03. Psychopharmacology

03.001 Chronic postnatal administration of methylmalonic acid provokes spatial memory deficits in rats

de Souza TL, Grauncke ACB, Della-Pace ID, Fiorin FS, de Castro M, Busanello G, Ribeiro LR, Royes LFF, Furian AF, Oliveira MS UFSM

Introduction: Methylmalonic Acidemia consists in a group of inherited neurometabolic disorders been characterized by accumulation of Methylmalonic Acid (MMA) in body fluids and tissues (2, 7). It has been demonstrated that MMA accumulation promotes neurological alterations including failure to thrive and psychomotor delay in patients (6). The objective of this project was to verify if an experimental model of this acidemia alters behavioral parameters in young rats. **Methods:** To develop this current study newborn Wistar rats were used to induce a model of chronic acidemia via subcutaneous injections of MMA, from 5th to 28th day of life, twice a day, from 0.72 to 1.67μmol/g as function of animal age (4). At the 29th day the animals were acclimatized to the open field and it was verified the number of crossing and rearing (3). 24 hours later, once again in the open field, was performed a training with two identical objects placed in a linear configuration. After 4 hours, the recognition test with spatial alteration, and 10 minutes later, the object recognition test were made to analyze the recognition index (RI) of memory on the moved or new object (1). At the 31st day the elevated plus maze test was conducted to verify anxiety-like behavior (5). Data were analyzed by unpaired t test and the value of t is presented if $P<0.05$. These were conducted in accordance with the approval of the Ethics Committee for Animal Research of the Federal University of Santa Maria (process #112/2010). **Results**: During all this study the animals were weight and there were no weight differences between the groups. In the acclimatization to the open field it was verified no difference in the number of crossing ($t=0.2775$) or rearing ($t=0.9153$). The object recognition with identical objects showed no preference for any of those by the animals. The spatial recognition test demonstrated that the animals treated with MMA presented a smaller RI of the dislocated object if compared to the control group ($t=2.929$; $P<0.01$). The object recognition test with different objects showed no differences between the groups. Furthermore, EPM test did not presented difference between the groups regarding the time in the open and closed arms ($t=0.9272$) or percentage of entries into the open or closed arms ($t=0.6234$). **Discussion**: We found that the chronic MMA administration at doses that raise its concentration in the blood and in the brain (4) causes memory deficits in spatial recognition, which can result from damage caused by even at the central nervous system (8, 9). However, this is only a supposition and would require more experiments to verify the possibility of neuroinflammatory or neurodegenerative processes in brain structures. **References**: 1. Bevins and Besheer. Nat Protoc 1:1306 (2006) 2. Chandler et al. Faseb J 23:1252 (2009); 3. Cippitelli et al. Neurobiol Learn Mem 94:538 (2010); 4. Dutra et al. Braz J Med Biol Res 24:595 (1991); 5. File and Gonzalez. Pharmacol Biochem Behav 54:123 (1996); 6. Manoli and Venditti. GeneReviews (1993); 7. Oberholzer et al. Arch Dis Child 42:492 (1967); 8. Pettenuzzo et al. Brain Res 976:234 (2003); 9. Royes et al. Pharmacol Biochem Behav 83:136 (2006); Work supported by CNPq and CAPES. The authors gratefully acknowledge the kind help of BioEx and LabNeuro.

03.002

03.002 Behavioral profile and brain molecular characterization of elastase 2A knockout mice. Diniz CRAF[1], Casarotto PC[1], Becari C[2], Guimarães FS[1], Guimarães AO[3], Salgado HC[2], Bader M[4], Pesquero JB[3], Salgado MC[1], Joca SRL[5] [1]FMRP-USP – Pharmacology, [2]FMRP-USP – Physiology, [3]Unifesp – Biophysics, [4]Max-Delbrück – Molecular Medicine, [5]FCFRP-USP – Physics and Chemistry

