limiting reward seeking even in periods of explicit non-availability. 3. Continuation of reward-seeking despite negative consequences. To incorporate the fact that addiction develops after chronic exposure to the drug, rats were tested after exposure to a binge diet consisting of alternating periods of food restriction and access to palatable food. Although no significantly distinct addictive subgroup could be identified, the model proved useful to measure loss of control over food intake. Animals that have less control over their food intake are more prone to reinstate food seeking behavior after extinction and are less sensitive to devaluation of the reward. Indicating that food seeking behavior in these animals may be stimulus directed (habitual) as opposed to goal-directed. Next we intend to down regulate the dopamine D2 receptor, using a AAV-mediated approach, to assess its role in uncontrolled feeding.

P065. JPC-077 interacts with VMAT2 to reduce the neurochemical and behavioral effects methamphetamine.

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FDA-approved treatments are not available for methamphetamine (METH) addiction. Support has been obtained for the vesicular monoamine transporter-2 (VMAT2) as a pharmacological target for the discovery and development of novel therapeutics for METH abuse. The current preclinical work provides support for JPC-077, a novel lead analog of lobelane, as a treatment for METH abuse. Effects of JPC-077 at VMAT2 and the dopamine transporter (DAT), and as an inhibitor of METH-evoked dopamine release from superfused striatal slices were determined. Also, JPC-077-mediated inhibition of responding for METH self-administration and for sucrose reinforcement was determined. JPC-077 exhibited a 6-fold increase in affinity (Ki=0.15 μM) for the [3H]dihydrotetrabenazine binding site on VMAT2, and a 5-fold increase in affinity (Ki = 9.3 nM) for the dopamine (DA) translocation site on VMAT2, relative to lobelane. JPC-077 acted to competitively inhibit DA uptake at VMAT2. JPC-077 evoked [3H]DA release (EC50 = 54 nM) from synaptic vesicles with 130-fold greater potency than lobelane or METH itself. JPC-077 had 370-fold greater selectivity for VMAT2 over the plasmalemma DAT, indicating that JPC-077 likely has low abuse liability. Importantly, JPC-077 inhibited (IC50 = 0.86 μM; Imax = 71.9%) METH-evoked DA release from striatal slices, while concurrently increasing extracellular dihydroxyphenylacetic acid. JPC-077 (56 mg/kg) decreased the number of methamphetamine infusions self-administered, but did not alter responding for food when given across repeated pretreatments. Thus, in vitro effectsof JPC-077 translated to in vivo efficacy, decreasing METH self-administration. As a result of these studies, JPC-077 has emerged as a lead compound in the discovery of a novel treatment for METH abuse.
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P066. The ethanol intake-reducing effect of acamprosate is associated with dopamine elevation.

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Recent studies indicate that the anti-craving substance acamprosate modulates accumbal dopamine levels via a dopamine-controlling nAc-VTA-nAc neurocircuitry. It was demonstrated that glycine receptors in the nucleus accumbens (nAc) are involved both in the dopamine-elevating effect and the ethanol intake-reducing effect of the drug. Furthermore, several rodent studies have reported a loss of effect of acamprosate on ethanol consumption after a few days of treatment. Here we wanted to explore the interaction of ethanol and acamprosate on nAc dopamine and investigate whether dopaminergic transmission may be related to the ethanol-reducing effects. By means of in vivo microdialysis we investigated nAc dopamine levels after acute and repeated administration of acamprosate in Wistar rats with and without the addition of ethanol as well as in ethanol medium- and high-preferring rats. Acamprosate was able to elevate nAc dopamine after acute and three days of treatment, an elevation that rendered ethanol unable to further influence dopamine output. Eleven days of acamprosate administration to ethanol preferring rats initially decreased their ethanol consumption, an effect that was lost after a few days despite continuation of the drug treatment. Interestingly, in rats were the acamprosate-induced decrease in ethanol consumption was lost, the dopamine elevating property of the drug was also lost and ethanol was able to produce a dopamine elevation. We suggest that acamprosate may partly substitute for the dopamine-elevating effects of ethanol but once this effect is lost, the ability to decrease ethanol intake is also lost.

P067. Effects of the monoamine stabilizer (-) OSU6162 on dopamine output in the nucleus accumbens in rats after long-term voluntary ethanol drinking.

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We have recently identified the monoamine stabilizer (-)-OSU6162 as a potential novel treatment for alcohol use disorder (AUD), by showing that it selectively decreases ethanol consumption, ethanol seeking, cue-induced reinstatement and withdrawal symptoms in rats chronically exposed to ethanol. Previously, (-)-OSU6162 has been shown to either increase or decrease dopamine levels depending on the prevailing dopaminergic tone. In ethanol-naïve rats, we have used microdialysis to show that (-)-OSU6162 blunts ethanol-induced dopamine output in the nucleus accumbens. To further investigate the mechanisms underlying (-)- OSU6162's effect in ethanol exposed rats, we here measured the dopamine output in the nucleus accumbens after a systemic injection of (-)-OSU6162 or ethanol, respectively, in two groups of rats that had been given voluntary intermittent access to 20% ethanol for approximately ten months. The injections were given after 23hrs alcohol abstinence. Ethanol (2.5 g/kg, i.p.) induced a slight increase in dopamine output compared to baseline, 15 and 30 minutes after injection. (-)-OSU6162 (30 mg/kg, s.c.) induced a slow increase in dopamine output which remained higher than baseline, and the ethanol-induced dopamine output, for several hours. These results indicate that (-)-OSU6162's ability to decrease ethanol consumption, cue-induced reinstatement and withdrawal symptoms rely on its capacity to normalize, or restore, the ethanol-induced dopamine deficits in the nucleus accumbens. Experiments with the combination treatment of (-)-OSU6162 and ethanol are ongoing.

P068. Dopamine D3 receptors and incubation of cocaine craving.

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Cue-induced drug-seeking behavior progressively increases over time after withdrawal from drug self-administration in rats - a phenomenon termed "incubation of craving." Although other brain mechanisms have been implicated in incubation of craving, the involvement of dopamine (DA) mechanisms has heretofore been unclear. Cue-induced cocaine-seeking behavior appears to parallel increases in DA D3, but not D1 or D2, receptor expression in the nucleus accumbens - suggesting involvement of DA D3 receptors in incubation of cocaine craving. We now report that systemic or local administration of SB277011A, a highly selective D3 receptor antagonist, into the nucleus accumbens core and shell or the central nucleus of the amygdala significantly inhibits expression of incubation of cocaine craving in rats after 2-30 days of withdrawal from cocaine self-administration. SB277011A microinjections into the dorsal striatum or basolateral amygdala had no effect. SB277011A microinjections into the nucleus accumbens core and shell or into the central nucleus of the amygdala had no effect on sucrose-seeking behavior in rats after 10-30 days of withdrawal. These findings suggest that DA D3 receptors in the nucleus accumbens and central amygdala play an important role in incubation of cocaine craving, and further support the potential efficacy of highly selective DA D3 receptor antagonists for treatment of cocaine addiction.

P069. Role of the Lateral Habenula and Tail of the Ventral Tegmental Area in Reinstatement of Cocaine Seeking.

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Increasing attention has focused on the pathways from the lateral habenula (LHb) to the ventral tegmental area (VTA), in part due to recent characterization of the tail of the VTA (tVTA). The tVTA receives input from the LHb and projects to the VTA. This pathway has major implications for motivated behavior, as the tVTA acts in an opposing manner, regulating dopamine release in the VTA (Kaufling et al., 2010). The current studies examined the role of the LHb/tVTA/VTA pathway in reinstatement of cocaine seeking. Rats self-administered cocaine (0.2 mg/50 ?l infusion) along an FR1 schedule of reinforcement (14 days), followed by extinction (7 days). Rats then underwent testing for cue-induced (tone+light) or yohimbine (2.5 mg/kg) + cue-induced reinstatement of cocaine seeking. LHb inactivation had no effect on cue-induced reinstatement, but it significantly reduced reinstatement of cocaine seeking during the yohimbine + cue condition. These results suggest that the LHb plays a key role during heightened anxiogenic states that modulate drug seeking. Previous work has shown that VTA DA cells respond to optogenetic phasic stimulation of the tVTA , but not tonic inhibition of the tVTA (Kaufling & Aston-Jones, 2012). As the LHb acts in opposition to the tVTA inhibitory efferent pathway to the VTA, a second study investigated the role of the tVTA in reinstatement of cocaine seeking. Preliminary data suggests that phasic activation of the tVTA will attenuates cocaine seeking during reinstatement.

P070. Orexin signaling in the ventral tegmental area is critical for cue-induced reinstatement of cocaine-seeking but not natural reward-seeking behavior.

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Introduction: Peripheral administration of the orexin receptor-1 antagonist SB-334867 blocks cue-induced reinstatement of drug-seeking. These effects may be mediated by orexinergic regulation of midbrain dopamine neurons. We investigated the effect of intra-ventral tegmental area (VTA) infusions of SB-334867 on cue-induced reinstatement of cocaine- and natural reward (sweetened condensed milk; SCM)-seeking. We also assessed whether VTA orexin-1 receptor blockade is associated with changes in Fos-protein expression within regions involved in reinstatement. Methods: Animals received intra-VTA administration of SB-334867 (cocaine group: 1,3µg; SCM group: 3,6µg) or vehicle before being exposed to stimuli previously associated with cocaine/SCM availability. Activation patterns within perifornical/lateral hypothalamus (PF/LH), paraventricular thalamus (PVT) and nucleus accumbens shell (NAcS) were assessed by counting Fos-positive neurons within these regions. Results: Intra-VTA administration of SB-334867 dose-dependently attenuated cue-induced reinstatement of cocaine- seeking, but had no effect on reinstatement of SCM-seeking. SB-treated animals exhibited fewer Fos- positive neurons in PVT and more Fos-positive neurons in NAcS. Conclusions: These data suggest that orexin signaling in VTA is important for cue-induced reinstatement of cocaine- seeking but not natural reward-seeking behavior. Whilst not directly assessed here, we suggest that blockade of orexinergic signaling in VTA results in reduced dopaminergic activation of VTA-efferents that are important for reinstatement behavior, including PVT and NacS. Further studies are investigating how this system is affected by early life stress.

P071. Kappa-Opioid Receptors in the Nucleus Accumbens Shell are Important for Pair Bond-induced Attenuation of Amphetamine-induced Conditioned Place Preference.

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A fundamental problem with addiction is the inability to stop using drugs despite adverse social consequences. Conversely, prior to the full development of drug addiction, positive social support increases an individual's ability maintain casual drug taking and not transition to compulsive drug seeking. Here, we examine the neurobiology that underlies a neuroprotection of drug reward by adaptive social behavior. We utilized the socially monogamous prairie vole, a rodent species that forms enduring pair bonds. When exposed to amphetamine, pair bonded prairie voles show resilience against the drug's rewarding effects while sexually naïve voles do not. Previous evidence supports that upregulation of D1 receptors in the nucleus accumbens (NAc), which also mediates the maintenance of a pair bond, partially underlies this resilience. Amphetamine stimulates D1 recepetors which then stimulates the activation of kappa-opioid receptors (KORs), a system known to blunt DA release and recently shown to also mediate pair bond maintenance. We therefore hypothesized that KOR activation in pair bonded voles attenuates drug reward. We first tested social housing conditions on amphetamine reward, establishing that only social interaction that produced a pair bond decreases amphetamine reward. We then antagonized KORs, both globally and in the NAc shell, which restored amphetamine reward in bonded voles, suggesting that KORs are indeed involved in the neuroprotection of drug reward provided by pair bonding.

P072. Delta9-THC and heroin exposure in adolescence differently affect heroin self-administration in adulthood

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Lewis (LEW) and Fischer (F344) inbred strains are differentially sensitive to the reinforcing properties of drugs of abuse. Using these strains we evaluated the influence of adolescent pre-exposure to Delta9-THC and heroin on heroin self-administration (SA) and reinstatement in adulthood. On the the 6th postnatal (PN) week rats were administered twice a day with increasing doses of heroin (Exp. I: 5,10,20 mg/kg, s.c.) and Delta9-THC (Exp. II: 2,4,8 mg/kg, i.p.) for three consecutive days. In adulthood (10th PN week), LEW and F344 rats were trained to acquire intravenous heroin SA (0.025 mg/kg dose unit) under fixed ratio (FR) schedules in Skinner boxes equipped with active and inactive nose-poking holes. In both experiments LEW rats showed higher operant responding activity and faster acquisition of opiate-reinforced behavior as compared to F344 rats. In LEW rats heroin pre-exposure in adolescence resulted in increased responding for heroin and increased heroin intake compared to controls rats as well as to F344 group on FR3 schedule (Exp.I). Adolescent pre-exposure of LEW rats to Delta9-THC resulted in a faster increase of responding when switching ratio schedule from FR-1 to FR-3 and FR-5 (Exp.II). During reinstatement LEW pretreated rats showed greater nose poking behavior compared to controls rats as well as to F344 group. These results demonstrate the importance of strain-related difference in the influence of adolescent heroin and Delta9-THC pre-exposure on heroin reinforcing properties in adulthood. Additional studies of heroin SA behavior under progressive ratio schedules in Delta9-THC pretreated LEW and F344 rats are still going on.

P073. Elevated activity of dopamine neurons during adolescence: implications for cocaine addiction.

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In humans, adolescence is a period of heightened propensity for cocaine addiction. It is unknown if this is due to greater access/exposure to cocaine at this age, or if the adolescent brain is particularly susceptible to addiction. We determined if adolescent rats show elevated activity of midbrain dopamine neurons, a trait associated with heightened susceptibility to addiction. Dopamine neuron activity showed an inverted U-shaped curve from weaning to adulthood, with peak activity during adolescence. Heightened dopamine neuron activity during adolescence was observed both in vivo, with extracellular recordings in anesthetized rats, and ex vivo, with cell-attached recordings from midbrain slices. Dopamine neurons fire irregularly, with interspersed bursts (clusters of high-frequency spikes). Relative to adults, adolescents exhibited more "non-bursting activity" and longer burst events, but no differences in burst frequency. Whole cell recordings showed that age groups did not differ in passive and active membrane properties, hyperpolarization-activated cation currents, or small conductance calcium-activated potassium currents. However, in adolescents, GABA-A receptor-mediated sIPSCs occurred at lower frequency and smaller amplitudes, suggesting a possible mechanism underlying heightened dopamine neuron activity during adolescence. Finally, the dopamine D2-class autoreceptor agonist quinpirole suppressed dopamine neuron activity to a similar extent across ages. Elevated dopamine neuron activity during adolescence was associated with elevated cocaine self-administration and could be reversed by administering drugs that modify dopamine neuron activity, suggesting a causal relationship between these electrophysiological and behavioral determinants of cocaine addiction. In conclusion, these studies demonstrate that neurophysiological differences during development underlie the heightened addiction liability observed during adolescence.

P074. Dopamine involvement in Acetaldehyde drinking behaviour: role of Ropinirole on.

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Rats self-administer acetaldehyde(ACD), ethanol's first metabolite, directly into cerebral ventricles (1), and multiple ICV infusions of ACD produce conditioned place preference (2). ACD, such as alcohol and other substances of abuse, interacts with dopaminergic reward system (3) and its reinforcing and addictive properties have been assessed through an operant-conflict conditioning procedure (4). Since dopamine D2receptor over-expression in the Nacc attenuates alcohol intake (5), this study aims at exploring the effects of ropinirole administration during abstinence, on ACD relapse. The protocol has been scheduled into 3 different periods: training (animals have been trained to self-administer ACD solution 3,2%v/v in order to obtain the induction and maintenance of an operant-drinking behavior), deprivation (rats have undergone repeated 1-week ACD abstinence during and received ropinirole at 0,03 mg/kg/day i.p.); and relapse (rats increase their operant behavior following deprivation). Our results indicate that ropinirole is able to reduce reinstatement of ACD operant-drinking behavior, confirming that hypo-dopaminergic activity is responsible for drug seeking behavior and relapse following abstinence. Further investigation could help considering ropinirole as a therapeutical tool for preventing alcohol drinking relapse. 1.Brown et al.(1979) Psychopharmacology 64:271-276. 2. Smith et al. (1984) Alcohol 1:193-195. 3. Melis M et al (2007)Eur J Neurosci. 26:2824-2833. 4.Cacace S et al., AlcoholClinExpRes 2012; 36(7):1278-87 5. Thanos et al. J Neurochem.2001;78:1094-1103.

P075. A Glucocorticoid Antagonist Shows Therapeutic Potential for Alcohol Dependence in a POC Human Laboratory Study.

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A focus for medication development in alcoholism is the post acute withdrawal, protracted abstinence phase. This phase may include cue-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis and impaired glucocorticoid receptor feedback and activity, which can activate the mesocorticolimbic dopamine system. We hypothesize that the glucocorticoid anatagonist, mifepristone, will show POC efficacy in non treatment-seeking outpatient alcoholics by significantly decreasing the number of drinks consumed relative to placebo. Subjects are medically healthy, male or female paid volunteers, 18-65 years of age, meeting DSM-IV criteria for current alcohol dependence and not seeking treatment. Subjects are randomly assigned to double-blind dosing for 1 week with mifepristone 600mg/d or placebo. Human laboratory cue reactivity procedures are conducted on the last day of dosing and salivary cortisol and VAS craving ratings collected. Subjects return after 1-week and 1-month to assess long term drug effects on drinking, cortisol, mood and sleep. Preliminary analyses found mifepristone was associated with a significantly greater reduction in drinking than placebo ($p=0.01$). Reduction in drinking was predicted by higher mifepristone plasma level, greater change in salivary cortisol levels from baseline to follow-up, and greater suppression of cue-induced craving in the human laboratory model. A positive signal in this non treatment-seeking, alcohol-dependent sample lends support to the potential utility of mifepristone in the treatment of alcohol dependence. A neurobiological explanation for the results may be related to normalization of the HPA axis/ brain extrahypothalamic stress systems and a subsequent normalization of the dysfunction of the mesocorticolimbic dopamine system in alcohol dependence.

P076. Dopamine system adaptations in alcohol abstinence: Translational evidence from humans and rats for a hyperdopaminergic state.

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Acute withdrawal from alcohol downregulatesdopamine(DA)ergic neurotransmission in the mesolimbic system, a finding that is consistently observed in animal models and that is also supported by some human neuroimaging studies. However, the state of the mesolimbic DA system in abstinence is less clear. Here we studied the dopamine receptors D1 and D2 as well as the dopamine transporter (DAT) in postmortem brain samples from human alcoholics and control subjects. To gain further insight into underlying mechanisms we compared these data to post-dependent rats after three weeks of withdrawal. We found a highly significant downregulation of D1 and DAT binding in the accumbens and nucleus caudatus of human alcoholics, while D2 was unaffected. After three weeks of withdrawal post-dependent rats showed a downregulation of D1 mRNA levels in the accumbens and caudate putamen with a corresponding reduction in binding in the nucleus accumbens shell and the substantianigra pars reticulata, while D2 binding was modestly but significantly upregulated in the ventral tegmental area (VTA) and the substantianigra pars reticulata. This was accompanied by reduced DAT binding in the VTA, and increased mRNA expression of the DA synthesizing enzyme tyrosine hydroxylase in the substantianigra pars compacta. Together, these data indicate increased DA levels during alcohol abstinence, which seems to contrast with the situation during acute withdrawal. Functionally, high tonic DA levels could blunt phasic DA signaling at D2 receptors resulting in difficulties in salience attribution to and discrimination of motivational stimuli.

P077. Characterization of input-specific innervation of the lateral habenula after exposure to drugs of abuse.

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The mesolimbic dopamine system plays a pivotal role in the processing of (natural) rewards, and drugs of abuse potently engage this system. The lateral habenula (LHb) in the epithalamus has emerged as a key regulator of activity of midbrain dopamine neurons. However, little is known about the contributions of the LHb to reward processing, or to the pathology of drug addiction. In the current study we set out to investigate how exposure to drugs of abuse alters synaptic inputs to the mouse LHb. We addressed this issue using a combination of optogenetics and slice electrophysiology, allowing for an input-specific investigation of the LHb. This is especially relevant since the LHb integrates signals originating from a great variety of nuclei, including ones coming from the neocortex and the pallidum. We stereotactically injected AAV2.1-CAG-Channelrhodopsin2-mCherry vectors into nuclei that send input to the LHb. After 2-3 weeks, whole-cell voltage clamp recordings were made in the LHb and input-specific synaptic responses were evoked by short light pulses. Prior to these recordings, animals had been exposed acutely or chronically to drugs of abuse. Our preliminary studies suggest that drugs of abuse indeed alter the synaptic strength of inputs to the LHb. Currently we are assessing the relevance of different types of afferents in this process.

P078. Zinc modulates ethanol-induced dopamine output in the rat nucleus accumbens.

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Alcohol abuse and alcoholism is a major health problem worldwide and imposes a high economic cost on society, although it is subject to extensive research, the role of changes in metabolism caused by alcohol consumption has been poorly investigated. Zinc (Zn2+) deficiency is a common metabolic aberration among alcoholics and Zn2+ influences the function of ligand-gated ion-channels, known pharmacological targets of ethanol (EtOH). We investigated whether manipulation of extracellular levels of Zn2+ modulates EtOH-induced increases of dopamine (DA) output, as measured by in vivo microdialysis in the rat, and whether voluntary EtOH consumption is altered by Zn2+-deficiency. Our findings show that short-term perfusion of the Zn2+- chelating agent tricine (10-100 uM in the perfusate) in the nAc fails to alter basal DA levels, whereas long-term perfusion significantly raises DA levels by 50%. We also show that short-term tricine-perfusion blocks ethanol- induced DA elevation. The present study indicates that Zn2+ status influences EtOH's interaction with the brain reward system, possibly by interfering with GlyR and GABAA receptor function. This also implies that Zn2+- deficiency among alcoholics may be important to correct in order to normalize important aspects of brain function.

P079. D-TMS in cocaine addiction: preliminary findings.

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Drug addiction is a brain disease with profound family, social, political implications. Cocaine abusers subjects, in various forms, cannot benefit from specific and effective therapeutic treatments. Recently,TranscranialMagneticStimulation(TMS)has emerged as non-drug therapeutic and non-invasive option in a wide range of brain disorders (Kobayashi &Pascual-Leone, 2003) with possible application in the field of drug addiction (Feil&Zangen, 2009). Recent theories (Melis et al., 2005; Koob& Volkow, 2010) attributed to the reduction of central dopaminergic tran- smission, an important role in behavioral changes related to substance abuse and speculate (Melis et al., 2005, Diana, 2011) that the "restoration" of a dopaminergic transmission can produce optimal therapeutic benefits. Therefore, we recruited (December 2011) cocaine-dependent patients (diagnosed according to the DMS IV) in Ser.T. ofMarsciano, and we have treated them with D-TMS. In short, we have 10 patients who started treat- ment. We asses-sed intake of cocaine before-during-after treatment through self-report and ve- rified through hair analysis. From this interim analysis, it appears that all subjects have reduced the intake of cocaine regardless of the frequency of stimulation and of the SHAM treatment which, however, was administered in 3 subjects. It is clear that it is essential to increase the number of patients involved in the study, as well as to "balance" the various groups in order to obtain numerical values appropriate to a optimal statistical analysis. Nevertheless, the data observed so far en- courage further and more in-depth analysis to fully assess the potential of dTMs in the supportive care of the cocaine abuse.

P080. Reversal of cocaine-evoked synaptic plasticity removes addiction related behaviors.

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Drug addiction is a brain disease that develops over time as a result of the initially voluntary consumption of substances and that can persist long after individual stops using them. Cocaine, a highly used addictive drug, causes a surge of extracellular dopamine release in nucleus accumbens; NAc. Dopamine may evoke synaptic plasticity at many synapses of the reward circuit that may underlie behavioral adaptations in mouse models of human addiction. A causal link has been missing however, and the positive consequences of restoring baseline transmission at identified synapses have not been tested. Here we first show that cocaine-induced ERK activation, a kinase required to addiction related behaviors, drives synaptic plasticity of excitatory transmission specifically onto D1R-expressing MSNs of the NAc. Moreover, optogenetic depotentiation of excitatory inputs to the NAc efficiently restores basal transmission and abolishes cocaine-induced locomotor sensitization. In recent work using self-administration in mice, we show that relapse to drug seeking also depends on the strengthening of glutamate transmission onto D1R-expressing neurons of the NAc. Indeed, prevention of relapse is observed when synaptic strength at the appropriate synapse is normalized by a specific optogenetic manipulation. To summarize, our results show that activation of dopamine-dependent signalling cascades in D1R-expressing neurons of the NAc is required to trigger strengthening of glutamate transmission, crucially involved in two animal models of relapse. Our data provide proof of principle that reversal of cocaine-evoked synaptic plasticity can reverse behavioral alterations caused by addictive drugs.

P081. Alpha-lipoic acid reduces ethanol self-administration in rats.

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Abstract Background - The main system of central ethanol oxidation is mediated by the enzyme catalase. By reacting with H2O2, brain catalase forms compound I (the catalase-H2O2 system), which is able to oxidize ethanol to acetaldehyde (ACD) in the brain. We have previously shown that ACD regu-lates ethanol motivational properties and possesses reinforcing effects by itself. In the present study, we investigate the effects of alpha- lipoic acid (ALA), a scavenging agent for H2O2, on oral ethanol self-administration. Methods - To this end, we trained Wistar rats to orally self-administer ethanol (10%) by nose pok-ing. The effect of intraperitoneal pretreatment with ALA was evaluated during a) maintenance of ethanol self-administration, b) ethanol self-administration under a progressive-ratio (PR) schedule of reinforcement, and c) oral ethanol priming to induce reinstatement of ethanol seeking behavior. Moreover, we tested the effect of ALA on saccharin (0.05%) reinforcement, as assessed by oral self-administration. Results - The results indicate that ALA dose-dependently reduced the maintenance, the break point of ethanol self-administration under a PR and the reinstatement of ethanol seeking behaviour with-out suppressing saccharin self-administration. Conclusions - These results support that ALA may have a potential use in alcoholism treatment.

P082. Differential effects of addictive drugs on dopamine release in shell and core of the nucleus accumbens in Hatano high and low-avoidance rats

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Hatano high- (HAA) and low-avoidance (LAA) strains were selected from Sprague-Dawley rats on the basis of their different performance in the shuttle-box task at the Hatano Research Institute (Japan) in 1985. Although HAA and LAA rats have displayed differences in the performance of other tasks and in some physiological parameters, a comparison of the effect of different drugs of abuse on dopamine (DA) transmission in Hatano strains is lacking. We used in vivo brain microdialysis to evaluate the effect of cocaine (5-10 mg/kg), amphetamine (0.1-0.20 mg/kg), morphine (0.5-1 mg/kg) and heroin (0.125-0.25 mg/kg) on DA transmission in the shell and core of the nucleus accumbens (NAc) of both strains. No differences were observed between strains in dopamine basal values or after saline injection (1ml/kg). The administration of psychostimulants induced a larger DA increase in the core of HAA compared to LAA rats, whereas the opposite was observed in the shell compartment. On the other hand, morphine (0.5-1 mg/kg) and heroin (0.25 mg/kg) increased extracellular DA to a larger extent in the shell of HAA than in LAA strain. Our results indicate that genetic backgrounds can produce different, and even opposite responses in DA transmission to drugs of abuse belonging to different pharmacological classes. These data suggest that comparative behavioural and neurochemical studies in Hatano rats may help to elucidate the role of the DA projections to NAc shell and core in the mechanism of drug abuse, and its relationship with genetic factors.

P083. A novel BAC transgenic mouse strain expressing GFP under control of the dopamine transporter promoter

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Dopamine (DA) plays a fundamental role in the central nervous system as a modulatory neurotransmitter, controlling different brain functions including locomotor activity, reward mechanisms, cognition and neuroendocrine functions. Dopaminergic neurons are primarily localized in substantia nigra (SN) and ventral tegmental area (VTA) of the midbrain and project to areas in the basal ganglia, prefrontal cortex and the limbic system. However, only a fraction of the cells in the VTA and SN are dopaminergic, therefore, markers permitting specific visualization and identification of dopaminergic neurons in this area are highly desirable. We have obtained a BAC transgenic mouse strain from GENSAT, which expresses enhanced green fluorescent protein (GFP) under control of the dopamine transporter (DAT) promoter (Slc6a3-GFP). The presynaptic DAT is responsible for sequestering released dopamine from the synaptic cleft and is selectively expressed in DA neurons. Initially, we found robust endogenous EGFP expression in ventral midbrain and striatal terminals that co-localized with dopaminergic markers (DAT and TH). Antibody labelling of GFP also showed extensive overlay with DAT in both cell soma and extensions of DA neurons in midbrain. DA uptake using striatal synaptosomes showed unchanged DA transport capacity in Slc6a3-EGFP mice compared to nontransgenic wild-type litter mates implying normal DAT levels. Basal locomotion and cocaine-induced hyperactivity was not significantly altered in Slc6a3-EGFP mice compared to wild-type litter mates. We further demonstrate using fluorescence activated cells sorting (FACS) that pure dopaminergic neurons can be isolated from Slc6a3-GFP mice as evidenced by enrichment of gene transcripts highly expressed in dopaminergic cells.

P084. The role of rostromedial tegmental nucleus in the regulation of dopamine neurons in sardinian alcohol preferring rats.

