Convegno Monotematico SIF

IN COLLABORAZIONE CON IL GRUPPO DI LAVORO SULLE DIPENDENZE PATOLOGICHE

Vecchie e nuove droghe d'abuso tematiche ed approcci dalla ricerca

Aula De Sandre, Policlinico G.B. Rossi
***VERONA*, 28-29 NOVEMBRE 2013**

Comitato Scientifico:
Cristiano Chiamulera
Daniela Parolaro
Paola Fadda
Patrizia Romualdi
Carla Cannizzaro

Comitato Organizzatore Locale:
Cristiano Chiamulera
Guido Fumagalli
Anna Benini
Marco Venniro
Thomas Zandonai

ABSTRACTS

SOCIETÀ ITALIANA FARMACOLOGIA

Indice Primo Autore

HYPERSTIMULATION OF PRELIMBIC mPFC D1 RECEPTORS MEDIATES THE SUPPRESSANT EFFECT OF DOPAMINE BETA-HYDROXYLASE INHIBITORS ON COCAINE-SEEKING REINSTATEMENT

Antinori S.[1], Fattore L.[2,3], Frau R.[1,4], Saba P.[1], Zanda M.T.[1], Fratta W.[1,2], Gessa G.L.[1,2,4], Devoto P.[1,2,4]

[1]Dept. Biomedical Sciences and [2]Center of Excellence "Neurobiology of Addiction", Univ. Cagliari, Italy; [3]CNR Neuroscience Institute-Cagliari, Italy; [4]"Guy Everett Laboratory", Univ. Cagliari, Italy.

The dopamine-beta-hydroxylase (DBH) inhibitors disulfiram and nepicastat were shown to suppress cocaine-primed reinstatement of cocaine-seeking behavior in rats (1) and to markedly potentiate cocaine-induced extracellular dopamine (DA) increase selectively in the medial prefrontal cortex (mPFC) (2,3). This study was aimed to clarify if the suppressant effect of these DBH inhibitors on cocaine-induced reinstatement of cocaine-seeking behavior was mediated by the high extracellular concentrations of DA in the mPFC leading to a supra-maximal stimulation of D1 receptors (D1Rs) in the prelimbic division of the mPFC. Male rats were trained to self-administer cocaine (0.5 mg/infusion) intravenously under a fixed-ratio 1 schedule of reinforcement. After extinction of self-administration behavior, acute intraperitoneal (i.p.) cocaine priming (10 mg/kg) promptly reinstated drug-seeking behavior, an effect reversed by pretreatment with either disulfiram or nepicastat (50 mg/kg, i.p.). Similarly to the DBH inhibitors, L-DOPA (50 mg/kg, i.p.) potentiated cocaine-induced DA release in the mPFC and inhibited cocaine-primed reinstatement. Moreover, both DBH inhibitors and L-DOPA potentiated cocaine-induced extracellular DA increase in the mPFC during the reinstatement test sessions. Finally, a separate group of rats received bilateral microinfusion of the dopamine D1R antagonist SCH23390 (SCH) into the prelimbic mPFC at doses (0.3 and 1 μg/0.5 μl/side) which *per se* inhibited drug-induced cocaine-seeking reinstatement. Notably, SCH reverted disulfiram and L-DOPA suppressant effect on cocaine-induced reinstatement and rescued cocaine-seeking behavior. These results suggest that the suppressant effect of DBH inhibitors and L-DOPA on cocaine-induced reinstatement is mediated through an excessive extracellular DA concentration that leads to a supra-normal D1R activation in the rat prelimbic mPFC, and that D1Rs blockade reduces the excessive D1R stimulation to the optimal level required for reinstatement of cocaine-seeking.

1. Schroeder *et al.* (2010) Neuropsychopharmacology 35:2440-9.
2. Devoto *et al.* (2012) Psychopharmacology 219:1153-64.
3. Devoto *et al.* (2013) Addict Biol., in press.

NERVOUS SYSTEM AND PSYCHIATRIC DISORDERS WITH KETAMINE (AB)USE: AN ANALYSIS ON THE WHO GLOBAL INDIVIDUAL CASE REPORTS DATABASE (VigiBase)

Arzenton E. [1], Auber A. [2], Opri S. [1], Chiamulera C. [1], Leone R. [1]

[1]Pharmacovigilance Centre of Veneto Region and NeuroPsiLab, Section of Pharmacology, Department of Public Health and Community Medicine, University of Verona, Verona, Italy.
[2]Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institute of Health, 251 Bayview Blvd, 21224 Baltimore, Maryland, USA.

Background: Ketamine is an anaesthetic drug with dissociative, analgesic and psychedelic properties[(1)]; it also has a role in pain management[(2)]. The dissociative experience (the "K Hole"), that patients coming round from ketamine-based anaesthesia have reported, is pleasurable to some and has led to ketamine gaining in popularity as a recreational drug[(3)]. Like all medicines, ketamine may have adverse drug reactions (ADRs). These can be spontaneously reported and then collected in an international database. The aim of this study is to analyze these reports to understand in which cases ketamine was non-medical used and was abused. Methods: Data were retrieved from the WHO Global Individual Case Safety Reports (ICSR)

database (VigiBase) in which ADRs are coded with MedDRA terminology and grouped in different System Organ Classes (SOC). In order to find cases related to ketamine recreational use, reports associated to Nervous system disorders SOC and Psychiatric disorders SOC were selected and analyzed. We did not considered reports referred to patient younger than 12 years. Results: Between January 1972 and December 2012 VigiBase contains 1,302 reports related to ketamine. According to the selection criteria, 457 cases were identified. These reports were then classified in 3 groups: A) sure indication of ketamine abuse (*n* = 55); B) sure indication of not ketamine abuse (*n* = 169); C) doubt cases because the reported information were not enough to associate the report to group A or group B (*n* = 233). The main ADRs reported in group A were: drug dependence, paranoid reaction, personality disorder, drug abuser, acute psychosis. Conclusions: The spontaneous reporting is an important source to provide further knowledge on the ADRs related to ketamine use. The analysis of each report allows to identify ADRs related to ketamine and contribute to increase the knowledge on the use of ketamine as a recreational drug.

1. Wolff K. and Winstock A. R.(2006) CNS Drugs 20 (3): 199-218;
2. Morgan C.J.A. and Curran H.V.(2012) Addiction 107 (1): 27-38;
3. Wood D. and al. (2011) BJU Intern 107: 1881-1884

SIGNIFICANCE OF A CORTICOTROPIN-RELEASING FACTOR RECEPTOR 1 GENE POLYMORPHISM IN ANXIETY AND STRESS-LIKE BEHAVIOURS OF MARCHIGIAN SARDINIAN ALCOHOL-PREFERRING RATS

Ayanwuyi L.O., Cippitelli A., Domi E., Ciccocioppo R. and Ubaldi M.

School of Pharmacy, Pharmacology unit, University of Camerino, Camerino, Italy

Marchigian Sardinian alcohol preferring (msP) rats exhibit high stress sensitivity along with anxious-like phenotype. Genetic analysis showed that over-expression of the CRF system of msP rats is linked to two single nucleotide polymorphisms occurring in the promoter region of the CRF1 receptor (CRF1-R). We examined whether these point mutations are associated to heightened anxiety and increased stress sensitivity. The msP rats were re-derived to obtain two distinct lines carrying the point mutations (AA) and wild type (GG), respectively. The phenotype of these two rat lines were assessed in comparison with those of unselected Wistar rats on preclinical models of anxiety. Both msP lines demonstrated higher anxiety-like behaviour in the elevated plus maze and fear conditioning paradigms compared to Wistars. Surprisingly, the AA rats showed a decreased burying in the defensive burying compared to GG and Wistar, likely due to stress hyper-sensitivity. Restrained Wistars performed in a similar manner as AA. The selective CRF1-R antagonist, antalarmin dose-dependently reduced the burying in Wistar rats. In the GG rats however, antalarmin at 10 mg/kg significantly increased burying while at 20 mg/kg, it decreased the burying behaviour. Similar results were obtained for AA although the differences were not statistically significant. These data indicate that the polymorphisms at CRF1-R are associated to increased sensitivity to stress and inhibition of active behavioural responses to stress. Our results also suggest that there is a general innate dysregulation of the brain stress system common to msP rats that are not directly related to the polymorphisms.

Keywords: defensive burying, anxiety, stress, alcohol preferring rats

D-TMS IN COCAINE ADDICTION: PRELIMINARY FINDINGS

Bolloni C.[1], Panella R.[2], Pedetti M.[3], Frascella A. G.[3], Cannizzaro C.[4], Diana M.[2]

[1]Dept. of Experimental Biomedicine and Clinical Neuroscience, University of Palermo; [2]Laboratory of Cognitive Neuroscience 'G. Minardi', Dept. of Chemistry and Pharmacy, University of Sassari; [3]Ser.T. Marsciano, USL1 Umbria; [4]Dept. of Health Promotion and Maternal Care, University of Palermo.

Drug addiction is a brain disease which leads to profound disturbances in an individual's behaviour. In spite of the progress made in the understanding of the neurobiological mechanisms underlying addiction, expectations from a therapeutic point of view have not been satisfying. Given the modest efficacy of therapeutic tools available, Transcranial Magnetic Stimulation (TMS) seems to be a promising "non-pharmacologic" aid in various neuropathologies (1) including addiction (2) which is characterized by a decrease of dopaminergic activity (DA) (3-4). Thus, 'restoring' pre-pathology DA activity may yield clinical benefits in addicts (5). In particular, it has been reported (6) that TMS reduces the craving for cocaine in cocaine addicts. Thus, the aim of the project is to apply bilateral dTMS to the PFC of cocaine abusers in order to deepen understanding the neural correlates of addiction; to identify optimal parameters of stimulation; and, above all, to evaluate short/long term therapeutic effects of dTMS. Since December 2011 we applied dTMS in thirteen cocaine abusers (average age: 35; F: 2; M: 11) selected on DSM IV criteria and randomly assigned to real/sham stimulation protocols. Ten of them are currently included in the study while three abandoned due to personal problems (3 drop-out). We assessed the intake of cocaine through self-reports and hair analysis at different times pre- (T_0) and post-treatment (T_1 T_2 $T_{3..}$). The interim analysis shows that all subjects have reduced intake of cocaine regardless of the frequency (1 or 10 Hz) of the stimulation protocol applied (sham condition was administered in only four subject). Six months after the treatment (T_2) all treated subjects show a reduction in cocaine intake with no distinction among groups (real vs sham nor 10 vs 1 Hz). 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. 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.

1. Kobayashi et al., (2003) Clin Neurophys 56:211-219
2. Feil et al., (2010) Neurosci & Biobe 35:248-275
3. Melis et al.,(2005) Inter Rev Neurob 63:101-154
4. Koob et al., (2010) Neuropsychoph 35(1):217-38
5. Diana, (2011) Front Psych i2:64
6. Politi et al, (2008) Am J on Add, Vol. 17 (4) : 345-346

PHARMACOLOGICAL MODULATION OF OPERANT BEHAVIOUR FOR ACETALDEHYDE. INVOLVEMENT OF D2 AND CB1 RECEPTORS.

Brancato A., Plescia F., Marino R.A.M., Gambino G. and Cannizzaro C.

Dept of Sciences for Health Promotion and Mater and Child Care "G.D'Alessandro", University of Palermo.

Acetaldehyde (ACD), the first metabolite of ethanol, has rewarding and motivational properties, as shown by behavioural studies specifically tailored for studying addictive-like behaviour (1, 2). The rewarding and incentive effects of alcohol and others addictive substances, result from their capability to enhance mesolimbic dopamine (DA) transmission, as well as to affect the cannabinoid system, which is able to fine-tune the activity of DA neurons (3). ACD directly increases DA neurotransmission (4), but the neural underpinning the operant behaviour for oral-self administered ACD still remains poorly understood. Since D2 and CB1 receptors are involved in alcohol addiction (3), as well as their interplay (5), in thus study we aimed at investigating their contribution in ACD operant behaviour, able to capture the key aspects of addictive-like behaviour, such as the acquisition and maintenance during training, drug-seeking during extinction, and relapse after a-week deprivation. In doing so, we took advantage from the administration of a D2 receptor agonist, quinpirole (0.03mg/kg, i.p.), and a CB1 receptor antagonist, AM281 (1 mg/kg, i.p.), both administered during extinction and relapse sessions. Our results show that oral ACD readily induced the acquisition and maintenance of an operant self-administration paradigm, and sustained a reinstatement behaviour. Quinpirole was able to significantly decrease the number of lever presses for ACD during extinction ($p<0.05$), and ACD intake during relapse ($p<0.01$; $p<0.001$). AM281 administration showed a similar reduction in the number of lever presses for ACD during extinction ($p<0.05$) and relapse sessions ($p<0.05$; $p<0.001$). Oral ACD elicits clear motivational properties, and displays addictive-like features, which are modulated by D2 and CB1 signalling, receptors involved in impulsivity and addiction development (6, 7). These findings further strengthen the mandatory need for taking into account ACD's crucial role in ethanol-related behaviours.