Introduction: The chymotrypsin-like elastase 2a (ELA2A) is an alternative pathway responsible for approximately 10-15% of angiotensin II (ANG-II) synthesis [1]. Acting through AT1 receptor, ANG-II, additionally to its cardiovascular role, has been related to behavioral changes [2,3]. In this study we evaluated the behavioral and molecular (in limbic structures) profile of ELA2A knockout (KO) animals. **Methods:** Male and female ELA2A KO and wild-type (WT; C57BL6/j) mice were used. Independent groups were submitted to the forced swimming test (FST), elevated plus maze (EPM), marble burying test (MBT), actimeter (ACTM) and barbering behavior analysis. The effects of repeated clomipramine treatment (CLM; 15mg/kg, orally, 14 days) in these animals were also assessed in the ACTM and EPM. Western blotting was used to compare limbic molecular expression in serotoninergic and cannabinoid receptors of KO animals. BDNF expression was also evaluated. Protocols were approved by the local Ethical Committee (protocol number: 146/2009). **Results:** We observed that only male ELA2A KO showed decreased immobility time in the FST [male: WT= 192 ± 17, KO= 137 ± 61; female: WT= 160 ± 28, KO= 125+/-39] and in the percentage of time spent in the EPM open arms (%OAT) [male: WT= 25 ± 13, KO= 8.6 ± 6; female: WT= 19 ± 9, KO= 11 ± 9] compared to WT. Male and female KO animals also showed increased barbering [chi-squared: 37.62] and repetitive behaviors, reflected as a higher number of buried marbles in the MBT [male: WT= 5.8 ± 2, KO= 9.8 ± 2; female: WT= 5.5 ± 3, KO= 11 ± 1] and in the percentage of repetitive movements measured in ACTM [male: WT= 38.36 ± 2.3, KO= 47.6 ± 9; female: WT= 37.8 ± 4.8, KO= 44 ± 4.2] from WT. CLM treatment reduced the percentage of repetitive movements [male: vehicle= 46 ± 3, CLM= 35 ± 4; female: vehicle= 45.7 ± 6, CLM= 37.9 ± 2.3] in KO animals, whereas an increase in the %OAT was only observed in males [male: vehicle= 1.8 ± 2.3, CLM= 11.9 ± 9.3; female: vehicle= 11.9 ± 7.7, CLM= 9.5 ± 5.6]. Male KO animals showed increased 5HT1A serotoninergic receptors in hippocampus and striatum [hpc: WT= 100.0 ± 9.576, KO= 155.2 ± 4.719; str: WT= 100,0 ± 11,88; KO= 177,4 ± 39,34], increased BDNF in frontal cortex [WT= 100.0 ± 13.50; KO= 169.5 ± 16.31], increased CB1 cannabinoid receptor in striatum [WT= 99.98 ± 13.64, KO= 186.9 ± 17.82] and decreased in hippocampus [WT= 99.98 ± 5.101, KO= 63.13 ± 13.09]. Behavioral results were expressed as mean+/-SD and molecular data as mean±SEM. **Discussion:** Our results suggest that ELA2A, allegedly by regulating ANG-II synthesis, can modulate antidepressant- and anxiety-like behaviors and repetitive movements. Molecular data meet the behavioral data. Behavioral and molecular data allow to shed light to a possible ANG-II system action on the neurobiology of anxiety spectrum disorders and mood disorders. **References:** [1]: Becari. C. Braz J Med Biol Res, 44: 914; 2011. [2]: Gard. P. R. Eur. J. Pharmacol, 438: 1; 2002. [3]: Saavedra. J. M. Regul. Peptides, 128: 227; 2005. **Finnancial Support:** FAPESP and CNPq.

03.003 Involvement of PI3K-class I gamma in acute effects of antidepressant drugs in mice. Vaz GN[1], Campos AC[1], Teixeira AL[1], Lima IVA[2] [1]UFMG – Tropical Medicine and Infectious Diseases, [2]ICB-UFMG – Pharmacology

Objectives: Several pieces of the literature have suggested the involvement of PI3K pathway in neuroprotective and neuroplastic events. The present work aimed to evaluate the behavioral profile of mice knockout (K.O.) for the PI3K-γ gene and its influence on the rapid behavioral effects of the antidepressant drugs. **Methods:** Male C57BL6 mice (wild type WT or PI3Kγ K.O.; 9-10 weeks old) were divided in 6 independent experimental groups and submitted to acute i.p. injections of Ketamine 10mg/Kg, Imipramine 15mg/Kg or vehicle. Thirty minutes after treatment animals were submitted to the forced swimming test (FST). Statistical differences were analyzed by ANOVA. All procedures were performed in accordance with the standards of the local Ethics Committee for Animal Experimentation (CETEA protocol number: 114/12). **Results:** The behavioral analysis of the FST revealed that vehicle-treated PI3K-γ K.O. group exhibited a higher percentage of immobility response when compared with WT-vehicle group (% of immobility K.O.-vehicle (n=7): 57.4+/-7.6; WT-vehicle (n=7) 46.7 +/-4.9; p=0.05). Moreover ketamine and imipramine treatments were effective in reducing the immobility percentage in WT animals (% of immobility WT-vehicle (n=7): 46.7 +/-4.9; WT-Ketamine (n=6): 14.5 +/-2.7 and WT-Imipramine (n=5) 26.4 +/-7.8; $p<0.05$) but not in PI3K-γ K.O. animals (% of immobility K.O.-vehicle (n=7): 57.4 +/-7.6; K.O.-Ketamine (n=7): 51.3 +/-7.7 and K.O.-Imipramine (n=5) 66.6 +/-4.9; p=ns). Results are expressed as mean+/- SEM. **Discussion:** Our results indicate that the PI3K-γ pathway plays a role in the modulation of rapid antidepressant-like effects of imipramine and ketamine in mice. PI3K-γ pathway can be regarded as a new pharmacological target for in psychiatry. **Financial support:** CNPq.