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Alcoholism is a psychiatric disorder, whose aetiology involves inherited predispositions and environmental factors. Alcohol activates the brain reward circuitry, which stems from the ventral tegmental area (VTA) where dopamine (DA) cells are located. DA neuron spontaneous activity tightly depends on afferent inputs. Among these, those arising from the GABAergic rostromedial tegmental nucleus (RMTg) play a major role in controlling their impulse activity, and in mediating the effects of drugs of abuse on DA cells. In this study we took advantage of significant differences in voluntary alcohol drinking between the selectively bred Sardinian alcohol-preferring (sP) and -nonpreferring (sNP) rat lines and investigated their electrophysiological properties in vivo. Extracellular single unit recordings revealed a difference in baseline DA cell firing activity between sP and sNP rats. Accordingly, the duration of inhibition elicited by electrical stimulation of the RMTg onto DA cells was reduced in sP rats. Consistently, RMTg neurons showed a reduced spontaneous activity in sP rats. When alcohol was systemically administered, we found an increased duration of inhibition from the RMTg on DA cells in sP rats. Given the crucial role played by RMTg cells in modulating DA cell activity, and given that sP and sNP rats are phenotypes of alcohol preference and aversion, respectively, we support the key role of RMTg nucleus in the regulation of the net reward signal encoded by the reward system and suggest its involvement in the individual vulnerability to excessive alcohol drinking.

P085. Alterations in cached learning in alcoholism: pilot data.

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In alcohol dependent patients, alcohol-associated stimuli elicit craving and conditioned responses and may potentially initiate drug-seeking behavior. This gives the attribution of value to formerly neutral stimuli through Pavlovian association a key role (Berridge, 2001). In animals, the adaptation of phasic dopaminergic prediction error signals over the course of learning appears to reflect this assignment of value to cues (Flagel et al., 2011). Chronic alcohol consumption reduces D2 receptors and increases responses to alcohol associated cues, suggesting a close parallel in addiction (Heinz et al., 2004). However, to what extent alterations in the Pavlovian learning process itself contribute to these findings, and indeed to the apparent faster habitization, is as yet unclear. We here describe a project that aims to comprehensively assess how learning alterations might contribute to the assignment of aberrantly high value in the development and recurrence of alcoholism. The project compares alcohol-dependent patients to healthy controls on a battery of tasks assessing Pavlovian, habitual and goal-directed reward-dependent learning behaviorally and with functional MRI. We here report preliminary data showing a shift towards cached learning and alterations in Pavlovian conditioning.

P086. Glutamatergic inputs to dopaminoceptive neurons affect novelty- seeking and sensitivity to alcohol.

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Persistence of drug-conditioned behaviors is associated with neuronal plasticity in the brain's dopamine system. In this study we investigate how glutamatergic inputs to dopaminoceptive neurons affect novelty- seeking and sensitivity to alcohol. We generated transgenic mouse lines with selective inactivation of either mGluR5 or NMDA receptors in neurons expressing dopamine D1 receptors. Mice with either mutation had no observable developmental impairments. Mutant mice showed normal anxiety-like behaviors, spatial learning and ability to learn instrumental food self-administration. However, while mice with NMDA receptor inactivation displayed normal responses to novelty and acquired instrumental responding in the operant sensation seeking task (OSS), loss of mGluR5 greatly reduced all behaviors associated with novelty and caused lack of responses in OSS. Conversely, while mice with mGluR5 inactivation in D1 neurons readily acquired conditioned place preference for alcohol (1.5 g/kg), animals with NMDA receptor inactivation were insensitive to alcohol conditioning. Nevertheless, even though mice with inactivated mGluR5 in D1 neurons had normal preference for alcohol, the did not escalate drinking after abstinence periods, unlike control littermates, which almost doubled their intake. These results show different and remarkably specific roles of glutamate signaling through mGluR5 or NMDA receptors on D1 neurons in control of reward-driven behaviors.
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P087. The dopamine activating properties of ethanol - neurocircuitry involved.

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Ethanol´s (EtOH) acute and chronic interactions with the mesolimbic dopamine (DA) system remain important for its reinforcing effects. We have published several papers implicating accumbal glycine receptors (GlyRs) in the DA activating/reinforcing effects of EtOH and have hypothesized that GlyRs in nAc via a nAc-VTA-nAcneurocircuitrytonically control mesolimbic DA activity and DA levels in nAc. Here we investigated using microdialysis if accumbalGlyRs modulate DA release after other drugs of abuse and to what degree GlyRs control impulse-driven DA release. We also studied on what cells in the nAcGlyRs are located using immunohistochemistry, and if cells retrogradely labeled from the aVTA express GlyRs. Field-potential recordings were used to study excitatory output in nAcSh.Accumbal strychnine perfusion blocked DA release after systemic EtOH and partly blocked that of nicotine and THC but did not influence cocaine or morphine effects. The majority of impulse-driven accumbal DA release was sustained by tonic GlyR activation. GlyRs were present mainly in the nAcSh and appeared co-localized with ChAT-staining. Retrograde tracing from the aVTA labeled numerous neurons in the nAcbutalso in the lateral septum. None of these co-stained for GlyRs. Strychnine reduced the electrophysiological effects of EtOH, nicotine and THC in nAc. GlyRs probably localized on cholinergic neurons in the nAcSh, control basal DA levels and seem crucial for EtOH-induced DA release, while they contribute to DA release after nicotine and THC. The proposed neurocircuitry may involve the lateral septum.

P088. Contribution of ventral and dorsal striatal dopamine to the reinforcing properties of alcohol.

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Alcohol is one of the most widely abused substances. With 76 million alcohol addicts worldwide, alcoholism forms a major burden to our society. Although the mesolimbic dopamine system has been implicated in alcohol consumption, the contribution of dopamine signaling in different striatal sub-regions to alcohol reinforcement is not entirely clear. The aim of this study was therefore to investigate the role of dopaminergic neurotransmission in the NAcbC, NAcbS and DLS in alcohol reinforcement. Male Lister Hooded rats were trained to drink alcohol for two months in the home-cage under an intermittent schedule. On average, they consumed 3.4 g/kg alcohol per 24h drinking session in the second month. Guide cannulas were then implanted bilaterally in the NAcbC, NAcbS or DLS. Subsequently, the rats were trained to respond for alcohol under a FR1 schedule of reinforcement, followed by a PR schedule of reinforcement, and the effects of infusion of the dopamine antagonist cis-(Z)-flupentixol (0-15 µg/side) were assessed in both paradigms. Preliminary results show that flupentixol into the NAcbS decreases responding for alcohol under both FR1 and PR schedules. Flupentixol infusions into the DLS tend to increase responding under a FR1 schedule, but have no effect during PR sessions. These results suggest that while dopamine both in the NAcbS and the DLS, contributes to alcohol reinforcement, NAcbS dopamine particularly mediates the motivation to obtain alcohol. These findings are largely consistent with our previous cocaine results. Hence, striatal dopamine may modulate cocaine and alcohol reinforcement in a comparable way.

P089. Implications for temporally precise optogenetic inhibition of reinstated cocaine seeking.

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The vulnerability to relapse has been linked to enduring adaptations in the structure and function of the brain's reward circuitry, including nucleus accumbens (NAc) neurons. Correlative evidence suggests that certain morphological and physiological changes are important mediators of addictive behaviors. Recent data from our laboratory indicate that these changes play a role in cocaine seeking in rats. It is not known, however, how or if these dynamic changes in cellular plasticity contribute to functional behavioral outcomes. Recent advances in optogenetics provide novel insights into the roles of the brain circuits that contribute to relapse. We demonstrated that optogenetic inhibition of the NAc or its afferents from the prefrontal cortex, basolateral amygdala or ventral tegmental area for the entire 2-hour reinstatement session attenuated reinstated cocaine seeking. Building on our recent discoveries that dynamic changes in NAc plasticity during the first 15 minutes of a reinstatement session parallel behavior, we hypothesize that the first 15 minutes of the reinstatement session may be the most critical for the initiation of the relapse event. The data to presented will determine if reinstatement can be suppressed by optogenetically inhibiting accumbens for the first 15 min after reinstating cocaine-conditioned cues. Male Sprague-Dawley rats underwent surgeries for viral microinjections of adeno- associated virus containing coding for the proton pump archaerhodopsin (ArchT) (CAG promoter), implantation of bilateral guide cannulae, and implantation of intra-jugular venous catheters. Animals then went through 12 days of cocaine self-administration followed by extinction training (2 hr/day). Following extinction, animals underwent cue-primed reinstatement of lever pressing along with the presence/absence of optical inhibition.

Dopamine and reward

P090. Dissociating the rewarding and motivational properties of social play behavior in adolescent rats: the role of dopamine, opioids and endocannabinoids.

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Natural rewards, such as feeding, drinking, sexual and social behavior are pleasurable behaviors in humans and animals, important for the survival of the individual, group or species. Interestingly, in comparison to feeding and sex, relatively little is known about the neurobiological underpinnings of the positive emotional properties of social behavior. Social play is the most characteristic social behavior displayed by adolescent mammals. It serves to facilitate social, physical and cognitive development and it is highly rewarding. Here, we dissociated the rewarding and motivational properties of social play behavior, using place conditioning and operant conditioning setups, respectively. Both social play-induced conditioned place preference (CPP) and operant responding were dependent on the animals' social motivation, as they were more pronounced in rats socially isolated during training and testing, compared to non-isolated or briefly isolated rats. Initial pharmacological characterization has shown that social play reward in the place conditioning setup relied on opioid, rather than dopaminergic or cannabinoid neurotransmission. On the other hand, dopaminergic neurotransmission did contribute to lever pressing for social play in the operant conditioning setup. Understanding the neural underpinnings of the rewarding and motivational properties of social play behavior may help to understand the physiology of this behavior, as well as its pathophysiology in psychiatric disorders characterized by social impairments, such as autism spectrum disorder and schizophrenia.

P091. Regulation of ethanol-induced dopamine release by inhibitory receptors in striatal subregions

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The nucleus accumbens is the primary target for the mesolimbic dopamine system and a key brain region for the reinforcing effects displayed by drugs of abuse, including ethanol. During the transition from recreational to compulsive consumption of reinforcing drugs, however, the dorsal striatum seems to be recruited. Understanding how dopamine-transmission is acutely regulated by ethanol in subregions of the striatum is thus important to understand synaptic underpinnings of ethanol-induced reward, and the transition to addiction. The extracellular level of dopamine was measured by reversed microdialysis in awake and freely moving Wistar rats subjected to local administration of ethanol in the nucleus accumbens or dorsolateral striatum. Ethanol (300 mM in the dialysis probe) significantly enhanced dopamine output in both the nucleus accumbens and the striatum. Local pre-treatment of the GABAA receptor antagonist bicuculline (50 μM) modulated the dopamine-elevating properties of ethanol in both the nucleus accumbens and the dorsolateral striatum, while the glycine receptor antagonist strychnine (20 μM) only inhibited ethanol-induced dopamine response in the nucleus accumbens. The data presented here suggests that the mechanisms underlying ethanol-induced dopamine-release vary within subregions of the striatum, which could be important for alcohol addiction and addictive behavior.

P092. DREADD-regulated modulation of behaviour in TH-CRE rats by increased VTA dopamine neuron activity

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Dopaminergic neurons in the ventral tegmental area (VTA) regulate dopamine (DA) transmission throughout the brain and thereby play a crucial role in normal functioning, but also in psychopathologies, including schizophrenia, anxiety, and depression. Although many pharmacological therapies for these disorders affect the brain monoamine systems, the role of dopaminergic projections in both the pathology and treatment are not completely understood. Through a novel approach, using DREADD (designer receptors exclusively activated by designer drugs) technology in TH-Cre rats (genetically modified rats expressing Cre in TH-positive cells), it is now possible to induce the expression of DREADD receptors exclusively on DA neurons in the VTA and modulate their activity through peripheral administration of 'designer-drug' CNO. This enables specific manipulation of dopaminergic activity in a pharmacological fashion. In this study, we aimed to set up and validate this DREADD technology in TH-Cre rats and examine the behavioural effects of DREADD activation by CNO. To do so, we injected double-floxed Cre-dependent DREADD viruses into the VTA. Subsequently, we activated the DREADD receptors by CNO, and monitored spontaneous behaviour in the homecage, as well as the motivation to work for palatable food in a progressive-ratio operant task. Preliminary data show a strong effect on both homecage locomotor activity and active leverpresses, suggesting that DREADD technology in TH-Cre rats indeed enables pharmacological manipulation of dopaminergic activity. By combining DREADD technology with conventional pharmacological interventions, we may further elucidate the neurobiological mechanisms underlying the treatment of divergent psychiatric symptoms.

P093. Anandamide interacts with the dopaminergic system to facilitate male rat sexual behaviour expression.

A. **Canseco-Alba**, G. Rodríguez-Manzo

Cinvestav

Sexual behaviour, like other rewarding behaviours, is modulated by the dopaminergic mesocorticolimbic system (MSL). Evidence suggests that endocannabinoids (eCN) play an important role in the regulation of rewarding behaviours through the modulation of MSL activity. CB1 receptor agonists have been found to inhibit male sexual behaviour. However, recent data from our laboratory showed that the eCN anandamide (AEA) exerts a dose-based biphasic effect on copulation, facilitating its expression at low doses both in sexually experienced and exhausted male rats. We hypothesized that AEA might exert these effects by interacting with the dopaminergic system. To test this hypothesis, we designed two experiments. In the first one, we recorded copulatory behaviour in independent groups of sexually experienced and sexually satiated male Wistar rats, i.p. injected with: 1) a facilitative dose of AEA (0.3 mg/kg), 2) the DA receptor antagonist haloperidol at a dose without effect on copulation (125 µg/kg), and 3) their combination. In the second experiment, independent groups of sexually experienced or sexually satiated rats received: 1) a suboptimal dose of AEA (0.1 mg/kg), 2) a suboptimal dose of the DA receptor agonist apomorphine (10 µg/kg) and 3) their combination. Results showed that the facilitative effects of AEA on sexual behaviour expression of sexually experienced and exhausted rats were blocked by haloperidol. Conversely, co- administration of suboptimal doses of AEA and apomorphine facilitated sexual behaviour expression in both types of animals. It is concluded that AEA exerts its facilitative effects on male rat sexual behaviour expression by interacting with the dopaminergic system.

P094. Choosing between sucrose consumption or running on a wheel depends on dopamine in the motivational circuitry: Involvement of D2 and adenosine A2A receptors.

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Dopamine (DA) in nucleus accumbens (Nacb) regulates effort-related decision-making. DA D2 receptors are colocalized with adenosine A2A receptors and they interact in an antagonistic manner. Wild type (WT) and A2A receptor knockout (A2ARKO) mice were used to explore DAergic involvement in the activational and directional components of motivated behaviors when multiple reinforcers are available. Haloperidol (D2 antagonist) was used in WT and in A2ARKO mice. A T-maze task was developed for the assessment of preference between physical activity (wheel running) in one arm and a dish with freely available sucrose pellets in the other. Control animals spent more time running and less consuming sucrose. WT animals that received haloperidol spent less time running, but increased time consuming sucrose and the amount consumed. A2ARKO mice did not shift to the less effort-requiring reinforcer, although, like the WT mice, they did reduce sucrose consumption when sucrose was devalued. WT animals showed a higher increase in c-Fos expression after haloperidol administration than A2ARKO mice in cingulate cortex and Nacb core. Thus, D2 antagonism reduced the choice of a reinforcer that involved vigorous activity but increased consumption of a reinforcer that required little effort. DA is involved in the regulation of behavioral activation and effort-based choice although not in the consumption of a primary food reinforcer such as sucrose. Adenosine A2ARKO mice show resistance to the effects of haloperidol. These results indicate that after DA antagonism, the preference for vigorous physical activity is reduced, while sucrose reinforcement remains.

P095. Neurochemical and behavioural responsiveness during sucrose self-administration

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Dopamine is implicated in the responsiveness to rewarding properties of natural and pharmacological stimuli. Conditioned stimuli (CS), linked to rewards such as food or drugs of abuse (uncontidioned stimuli, US), are essential to support the motivated behaviour. The role of mesocortical and mesolimbic DA in response to food CS after instrumental paradigm still remains unclear. The aim of our study was to investigate by microdialysis the impact of instrumental food CSs and US on behaviour and on basal DA in three terminal DA areas: shell and core of the nucleus accumbens (NAc) and medial prefrontal cortex (mPFCX), using a food self-administration paradigm. Rats were trained to acquire sucrose self-administration under a Fixed Ratio 1 (FR 1) or FR5 variable time out schedule of responding. After training, animals were able to self-administer sucrose pellets and microdialysis coupled with self-administration experiments were performed. The main finding of our study was that NAc shell DA has been activated not only by the conditioned cues but also by food after the instrumental conditioning. When both stimuli are presented in the same moment the increase of DA is strengthened and prolonged. DA response has been obtained in NAc core only during US presentation. mPFCX has been activated by each component of motivated behaviour, except for the response to CS using a FR5 schedule. We can conclude that DA in the NAc shell plays an important role on the acquisition and expression of motivated behaviour in food consumption, whereas NAc core and PFCX are less implicated.

P096. DNA methylation in the ventral tegmental area regulates associative reward learning

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UAB / Neurobiology

The formation and maintenance of reward-related memories is critical for adaptive behavior. This form of learning requires dopamine neurons located in the ventral tegmental area (VTA), which encode relationships between predictive cues and future rewards. Recent evidence suggests that epigenetic mechanisms, including DNA methylation, are essential regulators of neuronal plasticity and experience-dependent behavioral change. However, the role of molecular and epigenetic mechanisms in reward learning is poorly understood. Here, we reveal that the formation (but not maintenance) of stimulus-reward associations requires DNA methylation in the VTA. Inhibition of DNA methyltransferase activity in the VTA prior to conditioning site-selectively disrupted acquisition of learned approach behavior, but did not alter previously learned reward associations. Additionally, reward learning induced epigenetic alterations at key plasticity genes in the VTA, which are correlated with the development of learned behaviors in vivo and are blocked by inhibition of DNA methylation in vitro. These results provide the first evidence that epigenetic mechanisms regulate reward-related learning.

P097. Working activity for palatable caloric or non caloric food in non food-deprived and food-deprived rats

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Palatability is the hedonic food component that is considered to override the homeostatic mechanisms that control food intake. In this study we compared the amount of effort spent by non food-deprived and food-deprived rats in order to earn a palatable caloric (sucrose) or non-caloric (saccharin) snack. We performed neurochemical and behavioral experiments. Neurochemical studies: the dopaminergic response, in terms of extraneuronal dopamine levels and DARPP-32 phosphorylation pattern, to two consecutive palatable caloric or non-caloric snacks in the nucleus accumbens shell (NAcS) of non food-deprived and fasted rats was examined. Non food-deprived rats developed rapid habituation in the NAcS dopaminergic response following the first consumption of caloric and non-caloric palatable food, while food-deprived rats developed rapid habituation only to saccharin. Behavioral studies: in self-administration experiments, non food-deprived rats made a similar effort when operating for sucrose or saccharin. However, the same rats showed an increased response specifically for sucrose after 18-h fasting. After pre-feeding devaluation, rats reduced their response to sucrose but not for saccharin. These results strengthen the hypothesis that food intake is mainly controlled by palatability in non food-deprived rats and by caloric content in food-deprived rats. Moreover, they show that rapid habituation development was associated with a similar, basal working activity aimed at ingesting both caloric and non-caloric food, as observed in non food-deprived rats consuming sucrose or saccharin and in fasted rats consuming saccharin. Conversely, lack of habituation, as present in fasted rats consuming a caloric food, was associated with extra energy expenditure.

P098. Role of striatal indirect pathway dopamine D2 receptors in drug reward

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Located within the striatum are two populations of medium spiny neurons (MSNs) that provide opposing regulation of basal ganglia output and can be characterized by expression of either the dopamine D1 or D2 receptor. Direct pathway MSNs express the dopamine D1 receptor and opioid peptide dynorphin, while indirect pathway MSNs (iMSNs) express the dopamine D2 receptor and opioid peptide enkephalin. Data from our laboratory has shown that reduction of the dopamine D2 receptor on iMSNs is associated with reduced exploratory locomotor activity and reduced locomotor activity following acute cocaine. The goal of the current experiments was to examine whether a reduction in the dopamine D2 receptor on iMSNs alters cocaine reward, as indexed by conditioned place preference. Reduction in the dopamine D2 receptor on iMSNs was associated with attenuated locomotor activity in response to cocaine (15 mg/kg) or saline during conditioning trials and preference tests, compared to wild type mice. However, mice with a reduction in D2 receptors on iMSNs showed a conditioned place preference for cocaine similar to wild type mice. Previous literature suggests that enkephalin and the D2 receptor are involved in opioid reward. Dopamine depletion causes robust upregulation of striatal enkephalin levels, but the exact mechanism and specific role of D2 and D1 receptors remains controversial (Gerfen et al., Science, 1990; Sivam and Cox, 2006). Thus, we will test whether a reduction in D2 receptors on iMSNs affects the acquisition of morphine conditioned place preference and enkephalin expression and release within the striatum and projection areas.

P099. Conditioned saccharin avoidance induced by intra-accumbens shell amphetamine and intra-VTA morphine

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Saccharin avoidance conditioned by drugs of abuse (CSA) has been interpreted as an expression of the appetitive and dopamine-dependent, properties of the drug. In this study we investigated the effect of intra-Nucleus Accumbens (NAc) shell and core amphetamine and intra-ventral tegmental area (VTA)-morphine on CSA, at doses that classically are able to induce conditioned place preference (CPP). CSA was performed in a two bottle choice paradigm with two saccharin-drug associations. Amphetamine sulphate (10 and 20 µg/0.5 µl on each side), infused immediately after saccharin intake, significantly induced CSA when infused into the NAc shell, without differences between doses, while it was ineffective when infused into the NAc core. Morphine hydrochloride (0.5 and 1 µg/0.5µl on each side), infused immediately after saccharin intake, induce significant CSA at both doses tested. Amphetamine sulphate (20 µg/0.5 µl on each side) and morphine hydrochloride (1 µg/0.5µl on each side) did not induced significant CSA when infused 1.2 mm over the correspondent area (NAc shell and VTA respectively). Furthermore, morphine hydrochloride (1 µg/0.5µl on each side) infused into VTA induced a preferential increase of extracellular dopamine in the NAc shell compared with the core. The results obtained in this study, according with previous studies, suggest that drug of abuse induced-CSA, could be due to the rewarding properties of the drug which are, independently from its mechanism of action, dopamine-dependent.

P100. Spontaneous inhibitory synaptic currents mediated by D2 receptors

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Dopamine released through the activity of midbrain dopamine neurons is important in multiple physiological processes ranging from movement to reinforcement learning. This study describes spontaneous miniature inhibitory postsynaptic currents mediated by vesicular dopamine release acting locally on metabotropic D2 receptors leading to the activation of a G protein-coupled inwardly rectifying potassium conductance.

P101. Mesolimbic dopamine transmission during choices involving cost-benefit tradeoffs

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Mesolimbic dopamine transmission to the unexpected presentation of a single reward-predictive cue reflects the expected value of the reward associated with that cue but not the overall expected utility of the action required to obtain the reward. We investigated an unresolved discrepancy regarding whether cue-evoked dopamine transmission encodes the value of the subsequently chosen option or the greatest value available when choosing between concurrently available options of different value. We used fast-scan cyclic voltammetry to record dopamine release in the nucleus accumbens core of rats performing a decision- making task in which two levers were associated with different reward magnitudes and effort requirements. Each session included single-option "Forced" trials intermixed with two-option "Choice" trials. In Moderate Cost sessions, rats preferred a large reward / moderate effort option over a small reward / low effort option, and cue-evoked dopamine release demonstrated the expected encoding of reward value, with greater release for the larger reward option. In High Cost sessions, rats instead preferred the small reward / low effort option over a large reward / high effort option. We observed greater sustained dopamine transmission following cues for the non-preferred large reward / high effort option but no difference in peak dopamine release to the two options. Dopamine release during Choice trials was similar to that in the corresponding Forced trials in both session types. By representing the subsequently chosen option, mesolimbic dopamine transmission reflects the value of a decision that has already been made.

P102. The effect of LHb inactivation on Pavlovian versus Instrumental reversal learning

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Several lines of evidence implicate the lateral habenula (LHb) in the inhibition of reward and appetitively motivated behaviour. However, the role of the LHb in appetitively motivated learning tasks remains poorly understood. The experiments reported here examined the effect of LHb inactivation using bupivacaine, a sodium channel blocker, on two tasks involving the acquisition and extinction of appetitively motivated behaviour: Pavlovian reversal learning and instrumental reversal learning. Critically, Pavlovian reversal learning involves modulation of stimulus controlled responses, whereas instrumental reversal learning involves modulation of actions. In the Pavlovian reversal task, rats received CSA-Food and CSB-no Food pairings. Rats acquired conditioned magazine entry responses to CSA but not CSB. For Stage II, these contingencies were reversed so that CSA was presented alone while CSB was followed by a food reward. For the instrumental reversal learning task, rats were trained in Stage I on a Lever 1 - Food, Lever 2 - No Food task. Rats acquired lever pressing to the reinforced Lever 1 but not the non-reinforced Lever 2. In Stage II, this contingency was reversed so that responses on Lever 2 yielded a food reward whereas responses on Lever 1 did not. In both tasks, Bup was infused immediately prior to Stage II training. LHb infusions of Bup attenuated extinction in Pavlovian reversal learning and augmented instrumental reversal learning by increasing responding on the now, but never previously, reinforced Lever 2. Taken together, these findings implicate unique roles for LHb signals in the modulation of instrumental and Pavlovian behaviours.

P103. Protein Kinase C β and the Dopamine Transporter Regulate Surface D2-Like Dopamine Receptor Localization

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The dopamine transporter (DAT) and D2-Like dopamine autoreceptor (D2DR) regulate extracellular dopamine levels and signaling. D2DR agonists increase dopamine uptake, presumably through a physical coupling between the third intracellular loop of D2DR and the N-terminus of DAT. D2DR regulation of DAT is well known, but DAT regulation of D2DR is less understood. Protein Kinase Cβ (PKCβ) phosphorylates both D2DR and DAT to regulate the activity and/or trafficking of each protein. Here, our objective was to determine if DAT and PKCβ regulate surface D2DR localization. Changes in D2DR and DAT surface localization were measured using neuroblastoma N2a cells expressing D2DR with and without DAT. We found that DAT suppresses D2DR surface localization on a basal level, as compared to D2DR-Vector cells. Unexpectedly, D2DR agonist treatment increases both surface DAT and D2DR in DAT-D2DR cells, but not in D2DR-Vector cells, where this treatment internalizes D2DR as expected. Pretreatment with the PKCβ inhibitor LY379196 also increased D2DR surface localization, but only in DAT-D2DR cells. PKCβ inhibition has no effect on D2DR surface localization when using a mutant D2DR lacking three PKC phosphorylation sites regardless of DAT presence. Contrary to results with DAT, co-expression of deltaN22 DAT truncation with D2DR results in higher basal surface D2DR with no effect of PKCβ inhibition. These results suggest that DAT and PKCβ regulate D2DR surface localization through a physical complex. Supported by NIH grants DA 011697, DA 007267, and GM 07767.

P104. Role of nucleus accumbens dopamine in social play behavior in adolescent rats

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Social play is a form of social interaction that is crucial for proper development of physical, cognitive and social capacities of young mammals. It is highly rewarding; indeed, social play is modulated through neurotransmitters involved in reward processes such as dopamine, endogenous opioids and endocannabinoids. Our recent studies have shown that opioid neurotransmission in the nucleus accumbens (NAc) and endocannabinoids in NAc and amygdala mediate social play reward in adolescent rats. Dopaminergic neurotransmission in the NAc mediates certain aspects of the rewarding properties of food and drugs of abuse. Therefore, the present study had a twofold aim: 1. to investigate the role of NAc dopamine in social play behavior in adolescent rats; 2. to determine whether NAc dopaminergic neurotransmission underlies the play-enhancing effects of cannabinoid and opioid drugs. To this aim, we equipped four-week-old Wistar rats with bilateral guide cannulae aimed at the NAc, and briefly isolated them before testing. Intra-NAc infusion of low doses of amphetamine enhanced social play behavior in adolescent rats. These effects were antagonized by intra-NAc co-infusion of a non-effective dose of the dopamine receptor antagonist alpha-flupenthixol. Intra-NAc infusion of alpha-flupenthixol also antagonized the play-enhancing effects of systemic treatment with the anandamide hydrolysis inhibitor URB597, and attenuated the increase in social play induced by systemic administration of the opioid receptor agonist morphine. These findings suggest that NAc dopaminergic neurotransmission exerts an important role in social play and closely interacts with endocannabinoid and opioid systems.

P105. Isoflavone administration reduces cocaine self-administration responses and cue-induced cocaine seeking behavior and relapse in mice.

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Nowadays, no treatment for cocaine addiction is effective. Previous studies demonstrated that aldehyde dehydrogenase-2 (ALDH-2) inhibitors suppress cocaine rewarding effects by inhibiting dopamine synthesis (1). Daidzin, a natural isoflavone, is a potent ALDH-2 inhibitor (2). Thus, this study investigated the effects of 3 isoflavones: daidzin, daidzein and genistein, and a control compound, disulfiram, modulating cocaine-rewarding effects in a mouse model of self-administration. Mice were trained to nose-poke (FR1 schedule of reinforcement) to obtain cocaine (0.5 mg/kg/infusion). After a stable operant responding, the effects of a chronic treatment with daidzin (75 mg/kg, i.p.), daidzein (100 mg/kg, i.p.), genistein (100 mg/kg, i.p.) and disulfiram (75 mg/kg, i.p.) on cocaine self-administration were investigated. Our results show that these compounds decreased the number of active responses to obtain cocaine, being genistein the most effective one. At the end of the treatment, cocaine was substituted by saline, starting the extinction phase that lasted until responding on the active hole was lower than 40% of the mean response during the stable acquisition period. Then, mice were used to evaluate the effects of a single administration of daidzin, daidzein, genistein or disulfiram in cue-induced reinstatement of cocaine-seeking behavior and relapse. We have observed that daidzin, daidzein and disulfiram, but not genistein, decreased cue-induced cocaine relapse. In conclusion, isoflavones reduce cocaine-self-administration and relapse; and their pharmacological safety makes them good candidates for clinical applications.

