

Società Italiana di Farmacologia CONVEGNO MONOTEMATICO

in collaborazione con il

Gruppo Di Lavoro Sulle Dipendenze Patologiche

Addictive Disorders: from neurobiology to novel therapeutic strategies

Complesso Monumentale dello Steri
Università degli Studi di Palermo
Palermo, 27-28 Marzo 2015

Scientific Committee:

Daniela Parolaro, *University of Insubria*
Carla Cannizzaro, *University of Palermo*
Paola Fadda, *University of Cagliari*
Patrizia Romualdi, *University of Bologna*
Tiziana Rubino, *University of Insubria*
Cristiano Chiamulera, *University of Verona*

Local Organizing Committee:

Carla Cannizzaro
Anna Brancato
Fulvio Plescia
Emanuele Cannizzaro

Program

27.03.2015

- 14:00 – 15:00 **Opening and Congress Presentation – Sala Magna**
In the presence of Prof. Roberto Lagalla, Rector of the University of Palermo
- 15:00 – 17:00 **New targets and new drugs – Sala Magna**
Chair Daniela Parolaro, Insubria; Co-chair Cristiano Chiamulera, Verona
- Martina Palmisano**, Bologna – Innate and ethanol-induced differences in the Extended Amygdala gene expression between Marchigian Sardinian alcohol-preferring (msP) and Wistar rats
- Michela Rosas**, Cagliari – Effects of Withania somnifera Dunal on the motivational properties of ethanol: place conditioning and self-administration studies
- Luisa Lo Iacono**, Fondazione Santa Lucia, Rome – Adverse social experiences in juvenile mice increase cocaine-seeking behavior and alter blood gene expression
- Guendalina Olivero**, Genoa – Nicotinic $\alpha 7$ receptor activation induces NMDA receptors trafficking in glutamatergic terminals of the nucleus accumbens
- Esi Domi**, Camerino – Pioglitazone reduces nicotine withdrawal symptoms through PPAR γ stimulation
- Andrea Ossato**, Ferrara – Effect of acute administration of JWH-018 on cardiovascular functions in mice
- Isabella Canazza**, Ferrara – 2C-B and 251-NBOMe impair sensorimotor functions in mice
- 17:00 – 18:00 **Coffee Break and Poster Session – Sala delle Capriate**
- 18:00 – 19:00 **Honorary Lecture – Sala Magna**
Scott J. Russo, Icahn School of Medicine at Mount Sinai, New York
Motivational Neurocircuitry in Stress and Aggression
- 19:00 – 20:00 **Special event**
Complesso Monumentale dello Steri – Guided tour
- 20:30 **Social Dinner**

28.03.2015

08:30 – 10:00 **Effects of early exposure to addictive drugs** – *Sala delle Capriate*

Chair Patrizia Romualdi, Bologna; Co-chair Marco Diana, Sassari

Anna Brancato, Palermo – Drinking pattern matters: effects on maternal care and offspring vulnerability to alcohol in rats

Lucia Caffino, Milan – Repeated cocaine treatment during adolescence reduces spine density in the medial prefrontal cortex: a potential glucocorticoid receptor-mediated mechanism

Giuseppe Giannotti, Milan – Adolescent exposure to cocaine modulates BDNF in the medial prefrontal cortex of adult rats

Pamela Prini, Insubria – Role of Histone H3 Lysine 9 Try-Methylation in the development of the depressive/psychotic-like phenotype induced by adolescent THC exposure in rats

Erica Zamberletti, Insubria – Cortical neuroinflammation contributes to the development of cognitive deficits induced by adolescent THC exposure in rats

10:00 – 10:30 **Coffee Break**

10:30 – 11:30 Minisymposium – **The use of animals in research** – *Sala delle Capriate*

Chair Paola Fadda, Cagliari; Co-chair Liana Fattore, CNR, Cagliari

Gaetano Di Chiara, Cagliari

Alessandra Giarola, GSK, UK

Fabrizio Di Pietra, Palermo

11:30 – 12:30 **From the bench to the bedside** – *Sala delle Capriate*

Chair Tiziana Rubino, Insubria; Co-chair Walter Fratta, Cagliari

Elisabetta Gerace, Florence – Studies on the toxic and neuroprotective effects of cannabinoids in models of cerebral ischemia and ischemic tolerance

Federico Moro, Mario Negri Institute, Milan – Effects of single and repeated N-acetylcysteine on cue-induced nicotine-seeking behavior

Roberto Collu, Cagliari – Possible role of the Endocannabinoid System in the activity-based model of anorexia nervosa

Arianna Totti, Florence – Efficacy of anti-craving pharmacological therapy based on a psychobiological model in alcohol dependence

12:30 – 13:00 **Zardi Gori Foundation Awards**– *Sala delle Capriate*

Oral Presentation Award Committee: Patrizia Romualdi, Bologna, and Marco Pistis, Cagliari

Poster Presentation Award Committee: Miriam Melis, Cagliari, and Matteo Marti, Ferrara

Concluding Remarks

Carla Cannizzaro, Daniela Parolaro, Paola Fadda, Patrizia Romualdi, Tiziana Rubino, Cristiano Chiamulera

Poster presentations

- Acquas E**, Rosas M, Spina L, Peana A, Carboni E, Caboni PG, Melis M - Role of ethanol metabolites in its central effects
- Antinori S**, Fattore L, Fratta W, Gessa GL, Devoto P – L-DOPA inhibits cue induced reinstatement of cocaine-seeking behavior without altering cocaine-intake in rats
- Aroni S**, Sagheddu C, Pistis M, Muntoni AL – Cannabinoid withdrawal-induced hypodopaminergia: a role for rostromedial tegmental nucleus and lateral habenula?
- Bolloni C**, Piccoli T, Pedetti M, Frascella AG, Cannizzaro C, Diana M - Transcranial magnetic stimulation in cocaine addiction: preliminary findings
- Brancato A**, Vita C, Plescia F, Cannizzaro E, Morena M, Cavallaro A, Blanda G, Cannizzaro C - Environmental enrichment reverts the effects of continuous or intermittent perinatal alcohol exposure. Focus on alcohol vulnerability and affectivity in the offspring
- Caputi FF**, Carboni L, Candeletti S, Romualdi P – The Ubiquitin-26S Proteasome System as a common target for cocaine and ethanol exposure
- De Felice M**, Melis M, Cadoni C – Genetic and environmental factors influencing spontaneous activity of dopamine neurons in Lewis and Fisher rats
- Di Chio M**, Cavallieri L, Venniro M, Padovani L, Collo G, Chiamulera C – Acute effect of Ketamine on mTOR downstream: p70S6K and rpS6 expression in rat brain area related to depression and drug addiction
- Gambino G**, Rizzo V, Ferraro G, Schiera G, Cannizzaro C, Lentini E, Giammalva R, Sardo P, Carletti F - Cannabinoid-mediated modulation of hippocampal hyperexcitability: focus on the interplay with nitrenergic system in different rat models of temporal lobe epilepsy.
- Malta G**, Argo A, Brancato A, Roda G, Casagni E, Gambaro V, Procaccianti P, Cannizzaro C - Behavioural and pharmacological characterization of a novel cannabimimetic adamantane-derived indole, APICA, in C57BL/6J mice
- Malta G**, Argo A, Brancato A, Procaccianti P, Steven B. Karch, Cannizzaro C - The medical use of marijuana: use, abuse, prejudice, legal implication. A comparison between International and Italian laws
- Mameli A**, Scherma M, Fattore L, Fratta W, Fadda P – Chronic administration with nandrolone decanoate attenuates cocaine-induced conditioned place preference in rats
- Maniaci G**, Picone F, Dimarco T, Brancato A, Cannizzaro C - Alterations in the emotional regulation process in gambling addiction: the role of anger and alexithymia
- Padolecchia C**, Olivero G, Chen J, Pittaluga A, Grilli M, Marchi M – Receptor-receptors functional cross-talk: new evidences on nicotine-NMDA receptor interaction
- Piva A**, Caffino L, Di Chio M, Giannotti G, Venniro M, Mutti A, Cheung D, Yew DT, Fumagalli F, Chiamulera C – Different modulation of neuroplastic markers in the rat brain by acute or chronic ketamine self-administration
- Ponzoni L**, Braida D, Moretti M, Fasoli F, Canazza G, Zoli G, Gotti C, Sala M – Withdrawal from electronic cigarette vapour exposure elicits short and long term behavioural effects in mice
- Scuppa G**, Cippitelli A, Kallupi M, Domi E, Ciccocioppo R – Varenicline decreases nicotine self-administration, but not alcohol consumption in msP rats with concomitant access to both drugs
- Sutera FM**, Giunta Cardinale V, Brancato A, Vita C, Plescia F, Giannola LI, De Caro V, Cannizzaro C - DA-Phen as a new potential DA-mimetic agent for treatment of alcohol addiction: preclinical *in vivo* studies
- Zandonai T**, Diana M, Chiamulera C – A placebo controlled study on effects of smokeless tobacco (snus) administration on exercise endurance and on cognitive task in non-smoker men

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Role of ethanol metabolites in its central effects

Acquas Elio¹, Michela Rosas¹, Liliana Spina¹, Alessandra Peana², Ezio Carboni³, Pierluigi Caboni¹ and Miriam Melis³

¹Department of Life and Environmental Sciences, University of Cagliari ²Department of Chemistry and Pharmacy, University of Sassari

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The role of acetaldehyde, the first metabolite of ethanol, in the effects of its parent compound has gone a profound re-evaluation in the last five decades since the description made in the early 50's that patients treated with the aldehyde dehydrogenase inhibitor, antabuse, could perceive more pleasurable effects when drinking small volumes of alcoholic beverages. However, the suggestion that acetaldehyde could be a mediator of some of the effects of ethanol did never defeat the scepticism of the scientific community on the "acetaldehyde hypothesis" until the discovery of catalase, the enzyme that converts ethanol into acetaldehyde within the brain. Since then it has been demonstrated that either ethanol-derived acetaldehyde and acetaldehyde, on its own, share some critical behavioural and neurochemical features of its parent compound.

In this scenario the aim of our studies was to further investigate the role of acetaldehyde and of the acetaldehyde-dopamine (DA) conjugate, salsolinol, in the effects of ethanol by assessing 1) the ability of acetaldehyde to elicit Extracellular Signal regulated Kinases (pERK) in nuclei of the extended amygdala by immunohistochemistry experiments; 2) the role of pERK as well as of DA and opioid receptors in the motivational effects of acetaldehyde as determined by conditioned place preference (CPP) and self-administration (SA) experiments; 3) the role of acetaldehyde, catalase and salsolinol in the ability of ethanol to excite dopamine neurons in the posterior ventral tegmental area (pVTA) by *ex vivo* electrophysiology.

The results of these studies demonstrate that (1) in the immunohistochemistry experiments acetaldehyde (10 and 20 mg/kg), similarly to ethanol (1 g/kg), elicits pERK in the nucleus accumbens and other nuclei of the extended amygdala in a DA D₁-dependent manner. In addition, the effects of ethanol on pERK expression are prevented by blockade of peripheral metabolism of ethanol with 4-Methylpyrazole (90 mg/kg) and by acetaldehyde sequestration with D-penicillamine (50mg/kg). The results also show (2) that acetaldehyde (20 mg/kg) elicits CPP and this is prevented by either blockade of DA D₁ receptors and of Mitogen-activating Extracellular Kinase (MEK), the kinase responsible of ERK phosphorylation. In addition, the SA experiments confirmed that acetaldehyde (0.2%) is self-administered orally and that, similarly to ethanol oral SA, also acetaldehyde SA is subjected to an opioid receptor mediated control since naltrexone (0.4-0.8 mg/kg) prevents its acquisition, maintenance and deprivation effect. Finally, (3) the electrophysiological experiments brought to the demonstration that in order to excite DA neurons in the pVTA it is necessary that a two-step sequential mechanism takes place: a) conversion of ethanol into acetaldehyde by the action of catalase and b) condensation of acetaldehyde with DA to produce salsolinol. Overall these results provide evidence in further support of the view that the metabolism of ethanol plays a key role in some of its central effects and strongly suggest that this holds true also for its ability to excite DA neurons.

L-DOPA inhibits cue-induced reinstatement of cocaine-seeking behavior without altering cocaine-intake in rats

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We have recently shown that systemic administration of the dopamine-beta-hydroxylase inhibitors disulfiram and nopicastat suppresses cocaine-induced reinstatement of cocaine-seeking behavior in rats, and contextually increases cocaine-induced dopamine release in the medial prefrontal cortex (mPFC). The behavioral effects are mediated by a supra-maximal stimulation of dopamine D1 receptors that leads to their functional inactivation. Similarly, L-DOPA administration was demonstrated to suppress cocaine-induced reinstatement (Devoto et al., 2014).

In this follow-up study we verified whether the suppressant effect of L-DOPA on cue-induced reinstatement of cocaine-seeking behavior was as effective as on drug-induced reinstatement. To this aim, male rats were trained to daily self-administer cocaine (0.5 mg/kg/infusion) intravenously for 2 hours under a fixed ratio 1 schedule of reinforcement, each cocaine infusion being associated with visual (light) and auditory (noise) cues. Stimuli associated with drug intake play a key role in relapse to drug-seeking in humans. Thus, after a prolonged extinction training, animals underwent cue-induced reinstatement test sessions. Acute exposure to a cue priming immediately before starting the session promptly reinstated drug-seeking behavior to the pre-extinction responding level. This effect was efficaciously reversed by pretreatment with L-DOPA at the same dose (50 mg/kg) previously shown to be effective in preventing cocaine-induced reinstatement.

