Session 03 − Psychopharmacology

03.001

Activation of CB1 receptors reduces marble burying behavior in mice. Casarotto PC[1], Gomes FV[1], Resstel LBM[1], Guimarães FS[1] [1]FMRP-USP − Pharmacology

Introduction: obsessive-compulsive disorder (OCD) is an anxiety disorder characterized by the occurrence of obsessions (recurrent, excessive thoughts, attempted to be ignored and recognized as mind product) and compulsions (repetitive behaviors or mental acts). The marble burying test (MBT) was initially proposed as a screening model for anxiolytic compounds. But, contrary to most anxiety tests based on exploratory behavior, the observed effects (number of buried marbles) do not decrease following repeated test exposure. This fact led to the proposal that the MBT would rather evaluate a repetitive behavior potentially compulsive (Thomas 2009). Endocannabinoids (eCB), acting mainly through CB1 receptors, can reduce the release of several neurotransmitters. Glutamate is a major neurotransmitter in cortico-striato-thalamo-cortical circuits that has been implicated in the pathophysiology of OCD (Carlsson 2000). Drugs attenuating glutamate neurotransmission are effective in the treatment of OCD patients and in the MBT. Based on this evidence, the present study investigated the effects of CB1-activating drugs in mice submitted to the MBT. **Methods**: male C57BL6/J mice received ip injection of CB1 agonist (WIN55,212-2 at 0.3, 1, 3 mg/kg dose), FAAH inhibitor (URB597, 0.1 and 0.3 1mg/kg) or vehicle (2%Tween80 in sterile saline). An independent group received previous injection of the CB1 antagonist AM251 (1mg/kg) or vehicle, followed by WIN (1mg/kg) or URB (0.3mg/kg) injection. Another group were submitted to the open-field test to evaluate locomotor activity (ethical committee protocol number: 146/2009). **Results**: all drug treatments reduced the number of buried marbles in a dose-dependent manner [WIN: 22%, 34% and 91%; $F(3,20)=31.72$. URB: 49%, 71%, 94%; $F(3,21)=23.11$; from respective control groups]. Previous AM251 injection was able to block WIN and URB effect in the MBT. Only WIN at the 3mg/kg dose decreased the total distance travelled in the open-field test. **Discussion**: The results suggest that the endocannabinoid system could be a potential target in the therapy of obsessive-compulsive spectrum disorders. **Reference**: Carlsson ML. *Acta Psychiatr. Scand.* 102. 401. 2000. Thomas A. *Psychopharmacol (Berl).*204. 361. 2009. **Financial support**: Fapesp, CNPq.

03.002

iNOS knockout mice show increased expression of contextual fear conditioning: involvement of nNOS. Lisboa SF[1], Gomes FV[1], Cunha TM[1], Cunha FQ[1], Guimarães FS[1], Corrêa FMA[1], Joca SRL[2], Resstel LBM[1] [1]FMRP-USP, [2]FCFRP-USP − Física e Química

Introduction: The contextual fear conditioning (CFC) paradigm has been widely used as an animal model to study the neural substrate of defensive responses. The absence of the neuronal nitric oxide synthase enzyme gene (nNOS) in mice induces a decrease in the expression of CFC. In contrast, mice with deletion of the inducible isoform gene (iNOS-/-) present higher anxiety in the elevated plus maze, a trait likely to be related to a compensatory increased basal NOS activity in their central nervous system (CNS). Although the association between NO and stress-related behavior has been well documented, the participation of iNOS on the CFC has not been directly tested. Thus, the aim of this study was to investigate the behavior of iNOS knockout mice (iNOS-/-) in the expression and extinction of CFC. **Methods:** Immunofluorescence for iNOS was performed in the prefrontal cortex and hippocampus of iNOS-/- and their wild type (WT) littermates. Mice were submitted to an inescapable footshock conditioning session and were re-exposed to the same conditioning chamber 24h, 48h, 72h and 96h later, when the freezing response was recorded (Ceua: 166-2007). Independent groups of WT received systemic administration of a nNOS inhibitor, 7-nitroindazole (7-NI; 15, 30 or 60 mg/kg), 30 min before the test session. The effective dose (30mg/kg) was administered to iNOS-/- mice to CFC evaluation. **Results:** The qualitative analysis of prefrontal cortex and hippocampus of iNOS-/- showed no expression of iNOS, while there was basal expression of iNOS in their WT mice. The conditioned iNOS-/- animals (n=6) did not differ from the WT (n=6) on the expression of CFC (*Student's* t test; $P>0.05$), but spent more time in freezing behavior when freezing was evaluated 48h, 72h and 96h later ($F_{1,10}=8.12$; $p=0.02$), suggesting a deficit in the acquisition of aversive memory extinction. Furthermore, the systemic administration of 7-NI at dose of 30 mg/kg (n=9) decreased conditioned fear expression ($F_{3,27}=12.9$, $P<0.001$) while 15 (n=8) and 60 mg/kg (n=8) facilitated the acquisition of aversive memory extinction in WT animals ($F_{3,27}=11.4$; $P<0.0001$). The iNOS-/- presented increased expression of fear conditioning after administration of vehicle compared to vehicle-treated WT ($F_{1,18}=17.3$, $P<0.001$). Moreover, 7-NI (30 mg/kg; n=6) administration to iNOS-/- mice was also able to attenuate the increased expression of fear conditioning to levels similar to the vehicle-treated WT ($F_{1,18}=17.2$, $P<0.01$), suggesting that these animals present an overcompensation of NO production by nNOS. Furthermore, during extinction procedure there was a significant effect of genotype ($F_{1,28}=7.2$, $P=0.015$) and treatment ($F_{1,28}=20.1$, $P<0.001$), but no interaction between them ($F_{1,18}=0.8$, $P>0.05$), suggesting that although the iNOS-/- express more fear behavior, this decrease along the time and the treatment with 7-NI can facilitate the extinction. **Conclusion:** The iNOS-/- mice presented increased expression of fear conditioning behavior and impaired acquisition of extinction memory which could be a consequence of a compensatory increased NOS activity, since that nNOS pharmacological inhibition in the iNOS-/- mice attenuated the fear response. **Financial support:** CNPq, FAPESP and FAEPA.

03.003

Evaluation of the anxiolitic activity of the imidazolidinic derivative HPA-14. Carvalho FL[1], Mota VG[1], Nóbrega FFF[1], Salgado PRR[1], Fonsêca DV[1], Morais LCSL[1], Souza SA[2], Athayde-Filho PF[2] [1]UFPB − Pharmaceutical Technology, [2]UFPB − Chemistry

Introduction: About 62% of the drugs of synthetic origin used in modern medicine have a heterocyclic ring in their structure. Among these, we highlight the hydantoins because of its many pharmacological properties, such as antihypertensive, anticonvulsant, anticancer and antiparasitic. The hydantoin derivative HPA-14 (5-(4-isopropylphenyl)-3-phenyl-imidazolidin-2, 4-dione), obtained by organic synthesis was studied in this work in order to assess its possible anxiolytic effects in animal models. **Methods**: *Swiss* male mice (25-35 g, n = 8) were divided into groups and treated intraperitoneally with vehicle (distilled water and Tween 80), diazepam 0.5 mg / kg or HPA-14 at different doses (75, 150 and 300 mg / kg). The animals of diazepam group were evaluated in the tests of Elevated plus maze and Hole board 30 minutes after treatment, whereas the experimental and control groups were evaluated after one hour due to the results obtained previously in a pharmacological behavioral screening. All experiments were approved by the Ethics Committee for Animal Research of the Laboratory of Pharmaceutical Technology of UFPB (protocol number 0510/09). **Results and Discussion***:* In the elevated plus maze test only the dose of 150 mg/kg provided a significant reduction ($p < 0.05$) of the time spent in the closed arms (108.1 ± 27.2 s) compared to control (220.8 ± 12.2 s). None of the other parameters (number of entries in open or closed arms and time spent in open arms) was affected by the administration of HPA-14. In the hole board test, all doses (75, 150 and 300 mg / kg) were able to reduce ($p < 0.01$) the ambulation, recorded by the number of segments crossed (10.5 ± 3.4; 4, 9 ± 1.1 and 7.6 ± 2.4, respectively, vs control: 27.6 ± 3.7), and the number of dives (10.6 ± 2.5; 4.0 and 7.3 ± 1.1 ± 1.4, respectively, vs 19.9 ± 2.4) and increase ($p < 0.01$) the immobility time (81.9 ± 27.0; 86.6 ± 18.0 and 86, 8 ± 17.3 s, respectively, vs 1.0 ± 0.5 s). Only the dose of 150 mg / kg was able to increase ($p < 0.05$) latency to the first dive (43.8 ± 20.1 vs 6.3 ± 0.96 s). These results suggest that the imidazolidinic derivative HPA-14 has a pharmacological profile suggestive of sedative drug, requiring the achievement of other behavioral tests to confirm this profile and characterization of the mechanism of action of this substance. **Financial support***:* CAPES and FAPESQ.

03.004

Effects of hippocampal iNOS or nNOS inhibition in models of anxiety and depression. Sato VAH[1], Sales AJ[2], Joca SRL[2] [1]FMRP-USP − Farmacologia, [2]FCFRP-USP − Física e Química

Introduction: Nitric oxide (NO) and the NO synthases (NOS) are present in a wide range of brain regions involved in the modulation of defensive and adaptative behaviors, including in the dorsal hippocampus. Recent evidences have suggested that systemic or hippocampal administration of non-selective NOS inhibitors causes anxiolytic- and antidepressant-like effects in animal models. However, the NOS isoform involved in these effects are not clearly defined. Considering that mediators of the inflammatory response as well as upregulation of NO production can be induced by exposure to stress, the aim of the present study was to investigate the differential involvement of hippocampal inducible NOS isoform (iNOS) and neuronal NOS isoform (nNOS) in the modulation of defensive behaviors related to depression and anxiety, using the Forced Swimming Test (FST) and Elevated Plus Maze (EPM), respectively. **Methods:** Male Wistar rats with guide-cannulas aimed at the dorsal hippocampus were submitted to FST or EPM. In the FST, rats were submitted to pretest (PT: 15 min swimming) and received a local administration of n-propyl-L-arginine (NPLA, selective nNOS inhibitor: 0.001, 0.01, 0.1 or 1.0 nmol/0.5 µL), 1400W (selective iNOS inhibitor: 0.001 nmol/0.5 µL) or vehicle (0.5 µL). One day later, the immobility time (IT) was registered at a 5 min swimming test. In the EPM, rats received hippocampal administration of NPLA (0.01 nmol/0.5 µL), 1400W (0.001 nmol/0.5 µL) or vehicle (0.5 µL) 5 min before being exposed to EPM, where the time spent in the open and enclosed arms were scored for 5 min. Diazepam (2.5mg/kg, IP) was used as a positive control. All protocols were approved by a local ethical committee (Prot. 08.1.1133.53.4). **Results:** NPLA, but not 1400W, reduced the IT in the FST ($F_{55,5}$ = 11.22; $p<0.001$), an antidepressant-like effect in this model. In EPM, the treatments (NPLA, 1400W and Diazepam) increased the time spent in the open arms ($F_{30,3}$=5.35; $p<0.05$), indicating an anxiolytic-like effect. NPLA, 1400W or Diazepam did not induce significant locomotor effects since no differences between treatments were detected in the total number of entries in the arms of the EPM ($F_{30,3}$=1.526; $p=0.228$). Conclusions: These results indicate that hippocampal nNOS and iNOS may be differentially involved in the regulation of defensive behaviors related to anxiety and depression. **Financial support:** CAPES, CNPq and FAPESP.

03.005

Intra-hippocampal injection of cannabidiol induces antidepressant-like effect in the rat forced swimming test. Biojone C[1], Silva M[1], Moreira FA[2], Guimarães FS[1], Joca SRL[3] [1]FMRP-USP – Farmacologia, [2]ICB-UFMG − Farmacologia, [3]FCFRP-USP − Física e Química

Introduction: Cannabidiol (CBD) is a non-psychotomimetic compound from *Cannabis sativa* that induces anxiolytic- and antipsychotic-like effects in animal models. Recently, we reported *[Zanelati et al., Br J Pharmacol, 159(1), p.122, 2010]* antidepressant-like effect of systemically injected CBD, an effect mediated by 5-HT1a receptors. However, the brain sites involved in this effect have not been described so far. Considering the involvement of the dorsal hippocampus (DH) in the neurobiology of depression and that it has a high expression of 5-HT1a receptors, we aimed at investigating the involvement of this brain region in CBD-induced antidepressant-like effects. **Material and Methods:** Male Wistar rats (240g) with guide cannulas aimed at the dorsal hipocampal were submitted to the forced swimming pretest session (15 min), which was followed by the test session (5 min of forced swimming), 24h later. CBD (15, 30, 60 nmol/0.5 ul), 8-OH-DPAT 10 nmol or vehicle (grape seed oil) were microinjected in the DH 10 min before the test session. The latency for the first immobility and the total immobility time in the test session were scored by a trained and blinded observer. An independent group of rats received the same drug treatments and was tested in the open field test, where the total distance moved was recorded during 5 min, in order to investigate possible unspecific locomotor effects induced by the treatments. Data was analyzed by one-way ANOVA followed by Dunnet's test. The protocol used in this study was previously approved by Ethical Committee of the School of Medicine of Ribeirão Preto – USP (Proc. N. 08.1.1133.53.4). **Results:** CBD (15, 30 nmol) and 8-OH-DPAT decreased the total immobility time [$F_{4,44}$=4.93, $p<0.05$] and increased the latency to present the first episode of immobility [$F_{4,44}$=3.48, $p<0.05$]. In the open field test, no difference was observed between CBD 30, 60 nmol and vehicle group [$F_{2,12}$=0.087, $p>0.05$]. **Conclusion:** Intra-hippocampal CBD induces antidepressant-like effects similar to the 5-HT_{1A} agonist 8-OH-DPAT, an effect that is dissociated from unspecific locomotor effects. Therefore, the hippocampus seems to be an important brain site mediating CBD-induced antidepressant-like effects. **Financial support:** FAPESP, FAEPA.