P106. Inhibition of PKCbeta by enzastaurin attenuates amphetamine-stimulated efflux and behavior

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Amphetamine is a well-known drug of abuse that acts as a substrate for monoamine transporters on the plasma membrane of neurons and promotes reverse transport of monoamines into the synapse. The resulting increase in synaptic dopamine plays an important role in the rewarding and addictive properties of the drug. Amphetamine is known to activate PKC upon entry into the neuron. Previous work in our lab found that PKC inhibitors in general, and PKCbeta inhibitors specifically, decrease the amount of dopamine released by striatal slices following amphetamine treatment. Also, PKCbeta knock out mice show a decreased motor response to amphetamine compared to wild type controls, further indicating a role for this particular isozyme in amphetamine action. Here we demonstrate that enzastaurin, a PKCbeta selective inhibitor, attenuates amphetamine stimulated dopamine efflux in vitro and in vivo. Pretreatment of MN9D cells expressing the human dopamine transporter with enzastaurin results in a decrease in amphetamine stimulated efflux. Using in vivo microdialysis coupled with behavioral monitoring, we show that enzastaurin significantly attenuates the amphetamine stimulated increase in dopamine in the nucleus accumbens, and that this effect correlates with a decrease in amphetamine-stimulated locomotion. These results lend credence to the hypothesis that PKCbeta is a viable target for the treatment of amphetamine addiction. Supported by NIH grants R01 DA 011697, T32 GM007767, R37 EB003320, T32 DA07268

P107. Dopamine transmission and the role of D1- versus D2-like receptors in the nucleus accumbens during unexpected reward omission

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University of Michigan / Psychology

When necessary resources are no longer available, it is imperative that animals recognize this depletion and alter their behavior to obtain these resources. Using a laboratory model of foraging behavior, we have shown that when an expected reward is omitted, rats rapidly develop a behavioral preference for the rewarded option and avoid the omitted-reward option. Electrophysiology studies have shown that putative midbrain DA neurons decrease their firing rate when an anticipated reward is omitted, and this is hypothesized to decrease dopamine transmission and reduce D2-like receptor tone in terminal regions. We are investigating the transmission dynamics of dopamine in the nucleus accumbens during reward omission using fast-scan cyclic voltammetry and rapid-sampling microdialysis and also are examining the role of D1- versus D2-like receptors in mediating the development of a behavioral preference for the rewarded option. Specifically, we have found that the development of this behavioral preference is mediated by a reduction in D2-like, but not D1-like, receptor tone in the nucleus accumbens. Examining the transmission dynamics and role of dopamine receptors in altering behavior due to changes in reward availability is necessary for the ongoing understanding of the neurobiology of adaptive motivated behavior.

P108. Interactions between the Opioid and Dopamine System Regulate Pair Bond Formation and Maintenance in the Socially Monogamous Prairie Vole

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The socially monogamous prairie vole is an excellent animal model for studying the neurobiology of social attachment. Prairie voles form enduring pair bonds that begin with the development of an initial preference for their mating partner and this is associated with affiliative social interactions. However, following initial aspects of partner preference formation, there is a reduction in general affiliative behavior and an enhancement in general aversive social encounters, such as selective aggression toward novel conspecifics. Previous research from our laboratory has demonstrated that selective aggression is mediated by both kappa-opioid receptors (KORs) that mediate aversion and D1-like dopamine receptors that are important for motivated behavior. Importantly, activation of D1-like receptors is known to increase dynorphin, the endogenous ligand of KORs. We therefore hypothesized that D1-mediated selective aggression requires interactions with KORs. Indeed, activation of KORs within the NAc reversed blockade of selective aggression achieved by blockade of D1-like receptors. These data suggest that phasic increases in DA transmission activate D1-like receptors which enhances dynorphin release and its subsequent activation of KORs within the NAc shell directly drives selective aggression. This mechanism is supported by our next series of studies which measured 'real-time' DA release within the NAc shell of sexually naïve and pair bonded prairie voles and revealed that DA release is enhanced within the NAc shell of pair bonded males and this increase is positively correlated with measures of selective aggression. Together, this series of experiments demonstrates how opioid and DAsystems interact to regulate pair bond maintenance.

P109. Real-time dopamine release to food-predictive Pavlovian cues in rats with a history of cocaine self-administration

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Chronic cocaine self-administration can result in dramatic changes to both behavior and neural processing, often resulting in the chronic relapsing disorder of addiction. In addition, it has been shown that cocaine-experienced animals have deficits in appropriately guiding behavior for natural rewards that persist after abstinence from the drug, suggesting long-term changes in limbic circuits necessary for this type of goal-directed behavior. One possible mechanism for this dysfunction is alterations in the dynamics of dopamine (DA) signaling mechanisms following cocaine experience. In normal animals, phasic DA release in the nucleus accumbens (NAc) tracks the value of reward-predictive cues and is associated with the development of phasic neural encoding in NAc neurons. However, cocaine-experienced rats fail to develop this phasic NAc neural activity, suggesting a possible disconnection from normal DA signaling. To test this, rats were trained to self-administer either IV cocaine (0.33mg/inf; 2h sessions) or water to a foodcup (0.05ml) for 14d, and later learned a simple Pavlovian discrimination (CS+: sucrose; CS-: nothing). Using fast-scan cyclic voltammetry, we found that DA release to the Pavlovian cues in cocaine-treated animals was different than previously found in similar autoshaping studies, suggesting augmented DA signaling following cocaine exposure. This alteration may present a possible substrate for subsequent behavioral deficits seen following chronic cocaine experience.

P110. Effect of repeated administration of morphine and nicotine on 50-kHz ultrasonic vocalizations in male rats

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Significant evidence suggests that ultrasonic vocalizations (USVs) may index the emotional state in rats, and 50-kHz USVs have been proposed as a tool to investigate the rewarding properties of drugs. Apart from the evidence on the psychostimulants amphetamine and cocaine, little is known about the effects of other drugs with rewarding properties on emission of 50-kHz USVs. To further elucidate the neuropharmacology of 50-kHz USVs and their relevance in drug-induced reward, this study characterized the effects of different drugs possessing rewarding properties on 50-kHz USVs in adult male rats. Rats received the repeated administration of morphine (7.5 mg/kg, s.c.), or nicotine (0.4 mg/kg, s.c.) for 5 consecutive days. The number of 50-kHz USVs were then measured, together with locomotor activity, for 1 hour. As a comparison, additional rats received the repeated administration of amphetamine (2 mg/kg, i.p.), which strongly stimulates the emission of 50-kHz USVs, according to the same schedule. The results obtained demonstrated a robust effect of amphetamine on 50-kHz USVs emission and locomotor activity throughout the treatment. Conversely, morphine, and nicotine did not significantly elevate the total number of 50-kHz USVs, but induced motor stimulation. This study provides further evidence that that major differences exist in the effects of psychoactive drugs on 50-kHz USVs in rats, and confirm previous studies of our groups indicating that dopaminergic psychostimulants are more effective that other rewarding drugs in stimulating 50-kHz USVs

P111. Instrumental and Pavlovian conditioning in mice with inducible inactivation of NMDA receptors on cells expressing dopamine receptors D1.

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Neuronal plasticity of the brain's dopamine system plays a crucial role in reward processing and regulation of appetitive motivation which are key elements of adaptive behavior. Here we investigate ability to form Pavlovian and instrumental associations in a mouse model where NMDA-dependent plasticity of dopaminoceptive neurons is impaired. We generated NR1D1CreERT2 transgenic line with tamoxifen- inducible selective inactivation of NMDA receptors in neurons expressing dopamine D1 receptors. Effects of the mutation were assessed by measuring mRNA levels of glutamate receptor subunits and electrophysiological recordings of spontaneous excitatory postsynaptic currents on neurons in the nucleus accumbens. The mutant mice showed no obvious behavioral phenotype except of increased vertical activity in the open field. Animals were trained to perform instrumental food self-administration in Skinner boxes under fixed ratio schedule. Then flexibility of stable instrumental responding for food was assessed using various protocols, including progressive ratio and variable interval schedules, as well as extinction and reversal learning procedures. Pavlovian learning was tested in Skinner boxes in the conditioned reinforcement task. Mutant mice did not differ from controls in Pavlovian approach behavior or instrumental responding for Pavlovian stimulus, but showed a different rate of extinction of the conditioned response. Phenotyping of mice with inducible inactivation of NMDA receptors on dopaminoceptive neurons indicates that the mutation did not cause a major alterations in motivated behaviors, but might had specific effects on their flexibility. Support: Statutory funds of the Institute of Pharmacology of the Polish Academy of Sciences.

P112. Optogenetic Modulation of Ventral Tegmental Area Dopaminergic and GABAergic Neurons Affects Cue-reward Seeking.

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Ventral Tegmental Area (VTA) neurons play important roles in behavioural responses to rewards and environmental cues that predict them. Dopaminergic (DA) VTA neurons are well described in these processes, but recent work has shown that also y-aminobutyric acid (GABA) VTA neurons play an important role in this behaviour. We have previously shown that activation of VTA GABA neurons disrupts reward consummatory behavior. Yet, it remains uncertain to what extent this is mediated by local GABAergic inhibition of nearby VTA DA neurons. To investigate this, we now assess the effects of time locked inhibition of VTA DA neurons during different phases of task performance. To do this we use in vivo optogenetic approaches to manipulate these neural circuits in freely moving animals that perform a cue-reward seeking task. Specifically, we train adult mice to associate a cue with the delivery of a sucrose reward. During the task we monitor performance by assessing licking behavior before and after reward delivery. We hypothesize that transient inactivation of VTA DA neurons reduces conditioned and consummatory responses during this task.

P113. Neurosteroid agonist at GABAA receptor induces persistent neuroplasticity in VTA dopamine neurons

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Neurosteroids, such as allopregnanolone, are a subclass of steroids synthesized de novo in the brain, and they constitute an endogenous ligand group with the most potent positive modulatory actions known on ɣ-aminobutyric acid type A (GABAA) receptors. Neurosteroids have been implicated in several patterns of drug addiction including sensitization, withdrawal, and tolerance to different addictive drugs including alcohol. The precise molecular or neurobiological mechanism by which neurosteroids modulate the mesolimbic DA system is not well understood. Here, we have studied ganaxolone, a synthetic analog of allopregnanolone lacking nuclear steroid hormonal activity. We demonstrate for the first time that neurosteroids induce long-lasting potentiation of glutamate receptor currents in VTA DA neurons at least partly by inducing tonic inhibition of local VTA GABA interneurons. Tonic GABAA-mediated inhibition usually requires the expression of δ subunit, which we could detect immunohistochemically in the VTA neurons. We also studied conditioned behavioral responses in wild type C57BL/6J mice and in the δ subunit knockout mice and found that the aversive properties of ganaxolone are dependent on the δ subunit-containing receptors. These findings suggest that δ subunit-mediated tonic inhibition by neurosteroids plays important role in their aversive effects. Besides neurosteroids, also alcohol may target δ subunit-containing GABAA receptors. Modulations of the DA system in response to endogenous neurosteroid fluctuations during conditions such as stress and premenstrual syndrome might induce changes in alcohol behaviors and addiction. This study was supported by the Finnish Foundation for Alcohol Studies.

SYMPOSIA

TUESDAY, MAY 28TH

HIPPOCAMPAL OVER DRIVE OF THE MESOSTRIATAL DOPAMINE SYSTEM AS A BASIS FOR PSYCHOSIS IN SCHIZOPHRENIA

Organizer: A.A. Grace (USA)

Mapping the cortical and striatal response of the dopaminergic system to amphetamine: relationships to symptoms.

Judy Thompson, Mark Slifstein, Roberto Gil, Ragy Girgis, Liz Hackett, Larry Kegeles, Najate Ojeil, Rawad Ayoub, Xiayon, Raj Narendran, Anissa **Abi-Dargham**.

Columbia University-NY,Pittsburgh Medical Center-PA.

We have used PET and D2/3 radiotracers combined with an amphetamine challenge or a dopamine depletion paradigm in order to assess amphetamine induced dopamine release or baseline occupancy of D2/3 receptors by dopamine, in patients with schizophrenia, compared to matched controls. Studies have shown an increase in release and in baseline occupancy in drug free and drug naïve patients, most predominant in the associative striatum, in particular in the rostral caudate, predicting psychosis and its response to treatment. More recently we have examined amphetamine induced dopamine release in extrastriatal and cortical regions and their relationship to symptoms as well as cognitive performance. To date eighteen patients with schizophrenia (age 35 ± 11; 8 male, 10 female; 2 C, 7 AA, 6 H, and 3 multiracial); 5 naive to antipsychotic medication, and 13 were previously treated with antipsychotics, and 12 controls, age of 33 ± 9, 7 males and 5 females (1 C, 5 AA, 3 H, and 2 Asian, and 1 multiracial), have completed all procedures. These studies used the high affinity tracer [11C]FLB457 which allows measurements in limbic cortical and prefrontal cortical regions, as well as the thalamus and midbrain. This data set will allow us to examine relationships between cortical dopamine release and cognitive deficits as well as negative symptoms. We will also examine relationships between dopamine release across regions, such as cortical and subcortical regions, or hippocampal versus midbrain measurements. This comprehensive assessment across regions provides tools to probe the relationships between pathology in the hippocampus and the dopaminergic profile in patients with schizophrenia.

Functional imaging of the hippocampal-SN/VTA loop in humans

Toby Winton **Brown**

Otto-von-Guericke-University Magdeburg /

Human functional imaging studies provide evidence for the hippocampal-SN/VTA loop model by Lisman and Grace (2005) by showing that both regions are activated by novelty and that hippocampal activation to novel reward-predicting stimuli is correlated with striatal dopamine-release in PET. Functional connectivity analyses show that the SN/VTA responses to novel and rewarding stimuli functionally couple quite selectively with responses in the ventral striatum and the hippocampus. In healthy young adults, a single dose of L-DOPA reduces repetition suppression effects in the hippocampus (difference between novel and repeated items) while increasing hippocampal activity overall, akin to an "aberrant salience" effect. Furthermore, prolonged exposure to novel stimuli and ensuing prolonged activation of both the hippocampus and SN/VTA can lead to a situation in which the encoding of novel stimuli (memory tested after 30 minutes) is impaired but their long-term persistence (i.e. reduced forgetting; memory tested after 24 hours) is enhanced. Hence, increased dopaminergic stimulation, either pharmacologically or driven by hippocampal output signals (for novelty and/or reward) can lead to increased stimulus salience as well as impaired encoding. Thus, it is conceivable that pathological increases of hippocampal drive could account for some of the cognitive problems associated with schizophrenia.

Striatal dopamine & hippocampal glutamate in the development of schizophrenia

Oliver **Howes**

MRC CSC/ IoP KCL / Psychiatric Imaging

It is not known when dopaminergic dysfunction first occurs in the development of psychotic disorders such as schizophrenia, how it changes with the development of psychosis, or how it relates glutamatergic function. We have addressed these questions using PET and MRS imaging in longitudinal studies from the prodrome to the first psychotic episode, and in studies of patients with established schizophrenia. Method:The following age-matched groups have received [18F]-DOPA PET to measure dopamine synthesis capacity and MRS imaging to index glutamatergic function: A) two cohorts of individuals with at risk mental states who are at clinical high risk (CHR) psychosis; B) healthy controls; C) treatment responsive and resistant patients with schizophrenia. The CHR subjects received follow-up to determine who developed psychosis and repeat PET scans to determine change in striatal dopamine synthesis capacity with the onset of the first psychotic episode.
Results: Striatal dopamine synthesis capacity was elevated in the CHR cohort at baseline for the whole striatum ($F=3.7$, $df= 2,42$, $p=0.035$), and the associative striatal subdivision ($F=6.5$, $df=2,42$, $p=0.004$), which was confirmed in a second independent cohort. There was an altered relationship between dopaminergic and glutamatergic function in the CHR subjects compared to controls, which was most marked in the subjects who went on develop a psychotic disorder.There was a progressive increase in dopamine synthesis capacity with the development of psychosis ($t=3.01$, $df=7$, $p=0.020$).
Conclusions: These findings indicate that elevated dopamine synthesis capacity i) predates the onset of psychosis in people with prodromal symptoms, ii) increases further over time with the development of psychosis; and iii) shows an altered relationship with hippocampal glutamatergic function.

Aberrant hippocampal regulation of dopamine system function in the MAM model of schizophrenia

Daniel **Lodge**

UTHSCSA / Pharmacology

The dopamine hypothesis of schizophrenia is one of the oldest hypotheses of psychosis and suggests that an increase in dopamine system function underlies the positive symptoms of the disease. Interestingly, there does not appear to be any dysfunction within the dopamine system, per se, leading to the suggestion that an augmented dopamine system function is secondary to abnormal regulation by other brain regions. One such region is the hippocampus which has been demonstrated to increase the number of dopamine neurons available for phasic activation. Here we demonstrate, in the methylazoxymethanol acetate (MAM) model of schizophrenia, that hyperactivity within the ventral hippocampus is the cause of the aberrant dopamine neuron activity as well as the behavioral hyper-responsivity to psychomotor stimulant administration. Thus, aberrant hippocampal function may be a primary pathology in schizophrenia and further, the hippocampus may be a novel site for intervention for the treatment of psychosis.

DOPAMINE AND RESTLESS LEGS SYNDROME

Organizer: W. Paulus (Germany)

The non-human primate A11 diencephalospinal pathway is not dopaminergic

Imad **Ghorayeb**

Institut des Maladies Neurodegeneratives - Universite Bordeaux 2 - CNRS UMR 5293 /

The A11 diencephalospinal pathway is crucial for sensorimotor integration and pain control at the spinal cord level. Its dysfunction is thought to be involved in numerous painful conditions such as restless legs syndrome (RLS) and migraine. In the non-human primate (NHP), the A11 group appears to be the unique tyrosine hydroxylase (TH)-immunoreactive (IR) cell group projecting to the spinal cord with some fundamental differences compared to other dopaminergic systems, notably regarding the absence of dopamine transporter and aromatic amino acid decarboxylase (AADC) co-expression. The site(s) of action of the final end-product of these monoenzymatic TH-IR neurons, L-DOPA, remains to be determined. The demonstration, however, of AADC expressing neurons in the spinal cord supports the hypothesis of a local dopaminergic synthesis. As dopamine D1 receptor is not expressed in the NHP spinal cord, dopamine could modulate the sensorimotor processes mainly by decreasing the neuronal activity of the neurons expressing the D2 and D3 receptors. In RLS, dopamine agonists targeting the dopamine D2 and D3 receptor subtype have the higher efficacy on alleviating symptoms.

Functional Anatomy and Genetics Underlying Dopamine's Role in Restless Legs Syndrome

David **Rye**

Emory University Department of Neurology

Restless legs syndrome (RLS) is a common sensorimotor disorder around sleep whose expressivity is influenced by aging, female sex, pregnancy, deficient stores of mobilizable iron, genes. Pharmacological agents acting at D2 and D3 dopamine receptors are effective treatments but there is no clear consensus as to whether a hypo- or hyper-dopaminergic state lies at the core of RLS and which brain circuits are principally involved. Increasing attention has been focused upon a di-encephalospinal system which modulates spinal sensorimotor 'tone'. A single SNP in the BTBD9 gene increases risk of RLS (even in the absence of sensory symptoms) by 70-100%. From this knowledge, reverse genetics approaches in mice and Drosophila are revealing the first glimpses of molecular pathways that link iron and dopamine with RLS-like physiology and behavior. This progress will be reviewed and ongoing and future studies highlighted that seek to address persistent gaps in knowledge. David B. Rye M.D., Ph.D. Professor of Neurology Emory University School of Medicine Atlanta, GA 30322.

Treatment of the Restless Legs Syndrome (RLS)

Claudia **Trenkwalder**

Paracelsus Elena Klinik, Kassel, Germany and University Medical Center Göttingen, Department of Neurosurgery, Germany

The treatment of choice, currently licensed in many countries and with controlled trials available are the non-ergot-dopamine agonists ropinirole, pramipexole and rotigotine patch (partially licensed for RLS in as many countries as Europe, the US, Australia and Asia). Opioids, gabapentin and pregabalin are alternative treatments, but large trials are not provided for opioids. Large-scale controlled trials are available for the non-ergot DA pramipexole (Winkelman et al 2006) and ropinirole (Walters et al 2004, Trenkwalder et al 2004) and the rotigotine transdermal system (Trenkwalder et al 2008). Recommendations with low dosages mostly given before bedtime alleviate RLS nocturnal symptoms, improve subjective and objective QoSleep, reduce PLMS, and improve daytime symptoms including QoL. Interestingly, only small dosages are beneficial, dosage increase does not improve the condition over time, despite many patients tend to increase their dosage when loss of efficacy occurs. This phenomenon is striking and may point to the pathophysiological mechanisms of dopaminergic drugs in RLS. Metaanalysis of dopaminergic therapy point to better efficacy in drugs with longer half-life compared to pulsatile stimulation of i.e. L-DOPA. In augmentation, induced by dopaminergic treatment (see presentation W.Paulus), the circadian rhythm of RLS is changed or even omitted, another typical phenomenon only induced by dopaminergic treatment in RLS. From these characteristic phenomenons of dopaminergic treatment in RLS, hypothesis of the pathophysiology of action of the dopaminergic system in RLS will be discussed.

Dopaminergic Augmentation in RLS

Walter **Paulus**

University Medical Center Göttingen, Department of Clinical Neurophysiology, Germany

Augmentation in Restless Legs Syndrome (RLS) is defined as paradoxical long term increase in disease severity due to dopaminergic (over) medication. On a short-term base within the half-life of this medication nevertheless improves RLS symptomatology. Augmentation is difficult to separate from a particular severe RLS, the best criterion is a shift toearlier occurrence at daytime. In a hyperdopaminergic stateD2 receptors reduced in number by increased receptor degradation, unlike D1 receptors that are recycled to the plasma membrane. Augmentation will thus be related to a shift in the D2/D3 stimulation compared to D1/D5 receptor stimulation. Iron deficiency increases the risk to develop augmentation, partially by reducing the relative expression of D2 receptors more than those of the D1 receptors.

DUAL CONTROL OF DOPAMINE BY D2 AUTORECEPTORS AND DAT: REGULATION, EPISTASIS AND BEHAVIORAL IMPACT

Organizer: M.E. Gnegy (USA)

D2 autoreceptors stimulation enhances extracellular dopamine levels by acting synergistically on release and uptake mechanisms

Benoit-Marand Marianne

LNEC INSERM U-1084, Université de Poitiers

Salience is encoded by dopaminergic neurons as a phasic burst of activity contrasting with the basal tonic activity observed at rest. Phasic and tonic firing are translated in different extracellular dopamine concentrations. It is now well described how dopamine reuptake and dopamine autoinhibition by D2 autoreceptors shape this tonic and phasic levels of dopamine. However, little is known about the relationships between these two mechanisms. Here, we monitored dopamine uptake in the striatum of anesthetized mice using continuous amperometry. Dopamine overflow was evoked by electrical stimulation of the medial forebrain bundle. Dopamine uptake was slowed down by D2 antagonists haloperidol and eticlopride. This effect was not found in mice lacking D2 receptors. In order to evaluate the ability of tonic dopamine levels to regulate uptake, we blocked spontaneous dopamine release using gamma-butyrolactone. This manipulation had no effect on dopamine reuptake. However, in response to excessive dopamine release evoked by prolonged stimulation, dopamine uptake was saturated in mice lacking D2 receptors but not in wild type mice. Therefore, under physiological conditions, tonic dopamine levels do not regulate dopamine uptake. However, in conditions of excessive demand, D2 stimulation boosts dopamine uptake. Our results suggest that D2-like antagonists, by interfering with this mechanism, inhibit dopamine uptake. This inhibition synergizes with the potentiation of dopamine release induced by these drugs resulting in an increased dopamine overflow evoked by stimulation mimicking the bursting activity of dopaminergic neurons.

Association of genetic and epigeneticvariation of dopamine genes with phenotypesrelated to risk for schizophrenia

Alessandro **Bertolino**

Liability for schizophrenia is mainly provided by genetic variation, but environmental risk factors are also involved. Since diagnosis of schizophrenia is heterogeneous and does not represent a simple phenotype for genetic association studies, other phenotypes related to genetic risk and the pathophysiology of (so called intermediate phenotypes) can be evaluated. Prefrontal activity during working memory is indeed one such intermediate phenotype which is related to schizophrenia and dopamine dysregulation. Therefore, we have assessed how genetic variation in several genes related with dopamine signaling which include, COMT, DRD2, DAT, and AKT1. These studies have demonstrated both main effects of all these genes on prefrontal activity during working memory in humans, but also epistatic effects which are relevant to pre- and post-synaptic dopamine signaling. Consistently, these gene effects are also found on response to treatment with antipsychotics blocking the dopamine D2 receptor. In other studies we have also addressed the effects of epigenetic DNA methylation of COMT on prefrontal function demonstrating a clear gene by environment interaction. These studies represent a possible path to discover the effects of genetic and epigenetic variation on risk for intermediate phenotypes related to schizophrenia.

Protein kinase Cβ modulates trafficking and function of the dopamine transporter and the dopamine D2 autoreceptor

Margaret E. **Gnegy**, Rong Chen, Kathryn Luderman, Rheaclare Fraser, Stephanie Stokes, Michael Leitges, Conor Daining

University Michigan Med. Sch. / Pharmacology

Activation of presynaptic dopamine D2 autoreceptors (D2S) increases surface dopamine transporter (DAT) expression and dopamine uptake. Since DAT is a primary mechanism for terminating dopamine action, the interaction between DAT and D2S plays a key role in controlling extracellular levels of dopamine. We reported that DAT activity is modulated by protein kinase Cβ (PKCβ) but now demonstrate that PKCβ is integral for the interaction between DAT and D2S. In mouse striatal synaptosomes, either a specific PKCβ inhibitor or deletion of PKCβ abolished the interaction between DAT and D2S. This is not due to a loss in function of D2S. In the absence of PKCβ, two functional actions of D2S which reduce extracellular DA were enhanced: D2S -induced suppression of locomotor activity and of dopamine exocytosis. To elucidate the mechanism of PKCβ on the D2S-DAT interaction, DAT and D2S were co-transfected into mouse neuroblastoma N2A cells. Activation of D2S resulted in trafficking of both DAT and D2S to the cell surface. Upon inhibition of PKCβ, D2S-activated trafficking of DAT and dopamine uptake was completely abolished. Moreover, inhibition of PKCβ led to an up-regulation of surface D2S but not DAT expression, disrupting the trafficking of these two proteins. In both D2S-DAT-N2A cells and mouse synaptosomes, we show that D2S-stimulated DAT trafficking is mediated by a PKCβ-ERK signaling cascade. This study demonstrates that PKCβ has a substantial effect on coordinating the trafficking of both DAT and D2S. Funded by NIH grants DA011697 (MEG), DA025954 andand DA030890 (RC), University of Michigan Substance Abuse Research Center (RC), K.L. and R.F. T32-DA007267 (KL) and T32-DA007281 (RF).

Epistatic interactions between DRD2 and DAT genes: implications for clinical phenotypes.

Wolfgang **Sadee**

The Ohio State University / Pharmacology

We have identified and characterized several regulatory variants in the genes encoding the dopamine transporter (DAT) and the dopamine D2 receptor (DRD2) (1,2). The latter was significantly associated with increased risk of death as a result of cocaine abuse (3), whereas the former did not score significantly. However, we have observed a significant epistatic interaction between DAT and DRD2, conveying an eightfold enhanced lethal risk, an example of a clinically relevant gene-gene-interaction (epistasis), and more broadly a gene-gene-environment interaction) (4). We are now studying gene-gene interactions within the dopamine and related signaling pathways in a variety of behavioral disorders and therapies. Funded by the NIH/NIGMS Pharmacogenomics Research Network grant U01 GM092655. 1. Pinsonneault JK et al. Dopamine transporter gene variant affecting expression in human brain is associated with bipolar disorder. Neuropsychopharm. 8: 1644-1655 (2011). 2. Zhang Y et al. Novel polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc.Natl.Acad.Sci.USA. 104: 20552-20557 (2007). 3. Moyer RA et al. Intronic polymorphisms affecting alternative splicing of human dopamine D2 receptor are associated with cocaine abuse. Neuropsychopharm. 36: 753-762 (2011). 4. Sullivan D et al. Dopamine transporter DAT and receptor DRD2 variants affect risk of lethal cocaine abuse: A gene-gene-environment interaction. Translat.Psych., in press (2013).

NEUROPLASTICITY OF CORTICOLIMBIC DOPAMINE AND GLUTAMATE PATHWAYS IN DRUG ADDICTION

Organizer: R. E. See (USA)

Dynamic modulation of neuroplastic markers of dopaminergic and glutamatergic pathways following repeated exposure to cocaine during adolescence

Fabio **Fumagalli**

Department of Pharmacological and Biomolecular Sciences, University of Milan

The interaction between drug abuse and stress is a crucial component of drug addiction, although the underlying molecular mechanisms remain elusive. Dopamine and glutamate neurotransmission play indeed a role in cocaine abuse, although their interaction in the modulation of the stress response after chronic exposure to cocaine is still obscure. We exposed adolescent male rats to cocaine (20mg/kg/day) from PND 28 to PND 42; on PND 45, rats were subjected to swim stress (5 min) and sacrificed 15 min later. We focused on the medial prefrontal cortex (mPFC) that is still developing during adolescence and might be more vulnerable to stress. We found that the dopaminergic system is more affected by the exposure to cocaine alone as measured by changes in the expression of the main dopamine receptors and the dopamine transporters. Conversely, the glutamate system is more sensitive to the combination of cocaine and stress as measured by changes in the expression of glutamate transporters and post-synaptic receptor activation. These findings indicate that previous cocaine history affects the glutamate, but not the dopamine, response to stress whereas the repeated administration of cocaine alone targets primarily the dopaminergic system.