Extended access to self-administered cocaine may produce a number of symptoms characteristic of addiction that are not seen following more limited drug access, including persistent cognitive deficits (Paterson and Markou, 2003). Thus, the next step of our study was to allow animals to resume responding for cocaine during extended (6 hours) daily sessions for 1 week. As expected, cocaine self-administration was promptly reacquired and the mean number of active lever-presses/hour was roughly similar between the 2- and the 6-hours self-administration sessions (14.84 and 10.73). Once cocaine self-administration behaviour was stably re-acquired, rats were pretreated with L-DOPA either on the last day of cocaine self-administration training or during cue-induced reinstatement of drug-seeking behavior. Results showed that L-DOPA did not affect cocaine intake but was able to prevent cocaine-seeking reinstatement induced by re-exposure to an acute cue priming.

Overall, our findings showed that even though L-DOPA does not affect voluntarily cocaine intake in rats, it may be useful in the suppression of relapse to cocaine seeking since it is able to prevent both drug- and cue-induced reinstatement of cocaine-seeking.

References

Devoto P, Fattore L, Antinori S, Saba P, Frau R, Fratta W, Gessa GL (2014). Elevated dopamine in the medial prefrontal cortex suppresses cocaine seeking via D1 receptor overstimulation. *Addiction biology* [Epub ahead of print].

Paterson NE, Markou A (2003). Increased motivation for self-administered cocaine after escalated cocaine intake. *Neuroreport* 14(17):2229-32.

Cannabinoid withdrawal-induced hypodopaminergia: a role for rostromedial tegmental nucleus and lateral habenula?

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The hypoactivity of the mesolimbic dopamine (DA) system arising from the ventral tegmental area (VTA) has been implicated in the aversive consequences of withdrawal from several drugs of abuse including Cannabis [Diana et al., 1998]. These neuronal adaptations, which are characterized by imbalances between excitatory and inhibitory afferents onto DA cells, are thought to underlay the withdrawal-induced negative affective states that eventually lead to relapse into drug taking. The rostromedial tegmental nucleus (RMTg), a GABA structure which receives a strong input from the lateral habenula (LHb) and projects densely to midbrain DA neurons, is an important site involved in aversion and appetitive processes. In fact, both RMTg and LHb cells are activated by aversive/unpleasant events and inhibited by rewarding/positive stimuli. RMTg GABA neurons exert a negative control over the VTA, express CB1 receptors on their axon terminals impinging upon VTA DA neurons [Melis et al., 2014], and are a target for cannabinoid action on DA cells. Indeed, acute exposure to these drugs reduces RMTg neuron discharge activity and strongly suppresses the inhibition exerted by RMTg afferents, thus contributing to cannabinoid-induced DA neuronal excitation [Lecca et al., 2011; Lecca et al., 2012].

On these bases, we hypothesized that RMTg GABA projections to VTA might be causally involved in the hypodopaminergic state, which is one hallmark of cannabinoid withdrawal.

To address this issue, we used single unit extracellular recordings from RMTg and VTA neurons in anesthetized male Sprague-Dawley rats. To induce Δ^9 -tetrahydrocannabinol (Δ^9 -THC) dependence, rats were chronically treated with it (15 mg/kg, i.p.), or its vehicle, twice daily for 6.5 days [Diana et al., 1998]. Administration of the cannabinoid antagonist rimonabant (5 mg/kg, i.p.) precipitated an intense behavioral withdrawal syndrome ($p < 0.0001$ versus control (one-way ANOVA and Dunnett's test), whereas abrupt Δ^9 -THC suspension caused milder signs of abstinence ($p < 0.01$ versus control (one-way ANOVA and Dunnett's test)). Electrophysiological experiments confirmed that Δ^9 -THC withdrawal produced a marked decrease in the firing rate and burst firing of VTA DA neurons ($p < 0.01$ versus control (one-way ANOVA and Dunnett's test)). As expected, RMTg stimulation elicited a complete suppression of DA neuron discharge activity. Remarkably, in Δ^9 -THC withdrawn rats the duration of RMTg-evoked inhibition was prolonged when compared with controls, suggesting an augmented GABA inhibitory input onto DA cells. On the other hand, the spontaneous activity of RMTg GABA neurons was reduced in cannabinoid-withdrawn rats. Given the anatomical and functional role of the LHb, we are currently investigating whether LHb neuronal firing is altered after cannabinoid withdrawal.

Our findings support the idea that enhanced GABA output from RMTg might contribute to the hypodopaminergia induced by cannabinoid withdrawal, and confirm that the RMTg takes part in the neuronal circuits underlying drug dependence and addiction.

Transcranial magnetic stimulation in cocaine addiction: preliminary findings

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Cocaine-related disorders are currently among the most devastating mental disease as they leads to profound disturbances in an individual's behaviour resulting in tremendous economic, social, and moral costs. Imaging studies in human have shown a reduction of dopamine (DA) receptors accompanied by a lesser release of endogenous DA in the ventral striatum (AVT) of cocaine subjects thereby resulting in a 'dopamine-impooverished' brain¹⁻². This perturbations lead to neuroadaptations in several other circuits which are related to motivation, inhibitory control, and memory which finally determ compulsive-impulsive self drug administration³. The lasting reduction in physiological activity of the DA system leads to the idea that an increment on its activity, to restore pre-drug levels, may yield significant clinical improvements². There is a substantial need for therapeutic tools in addictive states and TMS appears to be a promising 'non-pharmacologic' candidate, since it can modulate the DA system and the function of related areas⁴. It has been just reported⁵ that rTMS over the left dorso-lateral prefrontal cortices (DLPfcx) temporarily reduces the craving for cocaine in cocaine addicts but there are no studies which have investigated the TMS effects in the cocaine intake. Considering that we applied bilateral deep-rTMS to the DLPfcx of cocaine abusers, in order (1) to evaluate the short/long term therapeutic effects of TMS in cocaine intake trough hair analysis during the time line ($T_0, T_1, T_2, T_3, \dots$), (2) to identify optimal parameters of stimulation (1HZ/10HZ), (3) to highlight possible correlation between cocaine intake and clinical parameters.

20 cocaine abusers have been recruited selected on the DSM-IV criteria and randomly assigned to real rTMS group (100% of motor threshold, 10 HZ/1 HZ, 5 second per train, 20 trains, 15 seconds of inter-stimulus) or to sham stimulation one. In order to investigate the effect of TMS on cocaine dependence we assessed the cocaine intake trough hair analysis before (T_0) and after treatment (T_1) and every 3 months for the follow-up data. The interim analysis shows that all subjects have reduced the intake of cocaine regardless of the frequency (10 Hz or sham condition) of the stimulation protocol applied. Six months after the treatment (T_2) all treated subjects show a reduction in cocaine intake with no distinction among groups (real vs sham). More cases are needed in 1 Hz and sham conditions to "balance" the groups. The follow-up data, however, shows a strong persistence of the effect in the real group, and decidedly weaker maintenance in sham. We hypothesize an initial placebo/sham effect which disappears over time in the sham patients group. More cases are needed in 1 hz and sham condition to balance the groups. Nevertheless these preliminary data encourage further investigation to evaluate the potential effects of dTMS in the treatment of cocaine abusers and in the prevention of relapses.

References

¹ Volkow et al., 2010 Bioessays 32(9):748-755; ² Diana, 2011 Front Psychi 2:64; ³ Volkow et al., 2004 Neurophar 47:3-13; ⁴ Feil et al., 2010 Neurosci&Biobe 35:248-275; ⁵ Politi et al., 2008 Am J on Add, Vol.17(4): 345-346

Environmental enrichment reverts the effects of continuous or intermittent perinatal alcohol exposure. Focus on alcohol vulnerability and affectivity in the offspring

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Alcohol consumption during perinatal periods is common, despite the warning of adverse effects on the foetal development. In female rats, the intermittent pattern of alcohol consumption is responsible for higher drinking levels and more profound disruption of maternal care than traditional continuous free-access paradigm, which can have persistent effects on the offspring. The environmental enrichment, a powerful form of experience-dependent plasticity that allows high cognitive, motor and sensory stimulations, is helpful for recovering from different neurological pathologies.

Thus, this study aimed at exploring the effects of environmental enrichment on alcohol vulnerability and affective behaviour in the offspring that was perinatally exposed to continuous or intermittent alcohol. Dams were given two-bottle choice to water and 20% alcohol with either continuous- or intermittent access (CA vs IA), along a 12-week period. They were alcohol-deprived during breeding and resumed alcohol self-administration from late gestation and throughout lactation. Alcohol-exposed offspring, reared in either standard- or enriched- conditions, were assessed for alcohol drinking behaviour in a free-choice paradigm and were also tested for the deprivation effect. Moreover, they were tested for behavioural reactivity in the open field; anxiety-like behaviour in the elevated plus maze and depressive-like behaviour in the forced swim test during the drinking paradigm.

Our results show that perinatal CA to alcohol did not increase alcohol-drinking behaviour with respect to controls. On the other hand, rats perinatally exposed to IA displayed a high vulnerability to alcohol, in terms of drinking behaviour and deprivation effect. The environmental enrichment was able to exert a protective role on alcohol vulnerability in perinatally IA exposed rats and controls, especially during relapse. Moreover, it was able to induce an increase in behavioural reactivity in the open field and a decrease in anxiety-like behaviour in the elevated plus maze, in both perinatally CA and IA exposed groups.

In conclusion, the pattern of alcohol consumption during pregnancy and lactation can influence long-term alcohol vulnerability in the offspring. Rearing conditions that promote high cognitive, motor and sensory stimulations improve resilience to alcohol abuse and affective tone, although they cannot be sufficient to full recovery from detrimental effects of perinatal alcohol exposure.

Drinking pattern matters: effects on maternal care and offspring vulnerability to alcohol in rats

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Alcohol drinking during pregnancy and post-partum period is a major concern because of the persistent neurobehavioral deficits in the offspring, which include increased vulnerability to substance abuse (1). The intermittent pattern of alcohol consumption induces higher drinking levels and deeper neurobiological changes in addiction-related brain regions, with respect to traditional free-access paradigms in male rats (2, 3). Nevertheless, no studies investigated on the effects of the drinking pattern on female subjects during pregnancy and perinatal time.

To this aim, this study explored the consequences of continuous vs. intermittent drinking pattern on maternal behaviour and on offspring vulnerability to alcohol, during adulthood.

Dams were given two-bottle choice to water and 20% alcohol with either continuous- or intermittent access (CA vs IA), along a 12-week period. They suspended alcohol drinking during breeding and resumed alcohol self-administration from late gestation throughout lactation, when they were assessed for home-cage undisturbed maternal behaviour. In the adulthood, alcohol-exposed offspring were assessed for alcohol drinking behaviour in a free-choice paradigm and tested for the deprivation effect.

Our results show that alcohol consumption and preference significantly decreased in IA group during pregnancy, returning to baseline during lactation. Alcohol drinking was able to disrupt spontaneous maternal behaviour, especially in IA exposed dams. On the other hand, perinatal CA exposure did not increase alcohol-drinking behaviour in the offspring with respect to controls, while rats perinatally exposed to IA displayed a high vulnerability to alcohol, in terms of drinking behaviour and deprivation effect.

In conclusion, this study indicates for the first time that the pattern of alcohol consumption can be responsible for different extents of maternal behaviour disruption and detrimental consequences in the offspring. Therefore gender- but also pattern-related differences should be taken into account for contrasting alcohol abuse and dependence, especially during perinatal time.

1. McMurray MS, Williams SK, Jarrett TM, Cox ET, Fay EE, Overstreet DH, Walker CH, Johns JM. *Neurotoxicol Teratol.* 2008;30(6):475-86.

2. Stuber GD, Hopf FW, Hahn J, Cho SL, Guillory A, Bonci A. *Alcohol Clin Exp Res.* 2008 Oct;32(10):1714-20

3. George O, Sanders C, Freiling J, Grigoryan E, Vu S, Allen CD, Crawford E, Mandyam CD, Koob GF. *Proc Natl Acad Sci U S A.* 2012;109(44):18156-61

Repeated cocaine treatment during adolescence reduces spine density in the medial prefrontal cortex: a potential glucocorticoid receptor-mediated mechanism.

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Objectives: Abstinence from drugs of abuse may cause an aversive emotional state that drives the negative reinforcement of addiction, presumably deriving from dysregulated neurochemical elements in the brain reward and stress systems. We decided to investigate the molecular mechanisms underlying short- and long-term withdrawal after prolonged exposure to cocaine during adolescence.

Methods: We exposed adolescent male rats to cocaine (20mg/kg/day subcutaneously) from post-natal day (PND) 28 to PND 42 and sacrificed them at PND 45 or 90. We focused our molecular analyses, using Real Time PCR and Western blot techniques as well as morphological analyses on dendritic spines, using a fluorescent dyolistic labeling technique, on the medial prefrontal cortex (mPFC), a brain region that is still developing during adolescence.