03.006

Systemic inhibition of inducible NO synthase (iNOS) evokes antidepressant-like effects. Montezuma K[1], Biojone C[2], Guimarães FS[2], Joca SRL[1] [1]FCFRP-USP − Física e Química, [2]FMRP-USP − Farmacologia

Introduction: Recent evidences have shown that systemic administration of NOS inhibitors induces antidepressant-like effects in animal models. However, the precise involvement of the different NOS isoforms in these effects has not been clearly defined yet. Considering that mediators of the inflammatory response as well as upregulation of NO production can be induced by exposure to stress, the aim of the present study was to test the hypothesis that iNOS-mediated NO production could be involved in the stress-induced behavioral consequences, such as the depressive-like behavior in the forced swimming test (FST). Therefore, we investigated the effects induced by the systemic injection of aminoguanidine (amg, preferential iNOS inhibitor), 1400W (selective iNOS inhibitor) or n-propyl-L-arginine (NPA, selective nNOS inhibitor) in mice submitted to the FST. **Methods:** Male Swiss mice (25-30g) received different doses of amg (75, 150 and 300 mg/kg), saline (10 ml/kg, ip) or the antidepressant desipramine (5 or 10 mg/kg). Thirty minutes later, the animals were submitted individually to 6 minutes of forced swimming into glass cylinders, when the immobility time was scored during the last 4 min by a trained and blinded observer. Independent groups received amg (75 mg/kg, effective dose), 1400W (0,75 µg/kg) or NPA (1,3 µg/kg) and were submitted to FST. 1400W and NPA doses were chosen based on the magnitude of their Ki values for iNOS and nNOS, respectively, when compared to amg Ki values, with the purpose to ascertain whether amg-induced effects would involve iNOS or nNOS inhibition, since it is known that nNOS inhibition induces antidepressant-like effects. Another independent group received the same treatments and was submitted to the open field test (6 min) in order to investigate possible unspecific drug-induced locomotor effects. The protocol used in this study was approved by the local Ethical Committee (CEUA no. 10.1.135.53.6). **Results:** Amg (75mg/kg) significantly decreased the immobility time when compared to the vehicle group (mean ± EPM: vehicle: 116.5±12.9, amg 75: 67.5±12.4*, amg 150: 126.7±12.46, amg 300: 89±11.2; $F_{3,20}$=4.8; p<0.05; *p<0.05, Dunnetts), similarly to desipramine (mean ± EPM: vehicle: 125±15.3, desipramine 5: 54.8±6.9*, desipramine 10: 58.6±15.3, $F_{2,11}$=6.61, p<0.05, *p<0.05, Dunnetts). 1400W, but not NPA, also reduced the immobility time (mean ± EPM: vehicle: 155.5±7.9, amg 75: 75.88±14.3*, 1400W: 102.5 ±16.5*, NPA: 128±17.18, $F_{3,28}$=5,58; p<0.01; *p<0.05, Dunnetts), indicating an antidepressant-like effect. Furthermore, none of the treatments induced significant locomotor effects in the open field test. **Discussion:** This is the first study to show that selective iNOS inhibition induces antidepressant-like effects. Our results thus suggest that iNOS could also be involved in the increased NO production that is thought to mediate the behavioral consequences of inescapable stress. Therefore, our results further support the involvement of NO in the neurobiology of depression. **Financial support:** FAPESP.

03.007

Shock intensity and duration: importance to induce learned helplessness in rats. Donadon MF, Padovan CM FFCLRP-USP − Psicologia e Educação

Introduction: Learned Helplessness (LH) is a classical animal model of depression based in impairment of avoidance to signalized escapable shocks (ES) after pre-exposure to inescapable footshocks (IS). These responses are attenuated/prevented by chronic treatment with antidepressant drugs, such as Imipramine (IMI), a 5-HT/NA reuptake blocker. Great attention is being given to ethical aspects involving animals in research, trying to minimize their suffering. Therefore, the aims of our work were: to validate an LH protocol (Experiment 1); and test whether chronic treatment with IMI would prevent LH in animals submitted to IS in the protocol defined in Experiment 1. The effects of chronic treatment with MK-801, an NMDA receptor antagonist, in this model were also investigated. **Methods:** Experiment 1: Male wistar rats (300g) were pre-exposed (PE) to protocols (P) with 40 IS or ES, which varied in intensity (0.6mA for P1 and P2 – or 0.4mA for P3) and duration in seconds (sec; 5 sec for P1 or 10 sec for P2 and P3). 24 hours later they received 30 ES (0.4mA or 0.2mA, respectively for P1/P2 and P3) signalized by a light stimulus (10 sec.) that remained on until the end of the shock. Shocks could be avoided/interrupted by crossing to the opposite side of the box. Control groups (P4, P5 and P6) received no shocks (NS) in PE. In Experiment 2 rats received 21 daily i.p. injections of Saline (1ml/kg; n=16); IMI (15mg/kg; n=17) or MK801 (0.05mg/kg; n=16) being the last injection immediately after pre-exposure to IS as in P2. Tests were performed 24 hours later. Helplessness (LH) was considered when number of failure to avoid/escape shocks was greater than 10. Percent of LH (%LH) animals per group were calculated and then analyzed by Chi-Square test. All procedures were approved by the local Animal Ethics Committee (CEUA) of the USP Ribeirão Preto (protocol 06.1.1131.53.0). **Results**: Pre-exposure to IS or ES (0.6mA; 5") increased %LH (92% and 71%) when compared to NS (27%) during test session (0.4mA; 5sec) (X_2^2=10.9; p<0.05). Decreasing the intensity of footshocks (IS or ES of 0.4mA) and increasing its duration (10sec.) induced similar results on %LH (50% for both conditions) when compared to NS rats (25%) (X_2^2=0.7; p>0.05). On the other hand, keeping 0.6mA of intensity but increasing its duration up to 10sec did change the performance of animals: %LH were lower on ES or NS groups (17% each) than in IS (100%) (X_2^2=71,9; p<0.05). Using this protocol chronic treatment with IMI prevented induction of LH (15%) when compared to Saline (83%) and MK801 (87,5%) treated rats (X_2^2=17.2; p<0.05). **Discussion:** Lower intensities of IS did not induce LH even when IS was longer in duration. IMI was able to prevent LH. **Affiliation:** SBFTE **Financial Support:** CAPES, CNPq and FAPESP, RUSP.

03.008

Cannabidiol attenuates MCPP-induced increase in marble burying behavior. Nardo M, Casarotto PC, Guimarães FS FMRP-USP

Introduction: the marble burying test (MBT) evaluates repetitive behaviors that can become compulsive. Since these behaviours are sensitive to serotonin reuptake inhibitors and repeated exposure to marbles does not lead to behavioral tolerance, this model has been proposed as a screening tool for potential drug treatment of obsessive-compulsive disorder (OCD). m-chloro-phenyl-piperazine (mCPP) is a non-specific serotonergic agonist (mainly 5HT1A, 5HT2C and 5HT1D receptors) reported to increase symptoms in OCD. Cannabidiol (CBD) is a non-psychotomimetic component of *Cannabis sativa* that shows anxiolytic and antidepressant effects in several animal models by acting as a 5HT1A receptor agonist. Based on these pieces of evidence the present study was aimed at investigating if mCPP could increase marble burying behavior and if CBD would prevent this effect. **Methods**: male S*wiss* mice weighting 25-30g and housed in groups of 20 mice/cage received ip injections of mCPP, CBD, paroxetine (PRX) or vehicle (Tween80 2% in saline) and were submitted to the MBT. The animals were put in the centre of a squared box (38 x 32 x 28 cm) with 5-cm sawdust layer covered floor where twenty-five green clear glass marbles had been evenly spaced. After 30 min the number of buried marbles (at least two thirds sawdust covered) was counted. Twenty or 30 minutes after mCPP (0; 0.1; 0.3 and 1mg/kg dose) or CBD (0, 5, 15 and 30 mg/kg), respectively, independent groups of animals were submitted to the MBT. In another experiment mice received a first PRX (10mg/kg), as positive control, or an ineffective CBD dose (15mg/kg) injection followed, 10 min latter, by vehicle or mCPP (0.1mg/kg) and submitted to the MBT 20 min after the last injection. Independent groups receiving mCPP (0.1mg/kg) or vehicle were submitted to the open field test 20 minutes after drug injection to evaluate locomotor activity (total distance travelled) and anxiety parameters (% time spent in central area). Ethical committee protocol number 146/2009. **Results**: mCPP caused a dual effect, increasing (Mean+/-SEM; at 0.1mg/kg: 18.7+/-0.68) or decreasing (at 1mg/kg: 5.1+/-1.2) the number of buried marbles compared to vehicle (10.56+/-0.91). Only the 30mg/kg CBD dose was able to decrease this behaviour (CBD: 5.00+/-1.32; vehicle: 11.9+/-0.83). However, PRX or an ineffective dose of CBD (15mg/kg) were able to block mCPP-induced increase in buried marbles. No effect was observed in the open-field analysis. **Discussion**: our results suggest that low doses of mCPP increase the number of buried marbles without affecting locomotor or anxiety-associated measures, reinforcing the idea that the MBT is more akin to repetitive than anxiety-related behaviours. In addition, CBD was able to block this effect, suggesting a possible role for this substance in the treatment of obsessive compulsive spectrum disorders. **Financial support**: Fapesp and CNPq

03.009

Withdrawal from methylphenidate increases neural reactivity of dorsal midbrain. Ferreira R, Shimizu-Bassi G, Nobre MJ FFCLRP-USP − Psicologia e Educação

Introduction: Ritalin (methylphenidate hydrochloride, MP) is a non-amphetamine psychostimulant and is the drug of choice to treat children and adults with attention deficit hyperactivity disorder (ADHD). However, concerns about the possible overuse of MP in young children have been disseminated both in the media and in scientific publications. There is also controversy over whether MP treatment has the potential to elicit drug dependence like other psychostimulants, such as cocaine and amphetamine. Behavioral studies have demonstrated that rats treated with MP during early developmental stages exhibit alterations in anxiety-related processes such as an increased response to stressful stimuli, elevated plasma levels of corticosterone and depression-like symptoms in adulthood. However, to date, few studies have addressed the consequences of chronic MP exposure or withdrawal after chronic treatment in adults. **Methods***:* Eighty-three male Wistar rats weighing 200-210 g at beginning of the treatment. Rats were submitted to 20 methylphenidate (Ritalin, Novartis) subcutaneous injections (days 1-3 = 5 mg, 4-6 = 10 mg, 7-11 = 20 mg/kg) twice a day. After initial exposure to an elevated plus-maze (EPM), brainstem neural activation elicited by exposure to EPM aversive cues was analyzed using a Fos-protein immunolabelling technique. Additional independent groups of animals were submitted to electrical stimulation of the dorsal periaqueductal gray (dPAG) and startle response procedures in order to verify the influence of MP or withdrawal on the expression of fear-related behaviors and motor response, respectively. (CEUA nº: 08.1.1547.53.3). **Results***:* Chronic treatment with MP, per se, did not promote any effects on the behavior of rats tested after the interruption of the long-term treatment in EPM, when compared with those obtained control animals. On the other hand, 48 hours of MP withdrawal enhanced significantly the expression of anxiety-like behaviors. MP chronic effects were effective in reducing Fos expression in median raphe nucleus and locus coeruleus. On the other hand, 48 hours of MP withdrawal resulted in significant increases of Fos immunolabeling in almost all areas in study, including all periaqueductal gray columns and the central nucleus of inferior colliculus. Forty-eight hours of MP withdrawal on the aversive thresholds of animals submitted to the electrical stimulation of the dPAG showed a decrease in freezing and a consequent increase in escape thresholds as compared to control rats. Rats tested under MP chronic effects have increased amplitude of startle, when compared with saline pre-treated animals. On the other hand, no effects were observed on the amplitude of startle of MP withdrawn-rats. **Discussion***:* Our results present new findings about the influence of MP treatment in adult rats, showing that after a sudden MP treatment-break increased anxiety and sensitization of anxiety-associated brainstem regions develop.

03.010

Truck drivers take indiscriminated use of amphetamines concomitant with toxic substances. Vieira BAC[1], Souza LA[2], Marques CD[2], Salomão PAV[2], Souza CL[3] [1]CEUNSP, [2]CEUNSP − Farmácia, [3]CEUNSP − Nutrição

Introduction: The amphetamines are stimulant drugs that acts in the central nervous system (CNS), popularly known as “rivet” by professional drivers in order to inhibit sleep. It is known that its use may occur concomitantly with psychoactive drugs like cocaine and cannabinoids. However, there are few reports in the literature related to this subject. **Objective**: The aim of this study was to analyze the profile of the truck drivers users of amphetamines, as well as their adverse effects and interactions with other drugs. **Method**: A total of 272 truck drivers from Iconha (ES), Aparecida do Norte (SP) e Araporã (MG) was analyzed. A questionnaire based on the report of this population was applied. Statistical analysis was performed using Excel. **Results**: The average age of the participants was 38.5 ± 10.6 years and the body mass index was 27.3 ± 4.5 kg/m². It was found that 87.5% travelled unaccompanied and 39,8% used some type of amphetamine, and of these, 65,7% used for more than two years. The participants reported buying the drug at gas station (60.18%) and at pharmacies (39.82%). The most frequently reported adverse effects were dry mouth (79.6%), euphoria (53.7%), loss of appetite (50,9%) and less frequently reports of headache and tachycardia. It was found that amphetamine was used concomitantly with alcohol (65.4%), tobacco (57.7%), marijuana (25.7%) and cocaine (23.8%). **Discussion and Conclusion**: The results of the study of Moreira Gadana (2009) showed that 65% of the population of drivers was amphetamine users, confirming the high rate of drug through this present study. This high number of users is because of the difficult profession, requiring many hours of work and a longer period of wakefulness, as well as easy acquisition of these drugs through illegal purchase, in other words, in unauthorized establishments and without prescription required by law. The adverse effects detected by this study caused no surprise to these users, as they had known these effects by many sources of information. High incidence of concomitant use with other drugs due to the fact that drug interactions increase the time required to be wakefulness. This study brings a reflection of the problems with accidents that these professionals can cause in traffic and disregard the traffic law.