1. Rodd-Henricks Z.A., Melendez R.I., Zaffaroni A., Goldstein A., McBride W.J. and Li T.K. (2002) Pharmacol Biochem Behav.
72(1-2):55-64;
2. Cacace S., Plescia F., Barberi I. and Cannizzaro C. (2012) Alcohol Clin Exp Res. 36(7):1278-87;
3. Vengeliene V., Bilbao A., Molander A. and Spanagel R. (2008) Br J Pharmacol. 154(2):299-315;
4. Melis M., Enrico P., Peana A.T. and Diana M. (2007) Eur J Neurosci. 26(10):2824-33
5. Fitzgerald M.L., Shobin E. and Pickel V.M. (2012) Prog Neuropsychopharmacol Biol Psychiatry. 38(1):21-9;
6. Trifilieff P. and Martinez D. (2013) Neuropharmacology http://dx.doi.org/10.1016/j.neuropharm.2013.06.031 [Epub ahead of
print];
7. Sidhpura N. and Parsons L.H. (2011) Neuropharmacology 61(7):1070-87.

STRESS RAPIDLY REORGANIZES THE GLUTAMATE SYNAPSE IN THE PREFRONTAL CORTEX OF COCAINE-WITHDRAWN ADOLESCENT RATS

Caffino L., Giannotti G., Racagni G. and Fumagalli F.

Dept. of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy and Collaborative Center of Dept. of Antidrug Policies, Presidency of the Council of Ministers, Rome, Italy.

Introduction: Adolescence is a developmental period characterized by impulsive choices which are associated with risky behaviours such as higher vulnerability to drug abuse. Among the different proteins that may contribute to such vulnerability, a critical role is played by glutamate. The main aim of our study was, therefore, to evaluate the response of the glutamate system to the long-term exposure to cocaine (20mg/kg/day) during adolescence [from postnatal day (PND) 28 to PND 42] and whether the rapid coping response of the glutamatergic synapse to an acute stress (5 minutes of swim stress and killing 15 min later) was influenced by the previous cocaine history.
Materials and methods: Critical determinants of glutamatergic homeostasis were measured in the medial prefrontal cortex (mPFC), which is still developing during adolescence and might be more vulnerable to stress, by means of Real Time PCR and Western blots. Circulating corticosterone levels were analyzed by ELISA and the time of immobility was measured by three independent investigators blind to the experimental design.
Results: The developmental exposure to cocaine influenced the response of the glutamatergic synapse to the acute stress by increasing the vesicular glutamate transporter, reducing glial glutamate transporters and increasing the activation of the NMDA receptor. This results in the activation of Cdc42 and phosphoPAK1 that may cause changes in spine structural plasticity. Notably, these effects were independent from the circulating levels of corticosterone. Additionally, cocaine-withdrawn rats exposed to stress spent more time immobile then their saline counterparts suggesting a pro-depressive phenotype.
Conclusion: These results indicate a coordinated series of changes, presumably through cocaine-induced reduction of baseline mPFC neuronal activity. This may result in a hyper-reactive glutamatergic synapse in the mPFC of rats with a prior cocaine history, when exposed to an acute stress. This cortical reorganization within a time frame of minutes may represent a contributing mechanism to the hypersensitivity to stress observed in abstinent cocaine addicts.

CHROMATIN REMODELING IN THE ENDOGENOUS OPIOID SYSTEM INDUCED BY MDMA

Caputi F. F. [1], Palmisano M. [1], Carretta D. [1], Carboni L., Candeletti S. [1], Romualdi P. [1].

[1]*Dept. of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Irnerio 48, 40126 Bologna, Italy.*

Introduction: The amphetamine 3,4-methylendioxymetamphetamine (MDMA, Ecstasy) is a psychostimulant addictive drug which induces several acute effects in humans: hyperactivity, hyperthermia, cognitive disturbances and elevated anxiety. MDMA acts primarily on the serotonin transporter, behaving as a substrate, blocking the serotonin reuptake. The effect induced by various psychostimulants can be modulated by the opioid system; even if the rewarding effects of MDMA have been well established in several species, the mechanisms leading to these effects are still unclear. Several studies have shown the involvement of the opioid system in the rewarding effects induced by MDMA and the epigenetic remodeling of chromatin has been already proposed as a mechanism underlying different addiction paradigms.
Aims and Method: The present study aimed to investigate epigenetic alterations induced by MDMA on the endogenous opioid system in an animal model. We performed acute (single injection, 8 mg/kg) or chronic (two injections a day for 7 days; 8 mg/kg) i.p. administration of MDMA in male Sprague-Dawley rats; 24h after last injection, nucleus accumbens (NA) and brainstem (BS) were dissected. MDMA effects on the epigenetic modifications at the promoter regions of prodynorphin, KOP, pronociceptin and NOP genes have been investigated using Chromatin Immuno Precipitation technique. The following histone modifications have been studied: H3K4me3 and H3K9Ac (activating markers), H3K27me3 and H3K9me2 (repressive markers).
Results: Specific histone modification changes at the promoter regions have been observed following both acute and chronic treatments. In NA, we observed a marked reduction in H3K9ac level after acute and chronic MDMA exposures, as well as in H3K9me2 level after acute treatment. No changes were observed in NOP promoter region, but the H3K4me3 level after acute MDMA exposure. H3K27me3 level showed a significant increase in prodynorphin gene promoter after chronic MDMA treatment, as well as a marked increase in H3K27me3, H3K9me2 and H3K9ac levels, after chronic MDMA treatment. In BS, a significant reduction in H3K27me3 and H3K9me2 levels at prodynorphin gene promoter was observed after both acute and chronic MDMA injection.
Discussion: Our data suggest that MDMA exposure evokes a chromatin remodeling in the endogenous opioid system, inducing a switch from hetero- to eu-chromatin in the promoter regions studied. These results are consistent with gene expression alterations, previously observed in our laboratory, therefore contributing to explain mechanisms underlying their regulation.
At last, these epigenetic mechanisms may contribute to clarify the gene expression modulation caused by MDMA exposure and, therefore, the long-lasting plasticity-related changes due to addictive drugs.

COULD THE UPS COMPLEX BE A NEW TARGET IN ADDICTION DISEASE ?

Caputi F.F., Carboni L., Candeletti S., Romualdi P.

Dept. of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Irnerio 48, 40126 Bologna, Italy.

One of the main issues in addiction disease studies is represented by the understanding of neuronal plasticity-related mechanisms and how the brain can change its structure and function in response to exposure to different drugs of abuse. Recent evidence demonstrates a role of the Ubiquitin-proteasome system (UPS) complex is not limited to the regulation of protein turnover but also in different neuronal activities, such as memory formation and reorganization (1). Moreover, among the new functions assigned to UPS complex is included a novel signaling role of ubiquitin in DNA repair and in protein kinases activation. Monoubiquitination and polyubiquitination have distinct signaling roles which make them as important signaling mechanisms that control distinct physiological and pathological processes (2). The 26S proteasome is a complex composed of two main structures: the core (20S), responsible for protein hydrolysis, and the regulatory part (19S), which is involved in substrate recognition (3). Specific genes encode for the different subunits in each of these structures. The aim of this study was to evaluate the gene expression regulation of specific proteasome subunits following exposure to different addictive drugs in human neuroblastoma SHSY-5Y cell line. To this purpose, cells were treated with 5μM cocaine or 40mM ethanol; 24 hours later the gene expression of three different proteasome subunits, namely 19S base subunit-Rpt2, 19S lid subunits-Rpn9 and 20S core subunit-ß1, was analyzed by Real time RT-PCR using specific TaqMan probes. Results showed an increase in Rpt2 subunit and a decrease of ß1 subunit gene expression after cocaine exposure; a decrease of ß1 subunit gene expression was observed after ethanol

treatment. No changes in Rpn9 subunit gene expression were detected following both treatments. These data suggest that cocaine and ethanol, despite displaying different mechanism of action, may share the proteasome as a common molecular target, in agreement with previous studies showing that the proteasome activity might be affected by different protocols of ethanol (4), morphine (5) or cocaine (6) exposures.

1. Jarome T.J. et al (2011) PLos One. 6:e24349.
2. Sun L. and Chen ZJ (2004) Curr Opin Cell Biol 16:119–126.
3. Gallastegui N. and Groll M. (2010) Trends BiochemSci 35:634–642.
4. LeCapitaine N.J. et al (2011) J Infect Dis. 204:1246-55.
5. Mouledous L. et al. (2005) Mol Pharmacol 68:467-476.
6. Ren Y-Z. et al.(2013) Neuropsychopharmacology. 38:778-790

PROLONGED " IN VITRO" EXPOSURE TO NICOTINE MODULATE THE FUNCTION OF PRESYNAPTIC GABA B AUTORECEPTORS PRESENT ON GABAERGIC NERVE ENDINGS IN RAT HIPPOCAMPUS.

Chen J., Salamone A., Zappettini S., Grilli M., Olivero G., Pittaluga A., Marchi M.

Dept. of Pharmacy., University of Genova, Genova, Italy

We investigated using different experimental models (1) on the functional interaction between presynaptic nicotinic receptors and other presynaptic receptors coexisting on the same terminal. We have provided evidence of the existence of a functional cross talk between nicotinic receptors and AMPA and NMDA receptors on dopaminergic nerve terminals in the nucleus accumbens. The in vitro short-term pre-exposure of synaptosomes to 30 μM nicotine or 5IA85380 caused ,as expected, a significant reduction of the 30 μM nicotine stimulatory effect but also significantly decreased the NMDA- and AMPA-evoked [3H]Dopamine (DA) overflow. This reduction was completely counteracted when synaptosomes were pretreated with nicotine plus mecamylamine or in presence of the selective antagonist DHβE indicating that the changes of the NMDA-dependent DA release reported were dependent to the activation of a β2* nAChR subtype. The in vitro short-term pre-exposure of synaptosomes to nicotine decreased also the AMPA-evoked release of noradrenaline but did not modify the functional responses of AMPA receptors present on GABAergic hippocampal nerve terminals. In this presentation we show that the exposure to different nicotinic agonists did not modify also the NMDA receptors present on GABA hippocampal synaptosomes. Immunocytochemical studies have shown that a significant percentage of hippocampal terminals were GABAergic and that some of them posses both NMDA and nACh receptors which contain the α4 and α7 nAChR subunits. Conversely, we have provided evidence that the in vitro short-term pre-exposure of synaptosomes to 30 μM nicotine caused a significant increase of the inhibitory effect of baclofen on the release of GABA suggesting an increase of the presence or of the function of GABA B autoreceptors on nerve terminals. In conclusion our results show that ACh, AMPA, NMDA and GABA B receptor function can be dynamically, positively or negatively, regulated, possibly in a different manner, in neurons in response to a brief incubation with nAChRs agonists.

1. Grilli M, Summa M, Salamone A, Olivero G, Zappettini S, Di Prisco S, Feligioni M, Usai C, Pittaluga A, Marchi M. Neuropharmacology 2012, 63(5):916-926.

This work was supported by grants from Italian MIUR (Ministero dell'Istruzione, dell'Universita` e della Ricerca, Projects n. 2009P7WHNR_003 AP; Projects n 2009R7WCZS_003 MM), from the European Community (Project Strategia d'Impresa in Settori di Nicchia per l'Economia Agro-Industriale del Mediterraneo, Pyrgi, project n° B51H10000000006 and project Progetto 7FP-KBBE BAMMBO

PPARγ AGONISM AS A NOVEL TREATMENT FOR OPIOID ADDICTION DEVOID OF ABUSE-LIABILITY.

de Guglielmo G.[1], Melis M.[2], De Luca M.A.[2,], Kallupi M.[1], Li H.W.[1] and Ciccocioppo R.[1]

[1]*School of Pharmacy, Pharmacology Unit, University of Camerino, Via Madonna delle Carceri, 62032 Camerino, Italy.*
[2] *Department of Biomedical Sciences, University of Cagliari, 09042 Cagliari, Italy*

Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-activated transcription factors of the nuclear hormone receptor superfamily. PPARγ is one of the three isoforms identified for the PPARs and is the receptor for the thiazolidinedione class of anti-diabetic medications including pioglitazone. PPARγ has been long studied for its role in adipogenesis and glucose metabolism, but the discovery of the localization in ventral tegmental area (VTA) neurons opens new vistas for a potential role in the regulation of reward processing and motivated behavior in drug addiction.
Using animal models of self-administration, here we demonstrate that activation of PPARγ by pioglitazone (30 and 60 mg/kg PO) reduces the motivation for heroin and attenuates its rewarding properties. These effects are associated with a marked and selective reduction of heroin-induced (0.4 mg/kg IP) elevation of extracellular DA levels in the nucleus accumbens (NAc) shell as measured by *in vivo* microdialysis. Through *ex vivo* electrophysiology in acute midbrain slices, we also show that stimulation of PPARγ attenuates opioid-induced excitation of VTA dopamine neurons via a reduction of presynaptic GABA release from the rostromedial tegmental nucleus (RMTg). Consistent with this finding site-specific microinjection of pioglitazone (5 µg/0.6 µL) into the RMTg reduced heroin taking.
Our data illustrate that activation of PPARγ by the insulin sensitizing agent pioglitazone may represent a new pharmacotherapeutic approach for the treatment of opioid addiction.