Results: We found reduced expression of some crucial effectors of the signaling pathways that regulate spine actin network, an effect associated with a reduction in cortical dendritic spine density and an enhanced formation of filopodia in cocaine-exposed animals sacrificed at PND45. Given that dendritic spines are known to be altered by stress exposure, we hypothesized that developmental exposure to cocaine may have altered the mechanisms involved in stress response. To this end, we analyzed the glucocorticoid receptor (GR) system and found increased GR transcription and translation as well as increased nuclear translocation of GR, associated with the reduced expression of the GR co-chaperone FKBP5 that, under physiological conditions, keeps the receptor in the cytoplasm, in the mPFC of cocaine-exposed PND 45 animals.

Conclusion: Based on our results, we speculate that short-term abstinence from adolescent exposure to cocaine alters stress-related mechanisms leading to changes in dendritic spine morphology, an effect that may contribute to the depressive-like behavior observed in early cocaine withdrawal. These findings may have implications for elucidating the role of drug abuse in the pathophysiology of stress-related disorders.

2C-B and 25I-NBOMe impair sensorimotor functions in mice

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2C-B and 25I-NBOMe are phenethylamines used for their hallucinogenic properties and easily obtainable over the Internet. In particular, 2C-B (2-(4-bromo-2,5-dimethoxyphenyl)ethanamine) is a well known compound [1] since it was synthesized in the '70s by Alexander Shulgin [2] while 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine) is a novel phenethylamine that has recently caused several cases of toxicity in Europe [3,4]. In particular, 25I-NBOMe is a potent agonist at the human 5-HT_{2A} receptors with a high hallucinogen potential similar to that of LSD [4]. In fact, agonist activation of 5-HT_{2A} receptors in the cortex is believed to be responsible for remarkable psychopharmacological effects exerted by hallucinogenic phenethylamines such as lysergic acid diethylamide (LSD) and psilocybin [5]. The hallucinatory effect induced by phenethylamines causes a profound alteration in the ability to perceive visual, acoustic and tactile inputs in human. In this study, we investigated the effect of 2C-B and 25I-NBOMe administration on sensorimotor functions and motor coordination in CD-1 male mice. Effects induced by 2C-B and 25I-NBOMe administration were investigated using a battery of behavioral tests widely used in studies of "safety-pharmacology" for the preclinical characterization of novel molecules [6,7]. In particular, we evaluated the effect of compounds on visual, acoustic, tactile (pinna, vibrissae and corneal reflex) sensorimotor responses and stimulated motor activity on the accelerated apparatus. The effects were monitored up to 300 minutes after drug administration. 2C-B at low doses (0.01-0.1 mg/Kg i.p.) impaired visual and acoustic responses in mice while at high doses (1-10 mg/Kg i.p.) it lost these effects. Whereas 25I-NBOMe (0.0001-10 mg/Kg i.p.) inhibited visual and acoustic responses up to 10 mg/Kg and the effects extend beyond 5 hours of observation. In particular, 25I-NBOMe inhibited more readily and with greater effectiveness than 2C-B auditory responses in mice. 25I-NBOMe (0.0001-10 mg/Kg i.p.) was ineffective in altering sensorimotor responses to tactile stimulation of pinna, vibrissae and cornea. While 2C-B (1-10 mg/Kg i.p.) transiently reduced only pinna responses. Both phenethylamines did not affect stimulated locomotion on the accelerated. For the first time these data demonstrate that 2C-B and 25I-NBOMe deeply impair visual and acoustic sensorimotor responses in mice. This aspect should be carefully evaluated to better understand the potential danger that phenethylamines may cause to health, with particular reference to performance in driving and hazardous works.

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[1] EMCDDA 2013, EDND database, 2C-B.

[2] Shulgin A, Shulgin A.PIHKAL: A Chemical Love Story. Transform Press; Berkeley, CA:1991.

[3] Rose SR, Poklis JL, Poklis A. (2013) A case of 25I-NBOMe (25-I) intoxication: a new potent 5-HT_{2A} agonist designer drug. *Clin Toxicol (Phila)*. 51(3): 174-177.

[4] EMCDDA 2013.-Europol Joint Report on a new psychoactive substance: 25I-NBOMe. <http://www.emcdda.europa.eu/publications/joint-report/25I-NBOMe>

[5] González-Maeso J, Weisstaub NV, Zhou M, et al., (2007) Hallucinogens recruits specific cortical 5-HT_{2A} receptor-mediated signaling pathways to affect behavior. *Neuron*. 53:439-452.

[6] Irwin S. (1968) *Psychopharmacology* 13: 222-257.

[7] S7A, Safety pharmacology studies for human pharmaceuticals, 2000.

The Ubiquitin-26S Proteasome System as a common target for cocaine and ethanol exposure

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The synaptic proteins proteolysis carried out by the Ubiquitin-26S Proteasome System (UPS) is recognized as a critical component in neural plasticity [1]. The UPS-mediated degradation system plays a crucial role in synaptic remodeling and in memory reconsolidation mechanisms after cocaine exposure [2]. Moreover, the UPS-complex is involved in ethanol-induced dysfunctions by changing the turnover of transcriptional factors and affecting epigenetic mechanisms [3]. Therefore, the UPS-complex could be a common target for different drugs of abuse.

This study aimed to evaluate the proteolytic proteasome activity and the gene expression regulation of specific proteasome subunits following exposure to cocaine or ethanol. To this purpose, neuroblastoma SHSY-5Y cells were treated with 5 μ M cocaine or 40mM ethanol at different time points: 2h, 24h and 48h. First of all, an opposite modulation by cocaine and ethanol was observed on proteasome activity. Indeed, while cocaine treatment increased 20S activity after 2h (131 \pm 10.63 vs. control 100 \pm 4.54, p <0.01), ethanol exposure induced an activity reduction which was statistically significant after 2h (86 \pm 1.96 vs. control 100 \pm 2.85, p <0.05). Subsequently, we analyzed the mRNA levels of specific 26S proteasome subunits. The β 1- β 2 and β 5 subunits belong to the proteasome-20S core containing the proteolysis active sites. These subunits were up-regulated by addictive substance treatment. Indeed, cocaine treatment increased β 1 gene expression after 2h (1.50 \pm 0.07 vs. control 1 \pm 0.04, p <0.001) and ethanol exposure induced similar alterations in β 1 and β 2 subunits. In particular the β 1 subunit gene expression was up-regulated at 2 and 24h (1.59 \pm 0.05 vs. control 1 \pm 0.04; 2.01 \pm 0.12 vs. control 1 \pm 0.07, p <0.001 respectively); β 2 mRNA levels increased after 24h (1.28 \pm 0.03 vs. control 1 \pm 0.07, p <0.01). The α subunits, which maintain open the gate between core region and 19S particle, displayed a different modulation after cocaine and ethanol exposure. Cocaine induced α 5 gene expression down-regulation at 24h (0.59 \pm 0.05 vs. control 1 \pm 0.04, p <0.05), followed by up-regulation after 48h (1.35 \pm 0.07 vs. control 1 \pm 0.08, p <0.05). A similar trend was observed for α 6 mRNA levels, which were down-regulated after 2h and 24h (0.41 \pm 0.05 vs. control 1 \pm 0.06; 0.38 \pm 0.04 vs. control 1 \pm 0.06, p <0.001, respectively), and up-regulated after 48h (1.38 \pm 0.04 vs control 1 \pm 0.08, p <0.001). In contrast, ethanol increased α 5 gene expression after 2h (1.55 \pm 0.11 vs. control 1 \pm 0.07, p <0.001) and 48h (1.49 \pm 0.15 vs. control 1 \pm 0.08, p <0.01); no change of α 6 mRNA was observed. Concerning the subunits located in the 19S regulatory particle, we observed that the Rpt3 subunit, involved in the mechanism of complex assembly, was strongly up-regulated by cocaine at 2h and 24h (1.48 \pm 0.06 vs. control 1 \pm 0.05; 3.87 \pm 0.07 vs. control 1 \pm 0.06, p <0.001, respectively). Conversely, ethanol exposure induced a marked down-regulation after 2h (0.59 \pm 0.09 vs. control 1 \pm 0.05, p <0.01). Rpn9 mRNA levels were not altered by cocaine or ethanol treatment at any time-point. In conclusion, our data showed that cocaine and ethanol exposures affect UPS activity and gene expression thus strengthening the hypothesis that these two addictive drugs, despite displaying different mechanisms of action, may share the 26S-proteasome machinery as a common molecular target.

[1] Yi and Ehlers (2005) *Neuron*. 47:629–632.; [2] Ren et al. (2013) *Neuropsychopharmacology*. 38:778-90.; [3] Bardac-Gorge (2009) *World J Gastroenterol*. 15:1163-7.

Possible role of the endocannabinoid system in the activity-based model of anorexia nervosa

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Anorexia Nervosa (AN) is a psychiatric pathology characterized by excessive body weight loss in most cases accompanied by physical hyperactivity difficult to control. Brain imaging studies have shown both neuroanatomical abnormalities and dysfunctional activation of brain areas modulating reward in AN patients (Kaye et al., 2009; Keating et al., 2012; Holsen et al., 2012). The endocannabinoid system (ECs) has been shown to contribute significantly in the modulation of the hedonic aspects of eating behaviour (Di Marzo et al., 2009), and a link between its dysregulation and AN symptoms could be possible (Di Marzo and Matias, 2005). In fact, plasma concentrations of the endogenous cannabinoid anandamide (arachidonylethanolamide, AEA) were found significantly enhanced in patients with AN (Monteleone et al., 2005). To further elucidate the role of the ECs in the pathophysiology of AN, the aim of our study was to investigate whether the pharmacological modulation of the ECs was able to modify the aberrant eating behavior present in a widely validated rodent model of AN. In the "activity-based anorexia" (ABA) paradigm, animals subjected to a restricted feeding schedule (two cycles of restriction separated by a period of recovery) and with free access to a running wheel show paradoxical increased running wheel activity (RWA) and a dramatic weight loss. Our data show that subchronic treatment (6 days of the second cycle restriction) with both the CB1/CB2 receptor agonist Δ 9-tetrahydrocannabinol (0.5 and 0.75 mg/kg) and the synthetic CB1 receptor agonist CP 55,940 (0.03 and 0.06 mg/kg) at the higher doses tested significantly reduced body weight loss. Moreover, each dose attenuated the RWA and produced a transient increase in food intake. However, subchronic treatment with the CB1 receptor inverse agonist/antagonist rimonabant at the doses tested (0.15 and 0.3 mg/kg) did not modify either body weight loss or RWA and produced a decrease in food intake. We have also found that plasma levels of leptin were significantly decreased in ABA animals in comparison with control group; on the contrary, circulating levels of ghrelin and corticosterone were increased. No effect was found on these levels after pharmacological treatments with both agonists and antagonists tested. Taken together our results demonstrate the involvement of the ECs in the pathophysiology of AN and suggest that pharmacological therapies based on the modulation of the endocannabinoid signaling might be effective in the treatment of AN.

Di Marzo (2009) *Int J Obes (Lond)*. Jun;33 Suppl 2:S18-24.; Di Marzo and Matias (2005) *Nat Neurosci*. May;8(5):585-9.; Holsen et al. (2012) *J Psychiatry Neurosci*. Sep; 37(5):322-32.; Kaye et al. (2009) *Nat Rev Neurosci*. Aug;10(8):573-84.; Keating et al. (2012) *Neuropsychologia*. Apr;50(5):567-75.; Monteleone et al. (2005) *Neuropsychopharmacology*. Jun;30(6):1216-21.

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Genetic and environmental factors influencing spontaneous activity of dopamine neurons in Lewis and Fischer rats

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Substance use disorder is a psychiatric illness, which depends upon interactions between inherited predispositions and environmental factors. Different variables often interact in the expression of vulnerable phenotypes, such as genetic background, gender, age and environmental settings. In fact, both adolescence, being a critical period for brain maturation, and environmental settings have been shown to increase susceptibility to drug experimentation and abuse. Addictive drugs share the property to activate the mesocorticolimbic dopamine (DA) system, which has long been implicated in processing reward and reinforcement. Such a system originates in the ventral tegmental area (VTA), where DA neurons are located, and projects to cortical and subcortical structures. VTA DA neurons play a key role in individual vulnerability to drug seeking behavior (Marinelli et al., 2003, Melis et al., 2009, Melis et al., 2013, Melis et al., 2014). Notably, dissimilarities in drug addictive behavior have also been shown by taking advantage of innate differences between inbred rat strains for their genetic components of vulnerability/resilience to addiction, i.e. Lewis (LEW) versus Fischer (F344) rats, respectively (Kosten TA, Ambrosio E., 2002).

The present investigation was sought to examine the followings: i) whether or not VTA DA cell spontaneous activity and their acute response to cocaine and heroin differed between LEW and F344 rats; ii) whether or not a chronic nicotine exposure (0.4 mg/kg s.c. for 5 days) during adolescence would affect VTA DA cell activity *per se* and/or to act as a gateway to drug abuse by modifying acute responses to cocaine and heroin in LEW and F344 rats, at adulthood.