03.011

Effects of anti-inflammatory and antidepressant strategies on depressive-like behavior in complete Freund's adjuvant (CFA)-treated mice. Maciel IS[1], Silva RBM[1], Calixto JB[2], Morrone FB[3], Campos MM[4] [1]PUCRS − Farmacologia, [2]UFSC − Farmacologia, [3]PUCRS − Farmácia, [4]PUCRS − Cirurgia-Odontologia

Introduction: Several studies have demonstrated a link between acute inflammation and behavioral alterations in animal models (Narita et al., J. Neur. 31, 739, 2006). It has been described that inflammation induced by bacterial lipopolysaccharide (LPS) is associated with development of sickness behavior, which is characterized by anhedonia, decreased food intake and locomotion, social isolation and changes in circardian cycle (Dantzer et al., Nature Neuro Rev. 9, 46, 2008). This work was aimed to characterize the depressive behavior in the mouse model of chronic inflammation induced by Complete Freund's Adjuvant (CFA). We also evaluated the effects of antidepressant and anti-inflammatory drugs in the responses induced by CFA. **Methods:** Male Swiss mice were used (8 per group, 25 − 30g). To induce chronic inflammation, animals received an intraplantar (i.pl.) injection of CFA (50 µl/paw). The control animals received 50 µl/paw of PBS. Two weeks after CFA administration, mice were submitted to the following tests: (i) *paw edema evaluation:* the increase in paw volume was measured with plethysmometer (in µl; Ugo Basile); (ii) *tail suspension test* (TST): this test considers the immobility time (during a 6-min period of evaluation), as an index of depressive state (despair behavior). Seven days after the i.pl. injection of CFA, animals were treated orally with imipramine (10mg/kg), fluoxetine (20 mg/kg), buprobione (20mg/kg), dexamethasone (0.5 mg/kg), celecoxib (30 mg/kg) or idomenthacin (10 mg/kg), once a day for 7 days. All the experimental protocols were approved by the local Ethics Committee (09/00104, PUCRS). **Results:** The chronic treatment with the antidepressant drugs imipramine, fluoxetine and bupropione was able to significantly prevent the depressive behavior induced by CFA (60 ± 7%; 66 ± 11% and 64 ± 7%, respectively), whereas it did not alter the paw edema formation. In addition, the chronic treatment with the anti-inflammatory drugs indomethacin and dexamethasone reduced the paw edema evoked by CFA (71 ± 5% and 62 ± 4%, respectively), without affecting depressive behavior. Interestingly, the treatment with celecoxib (30 mg/kg) was capable of significantly prevent both the depressive behavior induced by CFA (77 ± 6%) and the paw edema formation induced by this inflammatory agent (68 ± 4%). **Discussion:** The present study demonstrates that chronic inflammation induced by CFA is accompanied by depressive-like behavior, which is sensitive to the treatment with antidepressant drugs. However, among the tested anti-inflammatory drugs only the selective inhibitor of COX-2 celecoxib was able to reverse either the edematogenic response or the depressive behavior induced by CFA. Additional studies are under development to further characterize the mechanisms involved in this response. **Financial support:** CAPES, CNPq and FAPERGS.

03.012

One trial tolerance phenomenon to benzodiazepines: contribution of dorsal hippocampus protein synthesis. Gazarini L, Stern CAJ, Bertoglio LJ UFSC − Farmacologia

Introduction: The anxiolytic-like effect of drugs (e.g. benzodiazepines, barbiturates and ethanol) is no longer observed in rodents retested in the elevated plus-maze (EPM), a phenomenon described as one trial tolerance (OTT). The establishment of this phenomenon appears to be associated with the retrieval of aversive information acquired on EPM testing [1]. Brain regions implicated in these processes are now being investigated. Given that protein synthesis in dorsal hippocampus (DH) is generally necessary for memory consolidation [2], the objective of the study was to investigate whether the anisomycin (ANI)-induced DH protein synthesis inhibition would disrupt the aversive memory consolidation, and consequently prevent the midazolam OTT occurrence on EPM retesting. **Methods**: Male Wistar rats, aged three-months, were bilaterally implanted with guide cannulas aimed at the DH. One-week later, they were allocated to four groups (n=8-12/group) based on DH injections after EPM test [phosphate buffered-saline (PBS; vehicle) or ANI (80 µg) in 0.8 µl/side] and, 24 h later, systemic (*i.p.*) treatment 30 min before EPM retest [Saline (SAL, vehicle) or midazolam (MDZ; 0.5 mg/kg)]. Behavioral measures scored during 5 min in both test and retest were the percentage of open-arm entries (%OAE) and time (%OAT), stretched-attend postures (SAPs) and enclosed-arm entries (EAE). The experimental design aforementioned was approved by the local Ethical Committee in Animal Research (068/CEUA/PRPe/UFSC/2008). **Results:** Two-way analysis of variance (ANOVA) followed by Newman-Keuls test showed an increase in both %OAT and %OAE of MDZ/ANI-treated rats relative to MDZ/PBS-treated rats on EPM retesting [$F(1,38) = 9.44$, $p<0.001$; MDZ/PBS (mean ± S.E.M) = 3.4 ± 1.5 and MDZ/ANI = 16.1 ± 2.9 and $F(1,38) = 5.66$, $p<0.001$; MDZ/PBS = 9.9 ± 4.0 and MDZ/ANI = 36.9 ± 2.9, respectively]. As expected, the MDZ/PBS group did not increase ($p>0.05$) the retest open-arm exploration when compared to SAL/PBS-treated group. Importantly, these results were observed in the absence of changes in EAE, an EPM index of general exploratory activity. No statistically significant differences were observed for SAPs. Moreover, repeated-measures ANOVA showed that SAL-treated groups expressed further avoidance to open-arms, characterized by reduced %OAT [$F(1,38) = 10.4$, $p<0.01$; test = 13.7 ± 1.7 and retest = 2.1 ± 0.7] and %OAE [$F(1,38) = 7.63$, $p<0.01$; test = 31.5 ± 2.4 and retest = 10.6 ± 3.1], during the EPM retest when compared to their respective level on testing. **Discussion:** Confirming the memory trace underlying OTT, present results provide evidence that the DH protein synthesis contributes to this phenomenon, but not further avoidance to open-arms, exhibited by rats on EPM retesting. [1] Carobrez, A.P. et al. *Neurosci Biobehav Rev.*, 29(8), 1193, 2005. [2] Dudai Y. *Neuron.*, 17(3), 367, 1996. **Financial support:** CNPq and FAPESC.

03.013

Effect of transient reversible inactivation of the ventral hippocampus in rats submitted to the forced swimming test. Diniz CRAF, Casarotto PC, Joca SRL FCFRP-USP − Física e Química

Introduction: Studies of functional neuroanatomy indicate that the hippocampus can be divided into two functionally distinct subregions: the ventral hippocampus (VH), more closely related to anxiety and fear, and dorsal hippocampus, related to processes of learning, memory and hence the behavioral consequences of stress. However recent studies indicate an intercommunication between the dorsal and ventral regions. While inactivation of the dorsal hippocampus has been reported to induce antidepressant-like effects, the involvement of the VH in the modulation of depressive-like behaviors remains poorly explored. Therefore, the objective of this study was to evaluate VH involvement in the mediation of the stress-induced behavioral consequences associated to depression through its pharmacological inactivation with cobalt chloride ($CoCl_2$). **Methods:** seven days after the stereotaxic surgery, male Wistar rats (n = 8-10/group) with guide-cannulas aimed at the VH were submitted to a pretest session (PT: 15 min of forced swimming) and, 24h later, the immobility time (in seconds) were registered at a period of a 5 min test session. $CoCl_2$, a synaptic transmission inhibitor (1 nmol/0.5 µL dose) or saline (vehicle) were administered in 3 different periods in independent experiments: before the pretest (n=8-9 animals/group), after pretest (n=8-9) and before test (9-10). All protocols described herein have been approved by a local ethical committee (CEUA 09.1.441.53.8). **Results:** $CoCl_2$ administration before pretest did not induce significant effects [Mean +/- SEM: vehicle: 99+/-24, $CoCl_2$: 96+/-15]. On the other hand after pretest [vehicle: 55+/-18, $CoCl_2$: 127+/-27] and before test [vehicle: 71+/-9, $CoCl_2$: 121+/-21], $CoCl_2$ administration increased the immobility time. **Discussion:** Our results point to a differential and time dependent participation of ventral hippocampus in the modulation of stress-induced behavioral consequences related to depression. **Financial Support:** CNPq, FAPESP.

03.014

Involvement of local insular cortex neurotransmission on contextual fear conditioning. Alves FHF[1], Reis DG[2], Crestani CC[2], Corrêa FMA[3], Resstel LBM[3] [1]FMRP-USP − Farmacologia

Introduction and goals: The insular cortex (IC) is a limbic structure involved with both autonomic and behavior modulation during defensive responses. In rats, contextual fear conditioning evokes both freezing behavior and cardiovascular changes, mean arterial pressure (MAP) and heart rate (HR) increases, which are accompanied by IC activation. However, the IC roles on autonomic and behavior responses associated to fear conditioning to context has not been fully studied. **Materials and Methods**: Male Wistar rats (250g) had guide cannulae bilaterally implanted in the IC for microinjection, 100 nL each site, of 1mM of non-selective synapse blocker $CoCl_2$ or vehicle at different periods of the experimental procedure: 10 min before or immediately after the conditioning session or 10 min before the chamber re-exposition (test session). Twenty four hours before the test session, animals were submitted to conditioning session, when animals received 6 foot electrical shock (1.5 mA, 3 s). After the conditioning session a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. The Institution's Animal Ethics Committee approved the housing conditions and experimental procedures (process number: 215-2005). **Results:** Bilateral administration of $CoCl_2$ in the IC had no effect on both behavioral ($t=0.63$, $P>0.05$, $n=6$) and cardiovascular responses (MAP: $F_{(1,150)}= 0.38$, $P>0.05$ and HR: $F_{(1,150)}=0.75$, $P>0.05$) when administered before conditioning session. However, the animals which had received IC bilateral microinjection of $CoCl_2$ immediately after conditioning session or 10 min before the chamber re-exposition, test session, significantly reduced both behavioral ($t=7.46$, $P<0.0001$, $n=7$ and $t=5.6$, $P<0.001$, $n=5$ respectively) and cardiovascular (MAP: $F_{(1,150)}=101.5$, $P<0.0001$ and HR $F_{(1,150)}=102.9$, $P<0.0001$; MAP: $F_{(1,150)}=60.34$, $P<0.0001$ and HR: $F_{(1,150)}=87.8$, $P<0.0001$ respectively) responses. **Conclusion:** The present findings suggest that IC integrity is crucial for consolidation and evocation of contextual aversive memory. However, IC does not appear to be important to acquisition of this memory.

03.015

DNA demethylating agents: new antidepressant drugs? Sales AJ[1], Biojone C[2], Gomes MVM[3], Joca SRL[1] [1]FCFRP-USP − Física e Química, [2]FMRP-USP − Farmacologia, [3]UNOPAR − Genética

Introduction: Recent evidences have suggested that epigenetic mechanisms are thought to play a role in the plastic and behavioral changes induced by stress and antidepressant drugs. For example, histone acetylation, which is associated with transcriptional activation of specific genes, is increased in the hippocampus after chronic antidepressant treatment, what is thought to account for their therapeutic effects. On the other hand, DNA methylation, which is associated with transcriptional repression, is increased by stress exposure. Moreover, higher levels of methylation at specific genomic loci have been found in the hippocampus of suicide victims. Despite that, the direct involvement of DNA methylation in the regulation of depressive-like behaviors has not been investigated yet. Therefore, the aim of the present study was to test the hypothesis that DNA demethylation and the subsequent increase in gene expression would induce antidepressant-like effects. The effects induced by systemic or intra-hippocampal administration of different DNA demethylating agents (decitabine and 5-azacytidine) were then investigated in rats submitted to an animal model of depression, the forced swimming test (FST). **Methods:** Male Wistar rats (8-9/group) were submitted to a forced swimming pretest (PT) and received 3 ip injections (0, 5 and 23h later) of decitabine (0.1, 0.2, 0.3, 0.4 mg/kg), 5-AZA (0.4, 1.6, 3.2 mg/kg), imipramine (15 mg/kg) or vehicle. 24h after PT, the immobility time was registered at a 5 min swimming test. An independent group of animals underwent the same behavioral and pharmacological manipulations but were submitted to the open field test in order to assess drug-induced unspecific locomotor changes. A third group of rats (n= 6-8/group) were submitted to PT and received an intra-hippocampal injection of decitabine (50, 100 or 200 nmol/0.5 uL) or saline and were submitted to the test 24h later. After the behavioral tests, the hippocampus was removed for further analysis of the genomic DNA methylation (quantification of 5-methyl-2-deoxy cytidine using an ELISA kit). All behavioral protocols described herein were approved by our local ethical committee (CEUA, 10.1.136.53.2). **Results:** Systemic treatment with decitabine or 5-AZA reduced the immobility time in the forced swimming test ($F_{7,52}=8.19$, $P<0.01$; $F_{4,33}=10.86$, $p<0.01$; respectively), in a dose dependent fashion, similarly to the prototype antidepressant imipramine. Decitabine microinjection into the hippocampus also induced antidepressant-like effect, at the dose of 100 nmol/0.5 uL ($F_{3,28}=3.6$, $p<0.05$; Dunnets, $p<0.05$). None of the treatments induced significant locomotor effects in the open field test. The genomic DNA methylation profile in the hippocampus is under investigation. **Conclusion:** The present results indicate that the treatment with DNA demethylating agents induces antidepressant-like effects, similarly to imipramine. Therefore, DNA methylation might constitute an important pharmacological target for new antidepressant drugs. The results also point to the hippocampus as a structure where stress-induced DNA methylation might regulate depressive like-behaviors and contributes to a better comprehension about the neurobiology of depression. **Financial Support**: FAPESP, CNPq.