Methamphetamine-induced alterations in prefrontal cortex glutamate and dopamine function

RE **See**, A Parsegian, M Schwendt, DG See, CM Reichel; RE See

Medical University of South Carolina / Neurosciences; University of Tabuk

Methamphetamine (meth) addicts exhibit enduring cognitive and motivational abnormalities that likely reflect a dysregulation of prefrontal cortex (PFC) function. However, the impact of chronic meth on glutamate (GLU) and dopamine (DA) function within the PFC and their relationship to meth seeking remains unknown. To address these questions, we examined GLU and DA efflux and metabotropic GLU receptors in the medial PFC after meth self-administration in rats. Male rats self-administered daily meth (20 ug/50 ul infusion; FR1 schedule and 5 sec tone+light cue with each infusion), followed by daily extinction trials for 10-14 days. Control rats received yoked saline infusions. Measurement of GLU levels using no net flux microdialysis methods showed a meth-induced decrease in basal GLU levels in both the medial PFC and the nucleus accumbens core. In subjects tested during cue-induced, meth-primed, or cue+meth primed reinstatement of meth seeking, PFC GLU and DA efflux increased only in animals with a history of chronic meth. In a separate group of rats, measurement of surface and total levels of mGluR2/3 and mGluR7 receptors using surface biotinylation and immunoblotting showed a selective downregulation of surface mGluR2/3 receptors in the PFC, but not other regions. Together, these data suggest that the medial PFC exhibits persisting dysregulation of GLU and DA activity, consistent with the proposition of a hypofunctional basal state and a hyperfunctional activation state in the presence of stimuli that instigate meth seeking in drug addicted individuals.

mGluR2 loss in the corticoaccumbal neurocircuitry is a key pathophysiological mechanism mediating increased propensity to relapse

Rainer **Spanagel**

Institute of Psychopharmacology, Central Institute of Mental Health, Faculty of Medicine Mannheim, University of Heidelberg, Heidelberg, Germany

Our recent data show that mGluR2 loss in the rodent and human corticoaccumbal neurocircuitry may be a major consequence of drug/alcohol dependence leading to a hyper-glutamatergic state as demonstrated by microdialysis and animal/human spectroscopy studies and subsequently to increased propensity to relapse. Normalization of mGluR2 function within this brain circuit may be therefore of therapeutic value. Indeed restoration of mGluR2 function by infusing a lentiviral vector expressing the mGluR2 receptor into the infralimbic cortex attenuates relapse-like behavior. For pharmacological intervention studies we have used two DSM-IV/V based animal models - one for alcohol addiction (the deprivation model) and the 3-criteria model for cocaine addiction - and show that the application of the mGluR2/3 agonist LY379268 results in a pronounced reduction in relapse-like drinking behavior in the deprivation model. We further tested whether reinstatement of cocaine-seeking in addicted rats - deriving from the 3-criteria model - is sensitive to this treatment. Indeed, addicted rats that were treated systemically with the mGluR2/3 agonist LY379268 (0, 0.3, 3 mg/kg) showed a pronounced reduction in cue-induced reinstatement of cocaine-seeking behavior and any difference with non-addicted rats was abolished. In an attempt to further dissect the role played by mGluR2 and mGluR3 in cue-induced reinstatement, we used a knockout model. Because mGluR2 knockouts cannot be used in operant procedures due to motoric impairment, we only tested mGluR3 knockouts. These mice did not differ from controls in reinstatement, suggesting that mGluR2 receptors are critical in mediating addictive-like behavior.

Glutamatergic input from specific sources triggers psychostimulant-induced topographical and cell-type specific ERK activation in various nucleus accumbens shell subterritories.

Valjent E

Inserm U661, CNRS UMR5203, UM1& 2, IGF

Activated by a variety of therapeutic agents or drugs of abuse in physiological and pathological context, the ERK pathway has been proposed to play a critical role in the molecular mechanisms involved in dopamine- controlled striatal plasticity. In the nucleus accumbens (NAc) ERK phosphorylation induced by drugs of abuse is confined in D1R-expressing medium-sized spiny neurons, as a result of a combined stimulation of dopamine D1 and NMDA glutamate receptors. However, whether specific sources of glutamatergic inputs can trigger topographical and cell-type specific the regulation of the ERK pathway by drugs of abuse in the NAc is unknown. To address this issue, we first used a variety of BAC transgenic mice expressing enhanced green fluorescence (EGFP) or the Cre-recombinase (Cre) under the control of the promoter of dopamine D1, D2 and D3 receptors and of adenosine A2a receptor to dissect the microanatomy of the NAc. Moreover, using various immunological markers we characterized in detail the distribution of MSNs in the mouse NAc shell. Here we show that the inhomogeneous distribution of D1R-, D2R-, D3R- and A2aR-expressing MSNs allows defining several subterritories in the NAc shell, which exhibit particular neurochemical and inputs- specific features. We also provide evidence that various drugs of abuse generated a unique topography and cell-type specific activation of the ERK pathway within the shell subterritories. This study emphasizes the importance to precisely determine the neuronal populations in which signaling pathways are activated in order to better understand how they are regulated and what their corresponding functions are.

Striatal dopamine in compulsive drug use

Louk J.M.J. **Vanderschuren**

Utrecht University / Animals in Science and Society, Faculty of Veterinary Medicine

Drug addiction is a chronic relapsing disorder characterized by compulsive drug use, i.e., a loss of control over drug intake. Elucidating the neural substrates of compulsive drug use is one of the major challenges in contemporary drug addiction research. In the last decade, it has become clear that compulsive aspects of drug seeking and drug taking can be pertinently studied in animals. It has been hypothesized that the descent from casual to compulsive drug intake that characterizes addiction, is the result of two processes. First, there is increased dorsal striatal control over drug intake, causing drug use to become habitual, and second, there is reduced prefrontal cognitive control over drug use. Studies addressing this hypothesis, including the role of striatal and prefrontal dopamine in casual and compulsive drug use, will be discussed.

ALTERED ROLE OF DOPAMINE IN HUNTINGTON'S DISEASE

Organizer: M.S. Levine (USA)

Time-dependent alterations in dopamine modulation of glutamate synaptic transmission in mouse models of Huntington's disease

C. **Cepeda**, V.M. André, L. Galvan, G. Akopian, J.Y. Chen, E.A. Wang and M.S. Levine

University of California Los Angeles / Semel Institute

In animal models of Huntington's disease (HD), alterations in dopamine (DA) levels and receptor density have been observed. It is believed that high levels of DA in the early stages of the disease underlie hyperkinetic movements, whereas in the late stages DA levels drop and contribute to bradykinesia. In addition, a significant decrease in DA type 2 receptors is an early event in HD. DA modulates glutamate synaptic activity and changes in the DA system can affect the potential excitotoxic effects of glutamate in the striatum. Our present behavioral and electrophysiological studies use genetic mouse models of HD, including a fragment (R6/2) and two full-length (YAC128 and BACHD) models. Whole-cell patch clamp recordings in slices indicate time-dependent alterations in glutamate synaptic activity in direct (D1 receptor-containing) and indirect (D2 receptor-containing) pathway medium-sized spiny neurons (MSNs) of HD mice. In early symptomatic mice, glutamate transmission is increased in both D1 and D2 MSNs. In contrast, in fully symptomatic mice, glutamate transmission is decreased specifically in D1 cells. In addition, in the early symptomatic stage D1 receptor modulation of glutamate is absent in MSNs but treating the slices with tetrabenazine restores the glutamate balance and reduces the number of stereotypies. Currently, we are using optogenetic methods to manipulate selectively DA-containing neurons and fast-scan cyclic voltammetry to measure DA release in slices. Data from these studies will provide invaluable information to better modulate DA levels in the striatum.

Dopamine release and uptake alterations in Huntington's disease model rodents

M. A. **Johnson**, A. N. Ortiz, J. Kraft, G. L. Osterhaus, S. C. Fowler

Department of Chemistry and Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS 66045

The understanding of how dopamine release and uptake are altered in Huntington's disease is still evolving. Previously, we have shown that dopamine release is impaired in R6/2 Huntington's disease model mice. Moreover, this release impairment is due, in part, to incomplete loading of vesicles within dopaminergic presynaptic terminals. We show here also that dopamine release and uptake is impaired not only in R6/2 mice, but also in multiple genetically and chemically altered rodent models, including R6/1 mice, transgenic HD rats, and rats treated with 3-nitropropionic acid. Furthermore, our work addresses potential alterations in interactions between dopamine and other neurotransmitter systems, such as glutamate and gamma-aminobutyric acid, within the striatum. Overall, these findings suggest that neurotransmitter release impairment may be a general feature associated with Huntington's disease.

Altered Dopaminergic Function in the R6/1 Mouse Model of Huntington's Disease

Kerry **Murphy**

The Open University / Life, Health & Chemical Sciences

The advent of predictive gene testing for Huntington's disease (HD) combined with PET imaging has revealed an early loss of dopamine receptors in asymptomatic gene carriers. We have previously shown that synaptic plasticity is abnormal in the cortex of the R6/1 mouse model of HD and that this can be rescued by dopamine receptor agonists (Cummings et al. (2006) Human Molecular Genetics; Dallerac et al. (2011) Neurodegen. Dis.). We have now examined the properties of dopaminergic neurones in brain slices prepared from R6/1 mice carrying either 116 or 89 polyglutamine (Q) repeats (corresponding to manifest and pre-manifest motor phenotypes respectively) and littermate controls aged 9-11 months. Intracellular recordings were made from dopaminergic neurones located in the substantia nigra. Evoked release of striatal dopamine was measured using amperometry. Resting membrane potentials and input resistances were normal, however 116Q and 89Q transgenic dopaminergic neurones exhibited a statistically significant decrease in the amplitude and duration of the slow after hyperpolarisation and a marked increase in firing rate upon depolarisation. Evoked dopamine release was dramatically reduced in 116Q slices compared with controls. Unexpectedly, the converse was found for slices prepared from 89Q mice. Maximal dopamine release in these slices was significantly greater than controls. These data suggest that (i) dopaminergic neurones are abnormal from an early age and (ii) dopamine release undergoes a temporal biphasic shift from high to low; a profile that may underlie and contribute to the pathogenic complexity of HD.

Dopamine and serotonin innervation of the human striatum : A comparison between Huntington's chorea and Parkinson's disease

M **Parent**, S Petryszyn, MG Sanchez, C Bédard, E Pourcher, A Parent

Université Laval / Psychiatry and Neuroscience

In contrast to our vast knowledge of the dopamine (DA) system, much less is known about the involvement of serotonin (5-HT) in neurodegenerative diseases affecting the basal ganglia. Therefore, we designed a study that aimed at characterizing the status of the striatal DA and 5-HT systems in patients who suffered from either Parkinson's (PD) or Huntington's disease (HD), compared to age-matched controls. Antibodies against tyrosine hydroxylase (TH), 5-HT transporter (SERT) and proliferating cell nuclear antigen (PCNA) were used as specific markers of DA, 5-HT and cell proliferating activity, respectively. The results reveal a significant decrease of TH+ axon terminals throughout the striatum in both PD and HD, whereas the density of SERT+ axon varicosities was found to be slightly increased in the striatum of PD and HD patients compared to controls. An intense TH- immunoreactive zone along the ventricular border of the head of the caudate nucleus was observed in HD cases, but not in PD patients or age-matched controls. This thin (150-400 ?m) paraventricular band was composed of numerous small and densely packed DA axon varicosities and it overlapped the deep layers of the subventricular zone (SVZ). This finding concurs with the marked increase in neurogenesis noted in the SVZ of HD patients and suggests that DA plays a crucial role in mechanisms designed to compensate for the massive striatal neuronal loss that occurs in HD.

IMPULSIVITY IN PARKINSON'S DISEASE

Organizer: B. Averbeck (USA)

Jumping to conclusions in Parkinson's disease

A. **Djamshdidian**, S. Sullivan, T. Foltynie, P. Limousin, A. Lees and B. Averbeck

UCL, London, UK/ NIH, Bethesda, USA

A subgroup of treated patients with Parkinson's disease (PD) develop devastating behavioural side effects collectively termed impulsive compulsive behaviours (ICBs). We have tested PD patients with and without ICBs on the beads task, which is a validated information- sampling task, and compared results to substance abusers and pathological gamblers who do not have PD. We found that all patients gathered significantly less information and made more irrational choices than matched controls. Irrational choices where defined when participants would choose a cup which is according to the evidence they had most probably incorrect (eg. more blue beads have been drawn but the green cup has been guessed). Further PD patients without ICBs resembled patients with pathological gambling whereas PD patients with ICBs were similar to substance abusers. PD patients who were not taking dopamine agonists performed as well as healthy volunteers, even when treated with deep brain stimulation. Therefore, dopamine agonists are the single most important risk factor for impulsive choice in PD and results strengthen the link between ICBs in PD and substance abuse.

Impulse Control Problems in Parkinson's Disease: An Incentive Sensitization Account

Andrew D. **Lawrence**

Cardiff University / School of Psychology

A range of impulse control and related 'disinhibitory' disorders has been linked to dopamine therapies in Parkinson's disease, including disordered gambling, hypersexuality and the addiction-like compulsive use of dopamine replacement therapy, or dopamine dysregulation syndrome. The central role of dopaminergic drug therapy in these disorders suggests that they may be different manifestations of the same underlying causal mechanism, but what this mechanism is remains unclear. We have previously argued that alterations in brain reward circuitry are key to understanding impulsive control problems in Parkinson's disease. While debate continues over the precise role of mesolimbic dopamine systems in reward, one prominent theory holds that dopamine motivates the pursuit of rewards by attributing incentive salience to reward-related stimuli, triggering pursuit ('wanting'). Further, in the case of compulsive behavioural disorders, including addiction, reward cues may be attributed, as a result of incentive sensitization with pathological incentive salience. Here, we present evidence from neuroimaging (PET, fMRI) studies consistent with this hypothesis.

New approaches to treatment for impulsivity in Parkinson's disease

James **Rowe**

Parkinson's disease causes many types of impulsivity e.g. impaired motor inhibition, choice impulsivity with risk taking; and reflection impulsivity. These impairments occur even in the absence of clinically severe impulse control disorders (ICDs). The deregulation of dopaminergic functions is well established as a cause of impulsivity, especially in the context of dopaminergic therapies. However, impulsivity is multifactorial. In Parkinson's disease changes in noradrenergic and serotonergic systems, and changes in the structural connectivity of critical frontostriatal circuits, are also potential contributors to impulsivity, either directly or through interactions with dopamine. The changes in noradrenergic and serotonergic neurotransmitter systems are of particular interest, as they provide new targets for treatment of impulsivity. We have used pharmacological fMRI to study the effect of serotonin and noradrenaline reuptake inhibitors on motor impulsivity, using citalopram 30mg and atomoxetine 40mg respectively. These drugs are shown to have differential effects on stop-signal and no-go tasks, both in terms of better behavioral inhibition and enhanced regional brain activation. However, the neurocognitive effects of these two drugs depends on clinical factors (e.g. the stage of disease) while behavioral benefits also depend on individual differences instructural and functional brain connectivity. We discuss the role of noradrenaline and serotonin in inhibition in Parkinson's disease, including their direct effects and their potential interactions with dopamine at different stages of disease. Our results emphasize the importance of dopaminergic and non-dopaminergic neurotransmitters in the frontostriatal circuits required for regulation of behaviour and inhibition.

Impulse control disorders in Parkinson's disease

Valerie **Voon**

Department of Psychiatry, University of Cambridge, Cambridge

Dopamine agonist-related impulse control disorders such as pathological gambling, binge eating and hypersiexuality in Parkinson's disease are common and can be associated with significant dysfunction. The disorder provides an intriguing model to understand the interaction between dopaminergic medications and an underlying neural susceptibility. This talk reviews cognitive and neural mechanisms underlying these disorders in Parkinson's disease and discusses this within the context of other behavioural addictions in the general population.

MODULATION OF DOPAMINE SYSTEM FUNCTION BY NUCLEAR RECEPTORS: FOCUS ON ADDICTION

Organizer: R. Ciccocioppo (Italy)

Activation of PPARγ by pioglitazone reduces opioid reinforcement and opioid-induced activation of the mesolimbic dopamine system

Roberto **Ciccocioppo**[1]* Miriam Melis[2], Maria Antonietta De Luca[2], Serena Stopponi[1] Giordano de Guglielmo[1]

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Pioglitazone is a selective peroxisome proliferator-activated receptor-gamma (PPARγ) agonist approved for the clinical treatment of insulin resistance and Type 2 diabetes. Here we tested the effect of this drug on operant heroin self-administration paradigms. Microdialysis experiments to detect extracellular dopamine (DA) levels in the nucleus accumbens (NAcc) and ex vivo electrophysiological recordings from VTA DA neurons were also carried out.
Results showed that activation of PPARγ by pioglitazone (0, 10- 60 mg/kg, o.s.) selectively reduced FR1 and PR heroin self-administration ($p<0.01$). Saccharin self-administration was not modified by pioglitazone. In microdialysis experiments heroin elicited a significant increase of extracellular DA levels in the NAcc shell that was markedly reduced by pioglitazone. In a rat horizontal brain slice preparation whole-cell current-clamp recordings were performed from medial posterior VTA DA neurons investigating on the effects morphine. Acute application of morphine (0.3-3 µM) significantly increased the spontaneous activity of VTA DA neurons ($P<0.0001$). Whereas pre-incubation (2 hrs) with pioglitazone (3, 10 and 30 µM) prevented morphine-effects ($p<0.05$). The effect of pioglitazone was blocked by co-application of the selective PPARγ antagonist GW9662 (500 nM), which per se did not modify either the basal spontaneous activity or the morphine-induced excitation of VTA DA cells.
Altogether our results demonstrated that activation of PPARγ by pioglitazone attenuated the reinforcing effects of heroin and abolished the ability of opioids to stimulate mesolimbic DA system. These findings provide new information about the role of brain PPARγ receptors and identify pioglitazone as a candidate for treating opioid abuse.

Peroxisome Proliferator-activated nuclear receptor-alpha (PPAR-a): A novel taget for the development of anti-nicotine smoking Cessation Treatments

Steven R. **Goldberg**

Preclinical Pharmacology Section / NIDA IRP

Current anti-nicotine smoking cessation medications have limited efficacy and new approaches are needed. In a recent series of studies we have assessed inhibitors of the enzyme fatty acid amide hydrolase (FAAH), the primary enzyme responsible for degradation of the endocannabinoid anandamide and the endocannabinoid-related peroxisome proliferator-activated nuclear receptor (PPAR-?) ligands oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), as anti-nicotine smoking cessation treatments. Using intravenous nicotine self-administration and reinstatement models in rats and monkeys and in-vivo microdialysis and electrophysiology models in rats.We have shown that fatty acid amide hydrolase (FAAH) inhibitors URB597 and URB694 dose dependently block ongoing nicotine self-administration and prevent reinstatement of extinguished nicotine-seeking behavior by a nicotine prime or by nicotine-asociated cues in rats and squirrel monkeys. The blockade of nicotine self-administration and reinstatement of nicotine-seeking by FAAH inhibition appears to be primarily mediated by elevated levels of OEA and PEA acting on PPAR-? and not by elevated levels of anandamide. In squirrel monkeys and rats, the first generation lipid-lowering fibrate, clofibrate, selectively blocks self-administration of nicotine and reinstatement of nicotine-seeking behavior and this blockade is reversed by the PPAR-? antagonist MK886. Clofibrate and URB597 also block nicotine-induced activation of VTA dopamine neurons and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats, neurochemical actions of nicotine that underly nicotine addiction. These experiments have identified two novel targets for development of smoking cessation medications that can now be advanced to clinical trials. Supported by the Intramural Research Program of the National Institute on Drug Abuse, NIH, DHHS.

Bidirectional control of nicotinic cholinergic function by PPAR-α in dopamine neurons

M. **Pistis*** and M. melis

University of Cagliari / Department of Biomedical Sciences

Nicotinic acetylcholine receptors on dopamine neurons, particularly those containing the β2 subunits (β2*nAChRs), contribute to firing properties of VTA DA cells and mediate the addicting and motor-activating effects of nicotine. These receptors are targets for peroxisome proliferator-activated receptors-α (PPARα). PPARα, nuclear receptors activated by endogenous lipids of the endocannabinoid family, namely oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), affect discharge rate of DA cells and their response to nicotine by modulating β2*nAChRs. How PPARα endogenous ligands are synthesized by dopamine cells is currently unknown. Using ex vivo and in vivo electrophysiological techniques combined with biochemical and behavioral analysis, we show that activation of α7nAChRs on dopamine neurons increases levels of PPARα endogenous ligands in a Ca2+-dependent manner. Accordingly, in vivo production of OEA and PEA, triggered by α7nAChR activation, blocks nicotine-induced excitation of dopamine neurons and hyperlocomotion and displays antidepressant-like properties. Furthermore, tyrosine phosphorylation of the β2 subunits of nAChR was increased following in vivo PPARα activation. These data demonstrate that PPARα ligands are effectors of α7nAChRs and that their neuromodulatory properties depend upon phosphorylation of β2*nAChRs. This pathway is part of a homeostatic short loop feedback mechanism driven by nAChRs through of PPARα ligand synthesis and autocrine activation of PPARα. Our results unveil important physiological functions of nAChR/PPARα signaling pathway in dopamine neurons. Overall, the present study suggests new therapeutic targets for disorders associated with unbalanced dopamine-acetylcholine systems.

Glucocorticoid Receptors in dopaminoceptive neurons, key for the modulation of the dopamine system and stress-related behaviors.

Jacques Barik, Fabio Marti, Camille Baranowski, Carole Morel, Sebastian P. Fernandez, Christophe Lanteri, Jean-Pol Tassin, Cedric Mombereau, Arndt Benecke, Véronique Deroche, Alain Bailly, PierVi Piazza, Philippe Faure, François **Tronche**.

"Gene Regulation and Adaptive Behaviors" Team, UMR7224 CNRS/INSERM/UPMC /

Traumatic experiences and social stress may contribute to the onset of psychiatric disorders. These situations trigger stress responses including the activation of the HPA-axis and the release of glucocorticoid hormones. This mechanism is beneficial when normally working but disproportionate or excessively long-lived stress responses can precipitate the development of pathological anxiety, depression, inability to socially perform or addiction. The molecular and cellular mechanisms remain unclear. GCs activate the glucocorticoid receptor (GR), a ubiquitous transcription factor belonging to the Nuclear Receptor superfamily. To address its role in behavioral responses, we generated mice with GR gene ablation targeted to specific brain cell populations. We showed that selective GR gene ablation in mouse dopaminoceptive neurons expressing D1a receptor markedly decreased the motivation of mice to self-administer cocaine, sensitization and conditioned place preference to cocaine, dopamine cell-firing and the control exerted by dopaminoceptive neurons on dopamine cell-firing activity. Anxiety was unaffected, indicating that GR modify a number of behavioral disorders through different neuronal populations. Concerning depression-related behaviors, rodents subjected to repeated instances of aggression develop indeed enduring social aversion and anxiety. We showed that GR in dopaminoceptive neurons are essential for the appearance of a social aversion as well as dopaminergic neurochemical and electrophysiological neuroadaptations. Anxiety and fear memories remained unaffected. Acute inhibition of the activity of DA-releasing neurons fully restored social interaction in socially defeated wild-type mice. Our data suggest a GR-dependent neuronal dichotomy for the regulation of emotional and social behaviors, and clearly implicate GR as a link between stress resiliency and dopaminergic tone.

PARKINSON'S DISEASE:

FROM CAUSES TO NEUROPROTECTION

Organizer: M.J. Zigmond (USA)

Altered regulation of αSynuclein expression as a key determinant of "sporadic" Parkinson's disease risk

Asa **Abeliovich** and Herve Rhinn

Columbia University / PAthology and Cell Biology

Common genetic variants in the human population play a significant role in the pathogenesis of non-familial ('sporadic') Parkinson's disease (PD). Among such PD risk variants, the α-synuclein (αSyn) locus is of particular interest, as SNPs in this locus show the strongest and most robust impact on sporadic PD risk. Furthermore, very rare mutations in αSyn as well as triplication of the aSyn gene locus lead to familial inherited forms of PD. aSyn is thus an attractive therapeutic target for PD, with most strategies aimed at reducing its level or aggregation. Our findings point to a novel regulatory mechanism that impacts aSyn physiological and pathological functions: αSyn messenger RNA (mRNA) transcript differential 3' untranslated region (3'UTR) usage. Longer transcript isoforms (αSynL) correlate with increased protein accumulation, intraneuronal protein redistribution, and pathological functions, both in patient human brain and in model systems. This ultimately may provide a novel therapeutic approach by targeting specifically pathological rather than physiological functions of αSyn. aSyn 3'UTR usage is modified by dopamine exposure as well as by αSyn locus common genetic single nucleotide polymorphism (SNP) variants that increase PD risk. Thus, the 3'UTR regulation of αSynuclein is a convergence point of regulatory mechanisms that impact PD pathogenesis through aSyn. Whereas a small segment of the 3'UTR (sufficient to confer dopamine sensitivity) is conserved in rodent αSyn, most of the 3'UTR sequences are unique to humans.

Preclinical testing of neuroprotective strategies in Parkinson's disease.

Marie-Francoise **Chesselet**

UCLA / Neurology

"Neuroprotection" is usually understood as preventing cell death; yet "protecting" dysfunctional neurons form dying may not be clinically beneficial and evidence points to a long phase of dysfunction before neuronal death in most neurodegenerative diseases including Parkinson's disease (PD). In addition, models for testing neuroprotective strategies in PD have been problematic because studies of toxin-induced nigrostriatal DA neurons death have failed to predict clinical success, suggesting that the mechanisms by which DA neurons die in these experimental models differs from those occurring in the disease. Our laboratory has taken a different approach; we test strategies that may interfere with pathological mechanism of PD by measuring their ability to improve a range of behavioral, biochemical, and pathological evidence of neuronal dysfunction caused by alpha-synuclein, a protein involved in both genetic and sporadic forms of PD. Mice over-expressing wild-type, full length, human alpha-synuclein under the Thy1 promoter are used to mimic as closely as possible sporadic PD. The promoter leads to widespread over-expression of the transgene in neurons of the central and peripheral nervous systems thus producing widespread pathology as observed in PD. The end-point measures assess dysfunction in domains altered in PD, including nirostriatal dysfunction and DA loss, non-motor deficits, mitochondrial dysfunction, inflammation and protein aggregates but are not meant to exactly reproduce all facets of the human disease, which is not realistic or necessary. An overview of currently tested strategies and their effects on these endpoints will be presented. Support: MJFF, PHS P50 NS38367, ISIS Pharmaceuticals, gifts to UCLA CSPD.

Chaperone-Mediated Autophagy as a target for neuroprotection in Parkinson's Disease

Maria Xilouri1, Oystein Brekk1, Natalie Landeck2, Themistoklis Papasilekas1, Takis Pitichoutis3, Zeta Daifoti3, Deniz Kirik2, **Leonidas** Stefanis1,4

BRFAA / Basic Neurosciences (1), Lund University (2), Dept of Pharmacology, Univ of Athens Medical School (3), Second Dept of Neurology, Univ of Athens Medical School (4)

α-Synuclein (AS) levels are critical to Parkinson's Disease (PD) pathogenesis. Wild-type (WT) AS is degraded partly by chaperone-mediated autophagy (CMA), and aberrant AS may act as an inhibitor of CMA. To address whether the induction of CMA may represent a potential therapy against AS-induced neurotoxicity, we overexpressed lysosomal-associated membrane protein 2a (Lamp2a), the rate-limiting step of CMA, in human neuroblastoma SH-SY5Y cells, rat primary cortical neurons in vitro, and nigral dopaminergic neurons in vivo. Lamp2a overexpression in cellular systems led to upregulation of CMA, decreased AS turnover, and selective protection against adenoviral-mediated WTAS neurotoxicity. Protection was observed even when the steady-state levels of AS were unchanged, suggesting that it occurred through the attenuation of AS-mediated CMA dysfunction. Lamp2a overexpression via the nigral injection of recombinant adeno-associated virus vectors effectively ameliorated AS-induced dopaminergic neurodegeneration by increasing the survival of neurons located in the substantia nigra as well as the axon terminals located in the striatum, which was associated with a reduction in total AS levels and related aberrant species. We conclude that CMA induction may provide a novel therapeutic strategy in PD and related synucleinopathies through two different mechanisms: amelioration of CMA dysfunction and lowering of AS levels.

Targeting inflammatory pathways to protect DA neurons in Parkinson's Disease

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Emory University / Physiology and Georgia Institute of Technology/Biology, Bioscience and Bioengineering

Pharmacological and genetic studies suggest that neuroinflammatory mechanisms contribute to the pathology of Parkinson's disease (PD). Our group has previously demonstrated that neutralization of soluble tumor necrosis factor (TNF) by dominant-negative TNF (DN-TNF) inhibitors significantly attenuated the loss of DA neurons both in vitro and in vivo in rat models of parkinsonism, but the identity of the signaling pathways downstream of TNF that mediate neurotoxicity remain unclear. Using a mouse DA neuron-like cell line (MN9D) and rat primary neuron-glia cultures from ventral mesencephalon (VM), we have identified cellular and signaling mechanisms by which TNF induces DA neuron death. Ceramide is a sphingosine-based lipid signaling molecule downstream of TNF that is involved in the regulation of cellular differentiation, proliferation and apoptosis. Using lipidomics and high-content imaging approaches, we have discovered that soluble TNF- dependent generation of ceramide species and two novel atypical sphingoid bases exert toxic effects on primary DA neurons by triggering ER stress and disrupting mitochondrial respiration leading to their degeneration. Pharmacological inhibitors of sphingomyelinase (SMase), an enzyme that hydrolyzes active ceramide from inactive sphingomyelin pools attenuated solTNF-dependent toxicity in MN9D cells and primary DA neurons from VM. Ongoing studies to understand the links between ceramide metabolism and TNF-dependent death of DA neurons may reveal novel targets for drug discovery and may provide an opportunity to evaluate neuroprotective effects of compounds that prevent formation of toxic ceramide species or aid in their metabolism with the long-term goal of identifying new therapeutic strategies for PD.