To this aim, extracellular single-unit recordings from VTA DA cells were performed in urethane-anesthetized adult LEW and F344 rats. While statistical analysis revealed no differences in basal spontaneous activity of VTA DA cells in vehicle-treated rats (n=80 and n=74 cells from LEW and F344 rats, respectively), some parameters (i.e. number of spontaneously active cells, frequency of discharge, number of spikes in bursts, coefficient of variation) of VTA DA cell activity differed between nicotine-treated animals (n=55 and n=93 cells from LEW and F344 rats, respectively).

Acute cocaine (0.25-2 mg/kg i.v.) administration similarly and dose-dependently reduced spontaneous firing rate of VTA DA neurons in both strains irrespective of the nicotine pre-treatment during adolescence. Notably, when acutely administered, heroin (0.02-0.32 mg/kg i.v.) produced an effect that was larger in F344 when compared with LEW rats independently from nicotine exposure.

Overall, our results suggest that the exposure to nicotine during adolescence might alter basal electrophysiological parameters of VTA DA cells, thus revealing strain differences, and might contribute to susceptibility to onset of drug addiction.

Acute effect of Ketamine on mTOR downstream: p70S6K and rpS6 expression in rat brain area related to depression and drug addiction

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Recent research showed that a single infusion of ketamine induced a rapid antidepressant response; this acute effect has been related to the capacity of ketamine to trigger molecular mechanisms of neuroplasticity. Ketamine binds to the glutamate N-methyl-D-aspartic acid (NMDA) receptor and, at low doses blocks NMDA receptors located on inhibitory GABAergic neurons. There is evidence that enhanced glutamatergic transmission through AMPA and NMDA receptors after ketamine treatment may result in increased of mammalian target of rapamycin (mTOR) expression. mTOR is a protein kinase involved in translation control and long-lasting synaptic plasticity and its major downstream effect is the activation of p70 ribosomal S6 kinase (p70S6K). Numerous studies showed that acute ketamine dose-dependently increase rpS6 in rat brain areas related to drug addiction and to increased of dopamine level. Mice C57BL/6J Wilde-Type and mice Knock-Out DA-D3 gene, was divided in three groups (n=3-4mice/group) and each genotype injected with saline, ketamine 5 or 10mg/kg IP. At 60 minutes after injection, mice were anesthetized, transcardially perfused, and the brain was collected and processed for immunohistochemistry. The number of neurons positive for p70S6K and rpS6 immunostaining were analyzed from brains areas involved in the control of mood and/or involved in drug addiction as: prelimbic (PrL) and infralimbic (IL) prefrontal cortex, hippocampal (CA1, CA2 and CA3), basolateral amygdala (BL), nucleus accumbens core (AcbC) and shell (AcbSh), ventral tegmental area (VTA) and substantia nigra (SN). A main ketamine treatment effect on p70S6K phosphorylation was observed in PrL, IL, AcbSh, SN, CA1, CA3 and BL. Genotype effect on p70S6K was seen in IL, AcbSh, AcbC, VTA, SN and BL. A significant Genotype x Treatment interaction was observed in AcbSh, AcbC, SN, CA1, CA2, CA3 and BL. The main ketamine treatment effect on rpS6 expression was seen in PrL, AcbC, AcbSh, CA2, CA3. Genotype effect was observed in CA2, BL, whereas significant Genotype x Treatment interaction was seen in PrL, CA2, VTA, SN. Our study showed that: the neuroanatomical pattern of ketamine-induced p70S6K and rpS6 phosphorylation did not overlap, and, there was not significant differences in rpS6 phosphorylation between wild-type and DA-D3 KO, except for a significant Treatment x Genotype interaction in PrL, CA2, VTA, SN.

Pioglitazone reduces nicotine withdrawal symptoms through PPAR γ stimulation.

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Nicotine, a potent psychostimulant with high rewarding properties, is the main responsible of the addictive properties of tobacco smoking [1]. Nicotine discontinuation leads to negative abstinence symptoms characterized by somatic (physical) and affective (anxiety) components [2]. These aversive withdrawal symptoms increase the risk of relapse to smoking, thus therapies that prevent the insurgence of these negative state may represent valid candidates for smoking cessation. It has been recently shown in our laboratory, that pioglitazone, an agonist of peroxisome proliferator-activated nuclear receptor (PPAR γ), decreases alcohol consumption and attenuates the expression of alcohol withdrawal signs [3]. Here we studied the effect of pioglitazone on spontaneous withdrawal signs in Wistar rats following 7 days of nicotine treatment through implanted transdermal nicotine patches (5.2 mg/rat/day). The effect of pioglitazone (15.0, 30.0 mg/kg, p.o.) on somatic and affective withdrawal signs, assessed 16 hours and 6 days respectively, after the last nicotine administration. In addition, we analyzed the effect of pioglitazone on spontaneous nicotine withdrawal signs in conditional neuronal PPAR γ knock-out (KO) mice and their wild type counterparts (WT). Dependence was induced by injecting nicotine 2 mg/kg (s.c), 4 times per day for 8 consecutive days. The effect of pioglitazone and the effect of the antagonist, GW9662 (5mg/i.p) was evaluated on somatic signs and withdrawal-induced anxiety (20 hours and 6 days after the last injection). Furthermore, in order to elucidate the brain areas involved in these effects, we performed a Real-time qPCR analysis of PPAR γ gene expression in the amygdala, hippocampus and hypothalamus of nicotine-treated mice at 20 hours and 6 days of withdrawal. The results showed that pioglitazone was able to reduce the total abstinence score of somatic symptoms and nicotine-induced anxiety in rats. Moreover pioglitazone decreased the nicotine withdrawal somatic symptoms and anxiety in the WT but not in PPAR γ KO mice. The antagonist GW9662 blocked the effect of pioglitazone on somatic withdrawal signs and reversed its anxiolytic effect in the WT animals, confirming the involvement of PPAR γ in the effect. The gene expression results showed that 6 days of nicotine withdrawal induced a significant increase of PPAR γ mRNA levels in the amygdala and that pioglitazone treatment alone induced a significant overexpression of PPAR γ . Hippocampal PPAR γ mRNA levels were significantly increased at both 20 hours and 6 days into nicotine withdrawal. In the hippocampus, as well as in the amygdala, pioglitazone treatment caused an increase of PPAR γ gene expression, whereas no changes were observed in the hypothalamus. The results of this study indicate that the amelioration of nicotine withdrawal symptoms by pioglitazone engage anxiety-related neurocircuits. Above all, these findings demonstrate that pioglitazone treatment may offer a new pharmacological approach for the treatment of nicotine relapse.

[1] Crooks and Dvoskin, *Biochem Pharmacol.* 54:743-53; 1997; [2] Malin et al., *Pharmacol Biochem Behav.* 43:779-84; 1992; [3] Stopponi et al., *Biol Psychiatry.* 69:642-9; 2011

Cannabinoid-mediated modulation of hippocampal hyperexcitability: focus on the interplay with nitrergic system in different rat models of temporal lobe epilepsy.

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Temporal lobe epilepsy (TLE) is the most common type of partial complex seizure in adulthood [1]. Within the framework of hyperexcitability, growing interest has risen on the impact of cannabinoids on the control of paroxysmal phenomena [2], despite their reputation as psychotropic substances with addictive properties [3; 4]. In this regard, it was reported that the on-demand production of endocannabinoids from over-activated postsynaptic cells inhibits neurotransmitter release, hence protecting against excitotoxicity in the hippocampus [5; 6]. Nonetheless, the potential anticonvulsant action of cannabinoids has not been fully addressed. Indeed, CB-mediated effects in animal models are attributed not only to the functional involvement of the classical CB receptors-dependent signalling [7; 8], but also to the interaction with further synaptic processes concerning modulatory messengers. Among these, nitric oxide (NO) has caught our attention since it apparently acts as a mediator for cannabinoid effects [9; 10]. In the current study, we focused on two distinct rat models of TLE, the Maximal Dentate Activation and the Pilocarpine-induced acute seizures, providing electrophysiological and behavioural data on cannabinoid and nitrergic system interplay. We evaluated the antiepileptic effects of WIN 55,212-2 (WIN), a CB agonist, and of 7-Nitroindazole (7NI), a preferential inhibitor of neuronal NO synthase (nNOS), at different doses, alone and in combination. MDA study showed that these drugs protected animals from electrically-induced epileptiform discharges. In pilocarpine model, a dose-related activity of 7NI and WIN: a) decreased the behavioural scoring, used to describe the severity of chemically-induced acute seizures; b) affected latency of the onset of acute convulsions; c) dampened mortality rate. Interestingly, the combination of the treatments brought to light that individually ineffective doses of WIN turn into effective when nNOS activity is pharmacologically inhibited in both experimental conditions. Further MDA studies were conducted with a view to explore the pathway downstream NO. To achieve this, we modulated the activity of NO-dependent soluble Guanylate cyclase (sGC), a cGMP-synthase enzyme, which is the main target of NO signalling. We exploited ODO, a NO-dependent-sGC inhibitor, to evaluate its effect in co-administration with WIN. Results obtained suggested a possible involvement of cGMP pathway in the crosstalk between CB and NO. Taken all these into consideration, our findings suggest a putative antagonism between CB-activated pathway and NO signalling in the context of neuronal hyperexcitability and contribute to elucidate possible synaptic processes underlying neuroprotective properties of cannabinoids, with a view to better integrate antiepileptic therapy.

References

1. Wieser HG, 2004. *Epilepsia* 45:695-714; 2. Wallace MJ, 2001. *Eur J Pharmacol* 428:51-57; 3. Koob GF and Le Moal M, 2001. *Neuropsychopharm* 24(2): 97-129; 4. Gerra G, 2010. *Recent Pat CNS Drug Discov* 5:46-52; 5. Wilson RI and Nicoll RA, 2001. *Nature* 410:588-592; 6. Marsicano G, 2003. *Science* 302(5642):84-8; 7. Jones NA, 2012. *Seizure* 21(5):344-52; 8. Hill TD, 2013. *Br J Pharmacol* 170(3):679-92; 9. Bahremand A, 2009. *Epi Res* 84(2-3):110-9; 10. Rizzo V, 2014. *Epi Res* 108(10): 1711-1718

Studies on the toxic and neuroprotective effects of cannabinoids in models of cerebral ischemia and ischemic tolerance

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Cannabinoids (CBs) are implicated in a number of physiological and pathological mechanisms in the central nervous system, but their exact role in post-ischemic brain injury is unclear. The toxic and neuroprotective effects of synthetic and endogenous CBs were evaluated in rat organotypic hippocampal slices exposed to oxygen-glucose deprivation (OGD) and in gerbils subjected to bilateral carotid occlusion for 5 min. When present in the incubation medium, the synthetic CB agonists WIN 55212-2 and CP 55940 and the CB1 agonist ACEA exacerbated CA1 injury induced by OGD, whereas the CB1 receptor antagonists AM 251 and LY 320135 were neuroprotective. AM 251 also attenuated CA1 pyramidal cell death in gerbils *in vivo*. The endocannabinoid 2-arachidonoylglycerol (2-AG) reduced OGD injury in hippocampal slices, whereas anandamide (AEA) was neurotoxic at the same concentrations. The effects of WIN 55212-2, AEA and 2-AG in slices were all dependent on the activation of CB1 but not CB2 receptors, except for the toxic effects of AEA that were also dependent on vanilloid TRPV1 receptors. Our results suggest that exogenous administration of CB1 agonists and the production of endocannabinoids “on demand” may produce different, if not opposite, effects on the fate of neurons following cerebral ischemia.

We then studied the possible role of endocannabinoids in a model of ischemic tolerance, an endogenous neuroprotective mechanism by which exposure to a subtoxic insult results in resistance to a subsequent lethal stressor. For our study, we used a pharmacological model of preconditioning by exposing the slices to sublethal concentration of the mGluR1/5 agonist (*S*)-3,5-Dihydroxyphenylglycine (DHPG) (10 μ M for 30 min) and 24 h later, to excitotoxic exposure to 30 min OGD or to 10 μ M AMPA for 24 h, which leads to selective injury of the CA1 subregion 24 h later. After preconditioning, we observed a significant reduction in CA1 damage following our toxic insults. We performed whole-cell voltage-clamp recordings on control or preconditioned CA1 pyramidal neurons of organotypic slices. 24 h after exposure to preconditioning doses of DHPG, AMPA induced-currents were significantly reduced, while the expression of the postsynaptic proteins (GluA1, NR2A and NR2B subunits and of PSD-95) that we evaluated by Western blot analysis was not modified. To clarify the mechanisms whereby DHPG induces tolerance, we investigated the involvement of the endocannabinoid system. Exposure of slices to the CB1 antagonist AM251 during preconditioning and the subsequent 24 h recovery period prevented the development of tolerance to AMPA toxicity induced by DHPG. Accordingly, an increase in 2-arachidonoylglycerol (2-AG) induced by the MAG-lipase inhibitor URB602, but not in anandamide by the FAAH inhibitor URB597, was also able to induce tolerance suggesting that 2-AG is responsible for the induction of ischemic tolerance following DHPG preconditioning.

Adolescent exposure to cocaine modulates BDNF in the medial prefrontal cortex of adult rats

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Introduction: Drug addiction is a chronic, relapsing brain disease that results from the loss of control over drug intake, moving from a recreational to a compulsive use of the drugs. Although evidence exists that chronic cocaine exposure at adulthood is associated with changes in neuroplastic molecules, such as activity-regulated cytoskeleton-associated protein (Arc) and brain derived neurotrophic factor (BDNF), few data exist showing that cocaine exposure during adolescence modulates brain neuroplasticity. It is well established that adolescents are more sensitive than adults to drug abuse suggesting that adolescence is a factor of vulnerability for drug addiction. Nevertheless, the potential association between early onset of drug use and higher rates of addiction in adulthood is elusive, and so are the underlying molecular mechanisms.