KETAMINE ACUTE EFFECTS ON mTOR PATHWAY IN MICE AND RATS

Di Chio M. [2], Bono F.[1], Cavalleri L.[1], Atanasio S. [2], Tedesco V.[2], Collo G[1], Chiamulera C. [2]

[1]*Department of Molecular and Translational Medicine, Division of Pharmacology, University of Brescia, Italy.*
[2]*Department of Medicine and Public Health, Section of Pharmacology, University of Verona, Italy.*

It has been shown that a low acute dose of dissociative anaesthetic ketamine (KET) induces a rapid induction of neuroplasticity. We assessed the effects of KET on expression levels of mTOR downstreaming kinase p70S6K and of ribosomal protein S6 phosphorylation (rpS6P).
Our aim was to examine the effect of acute KET administration on these markers in drug addiction related cerebral areas.
Rats or mice were treated with KET 5, 10 mg/kg or vehicle i.p.. After 1 hour, brains were removed and processed for immunohistochemical assessement of p70S6K and rpS6P in prelimbic (PRL) and infralimbic (IL) cortices, nucleus accumbens core (NAcC) and nucleus accumbens shell (NAcS), hippocampus (CA1 and CA3), and basolateral amygdala (BLA).
A significant dose-related increase in rpS6P expression was found in PRL, IL, BLA, NAcC but not in the NAcS and hippocampus in rats, whereas only in PRL, IL, hippocampus and VTA in mice only at the lower dose. No effect on p70S6K was observed at either 5 or 10 mg/kg ip.
These data confirm acute KET-induced neuroplasticity effects, even if with some specie-dependence, and extend these findings to drug addiction-related brain areas.

ACTIVATION OF PPARγ PIOGLITAZONE PREVENTS SOMATIC AND AFFECTIVE NICOTINE WITHDRAWAL SIGNS IN RATS AND MICE.

Domi E., Ciccocioppo R., Scuppa G., and Ubaldi M.

School of Pharmacy, Pharmacology Unit, University of Camerino, Via Madonna delle Carceri, 62032

Camerino, Italy.

Nicotine is a potent psychoactive drug, worldwide abused. It's positive reinforcing effects, like mild euphoria, relaxation, and improved attention play a crucial role in the initiation of smoking and may lead to tobacco dependence. Discontinuation of smoking leads to negative affective symptoms such as depressed mood, increased anxiety and impaired memory performances. These severe affective withdrawal symptoms increase the risk of relapse to smoking. In our laboratory it has been recently shown that pioglitazone, a PPARg agonist, plays a critical role in alcohol and heroin dependence in rats.
Based on this background we studied the effect of pioglitazone on spontaneous withdrawal signs in Wistar rats following 7 days of nicotine treatment throught implanted transdermal nicotine patches (5.2 mg/rat/day). The effect of pioglitazone (15.0, 30.0 mg/kg, *via gavage)* was assessed on somatic and affective signs of withdrawal observed at 16 hours and 6 days, respectively. Furthermore we analyzed the effect of pioglitazone on spontaneous nicotine withdrawal signs on conditional neuronal PPARg knock-out (KO) mice and their wild type counterparts (WT). Dependence was induced by injecting nicotine (*s.c*) 2 mg/kg, 4 times per day for 8 consecutive days. The effect of pioglitazone was estimated on somatic withdrawal signs(20 hours after the last injection) and withdrawal-induced anxiety (6 days after the last injection) by the elevated plus maze test. Moreover we also tested the effect of the PPARg antagonist GW9662 (5mg/kg *i.p*) on the withdrawal-induced anxiety in the WT mice.
The results showed that pioglitazone was able to reduce somatic and affective nicotine withdrawal signs in the rat. Moreover pioglitazone reduced the somatic and affective nicotine withdrawal signs in the WT but not in PPARg KO mice. The antagonist GW9662 reversed the anxiolytic effect of pioglitazone in the wild type mice demonstrating that the reduction of nicotine withdrawal signs is mediated by PPARg. These preliminary findings suggest that pioglitazone may present a promising drug for the smoking cessation treatment.

ASSESSMENT OF DNA DAMAGE IN CANNABIS SMOKERS BY MICRONUCLEUS AND ALKALINE COMET ASSAY.

Doria D[a], Bosco O[b], Lanza A[b], Scotton A[a], Serpelloni G[c] and Fracasso ME[a].

[a] *Department of Public Health and Community Medicine, Section of Pharmacology, University of Verona*
[b] *Department of Addictions ULSS 20, Verona*
[c] *Chief of Department for Antidrug Policies, Presidency of the Council Ministers*

Introduction. The cannabis is the illegal substance most commonly used within many societies, mostly used by the young people, and widely perceived to be safe. Marijuana smoke contains several of the same chemical substances as those tobacco smoke (except nicotine), all producing reactive oxygen species (ROS). The aim of this study was to determine if the use of cannabis smoke induces direct DNA damage in oral mucosa cells and if the frequency of genotoxic events is related to the levels of the cellular oxidative status, to extent of the marijuana use, and/or to the combination of other illicit drugs. Materials and methods. The sample collection was performed at the Department of Addictions ULSS 20, Verona. The mucosa cells were collected by gentle brushing of the internal part of the cheeks of 55 marijuana smokers and 50 subjects (tobacco smokers), as controls. The comet assay, a microgel electrophoresis technique, was used for detecting DNA damage at level of the single cell. The DNA damage (TM, TI and TL) was evaluated. The cellular presence of micronuclei (MN) and other nuclear anomalies (binucleated cells and buds) were examined under microscope. The cellular oxidative status was evaluated by GSH content. Urine samples were collected to check other illicit drugs.Results. The comet parameters show a significant increase in tail intensity (TI) and a decrease in tail length (TL) in the marijuana- compared to the tobacco-smokers (controls), an increase of micronucleus and a decrease in GSH content in the marijuana cells. A significant negative correlation between TL and THC, and a good positive correlation between TI and THC. A negative significant correlation with tobacco smoke and a significant reduction in cellular GSH content in marijuana smokers.Conclusions. Both tests show that the marijuana smoke leads to significant increases in the parameters of DNA damage of oral mucosa cells. Comet and micronucleus test show significant increases in tail intensity (TI) and in micronucleus (MN), respectively. The great fall of intracellular levels of glutathione (GSH), important scavenger of reactive oxygen species (ROS), may be decisive in causing the DNA damage

ACETALDEHYDE MODULATES DENDRITIC SPINES IN THE NUCLEUS ACCUMBENS AFTER CHRONIC TREATMENT.

Fois G.R. [a], Muggironi G. [a], Mulas G. [b], Peana A.T. [a], Spiga S. [b], Diana M. [a]

[a] *'G.Minardi' Cognitive Neuroscience Lab., Department of Chemistry and Pharmacy, University of Sassari, 07100 Sassari, Italy.*
[b] *Department of Life and Environmental Science, University of Cagliari, Cagliari, Italy.*

Acetaldehyde (ACD), the first metabolite of ethanol (EtOH), appears to be involved in many EtOH psychoactive effects including activation of VTA dopamine (DA) neurons (1; 2) and motivational properties (3; 4). The aim of this study was to investigate possible ACD-induced changes in dendritic spines of medium spiny neurons (MSN) of the Nucleus Accumbens shell (Naccs). ACD was chronically administered to rats in a modified liquid diet for a total of 21 days. Rats were divided into two groups: 1) liquid diet without ACD; 2) liquid diet with ACD (0,15 %). Rats belonging to group 2 were further divided into 2 subgroups: a) sacrificed, without ACD suspension; b) sacrificed 12 hours after ACD suspension.
Subjects were then prepared for histology, utilizing a new method to visualize in the same slice spine's morphology, TH-positive fibers and PSD-95 positive. Confocal analysis reveals a loss of dendritic spines in MSN (37%), accompanied by a reduction of TH-positive terminals (73%) and PSD-95 positive elements (68,5%). Further analysis indicates that mature spines as long-thin are selectively affected. These changes occur only in the group b. The reduction of TH-positive terminals, PSD-95 and long-thin spines suggests a profound architectural remodeling of the accumbal synaptic triad. These results indicate functional consequences of these structural modifications and provide further evidence for an active role of ACD in synaptic plasticity in the Naccs.

Key Words: Acetaldehyde, Dendritic spines, Accumbens, Dopamine.

1. Foddai M, Dosia G, Spiga S, Diana M.(2004) Neuropsychopharmacology. 2004 Mar;29(3):530-6.
2. Melis M, Enrico P, Peana AT, Diana M. (2007) Eur J Neurosci. 2007 Nov;26(10):2824-33.
3. Peana AT, Assaretti AR, Muggironi G, Enrico P, Diana M.(2008) Alcohol Clin Exp Res. 2009 Jan;33(1):43-8. doi: 10.1111/j.1530-0277.2008.00809.x. Epub 2008 Oct 18.
4. Peana AT, Muggironi G, Diana M.(2010) Front Psychiatry. 2010;1:23. doi: 10.3389/fpsyt.2010.00023.

ADOLESCENT DELTA-9-TETRAHYDROCANNABINOL (THC) RESULTS IN ABNORMAL GLUTAMATE AND DOPAMINE NEUROTRANSMISSION WITHIN THE ADULT RAT PREFRONTAL CORTEX

Gabaglio M.[1], Zamberletti E.[1], Prini P.[1], Rubino T.[1] and Parolaro D.[1,2]

[1] *Dept. of Theoretical and Applied Sciences, Univ. of Insubria, Busto Arsizio (VA), Italy;*
[2] *Zardi-Gori Foundation, Milan, Italy.*

Exposure to THC during adolescence results in a complex schizoaffective-like phenotype in adulthood characterized by the presence of cognitive deficits, altered emotional reactivity and sensitization to acute phencyclidine (PCP) administration. Interestingly, no behavioral abnormalities are observed when THC is administered in adulthood, suggesting a specific vulnerability of the adolescent brain to the long-term adverse effects of cannabinoids. Since maturation of the prefrontal cortex (PFC) is one of the most important processes during adolescence, THC may predominantly affect the maturation of specific neurocircuits within this brain region, leading to abnormal behavioral responses in adulthood. Therefore, in the present study, we investigated the impact of adolescent THC exposure on the maturation of glutamate and dopamine systems in the PFC. To this aim, adolescent female Sprague-Dawley rats were treated with increasing doses of THC twice a day from PND 35 to 45 and in adulthood (PND 75) a series of biochemical investigations were performed in order to check for the presence of alterations in glutamate and dopamine markers. In the PFC of adult THC-treated rats significant changes in glutamate neurotransmission were

present. Indeed, basal glutamate release was significantly reduced by about 30% compared to controls. Intriguingly, a reduction of VGLUT and EAAT1 levels was also present, suggesting that alterations in both glutamate release and reuptake could be responsible for the observed reduction in glutamate release. Adolescent THC treatment induced significant changes also in NMDA and AMPA receptors. Specifically, the amount of GluN2B-containing NMDA receptors and GluA1-containing AMPA receptors was significantly higher in adult THC-treated rats compared to controls. Besides NMDA and AMPA receptors, metabotropic glutamate receptors (mGluR), in particular mGluR5 receptors, are also involved in the maintenance of synaptic plasticity and alterations in this receptor have been reported in drug addiction. We found that mGluR5 expression was significantly reduced within the PFC of adult THC-treated animals, possibly suggesting that the inhibitory feedback exerted by the endocannabinoid system is also impaired in these animals. Finally, as the PFC receives major dopaminergic innervation from the mesocortical dopamine projection from the ventral tegmental area (VTA) which are important in regulating pyramidal neuron activity, we checked whether alterations in dopamine markers were present in our experimental model. Interestingly, adolescent THC treatment leads to imbalances in dopamine D1 and D2 receptor densities within the PFC: in fact, a downregulation of D1 receptors was present, together with an upregulation of D2 receptors. In line with the observed changes in dopamine receptor densities, we found that also dopamine synthesis was affected by adolescent THC exposure, as tyrosine hydroxylase levels were significantly reduced in THC-treated rats compared to controls. As a whole, these findings indicate that adolescent THC exposure results in abnormal glutamate and dopamine neurotransmission within the adult PFC, which may be related to the schizoaffective-like phenotype observed in these animals.

Acknoledgements: THC was kindly provided by GW Pharmaceuticals (UK); This work was funded by Dipartimento delle Politiche Antidroga (Rome, Italy). GM has a pre-doctoral fellowship from Dipartimento delle Politiche Antidroga.

ETHANOL DEPENDENCE AND WITHDRAWAL: MOLECULAR MECHANISMS IN IMMATURE AND MATURE RAT ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES.

Gerace E.[1], [1]Landucci E.[1], Totti A.[1], Scartabelli T.[1], Moroni F.[2], Mannaioni G.[2] & Pellegrini-Giampietro D.E.[1]

[1]Department of Health Sciences, Clinical Pharmacology and Oncology Unit and [2]Department of Neuroscience, Psychology, Drug Research and Child Health (NeuroFarBa), University of Florence, Italy.

Chronic ethanol consumption causes persistent molecular alterations of neuronal circuits by mechanisms that are not yet fully understood. We investigated the mechanisms of ethanol dependence by exposing either immature (2 days *in vitro*) or mature (10 days *in vitro*) rat organotypic hippocampal slices to ethanol (100-300 mM) for 7 days. Ethanol was than withdrawn for 24 h and slices were incubated with propidium iodide to detect cell death. We found that ethanol withdrawal led to a dose-dependent CA1 pyramidal cell injury in mature but not in immature slices. To study the mechanisms for this differential response to ethanol, we analyzed the expression levels of presynaptic (vGlut1, vGlut2, CB1 receptor, synaptophysin) and postsynaptic (GluA1, GluA2, NR2A, NR2B) proteins in immature and mature slices after chronic treatment (dependence) or after ethanol withdrawal. Under both conditions, we observed a decrease in GluA1, GluA2 and synaptophysin expression levels in immature slices and a significant increase in the GluA1/GluA2 ratio in mature slices. Using whole cell voltage-clamp recordings from CA1 pyramidal cells of immature or mature slices, we measured the frequency and the amplitude of sEPSCs 7 days after exposure to ethanol and 24 h after ethanol withdrawal. Our electrophysiological results show a reduction in the frequency of sEPSCs in immature slices and a significant increase in the amplitude of sEPSCs in mature slices. Electron microscopy revealed disorganization of dendritic microtubuli in immature slices and signs of apoptotic cell death in mature slices. These results indicate that in immature slices ethanol induces an impairment of excitatory synaptic transmission similarly to what observed in fetal alcohol syndrome. In mature slices ethanol withdrawal leads to CA1 pyramidal cell death possibly due to expression of Ca^{2+}-permeable AMPA receptors.