ALTERING ADULT BRAIN FUNCTION: ENDURING EFFECTS OF EARLY-LIFE STIMULANT EXPOSURE

Organizer: H. Steiner (USA)

Juvenile Administration of Concomitant Methylphenidate and Fluoxetine Alters Behavioral Reactivity to Reward- and Mood-related Stimuli and Disrupts Ventral Tegmental Area Gene Expression in Adulthood.

BL Warren, Alcantara LF, Parise EM, CA **Bolaños**

Florida State University /

There is a rise in the concurrent use of methylphenidate (MPH) and fluoxetine (FLX) in pediatric populations. However, the long-term neurobiological consequences of combined MPH and FLX treatment (MPH+FLX) during juvenile periods are unknown. We administered saline (VEH), MPH, FLX, or MPH+FLX to juvenile Sprague-Dawley male rats from postnatal day 20-35, and assessed their reactivity to reward- and mood- related stimuli 24-h or 2-months after drug exposure. We also assessed mRNA and protein levels within the ventral tegmental area (VTA) to determine the effect of MPH, FLX, or MPH+FLX on the extracellular signal- regulated protein kinase-1/2 (ERK) pathway - a signaling cascade implicated in motivation and mood regulation. MPH+FLX enhanced sensitivity to drug (i.e., cocaine) and sucrose rewards, as well as anxiety- (i.e., elevated plus-maze) and stress- (i.e., forced swimming) eliciting situations when compared to VEH-treated rats. MPH+FLX exposure also increased mRNA of ERK2 and its downstream targets cAMP response element- binding protein (CREB), brain-derived neurotrophic factor (BDNF), cFos, early growth response protein-1 (zif268), and mammalian target of rapamycin (mTOR), and also increased protein phosphorylation of ERK2, CREB, and mTOR 2-months after drug exposure when compared to VEH-treated rats. Using herpes simplex virus-mediated gene transfer to block ERK2 activity within the VTA, we rescued the MPH+FLX-induced behavioral deficits seen in the forced swimming task 2-months after drug treatment. These results indicate that concurrent MPH+FLX exposure during preadolescence increases sensitivity to reward-related stimuli while simultaneously enhancing susceptibility to stressful situations, at least in part, due to long-lasting disruptions in ERK signaling within the VTA.

Molecular Maladaptations Underlying Behavioral Deficits in Prenatal Cocaine Exposed Mice

Z Kabir, A Katzman, A Rajadhyaksha, B **Kosofsky**

Department of Pediatrics, Weill Cornell Medical College

Prenatal cocaine exposure (PCE) in humans and animals has been shown to affect the behavior of exposed individuals, presumed to be a result of long-lasting molecular alterations in the brain. The prefrontal cortex (PFC), a brain region that receives strong dopaminergic input, shows persistent morphological and functional deficits in PCE animals consistent with cocaine's molecular site of action. Deficits in social development and cognition, processes that are heavily dependent on the PFC, have been reported following PCE, but have not been elucidated at the molecular level. In adult PCE mice, we identified spontaneous recovery of an extinguished cue-conditioned fear which could be rescued by infusion of recombinant BDNF protein into the mPFC after extinction learning. We identified a similar behavioral deficit in PCE adult mice that had a single copy of the BDNF Val66Met allele, a SNP in the BDNF gene that alters the synaptic release of BDNF protein. In both studies this was attributed to a decrease in induction of mature BDNF protein in the mPFC during extinction learning. We additionally observed a lack of plasticity of the BDNF gene in the mPFC of PCE animals evident as decreased constitutive binding of the transcription factors, methyl CpG binding protein 2 (MeCP2) and pCREB at the promoters of the BDNF activity-driven exons I and IV, that was unchanged after extinction learning. These findings extend our knowledge of the neurobiologic impact of PCE on the mPFC of mice, which may lead to improved clinical recognition and treatment of exposed individuals.

Morphological and molecular alterations associated with prenatal cocaine exposure in mice

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Prenatal cocaine exposure impairs fetal brain development and produces lasting alterations in brain structure and function. Using a mouse model, we show that prenatal cocaine exposure interferes with the development of GABA neurons of the forebrain. Specifically, tangential migration of GABA neurons from the basal to the dorsal forebrain is delayed in the embryo. The delay is associated with decreased expression of the brain derived neurotrophic factor (BDNF) in the embryonic basal forebrain. Although the neuronal migration deficit is transient, and can be restored by exogenous BDNF, there is a persistent reduction in the numerical density of GABA neurons in the medial prefrontal cortex (mPFC) of the prenatally cocaine exposed adult mice. The reduction selectively occurs in a subpopulation of GABA neurons that expresses the neuropeptide parvalbumin. There is also a significant decrease in the GABA-to-glutamate neuron ratio in the mPFC, which could underlie reductions in the inhibitory tone and impairment of long term potentiation. The mPFC regulates executive functions including attention, planning and impulse control. Alterations in mPFC GABA circuits induced by prenatal cocaine exposure could adversely these cognitive functions. More recently we have found that changes in BDNF mRNA expression in the prenatally cocaine exposed mice are transmissible from one generation to the next, illustrating heritability of the effects. Understanding the molecular mechanisms associated with alterations in brain structure and function, especially heritability of these features, could lead to improved methods of diagnosis, treatment and prevention of the adverse impact of prenatal cocaine exposure.

Cocaine-like gene regulation by methylphenidate in the adolescent striatum: Potentiation by SSRI antidepressants

H. **Steiner**

RFUMS/Chicago Medical School / Cellular and Molecular Pharmacology

Psychostimulants such as cocaine alter gene regulation in corticostriatal circuits, effects that are implicated in addiction. The psychostimulant methylphenidate (Ritalin), a dopamine reuptake inhibitor, is used in the treatment of medical conditions such as ADHD and sleep disorders, and is also taken as a cognitive enhancer. Research shows that methylphenidate affects the expression of a number of genes in ways similar to cocaine (dopamine/serotonin reuptake inhibitor), but other genes appear less affected. This may be related to the lack of methylphenidate effects on serotonin. Our recent findings, in adolescent rats, support this hypothesis. We show that concomitant treatment with selective serotonin reuptake inhibitors (SSRIs; fluoxetine, citalopram) together with methylphenidate potentiates methylphenidate-induced gene regulation in the striatum. This potentiation was demonstrated for acute induction of genes encoding transcription factors (c-fos, zif 268) and other immediate-early genes (homer 1a), as well as for the neuropeptides substance P and dynorphin (but not enkephalin). Moreover, concomitant treatment with SSRIs potentiates repeated methylphenidate-induced gene blunting (repression), an effect well established for prolonged exposure to illicit psychostimulants such as cocaine. On the other hand, the differential effects on neuropeptide markers (substance P, dynorphin in direct pathway vs. enkephalin in indirect pathway) suggest that methylphenidate-induced gene regulation (and the potentiation by SSRIs) may be more selective for the direct striatal output pathway than effects of psychostimulants such as cocaine. Overall, our findings of potentiated addiction-related gene regulation in the striatum suggest that SSRIs may increase the addiction liability of methylphenidate.

FUNCTIONAL CORRELATES OF DOPAMINE RECEPTOR SIGNALLING

Organizer: C. Missale (Italy)

Consequences of Functionally Selective Signaling at the D2 Dopamine Receptor

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Dopamine mediates many different physiological functions through activation of 5 distinct G protein-coupled receptors (GPCR) (D1-D5R). Dysfunctions in the brain dopamine system are thought to associate with psychiatric disorder symptoms since many therapeutic agents target these receptors. Classically, GPCR responses have been shown to be mediated through G-protein activation but recent evidence indicates that components of the desensitization machinery, namely β-arrestins, can act as scaffold for distinct signaling molecules. β-arrestin-dependent signaling displays distinct temporal and pharmacological properties and these signaling modes invariably mediate distinct cellular responses than G proteins-mediated effects. Thus, agonists and antagonists that can discriminate between these modes of signaling can thus lead to more selective therapeutic agents with less side-effects. We have shown that D2 dopamine receptors (D2R), the main target of clinically effective antipsychotics, can engage an Akt/GSK3 signaling pathway via the scaffolding of a β-arrestin2/Akt/PP2A/GSK3 complex. To further validate the physiological role of this signaling, we used genetic approaches like the neuron-selective deletion of β-arrestin and the downstream GSK3 β gene to show that these manipulations mimic the effects of antipsychotics. We have also developed novel aripiprazole-based D2R/ β-arrestin2 functionally selective ligands that show antipsychotic activity with fewer side-effects in animal models. In addition, we have developed mutant D2Rs that can selectively signal either through the G-protein- or β-arrestin-dependent pathways that can be re-expressed in vivo in neurons lacking the endogenous D2Rs that in combination with neuron-specific transcriptional profiling should facilitate identification of unique and previously unappreciated cellular, molecular targets of D2R signaling.

Signaling from dopamine receptors to the nucleus: striatal neurons epigenetics

Jean-Antoine **Girault**

Inserm UPMC / UMR-S 839

The striatum controls movements and motivation, and is involved in procedural memory. Classical models supported by recent experiments in transgenic mice emphasize the dopamine-modulated balance between the direct and indirect pathways. Dysfunction of these circuits participates in addiction, Parkinson's disease, schizophrenia and other neuropsychiatric diseases. Long-term changes in the properties of medium-size spiny neurons (MSNs), under the control of glutamate and dopamine, underlie the physiological and pathological plasticity of information processing in the basal ganglia. These long-term alterations involve gene transcription and epigenetic regulations. To specifically study the epigenetic alterations in the two populations of MSNs, we have set up in transgenic mice methods to analyze nuclei from neurons which express D1 or D2 dopamine receptors. We validated a rapid and efficient quantitative flow cytometry method for nuclear analysis by comparison with fluorescence-activated sorting of these nuclei and immunoblotting. We then compared changes in several histone post-translational modifications (acetylation and methylation) in D1R- and D2R-expressing neurons, after a single or repeated injections of cocaine. The time-dependent patterns of modifications were different in the two neuronal populations. Some modifications were persistent at least 1 day after the last cocaine injection, suggesting stable changes in the steady-state turnover of these modifications. These results allow a first cell type-specific approach of epigenetic regulations in striatal neurons. Further studies will examine their mechanisms and their contribution in the long lasting changes in gene expression.

D1 receptor signalling in basal ganglia function and dysfunctions.

Cristina **Missale**, Paola Savoia, Daria Savoldi and Chiara Fiorentini

Department of Molecular and Translational Medicine, University of Brescia

Dopamine (DA) plays a prominent role in motor control, attention and reward and malfunctioning of DA transmission is associated with various neurological disorders. Among the DA receptors, the D1 receptor (D1R) is the most abundant and widespread in the brain. Functionally, D1R interacts with Galphas and Galphaolf leading to cyclic AMP (cAMP) accumulation and protein kinase A (PKA) activation. Moreover, D1R stimulation activates the extracellular signal-regulated protein kinase (Erk1/2) pathway through a cAMP/PKA-dependent mechanism involving phosphoprotein-32 (DARPP-32) and protein phosphatase-1. We recently identified a novel mechanism for striatal D1R to activate Erk pathway that involves the recruitment and activation of the tyrosine phosphatase Shp-2. We established that in striatal neurons, D1R-mediated activation of Erk1/2 is mediated by a molecular complex resulting from the physical interaction of the D1R with the tyrosine phosphatase Shp-2. We found that D1R-induced Erk1/2 phosphorylation requires Shp-2 activation via its phosphorylation that is dependent on the cAMP/PKA pathway. Moreover, we established that in the striatum of a rat model of PD, phosphorylation of Shp-2 induced by D1R activation represents an upstream molecular event leading to persistent Erk1/2 phosphorylation during LID. In the same model, silencing of striatal Shp-2 prevented both Erk1/2 phosphorylation and dyskinesia, suggesting a crucial role of Shp-2 in LID development and maintenance. Therefore, modulating Shp-2 activation, in particular those interacting with the D1R and contributing to Erk1/2 activation, may represent a more targeted strategy for disorders of dopamine transmission.

Dopamine-mediated neural plasticity in reward and aversive learning behaviors

Shigetada **Nakanishi**

Osaka Bioscience Institute / Director

The nucleus accumbens (NAc) plays a pivotal role in reward and aversive learning, learning flexibility and decision making. Inputs of the NAc are transmitted through 2 parallel direct and indirect pathways and controlled by dopamine (DA) transmitter. To explore how the associative learning is controlled in the NAc, we developed 1) reversible neurotransmission-blocking (RNB), in which transmission of each pathway was selectively and reversibly blocked by the pathway-specific expression of transmission-blocking tetanus toxin and 2) asymmetric RNB, in which one side was blocked by RNB and the other side was pharmacologically manipulated by a transmitter agonist or antagonist. The activation of the direct pathway D1 receptors and the inactivation of the indirect pathway D2 receptors distinctly control reward and aversive learning, respectively. The D2 receptor inactivation is also critical for flexibility of reward learning. Furthermore, aversive learning is regulated by a set of selective receptors (NMDA, adenosine A2a and endocannabinoid CBI receptors), which are all involved in induction of long-term potentiation at cortico-accumbens synapses of the indirect pathway. The dynamic control of the NAc neural plasticity is thus essential for reward-seeking and aversion-avoiding decision making.

MRI AND PET INSIGHTS

INTO PARKINSON'S DISEASE

Organizer: P. Tuite (USA)

New PET imaging insights: Cholinopathy and gait and postural dysfunction in Parkinson Disease

Nicolaas I **Bohnen**, MD, PhD

University of Michigan / Radiology/Neurology

Gait dysfunction and postural instability are common in PD, are a significant cause of disability, and become progressively less responsive to dopaminergic therapy. Evidence is accumulating that cholinergic system dysfunction is a significant contributor to gait and balance in PD. The cholinergic system is implicated in mobility functions in PD because of degeneration of cholinergic neurons in the PPN and also secondary to loss of cholinergic basal forebrain (BF) neurons directing attention. Using [C-11]PMP acetylcholinesterase PET, we showed previously that cholinergic denervation, especially of the thalamus, reflecting PPN cholinergic afferent degeneration, is associated with falls in PD. The development of a novel vesicular acetylcholine transporter (VAChT) PET ligand, [F18]-FEOBV, shows dense cholinergic innervation of the cerebellar vermis that implicates an important cholinergic modulation of cerebellar control of posture. We have also preliminary data that basal forebrain cholinergic denervation is associated with slower gait speed in PD subjects without dementia, probably reflecting failing attentional capacities in PD. There is significant heterogeneity of cholinergic denervation in PD without dementia with about 1/3 of subjects having BF and about 1/6 PPN denervation. The distribution of findings suggests an intriguing "3-Hit" model where early and prominent striatal dopaminergic changes are followed by BF and ultimately PPN complex cholinergic neuron degeneration. This model posits that serial degeneration of nigrostriatal (first "hit") and BF/PPN cholinergic systems (second and third "hits") are responsible for the typically inexorable progression of dopamine-resistant gait and subsequent postural dysfunction in PD.

Functional brain imaging insights into cognitive and motivational disorders in Parkinson's Disease

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Although James Parkinson famously described Parkinson's Disease (PD) as "leaving the senses and intellect unaffected", cognitive and mood disturbances are now recognized as an integral part of the disease. Indeed, with the amelioration in the treatment of motor symptoms over the past few decades, it is now the cognitive and mood disorders that most impair quality of life. Dopamine deficiency in the striatum is the salient feature of PD and can be linked to many of the motor, cognitive and emotional symptoms and signs of the disease. Because the striatum is part of neural networks involving the entire cortex, striatal dopamine deficiency has widespread functional effects. A systems neuroscience approach based on the circuitry of the cortico-striatal system has influenced our understanding of the motor and cognitive symptoms and led to therapeutic advances such as deep brain stimulation. This understanding forms the basis of functional neuroimaging as applied to PD. By measuring cerebral blood flow (CBF), first with positron emission tomography (PET), and now with functional magnetic resonance imaging (fMRI), researchers have mapped the neurobiological substrates of cognitive and behavioral symptoms in PD. We will review the evidence that "frontal lobe" deficits in PD, affecting tasks such as planning, set-shifting, and executive control, may be due in part to dopamine deficiency in the neostriatum. We will also review brain imaging contributions to understanding the recent problem of dopamine agonist induced impulse control disorders in PD.

Resting State fMRI and other MR approaches to Parkinson's

M.J. **McKeown**

University of British Columbia / Neurology

Given the tight interconnectedness between the basal ganglia and cortex, disruption of functional networks in Parkinson's Disease (PD) individuals is perhaps unsurprising, suggesting network approaches to exploring the development of novel therapeutic strategies is critical. PD can be regarded as a fundamental disruption of network connectivity, and appropriate network assessments may eventually guide loci for treatments. The fact that many clinical features of the disease can be predicted on the basis of altered connectivity patterns further supports a systems-level approach to the disease. One way to assess system-level changes is to examine the co-activation of spontaneous fMRI BOLD time series from spatially distinct brain regions (i.e. "resting-state fMRI" - rs-fMRI). These studies in PD have consistently demonstrated increased connectivity between the basal ganglia and motor regions. We will also discuss other MRI-based methods to assess changes in PD, including Diffusion Tensor Imaging (DTI), Arterial Spin Labelling (ASL) and novel methods to assess myelin.

Novel MRI and MRS methods in Parkinson's disease

Paul **Tuite**, Melissa Terpstra, Shalom Michaeli, Uzay Emir, Gulin Oz, Christophe Lenglet and Noam Harel

University of Minnesota / Neurology

This talk will cover published data from myself and colleagues at the University of Minnesota Center for Magnetic Resonance Research Center and will include such methods as RAFF, T1rho, T2rho, Magnetization Transfer Imaging, Diffusion imaging, Susceptibility Weighted Imaging, Magnetic Resonance Spectroscopy (MRS) as they have been applied to Parkinson's disease (PD). A variety of imaging platforms and magnetic field strengths (3T, 4T and 7T) have been employed to improve the understanding of pathogenic factors in PD as well as possibly allowing for improved deep brain stimulation surgical planning and a means to track response to treatment and potentially disease progression. Specifically this will include the demonstration of MRS to track cortical glutathione in response to N-acetylcyteine administration in a PD study as well as other work showing structural and functional changes from disease.

THE DOPAMINE TRANSPORTER:

NEW INSIGHTS INTO PSYCHOSTIMULANT ACTIONS AND THE RELATIONSHIP

TO NEUROPSYCHIATRIC DISORDERS

Organizer: U. Gether (Denmark)

Direct activation of intracellular signaling pathways by amphetamines: a mechanism for modulating neurotransmitter transporter function

Susan G. **Amara**

National Institute of Mental Health, Bethesda, Maryland 20892 USA

Neurotransmitter transporters present at the plasma membrane mediate the clearance of neurotransmitters and have a profound impact on the extent of receptor activation during neuronal signaling. These carriers also are the primary targets for psychostimulant drugs of abuse and for drugs such as methylphenidate and amphetamines, which are used to treat attention deficit disorders in children. In recent studies we have observed that once amphetamine-like drugs enter dopamine neurons they activate multiple intracellular signaling pathways to trigger changes in the cellular trafficking of the dopamine transporter and other neuronal membrane proteins. Within the cell amphetamines activate the small GTPases, Rho and Rac1 and trigger endocytosis of the dopamine transporter (DAT) by a RhoA- and dynamin-dependent pathway. We have also found that increases in cAMP and PKA activity mediated by D1/D5 dopamine receptors or by amphetamine itself, serve as a break on DAT internalization, revealing interplay between PKA and RhoA signaling in the regulation of DAT activity. These studies show that amphetamine-like drugs act directly on specific cytoplasmic targets by a mechanism that is independent from their ability to elevate extracellular neurotransmitter concentrations and suggest novel drug targets for modulating the actions of amphetamines.

The dopamine transporter: new genetic mouse models and the relationship to psychostimulant addiction and neuropsychiatric disorders

Ulrik **Gether**

Department of Neuroscience and Pharmacology, Faculty of Health and Medical Sciences, Panum Institute, University of Copenhagen, Denmark

Psychotimulants, such as cocaine and amphetamine, exert their stimulatory action via binding to the presynaptic dopamine transporter (DAT). DAT mediates rapid reuptake of dopamine from the synaptic cleft and is believed to be a key player in dopamine-related diseases. We wish to gain insight into the molecular mechanisms underlying drug action in DAT, to reveal cellular mechanisms governing the activity and availability of DAT in the synaptic membrane and to understand how alterations in these processes might contribute to psychostimulant addiction and neuropsychiatric diseases. Our recent work has demonstrated an indispensable role of C-terminal PDZ (PSD-95/Discs-large/ZO-1) domain interactions in governing striatal distribution of DAT. In two different DAT knock-in mice with disrupted PDZ-binding motifs (DAT-AAA and DAT +Ala) we observe a dramatic loss of DAT expression in the striatum, in part as a result of increased constitutive internalization and subsequent degradation of the transport protein. Additional analyses of DAT-AAA mice revealed significant adaption within the dopamine system as evidenced by marked down-regulation of striatal dopamine D2 but not D1 receptors. DAT-AAA mice are characterized behaviorally by hyperlocomotion upon exposure to a novel environment, eliminated response to amphetamine and preserved response to cocaine. Notably, despite preserved locomotor response to cocaine, the mice display a marked decrease in cocaine self-administration. Of further interest, we have in parallel efforts identified new DAT mutations in patients suffering from both signs of Parkinson's disease and psychiatric-spectrum disorder. Summarized, our results should prove highly important for further dissecting the relationship between dopaminergic dysfunction, addiction and neuropsychiatric disorders.

Membrane microdomain localization of the dopamine transporter

Wendy M. Fong, Christopher W. Johnson, Kelvin Pau, Aurelio Galli, Jonathan A. Javitch, and Ai **Yamamoto**

Neurology,Pathology and Cell Biology/Columbia University

Cellular membranes act not only to segregate and demarcate different regions within a cell, but can also serve to scaffold and modulate protein function. The sub-compartmentalization within membranes is influenced by its lipid and protein composition, and the stability and of these compartments are modulated through protein-protein and protein-lipid interactions. One such compartment is known as membrane rafts; small, highly dynamic, cholesterol- and sphingolipid-rich domains that compartmentalize proteins to promote cellular signaling. Cell surface neurotransmitter transporter proteins such as the dopamine (DA) transporter (DAT) resides in membrane rafts, although the role for this localization is unclear. We recently reported that the localization of DAT to these microdomains is dependent on the protein Flotillin-1 (Flot1). We found that upon Flot1-depletion, DAT no longer resided in membrane microdomains. Moreover, while DAT-mediated DA uptake was unaffected, amphetamine induced efflux of DA through DAT was significantly attenuated. To determine if these changes can be observed in adult brain, we eliminated Flot1 expression in DAergic neurons using DATiresCre (Flot1 cKODATiresCre/+). We will report on our early characterization of these mice, and whether we modulate the impact of psychostimulants such as amphetamine and cocaine.

De novo and inherited rare variants identified from exome sequecing impact the dopamine transporter and its regulatory network.

Nicholas G. Campbell[1], Andrea N Belovich[2], Erin E Watt[1], Peter J. Hamilton[1], Kevin Erreger[3] and Aurelio Galli[1,2,3,5], and James S. **Sutcliffe**1[1,3,4]

[1]Center for Molecular Neuroscience, Departments of [2]Pharmacology, [3]Molecular Physiology & Biophysics, [4]Psychiatry, [5]Neuroscience Program in Substance Abuse, Vanderbilt University School of Medicine, Nashville, TN 37232-8548

Autism spectrum disorders (ASD) are a phenotypically and genetically complex disease. Data demonstrate a significant role for rare *de novo* and inherited sequence variants tocontribute to thegenetic liability of ASD. Despite profound genetic heterogeneity implicating hundreds of genetic loci, there is a highly significant increase in connectivity amongst proteins encoded by genes harboring genetic risk factors(e.g. functional *de novo* mutations). These networks provide an unparalleled opportunity for biologically informed investigation into the impact of ASD identified variations. Exome sequencing of ASD families by the NIH-ARRA Autism Sequencing Consortium identified a *de novo* missense mutation in the dopamine transporter gene (*SLC6A3*) and in several genes encoding other proteins that function in regulation of DAT, including syntaxin binding protein 1 (STXBP1)and an E3 ubiquitin ligase (NEDD4), and yet others are affected by rare *de novo* CNVs (e.g. syntaxin 1; protein phosphatase 2A).The finding of rare *de novo* variants in *SLC6A3* and in multiple loci regulating DAT, raises the possibility that inherited rare variants might confer genetic liability ASD by means of impacting dopamine homeostasis via DAT.Here we report an initial exploration of DAT rare variants and DAT-regulatory *de novo* variants identified in ASD probands. Results from ongoing studies are presented.

IMAGING AND THE ROLE OF DOPAMINE ACROSS ADDICTIONS: DIFFERENCES AND COMMONALITIES

Organizer: D. Martinez (USA)

Dopamine dysfunction in alcoholism

A. **Beck**

Charité - Universitätsmedizin Berlin / Dept. of Psychiatry and Psychotherapy

The disposition and maintenance of alcohol dependence has been associated with dopaminergic dysfunction associated with dysfunctional learning, particularly with increased salience attribution to alcohol-associated stimuli and Pavlovian-to-instrumental transfer, which establishes an effect of alcohol-associated cues on operant alcohol seeking and consumption.
Although microdialysis studies showed that dopamine release elicited by alcohol intake is rather low compared to other drugs of abuse, PET studies showed that chronic alcohol consumption leads to (potentially compensatory) long-term changes in the dopaminergic system (i.e. down-regulation of dopamine D2 receptors). Previous multimodal imaging studies showed that dopamine dysfunction in the ventral striatum is associated with increased brain activation elicited by alcohol-associated cues in brain areas associated with attention. Moreover, recent data suggest that this heightened cue-induced brain activation is also related to relapse, even if controlling for atrophic effects of alcohol intake. Furthermore, brain activation elicited by non-alcohol (e.g. monetary) reward was decreased in detoxified alcoholics and reward-dependent reversal learning was impaired compared to healthy controls, and that this impairment correlates with reduced functional connectivity between the ventral striatum and the dorsolateral prefrontal cortex.

Neural Response to Monetary Reward in Alcohol Dependence and Cannabis Use: Developmental Findings

E. **Forbes**

Models of addiction propose that function in neural reward circuitry changes with the development of addiction, but few fMRI studies in humans have examined the association of substance use with neural response to non-drug rewards. Furthermore, human research on reward circuitry and substance use has focused on adults with addiction, rather than adolescents, who have had shorter-term exposure to substances and may have yet to develop addiction. Knowledge of disrupted reward function is particularly sparse for cannabis, which is commonly used and has potential for addiction. This presentation will describe findings from two studies of neural response to monetary reward: one in adults with alcohol dependence (24 with alcohol dependence, 24 healthy control; age 27.2±4.28 years; 40% female), and one in adolescents with high rates of alcohol and cannabis use (N=33, all male, age 20). Participants underwent fMRI scanning during a monetary reward task that reliably engages the striatum and medial prefrontal cortex (mPFC). In addition, adolescents reported on their lifetime use of cannabis and their chronotype, or circadian preferences. In both adults and adolescents, alcohol dependence was associated with less mPFC response to monetary reward. For adolescents, this was particularly the case for those with greater eveningness, which reflects the tendency to be more active at night and is associated with addiction and affective disorders. For adults, alcohol dependence was associated with greater negative functional connectivity between the ventral striatum and mPFC. Adolescents with greater current frequency and earlier age of initiation of cannabis use exhibited altered response to monetary reward in the globus pallidus, an important output target for the striatum, and the parahippocampal gyrus, a region whose disrupted function has been implicated in cannabis effects on working memory. Together, these findings indicate that fMRI-measured frontostriatal response to monetary reward can inform our understanding of the development of addiction.

Dopamine and Addiction: imaging and the neurobiology of substance abuse

Diana **Martinez**

The involvement of dopamine in addiction has its origins in studies investigating reward and reinforced behavior in preclinical studies. Much of this research has been explored in the human brain using Positron Emission Tomography (PET) imaging of striatal dopamine transmission. These studies show that addiction is associated with a decrease in dopamine D2/3 receptors and a decrease in pre-synaptic dopamine release, and that this decrease occurs across different types of addiction, including cocaine, alcohol, and heroin dependence. However, these imaging studies also show that, in cocaine abuse, blunted dopamine transmission is predictive of cocaine seeking behavior. Low D2/3 receptor binding and low dopamine release are associated with the choice to self-administer cocaine over alternative reinforcers (such as money), which can be viewed as a failure to shift between competing rewards.

It is striking that addiction to different substances of abuse are accompanied by the same alteration in neurobiology, independent of their primary impact on the dopaminergic system. Moreover, similar alterations of the dopaminergic transmission and D2-like receptor system have been described in psychiatric diseases other than addiction. Although these psychiatric disorders differ in their phenomenology, they share a common deficit in reward-related behavior, particularly with respect to impulsivity and motivation. This presentation will describe the animal and human studies that link alterations in dopamine transmission and the D2 receptors with impulsive and motivated behavior. The hypothesis that these alterations in dopamine transmission represent the neurobiological underpinnings that facilitate impulsivity and undermine motivation, rather than the consequences of addiction itself, will be discussed.