Materials and methods: Male adolescent rats were exposed to cocaine during adolescence (20 mg/kg), from postnatal day (PND) 28 to PND 42 and sacrificed at PND 90. The molecular analyses on BDNF and its associated network were carried out using Real-Time-PCR technique. Protein levels were analyzed by western blot. Our analyses were performed in the medial prefrontal cortex (mPFC) that is still developing during adolescence.

Results: We found that developmental exposure to cocaine altered transcriptional and translational mechanisms governing neurotrophin expression. Total BDNF mRNA levels were enhanced in the mPFC of PND90 rats exposed to cocaine during adolescence, an effect sustained by increased BDNF exon IV levels through the transcription factors CaRF and NF- κ B. Enhanced BDNF mRNA levels resulted in an increase of the precursor and mature forms of BDNF protein, paralleled by a reduction of several miRNAs governing BDNF translation. Such effect results in the activation of the trkB/AKT pathway which, through increased S6 kinase phosphorylation, increases Arc protein levels in the nucleus and crude synaptosomal fraction. The investigation of the molecular mechanisms underlying such changes revealed that abstinence altered the inhibitory and degradative pathways regulating Arc expression via changes in FMR1, Ube3a and GRM5 mRNA levels. The up-regulation of Arc protein led, in turn, to reduced AMPA GluA1 mRNA and protein levels, indicating a widespread impact of abstinence on several markers of synaptic plasticity.

Conclusion: These findings point to BDNF and Arc/Arg3.1 as molecular signatures of the long-term abstinence from repeated exposure to cocaine during adolescence. Given that Arc/Arg3.1 may be a partner of BDNF in mediating such adaptive changes, we may speculate that an abstinence-induced alteration in the pathway of BDNF together with changes in the inhibitory and degradation pathways that regulate Arc/Arg3.1 synthesis may contribute to the incubation of cocaine craving, casting fresh light on Arc/Arg3.1 as a crossroad of different, but converging, neuroplastic mechanisms.

Adverse social experiences in juvenile mice increase cocaine-seeking behavior and alter blood gene expression

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Estimates suggest that approximately 40-50% of those who experienced childhood maltreatment (CM), will develop substance abuse problems. CM is associated with increased severity of Substance Use Disorder (SUD) and frequency of relapse to drug-seeking (RDS) after withdrawal (1,2). Among different types of CM, exposure to physical abuse is the experience that has the highest correlation with SUD. However, the molecular and neurobiological substrates engaged during early traumatic events and mediating the risk for SUD and RDS are poorly understood. To open perspective on new therapeutic targets and prevention of SUD and RDS, a deeper understanding of these phenomena is crucially required. Within this framework, the identification of non-invasive biomarkers that could predict subject's propensity to develop SUD after a traumatic childhood is highly warranted and an essential prerequisite to the development of effective prophylactic/therapeutic strategies. Recently we have established a mouse model of exposure to "hostile" social environment in early age. In our experiments pre-adolescent mouse pups are exposed to an adult aggressive male mouse (Early Social Stress, ESS), and tested for cocaine seeking behavior in adulthood by conditioned place preference (CPP; 3,4). These mice show high cocaine-seeking behavior and reinstatement to cocaine seeking at doses ineffective in control mice. Being the relapse into drug abuse considered a central problem in the treatment of addiction and the possibility of preventing it using measurable non-invasive biomarkers, considered a major clinical "advancement", we concentrated our studies on the reinstatement phenotype of ESS mice. We performed a genome wide transcriptome analysis of RNAs extracted from Peripheral Blood Mononuclear Cells of ESS and control mice in withdrawal from cocaine. This analysis revealed that more than 1000 transcripts were up-regulated in ESS group compared to controls. Interestingly the Gene Ontology/PANTHER pathway analysis revealed that these genes were enriched in the classes of blood coagulation, integrin signaling, and VEGF signaling pathways. A increased blood coagulation performance in ESS mice was also confirmed by Prothrombin time analysis. Finally we administrated a pharmacological treatment aimed to restore these functional/biological aspects during cocaine withdrawal. Surprisingly this treatment was able not only to improve parameters of the peripheral vasculature but it also rescued the increased susceptibility of the ESS mice to reinstate cocaine seeking.

Currently, our objective is to investigate how gene expression changes in the blood are connected with the brain functioning and to clarify how they associated with the propensity to relapse in cocaine-abstinent individuals.

References

1. Dube SR, Felitti VJ, Dong M, Chapman DP, Giles WH, Anda RF. Childhood abuse, neglect, and household dysfunction and the risk of illicit drug use: the Adverse Childhood Experiences Study. *Pediatrics*. 2003;111:564-572.
2. Van Dam NT, Rando K, Potenza MN, Tuit K, Sinha R. Childhood maltreatment, altered limbic neurobiology, and substance use relapse severity via trauma-specific reductions in limbic gray matter volume. *JAMA Psychiatry*. 2014;71:917-25.
3. Lo Iacono L, Visco-Comandini F, Valzania A, Viscomi MT, Aricò E, D'Amato FR, Puglisi-Allegra S, Carola V. Adverse social experiences in juvenile mice increase cocaine-seeking behavior and alter blood gene expression (under review).

Behavioural and pharmacological characterization of a novel cannabimimetic adamantane-derived indole, APICA, in C57BL/6J mice

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The novel adamantane derivative APICA (N-(adamantan-1-yl)-1-pentyl-1H-indole-3-carboxamide) was recently identified as a cannabimimetic indole of abuse (1, 2). Despite its novel structure, APICA recalls cannabimimetic indoles, such as representative member JWH-018. The emerging abuse problem, together with the paucity of information about the bioactivity of APICA (3) emphasize the need for further evaluation of the in vivo pharmacology of this novel indole-derived compound. In the present study, the effects of APICA (0 - 1 - 3 mg/Kg, i.p.) were tested in C57BL/6J mice, in a battery of tests that are sensitive to the effects of psychoactive cannabinoids, including body temperature; locomotor activity and behavioural reactivity in the open field test; nociception in the tail flick assay; motor coordination in the accelerating Rotarod; recognition memory in the novel object recognition test. Furthermore, the highest dose was also evaluated following the pre-treatment with the CB1 antagonist AM251 (3 mg/Kg, i.p.) or the CB2 antagonist AM630 (3 mg/Kg, i.p.). Our results show that APICA was able to dose-dependently decrease locomotor activity and behavioural reactivity in the open field, whereas only the highest dose was able to induce hypothermia, analgesia, motor ataxia and recognition memory impairment, with respect to vehicle ($p < 0.01$; $p < 0.001$). The pretreatment with the CB1 antagonist AM251 elicited an increase in body temperature, total distance travelled in the open field, descent latency in the Rotarod, and a decrease in tail flick latency ($p < 0.05$; $p < 0.01$). On the other hand, pretreatment with AM630 did not induced significant differences, resulting in hypothermia, antinociception and motor ataxia, with respect to vehicle. This study supports preliminary reports on APICA cannabimimetic properties, extending its detrimental effects on cognitive function. Moreover, these properties can be attributed to the CB1 receptor activity, indicating APICA as a selective CB1 receptor agonist. Selective CB1 receptor ligands may be useful in the pharmacotherapy of several pathological conditions; nevertheless, the increased use as designer drugs of abuse raises significant public health concerns.

(1) Uchiyama, N., Kawamura, M., Kikura-Hanajiri, R., and Goda, Y. (2012) *Forensic Toxicol.* 30, 114–125.

(2) Uchiyama, N., Kawamura, M., Kikura-Hanajiri, R., and Goda, Y. (2013) *Forensic Sci. Int.* 227, 21–32.

(3) Banister SD, Wilkinson SM, Longworth M, Stuart J, Apetz N, English K, Brooker L, Goebel C, Hibbs DE, Glass M, Connor M, McGregor IS, Kassiou M. (2013) *ACS Chem Neurosci* 4(7):1081-92.

The medical use of marijuana: use, abuse, prejudice, legal implication A comparison between International and Italian laws

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Cannabis sativa (CS) is a plant whose use, misuse and abuse is linked to the various possibilities, recreational and medical too. The principles of Cannabis (C) that confer pharmacological activity are delta-9-tetrahydrocannabinol, the isomer delta-8-tetrahydrocannabinol, tetrahydrocannabinol acid, cannabinal and cannabidiol. These principles have allowed the development of drugs that, according to recent studies, may replace or supplement the treatment of many diseases, especially cancer and neurological diseases. Alongside a therapeutic perspective it cannot be ignored the spread out of C as a drug of abuse, raising concerns on its medical use. In Italy, recent national legislation started in 2006 by the authorization to import drugs and active precursors, based on delta-9-tetrahydrocannabinol for therapeutic purposes, in the absence of therapeutic alternatives, in patients requiring those medicines, and ends to the law in force (issued January 23, 2013), which sets in scheduled drugs (Table 2 sec. B of DPR.309 / 1990) "herbal medicines based on cannabis." Lastly (September 2014), the Health Ministries and Government have assigned the cultivation and production of cannabis for therapeutic use to Military Chemical Pharmaceutical Institute.

In our contribution we reviewed jeopardized legal paramount in USA about medical use of CS and updated medical evidence about pro and cons to CS use. Since 2000, Colorado has allowed the domestic cultivation of C. and in 2010 products based on C. were admitted. The study n° 372/11 of "the New England Journal" published March 12, 2015 shows that the number of consumers grew from 4819 in 2008 to 115,467 in 2014. This increase, in association with authorization of trading substances containing marijuana for recreational purposes, has required a careful analysis of the adverse effects of C.

This study provides a comparative analysis among different regulations, helping in evidencing criticality and advantages on its use as a therapeutical alternative.

[1] Tista S. Ghosh M.D., Michael Van Dyke, Ph.D., Ali Mafey, M.S.W., Elizabeth Whitley, Ph.D., Dana Erpelding, M.A., Larry Wolk, M.D. (2015) The New England Journal of Medicine, 991-993;

[2] J. Michael Bostwick, M.D., Gary M. Reisfield, M.D., Robert L. DuPont, M.D. (2013) 1-3.

Chronic administration with nandrolone decanoate attenuates cocaine-induced condition place preference in rats

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As reported by the World Anti-Doping Agency, nandrolone decanoate is one of the most used prohibited anabolic steroid in all sports and often its use is associated with the consumption of other drugs of abuse such as cocaine (World Anti-Doping Agency, 2011; DuRant et al. 1995). Pre-exposure to nandrolone has been demonstrated to induce long lasting changes in neurochemical and behavioral response to cocaine in rats. For example, nandrolone inhibited cocaine-evoked dopamine release in the nucleus accumbens shell, a neurobiological marker of most drugs abuse by humans (Kurling et al. 2005). Moreover, stereotyped behavior and locomotor activity evoked by cocaine administration were attenuated by pre-treatment with nandrolone (Kailanto et al. 2011). Similar to other drugs of abuse, in animals studies cocaine can produce reward as measured by behavioral reinforcement test such as conditioned place preference (CPP) (Spiraki et al. 1982), a procedure widely used in rodents to assess drug effects related to reward or aversion, that can be used to explore motivational brain reward processes related to drugs abused by humans (Tzschentke, 1998). Using the CPP paradigm, the aim of this study was to investigate whether pre-exposure to nandrolone decanoate modulates the rewarding effects of cocaine in rats. We found that intramuscular (i.m.) injection of nandrolone decanoate (15mg/kg) given 1 hour before starting cocaine conditioning sessions, blocked the development of CPP induced by cocaine (10mg/kg i.p.). When given alone, nandrolone did not produce conditioned place preference or place aversion. Moreover, the expression of cocaine induced-CPP was attenuated when nandrolone decanoate was given daily for 14 days (15mg/kg, i.m.) before starting place conditioning. Our data showed that nandrolone decanoate decreased the rewarding effects of cocaine and suggest that chronic administration of this drug may induce dysfunction of the reward system.

Alterations in the emotional regulation process in gambling addiction: the role of anger and alexithymia

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Pathological Gambling is conceptualized as a behavioural addiction because of its neurobiologic, neurophysiologic and psychological features (American Psychiatric Association, 2013; Goudriaan et al., 2004; Petry et al., 2013). This study is aimed at the assessment of alexithymia and anger levels in 100 treatment-seeking pathological gamblers (PGs) compared with 100 healthy controls (HCs), matched for age, gender and education. Furthermore we aimed at evidencing a positive correlation between alexithymia, anger and severity of gambling disorder and showing a relationship between gambling behaviour and anger, after controlling for alexithymia. Finally the fourth aim was to explore the role that gender plays in PGs. Psychological assessment included the South Oaks Gambling Screen (SOGS), State-Trait Anger Expression Inventory-2 (STAXI-2) and Toronto Alexithymia Scale (TAS-20). Statistical analysis on the results shows a greater presence of anger levels in PGs, together with alterations in emotional processing. Severity of gambling behaviour positively correlates with alexithymia scores, State-Anger and Trait-Anger. Moreover, a significant contribution of anger in predicting gambling behaviour has been revealed after controlling for alexithymia. In conclusion, anger and alexithymia must be regarded as relevant components of the psychodiagnostic assessment of PGs, in order to select the best therapeutical strategies, to prevent attempted suicide and to reduce drop-out from the treatment.