03.004 Cannabidiol pretreatment reverses amphetamine-disruptive effects in the prepulse inhibition test in *Swiss* mice. Pedrazzi JFC[1], Issy AC[2], Guimarães FS[2], Del Bel EA[3]
[1]FMRP-USP – Neurociências e Ciências do Comportamento, [2]FMRP-USP – Farmacologia, [3]FORP-USP – Morfologia, Estomatologia e Fisiologia

Introduction: Schizophrenia is a highly disabling disease which would involve an imbalance in the dopaminergic neurotransmission and a glutamatergic hypofunction [1]. The Delta9-tetrahydrocannabinol (Delta9-THC), the main psychotomimetic constituent of *Cannabis sativa*, induces psychotic reactions and cognitive changes similar to schizophrenia symptoms [2]. However, cannabidiol, another component of cannabis, devoid of psychotomimetic properties and has been described as a compound with possible antipsychotic profile. Clinical studies have investigated the use of cannabidiol as an alternative treatment for schizophrenia and reported its therapeutic effects [3]. However, few preclinical studies have been performed to support possible clinical utility of cannabidiol and to reveal its mechanism of action. Sensorimotor deficit characterized by prepulse inhibition (PPI) disruption is presented by schizophrenia patients and is reproducible in the lab [4]. PPI disruption reproduces positive symptoms of schizophrenia. **Objective:** The aim of this study was to investigate the ability of cannabidiol to reverse the amphetamine disruptive effects in the PPI test, an antipsychotic predictive model. **Methods:** Male Swiss mice (25-35g) received intraperitoneal (i.p.) injection (one hour before test) of vehicle or cannabidiol (15, 30 or 60 mg/kg) followed, 30 minutes after, of a second i.p. injection of saline (10 ml/kg) or amphetamine (10 mg/kg) and were submitted to the PPI test 30 minutes later. The PPI test consist of 64 trials irregularly divided into pulse (P, white noise, 105 dB), prepulse (PP; pure tone; 7kHz; 80, 85 or 90 dB), prepulse+pulse (PP+P) and no-stimuli with white background noise level of 64 dB – %PPI=[100-(PP+P/P)*100]. The percentage of PPI was analyzed with repeated measures with the treatment as the independent factor and the prepulse intensity as repeated measure. Duncan´s post hoc test ($p<0.05$) was used to specify differences. **Results:** The acute treatment with amphetamine promoted significant PPI disruption at all prepulse intensities analyzed (80,85 and 90 dB) with means ± SEM: 32 ± 5,97; 43,4 ± 5,00 and 46,3 ± 5,07. Cannabidiol pretreatment (30 and 60 mg/kg) blocked the effect of amphetamine in the pre-pulse intensity of 80 dB, with means ± SEM: 51.4 ± 5.49 and 51.9 ± 5.23 respectively. The repeated measures ANOVA revealed a significant overall effect of treatment [$F(5,51)=12.23$, $p<0.001$], prepulse intensity [$F(2,102)=26.92$, $p<0.001$] and interaction between prepulse intensity and treatment [$F(10,102)= 3.127$, $p=0.002$]. By its own, cannabidiol did not produce any PPI change. **Discussion:** Our results demonstrate, for the first time, the ability of cannabidiol to reverse amphetamine-disruptive effects in the PPI test. These effects were dose and prepulse intensity dependent. Our findings support the hypothesis of cannabidiol antipsychotic profile. 1. Weiss, S.M.; Feldon, J.(2001) Environmental animal models for sensorimotor gating deficiencies in schizophrenia **Psychopharmacology** 156: 305-326. 2. Howlett, A.C. Barth, F.; Bonner, T.I.; Gabral, G.; Casellas, P.; Devane, W.A. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. **Pharmacol Rev** 54: 161-202. 3. 1. Zuardi, A.W.; Shirakawa, I.; Finkelfarb E.; Karniol I.G. (1982) Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. **Psychopharmacology** 76: 245-250. 4. Geyer, M.A.; Heinssen, R. New approaches to measurement and treatment research to improve cognition in schizophrenia. (2005) **Schizophr Bull.** 31: 806-809. **Financial support:** FAPESP Ethic committee number: 051/2012

03.005 Arcaine attenuates morphine-induced conditioned place preference in mice. Tomazi L[1], Mello CF[1], Schoffer AP[1], Girardi BA[2], Rubin MA[3] [1]UFSM – Pharmacology, [2]UFSM – Toxicological Biochemistry, [3]UFSM – Pharmacology