03.016

New *N*-phenylpiperazine derivatives with antipsychotic-like activity in rodents bind to α_{1a} and α_{1b} receptors. Betti AH[1], Antonio CB[1], HASSE DR[2], Vieira RO[3], Martins TS[4], Barreiro EJ[4], Fraga CAM[4], Noel F[5], Rates SMK[1] [1]UFRGS − Ciências Farmacêuticas, [2]UFRGS − Psicofarmacologia Experimental, [3]UFRJ − Farmacologia Celular e Molecular, [4]FF-UFRJ − LASSBio, [5]UFRJ – Farmacologia Básica e Clínica

Introduction: Considering the necessity of an antipsychotic more effective and safer to treat schizophrenia, a series of *N*-phenylpiperazine derivatives was planned through molecular hybridization between clozapine and L-741 prototypes (Menegatti *et al., Bioorg Med Chem* 11(22):4807, 2003). Among the substances, LASSBio-1412, LASSBio-1413 and LASSBio-1422 were selected, once they were active in an animal model predictive to schizophrenia positive symptoms (apomorphine-induced climbing) and did not affect motor coordination. In addition, they presented a multireceptor profile (D_2-*like*, 5-HT_{1A}, 5-HT_{2A}) characteristic of atypical antipsychotics (Betti, *SBFTE*, 2009). Cahir and King (*Eur Neuropsychopharmacol* 15:231, 2005) demonstrated that atypical and typical antipsychotics also bound to α_{1A} and α_{1B} receptors with relatively high affinity and this property could be relevant for the antipsychotic activity. The aim of this study was to verify if LASSBio-1412, LASSBio-1413 and LASSBio-1422 bind to α_{1A} and α_{1B}; to establish their minimal effective dose in reducing apomorphine-induced climbing behavior (4 mg/kg, s.c.), which is blocked by typical and atypical antipsychotics; to test them in the ketamine-induced hyperlocomotion (10 mg/kg, s.c.), which is impaired preferentially by atypical antipsychotics. **Methods:** Rabbit and rat livers were used for binding assays (α_{1A} and α_{1B} receptors) using [^{3}H]-prazosin 0.1 nM. Adult male CF1 mice were used for *in vivo* assays. All protocols used were approved by UFRGS Research Ethical Committee (project number 2008220). **Results and Discussion:** LASSBio-1412 and LASSBio-1413 showed a moderate affinity for α_{1A} and α_{1B} receptors. The *K*i for α_{1A} and α_{1B} receptors were: LASSBio-1412: 2.28 μM; 1.80 μM. LASSBio-1413: 0.784 μM; 0.804 μM. LASSBio-1422: >27.2 μM; 5.90 μM, respectively. At the minimal effective dose that inhibited climbing behavior (LASSBio-1412: 1 mg/kg, p.o.; LASSBio-1413: 15 mg/kg, p.o.; LASSBio-1422: 5 mg/kg, p.o.) these compounds were able to block the ketamine-induced hyperlocomotion (Veh+Veh: 265.8±39.4; Veh+KET: 620.1±42.2; CLO+Veh: 176±36.5; CLO+KET: 214.3±27.5; HAL+Veh: 243.4±39.3; HAL+KET: 673.4±73.9; LASSBio-1412+Veh: 144.5±17.8; LASSBio-1412+KET: 357.8±32.4; LASSBio-1413+Veh: 196.3±32.9; LASSBio-1413+KET: 406.1±54; LASSBio-1422+Veh: 164.2±23.6; LASSBio-1422+KET: 317.2±62), suggesting an atypical profile. In conclusion, these results demonstrated that the studied derivatives are promising molecules to antipsychotics development, once they were active in an animal model predictive to atypicality. Also, they presented a multireceptor profile characteristic of modern atypical antipsychotics. **Financial support:** CAPES, INCT-IM-INOFAR/CNPq.

03.017

Acute MDMA (Ecstasy) treatment induces a persistent leukocyte distribution change and enhances susceptibility to infection. Ferraz-de-Paula V[1], Ribeiro A[1], Souza-Queiroz J[2], Torello CO[3], Queiroz MLS[4], Moreau RLM[5], Palermo-Neto J[6] [1]FMVZ-USP − Patologia, [2]IP-USP, [3]UNICAMP − Farmacologia, [4]UNICAMP – Farmacologia / Hemocentro, [5]FCF-USP − Análises Clínicas e Toxicológicas, [6]FMZV-USP – Neuroimuno-modulation

Introduction: We have previously shown that MDMA (*Ecstasy*) decreases neutrophil activity and changes the leukocyte distribution in blood, spleen and bone marrow. The HPA axis activation and consequently the corticosterone released was shown to be responsible for these effects and the catecholamines seem not to be involved. It has been reported that Ecstasy users are often more susceptible to infectious diseases, and altered leukocyte distribution could be important in this issue. Therefore, the aim of this study was to search for the involvement of MDMA-altered leukocyte distribution in a model of infection by *Listeria monocytogenes* (LM). **Methods**: The animals were housed and used in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil, protocol: 1224/2007. Balb/C male mice (6 per group) were used and divided randomly 2 groups: Saline (C) and MDMA (10 mg/kg), 60 min after i.p. treatment, we inoculated i.p. $5X10^3$ LM per animal. Blood, spleen e bone marrow samples were harvested in order to evaluate the leukocyte distribution after 24, 48 and 72 hours. Previously to the LM inoculation, mice were divided randomly 2 groups: infected and not infected by LM. **Results**: We observed that the treatment with MDMA was able to decrease the bone marrow cellularity ($F(11,60)=8,408$; $p<0.01$), increase the spleen cellularity ($F(11,60)=3,438$; $p<0.001$) and increase spleen relative weight ($F(11,60)=13,49$; $p<0.0001$) following 24, 48 and 72 hours in infected mice. Furthermore, MDMA treatment decreased leukocyte in the blood after 72 hours ($F(3,20)=4,803$; $p<0.01$) in infected mice.
Discussion: We previously showed that MDMA acute treatment by activating HPA axis and consequently corticosterone release were responsible for decreseasing neutrophil activity and altering leukocyte distribution, and that catecholamines are not involved in these effects. Therefore, we showed for the first time a neuroimmune-dependent mechanism for the actions of MDMA in these parameters *in vivo*. In this work we searched for the MDMA effects in an animal model infection. We showed that MDMA induced a persistent alteration in leukocyte distribution following 72 hours and enhanced mice susceptibility to LM infection. Taken these data together we showed that MDMA can be considered an immunossupressor drug by neuroimmune-dependent mechanism. Financial Support: FAPESP and CNPq.

03.018

Evaluation of the antinociceptive effect of ethanolic extract of *Sida galheirensis* (Malvaceae). Salgado PRR, Fonsêca DV, Carvalho FL, Torres PA, Lima MRV, Morais LCSL, Almeida RN, Souza MFV UFPB − Pharmaceutical Technology

Introduction: The Malvaceae family comprises 243 genera and 4225 species. Members of this family occur in almost all parts of the world. Many species of Malvaceae are widely used in therapy as anti-fever, diuretics, anti-inflammatory drugs among other applications. Knowing this, *Sida galheirensis*, with endemic species in the semi-arid regions, was studied aiming to evaluate its antinociceptive activity through the acetic acid abdominal constriction test (writhing test) and formalin test. **Methods**: In both tests were used male Swiss mice (25-30g), n = 8, divided into three groups and treated intraperitoneally. The control group received vehicle (distilled water and Tween 80), the experimental group was treated with crude ethanolic extract of *S. galheirensis* (850 mg/kg) and the standard group received morphine (6 mg/kg). In the writhing test, 30 minutes after the initial treatments, the animals were treated with acetic acid solution 1% in distilled water (0.1 mL/10g) and placed in individual polyethylene boxes. After 5 minutes, was then recorded the number of contortions displayed by each animal during a period of 10 minutes of observation. A significant reduction in the number of contortions when compared to the control group is considered an antinociceptive response. In the formalin test, 30 minutes after the initial treatments, the animals were treated with 2.5% formalin intraplantar route, in hind paw of the animal. It was observed the reaction of pain (time to lick the hind leg) during the first phase, neurogenic (0-5 min), and the second phase, inflammatory (15-30 min). All experiments were approved by the Ethics Committee for Animal Research of LTF/UFPB (protocol number 0307/08). **Results and Discussion**: In the writhing test, after administration of the ethanolic extract of *S. galheirensis* (850 mg/kg), no significant changes were observed between the control group (18.4 ± 3.2) and experimental (10.6 ± 5.6). In the formalin test, during the first phase, the mice treated with ethanolic extract of *S. galheirensis* (850 mg/kg) showed no significant decrease in the time of paw licking (61.6 ± 15.3) compared to the control group (68.0 ± 7.2). The default group treated with morphine at a dose of 10 mg/kg significantly reduced ($p<0.05$) paw licking time (21.2 ± 10.1). In the second phase, the treatment of animals with the ethanolic extract of *S. galheirensis* at a dose of 850 mg/kg (27.2 ± 27.2) decreased significantly ($p<0.01$) the paw licking time compared to control (179.1 ± 30, 3). These results suggest that the ethanolic extract of *S. galheirensis* has antinociceptive activity due to inhibition of inflammatory mediators or cytokines, as demonstrated by significant reduction in licking time in the second phase of the formalin test. Will be given to further behavioral studies with this plant in order to pursue new activities in the same or confirm the already existing in central nervous system. **Financial Support**: FAPESQ.

03.019

Evaluation of central activity of essential oil *Lippia microphylla* (Verbenaceae) in mice. Monte LO, Mota VG, Pinheiro LS, Timóteo RNPD, Tavares JF, Morais LCSL, Alencar JL, Almeida RN UFPB − Pharmaceutical Technology

Introduction: The natural products including medicinal plants have been established as a main source for obtaining new drugs with potential therapeutic effect. Several advances in psychopharmacology have been obtained through experimental studies of behavior. This study investigated possible psychopharmacological effects of the essential oil of *Lippia microphylla* (EOLM) using investigative methodologies of activity in the central nervous system (CNS) in mice. The aim of this work was to contribute to the advancement of scientific knowledge of this species in the family Verbenaceae. All experimental protocols were approved by the local Ethics Committee for Animal Research (CEPA Nº: 1405/06). **Methods:** *Swiss* mice, male (n = 8), albino, weighing 30-40g, 3-month-old were treated intraperitoneally (i.p.) with EOLM at doses of 50, 100, 150, 200 and 300 mg / kg and vehicle in order to perform the behavioral pharmacological screening[1]. For open field test[2], and test on the revolving bar (Rota Rod test)[3], received the following treatments: vehicle, 50, 100 and 150 mg / kg of the EOLM. **Results**: Mice treated with EOLM showed behavioral changes, such as analgesia, decreased ambulation, decreased response to touch and sedation. In the open field test, animals treated with EOLM in the three doses tested (50 mg / kg: 39.4 ± 5.3; 100 mg / kg: 46 ± 6.5; 150 mg / kg: 19.3 ± 5.5) showed a decrease in ambulation compared to control (75.2 ± 5.5) and the rearing (control: 50.8 ± 6.1; 50 mg / kg: 22.2 ± 3 9; 100 mg / kg: 14 ± 5.5; 150 mg / kg: 0.75 ± 0.4), there was also reduction in the time of grooming in animals that received EOLM 150 mg / kg (0.6 ± 0.6 s.) compared to the control group (25.2 ± 8.4 s.); EOLM administration was unable to promote significant changes in the number of defecation in any of the doses tested. Thirty minutes after treatment with EOLM, animals treated with a dose of 150 mg / kg showed a significant decrease in time spent in the revolving bar (113.8 ± 19.8 s. versus 175.4 ± 3.7 s. of control). The other doses tested did not promote significant changes in this parameter. **Discussion**: EOLM presents psychopharmacological effects suggestive of psycholeptics drug with promising sedative activity and anxiolytic-like action. **Bibliographic references**: [1]Almeida, R. N. et al.; *Rev. Bras. Farm*., V. 80, p. 72, 1999. [2]Huang et al.; *J Ethnopharmacol*., V. 110 p. 471-475, 2007. [3]De Sousa et al.; *Rev. Bras. Farm*., V. 17, n. 1, p. 23-28, 2007. **Financial Support:** CNPq.