REPEATED COCAINE TREATMENT DURING ADOLESCENCE ALTERS BDNF SIGNALING IN THE PREFRONTAL CORTEX OF ADULT RATS

Giannotti G., Caffino L., Racagni G. and Fumagalli F.

Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy and Collaborative Center of Department of Antidrug Policies, Presidency of the Council of Ministers.

Introduction: Although evidence exists that chronic cocaine exposure at adulthood is associated with changes in BDNF expression, whether and how cocaine exposure during adolescence modulates BDNF is still unknown. Evidence exists that adolescents are more sensitive than adults to drug abuse suggesting that adolescence might represent a factor of vulnerability for drug addiction.
Materials and methods: Male adolescent rats were repeatedly exposed to cocaine during adolescence (20 mg/kg), from postnatal day (PND) 28 to PND 42, and the animals were sacrificed after 48 days of withdrawal, at PND 90. We focused our analyses on BDNF and its associated network. The molecular analyses were carried out using RT-PCR technique. Protein levels were analyzed using the western blot technique. Our analyses were performed in the medial prefrontal cortex (mPFC) that is still developing during adolescence and may, therefore, be a sensitive target of the repeated administration of cocaine.
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 PND 90 rats exposed to cocaine during adolescence, an effect sustained by changes in BDNF exon IV through the transcription factors CaRF and NF-kB. The enhancement of BDNF mRNA levels was paralleled by an increase of BDNF precursor and mature forms of the protein and by the preferential activation of the trkB/AKT pathway, that ultimately produced an up-regulation of Arc and a consequent reduction of GluA1 expression in the mPFC of PND 90 cocaine-treated rats.
Conclusion: These findings demonstrate that developmental exposure to cocaine dynamically dysregulates BDNF and its signaling network in the mPFC of adult rats, providing novel mechanisms that may contribute to cocaine-induced changes in synaptic plasticity.

CANNABINOID-INDUCED ALTERATION IN THE EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) SIGNALLING PATHWAY IN MALE, FEMALE AND OVARIECTOMIZED RATS

Giugliano V.[1]**,** Rosas M.[2], Antinori S.[1], Fratta W.[1,3,4], Acquas E.[2,3,4], Fattore L.[3,5]

[1]Dept. Biomedical Sciences, [2]Dept. Life and Environm Sciences-Pharmaceutical and [3]Centre of Excellence "Neurobiology of Addiction", Univ Cagliari, Italy; [4]National Institute of Neuroscience and [5]CNR Institute of Neuroscience, Italy

Drug addiction is considered as a neuroadaptive disorder characterized by dis-regulation of the mesocorticolimbic dopamine (DA) reward system. Phosphorylated Extracellur signal-Regulated Kinase (pERK) in dopamine-rich terminal areas plays a critical role in several psychopharmacological effects of addictive drugs, including cannabinoids. Accordingly, activation of cannabinoid CB1 receptors was found to induce a progressive increase of pERK in the striatum and nucleus accumbens (Acb) of male mice and it has been suggested that ERK activation in the mesocorticolimbic system plays a crucial role in mediating behavioral responses related to the addictive properties of drugs of abuse (1). Self-administration of the CB1 receptor agonist WIN55,212-2 (WIN, 12.5 microg/kg/infusion)) in Lister Hooded rats, depends on sex, intact female rats being more sensitive than males to the reinforcing properties of cannabinoids, and on oestrous cycle, ovariectomized (OVX) females being less responsive than intact females (2,3). In this study we investigated the influence exerted by sex and ovarian hormones on the degree of pERK expression following an acute intravenous infusion of WIN (0.3 mg/kg, 30 or 60 min before brain perfusion) or vehicle in the prefrontal cortex (PFCx) and Acb of female and male rats. Our data showed that, after treatment with WIN, in

male rats pERK expression increased in the PFCx and in the Acb, respectively, after 60 and 30 minutes. Conversely in both intact and OVX females, WIN did not induce significant effects on expression pERK.
These findings suggest that sex, but not the ovarian hormones, affects cannabinoid-induced activation of pERK in both areas in a time-dependent manner. This different expression of pERK could represent a possible molecular mechanism underlying the observed sex-dependent differences in cannabinoid addiction.

1.Valjent et al. (2004) *Eur J Neurosci* 19, 1826-36.
2. Fattore et al. (2007) *Br J Pharmacol* 152, 795-804.
3. Fattore et al. (2010) *Br J Pharmacol* 160, 724-3.

USE OF SYNTHETIC CANNABINOIDS TO CHEAT TOXICOLOGICAL SCREENINGS

Gottardo R., Sorio D., Bertaso A., Musile G., Tagliaro F.

Dept. of Public Health and Community Medicine, Unit of Forensic Medicine, University of Verona, Verona, Italy

Introduction: In its most recent annual report, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has pointed out an increasing diffusion of the "New Psychoactive Substances" (NPSs), as well as of the NPSs correlated intoxications [1-2]. The increasing availability of NPSs, and particularly of synthetic cannabinoids, in the illicit market is not paralleled by the possibility of identifying the penetration of their use in the population because of the lack of suitable analytical methods for their determination in the biological samples. The aim of the present study was test in real cases the application of the screening of NPSs in hair, based on LC-QTOF and LC-QQQ in order to study NPS-related histories and to support the epidemiological surveys on the penetration of the NPSs use in the population.
Methods: Hair samples (n= 600) were collected from subjects to whom the driving license had been suspended/withdrawn for "driving under the influence" of psychoactive drugs in years 2009-2012, who were controlled by toxicological analysis of the hair to exclude any relapse to drug abuse, in view of driving license re-issuing. Hair samples (~100 mg) were cut, incubated o.n. at 45° C under alkaline conditions and the mixtures underwent liquid-liquid extraction.
Results: Several of the synthetic cannabinoids available in the Italian clandestine market of NPS were found in the hair of a significant percentage of this group, although claiming long term abstinence from illicit drugs and resulting negative at the current hair/urine testing.
Conclusions: The presented data may suggest an intentional use of synthetic cannabinoids to cheat the current toxicological screenings, traditionally limited to the "NIDA five" (i.e. opiates, natural cannabinoids, cocaine and metabolites, amphetamines and methamphetamines, phencyclidine). This hypothesis greatly supports the need of further research to develop new analytical technology able to unravel the intricate problems of monitoring the diffusion of the NPS in the young generations and its consequences in terms of acute intoxications and traffic and occupational accidents.

1. Gibbons S, 'Legal highs'--novel and emerging psychoactive drugs: a chemical overview for the toxicologist. Clin Toxicol (Phila) 2012; 50: 15-24
2. European Monitoring Centre for Drugs and Drug Addiction, Annual report 2011 and 2012. Publications Office of the European Union, 2011 and 2012.

NICOTINE PRE-EXPOSURE MODULATES NMDA RECEPTORS FUNCTION IN DOPAMINERGIC TERMINALS OF THE RAT NUCLEUS ACCUMBENS

Grilli M., Salamone A., Zappettini S., Olivero G., Chen J., Pittaluga A., Marchi M.

Dept. of Pharmacy., University of Genova, Genova, Italy

The presynaptic control of dopamine release in the nucleus accumbens (NAc) by glutamate and acetylcholine has a profound impact on reward signaling. Here we provide immunocytochemical and

neurochemical evidence (1) supporting the co-localization and functional interaction between nicotinic acetylcholine receptors (nAChRs) and N-methyl-D-aspartic acid (NMDA) receptors in dopaminergic terminals of the NAc. Most NAc dopaminergic terminals possessed the nAChR a4 subunit and the pre-exposure of synaptosomes to nicotine (30 μM) or to the $\alpha4\beta2$-containing nAChR agonist 5IA85380 (10 nM) selectively inhibited the NMDA (100 μM)-evoked, but not the 4-aminopyridine (10 μM)-evoked, [^{3}H] dopamine outflow. This inhibition was counteracted by mecamylamine (10 μM). Nicotine and 5IA85380 also inhibited the NMDA (100 μM)-evoked calcium transients in single nerve terminals, an effect prevented by dihydro-β-erythroidine (1 μM). This indicates a functional interaction between $\alpha4\beta2$-containing nAChR and NMDA receptors within the same terminal, as supported by the immunocytochemical co-localization of $\alpha4$ and GluN1 subunits in individual NAc dopaminergic terminals. The NMDA-evoked [^{3}H]-dopamine outflow was blocked by MK801 (1 μM) and inhibited by the selective GluN2B-selective antagonists ifenprodil (1 μM) and RO 25-6981 (1 μM), but not by the GluN2A-preferring antagonists CPP-19755 (1 μM) and $ZnCl_2$ (1 nM). Notably, nicotine pretreatment significantly decreased the density of biotin-tagged GluN2B proteins in NAc synaptosomes. These results show that the recruitment of nAChRs dynamically and negatively regulate NMDA receptors in NAc dopaminergic terminals through the internalization of GluN2B receptors.

1. Grilli M, Summa M, Salamone A, Olivero G, Zappettini S, Di Prisco S, Feligioni M, Usai C, Pittaluga A, Marchi M. Neuropharmacology 2012, 63(5):916-926.

This work was supported by grants from Italian MIUR (Ministero dell'Istruzione, dell'Universita` e della Ricerca, Projects n. 2009P7WHNR_003 AP; Projects n 2009R7WCZS_003 MM), from the European Community (Project Strategia d'Impresa in Settori di Nicchia per l'Economia Agro-Industriale del Mediterraneo, Pyrgi, project n° B51H10000000006 and project Progetto 7FP-KBBE BAMMBO

BUPRENORPHINE REDUCES COCAINE SELF ADMINISTRATION IN RATS, THROUGH ACTIVATION OF THE NOCICEPTIN/ORPHANIN FQ-NOP RECEPTOR SYSTEM

Kallupi M., de Guglielmo G., Stopponi S., Ubaldi M. and Ciccocioppo R.

School of Pharmacy, Pharmacology Unit, University of Camerino, Camerino, Italy.

Buprenorphine is a partial agonist at mu-opioid and NOP receptors. It has mixed but primarily antagonistic actions on kappa-opioid and delta-opioid receptors. Buprenorphine's effect on the reduction of cocaine has been documented. Because of its opioid agonist effects, buprenorphine is abusable, therefore naloxone a mu-opioid receptor competitive antagonist is added in order to decrease its abuse liability. While the in vivo properties of buprenorphine have been characterized, the underlying pharmacology and signaling, particularly in cocaine addiction, remains poorly understood and needs more extensive pharmacological evaluation. Here we investigated in rodents, whether the potential reduction of cocaine intake induced by buprenorphine was mediated via activation of NOP receptors. Rats trained to self-administer cocaine 2 hours/day were injected with buprenorphine (0.3, 1 and, 3.0 mg/kg) intraperitoneally 90 min before access to cocaine. The effect of Naltrexone (0.25, 1.0 and 2.0 mg/kg) and of the selective NOP antagonist SB-612111 (10.0 and 30.0 mg/kg) was also tested on cocaine self-administration. The effectiveness of Naltrexone and of SB-612111 on the reduction of cocaine self-administration induced by Buprenorphine was subsequently explored. In conclusion, the concomitant administration of Naltrexone and SB-612111 was tested on the blockade of cocaine intake in rats induced by buprenorphine. Here we confirmed that buprenorphine decreased cocaine self-administration in rats in a dose dependent manner. Naltrexone and the selective NOP antagonism SB-612111 alone, did not affect cocaine self-administration, moreover, these drugs alone, failed to alter the reduction of cocaine self administration in rats induced by buprenorphine. Interestingly, the concomitant administration of Naltrexone and SB-612111 completely reversed the reduction of cocaine self-administration induced by buprenorphine. Taken together, these data lead to suggest that drugs with a NOP agonistic and mu-opioid agonistic profile, can decrease cocaine self-administration with minimal liability to produce opioid dependence and may be useful as a treatment for cocaine addiction.

INHIBITION OF PDE7 ACTIVITY AS A POTENTIAL NEW APPROACH TO TREAT NICOTINE ADDICTION

Li H.W., De Guglielmo G., Cippitelli A., Ayanwuyi L.O., Ciccocioppo R.

University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that regulate the cellular levels of the second messengers, cAMP and cGMP, by controlling their rates of degradation. PDE7 inhibitors raise cellular levels of cAMP by reducing degradation of cAMP through phosphodiesterase activity. Here we evaluated the role of PDE7 inhibitors in nicotine addiction.
Using operant self-administration paradigms, PDE7 inhibitor was tested for its effect on fixed ratio 3 (FR3) and progressive ratio (PR) of nicotine self-administration. Moreover we tested the effect of intra VTA injections of PDE7 on nicotine self-administration. The PDE7 inhibitor was also tested on nicotine seeking behavior after stress- and cue-induced relapse. Finally, we tested the effect of PDE7 inhibitor on food self-administration.
Results showed that PDE7 inhibitor (3.0 and 9.0 mg/kg) significantly reduced fixed ratio 3 (FR3) and progressive ratio (PR) of nicotine self-administration, but not food self-administration. The reinstatement experiments showed that PDE7 inhibitor exhibited significant effects in preventing stress- and cue-induced relapse to nicotine seeking behavior at all the doses tested (0.3, 1.0 and 3.0 mg/kg). Site-specific micro-injection of the PDE 7 inhibitor (1.0 µg/0.6 µL) into VTA significantly reduced nicotine self-administration. The VTA appears to be the brain site of action for these effects of PDE7 inhibitors. These results demonstrate a potential role for PDE7 inhibitors in the treatment of nicotine addiction.