Opioid addiction is a chronic, recurrent brain disease that is characterized by compulsive drug seeking and a high rate of relapse even after long periods of abstinence. Several studies have shown that the systemic administration of a variety of N-methyl-D-aspartate (NMDA) receptor antagonists can block the development of conditioned place preference (CPP) induced by rewarding drugs such as morphine. However, no study has investigated whether polyamines alter abuse-related effects of morphine. In this study we examined the effect of polyamines on CPP and on morphine-induced CPP. Adult male Swiss mice (25-30 g) from the animal house of the Federal University of Santa Maria were submitted to the CPP paradigm, an animal model serving to assess addictive potential of drugs in which environmental cues become associated with the subjective effects of drugs of abuse. On the first and the second days of experiments, each mice was pre-conditioned by placing it, once a day, in a CPP apparatus for 15 min, while they could freely access the three compartments of the apparatus. The time spent in each compartment was recorded. Conditioning phase consisted of four consecutive days, with sessions conducted twice each day with 6 h separating each. The duration of each session was 30 min and the mice were confined to the considered compartment, by isolating the compartment using a guillotine door. Animals received morphine, spermidine (endogenous polyamine that physiologically modulates the NMDA receptor) arcaine (antagonist of the polyamine-binding site at the NMDA receptor), or saline and immediately were confined to the non-preferred compartment. The post conditioning score was measured in the same way as the pre-conditioning score. The difference between post and preconditioning scores was considered as change in preference score. Morphine (2.5–10 mg/kg, i.p.) significantly increased the time spent in the drug-paired compartment. Spermidine (3–30 mg/kg, i.p.) and arcaine (0.3 – 3 mg/kg, i.p.) did not induce either CPP or conditioned place aversion. Arcaine (3 mg/kg) 15 minutes before of morphine (5 mg/kg) attenuated morphine-induced CPP (control: 2.77±11.49; arcaine 36.89±17.66; morphine: 146.2±22.39; arcaine+morphine: 77.78±26.41 means ± standard error). Spermidine (30 mg/kg) 15 minutes before arcaine (3 mg/kg) and morphine (5 mg/kg) prevented the attenuating effect of arcaine on morphine-induced CPP. Spermidine (30 mg/kg) 15 minutes before morphine (1.25 mg/kg, dose that did not induce CPP) did not induce CPP. These data show that arcaine attenuates the rewarding effects of morphine and suggests that the polyamine binding site may be a target to treat morphine abuse. The experiments were performed with the approval of the Ethics Committee of the Federal University of Santa Maria (process number 068/2011). Financial agencies: CNPq, FAPERGS, CAPES, PRPGP-UFSM.

03.006 Spermine reverses LPS-induced memory deficit in mice. Frühauf PKS[1], Ineu RP[1], Rossato MF[2], Mello CF[1], Rubin MA[1] [1]UFSM – Pharmacology, [2]UFSM – Toxicological Biochemistry

Neuroinflammation is implicated in several neurodegenerative diseases and may contribute to learning and memory deficits associated with these disorders. It is known that intraperitoneal injection of lipopolysaccharide (LPS) induces neuroinflammation and memory deterioration. Polyamines, like spermidine and spermine, are aliphatic amines with modulatory properties at N-Methyl-D-Aspartate receptors (NMDARs). Recent studies have shown that polyamines improve memory in cognitive tasks. Accumulating evidence suggests that endogenous spermine triggers antiinflammatory mechanisms at infection or injury sites, inhibiting the innate inflammatory response by restraining macrophages. The aim of this study was to investigate whether spermine reverses LPS-induced memory impairment in the object recognition task in mice. Adult male Swiss mice (25-30 g) from the animal house of the Federal University of Santa Maria were subjected to the object recognition task. The task consisted of three sessions: habituation, training and testing sessions. Each mouse was individually habituated to the box, with no objects for 10 minutes, and then returned to their home cages. Twenty-four hours later, training session took place, in which the animal was exposed to two equal objects, and the time spent exploring each object was recorded for 8 minutes. The test session was carried out 24 hours after training. In this session the animal was placed back in the behavioral chamber and one of the familiar objects was replaced by a novel object. The time spent exploring the familiar and the novel object was recorded for 8 minutes. LPS, spermine and/or saline were administered by i.p. route, immediately after training, in a 10 ml/kg injection volume. Since the discrimination score may be affected by locomotor alterations, we monitored the number of crossing responses in a subsequent open field trial Injection of LPS (250 µg/kg) caused object recognition impairment in mice. Spermine (1 mg/kg) significantly increased the discrimination score on novel object recognition task, indicating that spermine improved memory. A single injection of spermine at dose that did not alter memory (0.3 mg/kg), 5 minutes after LPS injection, reversed LPS-induced impairment of object recognition task (control: 10.73±1.39; LPS: -0.40±0.68; spermine: 9.33±1.41; LPS+spermine: 5.4±1,15; means ± standard error). Neither LPS nor spermine injections altered crossing responses in the open field. The results indicate that spermine protects from LPS-induced memory deficit in mice. The experiments were performed with the approval of the Ethics Committee of the Federal University of Santa Maria (process number 068/2011). **Financial agencies**: CNPq, FAPERGS, CAPES, PRPGP-UFSM.