03.020

Restraint stress changes temporal patterns of adenine nucleotides hydrolysis in rat's blood serum. Souza A[1], Detanico BC[1], Rozisky JR[1], Medeiros LF[2], Caumo W[2], Hidalgo MP[3], Battastini AMO[4], Torres ILS[2] [1]UFRGS − Farmacologia, [2]UFRGS − Anestesia, [3]UFRGS − Psiquiatria, [4]UFRGS − Bioquímica

Objectives: Adenosine 5´-triphosphate (ATP) and its breakdown products, ADP, AMP and adenosine can act as extracellular messenger in a range of biological processes through binding to the purinergic receptors, and are involved in a variety of pathological conditions including cerebral ischemia, neuroinflammatory, neuropsychiatric, neurodegenerative and cardiovascular diseases. Extracellular adenine nucleotides are metabolized to adenosine by a number of enzymes including NTPDases and ecto-5'-nucleotidase that are considered to be the major regulators of purinergic signaling in the blood. These enzymes may also have a protective function by keeping extracellular ATP/ADP and adenosine levels within physiological conditions. Previous work of our group demonstrate that ATPase and ADPase activities exhibit a 24 hour temporal pattern in blood serum rat. Circadian rhythms are present in a large number of organisms, representing an important mechanism for preparing the organism to environmental changes. Alterations of enzyme activities involved in nucleotide hydrolysis have also been reported after repeated and acute restraint stress. Moreover, it was demonstrated that stress can cause disruptions in biological circadian rhythms in humans and in rodents. Therefore, the aim of the present study was to examine the influence of acute restraint stress exposure upon temporal patterns in NTPDase and 5'-nucleotidase enzymes activities in rat blood serum. **Material and Methods:** Adult male Wistar rats the animals were divided into 4 groups according to daytime (ZT 0, ZT 6, ZT 12 and ZT 18) and each of these was subdivided in 4 groups according to time of death (control group, 0 hours, 6 hours and 24 hours after acute stress exposure). It was observed significant differences on effect of stress on 24-h profile on ATPase, ADPase and AMPase activities (n=7-11, One way ANOVA, $P < 0.001$ for all activities). The control groups showed significant higher ATPase (82.1%) and ADPase (64.0%) activities at ZT 12 and 18 when compared with control group. All stressed groups showed significant decrease in all enzymatic activities at ZT 12 and ZT 18 when compared with control group. Approved for Ethical Comitte/HCPA (08148) **Conclusion:** In conclusion, the activities of nucleotidase enzymes suffer a higher influence during night hours than at daylight hours by acute stress and this influence seems to persist at least 24 hours. The findings of this work suggest that stress can deregulates the circadian timing presents in nucleotidase enzymes. It may be proposed that altered levels of nucleotides in serum can be involved in cardiovascular events more frequently during the day in humans, and with its etiology induced by stress. Financial support: CNPq, GPPG/HCPA, CAPES.

03.021

Behavioral syndromes in experimental autoimmune encephalomyelitis. Rodrigues DH[1], Sousa LFC[1], Miranda AS[1], Lacerda-Queiroz N[1], Vilela MC[1], Campos RDL, Teixeira MM[1], Reis HJ[2], Teixeira AL[1] [1]UFMG − Imunofarmacologia, [2]UFMG − Neurofarmacologia

Introduction: Experimental autoimmune encephalomyelitis (EAE) is considered the animal model of multiple sclerosis. Besides motor impairment, some authors have reported behavioral changes in mice with EAE, which has been named "EAE behavioral syndrome" (Pollak *et al*. *J Neuroimmunol*. 2003; 137:94). In this study, we aimed to investigate behavioral parameters in animals with EAE using standardized behavioral tests. **Methods:** EAE was induced in 8-week female C57BL/6 mice with an emulsion of MOG_{35-55}, CFA and pertussis toxin. Behavioral symptoms were assessed using a screening battery called SHIRPA. Among the several parameters analyzed by SHIRPA, only the neuropsychiatric state was considered in this study. Memory and learning paradigms were assessed using the step-down inhibitory avoidance task and the object recognition test. Anxiety was evaluated by the elevated plus maze test. We performed all the analyses before the onset of EAE (days 8 and 9 post-induction) or 60 days after induction when there is a partial recovery of motor signs. All experimental procedures were approved by the local ethics committee (126/2006). **Results:** No differences were detected in any of the behavioral tests. In the SHIRPA screening battery, the neuropsychiatric state after 8 days of EAE induction was not distinct from controls (p=0.16; controls: 481.80±11.83, EAE: 436.80±26.16). Both controls and EAE animals had similar step-down latency after stimulation (p=0.42 after 1h30min; p=0.20 after 24h of stimulation). After resolution of the disease, control and animals with EAE exhibited similar time (s) spent exploring new objects (Controls: 53.75±4.29, EAE: 45.94±3.32, p=0.17 1h30min after animals were exposed to the first objects; Controls: 63.93±3.40, EAE: 50.87±8.86, p=0.15 24h after animals were exposed to the first objects). No differences were found in the exploration of open arms of the elevated plus maze between control and EAE groups both before onset (% time in open arms: Controls: 18.79±6.35, EAE: 7.77±2.54, p=0.13) and after 60 days of EAE induction (Controls: 22.59±7.21, EAE: 41.38±12.08, p=0.30). **Discussion:** We did not find any significant behavioral changes in the acute or in the resolution phase of EAE. These results strengthen the traditional view that only motor dysfunction is relevant in EAE. **Financial support**: CNPq, CAPES and FAPEMIG.

03.022

Chronic administration of medroxyprogesterone or clomifene, anti-estrogenic drugs, does not reproduce the antimanic-like effect of tamoxifen in an animal model of mania. Pereira M[1], Siba IP[1], Martynhak BJ[1], Correia D[1], Baretta IP[2], Andreatini R[1] [1]UFPR − Pharmacology, [2]UNIPar

Previous studies show that Tamoxifen, an anti-estrogenic drug that also inhibit PKC, have a clinical antimanic effect [1,3] and blocked psychostimulant-induced hyperlocomotion, an animal model for antimanic drugs[2].Medroxyprogesterone (MPA), which decreases estrogenic receptors, also exerts some clinical benefits on manic women patients[1].Thus, although other antimanic drugs (lithium and valproic acid) also inhibit PKC, the anti-estrogenic effect of tamoxifen could contribute to it antimanic effect.In this line, in our previous study we find that acute MPA (3.0 mg/kg) blocks partially amphetamine-induced hyperlocomotor [2].

Objective*:* Evaluate the effect of chronic and acute treatment of two anti-estrogenic drugs, MPA and clomiphene (an estrogenic receptor antagonist) in methylphenidate-induced hyperlocomotion in mice, an animal model of mania. Additionally, the effect of acute tamoxifen was also evaluated as positive control. **Methods***:* Adult Swiss male mice (3 months old) were treated with test drugs [MPA, 3.0 mg/kg or clomiphene (CLOM), 1.5 mg/kg for 21 days; acute MPA, CLOM or tamoxifen (TAM) 1.0 mg/kg, ip; for all n=8 per group].Spontaneous locomotor activity (number of beam interruptions) was measured in rectangular chambers equipped with three photocells on the walls. In test day, animals received MPA, CLOM, TAM or saline just before being placed into the individual chambers.After 30 minutes in the box, animals were given saline or methylphenidate (5mg/kg, dissolved in saline, s.c).Then, animals returned to the chambers for additional 70 min.The number of beam interruptions were recorded each 10 min.Antimanic-like effect was considered when test drug blocked hyperlocomotion induced by MPH without any effect in locomotor activity when administered alone. The data were analyzed with ANOVA followed by Newman-Keuls test. *Result:*The results showed that neither MPA and CLOM chronic treatment block the increase in locomotor activity induced by methylphenidate [$p<0.05$; MPA: 177 ± 18 and saline: 182 ± 14; CLOM: 173 ± 24 and saline: 208 ± 30 (mean ± SEM)], but MPA make this in acute one [$p<0.05$; MPA:150±39 and saline:324±44 ;].On the other hand, tamoxifen blocked methylphenidate-induced hyperlocomotion [$p<0.05$; TAM: 110 ± 9 and saline: 196 ± 19]. **Conclusions***:* The results do not indicate that reduction of estrogenic activity has an antimanic-like effect in the psychostimulant-induced hyperlocomotion model.The positive effect of tamoxifen replicates its antimanic-like effect on this animal model and also validates the procedure used. Therefore, the anti-estrogenic effect of tamoxifen probably did not contribute to its antimanic effect, which is probably related to its action over PCK activity [1,2].Accordingly, PKC inhibition would be relevant to the antimanic effect of mood stabilizers. *References:* [1] Kulkarni et al., *Psychoneuroendocrinol* 31, 543, 2006. [2] Sabioni et al., *Prog Neuropsychopharmacol Biol* Psychiatry 32, 1927, 2008. [3] Yildiz et al *Arch Gen Psychiatry* 65, 255, 2008. Financial support: CAPES, CNPq. **Ethics committee**: 306

03.023

Anxiolytic-like effect of benzodiazepine flurazepam is oestrous cycle-dependent. Silva Medeiros AG, Muniz GD, Figueiredo Neto JL, Arruda Junior WB, Costa RD, Carvalho MS, de Paula Soares V UFRN −. Biofísica e Farmacologia

Introduction: Behaviors related to anxiety may be experienced at the pre-menstrual syndrome that occurs during the late dioestrous (LD), when progesterone levels fall (Farage MA, Arch Gynecol Obstet 278(4):299, 2008). Electrophysiological studies showed that this falling increased the number of α4β1δ GABAA receptors immunoreactive neurones in the dorsal periaqueductal gray (dPAG), a midbrain area associated to anxiety, decreasing GABAergic tone at this area (Lovick TA, Pharmacol Biochem Behav 90(1):43, 2008). It would be hypothesized that this reduction of GABAergic tone would alter the effect of GABA-acting anxiolytic drugs, such as benzodiazepines. The objective of this work was to test this hypothesis by administering the benzodiazepine flurazepam to rats cycling at LD or proestrous phases submitted to the elevated plus-maze (EPM, Pellow S, J Neurosci Methods 14(3):149, 1985). At proestrous phase, there was no GABA receptors/tone alteration in the dPAG (Brack KE, Neuroscience 144(1):325, 2007). **Methods**: Female *Wistar* rats, 90 days, where submitted, 7 days before testing, to a vaginal smear and blue methylene dyeing to reveal the characteristic cytology of different stages of the oestrous cycle. Both vaginal smear and behavioral tests were performed at the same period (1 to 5 p.m.). In the experiment 1, proestrous-cycling rats (n=5-7 in each group) were injected with flurazepam (0, 7.5, 15 and 30 mg/kg, i.p.) 30 minutes before testing in the EPM for 5 minutes. In the experiment 2, flurazepam (0 and 7.5 mg/kg) was administered (i.p.) to both proestrous-cycling rats (n=7 in each group) and LD-cycling rats (n=5 in each group) submitted, after 30 minutes, to the EPM. All procedures were carried out in accordance with the Committee on Animal Research and Ethics of UFRN (Protocol 019/2010). **Results:** Flurazepam (flz) increased the % of time in the open arms (mean±SEM: saline=30.56±3.24, flz 7.5mg/kg=51.20±6.88, flz 15mg/kg=73.59±7.77 and flz 30mg/kg=85.66±6.69, $p<0.05$, Duncan test). At the doses of 15 and 30 mg/kg, flurazepam decreased the entries in the enclosed arms (mean±SEM: saline=9.28±0.60, flz 7.5mg/kg=9.25±1.30, flz 15mg/kg=4.2±1.24 and flz 30mg/kg=2.33±1.20, $p<0.05$, Duncan test). In experiment 2, flurazepam (7.5 mg/kg) increased the % time spent in the open arms by proestrous-cycling rats, but not LD-cycling rats (mean±SEM: proestrous-saline=22.42±7.64, proestrous-flz=53.23±4.70, LD-saline=31.33±11.75, LD-flz=33.66±3.22, $p<0.05$, Duncan test). **Discussion:** Our data showing that flurazepam at doses of 15 and 30 mg/kg decreased the entries in the enclosed arms suggest a sedative effect. This result is in accordance with the clinical prescription of this drug at 30mg/kg as sedative/hypnotic (Rosenberg RP, Ann Clin Psychiatry 18(1):49, 2006). At dose of 7.5 mg/kg, flurazepam administered to proestrous-cycling rats increased the % time spent by the animals in the open arms without altering the entries in the enclosed arms, suggesting an anxiolytic-like effect. Corroborating with the hypothesis that at the LD phase a reduction of GABAergic inhibitory tonus would reduce the efficacy of benzodiazepines, anxiolytic-like of flurazepam was not observed in LD-cycling rats. We thank Dr. Francisco Marcio Gomes Pinheiro. **Financial Support**: PROPESQ-UFRN

03.024

Role of median raphe nucleus 5-HT1a receptors on behavioral despair. Trovo MC, Almeida PVG, Pereira DHS, Padovan CM FFCLRP-USP − Psicologia e Educação