References

- American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Washington, DC: American Psychiatric Publishing.
- Goudriaan A.E, Oosterlaan J., de Beurs E., & Van den Brink W. (2004). Pathological gambling: a comprehensive review of biobehavioral findings. *Neuroscience Behavioral Review*, Apr;28(2):123-41.
- Petry N.M., Blanco C., Auriacombe M., Borges G., Bucholz K., Crowley T.J., ... O'Brien C. (2014). An Overview of and Rationale for Changes Proposed for Pathological Gambling in DSM-5, *Journal of Gambling Studies*, Jun;30(2):493-502.

Effects of single and repeated N-acetylcysteine on cue-induced nicotine-seeking behavior

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The inability of smokers to control relapse to drug-seeking behavior is a key feature of nicotine addiction. Although pharmacotherapy and psychosocial support can help smokers quit, the high relapse rates indicate a high unmet need for more effective therapies.

Recent studies have highlighted changes in glutamate (Glu) levels in circuitry from the prefrontal cortex to the nucleus accumbens as vital in the reinstatement of drug-seeking behavior. Restoring basal concentrations of extracellular Glu, thereby increasing tonic activation of the presynaptic group II metabotropic Glu receptors (mGluR2/3) by a single injection of N-acetylcysteine (N-AC) prevented conditioned cues-induced cocaine- and heroin-seeking behavior in rats after drug self-administration.

Although nicotine-associated cues reinstate drug-seeking and raise extracellular Glu in the nucleus accumbens, it is still not clear whether N-AC can inhibit cue-induced reinstatement in abstinent rats after nicotine self-administration. It is also not clear whether restoring Glu homeostasis by chronic N-AC treatment can enhance the outcome of cue-exposure therapy (CET) for smoking cessation.

To gain this information we used rats trained to associate discriminative stimuli (S^Ds) with intravenous nicotine or oral saccharin self-administration vs. no-reward in two-lever operant cages. Reinforced response was followed by cue signaling 20-second time-out (CSs).

Re-exposure to nicotine or saccharin S^D/CS⁺, but not no-reward S^D/CS⁻, revived responding at the previously reinforced lever. Acute N-AC, 100 mg/kg i.p., reduced cue-induced nicotine-seeking behavior without modifying cue-induced saccharin-seeking behavior. Pre-treatment with the selective mGluR2/3 antagonist LY341495, 1 mg/kg i.p., completely prevented the effect of N-AC on nicotine-seeking behavior.

When N-AC was given chronically during forced abstinence or in combination with CET, only in the latter condition N-AC not only reduced nicotine-seeking when biologically available during testing, but induced lasting anti-relapse activity that was still present two weeks later. Chronic treatment with N-AC, 100 mg/kg i.p. but not 60 mg/kg, during 14 days' exposure to nicotine-associated cues reduced drug-seeking without any tolerance. Chronic N-AC induced long-term anti-relapse activity that was still present 14 days after the end of treatment.

These results support and extend previous preclinical findings suggesting that N-AC might offer a therapeutic opportunity for acute cue-controlled nicotine-seeking and for promoting extinction of nicotine-cue conditioned responding. In view of the conflicting results so far in the clinical evaluation of N-AC's therapeutic activity for smoking cessation, the evidence that chronic N-AC enhanced the outcome of CET could have substantial impact on the design of future clinical trials.

Nicotinic $\alpha 7$ receptor activation induces NMDA receptors trafficking in glutamatergic terminals of the nucleus accumbens

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We here provide functional and immunocytochemical evidence supporting the co-localization and functional interaction between nicotinic acetylcholine receptors (nAChRs) and N-methyl-D-aspartic acid receptors (NMDARs) in glutamatergic terminals of the nucleus accumbens (NAc). Immunocytochemical studies showed that a significant percentage of Nac terminals were glutamatergic and possessed GluN1 and $\alpha 7$ -containing nAChR. A short-term pre-exposure of synaptosomes to nicotine (30 μ M) or choline (1mM) caused a significant potentiation of the 100 μ M NMDA-evoked [3H]D-aspartate ([3H]D-Asp) outflow, which was prevented by abungarotoxin (100 nM). The pre-exposure to nicotine (100 μ M) or choline (1 mM) also enhanced the NMDA-induced cytosolic free calcium levels, as measured by FURA-2 fluorescence imaging in individual NAc terminals, an effect also prevented by α -bungarotoxin. Pre-exposure to the $\alpha 4$ -nAChR agonists 5IA85380 (10 nM) or RJR2429 (1 μ M) did not modify NMDA-evoked ([3H]D-Asp) outflow and calcium transients. The NMDA-evoked ([3H]D-Asp) overflow was partially antagonized by the NMDAR antagonists MK801, D-AP5, 5,7-DCKA and R(-)-CPP and unaffected by the GluN2B/NMDAR antagonists Ro256981 and ifenprodil. Notably, pre-treatment with choline increased GluN2A biotin-tagged proteins. In conclusion, our results show that the GluN2A-NMDA receptor function can be positively regulated in NAc terminals in response to a brief incubation with $\alpha 7$ but not $\alpha 4$ nAChRs agonists. This might be a general feature in different brain areas since a similar nAChR-mediated bolstering of NMDA-induced ([3H]D-Asp) overflow was also observed in hippocampal synaptosomes.

Effects of acute administration of JWH-018 on cardiovascular functions in mice

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Synthetic cannabinoid (SCBs) have been used as recreational drugs for their psychoactive effects similar to those produced by cannabis [1]. These compounds, commercially known as K2 or "Spice", are easily purchased on the Internet market. SCBs are potent cannabinoid agonists that have high affinity for CB₁ and CB₂ receptors. Many recent reports indicate that SCBs create a serious public health issue due to medical and psychiatric toxicities [2, 3]. In particular, seizures, hyperreflexia, myoclonias and cardiac toxicity appear to be the main "atypical" side effects observed in emergency rooms [2, 3]. In particular, SCBs intake induces harmful effects on cardiovascular system such as palpitations, chest pain, arrhythmias and myocardial infarction [4]. The aim of this study was to investigate cardio-respiratory and -vascular alterations induced by acute administration of JWH-018 (0.3-6 mg/Kg i.p.) in awake and freely moving CD-1 mice. The detection of heart rate, breath rate, arterial saturation (SpO₂) and pulse distention, was provided by a non-invasive apparatus that utilizes a collar with an infrared sensor (Mouse Ox Plus). The determination of blood pressure was carried out by the BP 2000 that is a computerized non-invasive tail-cuffs system. JWH-018 (6 mg/Kg i.p.) induced relevant long-lasting (up to 5 hrs) cardio-respiratory changes characterized by deep bradycardia (heart rate reduction of about 50 %) alternated with episodes of tachyarrhythmias and tachycardia. Cardiac effects were accompanied by bradypnea (breathing rate reduction of about 50 %), mild and transitory reduction both in SpO₂ (SpO₂ reduction of about 25 %) and in pulse distention (reduction of about 35 %). Moreover JWH-018 (6 mg/Kg i.p.) increased the systolic blood pressure (+30 mm/Hg at 10 minutes after JWH-018 injection) and its effect persisted up to 1 hour. JWH-018 also increased the diastolic pressure (+20 mm/Hg at 10 minutes after JWH-018 injection) but the effect was transient and significant only in the first 20 min after the drug injection. The administration of the selective CB₁ receptor antagonist/inverse agonist AM 251 (6 mg/Kg i.p.) completely reverted cardio-respiratory and vascular changes. For the first time the present data reproduced in the mouse model the "atypical" cardiorespiratory and -vascular side effects induced by SCBs in humans and point out the greater cardiac toxicity induced by JWH-018 [3, 4, 5]. Further studies will be performed to investigate the neural mechanisms involved in the alterations caused by the administration of JWH-018 in mice.

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1) Italian National Early Warning System, Drug Policies Department, Presidency of the Council of Ministers, *National Report 2010*, Roma, 2010

2) Hermanns-Clausen M, Kneisel S, Szabo B, Auwaeter V (2012) Acute toxicity due to the confirmed consumption of synthetic cannabinoids: Clinical and laboratory findings. *Addiction*. 108: 534-44

3) Seely KA, Lapoint J, Moran JH, Fattore L. (2012). Spice drugs are more than harmless herbal blends: A review of the pharmacology and toxicology of synthetic cannabinoids. *Neuro-Pharmacology & Biological Psychiatry*. 39: 234-243

4) Mir A, Obafemi A, Young A and Kane C. (2011). Myocardial Infarction Associated With Use of the Synthetic Cannabinoid K2. *Pediatrics*, 128(6): 1622-1627.

5) Schneir AB, Cullen J and Ly BT. (2011). "Spice" girls: synthetic cannabinoid Intoxication. *The Journal of Emergency Medicine*. 40(3): 296-299.

Receptor-receptors functional cross-talk: new evidences on nicotine-NMDA receptor interaction

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In a recent study entitled "Prolonged nicotine exposure down-regulates presynaptic N-methyl-D-aspartic acid (NMDA) receptors in dopaminergic terminals of the rat nucleus accumbens" (Salamone et al., 2014) we have addressed the topic of the cross-talk between nicotinic receptors (nAChRs) and the NMDA receptors present on the same neuron. Moreover, we have demonstrated that a short pretreatment of dopaminergic nerve terminal to nicotine is able to reduce selectively the NMDA receptors function. Now we start with a new project focused on the identification of the mechanism involved in the nAChRs-NMDARs functional cross-talk. We study neurotransmitter release from rat nucleus accumbens synaptosomes prelabelled with [³H]Dopamine and [³H]γ amino butyric acid. Preliminary results show that different ionotropic pretreatment (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) 100μM, and Adenosine triphosphate (ATP) 100μM are able to mimic nicotine in the functional down regulation of NMDARs modulating Dopamine release. Conversely, metabotropic pretreatment (Oxotremorine 30μM and (S)-3,5-Dihydroxyphenylglycine (DHPG) are ineffective. Curiously, nicotine pretreatment fails to modify, in the same brain area, the function NMDARs modulating GABA release. Based on this evidences, we will test the possibility that NMDARs present on GABA terminals are pharmacological different from NMDARs modulating Dopamine release. Finally, using a biotinylation protocol coupled with western blot analysis, we will study whether the AMPA and ATP induced functional down regulation of NMDARs depend on the modification of glutamatergic receptors trafficking. All together, these data may help to understand the mechanisms that regulate the NMDRs plasticity.

Innate and ethanol-induced differences in the Extended Amygdala gene expression between Marchigian Sardinian alcohol-preferring (msP) and Wistar rats

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Several studies reported that alcohol preferring and non-preferring animals exhibit differences in gene expression in specific brain circuitries that may be decisive in shaping the vulnerability to alcoholism [1]. Genetically selected marchigian sardinian Preferring (msP) rats show natural ethanol preference and high sensitivity to stress together with anxious and depressive-like symptoms that ameliorate following ethanol drinking [2]. Among the circuitries involved in the regulation of stress-induced drug-seeking behaviour, the extended amygdala, comprising the bed nucleus of the stria terminalis (BNST), the central nucleus of the amygdala (AM) and the shell of the nucleus accumbens, represent a critical site [3]. In view of the crucial role played by the endogenous opioid system in ethanol intake [4], in this study we aimed to evaluate innate and ethanol-induced differences in dynorphin and nociceptin systems gene expression in the AM and BNST of Wistar and msP rats. Animals (n=6) were treated with chronic intermittent 10% alcohol (v/v) in a two-bottle free choice paradigm; after 30 days, rats were sacrificed and then AM and BNST were rapidly harvested and frozen.

Real-time qPCR analysis showed a down-regulation of prodynorphin (PDYN) mRNA level after ethanol intake (0.45±0.09 vs msP vehicle 0.95±0.11, p<0.05; two-way ANOVA, Bonferroni's post-hoc test) in the AM of msP rats, whereas no changes were observed in the BNST. A higher basal κ opioid receptor (KOP) gene expression was detected in the msP than in Wistar rats AM (1.26±0.05 vs 1.00±0.10, p<0.05); the ethanol intake caused a KOP down-regulation in the msP AM (0.65±0.04 vs msP vehicle 1.26±0.05, p<0.001) and an up-regulation in the msP BNST (1.41±0.09 vs msP vehicle 0.88±0.04, p<0.001). Higher pronociceptin (PNOC) mRNA basal levels was observed both in the AM (2.33±0.21 vs 1.00±0.20, p<0.001) and in the BNST (1.40±0.06 vs 1.00±0.11, p<0.05) of msP rats compared to Wistar animals. Ethanol intake caused a significant decrease of PNOC mRNA in both the investigated regions (AM: 1.67±0.17 vs msP vehicle 2.33±0.21, p<0.05; BNST: 0.94±0.09 vs msP vehicle 1.40±0.06, p<0.01) of msP animals only. Higher basal expression levels of nociceptin/orphanin FQ receptor (NOP) mRNA were observed in both the AM and the BNST of msP vs Wistar rats (AM: 1.81±0.11 vs 1.00±0.07, p<0.001; BNST: 1.57±0.09 vs 1.00±0.09, p<0.001). Moreover, ethanol induced a NOP mRNA down-regulation in the msP rats AM (0.65±0.13 vs msP vehicle 1.81±0.11, p<0.001) and an up-regulation in the msP BNST (2.06±0.10 vs msP vehicle 1.57±0.09, p<0.01). These findings suggest that, in the extended amygdala, the effect of ethanol is totally dependent on the genotype, since no alterations were observed for both systems in Wistar rats following alcohol exposure.