Imaging dopamine in prefrontal cortex

R. Narendran

University of Pittsburgh

Purpose:
To validate the use of [^{11}C]FLB 457 PET to measure dopamine (DA) release in the human cortex and use it in a clinical study in alcoholics.
Methods:
1.To compare [^{11}C]FLB 457 and [^{11}C]fallyprideand assess their vulnerability to amphetamine (AMPH) challenge
2. To evaluate the reproducibilityofbaseline and post-amphetamine [^{11}C]FLB 457 BP_{ND}
3. To evaluate the fractional contribution of specific binding to $D_{2/3}$ receptors in the human cerebellum, which is used as a measure of non specific binding for the derivation of [^{11}C]FLB 457 BP_{ND}
4. To evaluate the relationship between decrease in [^{11}C]FLB 457 BP_{ND} as measured with PET and peak increase in extracellular fluid (ECF) DA as measured with microdialysis in the primate cortex afterAMPH
Results:
1. AMPH induced DA release led to a decrease in [^{11}C]FLB 457 BP_{ND} in the cortical regions (ROIs) evaluated. No decrease in [^{11}C]fallypride BP_{ND} was detected in cortex following AMPH
2. The test-retest variability of baseline and post-amphetamine [^{11}C]FLB 457 BP_{ND} was ≤ 15%
3. The contribution of specific binding to $D_{2/3}$ receptors in the cerebellum was much lower than that in the cortex
4. Thedata suggest a linear relationship between ECF DA release and decrease in [^{11}C]FLB 457 BP_{ND} in the cortex
Conclusion: [^{11}C]FLB 457 PET allows for the measurement of DA release in the human cortex. In addition, preliminary data using this imaging paradigm in alcoholics and healthy controls will be included in the presentation.

REGULATION OF VTA NEURONS BY OREXIN/HYPOCRETIN: MECHANISMS, CIRCUITS AND BEHAVIORS

Organizer: G.S. Aston-Jones (USA)

Hypocretin modulation of morphine-induced synaptic plasticity in the ventral tegmental area

Corey Baimel and Stephanie L. **Borgland**

University of Calgary / Physiology & Pharmacology

Dopamine neurons in the ventral tegmental area (VTA) are a key target of addictive drugs and neuroplasticity in this region may underlie some of the core features of addiction. All drugs of abuse induce an LTP-like potentiation of excitatory inputs to VTA dopamine neurons. Hypocretin (hcrt), also known as orexin, is a lateral hypothalamic neuropeptide released into the VTA that exerts modulatory effects on a variety of behaviors produced by drugs of abuse. Acute application of hcrt potentiates excitatory synaptic transmission in the VTA, and inhibition of hcrt signaling blocks both cocaine-induced plasticity and behavioral sensitization. However, the role of hcrt on the plasticity induced by other classes of abused drugs is unknown. We determined if hypocretin action was necessary for morphine-induced synaptic plasticity in VTA dopamine neurons using whole-cell patch clamp electrophysiology in rat horizontal brain slices including the VTA. Morphine potentiated glutamatergic synapses by a pre-synaptic increase in glutamate release and by a post-synaptic change in AMPAR number or function, likely including a switch in subunit composition. Systemic or intra-VTA administration of SB 334867, a hcrt receptor-1 antagonist, blocked a morphine-induced increase in the AMPAR/NMDAR ratio, morphine-induced increases in AMPAR mEPSC frequency and amplitude, as well as morphine-induced AMPAR redistribution measured by a change in rectification. These results support a role for hcrt signaling in both pre-and post-synaptic potentiation of glutamatergic transmission in the VTA by morphine. Because hcrt signaling is required for plasticity induced by both morphine and cocaine, hcrt may function as a gate keeper for drug induced plasticity of dopamine neurons.

Evidence for drug-induced modulation of the hypothalamic orexin to VTA dopamine neuron pathway and relevance for relapse-like behavior

Yeoh, JW, James MH, Bains JS, Graham BA and **Dayas** CV

School of Biomedical Sciences and Pharmacy, Centre for Translational Neuroscience and Mental Health, HMRI, University of Newcastle, NSW, Australia

The lateral hypothalamic area (LHA) orexin (hypocretin) system is important for drug-seeking behaviour. Our recent work has focused on understanding how the orexin system interfaces with other key brain regions that control relapse and how orexin circuitry is 'rewired' by drugs. With regard to where orexin(s) may trigger drug-seeking, we focused on two brain structures, the paraventricular thalamus (PVT) and ventral tegmental area (VTA), both of which receive significant orexinergic innervation and have well characterized roles in modulating drug-seeking. We found that intra-PVT injections of SB-334867 (an OrX-1 receptor antagonist) did not alter drug-seeking. In contrast, intra-VTA SB-334867 significantly suppressed cocaine-seeking but failed to alter natural reward-seeking or locomotor activity. VTA dopamine neurons and NAC neurons undergo significant drug-induced synaptic plasticity, however, far less is known about drug-induced changes to LHA-orexin circuits. We found that self-administered cocaine significantly increased the frequency of miniature excitatory post-synaptic currents (mEPSCs) in LHA-neurons but had no effect on mEPSC amplitude. In further experiments in which we electrically activated synaptic inputs to the LHA, we found that neurons from cocaine-trained animals had significantly lower paired-pulse ratios compared to controls but the AMPA/NDMA ratio was unchanged. Together these findings indicate an action of cocaine at presynaptic locations on orexin neurons. The implications of these findings are that the increased excitatory drive to LHA-orexin circuitry leads to a state in which relapse-relevant targets of this signaling (e.g. the VTA) are more easily recruited by stimuli such as drug-linked cues.

Hypocretin/orexin regulation of dopamine signaling: implications for motivated behavior

Rodrigo A. **España**

Neurobiology and Anatomy / Drexel University College of Medicine

The hypocretins/orexins are comprised of two neuroexcitatory peptides that are synthesized exclusively within a circumscribed region of the lateral hypothalamus. These peptides project widely throughout the brain and interact with a variety of regions involved in the regulation of arousal-related processes including those associated with motivated behavior. Amassing evidence indicates that the hypocretins have been implicated in the regulation of reinforcement and reward processes, likely via actions on the mesolimbic dopamine system. For example, previous studies indicate that blockade of hypocretin neurotransmission reduces conditioned place preference for morphine, cue- and stress-induced reinstatement of ethanol and cocaine-seeking, and blocks behavioral sensitization to cocaine. Using a combination of behavioral and neurochemical techniques we have embarked on a series of studies to examine the influence of the hypocretin system on cocaine-induced alterations in reward/reinforcement behaviors as well as dopamine signaling in the nucleus accumbens. In those studies we have shown that treatment with a hypocretin peptide promotes cocaine self-administration across a variety of self-administration procedures. Additionally, using both microdialysis and voltammetry techniques, we have shown that a hypocretin peptide enhances dopamine responses to cocaine. In contrast, blockade of hypocretin 1 receptors, but not hypocretin 2 receptors, reduces cocaine self-administration and attenuates cocaine-induced elevations in dopamine. Consistent with these observations, our voltammetry studies indicate that knockout mice lacking the ability to produce hypocretin, display lower levels of dopamine release and reduced dopamine uptake rates under baseline conditions. Furthermore, hypocretin knockout mice have attenuated dopamine responses to cocaine and do not display conditioned place preference for cocaine. When considered together, these studies demonstrate that the hypocretin system participates in the regulation of dopamine signaling under baseline conditions and in responses to cocaine and provide additional evidence for the hypothesis that the hypocretins are involved in reward and reinforcement processes through actions on the mesolimbic dopamine system.

Functions of VTA orexin, and orexin/glutamate interactions in reinstatement of cocaine seeking

Stephen V. **Mahler**, Rachel J Smith, Gary Aston-Jones

Med. Univ. South Carolina / Neurosciences

Work over the last several years has shown that orexin input to the ventral tegmental area (VTA) importantly regulates stimulus-induced reward seeking by modulating midbrain dopamine (DA) neurons. I will describe studies showing that VTA Orexin1 receptors are necessary for stimulus-induced, but not primed relapse to drug seeking in a cocaine self-administration/reinstatement paradigm. In addition, I will describe recent studies showing that an important action of orexin in VTA may be to modulate the impact of cue-related glutamate inputs acting at AMPA receptors. I will also discuss how the anatomical localization of orexin neurons within hypothalamus may impact functional effects of the peptide, and how this issue relates to the self-administration/reinstatement model of relapse in addiction.

Orexin/dynorphin neuronal contributions to reinstatement and extinction of alcohol seeking

Gavan P. **McNally**

The University of New South Wales /

Orexin/dynorphin neurons promote and prevent relapse to drug seeking by serving important roles in the reinstatement and extinction of drug seeking. Experiments will be reported, using alcohol self administration in rats, that study the role of orexin/dynorphin neurons, their control by prefrontal cortex and nucleus accumbens shell, and their interactions with midbrain ventral tegmental area, as well as paraventricular thalamus. These experiments suggest that context-induced reinstatement of alcohol seeking depends on accumbal shell control over lateral hypothalamic orexin neurons that may, in turn, depend on midbrain dopamine neurons whereas context-specific expression of extinction depends on prefrontal inputs to orexin/dynorphin neurons in the perifornical and dorsomedial hypothalamus and their projections to paraventricular thalamus.

NEW MECHANISMS BY WHICH TRACE AMINE-ASSOCIATED RECEPTORS MODULATE DOPAMINE NEURON ACTIVITY

Organizer: D.K. Grandy (USA)

Neurochemical mechanisms involved in the modulation of dopamine transmission by Trace Amine Associated Receptor 1 (TAAR1)

Tatyana D. Sotnikova, Stefano Espinoza, Damiana Leo, Ilya Sukhanov, Liudmila Mus

and Raul R. **Gainetdinov.**

Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Genova, Italy

Recent observations have indicated that Trace Amine Associated Receptor 1 (TAAR1) may represent a novel target for the pharmacology of dopamine-related disorders such as schizophrenia, ADHD and Parkinson's disease. To investigate the mechanisms of TAAR1-dependent modulation of physiological functions mediated by dopamine we performed series of experiments by using both in vivo and in vitro approaches. By applying various experimental paradigms aimed to model dopaminergic dysregulation in mice lacking TAAR1 and newly developed selective TAAR1 ligands, we investigated the potential role of TAAR1 in modulating dopamine-related functions such as movement control. Furthermore, we investigated the biochemical mechanism of interaction between TAAR1 and D2 dopamine receptors and the role this interaction plays in D2R-related signaling and behaviors. Finally, we applied in vivo microdialysis and fast scan cyclic voltammetry (FSCV) to investigate neurochemical mechanisms involved. In TAAR1 knockout (KO) mice, we observed that TAAR1 generally exerts an inhibitory influence on the locomotion, so TAAR1 agonists inhibit dopamine-dependent locomotor activity, while effects of dopaminergic stimulation is enhanced in TAAR1 KO mice. In biochemical studies, we observed close functional interaction between TAAR1 and D2 dopamine receptors. A significant modulation of dopamine dynamics following TAAR1 activation was also observed in neurochemical studies. These data indicate that TAAR1 can affect dopamine neurotransmission via several mechanisms and this modulatory influence can have important functional consequences in vivo.

In Vivo Studies Reveal Trace Amine-Associated Receptor 1 Mediates a Novel Interaction Between Methamphetamine and Bupropion

D.K. **Grandy**

Oregon Health & Science University / Physiology & Pharmacology

The abuse of methamphetamine (METH) is still on the rise worldwide yet its burden on society has already outpaced available resources making it a public health challenge of major proportions for which there is no treatment. Although much attention has focused on the dopamine transporter (DAT) as the mediator of METH's psychotropic effects targeting DAT has not resulted in an effective pharmacologic intervention that prolongs abstinence or prevents relapse to METH abuse. In 2001 we reported an orphan G protein-coupled receptor, now referred to as Trace Amine-Associated Receptor 1 (TAAR1), is directly activated by nanomolar concentrations of amphetamine and METH in vitro. This finding revealed a previously unrecognized gap in our knowledge about the pharmacodynamics of METH and led us to conceive of the two-hit hypothesis of METH action: The behavioral effects of METH are manifestations of its interaction with DAT and TAAR1. To test our hypothesis in vivo we began by monitoring the locomotor activity of adult male wild type (WT) and taar1 knockout (KO) mice exposed to METH in the absence and presence of the TAAR1-selective antagonist EPPTB, the DAT blocker bupropion (BUP) or the two combined. Surprisingly, coadministration of BUP and METH stimulated the activity of WT mice to a significantly higher level than either drug alone. Since this response was not observed in WT mice treated with EPPTB plus BUP prior to receiving METH or KO mice treated with BUP followed by METH we conclude this synergistic response is TAAR1 mediated.

Discovery and characterization of selective TAAR1 agonists as potential therapeutic drugs in the field of mental illness

Marius C. **Hoener**, Florent G. Revel, Anja Harmeier, Roger D. Norcross, Tanya L. Wallace, Céline Risterucci, Jean-Luc Moreau and Joseph G. Wettstein

F. Hoffmann-La Roche, Basel, Switzerland

Dysregulation of monoaminergic neurotransmission is a hallmark of major neuropsychiatric disorders. The trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor activated by trace amines like p-tyramine and beta-phenylethylamine, endogenous compounds with structural similarity to biogenic amines. TAAR1 agonists were developed, characterized and, together with TAAR1 transgenic animals, used to evaluate TAAR1 as a potential drug target for neuropsychiatric disorders. By manipulating TAAR1 activity by use of either Taar1 knock-out animals or selective ligands, we show that TAAR1 modulates dopaminergic, serotonergic and glutamatergic neurotransmission and thus reveal that TAAR1 activation represents a novel therapeutic option for neuropsychiatric disorders. We identified the first potent and selective TAAR1 ligands through a medicinal chemistry program taking advantage of the considerable overlap between the pharmacophore space occupied by TAAR1 ligands and ligands of other biogenic amine receptors. In rodents, activation of TAAR1 by potent, selective and pharmacologically distinct compounds blocked psychostimulant-induced hyperactivity and produced a brain activation pattern in phMRI reminiscent of the antipsychotic drug olanzapine, suggesting antipsychotic-like properties. Importantly, TAAR1 agonists did not induce the typical side-effects produced by current antipsychotic drugs such as catalepsy or weight gain. TAAR1 partial agonism even reduced haloperidol-induced catalepsy and, remarkably, prevented olanzapine from increasing body weight and fat accumulation. Finally, TAAR1 agonists produced pro-cognitive effects as well as antidepressant-like properties in rodents and monkeys. These data suggest that TAAR1 agonists have promise as novel and differentiated medicines for the potential treatment of schizophrenia and perhaps other mental disorders.

Trace Amine Associated Receptor 1 (TAAR1) Signaling Differentially Regulates Dopamine and Norepinephrine Transporter Internalization

Gregory **Miller**

Harvard Medical School / Neuroscience

A recently discovered function of Trace Amine Associated Receptor 1 (TAAR1) is its capacity to attenuate the behavioral effects of psychostimulants that function as substrates for the dopamine (DAT) and norepinephrine (NET) transporters.This study investigated TAAR1 influence on endocytic trafficking of DAT and NET using methamphetamine (METH), which is both a potent TAAR1 agonist as well as a substrate for DAT and NET. Findings were compared in METH-treated HEK293 cells transfected with DAT, with or without TAAR1, and in METH-treated synaptosomes derived from wild-type (WT) or TAAR1 knockout (KO) mice, and in synaptosomes isolated from WT and KO mice administered METH (1 mg/kg, i.p.) in vivo. In all three preparations expressing TAAR1, METH induced DAT internalization, but failed to promote DAT internalization in the absence of TAAR1. METH did not promote NET internalization in transfected HEK293 cells or in synaptosomes isolated from NET-rich thalamus even in the presence of TAAR1. METH induced CRE- and NFAT-regulated signal transduction pathways in vitro, and increased phosphorylated PKA and PKC levels in transfected cells and synaptosomes, but signal transduction required TAAR1. Direct activation of PKC promoted DAT and NET internalization, but NET (and not DAT) internalization was blocked by concurrent PKA activation. Conversely, PKA inhibition enabled METH/TAAR1- or direct PKC-mediated NET internalization. These findings implicate TAAR1 as a principal modulator of DAT and NET function and mobility, with relevance to the pathophysiology and treatment of neuropsychiatric and addictive disorders associated with dopamine dysfunction.

POSTER SESSION III

Tuesday,MAY28th

Dopamine and signal transduction

P114. Combining DA application and time resolved FRET to investigate effects of physiological DA fluctuations on downstream signaling cascades in medium spiny neurons.

Thorvald **Andreassen**, Kenneth Lindegaard Madsen, Ulrik Gether

University of Copenhagen / Department of Neuroscience and Pharmacology

The major target for dopamine (DA) signals is the medium spiny neurons (MSNs) in the nucleus accumbens and striatum. The majority of MSNs are categorized as belonging to either the direct or indirect pathway; MSNs in the direct pathway express dopamine D1 receptors whereas neurons in the indirect pathway express D2 receptors and the two pathways appear to be affected differently by local changes in DA levels. The sensitivity towards cortical inputs is regulated by, among others, DA, which acts to increase cAMP and protein kinase A (PKA) signaling in D1 positive neurons but to decrease cAMP/PKA signaling in D2 positive neurons. Dopaminergic neurons exhibits tonic and phasic DA release, linked to distinct behavioral states. The causal relationship between distinct behavioral states due to fluctuating levels of DA, receptor binding and second messenger system activation leading to changes in AMPA receptor activation/inactivation is currently unresolved. Thus greater insight into these fundamental mechanisms of DA transmission holds promise to improved treatments of drug addiction, ADHD, Schizophrenia and Parkinson's disease. Using novel FRET based biosensors we are able to distinguish downstream effects of D1R activation in MSNs by measuring intracellular time-dependent FRET ratio changes in single cells. The technique will hopefully provide a means to distinguish the downstream signaling cascade via the D1 and D2 receptors, and ultimately the cellular mechanisms of the direct and indirect DA signaling pathway.

P115. Growth Associated Protein-43 Regulates Dopamine Transporter Mediated Amphetamine-induced Reverse Transport

Bipasha **Guptaroy**, Aalisha Desai, Katharyn Luderman, Karina Meiri and Margaret E. Gnegy

Department of Pharmacology / University of Michigan and Program in Cell and Molecular Biology and Neuroscience/ Tufts University School of Medicine

Increase in synaptic DA level through the dopamine transporter (DAT) is critical for psychostimulatory effect of drugs of abuse such as amphetamine, but underlying mechanisms remain unknown. Amphetamine (AMPH) is a substrate of DAT and increases synaptic dopamine (DA) by competitively inhibiting DA uptake and eliciting reverse transport (efflux) of DA through DAT. Protein kinase C (PKC), specifically PKCbeta, is important in AMPH-stimulated DA efflux through DAT. Phosphorylation of a presynaptic PKC substrate - growth associated protein-43 (GAP-43) at its PKC phosphorylation site (ser41) is increased in rat synaptosomes upon AMPH stimulation. Here we show that GAP-43 regulates AMPH-stimulated DA efflux through DAT. GAP-43 associated and co-localized with surface DAT. Overexpression of GAP-43 in heterologous cells (hDAT-HEK), which contain no endogenous GAP-43, enhanced AMPH-stimulated DA efflux compared to vector transfected cells without any effect on DA uptake or surface level of DAT. Conversely, GAP-43 gene knock down in mice compromised AMPH-stimulated DA efflux. Specific inhibition of PKCbeta blocked amphetamine stimulation of GAP-43 phosphorylation at its PKC phosphorylation site (ser41) and GAP-43 enhancement of amphetamine stimulated DA efflux in hDAT-HEK cells. In PKCbeta knockout mice basal phosphorylation of GAP-43 at ser41 was reduced compared to wild type mice. Repeated intermittent amphetamine treatment increased phophsoser41 GAP-43 levels in wild type but not in PKCbeta knockout mice. Our results support a role for GAP-43 in PKCbeta modulation of amphetamine action on DAT. Funded by DA011697.

P116. Erk1 map kinase regulates erk2 dependent signalling in the striatum

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ERK1 and ERK2 are the main isoforms belonging to the Extracellular signal Regulated Kinase (ERK)/Mitogen Activated Protein Kinase (MAPK) superfamily. ERK1 and ERK2 have previously been implicated in cell proliferation and cancer, differentiation and behavioural plasticity. However, their role in the control of brain function is still controversial since conflicting results have been recently reported. Here we show converging evidence in vitro and in vivo that the ratio between ERK1 and ERK2 activity is the main determinant of the biological readout. Altogether, our results demonstrate a unique and unexpected role for ERK1 MAP kinase in regulating multiple cellular functions, particularly in the mature brain, with potential relevant implications for the treatment of striatum-dependent disorders.

P117. SorCS2 is critical for dopaminergic firing pattern and the response to drugs of abuse

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The sortilin-related receptor SorCS2 is highly expressed during development of the dopaminergic system and has been genetically linked to bipolar disorder. Using in vivo single-unit recording we have measured dopaminergic transmission in the VTA of SorCS2 deficient mice and wildtype mice. We found that DA neurons fires in a more regular manner than DA neurons in the wildtype mice. To test if the change in firing is due to different levels of dopamine, we dissected out striatum and the midbrain from SorCS2 deficient and wildtype mice, and conducted HPLC. Interestingly, we found a significant reduced level of striatal dopamine, while no differences in the dopamine levels in the VTA was observed. Due to the lower level of dopamine, we speculated that SorCS2 deficient mice might have abnormal response to psychostimulants. Thus, we tested the locomotor activity in 17-20 weeks old SorCS2 deficient mice. Strikingly, vehicle-treated SorCS2 deficient mice were significantly more active than their wildtype controls. Further, SorCS2 deficient mice responded significant less to amphetamine than their wildtype controls. All together, our results strongly suggest an important role of SorCS2 as a regulator of the dopaminergic system.

P118. Modulation of translational machinery in the striatum by d-amphetamine

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The dorsal striatum and the nucleus accumbens are two major structures critically involved in adaptive control of behavior. The molecular and cellular mechanisms of dopamine-controlled striatal plasticity are yet poorly understood. We hypothesize that the regulation of local mRNA translation could be an essential actor. However, how and in which neuronal population dopamine regulates mRNA translation is largely unappreciated. Previous studies showed that the mTORC1 (Mammalian Target of Rapamycin Complex 1) and ERK (Extracellular signal-Regulated Kinase) pathways regulate protein translation in various models of hippocampal plasticity. Therefore, we investigated whether these two pathways could act in concert to regulate translation following in vivo d-amphetamine (d-amph) administration. We found that a single exposure of d-amph increases the phosphorylation of the ribosomal protein S6 (rS6) mainly in striatal synaptoneurosomes, suggesting that local translation might occur. This phosphorylation takes place selectively in striatonigral medium-sized spiny neurons (MSNs) and depends on both dopamine D1 and mGluR1 receptor stimulation. Surprisingly, our results revealed that d-amph-induced rS6 phosphorylation does not involve mTORC1 and ERK, which are the main kinases atributted to rS6 regulation. In parallel, we observed that d-amph exposure triggers the phosphorylation of eIF4E (eukaryotic initiation factor 4E), a key player in the initiation step of translation. Altogether our findings suggest that d-amph modulates the translational machinery selectively in striatonigral MSNs and thus provide new insights to better understand dopamine-controlled striatal plasticity.

P119. Therapeutic approaches against Ras-ERK signaling for the treatment of L-DOPA induced dyskinesia and drug addiction

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In the central nervous system, the Ras-ERK signaling pathway is implicated in a variety of processes, from cell survival and differentiation to synaptic plasticity and memory formation. Compelling evidence indicates that the alteration of the Ras-ERK signaling pathway in the basal ganglia and in particular in the striatum is responsible for the onset of brain pathologies such as L-DOPA Induced Dyskinesia (LID) and drug addiction. In LID, abnormal dopamine signaling associated to prolonged L DOPA administration for treating Parkinson Disease results in a hyperactivation of the ERK cascade in the striatal circuitry. In a 6-OHDA mouse model which mimics such aberrant state, a therapeutic approach has been tested with both pharmacological and genetic manipulations. Cell Permeable Peptides (CPPs) against ERK and Ras- GRF1, a key brain specific component of the Ras-ERK pathway, and Lentiviral Vectors (LVs) causing downregulation of Ras-GRF1, result in a significant attenuation of dyskinetic symptoms and reduced ERK signaling in the striatum. Similarly, in response to cocaine administration an upregulation of Ras-ERK pathway appears to be a key pathogenetic factor of addiction. Here we show that CPPs against Ras-ERK cascade are able to attenuate cocaine dependent behavioral responses in mice and the associated molecular changes in the striatum. Altogether these data confirm the validity of a therapeutic approach based on Ras-ERK inhibition to treat hyperdopaminergic disorders of the basal ganglia.

Dopamine and schizophrenia

P120. Dysfunction in metabolic mTORC2/Akt signaling disrupts brain D2R signaling and DA homeostasis

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There is a growing appreciation for the importance of the comorbidity between mental illness and metabolic disorders. Accumulating evidence supports the key role of aberrant central dopamine (DA) signaling in both schizophrenia, a devastating brain disease, and obesity, a metabolic epidemic of modern time. Dysregulated nigrostriatal DA neurotransmission is thought to be critical in promoting the positive symptoms of schizophrenia. However, the molecular mechanisms underlying this dysregulation have yet to be completely elucidated. Recently, dysregulation of metabolic kinase Akt has been linked to the etiology of schizophrenia. Data from our laboratory suggest that Akt signaling regulates brain DA homeostasis, providing a possible molecular mechanism connecting metabolic dysfunctions to mental illness. To study how aberrant Akt signaling influences central DA homeostasis, we disrupt Akt function by neuronal deletion of the rictor protein, which results in severe impairment in Akt Ser473 phosphorylation. The transgenic mouse model with disrupted brain Akt function exhibits hypersensitivity to psychostimulant effects of amphetamine (assessed by open field locomotor activity). Our data also suggest that dysfunctional Akt signaling causes aberrant expression and/or function of major DA homeostasis markers: tyrosine hydroxylase, DA transporter (DAT), as well as D2 DA receptor and its downstream kinase ERK1/2. Our in vivo studies show that alterations in striatal DA neurotransmission supported by impaired Akt function are reduced by D2 DA receptor blockage. Our data points to Akt signaling as a novel molecular mechanism regulating central DA tone and D2 DA receptors signaling.

P121. Antipsychotic-like properties of antiandrogenic drugs: focus on dopaminergic system

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Cogent evidence has shown that male gender confers a higher vulnerability for several neuropsychiatric disorders related to dysregulations of dopamine neurotransmission, including schizophrenia. To elucidate the underpinnings of gender differences in this disorder, we examined the impact of antiandrogenic drugs in the rat model of prepulse inhibition (PPI) of the acoustic startle reflex, a well-validated paradigm for the measurement of sensorimotor gating. PPI deficits are observed in schizophrenic patients, and can be induced in rodents by dopaminergic agonists, in an antipsychotic-sensitive fashion. To assess the potential implications of steroidogenic pathways in schizophrenia, we investigated the role of 5α-reductase (5AR), the enzyme that converts testosterone into its potent androgenic metabolite 5α-dihydrotestosterone (DHT), in the modulation of dopaminergic responses. We found that systemic and intra-accumbal injections of finasteride, the prototypical 5AR inhibitor, reversed the PPI deficits induced by apomorphine. Similar effects were observed following administration of abiraterone and ketoconazole, the inhibitors of CYP450-C17, the rate-limiting enzyme in testosterone synthesis. Conversely, the androgen receptor antagonist flutamide failed to elicit significant antidopaminergic and PPI-restorative properties in rats. To verify whether 5α-reduced testosterone derivatives may be implicated in the antipsychotic-like mechanisms of antiandrogens, we tested the effects of DHT and its metabolite 3α-diol in PPI. Both compounds induced significant PPI deficits after intra-accumbal injections. These findings suggest that 5α-reduced androgens play a central role in the regulation of dopaminergic signaling in the nucleus accumbens, and afford an interesting platform to theorize the mechanisms of androgenic implication in schizophrenia.

P122. Intranasal Oxytocin effects in mice

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Intranasal administration of oxytocin (OXT) has generated interest in recent years as a route to deliver into the brain, biologically effective concentrations of this neuropeptide, for treatment of brain diseases, without triggering systemic hormone-like side effects. OXT has been implicated in social behaviours such as social recognition, social approach, pair bonding, paternal and maternal behaviour. Thus, this evidence has led to propose its potential use as an adjunctive therapeutic treatment in neuropsychiatric diseases characterised by impaired social behaviours such as autism spectrum disorders and schizophrenia. Promising preliminary results with intranasal OXT in schizophrenic and autistic patients have been obtained, however, it is not yet clear the specificity and durability of the behavioural effects induced by chronic intranasal OXT. Our present study aims to elucidate effects of chronic intranasal administration of OXT in mice, in order to dissect OXT-dependent behavioural effects and its neurobiological basis. Social interaction experiments of OXT-treated C57BL/6J male mice with female stimulus mice and with their male cagemates were peformed, after which, locomotor activity tests, to check for locomotor effects of the treatment. In order to check for cognitive effects of OXT in these mice, the Temporal Order Object Recognition (TOR) test was carried out, finally followed by the prepulse inhibition (PPI) test. Chronic OXT intranasal treatment selectively reduced social behaviours in C57BL/6J male mice. These effects appear to depend on the reduction in the number of OXT receptors in various brain regions as a consequence of the chronic intranasal OXT treatment.

P123. D-cell hypothesis for mesolimbic dopamine hyperactivity of schizophrenia

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Abstract: Recent pharmacological discovery on trace amine-associated receptor, type 1(TAAR1) has emphasized the importance of trace amines in pathogenesis of psychoses, such as schizophrenia. TAAR1 modulates the functions of dopamine (DA). So-called D-neurons are putative producer of trace amines, endogenous ligands of TAAR1. The D-neuron is defined "the aromatic L-amino acid decarboxylase (AADC)-containing neuron, but neither dopaminergic nor serotonergic", i.e. neither containing tyrosine hydroxylase nor tryptophan hydroxylase. AADC is an enzyme, also called dopa decarboxylase (DDC). The localization of D-neurons in the central nervous system has been specified into 15 groups, from the spinal cord (D1) to striatum (D15). We showed the decrease of D-neurons in D15 in postmortem brains of schizophrenia, where midbrain DA neurons are heavily innervated. Decrease of D-neurons may cause reduction of trace amines in the striatum, and may also decrease stimulation of TAAR1 on striatal terminals of ventral tegmental area (VTA) DA neurons. This leads increase of firing frequency of VTA DA neurons, and also DA hyperactivity in the striatum and nucleus accumbens. The author shows the novel theory, "D-cell hypothesis", for mesolimbic DA hyperactivity of schizophrenia, and some clinical and/or experimental evidences that support this hypothesis.