Introduction: The forced swim test (FST) is a widely used animal model of depression in which animals previously submitted to fifteen minutes of forced swim display increased immobility when re-tested twenty four hours later. This phenomenon is known as behavioural despair and can be attenuated by chronic treatment with antidepressants and intra-hippocampal injections of serotoninergic agonists. Since hippocampal serotoninergic afferents arrives from the Median Raphe Nucleus (MnRN) and this pathway has been involved in the development of tolerance to stress, the aim of this work was to investigate the role of 5-HT1a receptors (5-HT1aR) within the MnRN in the FST. All procedures were approved by local ethical comitte on animal research (CEUA 2006.1.1131.53.3). **Methods:** Male wistar rats with cannulas aimed to the MnRN were submitted to fifteen minutes (Pre-exposure, PE) of forced swim and tested twenty four hours later. All animals received two intra-MnRN injections (0.2µL each; five minutes interval) of Saline (Sal), 8-OH-DPAT (DPAT) and/or WAY100635 (WAY), administered as follows: Sal+Sal, Sal+DPAT (3nmols/0.2µL), WAY+Sal (0.3nmols/0.2µL) and WAY+DPAT. Animals received the injections immediately before or after PE. Another group of animals was treated twenty four hours after PE. Control rats received treatment twenty four hours or five minutes before test. Latency to display immobility (LAT) and total time spent immobile (TSI) were registered and analyzed by One-way ANOVA followed by Duncan for each experimental protocol. **Results:** When given after PE, DPAT increased LAT (185±45) and decreased TSI (26.8±10.8) when compared to saline (LAT=58.9±14.1; TSI=91.2±7.2) or WAY (WAY+Sal: LAT=58.7±23.2; TSI=123.5±12.3) treated rats (LAT:$F_{3,49}$=4; p<0.05; TSI: $F_{3,49}$=6.2; p<0.05). This effect of DPAT was prevented by previous treatment with WAY (WAY+DPAT: LAT=131.3±36.4; TSI=80.4±22.6).Similar results were observed when treatment was performed before PE (LAT: Sal+DPAT=103±19.8; Sal+Sal=74.8±12.4; WAY+DPAT=184±20.7; WAY+Sal=76.7±11.7; $F_{3,39}$=10.3; p<0.05) (TSI: Sal+DPAT=76.9±8.8; Sal+Sal=133.1±9.6; WAY+DPAT=51.1±11.6; WAY+Sal=102.8±33.8; $F_{3,39}$=12.4; p<0.05).Although DPAT also increased LAT (172.3±21.9) and decreased TSI (27.1±9.7) when given before test in stressed rats, previous treatment with WAY did not block DPAT effects (LAT: Sal+Sal=108.4±15.5; WAY+DPAT=203.5±19.9; WAY+Sal=91.1±19.5; $F_{3,40}$=7.5; p<0.05) (TSI: Sal+Sal=83.4±4.1; WAY+DPAT=17.6±4.1; WAY+Sal: 58.9±17.5; $F_{3,40}$=8.8; p<0.05;).No effects of drugs were observed when treatment was performed in non-stressed rats (PE4: LAT: $F_{3,29}$=1.7; p>0.05; TSI: $F_{3,29}$=0.7; p>0.05; PE5: LAT: $F_{3,22}$=1.7; p>0.05 TSI: $F_{3,22}$=0.5; p>0.05). **Conclusion:** Our results suggest that 5-HT1aR localized in the MnRN are important in processes involving acquisition and consolidation of stressful memories, but not for retrieval. **Financial Support:** CAPES, CNPq and FAPESP.

03.025

Nitric oxide modulates glutamatergic control of sensorimotor gating in rats. Henriques-Santos NF[1], Brosco MC[1], Del Bel EA[2], Salum C[1] [1]UFABC − Matemática, Computação e Cognição, [2]FORP-USP − Morfologia, Estomatologia e Fisiologia

Introduction: Behavior evidence of a nitric oxide (NO) modulatory role suggests that a hyperactivity of the nitrergic system may occur in parallel to the high dopaminergic and low glutamatergic neurotransmissions in schizophrenia. Prepulse inhibition (PPI), a model to access the deficits on the sensorimotor gating that occur in several mental disorders, is characterized by a normal reduction on the startle reflex in response to an intense stimulus (pulse) when this is preceded by a low intensity stimulus (prepulse). Glutamate receptor antagonists elicit deficits in PPI. We have previously shown that deficits on PPI caused by the indirect DA agonist, amphetamine, were prevented by the previous injection of a NO synthase (NOS) inhibitor. The present study investigated the ability of a NOS inhibitor in preventing the PPI disruptions and the locomotor hyperactivity caused by a NMDA receptor antagonist. **Methods:** Male Wistar rats received two systemic injections of saline or NG-L-nitro-arginine (LNO, 40 mg/kg, i.p.) and saline or memantine (10 or 17 mg/kg, i.p.), one hour and thirty min before testing, respectively. Each animal was individually submitted to a session consisting on 5 min of acclimatization (no stimuli), 10 presentations of pulse (white noise, 100 dB), 64 randomly distributed presentations with 30 s inter-stimuli-interval of prepulse alone (69, 73 or 81 dB, pure tone, 3 kHz), pulse alone, no stimulus or prepulse+pulse. Immediately after PPI test, each animal was placed on the center of an acrylic open field and the locomotor activity was recorded. These procedures were previously approved by the animal Care and Use Committee of University of São Paulo (229/2005). **Results:** The repeated analyses of variance (ANOVA) on the %PPI, which refers to the amplitude of startle response (ASR) to prepulse+pulse relative to the ASR to pulse alone, with the factors Treatment (between subjects) and Intensity of prepulse (within subjects) presented significant main effects of Treatment (P<0,001) and Intensity (P=0,005). The *post-hoc* Duncan test revealed that memantine at both doses significantly disrupted PPI and the previous injection of LNO prevented these effects (P<0,05). Another two-way ANOVA of the distance travelled by the rat on the open field, with factors Pre-Treatment and Treatment, detected a significant main effect of Treatment (P<0,05). The *post-hoc* Duncan test revealed a dose dependent effect of memantine, since it significantly increased the distance travelled at the lower dose and reduced it at the higher dose (P<0,05). These effects were not verified when memantine was preceded by LNO. **Discussion:** The present results suggest a nitrergic modulation of glutamatergic neurotransmission on sensorimotor gating and demonstrate for the first time the interaction between a NOS inhibitor and memantine and LNO on the control of PPI test. **Financial Support:** Federal University ABC **Reference:** [1] Wiley, J.L., 1998. Nitric oxide synthase inhibitors attenuate phencyclidine-induced disruption of prepulse inhibition. *Neuropsychopharm*. 19, 86-94.

03.026

Effects of nNOS Inhibition on forced swimming-induced FOS expression in the rat brain.

Silva M, Aguiar DC, Guimarães FS, Joca SRL FMRP-USP − Farmacologia

Introduction: Systemic inhibition of NO synthesis is reported to induce antidepressant-like effects in animal models. However, the brain pathways involved in such effects are unknown. Therefore, we aimed at investigating Fos expression, a marker of neuronal activity, in different brain regions of rats treated with 7-NI (nNOS inhibitor) and submitted to an animal model of depression. **Methods**: Male Wistar rats (200-220 g) were submitted to a forced swimming for 15 min pretest (PT) and received three ip injections (0, 5 and 23h after PT) of Fluoxetine (FLX:10 mg/kg), Venlafaxine (VLX:10 mg/kg), 7-NI (30 mg/kg) or respective vehicles (1mL/kg). One hour after the last drug injection, the immobility time was recorded in a 5 min swim test. Two hours after test or 3h after the last drug injection (unstressed treated group), rats were sacrificed to have their brains removed and processed for Fos immunohistochemistry. The number of Fos-positive nuclei was counted using a computerized image analysis system. All protocols described herein were approved by a local ethical committee (CETEA, Prot. N.145/2008). **Results**: 7-NI, VLF or FLX reduced immobility time ($F_{4,29}$=8,59, p<0,05). Although these treatments did not induce significant effects per se, they attenuated the stress-induced fos expression in several brain regions. *PFC* (CG: naive 0.36 ± 0.27 vehicle 9.98 ± 2.56 *; FLX 1.37 ± 0.39 **; VLF 0.87 ± 0.35 **; 7-NI 1.38 ± 0.58 **. PL: naive 0,32 ± 0.15; vehicle 11.61 ± 4.26 *; FLX 1.24 ± 0.70 **; VLF 0.58 ± 0.27 **; 7-NI 0.73 ± 0.26 **. IL: naive 0.40 ± 0.35; vehicle 7.28 ± 3.77 *; FLX 0.89 ± 0.40 **; VLF 0.16 ± 0.16 **; 7-NI 0.73 ± 0.26 **), *Raphe* (Dorsal: naive 0.60 ± 0.33; vehicle 7.38 ± 1.44 *; FLX 2.03 ± 0.51 **; VLF 0.96 ± 0.24 **; 7-NI 1.29 ± 0.25 **. Median: naive 0.00 ± 0.00; vehicle 77.17 ± 12.82 *; FLX 17.02 ± 6.81 **; VLF 14.56 ± 7.51 **; 7-NI 25.16 ± 5.59 **), *Locus Coeruleus* (naive: 0.66 ± 0.38; vehicle: 23.30 ± 4.18 *; FLX: 8.07 ± 2.08 **; VLF: 6.14 ± 2.33 **; 7-NI: 8.93 ±2.86 **), *Thalamus* (naive: 0.44 ± 0.11; vehicle:19.06 ± 6.43 *; FLX: 2.91 ± 1.43 **; VLF: 3.15 ± 1.62 **; 7-NI:2.41 ± 0.10 **), *PVN* (naive: 0.60 ± 0.29; vehicle: 19.32 ± 4.14 *; FLX: 2.16 ± 0.93 **; VLF: 1.74 ± 0.88 **; 7-NI: 2.57 ± 0.47 **), *amygdala medial* (naive: 0.00 ± 0.00; vehicle: 2.53 ± 0.88 *; FLX: 0.35 ± 0.17 **; VLF: 0.15 ± 0.10 **; 7NI: 0.27 ± 0.17 **), *bed nucleus* (naive: 0.00 ± 0.00; vehicle: 0.24 ± 0.11 *; FLX: 0.05 ± 0.02 **; VLF: 0.05 ± 0.03 **; 7NI: 0.04 ± 0.03 **).
The results are shown as mean ± SEM of the number of fos positive cells. *different from naive
**different from vehicle; ANOVA followed by Tukey.
Discussion and Conclusions. Antidepressant-like effect induced by nNOS inhibition shares common neurobiological substracts with conventional antidepressants in the FST.
Financial Support: FAPESP, FAEPA

03.027

Antidepressant-like activity of *Hypericum caprifoliatum* Cham. & Schltdl (Guttifereae) is not due to NMDA receptor activation neither to neuronal glutamate transport. Centurião FB[1], Stein AC[1], Gay BM[2], Prigol M[2], Viana AF[3], Nogueira CW[2], Rates SMK[3] [1]UFRGS − Farmácia, [2]UFSM − Química, [3]UFRGS – Ciências Fisiológicas

Introduction: It has already been demonstrated by our group that the lipophilic fraction of *Hypericum caprifoliatum* and its main phloroglucinol derivative (HC1) display antidepressant-like activity in rodents and inhibits the synaptossomal uptake of dopamine, noradrenaline and serotonin without interacting with their respective neuronal carriers (Viana et al., Neuropharmacol. 49, 1042, 2005). Therefore, the manner by which *H. caprifoliatum* acts is different from classical antidepressants. The involvement of glutamatergic neurotransmission on mood disorders etiology has been demonstrated (Petrie et al., Pharmacol. Ther., 87, 11, 2000). Since the phloroglucinol derivative hyperforin is able to inhibit the synaptosomal uptake of L-glutamate (Müller et al., Pharmacopsychiatry, 30, 102, 1997) and also enhances extracellular L-glutamate levels in rat brain (Kaehler et al., Neurosci. Lett., 262, 199, 1999), in this work, we carried on the investigation of *Hypericum caprifoliatum* mechanism of action by evaluating the effects of HC1 on glutamatergic neurotransmission in rodents. **Methods:** This study assessed the effects of MK-801 (a NMDA glutamate receptor antagonist) on antidepressant effect of HC1, using mice forced swimming test (FST). MK-801 (0.05 and 0.1 mg/kg i.p.) was administered alone or 30 min after HC1 (360 mg/kg, p.o.) treatment . Thirty min or 60 min after the adminstration of MK-801 or HC1 respectively the animals were exposed to the forced swimming and immobility time (in seconds) was registered during six minutes. We also investigated the effects of HC1 (10 and 100 ng/ml) on [^{3}H]glutamate uptake and release using rat brain synaptosomes. All results are cited as mean immobility time $\pm$ SEM (n = 8). Statiscal analysis was performed by one-way ANOVA followed bu Dunnett´s test for comparison with control group. The protocols were approved by UFRGS Research Ethical Committee (project number 01-588). **Results and Discussion:** MK-801 given alone at the dose of 0.1 mg/kg reduced the immobility time in mice (p< 0.05) but at the dose of 0.05 mg/kg it was ineffective (control: 183.6s $\pm$ 9.5; MK-801 0.05: 128.6 s $\pm$ 18.9; MK-801 0.1: 99.9s $\pm$ 24.5). The combined administration of HC1 (360 mg/kg) with MK-801 in the two concentrations tested (0.05 and 0.1 mg/kg) had no effect on the anti-immobility effect of HC1 in the FST (HC1 = 103.15s $\pm$ 18.7; HC1 + MK-801 0.05 = 89.7s $\pm$ 18.7; HC1 + MK-801 0.1 = 89.4s $\pm$ 17.9). In vitro, HC1 (10 and 100 ng/ml) had no significant effect on [^{3}H]glutamate uptake and release by rat brain synaptosomes. These results suggest that glutamatergic neurotransmission, mainly via NMDA receptors, is not involved on the antidepressant-like activity of *H. caprifoliatum*. **Financial support:** CNPq, CAPES

03.028

Repeated morphine administration in early life promotes anxiolytic effect on elevated plus-maze. Nonose Y[1], Rozisky JR[1], Santos VS[1], Medeiros LF[1], Souza A[2], Caumo W[3], Torres ILS[1] [1]UFRGS − Farmacologia, [2]UFRGS − Bioquímica, [3]UFRGS − Anestesia