EXPOSURE TO ADVERSE ENVIRONMENT DURING PRE-ADOLESCENCE INDUCES VULNERABILITY TO DRUG-ADDICTION BEHAVIOR IN ADULT MICE

Lo Iacono L[1], Visco Comandini F[1,2], Caputo P[1], Alari MG[1], Furfaro R[1], Cabib S[1,3], Puglisi-Allegra S[1,3] and *Carola V[1]*

1. *Santa Lucia Foundation, Via Fosso del Fiorano 63,64, Rome, Italy*
2. *Department of Physiology and Pharmacology, University "La Sapienza", Rome, Italy*
3. *Department of Psychology, University "La Sapienza", Rome, Italy*

Drug addiction is a chronic relapsing pathology that emerges only in a small proportion of drug users. A great deal of interest has been placed on the quality of the environment experienced during early age as a modulator of the susceptibility to drug-addiction in adulthood (1). Whilst several animal studies showed a similar modulation of acute and/or chronic stress on the escalation of drug abuse, the long term effects of a potential "adverse/aggressive" environment during pre-adolescent period in inducing vulnerability to addiction and relapse to drug use has never been modeled in rodents. Recently, we have explored the effect of the exposure to two different adverse experiences during pre-adolescent age on susceptibility to drug-addition and relapse to drug-seeking in adult mice. In our study, a first group of pups experienced social isolation (SI), whereas a second one was separated from the mother and exposed to an adult male mouse in its resident cage (Social Stressed, SS) for 30' per day from postnatal day 14 to 22. SI, SS and control unhandled mice were then tested for cocaine induced Conditioned Place Preference (CPP) in adulthood as previously described (2). Interestingly while both SI and SS groups showed increased cocaine induced CPP compared to control mice, only SS mice were susceptible to cocaine-induced reinstatement. To identify the molecular substrates underlying this susceptibility, we performed genome-wide RNA sequencing analysis in nucleus accumbens (NAC) punches of mice sacrificed after reinstatement. Preliminary results reported a number of transcripts that are differentially expressed between SS and SI mice in NAC, and need now to be further investigated. Overall our data demonstrate for the first time that exposing pre-adolescent mice to an "adverse/aggressive" environment can impact the adult susceptibility to drug-addiction behavior and relapse to drug seeking, and that this effect is regulated in a "trauma" dependent manner. The identification of molecular pathways underlying the early-"trauma" induced susceptibility to drug reinstatement could help discovering new pharmacological targets for prevention of

relapse to drug-seeking.

1. Sinha, R., (2008) Ann NY Acad Sci 1141:105-130.
2. Orsini, C., Bonito-Oliva, A., Conversi, D., Cabib, S. (2008.) Psychopharmacology (Berl) 198:287-96

JWH-018 AND ITS N-PENTYL-ALOGENATED DERIVATES IMPAIR SENSORY MOTOR FUNCTIONS IN MICE.

Marti M.[1,2,3], Ossato A.[1], Trapella C.[3,4], Seri C.[5], Rimondo C.[5], Serpelloni G.[6]

[1]*Department of Life Sciences and Biotechnology (SVeB), University of Ferrara, Italy*
[2] *Center for Neuroscience and Istituto Nazionale di Neuroscienze, Italy*
[3] *Collaborative Center for the Italian National Early Warning System, Drug Policies Department, Presidency of the Council of Ministers*
[4]*Department of Chemistry and Pharmaceutical Sciences, University of Ferrara, Italy*
[5] *Italian National Early Warning System, Drug Policies Department, Presidency of the Council of Ministers*
[6] *Drug Policies Department, Presidency of the Council of Ministers*

JWH-018 (1-pentyl-3-(1-naphthoyl) indole) is a synthetic CB1 and CB2 cannabinoid agonist illegally marketed in "Spice" and "herbal blend" for its psychoactive effects similar to those produced by cannabis (1). Recently, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported to the Italian Drugs Early Warning System (NEWS) the seizure of plant material containing halogenated derivatives (N-(5-chloro-pentyl)- and N-(5-bromide-pentyl)) of the synthetic cannabinoid JWH-018. In rodents JWH-018 reproduces the typical effects of THC as hypothermia, analgesia, hypolocomotion and akinesia (2), while its effects and that of the halogenated derivatives on sensory motor functions are still unknowns. This aspect should be carefully evaluated to better understand the potential danger that these substances can cause if taken when driving or performing hazardous work (public drivers, civil aviation pilots, military). Therefore, the aim of this study was to evaluate the effect of JWH-018, JWH-018-Cl and JWH-018-Br on sensory motor function in mice and to compare their action with that induced by THC. A specific functional observatory battery test (3) was adopted to investigate the effects of cannabinoid compounds on overall sensory motor functions (auditory, tactile, visual). Sensory motor functions were rapidly inhibited in mice already after 5 minutes from the administration of a dose (6 mg/Kg, i.p.) of JWH-018 (~90%), JWH-018-Cl (~80%) and JWH-018-Br (~70%), while the THC appeared less effective (~50% inhibition). The effects persisted steadily until the second hour and tended to decline over the following three hours of observation. At 5 hours the effects were still present (~40% inhibition). Sensory motor impairments were prevented by the administration of the selective CB-1 receptor antagonist AM251 (3 mg/Kg). These results show that JWH-018 and its N-pentyl-halogenated derivatives impair overall sensory motor functions in mice. This aspect should be carefully investigated since the powerful-THC like action of synthetic cannabinoids on sensory motor function should be considered in the prevention of occupational accidents and traffic injuries.(4).

1. Sistema Nazionale di Allerta Precoce, Dipartimento Politiche Antidroga, Presidenza del Consiglio dei Ministri, *Report Nazionale 2010*, Roma, 2010
2. Wiebelhaus J.M., Poklis J.L., Poklis A., Vann R.E., Lichtman A.H., Wise L.E. (2012) Drug Alcohol Depend. 126: 316-323.
3. Irwin S. (1968) Psychopharmacology 13: 222-257
4. http://www.dronet.org/monografia.php?monografie=72

POWERFUL COCAINE-LIKE ACTION OF MDPV ON AGGRESSIVE BEHAVIOUR IN ISOLATED MICE

Marti M.[1,2,3], Vigolo A.[1], Seri C.[4], Rimondo C.[4], Serpelloni G.[5]

[1]*Department of Life Sciences and Biotechnology (SVeB), University of Ferrara, Italy*
[2] *Center for Neuroscience and Istituto Nazionale di Neuroscienze, Italy*
[3] *Collaborative Center for the Italian National Early Warning System, Drug Policies Department, Presidency of the Council of Ministers*
[4] *Italian National Early Warning System, Drug Policies Department, Presidency of the Council of Ministers*
[5] *Drug Policies Department, Presidency of the Council of Ministers*

MDPV (3,4-methylenedioxypyrovalerone) is a synthetic cathinone illegally marketed as "bath salts" or "plant food" and consumed for its psychostimulant effects similar to those produced by cocaine, amphetamines and MDMA. Clinical reports indicate that MDPV produce euphoria, increase alertness and at high doses it causes agitation, psychosis, tachycardia and even death (1). In particular, the second leading cause of death induced by MDPV and other cathinones is associated with self-harm, risky and violent behavior (2, 3). Anecdotal reports suggests that MDPV increases violent aggressive behavior in men similar to that reported for alcohol and cocaine consumption (4).
In rodents, MDPV reproduces the typical physiological effects of psychostimulant drugs, showing a greater potency compared to cocaine (5). Nevertheless, its role on aggressive behavior is still unknowns. Therefore, the aim of this study was to evaluate the effect of MDPV on aggressive behavior in mice and to compare its action with that induced by cocaine.
The Resident-Intruder paradigm in isolated mice (6) was undertaken to investigate the effect of MDPV and cocaine on aggressive behavior. Saline administration causes an increase in aggressive behavior in 7% of total mice (n=50) with an increase of bite frequency of +52±1% respect to control baseline. Systemic MDPV administration (i.p.) at 0.1 and 10 mg/Kg causes an increased aggressive behavior in 46% and 55 % of total mice (n=50 for each treatment) with an increased frequency of bites of +134±22% and +155±18 % respectively. Similarly, cocaine administration (i.p.) at 0.1 and 10 mg/Kg causes an increased aggressive behavior in 13% and 27 % of total mice (n=50 for each treatment) with an increased frequency of bites of +82±8% and +132±11 % respectively, proving to be less potent than MDPV in inducing aggressive behavior in mice. The aggressive effect caused by MDPV is consistent with the positive modulation on catecholamine release (5) and its powerful action may reflect its stronger ability to inhibit dopamine and norepinephrine uptake respect to cocaine (5). These results show for the first time that MDPV enhance aggressive behavior in mice with grater potency compared to cocaine. This aspect should be carefully investigated for the prevention of interpersonal violence in human induced by novel psychoactive drug consumption.

1. Prosser J.M. and Nelson L.S. (2012) J Med Toxicol. 8: 33-42.
2. Marinetti L.J. and Antonides H.M. (2013) J. Anal. Toxicol. 37: 135-146.
3. Schifano F., Corkery J. and Ghodse A.H. (2012) J. Clin. Psychopharmacol. 32: 710-714.
4. Chermack S.T. and Blow F.C. (2002) Drug Alcohol Depend. 58: 43-54.
5. Baumann M.H., Partilla J.S., Lehener K.R. et al., (2013) Neuropsychopharmacology 38:552-562.
6. Miczec K.A. and O'Donnel J.M. (1978) Psychopharmacology 57: 47-55

PREVENTION OF NICOTINE-SEEKING BEHAVIOUR BY GLUTAMATERGIC THERAPY: ROLE OF mGlu2/3 RECEPTORS

Moro F., Di Clemente A., Marzo C.M. and Cervo L.

IRCCS – Mario Negri Istituto di Ricerche Farmacologiche,
Experimental Psychopharmacology, Department of Neuroscience,
Via La Masa 19, 20156 Milan, Italy

Tobacco smoking is a chronic relapsing disorder marked by recurrent resumption after abstinence. Although various pharmacological approaches have been proposed to help people stop smoking, the high relapse rates indicate a need for more efficacious treatments. Recent studies have highlighted dysregulations in the glutamatergic (Glu) system, and in particular a decrease in the function of the cystine-Glu antiporter system xc- in the nucleus accumbens. N-acetylcysteine (N-AC), a cystine pro-drug, by increasing the activity of the cystine-Glu antiporter system xc- may reverse nicotine-induced alterations in Glu transmission and decrease nicotine-seeking. The present study investigated whether N-AC modulates reinstatement of nicotine-seeking behavior in a rat model of drug-seeking relapse by drug-associated cues with high face

and construct validity. Separate groups of 7-8 male Wistar rats were trained, with or without any previous confounding training for food reinforcement, to associate discriminative stimuli (S^Ds) with the availability of i.v. nicotine (0.03 mg/kg/65µL/2s/infusion) *vs.* non-reward in two-lever operant cages. Reinforced response was followed by cue signaling 20-s time-out (CSs). Once the training criterion was met, rats underwent extinction of lever presses, in the absence of reinforcers, S^Ds and CSs. Re-exposure to nicotine S^{D+}/CS^+, but not non-reward S^{D-}/CS^-, revived responding at the previously reinforced lever. Within-subjects experimental designs revealed that 100 mg/kg i.p. NA-C, but not 30 and 60 mg/kg, attenuated or completely antagonized reinstatement of nicotine-seeking depending on previous training for food reinforcement. NA-C (100 mg/kg i.p.) did not modify conditioned reinstatement by saccharin associated cues (100 µL/2s of a 50 mg/L solution of saccharin in water), and did not modify spontaneous locomotor activity of rats with a similar history of nicotine self-administration. The efficacy of 1 and 2 mg/kg i.p. LY341495, a potent and selective antagonist at presynaptic group II metabotropic Glu receptors (mGluR2/3), in preventing N-AC anti-reinstatement activity, suggests that the N-AC activation of xc- prevents nicotine-seeking by increasing Glu tone on mGluR2/3 and thereby inhibiting excitatory transmission.

STRUCTURAL PLASTICITY CHANGES IN THE NUCLEUS ACCUMBENS OF ETHANOL DEPENDENT RATS.

Muggironi G.[a], G. Mulas G.[b], Fois G.R.[a], Peana A.T.[a] , S. Spiga S.[b], Diana M.[a]

[a]'G.Minardi' Cognitive Neuroscience Lab., Department of Chemistry and Pharmacy, University of Sassari, 07100 Sassari, Italy. [b]Department of Life and Environmental Science, University of Cagliari, Cagliari, Italy.