P124. Specific knockdown of the Drd2 gene in the NAcc reproduces the social novelty discrimination deficit induced by a neonatal treatment with phencyclidine.

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Dopamine (DA) and Glutamate (GLU) neurotransmission imbalance in the Nucleus Accumbens (NAcc) play a pivotal role in the symptomatology of Schizophrenia (SZ). In order to specifically evaluate the role of the NAcc Drd2 in the cognitive symptoms characterizing SZ, we have inhibited (knock down = KD) the expression of the Drd2 gene by lentiviral mediated siRNA transfer in the NAcc. Then, we have compared the effects of the Drd2-KD in adult rats treated neonatally either with phencyclidine (PCP) or saline. The treatment with PCP, a non-competitive NMDA receptor antagonist, generates a validated pharmacological model of SZ mimicking negative, positive and cognitive symptoms. We investigated the cognitive performances of these rats with the social novelty discrimination (SND) test. We have established that: a) neonatal PCP treatment results in a deficit in SND in adult rats; b) the KD of the Drd2 gene in the NAcc reproduces to the same extent the SND deficit induced by the PCP treatment in the saline-treated group of rats; c) this deficit is neither increased nor reduced by the coupling of the neonatal PCP treatment with the Drd2 gene KD. These results suggest that the specific inhibition of Drd2 in the NAcc is sufficient alone to reproduce a behavioral deficit that is induced by the sub-chronic neonatal treatment with PCP. Thus, the specific and localized KD of the Drd2 gene by lentiviral-mediated siRNA transfer may greatly contribute to dissecting the precise role of Drd2 in the NAcc in SZ-related symptoms.

P125. Cariprazine preferentially induces c-fos mRNA in prefrontal cortical regions of rat brain: comparison with aripiprazole, SV-156 and SB-277011.

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Cariprazine is a potent dopamine D_3/D_2 receptor partial agonist antipsychotic in development for the treatment of schizophrenia and bipolar mania. The induction of c-fos mRNA (determined by *in situ* hybridization) by cariprazine was evaluated in selected rat brain areas; results were compared with aripiprazole, SV-156 and SB-277011.

Treatment with cariprazine (0.1-1 mg/kg, PO) similar to SB-277011 (20 mg/kg, PO) induced c-fos mRNA in the islands of Calleja, anterior cingulate and prelimbic cortex. Aripiprazole (3-30 mg/kg, PO) exerted moderate enhancement (with no clear dose dependency), SV-156 (3 mg/kg) did not induce c-fos in these regions. Cariprazine, but not SV-156 or SB-277011, induced c-fos mRNA in the n. accumbens shell region; slight induction was seen with aripiprazole. None of the tested compounds induced c-fos in the striatum.
These results demonstrate that acute administration of cariprazine induces c-fos mRNA expression in rat brain prefrontal cortical regions. The magnitude and location of cellular activation by cariprazine was most similar to SB-277011, a selective D_3 antagonist, but distinct from aripiprazole and SV-156. Induction of c-fos by cariprazine in the prefrontal cortex may be mediated by the D_3 receptor and may play a role in cariprazine's procognitive effects.

P126. Cathecol-O-methyltransferase (COMT) modulates long-term memory in mice.

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COMT is involved in the degradation of dopamine, especially in the prefrontal cortex (PFC) of both humans and rodents, probably due to low levels of DA transporters at synapses of the PFC. We previously reported that increased COMT enzymatic activity following overexpression of the human COMT-Val polymorphisms (COMT- Val tg) in mice, result in disrupted object recognition and working memory abilities. To further investigate the role of COMT genetic modifications in other cognitive domains such as long-term memory, we tested COMT- Val tg mice using a fear conditioning paradigm. COMT-Val-tg mice showed higher levels of freezing compared to control, when re-exposed to the conditioning chamber 50 days, but not 24 hours later. This was reverted by turning off the COMT-Val tg, 20 days before the remote memory test. This evidence implicate COMT overexpression in altered extinction and/or memory for fearful events. Interestingly, using microarray experiments, we found that mRNA CB1 receptors levels in PFC were increased in naive male COMT-Val tg mice compared to their controls. This might be involved in the altered fear conditioning long term memory abilities of COMT Val tg mice as disruption of the endogenous cannabinoid system has been consistently implicated in extinction, delay and contextual fear conditioning models.

P127. Arc involvement in Schizophrenia-related symptoms in mice

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Activity-regulated cytoskeletal-associated protein (Arc) has been demonstrated to play a critical role in synaptic plasticity and hippocampal-dependent memory storage. However, whether deficiencies in Arc expression may contribute to the development of psychiatric disorders remain largely unknown. Using genetically modified Arc knockout mice, we conducted a battery of behavioral assays to interrogate the role of Arc in brain functions commonly affected in psychiatric disorders. Here, we report that Arc knockout mice have defective sensorimotor gating abilities in prepulse inhibition tasks, impaired cognitive abilities in discriminating between objects presented in different temporal orders, reduced sociability and social preference for novel conspecifics, and heightened locomotor responses to psychostimulant amphetamine. Interestingly, these alterations might implicate an altered dopaminergic system following Arc genetic disruption. Indeed, we are accumulating data indicating that Arc knockout mice show altered dopamine level in the hippocampus. Taken together, our results have revealed that Arc knockout mice are specifically impaired in behavioral tasks suggested to model the cognitive, negative and positive symptoms in schizophrenia. These findings raise the possibility that dysfunctions in Arc mediated molecular pathways may contribute to the pathogenesis of psychiatric disorders including schizophrenia.

P128. Dopamine and Other Monoamines Systems are Affected by Histamine(H3) Mechanisms: Combined Microdialysis and Electrophysiological Approaches.

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The histamine-3 (H3) receptor agonist immepip and the inverse agonist thioperamide regulate central histaminergic neurotransmission (Folgering et al, 2009; Eur Neuropsychopharmacol 19: S279). Thus, thioperamide stimulated histaminergic cell firing and increased histamine levels, while immepip had an opposing effect. Effects on downstream neurotransmitters are, however, unclear. Therefore, we compared systemic immepip and thioperamide vs. local histamine on dopamine (DA), serotonin (5-HT) and norepinephrine (NE) neurotransmission in the rat brain. Briefly, microdialysate monoamine levels were assessed from the prefrontal cortex (PFC). Additionally, electrophysiological recordings were performed from DA, 5-HT and NE neurons of the ventral tegmental area (VTA), dorsal raphe nucleus (DRN) and locus coeruleus (LC), under anesthesia. Thioperamide increased extracellular DA, 5-HT and NE levels in the PFC. Thioperamide also increased the mean firing rate of DA neurons in the VTA, which was blocked by immepip. 5-HT DRN and NE LC neurons were unaffected by these ligands. Interestingly, iontophoretic histamine increased the firing rate of dopamine VTA DA neurons. Taken together these data support a role for H3-receptors in the modulation of monoaminergic systems, further suggesting the importance for H3-receptors in the modulation of neuropsychiatric disease.

P129. Midbrain dopamine neuron dysfunction in an infection-based rat model of schizophrenia: interaction with adolescent Cannabis exposure.

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Prenatal viral infection is associated with increased risk of schizophrenia. On the other hand, a large body of literature shows an association between adolescent Cannabis use and adult onset of psychosis. The aim of this study was to evaluate the interaction between these factors in the occurrence of psychoses, and whether they produce functional changes on ventral tegmental area (VTA) dopamine (DA) neurons, that play a crucial role in their pathophysiology. Single cell extracellular electrophysiological recordings were performed in adult anaesthetized rats, which were divided into different groups according to the prenatal, postnatal treatments or both. Pregnant rats were injected on gestational day 14 with the inflammatory agent polyriboinosinic-polyribocitidylic acid (poly I:C, 4mg/kg i.v.) or vehicle. To mimic a model of adolescent Cannabis consumption, adolescent rats (from postnatal day 45 to 55) were treated with increasing doses of ?9-tetrahydrocannabinol (THC) (2.5-10 mg/kg i.p.). Rats exposed in utero to poly I:C (poly I:C rats) showed reduced mean frequency and number of spontaneously active VTA DA neurons as compared to controls, whereas percent of burst firing and coefficient of variation (CV) were not different. However, poly I:C rats displayed increased mean intraburst frequency and reduced mean burst duration. In contrast, only the percent of burst firing and CV were altered in THC-treated rats. Interestingly, in poly I:C rats adolescent administration of THC normalized rather than further disrupted several electrophysiological parameters. Overall, our findings provide electrophysiological evidence of altered activity of DA neurons following exposure to pre- and postnatal risk factors for psychoses.

P130. Pathophysiological alterations of midbrain dopaminergic neurons in their unit activity and channel properties in a schizophrenia model established by epidermal growth factor.

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A cytokine, epidermal growth factor (EGF), is implicated in dopamine-associated brain diseases such as schizophrenia. We found that the animals transiently exposed to EGF during neonatal stage later exhibit schizophrenia-like behavioral abnormality at post-pubertal stages with enhanced morphological and neurochemical development of midbrain dopaminergic neurons. However physiological impact of neonatal exposure to EGF on this neuronal population was not assessed. Using patch clamp and unit recording techniques, here, we analyzed intrinsic firing and channel properties of adult dopaminergic neurons in midbrain slice preparations or in an anesthetized condition. Whole-cell patch clamp recordings from the ventral tegmental area (VTA) reveals that EGF-treated mice exhibited the attenuation of spike afterhyperpolarization as well as a decrease in outward tail currents elicited by prolonged depolarization, both suggesting the prolonged influences of EGF on the electrophysiological characteristics of dopaminergic neurons. To explore how these alterations are reflected in their firing in vivo, we performed single unit recordings in chloral hydrate-anesthetized mice. In EGF-treated mice, there were enhanced burst activities but no changes in the mean firing frequency. These results suggest that the excess neurotrophic actions on perinatal dopamine neurons persistently impair their excitability and firing properties, potentially leading to the abnormal behaviors relevant to schizophrenia.

P131. Modulation of dopaminergic signalling in the striatum by phosphodiesterase 10A (PDE10A) inhibitors.

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Phosphodiesterase 10A (PDE10A) is a dominant phosphodiesterase in striatal medium spiny neurons (MSN) that hydrolyse the second messengers cAMP and cGMP. Preclinical evidence suggests that PDE10A inhibitors are anti-psychotic, pro-cognitive and hold potential for treatment of negative symptoms. PDE10A inhibitors are currently being evaluated in clinical settings for the treatment of schizophrenia. Inhibition of PDE10A leads to increased cyclic nucleotide levels in the striatum and is expected to negatively modulate dopamine D2 receptor signalling in the indirect pathway MSN and positive modulate dopamine D1 receptor signalling in the direct pathway MSN. In behavioural assays such as phencyclidine-induced hyperactivity and conditioned-avoidance response, the impact of PDE10A inhibitors is quite similar to dopamine D2 antagonists. However, there are also differences, some of which can be attributed to positive modulation of dopamine D1 receptor signalling. Comparative behavioural data for structurally unrelated PDE10A inhibitors are presented along with PDE10A occupancy assessed with a novel PDE10A in vivo binding ligand. Interpretation of the relative impact of PDE10A inhibition on dopamine D1 and D2 receptor signaling in different paradigms is discussed as well as the impact on the potential for treatment of schizophrenia.

P132. Perinatal exposure to the cytokine EGF produces pallidal hyperinnervation of dopaminergic neurons and the indirect pathway dysfunction in the schizophrenia animal model

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Epidermal growth factor (EGF) is one of ErbB receptor ligands and possesses a neurotrophic activity on brain neurons. Excessive perinatal signaling of this cytokine developed schizophrenia-like behavioral endophenotypes in various animals, such as PPI deficit. According to the dopamine hypothesis for schizophrenia, we addressed the question of whether the neurotrophic action of this cytokine targets the dopamine system and contributes to the behavioral abnormalities of this model. We found the persistent increase in tyrosine hydroxylase level and dopamine content in the globus pallidus of the EGF model rats. In parallel, pallidal dopamine release was elevated in EGF-treated rats but normalized by subchronic treatment with risperidone. Pallidal infusion of reserpine (VMAT inhibitor) or raclopride (D2-like receptor antagonist), but not SCH23390 (D1-like receptor antagonist), ameliorated PPI deficits of the EGF model rats. Conversely, local infusion of quinpirole (D2-like agonist) to normal rats disrupted PPI. We also found that the hyperdopaminergic state increased firing rates of pallidal neurons and resulted in the enhancement of GABA release in the substantia nigra, one of the targets of pallidal efferrents. These findings suggest that the pallidal dopaminergic innervation is vulnerable to circulating EGF and its abnormality provides crucial impact on basal ganglia function and cognitive behaviors.

Dopamine receptors

P133. Testing drugs for structural plasticity in dopaminergic neurons: translation from mouse primary cell culture to human iPSC-derived neurons

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Innovative treatments for neurological and psychiatric disorders can be discovered by interfering with the molecular mechanisms operating in neurons that are present in dysfunctional brain circuits described in patients. This is the case for mesencephalic dopaminergic neurons, whose susceptibility to damage or capacity to adapt with structural plasticity is well established in Parkinson's Disorder (PD) and, more recently, in Substance Abuse. Converging findings indicate that dopamine D2D3 receptor (R) agonists reduces the progression of PD by acting on D2D3R but it is still partially unclear which cellular processes and intracellular pathways are involved. In our laboratory we used primary cultures of mesencephalic neurons from wildtype and KO mice. In the present work we investigated the neurotrophic-like effects of D2D3R agonists such as quinpirole, ropinirole, 7OHDPAT and S325045, the indirect dopamine agonists amphetamine and cocaine, and other substances including nicotine. Primary mesencephalic neuronal cultures from D3KO and wildtype mice were prepared at 12.5 embryonic day. Morphometric assessments showed that DA agonists produced a significant increase of dendritic arborisation and soma size in DA neurons when compared to vehicle. These morphological features were associated with the activation of ERK and Akt-mTOR pathways, known to be involved in neuronal plasticity. These effects were completely blocked by pretreatment with D3R antagonists SB277011A and S33084. No effects were observed in neuronal cultures from D3KO mice, suggesting a key role for D3R in dopamine-induced structural plasticity. Recently, we established protocols to obtain neurons from the human iPSC we have generated.

P134. Striatal D2 receptors in stress coping: genetic and environmental influences

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Mice of the inbred DBA /2J strain exposed to an experience of reduced food availability (12 days) ending 3 days before testing do not develop a lasting helplessness in the forced swimming test (FST) , accumulation of DeltaFos in the shell of the nucleus accumbens, a known marker of stress resilience. These effects were strain- specific because they were absent in mice of the standard inbred C57BL/6J strain. Control DBA/2J mice show FST-induced enhanced c-Fos expression in the left dorsolateral striatum (DLS) and this response was absent in food-restricted mice. Finally, temporary inactivation of the left DLS or blockade of D2 (but not D1) dopamine receptors by local infusion of sulpiride immediately after a first experience with FST prevented stabilization of helplessness in continuously free-fed mice. Control mice of the C57BL/6J strain showed enhanced FST-induced c-Fos expression in several brain areas and in these mice stabilization of helplessness was prevented by bilateral inactivation of the hippocampus. Finally, whereas expression of the long isoform of the D2 receptors was predominant in C57BL/6J mice, as reported in rats, DBA/2J mice were characterized by high levels of the short isoform of the dopamine receptor. Together, these results support the hypothesis that gene-environment interaction moderate the pro-depressant effects of stress experiences possibly through modulation of striatal D2 receptors.

P135. Chronic alcohol disrupts dopamine receptor activity and the cognitive function of the medial prefrontal cortex

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Dopamine (DA) exerts powerful effects on cognition via modulation of excitatory and inhibitory neurotransmission in mPFC. We examined the impact of chronic intermittent ethanol (CIE) exposure on cognitive function and DA-mediated neurotransmission in the rat mPFC. Consistent with alterations in executive function in alcoholics, CIE-exposed rats exhibited deficits in behavioral flexibility in an operant set- shifting task. Since alterations in dopaminergic neurotransmission in the mPFC have been shown to influence cognition, an adult acute slice preparation was used to examine changes in DA receptor function in the mPFC following CIE. In slices from control rats, D1, D2, and D4 receptor stimulation exerted complex actions on neuronal firing and synaptic neurotransmission that were dependent upon both receptor subtype and cell type (pyramidal cell or fast-spiking interneuron). In CIE rats, there was a near complete loss of D2 and D4 receptor modulation on evoked firing and neurotransmission. CIE did not alter D1 or mGluR1 modulation of firing. In contrast to CIE effects, adolescent intermittent ethanol (AIE) did not alter D2 modulation of pyramidal cell firing in the adult mPFC. There was, however, a decrease in COMT expression, suggesting possible disruption of the mPFC DA system. The observed CIE-induced disruption in D2/D4 receptor signaling is consistent with the suggestion that chronic alcohol exposure disrupts cognition by disrupting D2/D4 signaling in mPFC. In contrast, a transient alteration in DA receptor signaling during adolescence might indirectly alter cognitive functioning in adulthood by disrupting the maturation of cortical networks.

P136. Distinct roles of dopamine D2 receptors in dorsal and ventral striatum on motor and drug-related behaviors

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This project utilized a multi-pronged approach to study the role of striatal D2Rs in motor and drug-related behaviors, considering the effects of both a life-long reduction of D2R expression in indirect-pathway medium spiny neurons (iMSNs) and an acute reduction of D2R expression in the ventral striatum (NAc). A Cre-loxP system was used to induce life-long reduction of D2R expression by 30% and 70% in iMSNs. Reduction of D2Rs in the striatum was confirmed by qPCR and binding experiments. In mice with a life-long reduction of D2R expression in iMSNs, exploratory and home cage locomotion was reduced, and acute locomotor response to cocaine was blunted. However, these mice displayed unimpaired locomotor sensitization to cocaine. Reduction of striatal D2R impaired rotarod performance, implicating the importance of striatal D2R expression in motor learning. To acutely reduce D2R expression in the NAc, viral vectors expressing Cre recombinase under a strong promoter were stereotaxically injected into the NAc of mice with floxed Drd2 genes. Cre-injected mice displayed markedly reduced (85%) NAc Drd2 mRNA levels, showed reduced locomotion in novel and home cage environments, and had a blunted acute locomotor response to cocaine. Despite these impairments, mice lacking NAc D2R showed locomotor sensitization to cocaine and normal motor learning when trained on the rotarod. Taken together, these findings suggest that NAc D2Rs are essential for locomotor behaviors and acute behavioral responses to drugs of abuse, whereas D2R expression in the dorsal striatum is important for motor learning.

P137. Normalizing Dopamine D2 Receptor-Mediated Responses in D2-KO Mice by Virus-Mediated Restoration of D2 Receptors: Comparing D2L and D2S

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D2 receptor null-mutant (D2-KO) mice have altered responses to the rewarding and locomotor effects of psychostimulant drugs, evidence of a necessary role for D2 receptors in these behaviors. Work with mice that constitutively express only the D2 receptor short form, D2S, as a result of genetic deletion of the long form (D2L), has supported a model in which D2L is thought to be the postsynaptic D2 receptor on medium spiny neurons in the basal forebrain, and D2S the autoreceptor that regulates the activity of dopamine neurons and dopamine synthesis/release. Because constitutive deletion of the D2 or D2L receptor may cause compensatory changes that influence functional outcomes, our approach is to determine that D2-KO mice are deficient in a behavioral, biochemical, or electrophysiological response, and then to determine if the response can be normalized by virus-mediated D2 receptor expression. We confirmed that D2-KO mice are deficient in methamphetamine-induced locomotion and lack D2 receptor agonist-induced activation of potassium channels (GIRKs) in dopaminergic neurons. Virus-mediated expression of D2L in the nucleus accumbens restored amphetamine-induced locomotion compared to mice receiving control virus. Furthermore, the effect of expression of D2S was indistinguishable from D2L. Similarly, virus-mediated expression of either D2S or D2L in substantia nigra neurons restored D2 agonist-induced activation of GIRKs. In this acute expression system, the alternatively spliced forms of the D2 receptor appear to be equally capable of acting as postsynaptic receptors and autoreceptors. (MH045372, DA018165, and VA Merit Review)

P138. Role of D1 and D3 dopamine receptor heterodimerization in the regulation of the receptor signaling

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It has been already accepted that G protein-coupled receptors (GPCRs), through a protein-protein interaction at the cell membrane, form heterodimers with closely related or structurally divergent receptors with pharmacological, signaling and trafficking properties different from those of their constituent receptors. The most relevant implication of heterodimerization is the observation that GPCR heterodimers offer a novel set of pharmacological targets. We recently provided strong evidence that in the striatum, the GPCR dopamine D1 (D1R) and D3 (D3R) physically interact to form a heterodimeric complex that displays unique pharmacological and trafficking properties that are different from those of D1R and D3R homo-oligomers. In this study, the intracellular signals specifically mediated by D1R-D3R heterodimers activation was investigated. We found that in HEK293 cells expressing D1R, activation of D1R cells induced ERK1/2 phosphorilation, but not AKT, in a cAMP/PKA-dependent way. By contrast, in HEK293 cells expressing theD3R cells, D3R stimulation resulted in both ERK1/2 and AKT activation, in a Gi protein/PI-3K-dependent mechanism. Interestingly, in cells expressing D1R-D3R complexes, the coincident stimulation of both interacting receptors resulted in ERK1/2 activation that involved PKA, but not Gi protein/PI-3K. By contrast, D1R-D3R complexes activation did non resulted in AKT phosphorilation, suggesting that heterodimerization of D1R with D3R deeply affect D3R capability to activate Gi protein-dependent signaling pathways. Taken together, our data give a novel insight into how D1R and D3R may function in an integrated way. Supported by MIUR and University of Brescia (PRIN 2008 to CM).

Dopamine and affective disorders

P139. Different classess of antidepressants increase dopamine and norepinephrine release in the bed nucleus of stria terminalis: an "in vivo" microdialysis study.

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Antidepressants include a relatively wide spectrum of drugs that increase the synaptic concentration of monoamines, mostly through neurotransmitter reuptake blockade.

The bed nucleus of stria teminalis (BNST) is considered a relay station in mediating the activation of stress response but also in the acquisition and expression of emotions. BNST is richly innervated by monoamines and sends back projections to the nucleus of monoamine origin. The administration of selective blockers of norepinephrine transporter (NET) increases the extracellular concentration (output) of norepinephrine but also of dopamine in the BNST, suggesting that catecholamine transmission in BNST could be involved in antidepressant mechanisms. The aim of this study, carried out through the in vivo microdialysis technique, was the characterization of the acute effect of various antidepressants, belonging to different monoamine reuptake blockers categories, on dopamine and norepinephrine ouput in the BNST, We observed that all the tested antidepressants (5 to 20 mg/kg i.p.) increased, dose dependently catecholamines. In particular, the maximum increases (in % of basals), for norepinephrine and dopamine respectively, were as follows: desipramine, 239 and 137; reboxetine 185 and 128; imipramine 512 and 359; citalopram 95 and 122; fluoxetine 122 and 68; bupropion 255 and 164. These results suggest that catecholamine transmission in the BNST might be part of a common downstream pathway that is involved in the mechanism of action of antidepressants and consequently it can be hypothesized that a dysfunction of neuronal transmission in this brain area might have a role in the aetiology of affective disorders.

P140. The role of extended amygdala dopamine D2 receptor-expressing neurons in fear expression and fear generalization

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Using BAC transgenic mice expressing EGFP under a Drd2 promotor sequence we observed that D2R-expressing neurons constitute half of the neuronal population of the dorsolateral BST (BSTDL) and the lateral CEA (CEAL) We performed immunohistochemical analysis of extracellular signal-regulated kinase (ERK1/2) phosphorylation to study plasticity-related activity following auditory fear conditioning. We observed a striking decrease followed by a sustained increase in ERK1/2 phosphorylation in the extended amygdala of mice subjected to an auditory fear conditioning protocol. These biphasic changes occurred specifically in D2R-expressing neurons of the BSTDL and CEAL. Systemic administration of the D2/3 receptor antagonist raclopride immediately following fear conditioning prevented the sustained increase in ERK1/2 phosphorylation in exteded amygdala D2R-expressing neurons. Moreover, systemic raclopride did not affect freezing in response to a conditioned auditory stimulus but induced a generalized freezing response towards non-conditioned auditory stimuli. Similarly, we fear generalization following local infusion of raclopride in either the BSTDL or CEAL. Our data suggest that ERK1/2 phosphorylation in D2R-expressing neurons of the lateral extended amygdala is required to prevent fear generalization.

P141.Molecular mechanisms behind inflammation-induced malaise and aversion.

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Systemic inflammation triggers a range of central nervous symptoms including fever, loss of appetite and sleepiness. A prominent feature of inflammatory conditions is that they cause feelings of malaise and discomfort. However, the molecular mechanisms behind the fact that inflammation is perceived as something negative and uncomfortable remain elusive. Here we show a pathway by which inflammatory signals can reach the brain and trigger negative affect and we identify a neuronal population on which they act. To assay inflammation-induced discomfort and aversion we used conditioned place aversion in mice. We show that inflammation-induced aversion depends on a collaborative action of the cytokines IL-6, IL-1 and TNF-a. Further, cytokine binding to the brain endothelium, leading to prostaglandin E2 synthesis dependent on cyclooxygenase-1 and microsomal prostaglandin E synthase 1, is critical for the aversion. The prostaglandin E2 subsequently target EP1 receptors on dopamine D1 receptor expressing neurons to mediate the aversive effect. Also, the aversion is blocked in mice lacking NMDA-receptors on dopaminergic neurons. This pathway is in many ways different from the pathways identified for other inflammatory symptoms and strongly suggests that prostaglandin dependent modulation of dopaminergic reward circuitry is a key mechanism by which inflammation is detected as something negative and aversive. Our findings may have implications both for acute feelings of discomfort and more chronic depressive symptoms related to inflammation.

P142. Initial pharmacotherapy by dopamine stabilizer, aripiprazole, for inpatients due to suicidal attempt

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Aripiprazole, a dopamine stabilizer, is used for the treatment of wide spectrum of psychotic symptoms. In our facility, aripiprazole has often been prescribed for cases with suicidal attempts. Rapid improvement of psychotic symptoms, including depression, anxiety, hallucination or delusion, and fewer incidence of over-sedation may enable clinicians to shorten the duration of admission. For cases with acute intoxication by psychoactive substances such as benzodiazepines or antidepressants, replacement therapy by aripiprazole may be a choice to simplify pharmacotherapy. The replacement from benzodiazepines including quazepam to aripiprazole might also be effective for preventing and/or improving the symptoms of sleep apnea syndrome. For side effects of akathisia and/or insomnia, during aripiprazole administration, addition of histamine H1 receptor antagonist such as promethazine hydrochloride or hydroxyzine, or valproate has been shown to be effective. We would like to show our clinical experiences of initial pharmacotherapy using aripiprazole (3-30mg/day) for 20 inpatients due to suicidal attempt, who admitted through the Tertiary Emergency Center at Nuclear Heart Hospital.

P143. Tyrosine hydroxylase dysfunction and dopamine deficiency in phenylketonuria

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Hyperphenylalaninemia (HPA) caused by the hepatic phenylalanine hydroxylase (PAH), has severe consequences on brain neurotransmitter metabolism. Such changes can be studied in ENU1/2 and ENU2/2 mice, with mild/BH4-responsive or severe/phenylketonuric HPA, respectively. Brain phenylalanine (Phe) levels and Phe/tyrosine (Tyr) ratios levels were elevated 5-6-fold in ENU1/2 and up to 10-20-fold in ENU2/2 mice compared to normal mice, whereas tetrahydrobiopterin (BH4) was only slightly elevated in the brains of ENU2/2 mice. On the other hand, L-Dopa, dopamine, noradrenaline, HVA, serotonin and 5HIAA are all reduced in the ENU2/2 mice. Moreover, as seen by immunoquantification, the level of tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of dopamine, noradrenaline, and adrenaline, was 1.5-fold decreased in untreated ENU2/2 mice. We have investigated the effect of different treatment modalities for HPA on the level of BH4, monoamine neurotransmitters and their metabolites, as well as TH content in brain. The ENU1/2 mice were supplemented with BH4 at 50 mg/kg/day for 10 days, a treatment that has previously been found to have a pharmacological chaperone effect on TH in normal mice (Thöny, et al., 2008)), and ENU2/2 mice with gene therapeutic viral and non-viral vectors, or with low (Phe) diet. Supplementation of ENU1/2 with BH4 only showed a trend towards a small increase in TH activity in the ENU1/2 and no change in the neurotransmitter and metabolite profile.

P144. Lateral Habenula Modulation Of Ventral Tegmental Area Dopamine Neurons in a Rodent Model of Depression

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Anhedonia, the inability to derive pleasure from normally pleasurable stimuli, is a core symptom of major depressive disorder (MDD) and may be the result of abnormal mesolimbic dopamine (DA) system signaling. The lateral habenula (LHb) has a prominent inhibitory action over the ventral tegmental area (VTA) midbrain DA center via the rostral medial tegmentum, and has been shown to be overactive in humans with MDD and in animal models of depression. However, the specific changes in VTA DA neuron activity states driving the observed decrease in DA system function following LHb stimulation have yet to be fully characterized. We used single-unit extracellular recordings from identified VTA DA neurons in rats in which the LHb was activated by injection of low or high-dose NMDA or vehicle and compared measures of tonic and phasic DA neuron activity between groups. Pharmacological activation of the LHb at high levels resulted in a prominent (>50%) decrease in the overall number of DA neurons spontaneously active (tonic activity), resembling the changes in VTA DA neuron population activity seen following chronic mild stress (CMS) in rodents. These data suggest that the LHb may play a role in the pathophysiology of MDD by reducing the amount of DA available to reward centers downstream of the VTA for use during reward processing and thus limiting their ability to properly respond to a rewarding stimulus. This interpretation is consistent with patient reports of inability to enjoy pleasurable stimuli.