03.007 Evaluation of the effect of acute administration of agomelatine on the behavior of female rats in the elevated plus maze and forced swimming tests. Mendes CRM[1], Andrade AS[1], André E[2], Gavioli EC[1], Maia JP[3], Soares-Rachetti VP[1] [1]UFRN – Biofísica e Farmacologia, [2]UFPR – Farmacologia, [3]UFRN – Clínica Médica

Introduction: Agomelatine is a melatonin MT1 and MT2 receptors agonist and a serotonin 5-HT2C receptors antagonist with clinical efficacy in alleviating symptoms of depression, anxiety and in the reestablishment of the sleep-wake cycle in patients [Loo H. Int. Clin. Psychopharmacol. 17(5), 239, 2002; Millan M. Psychopharmacology (Berl) 177(4), 448, 2005; Olié J.P. Int J Neuropsychopharmacol. 10(5), 661, 2007**]**. Studies exploring the acute effect of agomelatine are scarce and divergent in the literature. The objective of this study was to investigate whether acute treatment with agomelatine promotes behavioral changes related to anxiety and depression in female rats. **Methods:** Female *Wistar* rats (90 days) were submitted to the oral administration of saline or agomelatine (12.5, 25 and 50 mg/kg/ml). Sixty minutes after the administration, the animals were tested in the elevated plus maze (EPM) for 5 minutes, the open field test for 15 minutes and in the forced swimming test (FST) for 5 minutes. The experiments were approved by the Ethics Committee on Animal Use - CEUA/UFRN under protocol number 007/2012. Results: Acute administration of agomelatine did not affect the exploration of the open arms in the EPM by females [% time spent in the open arm (mean±SEM): saline: 34.32±4.56; 12.5mg: 36.13±4.04; 25mg: 44.47±4.10; 50mg: 31.35±5.80, NS, ANOVA, n=9-11] compared with saline group. Also, this drug altered neither the distance traveled (m) nor the mean speed (m/s) in the open field test [distance traveled (mean±SEM): saline: 31.41±0.00; 12.5mg: 34.95±4.60; 25mg: 24.20±1.54; 50mg: 41.09±16.06), NS, ANOVA, preliminary data]. In the FST agomelatine did not affect the time (in seconds) of immobility and climbing of rats [time of immobility (mean±SEM): saline: 204.70±11.16; 12.5mg: 202.80±12.94; 25mg: 179.72±16.89; 50mg: 208.45±11.57, NS, ANOVA, 9-11] compared with control animals. Discussion: Data here presented suggest that acute treatment with agomelatine in the doses here employed has no significant effect on behaviors related to both anxiety and depression in females. This lack of effect was not influenced by the effect of agomelatine on locomotion of the animals, since this drug did not altered the distance traveled in the open field test. Financial support: PROPESQ-UFRN/PIBIC, CNPq.

03.008 Depressive-like behavioral phenotype of galectin-1 and 3 knock-out mice. Sartim AG[1], Joca SRL[1], Baruffi MD[2] [1]FCFRP-USP – Física e Química, [2]FCFRP-USP – Análises Clínicas, Toxicológicas e Bromatológicas

Introduction: Galectins are carbohydrate-binding proteins with affinity for β-galactoside glycans. Several galectins have been identified. Galectin-1 (Gal-1) and galectin-3 (Gal-3) are expressed in several tissues, including the brain, where they can play important roles in physiological and pathological processes. In the central nervous system, it has been shown that Gal-1 promotes brain-derived neurotrophic factor (BDNF) expression and adult neural stem cells proliferation. Additional evidence has shown that the behavioral effect of antidepressant drugs relies on their ability to increase BDNF expression and hippocampal neurogenesis. Considering that BDNF and neurogenesis are important in the neurobiology of depression and in the mechanism of action of antidepressants, the aim of this work was to test the hypothesis that Gal-1 and Gal-3 would also be involved in the neurobiology of depression. In order to investigate that the behavioral phenotype of Gal-1 and Gal-3 knock-out mice was analyzed in animal models predictive of antidepressant-like behaviors, the Forced Swimming Test (FST) and the Tail Suspension Test (TST). **Methods:** male C57BL-6 knock-out mice to Gal-1 and Gal-3 and wild-type (WT) were used. Independent groups were submitted to the FST and the TST, during 6 minutes. Conventional antidepressant drugs decrease the immobility time in these tests, while stress exposure does the opposite. Additional groups were submitted to open field test (OFT), in order to have their locomotor activity measured. The immobility time in the FST and in the TST and the number of crossings in the OFT was measured during the last 4 minutes of the test. The experimental protocols were approved by the local Ethical Committee of University of Sao Paulo- Ribeirão Preto (protocol number 10.1.136.53.2). **Results:** Gal-1 and Gal-3 Knock-out mice demonstrated an increased immobility time in the FST (170.1±8.62 and 189.8±9.14 seconds, respectively) ($F_{3,31}=10,36$, $P<0.05$) and TST (187.1±5.88 and 183.6±5.88 seconds, respectively) ($F_{3,21}=8,088$, $P<0.05$) compared to WT animals (132.8±7.41 for FST and 148.6±6.87 for TST), a depressive-like phenotype. Furthermore, no differences in the locomotor activity were found between WT (25.53±1.89 seconds) and KO mice (27.48±2.35 for Gal-1 KO and 25.33±3.88 for Gal-3 KO) ($F_{3,30}=1,746$, $P>0.05$). **Discussion:** The present work shows for the first time that the Gal-1 and Gal-3 Knock-out mice present a depressive-like behavioral phenotype in two widely used animal models of depression. These dates suggest that Gal-1 and Gal-3 may be involved in the neurobiology of depression. **Acknowledgements:** The authors wish to thank CNPq and FAPESP for financial support.