In conclusion, this study indicates that PDYN/KOP and PNOC/NOP systems contribute to the peculiar characteristic of msP in which an innate preference for alcohol has been co-segregated with an anxious and depression-like phenotype and that, in these animals, alcohol intake allows recovery from negative mood states acting through opioid system.

[1]Prakash et al., Alcohol Clin Exp Res. 32:909-20;2008; [2]Ciccocioppo et al., Addict Biol. 11:339-55;2006; [3]Koob, Eur Neuropsychopharmacol. 13:442-52;2003; [4]D'Addario et al., Addict Biol. 18:425-33;2011

Different modulation of neuroplastic markers in the rat brain by acute or chronic ketamine selfadministration

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Ketamine was shown to own reinforcing properties, i.e. to initiate and maintain selfadministration as well as anti-depressant effects after a single dose. Evidence exists showing that a single ketamine injection induced neuroadaptive changes in addiction-related brain areas, such as release of BDNF and stimulation of AKT/mTORC1 signalling cascade, both involved in synaptic strengthening and LTP. The objective of this study was to assess the expression of neuroplasticity markers such as zif-268, rpS6 and BDNF in brain areas related to addiction and mood, with the aim to possibly discriminate between the reinforcing and antidepressant ketamine dose-regimen effects.

Rats were daily trained to self-administer IV ketamine S/A (group 12x30) or to receive a single ketamine 0.5 mg/kg IV (group 1x1) or vehicle (group 0x1) injections. At endpoint of the 12x30 group the total ketamine intake was 191.2 ± 82.1 mg/kg IV, with an average of 6.4 mg/kg ± 2.7 mg/kg IV of daily intake (mean ± SD). Brains were perfused and processed for immunohistochemistry against zif-268 and rpS6 in prelimbic (PRL) and infralimbic (IL) cortex, nucleus accumbens core (NACC) and shell (NACS), ventral tegmental area (VTA), hippocampus CA1, CA2 and CA3, and basolateral amygdala (BLA). The BDNF protein levels in ventral striatum (VS) and hippocampus (HP) were processed by Western blotting and analysed with a one-way ANOVA followed by Fisher's LSD test.

Zif268 cell counting showed a significant main effect of ketamine IV in all the brain areas except for hippocampal CA3. Tukey's multiple comparisons, showed a significant effect in the 1x1 vs. 0x1 group in PRL, NACC, NACS. Group 12x30 showed a significant increase vs. 0x1 group in all brain regions except CA3. Pearson's multiple comparison showed correlations in the 12x30 group between hippocampal CA areas and dopaminergic nerve terminals areas. In group 1x1, significant correlations were found within the mesocorticolimbic pathway, and between CA3 and NAC areas.

For rpS6, a significant main effect of ketamine IV was seen in all the analysed brain areas. Tukey's multiple comparisons showed a significant difference in 1x1 vs. 0x1 group in all brain areas, with higher activation of rpS6 in the vehicle group. 12x30 group showed a significant decrease of rpS6 in all brain regions except for CA2 vs. vehicle and a significant increase in CA1, CA2, CA3 and BLA vs. 1x1 group.

Mature BDNF levels in hippocampus showed a significant increase in 1x1 vs. 0x1 and 12x30 groups, and a significant decrease in 12x30 vs. 0x1 group; proBDNF level was significant lower in 1x1 and 12x30 groups vs. vehicle group. In VS conversely, we observed a significant increase of proBDNF in 12x30 vs. 0x1 and 1x1 groups, and a significant decrease of mBDNF in 1x1 and 12x30 groups vs. vehicle group level.

Our study report significant differences in pattern and intensity of zif268 and rpS6 expression due to ketamine S/A vs. the single ketamine injection. These differences, together with results on BDNF expression levels, might suggest a clear molecular discrimination between reinforcing and antidepressant dose-regimen effects of ketamine.

Role of Histone H3 Lysine 9 Try-Methylation in the development of the depressive/psychotic-like phenotype induced by adolescent THC exposure

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We recently demonstrated that adolescent female rats treated with the psychoactive ingredient of marijuana delta-9-tetrahydrocannabinol (THC), develop a depressive/psychotic-like phenotype in adulthood. Moreover, we observed that adolescent, but not adult, THC exposure leads to this phenotype, suggesting that adolescence may represent a vulnerable period for the psychiatric consequences of THC exposure. However, the neurobiology of this vulnerability is not clear. Recently, several papers support an involvement of epigenetic mechanisms in the pathogenesis of psychiatric illnesses. In line with this data, in the PFC of THC-treated animals we observed increased tri-methylation of Lysin9 on the histone H3 (H3K9me3, associated with transcriptional repression) 2 hours after the end of the THC treatment. Moreover, 24 hours later, this increase was still present together with increased di-methylation of Lysin9 and acetylation of Lysin14 on histone H3 (H3K9me2 and H3K14Ac, associated with transcriptional repression and activation respectively) and these alterations returned to control levels after 24 hours. In contrast, adult THC exposure induced only an increase of H3K9me3, 2 hours after the last THC injection. Therefore, these biochemical data confirm the major vulnerability of the adolescent brain.

Since the histone modification mainly disrupted by the adolescent THC exposure was the H3K9me3, the goal of the present work was to investigate the enzyme responsible of this modification, SUV39H1 after adolescent THC exposure, by Western Blot. To this aim, adolescent female rats were treated with increasing doses of THC twice a day from PND 35 to 45 and the analysis was performed in the PFC 2, 24 and 48 hours after the last THC injection.

Two and 24 hours after the end of the treatment, we observed a significant increase in SUV39H1 protein levels that return to control 24 hours later. These data fit well with the increase in H3K9me3 observed at the same time-point.

In order to understand the possible role of the H3K9me3 in the development of the depressive/psychotic-like phenotype induced by the adolescent THC exposure, we next administered Chaetocin (0.1 mg/Kg, one a day, i.p.), a selective inhibitor of SUV39H1, during the adolescent THC treatment and we performed behavioral tests at PND 75.

Chaetocin administration significantly prevented the cognitive deficit induced by the adolescent THC exposure in the Novel Object Recognition test. On the contrary, Chaetocin administration did not prevent social deficit in the Social Interaction test and behavioral despair in the Forced Swim Test.

As a whole, these data suggest that pharmacological modulation of SUV39H1 prevents cognitive deficits, but not the alterations of emotional behaviors, suggesting that the increase of H3K9me3 might play a role in the development of some signs, but not all, of the depressive/psychotic phenotype induced by adolescent THC exposure.

Withdrawal from electronic cigarette vapour exposure elicits short and long term behavioural effects in mice

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Nicotine is known to induce physical and psychic dependence. Although born in 2004, the electronic cigarette (e-cigarette) recently has rapidly spread worldwide. Until now only few controlled toxicological studies are available and so far no studies on the effect of e-cigarettes at the molecular and behavioral levels have been performed. On this basis, the aim of our study was to investigate the behavioural and biochemical changes induced by e-cigarette in comparison with standard tobacco cigarette, during exposure and withdrawal, using an innovative mouse model of vapour or smoke exposure. Balb/c male mice were exposed in groups of 30 animals at a time, to the product of vapour of the e-cigarette liquid (containing 5.6 mg of nicotine) or to the smoke of 7 standard cigarettes (Chesterfield Red) or to air (control group), 3 times a day, 5 days a week for 7 weeks. One hour after the last session, half of the animals were sacrificed for neurochemical analysis, and the others underwent mecamylamine-precipitated or spontaneous withdrawal for the purposes of behavioral analysis. Receptor binding studies showed a significant up-regulation of $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptor subtype in different brain areas of animals exposed to both e-cigarette and standard cigarette smoke. Similar brain cotinine and nicotine concentrations and comparable urine cotinine levels were found. During mecamylamine-precipitated withdrawal there was a decrease in horizontal and vertical movements and the presence of typical withdrawal symptoms and signs in mice exposed to standard cigarette smoke which were more pronounced than those elicited by e-cigarette. A milder withdrawal syndrome was observed in animals exposed to e-cigarette. Spontaneous nicotine withdrawal syndrome evaluated from 24 hours up to 90 days after exposure evidenced cognitive impairment and increased anxiety in both groups. Surprisingly, e-cigarette vapour exposure elicited early onset of depressive-like behaviour and a more severe compulsive behaviour. In conclusion, although e-cigarettes are rapidly gaining acceptance as aid for smoking cessation, it is necessary to consider their possible long-term withdrawal effects on anxiety and depression.

Effects of *Withania somnifera* Dunal on the motivational properties of ethanol: place conditioning and self-administration studies

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Ethanol is responsible for the effects of alcoholic beverages whose uncontrolled use represents a serious risk factor for a great number of diseases and premature deaths. However, in spite of intense research on the mechanisms by which ethanol affects behaviour and may bring to development of alcoholism, an enormous gap has yet to be filled in order to provide pharmacological tools that may interfere with such mechanisms. Furthermore, the achievement of such task is made particularly difficult by the complex phenomenology of alcoholism as well as by its multifactorial determinants. Thus, besides the pharmacological approaches that extend from the use of disulfiram, naltrexone and acamprosate, recent investigations have also pointed to herbal remedies used by traditional and folk medicine. Interestingly, among the plants that are widely used also for different indications, *Pueraria lobata* (Kudzu), *Panax ginseng* and *Salvia miltiorrhiza*, have been reported to be of some interest and have been investigated to be suggested as possible adjuvants for prevention and treatment of alcoholism. Recent evidence has shown that *Withania somnifera* Dunal, a herbal remedy used in traditional medicine for its anti-inflammatory properties, reduces ethanol withdrawal-induced anxiety, potentiates ethanol-induced anxiolysis and impairs morphine-elicited place conditioning. As an extension of these observations, aim of this study was to investigate the effects of *Withania somnifera* roots extract (WSE) on ethanol-elicited place conditioning (both preference and aversion) and on motivation for drinking ethanol by operant self-administration paradigms. To this end, in place conditioning experiments male CD-1 mice underwent backward (conditioned place preference, CPP) and forward (conditioned place aversion, CPA) conditioning and the effects of WSE (50 and 100 mg/kg) were evaluated on acquisition and expression of ethanol-elicited CPP and CPA. In self-administration experiments male Wistar rats were trained to self-administer ethanol (10%) by nose-poking and the effects of WSE (25 and 75 mg/kg) were evaluated on acquisition and maintenance of ethanol self-administration, on ethanol breakpoint under a progressive-ratio schedule of reinforcement, on ethanol deprivation effect and on reinstatement of seeking behaviours. The results of these experiments confirm 1) that under appropriate experimental conditions, ethanol is able to elicit both CPP and CPA; 2) that the administration of WSE significantly impairs both the acquisition and the expression of CPP and CPA, without affecting spatial memory, as determined by a two-trial memory recognition task; 3) that WSE reduces acquisition, maintenance and breakpoint of ethanol self-administration as well as the deprivation effect and reinstatement of ethanol-seeking behaviour. Although further studies are necessary to investigate in detail the mechanism(s) by which WSE interferes with the motivational properties of ethanol and affects ethanol taking behaviour, that these results provide support to the suggestion that the use of WSE could represent an interesting phytotherapeutic approach for the treatment of excessive alcohol drinking and to prevent alcohol relapse.

Varenicline decreases nicotine self-administration, but not alcohol consumption in msP rats with concomitant access to both drugs

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Background: Alcohol and nicotine are largely co-abused (1). Environmental and psychosocial factors are responsible for the strong association between alcohol and nicotine addiction, however a variety of studies have also demonstrated pharmacological interactions of the two drugs at the level of the neuronal nicotinic receptors (nAChRs).

Here we used Marchigian Sardinian alcohol preferring (msP) rats (2) to study whether alcohol consumption could influence nicotine seeking and craving and vice-versa. Then, we investigated if the concomitant exposure to nicotine and alcohol could affect the well-known efficacy of the selective $\alpha_4\beta_2$ nACh receptor partial agonist varenicline on nicotine and alcohol consumption.

Methods: We performed two different models of co-abuse: the first one consisted of co-administration of nicotine (30 mg/kg/0.1 ml) and ethanol (10% v/v) together in the same operant session using the classic Skinner boxes (3). In the second one, msP rats were concurrently exposed to daily 2-hours nicotine sessions, while being given unlimited access to ethanol, 22-hours per day, through the two bottle choice paradigm.

Results: Consistent with previous results, we found that a history of alcohol drinking did not alter the motivation to self-administer nicotine as measured under a Progressive Ratio schedule of reinforcement. As expected, varenicline (0.3, 1.0 and 3.0 mg/kg) significantly decrease nicotine self-administration in msP (** $p < .01$) independently from co-exposure to alcohol or not. Interestingly, varenicline did not alter alcohol consumption either in home-cage two bottle choice condition or following operant self-administration paradigm.