P145. The impact of co-treatment with antidepressant drugs and risperidone on the extracellular level of dopamine and its metabolites in rat frontal cortex

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A combination of atypical antipsychotic drugs (AADs) and antidepressants (ADs) has recently been found to be beneficial to a number of neuropsychiatric disorders such as major depression or schizophrenia. A few clinical reports have suggested that a combination of a low dose of AAD, e.g. risperodone, and ADs has a beneficial effect on the treatment of drug-resistant depression. In the present study we investigated the effect of fluoxetine or mirtazapine and risperidone, given separately or jointly, on the extracellular levels of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) in rat frontal cortex using a microdialysis in freely moving animals. Risperidone (0.1 and 1 mg/kg) increased the extracellular levels of DA, DOPAC and HVA. The increase in DA level induced by fluoxetine (10 mg/kg) was weaker than that evoked by risperidone. Mirtazapine (10 and 20 mg/kg) also increased DA level, but its effect on DOPAC and HVA was weaker compared to risperidone. A combination of fluoxetine or mirtazapine and the higher dose of risperidone produced a stronger effect on the extracellular levels of DA, DOPAC and HVA than did those drugs given separately. The above findings suggest that the effect of combined administration of risperidone and ADs on DA cortical release may be of crucial importance to the pharmacotherapy of drug-resistant depression. Acknowledgemens: This study was financially supported by grant POIG. 01.01.02-12-004/09-00 from the European Regional Development Fund.

Dopamine neurons

P146.Dopamine neurons in opiate withdrawal: a computational perspective.

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Dopamine (DA) neurons of the ventral tegmental area (VTA) are involved in the neurobiological mechanisms underlying addictive processes. It has been shown that withdrawal from drugs of abuse, cause profound modifications in the morphology and physiology of these neurons, but the mechanisms underlying these modifications are poorly understood. Because of their high predictive value, computational models are a powerful tool in neurobiological research, and have been used to gain further insights and deeper understanding on the molecular and physiological mechanisms underlying the development of various psychiatric disorders. Here we present a biophysical model of a DA VTA neuron based on 3d morphological reconstructions and electrophysiological data from literature, showing how opiate withdrawal-driven morphological and electrophysiological changes could affect the firing rate and pattern of these neurons. The model is composed by 89 membrane segments, with sodium and calcium dynamics responsible for the basal in vivo activity of these neurons; the set of inputs is modeled adding GabaA synapses and AMPA/NMDA, activated in such a way to model the behavior of glutamatergic and gabaergic inputs. We modeled the opiate withdrawal state applying the morphometric modifications and changing the balance of Glu/Gaba inputs as described by electrophysiological data (Bonci and Williams, 1997; Manzoni and Williams, 1999). Our results suggest that changes in the balance of Glu/Gaba inputs could explain the hypofunction of VTA DA neurons observed during opiate withdrawal while morphological changes could be responsible for the hypersensitivity to drug administration showed by these neurons during withdrawal (Diana et al., 1999).

P147. Functional and topographical analysis of the brainstem cholinergic innervation of the ventral tegmental area

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Dopaminergic neurons of the ventral tegmental area (VTA) fire in a regular (tonic) or in an irregular (phasic or bursting) mode, and this has been associated with different forms of dopamine release. The pontine laterodorsal tegmental nucleus (LDTg) and the pedunculopontine nucleus (PPN) provide cholinergic innervation of the VTA and play an important role in the control of dopaminergic neuron firing. Tracer studies have suggested a widespread topographical cholinergic innervation of the VTA. Moreover, it is known that VTA projection neurons are topographically organized, which raises the possibility that LDTg/PPN neurons are involved in the modulation over distinct sub-regions and possibly distinct cell types of the VTA. We have examined the topographical organization and properties of these brainstem projections to the VTA using retrograde and anterograde tracing, and in vivo optogenetic stimulation of the PPN or LDTg in anaesthetized ChAT-cre rats, in combination with juxtacellular recordings and labelling of VTA neurons. Optical stimulation of cholinergic fibres from the PPN produced an increase in the firing rate of both dopaminergic and non-dopaminergic neurons in the VTA, whereas stimulation of cholinergic fibres from the LDTg resulted in a more heterogeneous response. The tracing studies revealed differences in the pattern of innervation of cholinergic axons within the VTA, such that the PPN innervates the whole extent of the VTA whereas the LDTg innervation is spatially restricted to the dorsal regions. These data suggest that the cholinergic innervation of the VTA arising in the PPN and LDTg are both functionally and topographically distinct.

P148. Neural bases for the excitatory control of VTA dopamine neurons by the ventral hippocampus

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The hippocampal formation including the ventral subiculum and the ventral CA1 area (vSUB/CA1) is a brain region that is involved in context-dependent processes, and also is considered as a regulator of emotion. The ventral tegmental area (VTA) plays a role in the acquisition of learned appetitive behaviors and in the development of drug addiction. It is now well accepted that the vSUB/CA1 activates the dopamine system, however, the mechanism by which the vSUB/CA1 regulates the activity of the VTA DA neurons is still unclear. We used electrophysiological approaches in anesthetized rats to demonstrate that the vSUB/CA1 has an excitatory effect on the tonic activity of VTA DA neurons .The electrical stimulation of the vSUB/CA1 evoked responses of DA neurons whose mostly respond by excitation. Moreover, we have previously shown that the Bed nucleus of the stria terminalis (BNST) stimulation increases VTA dopamine neuron activity and that the BNST receives excitatory inputs from the vSUB/CA1. Here, we blocked the excitation driven by the electrical stimulation of vSUB/CA1 by infusing locally in the BNST glutamatergic receptors antagonists. We conclude that the BNST relays the excitatory drive between the vSUB/CA1 and VTA. We found that BNST received inputs from the vSUB/CA1 and projected glutamatergic efferences to the VTA by using tracing experiment coupled with RT PCR -laser microdissection. All together, these results strongly suggest that the BNST relays the excitatory drive between the vSUB/CA1 and DA neurons of the VTA.

P149. SorLA Controls Neurotrophic Activity by Sorting of GDNF and its Receptors GFRa1 and RET

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GDNF is a potent neurotrophic factor that has reached clinical trials for Parkinsons's disease. GDNF binds to its co-receptor GFRa1 and signals through the transmembrane receptor tyrosine kinase RET, or RET-independently through NCAM or syndecan-3. Whereas the GDNF signaling cascades are well described, cellular turnover and trafficking of GDNF and its receptors remains poorly characterized. Here, we find that SorLA acts as sorting receptor for the GDNF/GFRa1 complex directing it from the cell surface to endosomes. Through this mechanism, GDNF is targeted to lysosomes and degraded while GFRa1 recycles, creating an efficient GDNF clearance pathway. The SorLA/GFRa1 complex further targets RET for endocytosis but not for degradation, affecting GDNF-induced neurotrophic activities. SorLA-deficient mice display elevated GDNF levels, altered dopaminergic function, marked hyperactivity, and reduced anxiety. All of which are phenotypes related to abnormal GDNF activity. Taken together, these findings establish SorLA as critical regulator of GDNF activity in the CNS.

P150. High throughput single-cell expression analysis of midbrain dopamine neurons reveals Aldh1a1 as marker of vulnerability in a model of Parkinson's disease

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The midbrain dopaminergic neurons are involved in diverse physiological pathways including motor control, learning, cognition, and reward behaviors. In the last thirty years, several lines of evidence have suggested that there may be additional levels of dopamine heterogeneity, that may be even more meaningful than their classification into three anatomically defined clusters. In order to assess the extent of midbrain dopamine diversity, we used a high throughput gene expression platform based on microfluidic dynamic arrays to evaluate the expression of 96 genes in single dopamine cells. This approach revealed the existence of multiple genetically distinct populations of dopamine neurons, with distinct molecular signatures. One of these population is defined by high expression of Aldh1a1 and low expression of Calb1. This population is localized in the ventral tier of the substantia nigra. Moreover, Aldh1a1 expressing neurons of the substantia nigra are more vulnerable in the MPTP model of Parkinson's disease. This gene might provide additional cues to the vulnerability of a subset of dopamine neurons to Parkinson's disease, as well as provide an avenue for an earlier diagnostic of the disease.

P151. Metabolic state affects the encoding of reward-related information by VTA dopamine neurons.

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The mesolimbic dopamine (mesDA) system is known for its role in associative learning, reward-seeking, and signaling of reward-related information. As such, increased activity of mesDA neurons drives motivated behavior and promotes operant responding for food. The fact that this food-seeking behavior resembles that of animals in a negative energy-balance and in light of the sensitivity of ventral tegmental area (VTA) dopamine (DA) neurons to feeding hormones like leptin and ghrelin (that signal information about the current metabolic state), this system may be important in the regulation of food intake. However, the nature of mesDA's role in energy balance remains unclear. To investigate how energy balance affects reward-signaling by mesDA neurons, in vivo electrophysiological recordings were made of (putative) DA neurons in the ventral tegmental area (VTA) of adult Wistar rats during the execution of a behavioural task. In this task, animals were able to obtain food rewards that differed in their rewarding value. Neuronal activity was subsequently related to cue-presentation and the delivery of food rewards. To manipulate energy balance, animals were either food-deprived or free-fed preceding the recording session. In addition, the effect of peripheral injections of leptin and ghrelin on reward-encoding and task performance was measured. Preliminary data shows that leptin, but not ghrelin, reduces reward-signalling during operant responding for food. Elucidation of the role of mesDA neurons in feeding behavior might provide important insights into the role of this neural circuit in obesity and anorexia nervosa.

P152. Alpha-synuclein regulates dopaminergic synapse arrangement and functionality by modulating synapsin III and the dopamine transporter

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Besides being involved in Parkinson's disease (PD) pathophysiology, alpha-synuclein (AS) plays a key role in the regulation of dopaminergic synaptic function (Bellucci et al., 2012, Brain Res. 1476: 183). For instance, we recently observed that AS aggregation into insoluble inclusions affects dopaminergic synapse arrangement as it interacts with a key protein involved in the control of dopamine (DA) release and reuptake: the dopamine transporter (DAT) (Bellucci et al., 2008, J. Neurochem. 106:560-577; Bellucci et al., 2011, PLoS ONE 6: e27959). Here, we aimed at investigating whether and how AS may affect nigrostriatal dopaminergic synapse arrangement and functionality by regulating another negative modulator of DA release: synapsin III (Kile et al., 2010, J. Neurosci. 30 (29):9762-70). We used AS-null C57BL/6S (Specht and Schoepfer, 2001, BMC Neurosci. 2: 11-19) and C57BL/6J control mice to investigate the occurrence of interactions between AS, synapsin III and DAT as well as to assess their levels and distribution in striatal dopaminergic synapses. We found that AS differentially affected the subcellular localization and expression of synapsin III and DAT in the striatum of 3-8-12 month-old C57BL/6S mice when compared to control mice. Moreover, by analyzing the acute locomotor response to the administration of cocaine (10mg/Kg i.p.) or the selective DAT inhibitor GBR 12935 (10mg/Kg i.p.) in the open field behavioural paradigm, we found significant differences between AS null and control mice. Our data indicate that AS affects nigrostriatal dopaminergic synapse arrangement and DA turnover by modulating the subcellular distribution and the levels of synapsin III.

Dopamine and neuroplasticity

P153. Characterization of neural activity of midbrain dopamine and rostromedial tegmental neurons in a rat model of neuropathic pain.

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Several brain regions have been involved in the mediation of affective and cognitive components of chronic pain. Some of the most invalidating consequences of chronic pain like hyperalgesia, anhedonia, as well as anxiety and depression, have been shown to be associated with changes of the dopaminergic reward system. The rostromedial tegmental nucleus (RMTg), a structure located caudally to the dopaminergic ventral tegmental area (VTA), is an important site involved in the mechanisms of aversion. RMTg contains γ-aminobutyric acid (GABA) neurons which respond to noxious stimuli and provide a major inhibitory projection to VTA dopamine neurons. Little is known on how these brain regions are affected by pain. To this aim, we investigated on how neural activity is remodeled within these brain regions in a rat model of neuropathic pain, the *spared nerve injury* (SNI). The spontaneous activity of VTA dopamine cells was examined by using *in vivo* extracellular single unit recordings from SNI animals and sham-operated controls, one and two weeks following surgery. After one week or two weeks VTA dopamine neurons from SNI rats did not show any difference in the baseline firing activity. However, after two weeks, the duration of inhibition elicited by electrical stimulation of the RMTg onto dopamine cells was reduced in SNI rats. Our data suggest a possible role of the brain reward and aversion circuitry in processing affective and cognitive components of neuropathic pain.

P154. Cellular mechanisms underlying changes in eCB-signaling during the switch from the goal-directed to habitual behaviour.

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Goal-directed control of actions is the ability to adapt behavior to obtain outcomes and to efficiently respond in changing situations. With repetition, actions become more automatic and habitual. However, maladaptive habits can appear under pathological conditions. In a model of cannabinoid tolerance, we have shown that the expression of habitual behaviour associates to adaptations in the endocannabinoid(eCBs)-mediated plasticity (eCB-LTD) in the lateral part of the dorsal striatum (DLS) (Nazzaro & Greco, et al, 2012). However, the mechanisms underlying changes in eCB-signaling during the physiological switch between goal-directed vs habitual responding are not known. To address this question, we tested the impact of different training regimes of instrumental conditioning, promoting either goal-directed (short-training) or habitual behaviour (overtraining), on striatal eCB signaling. In the DLS, LTD depends on eCBs released upon activation of groupI metabotropic glutamate receptors (mGlur1/5) and activation of dopamine D2 receptors. Overtrained mice showed a reduction of eCB-mediated LTD induced both by cortical stimulation and by pharmacological activation of mGlur1/5 receptors, compared to short-trained animals. This raises the possibility that adaptations in mGlur5 signaling may contribute to habit formation. Systemic administration of the mGlur5 antagonist MPEP during training restored the sensitivity to contingency degradation in overtrained mice, suggesting a rescued goal-directed behaviour. Similar results were obtained upon administration of molecules regulating the activity of dopamine signaling. Our data indicate that the functional interplay between mGlur5- and dopamine signaling modulates the switch from goal-directed to habitual behaviour.

P155. An increase in the natural GDNF expression enhances and protects the nigrostriatal dopaminergic system

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Glial cell line-Derived Neurotrophic Factor (GDNF) protects and promotes the survival of dopamine (DA) neurons, when administered to cell cultures in vitro or to neurotoxin based in vivo models of Parkinson's disease. Despite this well established role for exogenous GDNF, the exact role of endogenous, physiological GDNF remains largely unknown, mainly due to the lack of proper animal models. For studying the role of endogenous GDNF in the development and function of nigrostriatal DA system, we created a knock-in, hypermorphic mouse model, where natural GDNF expression is increased. Heterozygous (hetz) and homozygous (homoz) hypermorphic mice have 30% and 70% increased brain GDNF mRNA levels. Stereological estimation of the number of TH- and VMAT2-positive cells in substantia nigra pars compacta (SNpc) revealed a 15% increase in DA cell number in the age groups of 7.5 days, 3-4 months and 17 months. Similarly striatal tissue DA levels were increased 20% in the same age groups, when measured by HPLC. Consequently amphetamine-induced locomotor activity (1 mg/kg; i.p.) and striatal DA response to local amphetamine stimulation (100 µM for 1 hour, in vivo microdialysis) were augmented by about 30%. The young Hetz mice demonstrated enhanced motor performance and the old (15-17 m) improved motor learning in accelerating rotarod test. Finally, we saw a protection from proteasome inhibitor lactacystin-induced toxicity after a stereotactic injection above SNpc. In conclusion, our results demonstrate that already a modest increase in the natural GDNF expression functionally enhances and protects the nigrostriatal DAergic system with persistent changes until old age.

P156. Lateral habenula neurons encoding aversion

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An aversive event affects the activity of dopamine (DA) neurons in the midbrain. However, the neuronal mechanisms that underlie this effect are still poorly investigated. Convergent anatomical and functional evidence show a pivotal role of Lateral Habenula (LHb) in regulating midbrain DA activity. Moreover, in vivo single unit electrophysiological studies indicate an inverse relationship between LHb and DA neurons in response to a single unpredicted aversive stimulus. LHb neurons encode these events by phasically increasing their firing rate (Matsumoto and Hikosaka 2007, 2009). Interestingly, the activation of LHb terminals onto midbrain neurons induces avoidance behavior in mice (Stamatakis and Stuber 2012, Lammel et al 2012). Despite these data little is known about the cellular changes that occur after a single session to an aversive event. Here, we asked whether the properties of LHb neurons are affected early after an unpredicted aversive experience. We take advantage of a behavioral paradigm that elicits aversion, the inescapable footshock paradigm and electrophysiological ex-vivo recordings in mice. Patch-clamp recordings were performed in LHb neurons from sagittal slices one hour after the end of the behavioral procedure. We characterized the spontaneous firing activity in LHb neurons in cell-attached mode in control vs footshock exposed mice. We find that footshock exposure increases the frequency of spontaneous action potential and the number of LHb neurons spontaneously active. These data indicate that an aversive experience drives an increased excitability onto LHb neurons, providing a cellular mechanism for the encoding of aversive stimuli in LHb.

P157. Transcription factors Lmx1a and Lmx1b cooperatively regulate axon guidance of midbrain dopamine neurons.

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Degeneration of midbrain dopaminergic neurons (mDA) is the principal cause of Parkinson's disease. Graft of dopaminergic neurons newly generated from stem cells represent a promising therapeutic avenue. However, a major factor limiting success in transplantation studies is the inappropriate re-innervation of the grafted neurons. It is thus primordial to identify factors regulating axon projection and connectivity of mDA neurons. We recently discovered that transcription factors Lmx1a and Lmx1b were essential for mDA progenitor specification, proliferation and differentiation. In this new study we investigate the role of Lmx1a and Lmx1b in postmitotic mDA neurons. Analysis of dopaminergic axon projections of Lmx1a/b double conditional mutant mice reveals a striking axon guidance defect and confirms the essential role of Lmx1a/b in the establishment of dopaminergic circuit formation. We also identified target genes regulated by these transcription factors and assess how these genes control appropriate mDA neurons connectivity. At the light of our data, we propose a novel model to explain the segregation of the nigrostriatal and mesolimbic pathways.

P158. Acetaldehyde modulates dendritic spines in the Nucleus Accumbens after chronic treatment.

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Acetaldehyde (ACD), the first metabolite of ethanol (EtOH), appears to be involved in many EtOH psychoactive effects including activation of VTA dopamine (DA) neurons (Foddai et al., 2004; Melis et al., 2007) and motivational properties (Peana et al., 2008; 2010). The aim of this study was to investigate possible ACD-induced changes in dendritic spines of medium spiny neurons (MSN) of the Nucleus Accumbens shell (Naccs). ACD was chronically administered to rats in a modified liquid diet for a total of 21 days. Rats were divided into two groups: 1) liquid diet without ACD; 2) liquid diet with ACD (0,15 %). Rats belonging to group 2 were further divided into 2 subgroups: a) sacrificed, without ACD suspension; b) sacrificed 12 hours after ACD suspension. Subjects were then prepared for histology, utilizing a new method to visualize in the same slice spine's morphology, TH-positive fibers and PSD-95 positive. Confocal analysis reveals a loss of dendritic spines in MSN (37%), accompanied by a reduction of TH-positive terminals (73 %) and PSD-95 positive elements (68,5%). Further analysis indicates that mature spines as long-thin are selectively affected. These changes occur only in the group b. The reduction of TH-positive terminals, PSD-95 and long-thin spines suggests a profound architectural remodeling of the accumbal synaptic triad. These results indicate functional consequences of these structural modifications and provide further evidence for an active role of ACD in synaptic plasticity in the Naccs. Key Words: Acetaldehyde, Dendritic spines, Accumbens, Dopamine.

P159. Toward a calcium-based account of dopamine-dependent STDP

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Spike-timing dependent plasticity (STDP) has provided an attractive framework for experimental and theoretical accounts of how pre- and post-synaptic neuronal spike timing influence synaptic plasticity. However, the generality of the STDP construct has been questioned, and Shouval et al. have offered more parsimonious accounts based on intracellular calcium dynamics (Shouval et al. 2002 PNAS; Shouval et al. 2010 Front Comput Neurosci). Additionally, evidence for the influence of neuromodulators on synaptic plasticity dates back to some of the earliest experimental demonstrations of LTP and LTD. A rapidly expanding body of experimental evidence suggests myriad ways in which the tripartite factors of pre- synaptic activity, post-synaptic activity, and dopaminergic signaling combine to produce a complex function for synaptic plasticity rules. Yet several other factors also influence the net changes in synaptic strength, such as the class of dopamine receptors (D1- versus D2-type), experimental conditions (e.g. whether inhibition is blocked), and the specific synapse (different classes of pre- and post-synaptic neurons). We have sought to determine whether a simple computational model of calcium-dependent long term synaptic plasticity rules, analogous to Shouval's earlier work, can be extended to incorporate the influence of dopamine. Our preliminary results for synapses in the striatum suggest that we can accommodate the differential influences of dopamine on the D1- and D2-type receptor expressing neurons in a variety of commonly used experimental conditions. The results suggest that a calcium-based plasticity rule may provide a more parsimonious account of dopamine-dependentsynaptic plasticity.

P160. Structural plasticity changes in the shell of the Nucleus Accumbens of ethanol dependent rats.

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Neuronal refinement and stabilization are hypothesized to confer resilience to poor decision-making and addictive-like behaviors, such as excessive ethanol drinking and dependence. Accordingly, structural abnormalities are likely to contribute to the appearance of alcohol withdrawal signs and symptoms that occur from suddenly ceasing the use of alcohol after chronic ingestion. Here we show that ethanol dependent rats display a loss of dendritic spines in medium spiny neurons of the Nacc, accompanied by a reduction of TH-positive terminals and PSD-95 positive elements. Further analysis indicates that 'long thin', but not 'mushroom', spines are affected. These changes are restricted to the withdrawal phase of ethanol dependence suggesting their relevance to the genesis of signs and/or symptoms affecting specifically ethanol withdrawal, and thus the whole addicting cycle. Overall these results highlight the importance of spine function on the evolution of alcohol dependence and suggest that the selective loss of 'long thin' spines may significantly contribute to further 'impoverish' the already deficient dopaminergic transmission whose hypofunctionality is a major factor for the emergence of the harmful consequences of alcohol abuse/dependence.

P161. Dopamine Induces Gap-Junctions-Mediated Anti-Synchronous Action Potential Firing in Striatal Fast-Spiking Interneurons.

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Fast-spiking interneurons (FSIs) play a central role in organizing the output of striatal neural circuits, yet functional interactions between these cells are still largely unknown. Here we investigated the interplay of action potential firing between electrically connected pairs of identified FSIs in mouse striatal slices. In addition to a loose coordination of firing activity mediated by membrane potential coupling, gap junctions induced a fast, repetitive inhibition of postsynaptic action potentials at frequencies >20-30 Hz, resulting in anti-phase firing. Spike silencing occurred even in the absence of GABAergic synapses or persisted after complete block of GABAA receptors. Bath application of dopamine (100uM) induced supra-threshold depolarization and anti-correlated burst firing in FSI pairs. The complex pattern of functional coordination mediated by gap junctions endows FSIs with peculiar dynamic properties that may be critical in controlling striatal-dependent behavior.

P162. Endocannabinoid / dopamine interact to mediate bidirectional spike-timing dependent potentiation and depression

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The extended capabilities of the brain for learning and memory admittedly rely mainly on synaptic plasticity. Yet, our knowledge of synaptic plasticity often depends on dedicated experimental protocols that imply a high number (hundreds) of stimulations to induce plasticity. However, it is still unknown if small numbers of paired stimulations can trigger spike-timing-dependent plasticity (STDP). Here, we investigated the frontiers of STDP and provide evidence that few (even 5 to 10) stimulations can trigger reliable and robust LTP at corticostriatal synapses. This LTP is NMDA receptor independent but endocannabinoid-mediated through activation of the type-1 cannabinoid receptor (CB1R) and the transient receptor potential vanilloid type-1 (TRPV1). In contrast to the widespread belief that endocannabinoids are only able to depress synaptic transmission, our data show that they in fact can also potentiate it. In addition, our study provides an exhaustive mapping of the molecular signaling pathways involved in this process using a sound combination of experimental and computational modeling approaches. We show it depends on the activation of metabotropic glutamate receptor type-5, dopaminergic receptors, voltage-sensitive calcium channels, phospholipase C, diacylglycerol lipase, CB1R, TRPV1 and presynaptic PKA. Finally, we demonstrate that this endocannabinoid-mediated LTP is a widespread process that we observed in the cortex and in the striatum, and occurs in juvenile adult rodents. Our results considerably enlarge the spectrum of action of endocannabinoids as (i) promoting not only depression but also potentiation, i.e. acting as a genuine bidirectional system and (ii) supporting STDP at low numbers of paired stimulation.

P163. Calcium-impermeable, GluN3A-containing NMDARs in cocaine evoked synaptic plasticity

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Cocaine alters excitatory transmission onto synapses of dopamine (DA) neurons in the ventral tegmental area (VTA). Recently we have shown that the increase in AMPA/NMDA ratio observed 24 hours after a single cocaine injection is accompanied with decreased function of NMDARs. To assess the contribution of NMDAR subunit composition changes on the induction and expression of cocaine-evoked plasticity in the VTA we performed electrophysiological recording on DA neurons in saline and cocaine treated mice one day after a single injection. Dendritic calcium imaging revealed the existence of calcium impermeable NMDA receptors at the synapses of cocaine treated mice. This change was associated with reduced magnesium sensitivity of NMDARs, increased ifenprodil sensitivity and increased decay time kinetics of NMDAR-EPSCs. Furthermore all these cocaine-induced changes in NMDAR-transmission were absent in GluN3A knockout mice. These data suggest the incorporation of GluN3A-containing NMDARs together with a decrease in the GluN2A/GluN2B ratio. In addition we found that Group-I mGluR receptor activation re-potentiated the NMDAR mediated transmission and reversed the subunit composition changes. This potentiation relied on intracellular calcium signalling and PKC-dependent NMDA receptor trafficking. All together our data reveal an unexpected role for these receptors in drug-evoked plasticity.

P164. Dopamine 1 Receptor binding to NMDA receptors in striatal neurons is critical for cocaine-induced adaptations

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We recently showed that long-term behavioral responses to cocaine involve a cAMP-independent potentiation of calcium influx through NMDAR by the Dopamine 1 Receptor (D1R) in the striatum. This potentiation is mediated by the D1R-dependent phosphorylation of GluN2B NMDAR subunits and triggers ERK activation by cocaine, which is essential for the establishment of addictive like behavior in rodents. The D1R also directly interacts with the GluN1 subunit of NMDAR and a D1R/NMDAR complex mutually potentiates both receptors' functions. Here, we combine in vitro, ex vivo and in vivo approaches to study the role of the D1R/GluN1 direct interaction in cocaine induced adaptations. In vitro, using Proximity Ligation Assays, we found that the concomitant stimulation of both receptors favors the formation of endogenous D1R/GluN1 complexes. We then designed an interfering cell-penetrating peptide corresponding to the domain of GluN1 that binds to D1R. The uncoupling of D1R and GluN1 does not alter the cAMP/PKA pathway downstream from D1R or glutamate-induced calcium influx through NMDAR. By contrast, when both receptors are co-stimulated, disruption of D1R/GluN1 oligomers blocks D1R-dependent potentiation of calcium influx through GluN2B-NMDAR and subsequent ERK activation. Using electrophysiology in striatal slices from adult mice, we establish that D1R/GluN1 oligomers are crucial for the positive modulation of NMDAR currents by D1R and for long-term potentiation in D1R mediums sized spiny neurons. Finally, intra-striatal delivery of TAT-GluN1-C1 to adult mice alters cocaine-induced locomotor sensitization. Overall, this work highlights a critical role for D1R/GluN1 oligomers in cocaine induced adaptations in the striatum.

P165. Impact of hippocampal theta burst stimulation on VTA dopamine neurons

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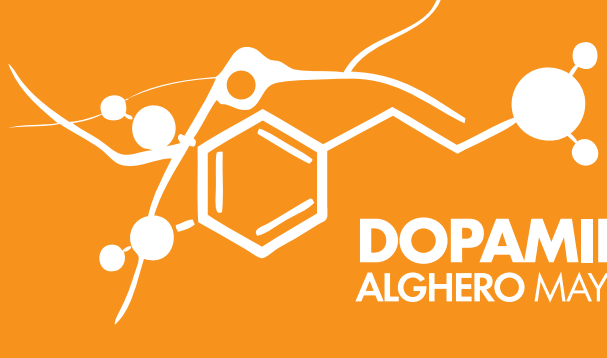
The hippocampus (HPC) is recognized as an important structure in learning and memory, including the integration of relevant informations to modulate the behavior. Theta frequency activity of the HPC has been shown to generate adaptive synaptic changes, like long term potentiation, outside the HPC. Using in vivo extracellular recording techniques in anesthetized rats, we have investigated how the firing of dopaminergic neurons is affected by the theta frequency stimulation of the ventral subiculum of hippocampus (vSub/CA1). This work shows that 24 hours after the induction of a theta burst in vSub/CA1, there is an increase of the activity (firing rate, bursting activity) of DA neurons and there is an increase on the number of DA neurons activated in VTA (cells/track). Moreover, we have previously shown that the BNST stimulation increases VTA dopamine neuron firing rate and bursting activity and that the Bed nucleus of the stria terminalis (BNST) receives excitatory inputs from the vHPC. Here we demonstrated that an intra-BNST infusion of AP5 (a NMDA receptors antagonist) prior the hippocampal tetanus blocks the effect of theta burst stimulation on VTA dopamine neurons bursting parameters. These results contribute to clarify important points concerning the regulation of VTA DA neurons activity by the vSub/CA1, probably through the BNST.

AUTHORS



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