03.009 Acute systemic administration of DNA methylation inhibitors induces antidepressant-like effects: **Involvement of cortical BDNF-TRKB-mTOR pathway**. Romano ACD, Pereira VS, Joca SRL USP

Introduction: DNA methylation, catalyzed by DNA methyltranferases (DNMTs), is an epigenetic mechanism that is associated with transcriptional repression. It was shown by our group that the DNMT inhibition induces antidepressant-like effects in preclinical models. However, the molecular mechanism and the brain regions involved in such effects remain poorly investigated. Evidence has shown that stress exposure decreases while chronic antidepressant treatment increases cortical BDNF (brain derived neurotrophic factor) levels. The behavioral effect of conventional antidepressant treatment has been attributed to their ability to restore BDNF levels in limbic regions. Additional evidence has shown that stress increases DNA methylation in BDNF promoter regions and higher levels of DNA methylation in this gene have been found in depressed individual. Therefore the aim of this work was to test the hypothesis that DNMT inhibitors would induce acute antidepressant-like effect and that such effects would be associated to increase BDNF signaling in the vmPFC. **Methods:** Firstly, male wistar rats (280g) were submitted to a 15min session of forced swimming (FS) and, 24h later, they were submitted to a 5min FS test when the immobility time (IT) was measured (Ethic Committee Number:12.1.235.53.2). In this test was evaluated the antidepressant-like effect acute of DNA methylation inhibitor systemically, RG108 (0.2 and 0.4 mg/kg both). Secondly, with guide cannulas aimed at vmPFC were submitted to a 15min session of forced swimming (FS) and, 24h later, they were submitted to a 5min FS test when the immobility time (IT) was measured (Ethic Committee Number:12.1.235.53.2). In this step the animals received an injection of BDNF (0.1 or 0.2nmol/0.2µL) or vehicle. An independent group an injection of K252a (TrkB antagonist at 0.01nmol/0.2µL) or Rapamycin (RAPA - mTOR inhibitor at 0.2nmol/0.2µL) or vehicle was realized 10 min before BDNF (0.2nmol/0.2µL) or vehicle. The animals were exposed to the test session 30 min after the last injection. Finally, in an independent group an injection K252a or RAPA or vehicle and an administration of RG108 systemically (DNA methylation inhibitor - 0,2mg/kg i.p.) or vehicle was realized 1 hour before test. All data were analyzed by ANOVA followed by Dunnet post-hoc test. **Results**: The DNA methylation inhibitor, RG108, reduced the IT in FS (vhl: 141,5 ±61,54; imi; 8± 11,31; RG108 0,2: 18±27,65; RG1080,4: 141,8±36,85; $F_{(3,10)}$=10.34; *p<0.05). BDNF administration into vmPFC reduced the IT (vhl: 203.0±17.41; BDNF 0.1nmol: 129.0±22.28; BDNF 0.2nmol: 38.14±16.65*; $F_{(2,15)}$= 21.67; *p<0.05). K252a did not reduce the IT *per se* but it abolished the effects of BDNF in the FST (vhl+vhl: 177.1±18.29; vhl+BDNF: 66.33±24.58*; K252a+vhl: 156.2±23.65; K252a+BDNF: 173.1±24.76; $F_{(3,22)}$= 5.119; *p<0.05). The effect of BDNF was also blocked by RAPA pre-treatment (DMSO+vhl: 213.5±16.62; DMSO+BDNF: 31.29±3.517*; RAPA+vhl: 182.4±4.781; RAPA+BDNF: 175.3±22.77; $F_{(3,25)}$= 29.03; *p<0.05). K252a and RAPA abolished the antidepressant-like effects of RG108 (vhl+vhl: 165.6±11.83; vhl+RG108: 72.57±24.19*; RAPA+RG108: 167.3±23.05; k252a+RG108:137.0±16.19; $F_{(3,24)}$= 5.161; *p<0.05). **Discussion**: Our present data shows that antidepressant-like effect of RG108 is dependent of BDNF-TRkB-mTOR pathway in vmPFC. Beyond that we also show that BDNF antidepressant-like effect is dependent of mTOR. Therefore, our data suggest that it might exist an interaction between DNA methylation inhibitor and BDNF antidepressant-like effects, since TRkB and mTOR blockade is able to abolish the antidepressant-like effects of both agents. **Financial agencies**: FAPESP.