Neuronal refinement and stabilization are hypothesized to confer resilience to poor decision- making and addictive-like behaviors, such as excessive ethanol drinking and dependence. Accordingly, structural abnormalities are likely to contribute to the appearance of alcohol withdrawal signs and symptoms that occur from suddenly ceasing the use of alcohol after chronic ingestion.
Here we show that ethanol dependent rats display a loss of dendritic spines in medium spiny neurons of the Nacc, accompanied by a reduction of TH-positive terminals and PSD-95 positive elements. Further analysis indicates that 'long thin', but not 'mushroom', spines are affected. These changes are restricted to the withdrawal phase of ethanol dependence suggesting their relevance to the genesis of signs and/or symptoms affecting specifically ethanol withdrawal, and thus the whole addicting cycle. Overall these results highlight the importance of spine function on the evolution of alcohol dependence and suggest that the selective loss of 'long thin' spines may significantly contribute to further 'impoverish' the already deficient dopaminergic transmission whose hypofunctionality is a major factor for the emergence of the harmful consequences of alcohol abuse/dependence.

Key Words: Alcoholism, Withdrawal, Dendritic spines, Accumbens, Dopamine.

WEEKLY KETAMINE SELF-ADMINISTRATION IN RATS AS A MODEL OF INTERMITTENT KETAMINE USE

Mutti A., Venniro M., Chiamulera C.

Neuropsychopharmacology Lab, Dept. Public Health & Community Medicine, Univ. of Verona, Verona, Italy.

Ketamine (KET) is a dissociative anesthetic, which became a drug of abuse in many parts of the world at sub-anaesthetic doses. The frequency of its use in humans is often intermittent but KET rewarding properties were shown only on a daily basis in laboratory animal self-administration (S/A) studies. The aim of our study was to mimic human intermittent consumption developing a model of KET S/A in rats on a weekly basis.
Two groups, Daily and Weekly, of Sprague Dawley adult male rats were trained in operant boxes to self-

administer intravenous KET (FR1: 0.5 mg/kg/infusion; TO = 40 sec) respectively during daily or weekly 1-hour sessions. Both groups acquired KET S/A within 10 sessions, with a similar mean number of KET infusions/session at stability (Weekly 15.2 ± 0.4 S.D.; Daily 16.8 ± 1.3 S.D.). The discrimination for active vs. inactive lever was similarly obtained in the two groups.
After saline substitution for KET infusion, there was a decrease of rate of responding in both groups, but more sessions were required to extinguish responding in Weekly (11-21 sessions) than in Daily (4-7 sessions) group. Then, when KET infusion was represented (reacquisition), Weekly group exhibited a slower rate of responding during the following 10-session reacquisition period.
In conclusion, we confirmed the reinforcing properties of KET on a weekly basis. However, the longer extinction and the slower reacquisition of KET S/A in Weekly group suggest a potential use of extinction therapy as an intervention for intermittent, occasional, KET use in humans.

INHIBITORY EFFECTS OF BETA-AMYLOID 1-40 ON THE NICOTINE-EVOKED NEUROTRANSMITTER RELEASE IN THE RAT CENTRAL NERVOUS SYSTEM

Olivero G[1]., Mura E[2]., Chen J[1]., Salamone A[1]., Preda S[2]., Zappettini S[1]., Grilli M[1]., Govoni S[2]., Marchi M[1].

Dept. of Pharmacy, University of Genova, Genova, Italy
Dept. of Drug Sciences, University of Pavia, Pavia, Italy

It is well established that cholinergic system modulates the function of several neurotransmitter systems through the activation of different nicotinic acetylcholine receptor (nAChR) subtypes. Recent studies *in vivo* and *in vitro* have shown that beta-amyloid (Aß) is able to modulate the function of these receptors. The relevance of the activation of nAChR subtypes which stimulate *in vivo* amino acids (AA) release and the role of Aß at this regard have been so far poorly investigated. It has to be also noted that nAChRs are present in several non-neuronal cells, including astrocytes, and therefore part of the AA released *in vivo* might result also from these cells.
In this work we found that nicotine was able to enhance the *in vivo* hippocampal overflow of three AA (GLU, glutamate; ASP, aspartate; GABA, γ amino butyric acid) being more potent in stimulating GLU overflow. The α7 selective agonist PHA543613 induced an overflow very similar to that of nicotine. The α4β2 selective agonist 5IA85380 was significantly less potent in inducing GLU overflow, while the overflows of ASP and GABA were almost inconsistent. Aβ1-40 inhibited the neurotransmitter overflow of GLU stimulated by PHA543613 but not the one evoked by 5IA85380. In hippocampal gliosomes nicotine elicited selectively GLU overflow which was also evoked by 5IA85380 and by the α7 selective agonist choline. Nicotine- and choline-induced glutamate overflow in gliosomes was inhibited by Aβ1-40. Interestingly, Aβ1-40 was able to inhibit also the nicotine-evoked dopamine release in the nucleus accumbens but not the nicotine-evoked release of noradrenaline from hippocampal synaptosomes, suggesting a specificity of action on different nAChRs. The inhibitory effect of Aβ1-40 was absent when Aβ1-40 was introduced into the nerve endings suggesting that the modulatory effect on nAChRs is due to an interaction with targets located on the terminal external surface. From our results we can conclude that (a) nicotine administration *in vivo* elicits hippocampal GLU release mostly through α7 nAChRs likely present both on neurons and astrocytes; (b) the inhibitory effect of Aβ1-40 on the nicotinic-control of GLU release seems to depend primarily to the inhibition of α7 nAChRs functional responses and (c) Aβ1-40 is able to inhibit selectively only some nAChR subtypes through a mechanism which involves its interaction with targets present on the external surface of nerve endings.

This work was supported by from the Italian Ministero Università Ricerca to Prof. Mario Marchi (Prot. N° 2009R7WCZS_003), by the European Community Project PYRGI, project n° B51H10000000006 and by the project 7FP-KBBE BAMMBO and to Prof. Govoni (MIUR 2009). This work is supported by a grant of the Alzheimer's Association (NIRG-11-205183) to Elisa Mura.

RATS AS A MODEL OF INTERMITTENT DRUG USE: WEEKLY COCAINE AND NICOTINE SELF-ADMINISTRATION

Padovani L., Mutti A., Venniro M., Chiamulera C.

Neuropsychopharmacology Lab., Dept. Public Health & Community Medicine, Univ. of Verona, Verona, Italy.

The reinforcing properties of psycho-stimulant drugs like cocaine (COC) and nicotine (NIC) have been extensively studied in animal models, e.g., the drug self-administration (S/A) model performed on a daily session basis. However, it is also well-known that intermittent use of both drugs often occurs in humans, and that this occasional frequency of drug-taking may be maintained in the long term.
The aim of our study was to assess whether COC and NIC S/A in rats was acquired and maintained on a weekly basis, in order to mimic the occasional drug-taking behaviour in humans.
For each drug, two groups (Daily and Weekly) of Sprague Dawley adult male rats were trained in operant boxes to intravenously self-administer COC (FR1: 0.5 mg/kg/infusion, TO = 20 sec) or NIC (FR1: 0.03 mg/kg/infusion TO = 60 sec) during daily or weekly 1-hour consecutive sessions.
Weekly COC number of infusions/h significantly increased starting from the session 3 even if COC S/A was significantly acquired only at session 5 according to significant discrimination between active vs. inactive lever. On the other hand, Daily COC S/A was acquired at session 6.
For each NIC group, the number of infusions/h increased along the 8 sessions, significantly at session 5 (Daily) and 6 (Weekly). For Daily NIC, two-way ANOVA showed a main effect of lever, i.e., discrimination. For Weekly NIC, two-way ANOVA showed a main effect of sessions and lever, i.e., discrimination.
In conclusion, we confirmed that rats are able to self-administer COC and NIC on a weekly regimen. Although for both drugs there was an increasing number of infusions over sessions, only COC groups however acquired and maintained the S/A. The lack of discrimination for NIC groups was probably due to hyperactivity induced by the drug.

EFFECTS OF ACUTE COCAINE ON ERK PHOSPHORYLATION IN THE LIMBIC SYSTEM OF TWO LINES OF RATS DIFFERING IN THE SUSCEPTIBILITY TO SELF ADMINISTER COCAINE.

Piludu M.A., Rosas M., Giugliano V., Sabariego M., Giorgi O., Acquas E.

Department of Life and Environmental Sciences, University of Cagliari;
Centre of Excellence for the Neurobiology of Addiction, University of Cagliari;
CNR Institute of Neuroscience, Cagliari, Italy.

Introduction. Drugs of abuse induce plastic adaptations in the cellular mechanisms underlying learning and memory. Such mechanisms involve signaling by extracellular signal-regulated kinase (ERK). Both acute and chronic administration of cocaine increase ERK phosphorylation in mesolimbic areas like the prefrontal cortex (PFCx) and the nucleus accumbens (NAc) which play a key role in drug addiction. The Roman low- (RLA) and high- (RHA) avoidance rats, selectively bred for, respectively, poor vs. rapid acquisition of active avoidance in a shuttle box, differ markedly in the propensity to self-administer cocaine. Hence, the present study was aimed at comparing the effects of the acute administration of cocaine on ERK phosphorylation in the PFCx (infralimbic and prelimbic) and in the NAc (shell and core) of RHA and RLA rats.
Materials and Methods. Rats were administered with cocaine (5 mg/kg, i.p.) or vehicle and transcardially perfused 20 min after injection. Brains were removed and coronal sections were cut with a vibratome. After immunohistochemical staining, the slices were examined with an optic microscope to count the pERK-positive neurons in the regions of interest.
Results. Acute cocaine induced a larger increase in the number of p-ERK positive neurons in the NAc shell and in the infralimbic portion of the PFCx (PFCx IL) of RHA vs. RLA rats, but failed to modify p-ERK immunoreactivity in the NAc core and in the prelimbic PFCx (PFCx PrL) of either line.
Conclusions. Cocaine increased ERK phosphorylation in the PFCx IL and the Nac shell, two brain areas

belonging to the limbic subcircuit which controls motivated behaviors that play a crucial role in the initial response to the drug, but did not affect pERK in the PFCx PrL and NAc core, two areas that are part of a motor subcircuit involved in habit acquisition upon long term exposure to cocaine. Notably, cocaine increased ERK phosphorylation only in RHA rats which are more susceptible to develop cocaine self administration (SA) than RLA rats. These results are consistent with the view that RHA and RLA rats represent a valid model of individual vulnerability to drug addiction.

AN INNOVATIVE MOUSE MODEL TO NICOTINE ADDICTION: BEHAVIOURAL AND BIOCHEMICAL CORRELATES

Ponzoni L.[1,2], Braida D.[1], Martucci R.[1], Gotti C.[3], Sala M.[1,3]

[1] Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano; [2] Fondazione Fratelli Confalonieri Milano; [3] Istituto di Neuroscienze, CNR, Milano

Nicotine is the main addictive substance in tobacco smoke. The way in which it is given to animals is very different from that used by humans (smoking cigarettes), thus making the animal results less reliable and not completely overlapping (1). On this basis, the aim of the present study was to investigate the behavioural and biochemical changes after exposing mice to nicotine smoke. Balb/C male mice, in groups of 30, were daily exposed to the smoke of 7 Chesterfield Red cigarettes (3 times a day for 7 weeks). We used a mechanical ventilator to vaporize cigarette smoke or only air in an air-controlled cage. After 3 and 7 weeks animals were killed and brains removed for binding studies. Our results demonstrate a significant up-regulation of α4β2 neuronal nicotinic acetylcholine receptors subtype at 7 but not at 3 weeks. Thus, this time was chosen for the behavioural analysis. Immediately after smoking cessation, withdrawal syndrome was precipitated by mecamylamine injection (1mg/kg). Signs and symptoms of abstinence were significantly increased in the smoke-exposed group compared to controls exposed to air. Spontaneous motor activity, evaluated through an automated activity cage, decreased only 24 hours after smoking cessation. Spatial object recognition test revealed a significant impairment in recognizing the new spatial location of a familiar object, at least up to 30 days after smoking cessation. Emotional profile was measured using elevated plus maze test for anxiety-like behaviour and tail suspension and anhedonia for depressive-like behaviour. Compared to controls, mice exposed to cigarette smoke, showed, after 48 hours, an anxious state and, after 30 days, a depressive like behaviour which lasted 90 days after smoking cessation. In humans tobacco smoke is frequently associated with marijuana smoke (2). Thus, after smoking cessation, in a further group of animals, we verified, using the conditioned place preference test, whether mice were cross-sensitized to the reinforcing effect of Δ^9-tetrahydrocannabinol (Δ9-THC). Δ^9-THC (0.01-0.3 mg/kg) induced a greater CPP in nicotine pre-exposed animals compared to control group. In conclusion, we propose an innovative method for studying nicotine dependence to investigate short- and long- term alterations induced by prolonged exposure to tobacco smoke.