Objectives: management of opiate tolerance and withdrawal symptoms in opiate-exposed infants remains a major medical challenge. Thus, basic and clinical research that targets the mechanisms that underlie the development of opiate dependence and withdrawal in the infant is needed. Our group has demonstrated that animals treated with morphine in early life present some withdrawal symptoms in the open field test, such as the increase of exploratory activity at P30 (Rozisky et al., 2008). Thus, the objectives of this study were to determine the effect of repeated morphine administration in early life on behaviors in the plus maze test at P30. **Methods and Results: t**he protocol of this experimental study was approved by the Ethics Committee of Hospital of Clinics of Porto Alegre- HCPA (GPPG/HCPA-08345). In this study, 8-day-old male *Wistar* rats were used and divided into two groups: control (**C**, n= 8) and morphine (**M**, n=7) at postnatal day 8 (P8), which received saline or morphine (5 µg s.c. in the mid-scapular area) once a day for one week. Behavioral responses were performed at P30 by elevated plus maze test, and it was analyzed the following behaviors: duration of time spent in the open-arms (OT) and in the closed-arms (CT), number of open-arm entries (OE) and number of closed-arm entries (CE), number of protected head dipping (PHD) and number of unprotected head dipping (NPHD). Data were analyzed by Student's *t* test and results were expressed as mean ± standard deviation of the mean. Differences were considered statistically significant with $P<0.05$. The morphine group showed increase in the time spent in the open-arms, in the number of open-arm entries and in the number of unprotected head dipping in comparison to the control group (OT**:** Control= 1.0±0.9 seconds, Morphine= 28.57±11.3 seconds; OE: Control= 0.25±0.2, Morphine= 2.43±1.2; NPHD: Control= 0.5±0.4, Morphine= 6.14±5, Student's *t* test, $P<0.05$). There were no differences between the groups in the other behaviors analyzed (CT: Control= 276.25±20 seconds, Morphine= 240.7±26.5 seconds; CE: Control= 4±2.3, Morphine= 4.14±1.6; PHD: Control= 4.12±3, Morphine= 3.14±1.21, Student's *t* test, $P>0.05$). **Conclusions:** these findings indicate that morphine exposure in early life can result in anxiolytic-like effect on exploratory activity in the plus maze test, and it was expressed after two weeks of treatment. Morphine is known to stimulate dopaminergic transmission, most extensively regarding the mesocorticolimbic system and its neural inputs (Cadoni and Di Chiara, 1999). This effect is regarded as the substrate for modulation of anxiolytic-like behavior in rat induced by morphine (Rezayof et al., 2009). Our results corroborate this previous study since they showed that young animals develop this behavior two days after morphine withdrawal and that this response remained up to 30 days of age. Further studies with dopaminergic antagonist receptors are needed for to elucidate our hypothesis. In addition, these behavioral changes in response to sustained exposure to morphine during early life indicate the importance in evaluating clinical consequences of long-term opioid administration. **Financial Support:** CNPq, CAPES, FIPE at Hospital de Clínicas de Porto Alegre, PROPESQ/UFRGS, FAPERGS.

03.029

Evaluation of intestinal motility tolerance after repeated diethylpropion administration in rats. Dalpra WL[1], Caletti, G[1], Olguins, DB[2], Barros HMT[1], Gomez, R[1] [1]UFCSPA − Farmacologia, [2]IPA − Farmacologia

Introduction: Diethylpropion (DP), also known as amfepramone, is an anorectic and stimulant of the central nervous system (SNC) similar to amphetamines. It is widely used for obesity treatment and weight management, representing one of the most prescribed drugs. The DP acts in the SNC by inhibiting the reuptake and increasing the release of noradrenaline and dopamine. It is already known that chronic administration of DP is associated with central tolerance to its anorectic effect, but few studies show tolerance to its non-central effects. Because one of the most common side effects from this drug is the constipation we evaluated, in this study, the effect of chronic administration of diethylpropion on intestinal motility of rats. **Methods:** Adult female Wistar rats from the animal facility of UFCSPA, were divided in three groups: control (CTR, n = 20), acute (ADP, n = 20) and chronic (CDP, n = 20). The CTR and the ACT group received, daily, for 21 days, saline i.p. injections (1 mL/kg), and CDP group received diethylpropion at the doses of 15 mg/kg, ip. In the last day of the treatment, and after 18 hour of fasting, rats from the CTR group received saline, while rats from the ADP and CDP groups received diethylpropion. After 30 minutes from this administration, rats were orally administered with 1 mL of a charcoal solution (20%). They remained in their home cages and after 30 minutes from the charcoal, they were euthanized by cervical dislocation. Abdomen was opened and the leading front of marker was identified in the small intestine. The entire length of small intestine was carefully isolated by cutting at pyloric and ileocaecal ends. The distance travelled by charcoal and the total length of the intestine were measured in cm(s). An ANOVA-one way was run to identify differences among treatments, followed by the Bonferroni post hok test. Values were presented as mean ± standard deviation and were considered significant when $p < 0.05$**.** All procedures were approved by the Ethics Committee for Animal Experimentation of UFCSPA, license number 423/09. **Results:** Our results showed that diethylpropion decreases the intestinal motility in both acute and chronic treated groups (CTR: 53.3 ± 15.6, ADP: 25.7 ± 13.0 and CDP: 32.4 ± 18.4 cm, $p < 0.001$). Although rats from the CDP group showed a tendency to reverse the lower intestinal motility found in the ADP group, this difference did not reach statistical significance. When we considered the total length and the percentage of migration we found the same differences from above (CTR: 43.4 ± 9.6, ADP: 22.8 ± 3.8 and CDP: 30.5 ± 13.7 % $p<0.001$) **Discussion:** According to our results there is no tolerance for the constipant effect after repeated administration of diethylpropion. Thus, patients chronically administered with this drug should be considered as potential users of laxatives during programs to lose weight. **Support:** UFCSPA

03.030

Blockade of glutamate NMDA receptors in the rat ventromedial prefrontal cortex (vMPFC) induces antidepressant-like effects. Pereira VS[1], Joca SRL[2] [1]FMRP-USP − Farmacologia, [2]FCFRP-USP − Física e Química

Introduction: Recent evidences have proposed a role for glutamate in the neurobiology of depression. For example, chronic antidepressant treatment modulates the expression of glutamate NMDA receptors in some encephalic structures, such as the medial prefrontal cortex (MPFC) (Skolnick P, et al.; 2009). Moreover, systemic injection of glutamate NMDA antagonists induces antidepressant-like effects in rodents. In depressed humans, the activity of the MPFC and the levels of glutamate are elevated, thus suggesting the involvement of such brain structure in depression pathophysiology (Skolnick P, et al.; 2009). However, lesion and inactivation of the MPFC have produced contradictory results, what can be attributed to the differential participation of MPFC subregions targeted in each of the different studies (Price JL, et al.; 2009). We have recently shown that transient inactivation of the ventral portion of MPFC induces antidepressant-like effects. However, the involvement of the glutamatergic neurotransmission in the vMPFC in the modulation of depressive-like behavior remains to be investigated. **Methods:** Male Wistar rats (240-250g) were submitted to stereotaxic surgery to insert guide cannulas bilaterally into the vMPFC. A week later, they were submitted to a session (15 min) of forced swim. Twenty four hours later, the animals received a bilateral injection of the glutamate NMDA antagonist, LY235959 (LY; 1, 3 and 10nmol/0.2μL) and were submitted to a 5 min test session of forced swim, when the immobility time was measured. The behavioral protocols described herein are approved by our local ethical committee (CEUA, 09.1.1617.53.2). **Results:** The administration of LY into the vMPFC, at the dose of 3 nmol/0.2 μL, induced antidepressant-like effects (Mean±SEM: vehicle:116,3±21,17; LY 1nmol: 142,3±25,34; LY 3nmol: 45,38±18,86*; LY 10nmol: 60,30±16,75; $F_{(3,33)}$=4,74; p<0,05; *p<0,05, Dunnett). **Discussion:** Ours results show the participation of the glutamatergic system of the vMPFC, through NMDA receptor, modulates the behavioral responses to the stress and the depressive-like behavior in rats. Previous data from our group showed that LY235959 administration into the vMPFC at the given doses does not cause any alteration in the locomotor activity and do not act trough a non-NMDA receptor pathway (Resstel LB, et al.; 2008). Therefore, this is the first data showing that NMDA blockade in the vMPFC promotes antidepressant-like effects, thus corroborating the proposed involvement of the local glutamatergic system as an important target to drugs with antidepressant effect.

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03.031

Ayahuasca repeated treatment inhibits behavioral sensitization previously developed to ethanol in mice. Marinho EAV[1], Gerardi-Junior CA[2], Santos R[3], Baldaia MA[4], Oliveira-Lima AJ[5], Wuo-Silva R[6], Hollais AW[7], Malpezzi-Marinho ELA[2], Fernandes HA[8], Frussa-Filho R[5]
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Introduction: drugs of abuse including ethanol, increase dopamine levels in the nucleus accumbens (Nacc) producing, in rodents, locomotor stimulation, a behavior which is sensitized after repeated administration (ARAÚJO et al., Pharmacol. Biochem. Behav., 82, 40, 2005). This sensitization has been proposed to share neuronal mechanisms with drug craving. The Ayahuasca is a beverage used by indigene tribes in your rituals. The beverage now is used to in rituals of urban religions. The aim of this study was to investigate if repeated treatment with the Ayahuasca is able to inhibit previously developed locomotor sensitization to ethanol in mice (Ethics Committee number: 176/2008). **Methods:** Swiss mice received intraperitoneally (ip) 1.8 g/kg of the ethanol (Eth) or saline (Sal) every other day for 15 days (8 injections). Five minutes after each injection the animals were exposed to an open field (OF) for 10 min. Locomotor activity (LA) was measured by the number of squares crossed in the OF, on the 1st and 15th days. On the 17th day animals began to be treated with vehicle or Ayahuasca (Aya 100 or 300 mg/kg) by oral route, every other day for 7 days (4 injections). One hour after each injection were exposed to OF for 10 min, being their LA quantified on the 17th and 23th days. On the 29th day all the animals were challenged ip with 1.8 g/kg of ethanol and their LA was quantified for 10 min in the OF. Therefore, the following groups (n=10) were formed: Sal-Sal-Eth; Sal-Aya100-Eth; Sal-Aya300-Eth; Eth-Sal-Eth; Eth-Aya100-Eth and Eth-Aya300-Eth. **Results:** The 1st ethanol injection produced a significant increase in LA of ethanol groups (Eth-Sal-Eth: 316±44; Eth-Aya100-Eth: 290±31 and Eth-Aya300-Eth: 311±31), compared to saline groups (Sal-Sal-Eth: 121±11; Sal-Aya100-Eth: 106±10; Sal-Aya300-Eth: 126±10) (two way ANOVA, $p<0,05$). In the 7th day the same observations were realized (377±24; 375±52; 360±37 compared to 138±9; 134±17; 117±28) as well as on the 15th day (387±41; 364±48; 391±68 compared to 124±18; 124±19; 122±10). Do not were observed significantly differences when the same groups were compared between itself in the different days [repeated measures ANOVA, $F(8,89)=0.44$; $p=0.90$]. The Ayahuasca (at both doses) 1st injection did not modify LA of either saline- or ethanol- previously treated animals. However, after ethanol challenge injection (day 27), two-way ANOVA revealed significant effects of ethanol previous treatment and Ayahuasca previous treatment ($p<0.05$). Indeed, ethanol previous treatment potentiated the hyperlocomotion induced by ethanol challenge injection (Sal-Sal-Eth: 342±51; Eth-Sal-Eth: 524±25), once again demonstrating the expression of the behavioral sensitization phenomenon. This behavioral sensitization to ethanol was inhibited by Ayahuasca previous treatment (Eth-Aya100-Eth: 359±34; Eth-Aya300-Eth: 352±29). **Conclusion:** Ayahuasca treatment was able to inhibit locomotor sensitization previously developed to ethanol treatment. **Financial Support**: UBC

03.032

Acute effect of *Dioclea violacea* M. (aqueous extract) on general activity observed in the rats on elevated plusmaze and its dyskinetics movements. Gemignani S, Silva FO, Biscaro MDA, Santos NSS, Rassam E, Gonçalves RB, Pedroso-Mariani SR FMJ − Farmacologia

Introduction: *Dioclea violacea* M. (DVM), also known as coronha, found from the south of the Guianes to São Paulo and Mato Grosso do Sul (Corrêa, M.P., 1984), is used in folk medicine to "prevent and remove sequelae of stroke, in the treatment of epilepsy and Parkinson´s." It's indicated as "soothing" in the form of infusions prepared from powder of the seed. The goal of this work was to study the behavioral effects of acute administration of aqueous extract of DVM on the general activity observed in rats in the plusmaze and the effects on jaw movements (JM) and protrusion of the tongue (PT). Methods: (EAEC-FMJ number: 165/09) male Wistar Rats (N = 12), weighing 350 g on average, were divided into 2 groups. A DVM group was treated with a suspension of DVM seed powder, with a dose of ip 36 mg/kg, prepared in the form of infusion. The animals in the control group (C) were injected with distilled water (1 ml/kg, i.p.) and Tween 80 (2 drops/ml of water). After twenty minutes it was recorded the activity of these animals in the plusmaze, during 5 minutes (frequency of locomotion). Then, the animals were isolated in metal cages for observation for ten minutes and recorded the frequencies of JM and PT. These equipments are suitable for the evaluation of substances that act on the central nervous system, in particular in nucleus acumbens (voluntary movement) and striatus (involuntary movements, balance, dopaminergic/cholinergic, dyskinesias) (WOLF, M.E. et alii, 1983). In the group treated with DVM, it was not noticed significant changes of general activity (frequency of locomotion). It was not observed effects on the movements of tongue protrusion in dose employed. The frequency of jaw movements JM in rats administered with DVM presented significant increase (average ± standard deviation = 81 ± 31.83) when compared to the control animals (27.17 ± 13.82) ($p<0,05$ test t-student). The effect of DVM (aqueous extract) promotes increased jaw movement, suggesting an effect on the involuntary motor activity and no expression in locomotive activity, in the dose studied. A chronic study with the infusion must be used to reproduce the folksy tea uses.