These results confirm that modulation of $\alpha_4\beta_2$ nAChRs by varenicline represents an efficacious treatment to attenuate nicotine consumption and expand this finding in animals genetically selected for excessive alcohol drinking. On the other hand, data demonstrate that this drug is not able to reduce alcohol intake alone as well as in the presence of concomitant nicotine. This latter finding do not support the use of varenicline in alcohol abuse. Noteworthy mixed preclinical and clinical results have been so far reported on the efficacy of varenicline in alcohol abuse treatment.

(1) Miller and Gold (1998). "Comorbid cigarette and alcohol addiction: epidemiology and treatment" *J Addict Dis* 17(1):55-66

(2) Ciccocioppo (2006). "Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats: an animal model to study the neurobiology of alcoholism." *Addict Biol* 11(3-4): 339-355.

(3) Le (2010). "Co-administration of intravenous nicotine and oral alcohol in rats." *Psychopharmacology*. 208(3): 475-486

DA-Phen as a new potential DA-mimetic agent for treatment of alcohol addiction: preclinical *in vivo* studies

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Rewarding and reinforcing properties of alcohol are mediated by activation of the mesolimbic dopaminergic system¹. This neurosystem is hypofunctional in the addicted brain, even beyond somatic and psychological signs of withdrawal. Boosting strategy on the dopaminergic tone could represent a valid approach to alcohol addiction treatment². The effects of a new dopamine conjugate³ (2-amino-N-[2-(3,4-dihydroxyphenyl)-ethyl]-3-phenyl-propionamide, DA-Phen) on operant behaviour and on withdrawal behaviour, following alcohol deprivation, were evaluated. The concentration of acetaldehyde (ACD), ethanol's first metabolite, as an indirect measure of the possible DA-Phen modulation in alcohol consumption, was also assessed. Male Wistar rats were tested in an operant paradigm, made by different phases: Habituation (Ethanol 5%), Training (Ethanol 20%), Extinction and Deprivation/Relapse (3 cycles). Rats were treated with DA-Phen (0.03 mmol/kg i.p.) during abstinence, or during relapse. Behavioural reactivity and anxiety-like behaviour during withdrawal were also evaluated. At the end of described paradigm, animals were sacrificed, and DA-Phen distribution in organ homogenates was detected and quantified. DA-Phen promoted a reduction in alcohol intake by 50% from the second day of relapse ($p < 0.001$). DA-Phen administration was also able to induce significant reductions in chronically alcohol-treated rats on main OF (total distance travelled, $p < 0.001$; percentage of time on centre, $p < 0.05$) and EPM parameters (percentage of open time, $p < 0.001$; open arm entries, $p < 0.05$; and head dippings, $p < 0.001$). Quantitative DA-Phen analysis indicated a distribution in kidneys (0.806±0.088 mg/g), spleen (0.432±0.035 mg/g), plasma (0.223±0.065 mg/g), liver (0.138±0.006 mg/g) and cerebral region (0.101±0.008 mg/g). The quantification of ACD in brain and liver homogenates of ethanol drinking rats showed a reduction in ACD levels when DA-Phen was administered (25.24±1.30 mg/g in the brain and 3.20±0.60 mg/g in the liver), with respect to untreated subjects (30.28±2.80 mg/g in the brain and 2.25±0.40 mg/g in the liver). In conclusion, DA-Phen reduces ethanol intake, likely enhancing dopaminergic tone, and reduced withdrawal behaviour. Our data also suggest that DA-Phen is able to produce the decrease of ACD concentration in both brain and liver, probably due to a condensation between ACD and DA-Phen. Further studies are ongoing in order to verify this hypothesis.

¹Koob GF. (2013) Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. *Curr. Top. Behav. Neurosci* 3:3-30.

²Diana M. (2011) The dopamine hypothesis of drug addiction and its potential therapeutic value. *Front. Psychiatry*. 2:64. doi:10.3389/fpsy.2011.00064.

³Giannola LI, De Caro V, Giandalia G, Siragusa MG, Lamartina L. (2008) Synthesis and *in vitro* studies on a potential dopamine prodrug. *Pharmazie*. 63:704-10.

Efficacy of anti-craving pharmacological therapy based on a psychobiological model in alcohol dependence

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Craving is a prominent feature of alcohol dependence and frequently a cause of relapse. Aim of our study has been to assess the efficacy of specific anti-craving drugs for the treatment of a cohort of patients diagnosed as alcohol-dependent. Pharmacotherapy has been chosen according to the typology of craving, which was identified by the three-pathway psychobiological model proposed by Verheul et al., [1]: relief craving, reward craving and obsessive craving. Currently available pharmacological options were acamprosate, nalmefene and topiramate for relief craving, reward craving and obsessive craving, respectively. These medications have been used as an adjunct, not an alternative, to the clinic's usual psychosocial and pharmacological treatment program. Sixty six patients (25 women and 41 men) recruited from November 2013 to November 2014 and follow-up visits to assess drinking status at 1 and 3 months after inclusion have been performed. Patients were divided into two groups: treated group (n=34) who underwent craving evaluation and consequently received specific pharmacotherapy, and control group (n=32). Drops out were n=6 (17%) and n=8 (25%) in treated and control group, respectively. Craving was identified as relief (n= 21; 75%), reward (n= 5; 17.8%) and obsessive (n= 2; 7.2%) and treated with acamprosate, nalmefene and topiramate, respectively. During the observation period, patients of the treated group decreased craving and improve the Visual Analogic Scale (VAS) score from 37.1% to 68% and Obsessive Compulsive Drinking Scale (OCDS) score from 62.7% to 76.5% at 1 and 3 months of treatment. Moreover, relapsing patients in treated group were 32% (n=9) and 8.6% (n=2) at 1 and 3 month comparing to 37.5% (n=9) and 33% (n=5) of control group at the same time period. Furthermore, relapsing reduction of alcohol consumption (g/day) was greater in the treated group (58%) compared to the control group (37.8%) thus showing a significant increase in the relapse severity in the latest.

In conclusion, these findings suggest that an anti-craving therapy based on a psychobiological model could be effective for retention in treatment and relapse prevention in alcohol dependence.

1. Verheul R, Van Den Brink W, Geerlings P, A three-pathway psychobiological model of craving for alcohol. *Alcohol Alcoholism*. 1999; 34:197-222.

Cortical neuroinflammation contributes to the development of cognitive deficits induced by adolescent thc exposure in rats

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Emerging evidence suggests that microglia might play a crucial role in brain development, regulating synaptic maturation and function, possibly suggesting that deficits in microglia function may contribute to synaptic abnormalities seen in some neurodevelopmental disorders.

In the present study, we evaluated whether chronic exposure to delta-9-tetrahydrocannabinol (THC) during adolescence could have long-term consequences on microglia functions and we explored their possible contribution to the development of THC-induced alterations in mood and cognition in adult female rats.

To this aim, chronic THC (or vehicle) treatment was performed between PND 35 to 45 and we examined whether chronic THC exposure during adolescence would affect microglia in the long-term in brain regions expressing CB1 receptors and involved in the modulation of mood and cognition.

We found that adolescent THC administration significantly increased the expression of the microglial marker, Iba1, by about 40% when compared to vehicle-treated controls, specifically in the PFC and not in the other brain areas under investigation.

The observed alteration in Iba1 expression was associated with increased levels of pro-inflammatory mediators, that is TNF- α , iNOS, COX-2, and decreased expression of the anti-inflammatory cytokine, IL-10, specifically in the PFC. Furthermore, a significant up-regulation of CB2 receptors was observed on microglia cells in the adult PFC following adolescent THC exposure.

These data suggest that THC administration during adolescence produces persistent molecular signs of cortical neuroinflammation.

In order to assess whether microglia activation could play a role in determining the behavioral phenotype observed in adult THC-exposed animals, we inhibited microglia activation by co-administering Ibudilast, a non-selective phosphodiesterase inhibitor, concomitantly to THC treatment and animals were then submitted to behavioral testing in adulthood.

Intriguingly, co-treatment with Ibudilast and THC during adolescence prevented microglia activation in the PFC of THC-exposed animals, as demonstrated by the normalization of Iba1 levels and cytokine expression. More interestingly, Ibudilast administration significantly attenuated the development of cognitive deficits associated with adolescent THC exposure, thus suggesting that microglia activation could contribute to determine some of the behavioral alterations observed in adult THC-treated rats.

The present findings demonstrate that adolescent THC administration is associated with long-term microglia activation within the PFC and experimental evidence indicate a causal role for microglial activation in the development of THC-induced cognitive deficits.

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A placebo controlled study on effects of smokeless tobacco (snus) administration on exercise endurance and on cognitive task in non-smoker men

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Introduction

A proliferation of nicotine use in the sport environment has been observed in recent years mainly as smokeless tobacco (snus, SS). For this reason, nicotine has been listed on World Anti-doping Agency's Monitoring Program from 2012 to 2015 in order to detect potential patterns of abuse. However, little is known regarding SS potential performance-enhancing effects, especially on fatigue perception during endurance performance and cognitive tasks. The aim of this research was to assess SS effects on, i), exercise until exhaustion, and, ii), on the Iowa Gambling Task (IGT) test for decision-making processing.

Methods

Study 1. The study was a double-blind placebo controlled (SP) crossover design. We recruited 14 male non-smokers. Subjects were studied during three sessions on cycle-ergometer: experimental session 1 (Exp1) consisted on an incremental exercise test to determine maximal aerobic power output (Wmax); Exp2 and Exp3 consisted in exercise at 65% Wmax until exhaustion in SS or SP conditions. During the Exp2 and Exp3 muscle and cerebral oxygenation by means of NIRS (near-infrared spectroscopy) and the global rating of perceived exertion (RPE) were recorded. Before and after all experiments, subjects were administered the Profile of Mood of State questionnaire (POMS) and tested by means of Transcranial Magnetic Stimulation (TMS) to assess changes in cortico-motor excitability due to the prolonged exercise.

Study 2. The aim of this study was to measure the effect of SS administration with the Iowa Gambling Task (IGT) an experimental test to study decision-making. We recruited 40 male non-smokers. Subjects were randomized to blindly receive SS or SP on two different days according to a cross-over design. The Profile of Mood of State questionnaire (POMS) was administered before SS or SP administration and at the end of IGT.

Results

Study 1. Time to exhaustion (TTE) was 64.4 ± 41.5 min after SS and 51.6 ± 17.2 min after SP; paired Student's t-test showed a not significant difference (19.2%). RPE in the first 30 minutes during both sessions showed a significant difference after 10 minutes from start exercise. We found significant differences in the cerebral and muscular tissues oxygenation levels in the first 30 minutes of the exercise during SS and SP tests.

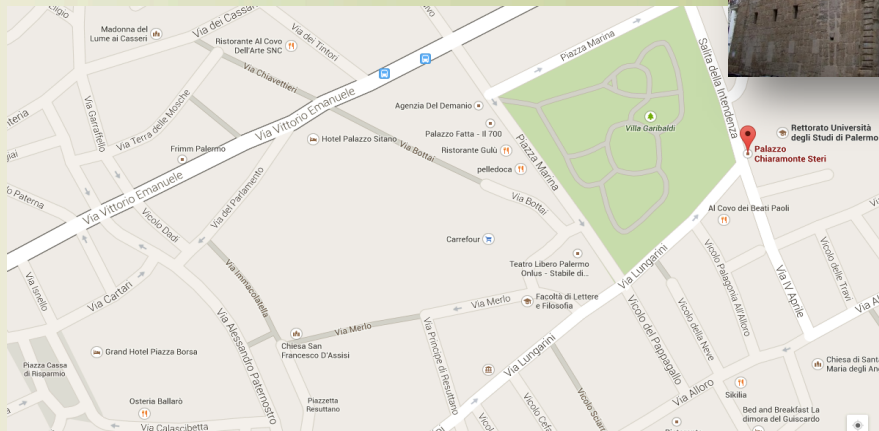
Study 2. No differences were observed in overall net score (SP vs. SS). A significant difference was observed for net scores in T1 (1-20 choices) between the SS and the SP condition ($p=0.0499$; paired Student's t-test). Twenty-three subjects reported adverse events at the end of SS session.

Conclusions

The study showed that the SS effect, compared to placebo condition, could not be an improvement of fatigue during an endurance exercise until exhaustion despite of an increased tissue muscular and cerebral oxygenation (*Study 1*). It seems that the SS may produces an early but transient cognitive improvement at IGT. However, adverse SS effects could have detrimental effects on performance in naïve users reducing the probability of the doping liability risk (*Study 2*).

General Informations

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Registration *Sala Magna, 2nd floor*
Friday, March 27th - 13:00 – 14:00

Poster Presentations *Sala delle Capriate, 3rd floor*
Friday, March 27th - 17:00- 18:00
Posters (max 70 cm wide, 100 cm long, portrait format) can be affixed starting from 14:30. Authors are requested to be in attendance at their poster for discussion during the Poster Session, and to collect it before leaving the venue.

Certificates of Attendance *Sala delle Capriate, 3rd floor*
Saturday, March 28th - 12:30-13:00

Program Book online Scientific Program and abstracts will be available online on the website of the Italian Society of Pharmacology (SIF)
http://www.sifweb.org/eventi/eventi_sif_monotematici.php



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