03.010 Environmental enrichment and its protective effect on stress-induced anxiety: **implications of glucocorticoid signaling**, **MAPK pathway and CREB in basolateral amygdale**. Novaes LS, Santos NB, Lopes DCF, Duque EA, Wiezel G, Munhoz CD ICB-USP – Farmacologia

Introduction: Environmental enrichment (EE) is an experimental model that enhances the animal opportunity to interact with sensory, motor, and social stimuli comparing to the standard conditions. Among the benefits of EE are the improvements in learning and memory, as well as reduction in stress-induced behaviors, including anxiety. Overall, the most conclusive findings on the latter issue show a casual relationship between stress-induced changes in the hippocampus and amygdala and long-lasting anxiety symptoms. In this regard, EE has gained attention for promoting or restoring the normal adult hippocampal neurogenesis, as well as by modulating the systemic release of glucocorticoid hormones (GCs). However, the relationship among stress, anxiety and the mechanisms by which EE exerts its protective role remains inconclusive, especially considering the immediate effects exerted by acute stress on animal behavior. **Objective**: The present study aimed to verify whether EE influences the process of a stressor stimulus in order to modify the course of behavioral response in rats, and which bio-molecular changes would be related to this process. **Method**: Male *Wistar* rats were housed in EE (14 days) prior to restraint stress (1h) and, just after the stressor stimulus, we measured the anxiety-like behavior (elevated plus maze) and changes of intracellular pathways in the basolateral amygdala (BLA) of these animals (*Westernblot* analysis). All experiments were conducted in accordance with the ethical principles in animal research adopted by the Institute of Biomedical Sciences local Animal Care Committee, University of São Paulo (n. 006/book 02/page 98 CEUA ICB-USP). **Results**: Briefly, we found that EE is able to prevent the emergence of anxiety-like symptoms triggered immediately after stress and that this effect is not due to changes in systemic release of corticosterone (a rat GCs). On the other hand, our results showed that stress promotes a rapid increase in the nuclear activity of glucocorticoid receptor (GR) in the BLA, effect prevented by previous EE submission. Furthermore, we observed an acute reduction in the activity of ERK (a MAPK protein) and CREB in the BLA of stressed animals, while in the EE animals this effect seemed prolonged. Finally, we found that the acute stress promotes an augment in the protein expression of immediate-early gene Egr-1 in the BLA, indicating a possible increase of neuronal activity in this region as a result of stress, while no changes in the expression of this protein was found in EE animals, even the stressed ones. **Conclusions**: The hyperactivity of BLA neurons is a phenotype that is in strong association with the anxiety-like behavior and changes in this process may be one of the mechanism by which EE exerts its protective effects against stress-induced anxiety. In this regard, modulation of GR, ERK and CREB activity by EE in the BLA can help us to understand in which way this phenomenon occurs. Financial support: FAPESP and CNPq

03.010 Environmental enrichment and its protective effect on stress-induced anxiety: **implications of glucocorticoid signaling**, **MAPK pathway and CREB in basolateral amygdale**. Novaes LS, Santos NB, Lopes DCF, Duque EA, Wiezel G, Munhoz CD ICB-USP – Farmacologia

Reported evidence suggests that panic patients have a deficient opioid modulation [1] and shows that the activation of μ e κ opioid receptors localized in the dorsal periaqueductal grey matter (DPAG) has antiaversive and aversive behavioral effects, respectively [2]. Previous results showed that the intra-DPAG microinjection of the μ-antagonist CTOP (1 nmol/0.2 μL/120 s) has no effect by itself ($p>0.05$) in the elevated T maze (ETM) [3].The intra-DPAG injection of the κ-receptor opioid antagonist Nor-BNI (3.4 nmol/0.2 μL/120 s) significantly decreased inhibitory avoidance latency compared to control at avoidance 2 ($p<0.05$) in the ETM, interpreted as an anxiolytic-like effect, without affecting escape (unpublished). This study investigated the participation of μ and κ opioid receptors in the rat DPAG on escape elicited by the electrical stimulation of the same brain area. Male Wistar rats (UEM Ethics Committee 028/2013) were microinjected (0.2 μL/120 s) intra-DPAG with the μ-opiod receptor antagonist CTOP (0.5, 1.0, 2.0 nmol), or the κ-opiod antagonist Nor-BNI (3.4 and 6.8 nmol) and the threshold intensity of electrical current applied to the DPAG that evoked escape behavior was measured 20 min before and 10min after drug treatment. Drug effects were expressed as the difference between post- and pre-treatment escape thresholds. Data were analyzed by one-way ANOVA. When appropriate, *post hoc* comparisons were performed by the Duncan's test. Compared to saline-injected animals, escape thresholds were significantly increased Nor-BNI (Saline = 4.00±5.14; Nor BNI 3.4 nmol = 12.00±6.64; NorBNI 6.8 nmol = 46.40±7.27) [$F_{(1,21)}$= 15.39, $p<0.01$)]. The *post hoc* Duncan's test showed that 6.8 nmol of Nor-BNI significantly ($p<0.01$) raised the escape threshold, indicating a panicolytic-like effect. At the doses studied CTOP [$F_{(1,13)}$= 1.38, $p>0.05$)] was ineffective when compared to saline group. In conclusion, these results confirm the results obtained in the ETM, showing that the activation of κ-opiod receptors tonically enhances aversion in the DPAG. [1] Preter, M., Klein, D.F., Prog Neuropsychopharmacol Biol Psychiatry 32, 603–612, 2008. [2] Motta, et al.. Psychopharmacology, v. 120, p. 470-474, 1995. [3] Roncon, et al. J. Psychopharmacology, 2013. DOI: 10.1177/0269881113485144 **Financial Support:** Capes and CNPq