03.033

Role of L-arginine-nitric oxide pathway and possible implications for cardiovascular disease in depressed patients. Pinto VLM[1], Fontoura PCS[1], Brunini T[2], Mendes Ribeiro AC[1] [1]UERJ − Farmacologia e Psicobiologia, [2]UERJ − Farmacologia

Introduction: Depression is an independent cardiovascular risk factor which is comparable to conventional risk factors. It has been suggested that a reduction of NO bioavailability may be the link between depression and cardiovascular disease (CVD), through platelet activation, endothelial dysfunction and elevated concentration of pro-inflammatory circulating cytokines (1). Patients suffering from CVD who are also diagnosed with major depressive disorder (MD) have a 2.5 to 4-fold increase in the occurrence of CVD events (2). The aim of study was to investigate the role of the L-arginine nitric oxide pathway in patients with MD without any medication and try to elucidate the mechanisms that could be implicated in the increased cardiovascular risk associated with MD. **Methods**: 10 patients with depression meeting DSM IV criteria (4 males and 6 females, mean age: 38±11years), were paired with 10 normal control subjects. Healthy control (HC) subjects did not have a diagnosis of a current or past psychiatric Axis I disorder. The exclusion criteria were heart and renal failure, mental retardation, diabetes mellitus, ischemic heart disease, infection, dyslipidemia, CVD and smokers. Neither HC nor MD were receiving any medication with the exception of psychotropics and antiagregants. Ethical approval was obtained (1436 − CEP/HUPE). Extracellular L-arginine transport into platelets was measured by kinetic methods, using increasing concentrations of [^{3}H] L-arginine. NOS activity was evaluated by the conversion of [H^3]L-arginine into [^{3}H]L-citrulline and NOS expression was evaluated using the Western Blotting technique. Platelet aggregation was induced by collagen (2, 4 and 8 µg/ml). The Mann-Whitney *U* test or unpaired test was used to analyse the differences between MD and (HC), in accordance with the Kolmogorov-Smirnov test. Values are expressed as means ± SEM. A *p* value of less than .05 was considered significant. **Results:** L-arginine influx in platelets − L-arginine transport via system y+L was decreased in MD compared to controls (MD: 20±2, HC: 46±9, p=0,019) **.** Basal NOS activity, assaying the production of L-[3H]-citrulline from L-[3H]- arginine, was decreased in platelets from MD in relation to HC (HC: 0,16±0,01, MD: 0,09±0,01, p=0,02). MD did not affect platelet eNOS and iNOS expression in platelet lysates. A dose-related effect was observed in the platelet aggregation assays induced by increasing concentrations of fibrillar collagen. However, no difference was seen when patients with MD were compared with controls. **Discussion**: Despite evidence that cardiovascular disease and depressive disorder are associated, accurate mechanisms to explain this relationship remain unknown. Our findings showed an inhibition in the L-arginine-NO pathway in MD which may play a role in these diseases.

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03.034

Chronic imipramine treatment enhances the panicolytic-like effect caused by the stimulation of 5-HT_{1A} and 5-HT_{2A} receptors in the dorsomedial hypothalamic nucleus. de Bortoli VC, Zangrossi Jr H FMRP-USP − Farmacologia

Introduction: Electrical or chemical stimulation of the dorsal periaqueductal gray matter (DPAG) or the dorsomedial hypothalamic nucleus (DMH) induces defensive reactions, such as escape behavior, that are suggestive that the experimental animal is undergoing a markedly aversive experience. Give the striking similarities between the autonomic and behavioral effects of the DPAG or DMH stimulation and the symptoms of panic attacks, it has been suggested that these areas are involved in the genesis of panic disorder in humans and that their stimulation in animals can model panic attacks [1]. Intra-DPAG injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT or the preferential 5-HT_{2A} receptor agonist DOI inhibits escape induced by the electrical/chemical stimulation of this brainstem area. Long-term treatment with antipanic drugs such as imipramine, alprazolam and fluoxetine facilitates the inhibitory effect of these 5-HT receptor agonists on escape performance [2]. This chance has been implicated in the mode of action of antipanic drugs. In the present study we investigated whether long-term treatment with imipramine may also interfere with the effect of intra-DMH injection of 8-OH-DPAT and DOI on the escape reaction induced by the electrical stimulation of this hypothalamic mucleus. **Methods:** Male Wistar rats (250-280 g) chronically (21-23 days) treatment with imipramine (15 mg/kg, i.p.) were intra-DMH injected (0.2 ul) with 8-OH-DPAT (8 nmols), DOI (16 nmols) or saline. The threshold of aversive electrical stimulation that applied to the DMH evokes escape behavior was measured before and after the microinjection of these agonists. Commission ethical protocol nº 077/2008 – CETEA-FMRP/USP. **Results**: Our data showed that in rats chronically injected with saline, intra-DMH administration of 8-OH-DPAT or DOI significantly raised the threshold of aversive electrical stimulation for inducing escape [Δ threshold (mean ± EPM; μA): saline = 5.50 ± 2.13; 8-OH-DPAT = 21.50 ± 1.84 or DOI = 28.00 ± 2.39]. Futhermore, the inhibitory effect of serotonergic agonists was significantly higher in animals receiving long-term treatment with imipramine: [8-OH-DPAT = 35.11 ± 5.11 or DOI = 57.33 ± 6.57]. **Discussion:** As observed in the DPAG, imipramine facilitates 5-HT_{1A}- and 5-HT_{2A}-receptor-mediated neurotransmission in the DMH, implicating this effect in the mode of action of antipanic drugs. References: [1] Graeff FG and Zangrossi JrH. *Textbook of biological psychiatry: animal models of anxiety disorders*, p. 879, 2002. [2] Guimarães FS et al. *Handbook of the behavioral neurobiology of serotonin*, p. 667, 2010. **Support:** FAPESP.

03.035

Bipolar disorder and cardiovascular disease. Fontoura PCS[1], Pinto VLM[1], Cheniaux Jr E[2], da Silva O[1], Brunini T[3], Mendes Ribeiro AC[1] [1]UERJ − Farmacologia e Psicobiologia, [2]IPUB-UFRJ − Psiquiatria, [3]UERJ − Farmacologia

Introduction: Bipolar disorder (BD) is a mood disorder, according to the DMS-IV (Diagnostic and Statistic Manual of Mental Disease), and can be subdivided into: Bipolar I Disorder, Bipolar II Disorder, Cyclothymic Disorder and Psychotic Disorder Not Otherwise Specified. BD affects approximately 1.5% of the population and it is characterized by the presence of mania and depression episodes. Patients with BD without these symptoms are diagnosed as euthymic BD. Similarly to other psychiatric disorders, such as depression, anxiety and schizophrenia, BD represents an important cardiovascular risk factor[2,3], and the mortality is twice that observed in the general population[4]. However, the exact mechanisms underlying this relationship remain unknown. Recent studies suggest the involvement of the L-arginine-nitric oxide pathway in the physiopathology of BD[5]. Nitric oxide (NO) is a lipophylic gas which is produced by different blood cells. L-arginine, its precursor, is transported into platelets by system y^+L, and activates the enzyme NO synthase (NOS), which produces NO and L-citrulline. NO has many physiological functions, including vasodilatation, neurotransmission and platelet aggregation inhibition. Although the intracellular concentration of L-arginine is above the K_m of NOS, the extracellular transport of L-arginine is necessary for NO production[6]. The aim of this study was to investigate L-arginine transport and NOS activity and expression in the platelets of patients with bipolar disorder I (BD I). **Methods**: Twenty-five patients with BD I (9 in euthymic, 8 in depression and 8 in the manic phase) and 10 healthy controls were included in this study. Extracellular L-arginine transport into the platelets was measured by kinetic methods, using increasing concentrations of [^{3}H] L-arginine. NOS activity was evaluated by the conversion of [H^3]L-arginine into [^{3}H]L-citrulline and NOS expression was evaluated using the Western Blotting technique. One-way ANOVA was used for statistical analysis. Data were presented by mean and standard error and statistic difference was considered when $p<0.05$. This study was approved by the Ethics Committee of the Pedro Ernesto University Hospital (1436-CEP/HUPE/UERJ). **Results:** Total L-arginine transport (pmol L-arginine/10^8 cells/min) was similar in bipolar patients and healthy controls. L-arginine transport via system y^+L did not differ between bipolar patients compared with healthy controls. NOS activity (pmol L-citrulline/10^9 cells) was reduced in all BD groups (euthymic, depressive and manic) compared with controls ($p<0.0001$). **Discussion:** These results suggest that NO production by NOS is reduced in all groups of BD patients in the presence of normal concentrations of its substrate and the unaltered expression of eNOS and iNOS. This lower production of NO can contribute, at least in part, to the high cardiovascular risk factor seen in patients with BD. **References:** [1]Diagnostic and Statistical *Manual of Mental Disorders* 4th edition. Artmed, 2002. [2]Sowden GL, *Int J Cardiol* 132(1):30-7, 2009. [3]Garcia-Portilla MP, *J Affect Disord* 115(3):302-8, 2009. [4]Murray DP, *Curr Psychiatry Rep* 11(6):475-80, 2009. [5]Yanik M, *Eur Arch Psychiatry Clin Neurosci* 254(1):43-7, 2004. [6]Brunini TM, *Pflugers Arch* 445:547-50, 2003. **Financial support**: FAPERJ

03.036

Memory impairment is associated with inflammatory changes in the hippocampus of DENV-3 infected mice. Campos RDL[1], Amaral DCG[2], Cisalpino D[3], Vilela MC[4], Rodrigues DH[5], Miranda AS[1], Lacerda-Queiroz N[5], Souza KPR[3], Kroon EG[3], Reis HJ[6], Teixeira, AL[2] [1]UFMG − Bioquímica e Imunologia, [2]UFMG − Clínica Médica, [3]UFMG − Microbiologia, [4]UFMG − Neurociências, [5]UFMG − Biologia Celular, [6]ICB-UFMG − Farmacologia

Introduction: Dengue virus causes the most frequent human arboviral disease. The involvement of the central nervous system (CNS) in dengue infection has been reported, but its frequency has not been established yet. Dengue virus may actively invade the CNS during acute infection, leading to mental confusion as part of diffuse encephalitis. Previous histopathological observation from our group has shown hippocampal damage after human DENV-3 intracerebral inoculum in mice. In the present study, we aimed to investigate further the effects of Dengue in mice hippocampus, assessing the expression of cytokines and the effects in a learning and memory test. **Methods:** C57BL/6 8-week male mice were intracerebrally inoculated with $4x10^3$ PFU of DENV-3 or PBS. Animals were observed daily. Memory and learning paradigms were assessed using the step-down inhibitory avoidance task (1) 4 and 5 days post-infection (dpi). Levels of IL-6, CXCL1 and TNF-α mRNA were measured in hippocampus of mice by RT-PCR at 3 and 5 dpi. Statistical analysis was performed using Student's t-test for the step-down inhibitory avoidance task and Kruskal-Wallis test for PCR-RT data. All experimental procedures were approved by the local ethics committee (104/2009). **Results:** Infected animals presented increased levels of IL-6 mRNA when compared to control (mean SE; control: 1.13 ± 0.32; 3dpi: 10.73 ± 4.66; 5dpi: 78.00 ± 26.69). Expression of CXCL1 was also increased at 5dpi when compared to control and 3dpi (control: 1.0.31; 3dpi: 2.10±0.77; 5dpi: 11.40±3.27). TNF-α gene was overexpressed at 5dpi when compared to controls and 3dpi (control: 1.32±0.33; 3dpi: 5.31±0.68; 5dpi: 42.01±14.00). Infected animals at 5dpi presented decreased step-down latency (seconds) 1h30min after practice (control: 179.90±0.11; infected: 104.40±18.72 after 1h30min; p=0.11 after 24h practice). **Discussion:** Our data shows that infected mice have impaired short term memory. Levels of proinflammatory molecules were also altered. Increased expression of IL-6 and TNF-α in brain has been shown to synergize with IL-1 to cause sickness behavior (2). Emerging evidence, however, suggests another role for brain IL-6 in CNS function, facilitating disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cells (3). High levels of TNF-α mRNA may also point to increased glutamate neurotoxicity and possible induction of apoptosis (4). Increased expression of CXCL1 suggests neutrophils recruitment with consequent tissue damage (5). Altogether, our results indicate that an impaired short term memory observed in mice infected with human DENV-3 may be partially explained by inflammatory changes in hippocampus, including increased expression of IL-6, TNF-α and CXCL1. Financial support: CNPq, CAPES and FAPEMIG. (1) Ahmadi S *et al*., Arch Iran Med. 2010; 13(3):209-16. (2) Bluthe RM *et al.*, *Physiol. Behav.* 70 (2000), pp. 367–373. (3) Sparkman NL *et al*., *J Neurosci* (2006); 26:10709–10716. (4) Pickering M *et al*., *Exp. Physiol.* 90 (2005), pp. 663–670