1. Cohen A. and George O. (2013) Front Psychiatry 4:41
2. Degenhardt L., Hall W. and Lynskey M. (2001) Addiction 96(11):1603-14

RECEPTOR-INDEPENDENT INHIBITION BY SR141716 OF BASAL AND AGONIST-STIMULATED G PROTEIN ACTIVITY

Porcu A.[1], Casti A.[1], Madeddu C.[1], Mascia M.P.[2], Melis M.[1,2], Gessa G.L.[2], Castelli M.P.[1]

1Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria di Monserrato, Cagliari
[2]CNR National Research Council of Italy, Neuroscience Institute - Cagliari

Initial events leading to drug addiction involve acute effects at the specific sites of action of the abused drug. These sites (e.g., G-protein coupled receptors and ligand-gated ion channels) typically activate neural circuits associated with reward, particularly the mesocorticolimbic dopaminergic (DA) system. The

endocannabinoid system was shown to greatly modulate DA reward circuits, which implies it may play a major role in addiction processes (1). The CB1 receptor (CB1R) antagonist/inverse agonist SR141716 (SR) decreases self-administration of palatable food, heroin, ethanol and nicotine, and inhibit basal G-protein activity (2). *In vitro* and *in vivo* evidence suggest that inverse agonist effects of SR are both CB1R-dependent and independent, but the exact mechanism has not been clarified yet. Here we investigated the effects of SR in i) systems containing CB1Rs, $GABA_B$ receptor ($GABA_BR$) and μ-opioid receptor (MOR) populations (rat membrane homogenates), ii) systems lacking CB1Rs (CB1-knockout mice), iii) oocytes containing homogeneous populations of expressed recombinant $GABA_BR$, iv) acute brain slices containing the midbrain by performing whole cell patch clamp recordings from DA neurons *ex vivo*. SR (10 and 25 μM) significantly decreased basal [^{35}S]guanosine 5'-3-O-(thio)triphosphate (GTPγS) and [^{35}S]GTPγS binding in the abovementioned system. The inhibitory effect of SR on basal GTPγS activity was not blocked either by the CB1R, MOR or $GABA_BR$ antagonists, respectively, suggesting that SR reduces basal GTPγS activity by a mechanism independent from CB1Rs, MORs or $GABA_BRs$. Increasing fixed concentrations of SR produced a rightward shift of the concentration-response curve of baclofen with only a slight concomitant decrease in the maximal stimulation, displaying at the highest concentration tested (5 μM) a significant decrease of potency of baclofen rather than its maximal efficacy. SR blocks baclofen-induced outward current in DA neurons via a mechanism independent from CB1 receptor activation. In *Xenopus laevis* oocytes expressing $GABA_{B(1a,2,\ GIRK)}$ receptors, SR inhibits GABA-induced inward currents. We provided evidence that SR inverse agonist effects observed at higher concentrations are not CB1R-mediated.

1. Justinova Z, Panlilio LV, Goldberg SR (2009) *Curr Top Behav Neurosci.* 1:309-346
2. Ward SJ, Rosenberg M, Dykstra LA, Walker EA, (2009) *Drug and Alcohol Dependence* 105:248-255

ADOLESCENT THC EXPOSURE IMPAIRS GENE EXPRESSION INVOLVED IN SYNAPTIC PLASTICITY IN THE RAT PREFRONTAL CORTEX

Prini P.[1], Zamberletti E. [1], Gabaglio M. [1], Parolaro D. [1,2] and Rubino T. [1]

[1] *Dept. of Theoretical and Applied Science, University of Insubria, Busto Arsizio (Va), Italy*
[2] *Zardi-Gori Foundation, Milan*

We recently demonstrated that adolescent female rats treated with delta-9-tetrahydrocannabinol (THC) develop a schizoaffective-like phenotype in adulthood. Moreover, according to the increasing number of papers supporting the involvement of epigenetic mechanisms in the development of psychiatric illnesses, we observed in the prefrontal cortex of these animals increased acetylation of Lysin14 on histone H3 (H3K14Ac, associated with transcriptional activation) 24 h after the end of THC treatment.
Since histone modifications impact transcriptional activity, the aim of this work was to investigate the effect of adolescent THC exposure on gene expression in the prefrontal cortex. Adolescence is characterized by intense processes of neuronal refinement in which the endocannabinoid system (ECS) seems to play a crucial role, thus we focused our attention on genes closely related to the ECS or involved in synaptic plasticity. To this aim, adolescent female rats were treated with increasing doses of THC twice a day from PND 35 to 45 and 24 hours after the last THC injection Real-Time PCR array analysis was performed in the prefrontal cortex. Besides genes related to the ECS, we investigated 34 genes involved in synaptic plasticity (e.g. belonging to the glutamatergic and gabaergic system as well as coding for proteins or pathways related to plasticity). To clarify whether adolescence represents a more vulnerable time window for the adverse effect of THC, the same protocol of administration as well as analysis were carried out in adult female rats (PND 75-85). THC exposure induced an intense and wide spread decrease in mRNA levels of the considered genes only in adolescent rats, thus supporting the vulnerability of the adolescent brain towards THC effects. However the reduction in gene expression did not correlate with the increase of H3K14Ac. Thus, to investigate the time course of the altered gene expression, the same Real-Time PCR array analyses were performed 2 and 48 hours after the last THC injection. Adolescent THC exposure induced a global decrease of the mRNA levels analyzed immediately after the treatment. In contrast, all the mRNA analyzed returned to control levels or even increased above them 48 hours after the end of the treatment. This effect might be related to the enhanced H3K14 acetylation observed at 24hours. To understand the stability of the alterations in gene expression, we performed the same Real-Time PCR array analyses at PND 60 and 75. An increase of several mRNA levels was still present at PND 60, while only the

expression of two genes resulted to be altered at PND 75. They were Gria1 and Gad1, both possibly related to the schizo-affective phenotype present in adult animals pre-exposed to THC during adolescence. As a whole, these data suggest that adolescent THC treatment impaired the steady state expression of a set of genes involved in brain plasticity. These alterations might play a role in the development of the schizo-affective disorder induced by adolescent THC exposure.

Acknowledgements: this work was supported by Dipartimento delle Politiche Antidroga, progetto EpiCa

DIFFERENTIAL EFFECTS OF MORPHINE ON ERK PHOSPHORYLATION IN RAT AND MOUSE NUCLEUS ACCUMBENS SHELL AND CORE

Rosas M.[1], Longoni R.[1], Spina L.[1], Acquas E.[1,2,3]

[1]Dept. of Life and Environmental Sciences - Pharmaceutical, Pharmacological and Nutraceutical Sciences Section; [2]Centre of Excellence on Neurobiology of Addiction and [3]National Institute of Neuroscience - INN, University of Cagliari, Cagliari, Italy.

Phosphorylated Extracellular signal Regulated Kinase (pERK) is critically involved in the mechanism of action of addictive drugs, although some controversial findings concerning the effects of morphine on ERK phosphorylation in the nucleus accumbens (Acb) have been reported. The present study was aimed to investigate this issue by assessing the ability of morphine to activate ERK phosphorylation in the Acb shell (AcbSh) and core (AcbC) of Sprague-Dawley and Wistar rats as well as of CD-1 and C57BL6J mice.
To this end, upon acute morphine (1 and 5 mg/kg) administration, pERK expression was measured using two immunohistochemical approaches, namely, % pERK values corrected for background (VCB) (i.e., optical density/neuron) and % pERK-positive neurons/area. The results of these experiments demonstrate that, in both Sprague-Dawley and Wistar rats, morphine a) increased % pERK VCB but b) decreased % pERK-positive neurons in the AcbSh and AcbC. In addition, pre-treatment with the dopamine D_1 receptor antagonist, SCH 39166 (50 mg/kg), and the μ opioid receptor antagonist, naltrexone (1.5 mg/kg), prevented the increases of % pERK VCB, while only pre-treatment with naltrexone, but not SCH 39166, prevented the reduction of % pERK-positive neurons. In contrast, in CD-1 and C57BL6J mice, morphine increased preferentially in the AcbSh a) % pERK VCB and b) % pERK-positive neurons/area. These effects were both antagonized by either SCH 39166 and naltrexone. Overall, these findings confirm previously reported differential effects of morphine on ERK phosphorylation in the Acb of rats and mice. In addition, these results indicate that D_1 and m opioid receptor-mediated processes are differentially involved in the mechanism(s) by which morphine affects pERK expression in the Acb and call for further experiments to elucidate the significance of the property of morphine to either increase and decrease distinct measures of pERK in the Acb.

ELEVATION OF KYNURENIC ACID LEVELS SUPPRESSES DELTA-9-TETRAHYDROCANNABINOL-INDUCED EXCITATION OF MESOLIMBIC DOPAMINE AND PREFRONTAL CORTEX PYRAMIDAL NEURONS.

C. Sagheddu[1], S. Lecca[1], A. Luchicchi[1], S.R. Goldberg[2] and M. Pistis[1]

[1] Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Monserrato, Italy.
[2] Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch and Integrative Neurobiology Section and Psychobiology Section, Molecular Targets & Medications Discovery Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, Maryland, USA.

Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of Cannabis extracts, like most drugs of abuse, enhances dopaminergic (DA) transmission by increasing both DA neuron firing rate and DA release in the nucleus accumbens shell (shNAc). This effect, that is mediated by cannabinoid CB_1 receptors, presumably underlies the rewarding and dependence-inducing effects of marijuana behavioral and neurochemical studies. Justinova *et al.* (*in press*), have very recently demonstrated that elevations of brain levels of kynurenic acid (KYNA), an endogenous product of the normal metabolism of amino acid L-tryptophan, suppresses THC-induced effects in rats and monkeys.
On these bases, we carried out in vivo electrophysiological single cell recordings in anesthetized rats to investigate how KYNA modulates THC-induced electrophysiological actions on DA neurons in the ventral tegmental area (VTA) and pyramidal neurons in the medial prefrontal cortex (mPFC). Neurons were selected as projecting to the shNAc by antidromic stimulation.
According with previous studies, we confirmed that intravenously administered THC (0.3 – 2.4 mg/Kg), increased firing activity of DA (137.1 ± 4.1 %, n = 13, $p<0.05$) and mPFC (306.2 ± 75.6 %, n = 6, $p<0.01$) cells projecting to the shNAc. To enhance brain levels of KYNA, the kynurenine-3-monooxygenase inhibitor, Ro 61-8048 (Ro, 30 mg/kg, i.p.) was administered 40 minutes before recordings. Consistent with microdialysis and behavioral studies, THC-induced increase in firing activity was completely abolished in DA (103.6 ± 3.3 %, n = 7) as well in mPFC (119.4 ± 28.1 %, n = 6) cells recorded from rats pretreated with Ro ($p< 0.01$). KYNA was suggested to act as a negative allosteric modulator of α7 nicotinic acetylcholine receptors (α7-nAChRs), therefore, in the attempt to prevent Ro effects we administered the positive allosteric modulators of α7-nAChRs galantamine (3 mg/kg, i.p.) or PNU120596 (1 mg/kg, i.p.). However, these drugs were unable to prevent the effects of Ro, suggesting that the electrophysiological effects of KYNA might be dependent on sites of actions different from α7-nAChR.
Patients seeking help for Cannabis dependence are increasing worldwide but specific pharmacological treatments are lacking and urgently needed, especially after the failure of CB_1 antagonists due to psychiatric side effects. Modulation of brain KYNA levels might represent a novel therapeutic approach to treat Cannabis dependence.

CB_2 ANTAGONIST AM630, MONOACYLGLYCEROL LIPASE INHIBITOR JZL184 AND CANNABIDIOL DID NOT AFFECT HEDONIC PROPERTIES OF FOOD IN RATS WITH BINGE EATING BEHAVIOR

Satta V.[1], Scherma M.[1], Fattore L.[2,3], Collu R.[1], Fratta W.[1,2] and Fadda P.[1,2]

[1]Dept of Biomedical Sciences, University of Cagliari, Italy; [2]Center of Excellence "Neurobiology of Dependence", University of Cagliari, Italy; [3]CNR Neuroscience Institute - Cagliari, National Research Council, Italy.

Binge eating disorder (BED) is characterized by repetitive episodes of intermittent, uncontrolled and excessive food consumption of highly palatable foods within a short period of time (1). The endocannabinoid system has been shown to contribute significantly to the hedonic valuation of food: infact, cannabinoid type-1 receptors (CB1R) are expressed in several areas involved in brain reinforcement and reward processes. Various animal studies showed that CB1 receptor agonists increase food intake, particularly the consumption of palatable foods. By contrast, CB1 receptor antagonists decrease food intake by preferentially reducing the consumption of palatable food. We have recently shown that pharmacological modulation of the endocannabinoid system, by the natural CB1/CB2 receptor agonist THC and by the CB1 inverse agonist/antagonist rimonabant, was able to modify the aberrant eating behavior present in a validated rat model of BED, acting preferentially on the intake of palatable food (2). This follow-up study was undertaken to investigate whether the cannabidiol (CBD), a major nonpsychotropic constituent of cannabis, the monoacylglycerol lipase inhibitor JZL184, which prolongs the effect of the endogenous 2-AG, and the CB2 antagonist AM630 were effective under the same experimental conditions. Binge eating behaviour was induced in animals by giving them a sporadic (3 days/week) and limited (2h) access to a high fat diet (margarine) in addition to a continuous access to chow and water (HR group). In these animals, the intake of margarine becomes significantly greater than in animals with limited daily access to margarine (LR group), and remains stable over prolonged periods of time. CBD was not able to affect the margarine intake, although we observed a positive trend. However, JZL184 significantly increased the margarine intake only in LR group at both doses tested. Finally, no effect was found with AM630. These data seems to underline

the possible role of CB1R in binge eating behavior.

1. American Psychiatric Association (2013). Washington, DC: American Psychiatric Press.
2. Scherma et al. (2013) British Journal of Pharmacology 169:820–833.

ACTIVATION OF PPARγ BY PIOGLITAZONE ATTENUATES REINSTATEMENT OF HEROIN SEEKING IN THE RAT

Scuppa G.[1], de Guglielmo G.[1], Kallupi M.[1], Stopponi S.[1] and Ciccocioppo R.[1]

[1]School of Pharmacy, Pharmacology Unit, University of Camerino, via Madonna delle Carceri, 62032, Camerino, Italy

Addiction is a chronic relapsing disease characterized by loss of control over drug use, compulsive seeking and craving for a substance of abuse and use that persists or resumes despite negative consequences. Dependence to opioid is recognized as one of the most pervasive form of addiction worldwide. In human addicts, reinstatement of heroin seeking could be triggered by stress, heroin associated cues or acute re-exposure to the drug. In addition, cue-induced reinstatement increases progressively after withdrawal, a phenomenon called incubation of heroin craving.
Recent evidence from our laboratory has demonstrated that activation of peroxisome proliferator-activated receptors (PPARγ) by pioglitazone, a drug currently used to treat insulin resistance in Type II diabetes, reduced the abuse liability associated with heroin and alcohol in rodents.
Based on this finding we used four different animal models of relapse I) yohimbine stress-induced reinstatement, II) discriminative-cue induced reinstatement, III) drug priming induced reinstatement and IV) incubation of heroin craving to evaluate the effect of pioglitazone (10, 30 and 60 mg/kg), on attenuating the resumption of the heroin-reinforced operant response in the rat.
Results demonstrated that activation of PPARg by pioglitazone abolished the reinstatement of heroin seeking induced by yohimbine and reduced the priming induced relapse and the incubation of heroin craving. However, pioglitazone was not effective in attenuating the reinstatement induced by the re-exposure to the previously heroin associated cues.
The present study provides pre-clinical evidence of the efficacy of pioglitazone on reducing the vulnerability to relapse to heroin seeking. Based on these findings, we hypothesize that pioglitazone may represent a new therapeutic approach for the prevention of various forms of heroin relapse.

CLINICAL OUTCOME OF METHADONE MAINTENANCE TREATMENT: DOSE ADJUSTMENTS AND PLASMA R-METHADONE LEVELS (RANDOMIZED STUDY EUDRACT : 2008-005028-10)

Sili M[1,2], Mannaioni G[1,2,] , Lanzi C[1,2] , Lotti M[1,2], Galli V[1,2], Totti A[1,2], Pacileo I[1,2], Quaranta MR[1,2], Pracucci C[1,2], Orsini F[1,2], Dilaghi A[1,2], Bertieri L[1,2], Occupati B[2], Michahelles A[3], Ciuti R[3], Bianchini E[4], Fabbro G[4], Biggeri A[4] and Moroni F[1,2].

[1] Neurofarba Department, Pharmacology Section, University of Florence; [2] Universitary Clinic of Careggi, Florence, Medical Toxicology Unit; [3] Universitary Clinic of Careggi, Florence, General Toxicological Laboratory ; [4] Instute for Oncologic Studies and Prevention (ISPO), Biostatistic Unit.

Background: Opioid dependence is a chronic relapsing disease that is difficult to cure, but effective treatments are available to stabilize patients and reduce harm, thereby increasing life expectancy and quality of life. Although methadone maintenance treatment (MMT) is recognized as the treatment of reference for opioid dependence many controversies still exist on the daily methadone doses necessary for therapy optimization. R/S MT is a 50:50 mixture of two enantiomers but R/MT accounts for the majority of its pharmacological effects. Due to the variability of methadone concentration-to-dose-to-weight ratios with

regard to the consumption of illicit opiate, a therapeutic response has been recently associated with (R)- (at 250 ng/ml) and (R,S)-methadone (at 400 ng/ml) but not with (S)-methadone concentrations.
Methods: In an open-label, phase 4, randomized controlled trial involving 13 outpatient clinics in Italy, we compared two populations: the first (153 patients) with daily doses of R/S MT in order to obtain or maintain R/MT plasma concentration in a range between 80 to 250 ng/ml (experimental group), the second (155 patients) treated with daily doses of R/S MT with no control of R/MT plasma concentration (control group). The primary outcomes, assessed at 6 and 12 months, were retention in addiction treatment, reduction in illicit drug use and amelioration of social aspects as defined by the Opiate Treatment Index (OTI). Results: We observed a significant reduction trend in the intensity of abuse (Q) of heroin in both groups from the enrollment to the last interview. The reduction trend was noticed also for cocaine abuse. We recorded therefore an amelioration of the social score and so the quality of life of patients from the beginning of the study to the end. In the "per protocol" approach the social score was better in a statistically significant way in the experimental group. Linear relationship between R/S MT daily dose and MT enantiomers plasma concentrations was low, outlining the necessity of measuring plasma R-MT levels. In the "intention to treat" statistical approach the results we obtained did not support our primary goal. In fact, a lower number of patients (81%) randomized in the experimental group remained in treatment after six months from enrollment compared to control group (91%, OR 0.42; 95%, confidence interval 0.21/0.83, P>chi2 0.012). This difference is not present at 12 months (80% control group versus 72% experimental group, OR 0.64; 95%, confidence interval 0.38/1.09, P>chi2 0.096). In the "per-protocol" analysis instead, 100% of adherent patients in experimental group remained in treatment compared to 90.97% of non-adherent in control group after six months (P>chi2 0.035) and 93.48% compared to 80% after twelve months (OR 3.58; 95%, confidence interval 0.37-1.09, P>chi2 0.009).
Conclusion: Although "intention to treat" approach failed the primary outcome, a subsequent "per protocol" analysis suggested that methadone dose adjusted patients (experimental group) showed a significantly better retention in treatment, with a consequent amelioration of both clinical outcome and quality of life.

WEEKLY KETAMINE SELF-ADMINISTRATION IN RATS: IS A MODEL OF KETAMINE INJECTION PATTERN?

Venniro M., Mutti A., Chiamulera C.

Neuropsychopharmacology Lab, Dept. Public Health & Community Medicine, Univ. of Verona, Verona, Italy.

Ketamine (KET) is a NMDA receptor antagonist which became increasing popular in clubs and rave scene. Its human recreational use was also reported as intermittent (i.e. occasional injections instead of daily). The aim of our study was to assess whether KET self-administration (S/A) in rats was acquired and maintained on a weekly basis in order to mimic the intermittent consumption in humans. KET was shown to own reinforcing properties, i.e. to initiate and maintain daily S/A in laboratory animals. Two groups of Sprague Dawley adult male rats were trained in operant boxes to self-administer intravenous KET (FR1: 0.5 mg/kg/infusion; TO=40 sec) during daily or weekly 1-hour sessions. Both groups acquired KET S/A within 10 sessions. Mean number of KET infusions/session and rate of responding for active vs inactive lever in weekly group was equal to daily group. Reinforcing properties of weekly KET were confirmed when saline substitution for KET infusion significantly decreased rate of responding during daily extinction sessions. However, more sessions were required to extinguish responding in the weekly (11-21 sessions) than in daily (4-7 sessions) group. Then KET infusion was represented and weekly group exhibited a slower reacquisition than in daily. In conclusion, robust KET S/A was rapidly acquired on a weekly basis but it showed a slower extinction of responding that was followed by a slower KET S/A reacquisition. We modelled the frequency of KET injection in humans, however for a more complete face validity is necessary understand the enviromental factors that impact on patterns of KET injection.

ADOLESCENT DELTA-9-TETRAHYDROCANNABINOL (THC) TREATMENT RESULTS IN PERSISTENT MICROGLIAL ACTIVATION AND UP-REGULATION OF GLIAL CB2

RECEPTORS WITHIN THE ADULT RAT PREFRONTAL CORTEX

Zamberletti E.[1], Gabaglio M.[1], Prini P.[1], Rubino T.[1] and Parolaro D.[1,2]

[1] *Dept. of Theoretical and Applied Sciences, Univ. of Insubria, Busto Arsizio (VA), Italy;*
[2] *Zardi-Gori Foundation, Milan, Italy*

Delta-9-tetrahydrocannabinol (THC) exposure during adolescence leads to the development of a complex schizoaffective-like phenotype in adult female rats, characterized by the presence of cognitive deficits, altered emotional reactivity and sensitization to the locomotor-activating effects of psychostimulants. We hypothesize that adolescent THC abuse might disrupt normal brain maturation, thus increasing the risk of developing psychotic-like symptoms in adulthood.
Emerging evidence suggests that microglia might play a crucial role in brain development, regulating synaptic maturation and function in the uninjured brain, suggesting that deficits in microglia function may contribute to synaptic abnormalities seen in some neurodevelopmental disorders. Therefore, in the present study we assessed whether the psychotic-like phenotype induced by adolescent THC exposure was associated with changes in microglia activation. To this aim, chronic THC (or vehicle) treatment was performed between PND 35 to 45 and ionized calcium-binding adaptor molecule 1 (Iba1) expression, a marker of activated microglia, was monitored 24 hours (PND 46), 15 days (PND 60) and 30 days (PND 75) after discontinuing THC treatment. The effect of chronic THC treatment on microglia was also morphologically determined in the rat prefrontal cortex (PFC) by using Iba1 immunofluorescence.
In control animals, Iba1 expression significantly increased from PND 46 to PND 60 whereas stable protein expression levels were present from PND 60 to PND 75. These changes might reflect the presence of synaptic remodeling events between mid and late adolescence that are not occurring in the adult brain. Intriguingly, at PND 46 Iba1 levels were significantly higher in THC-pretreated rats compared to controls and a further increase in Iba1 expression was observed from PND 60 to PND 75, suggesting the presence of aberrant glia activation following adolescent THC exposure. This hypothesis was supported by morphological studies revealing that in THC-pretreated rats glial cells were maintained in a chronic activated state (amoeboid morphology) throughout all the time window considered. As very recent data indicate a possible role for CB2 receptors in the pathogenesis of schizophrenia and microglial cells express CB2 receptor at detectable levels when activated, in the second part of this study, we checked whether adolescent THC treatment could alter CB2 receptor expression on glial cells. CB2 expression was very low in control animals, in line with the idea that glial cells express very low levels of CB2 receptor in resting homeostatic conditions In contrast, western blot analyses revealed that CB2 expression was significantly up-regulated in the PFC of THC-pretreated rats at all the considered time points. Immunofluorescence studies clearly demonstrated that the up-regulation of CB2 receptors involved only microglial cells, as it was not present on neurons nor astrocytes. As a whole, the present findings demonstrate that the psychotic-like phenotype induced by adolescent THC exposure is associated with changes in microglia activation as well as with enhanced CB2 expression on glial cells.
Further studies are needed in order to clarify the role of microglia activation and CB2 receptor up-regulation in the development of THC-induced psychotic-like phenotype.

Acknowledgements: Funded by Dipartimento delle Politiche Antidroga (Rome, Italy). MG has a pre-doctoral fellowship from Dipartimento delle Politiche Antidroga. Delta-9-tetrahydrocannabinol was kindly supplied by GW Pharmaceuticals (Salisbury, UK).

ENHANCED CANNABINOID SELF-ADMINISTRATION IN OLFACTORY BULBECTOMIZED RATS: EVALUATION OF POSSIBLE SEROTONERGIC AND DOPAMINERGIC UNDERLYING MECHANISMS

Zanda M.T.[1], Amchova P.[2], Kucerova J.[2], Giugliano V.[1], Sulkova A.[2], Fratta W.[1,3], Fadda P.[1,3], Fattore L.[3,4]

[1]*Dept. Biomedical Sciences, Univ Cagliari, Italy;* [2]*CEITEC - Masaryk University, Brno, Czech Republic;*
[3]*Centre of Excellence "Neurobiology of Dependence", Univ Cagliari, Italy;* [4]*CNR Neuroscience Institute-*

Cagliari, Italy.

Depression often co-occurs with the use of drugs and a link between heavy cannabis use and depressive states has been reported. In this study we combined the olfactory bulbectomy (OBX) model of depression with the intravenous cannabinoid self-administration procedure to verify whether OBX rats display higher voluntary intake of the cannabinoid CB_1 receptor agonist WIN 55,212-2 (WIN, 12.5 mμg/kg/infusion). To this aim, Lister Hooded rats were olfactory-bulbectomized (OBX) or sham-operated (SHAM) and after 21 days were tested for the development of anhedonia (sucrose preference test) and hypermotility in response to a novel aversive environment. Only rats displaying a depressive-like phenotype were allowed to self-administer WIN by lever-pressing and under a continuous schedule of reinforcement in 2h daily sessions, as previously described (1). Data showed that both OBX and SHAM rats develop stable cannabinoid intake; yet, rates of responding for WIN was constantly higher in OBX than in SHAM rats starting soon after the first week of training. This finding is in line with previously reported higher intake of methamphetamine (2) and oral nicotine (3) in OBX than in SHAM animals, thus confirming the hypothesis of higher drug intake in depressive-like conditions. Moreover, OBX rats needed more sessions than SHAM rats to extinguish operant responding thus displaying a higher propensity to retain cannabinoid-seeking behaviour. Notably, acute pre-treatment with the serotonin 5-HT_{1B} receptor agonist, CGS-12066B (2.5-10 mg/kg) did not significantly modify WIN intake in both OBX and SHAM rats. However, contrary to SHAM, OBX rats did not respond to a challenge of WIN 0.3 mg/kg by increasing the level of dopamine in the nucleus accumbens shell. Our results demonstrated that cannabinoid self-administration is not affected by acute stimulation of the serotonin 5-HT_{1B} receptor and that OBX rats display alterations in cannabinoid-induced brain reward-function, which rats seem to compensate by voluntary increasing cannabinoid intake.

1. Fattore *et al.* (2001) Psychopharmacology 156:410-6.
2. Kucerova *et al.* (2011) Int J Neuropsychopharmacol. 15:1503-11.
3. Vieyra-Reyes *et al.* (2008) Brain Res. Bull. 77:13-8.

SOCIETÀ ITALIANA FARMACOLOGIA