03.012 Involvement of induced nitric oxide synthase in anxiety-like effects during ethanol withdrawal in mice. Bonassoli VT, Milani H, de Oliveira RM UEM – Farmacologia e Terapêutica

Introduction: Experimental evidence has indicated the involvement of nitric oxide (NO) on the behavioral manifestation of ethanol withdrawal. NO producing neurons have been shown to be activated in several brain structures related to anxiety such as the hypothalamic and amygdaloid nuclei and the dorsolateral periaqueductal gray matter (DLPAG). Intra-DLPAG injections of the non-selective NO synthase (NOS) inhibitor L-NAME and of the selective inducible NOS (iNOS) inhibitor 1400W decreased the anxiogenic-like effects of ethanol withdrawal in rats exposed to the light/dark box test. However, little is known about the systemic effects of these drugs during ethanol withdrawal in mice. The objective of the study was to evaluate the effects of systemic intraperitoneal (i.p.) of L-NAME and 1400W in ethanol withdrawal-induced anxiety in mice. **Materials and Methods:** Male Swiss mice (25-30g) were subjected to an oral ethanol self-administration procedure, in which they were offered 5.6% (vol/vol) nutritionally balanced ethanol solution as the only source of food, for 6 days followed by abrupt ethanol discontinuation. Twenty four hours after ethanol discontinuation, mice received i.p. injections of saline, L-NAME (20 mg/kg and 40 mg/kg) or 1400W (3.0 µg/kg). Thirty min after the injections, the animals were placed in the open field (5 min) where the travelled distance was accessed. Subsequently, the animals were evaluated in the elevated plus maze (EPM) for 5 min. The percentage of time spent, the percentage of entries in the open arms and number of entries in closed arms of the EPM were recorded and analyzed using the ANYMAZE software®. ANOVA or test Student t test were used for statistical comparisons. All procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 031/2010). **Results:** Data were expressed as mean±S.E.M. L-NAME 20 mg/kg increased the percentage of time ($F_{2,20}$=4.4, P=0.03; saline=28.1±2.6; L-NAME 20 mg/kg=45.7±4.9; L-NAME 40 mg/kg=35.9±7.2), and of entries ($F_{2,20}$=5.9; P=0.01; saline=38.0±2.2; L-NAME 20 mg/kg=51.9±4.4; L-NAME 40 mg/kg=47.8±3.2) into the open arms of the EPM compared to saline. No significant difference was observed on number of entries in closed arms of the EPM (P>0.05) or the traveled distance in the open field test (P>0.05).Significant increase in the percentage of time (t=2.3, df=13, P=0.04 saline=28.1±2.6; 1400W=38.4±3.4) and of entries in the open arms (t=3.2, df=13, P<0.01; saline=38.0±2.2; 1400W=50.2±3.2) were also detected after 1400W administration. No significant difference was observed in the closed arms entries in the EPM (P>0.05) or the traveled distance in the open field test (P>0.05). **Discussion:** Pharmacological inhibition of NOS by the non-selective inhibitor L-NAME or by the selective iNOS inhibitor 1400W decreased ethanol withdrawal-induced anxiety-like behavior in the EPM without altering locomotor activity of the animals. These findings support the involvement of NO in anxiety-like behavior observed during ethanol withdrawal and suggest that, at least in part, the observed anxiolytic-like effects should be mediated by iNOS inhibition. **Financial support**: CAPES and Fundação Araucária.

03.013 Effects of ethanol withdrawal on anxiety and locomotor activity of mice evaluated in the open field and light/dark box tests. Coltri LP, Bonassoli VT, Milani H, de Oliveira RM
UEM – Farmacologia e Terapêutica

Introduction: Animal models of ethanol withdrawal-induced anxiety have been used to explore the neurobiology underlying withdrawal and to evaluate the utility of therapeutic agents aimed at reducing withdrawal severity. The elevated plus maze, light/dark box (LDB), and open field (OF) tests are the most commonly used tests. However, ethanol withdrawal effects, especially those dependent on spontaneous motor activity, are difficult to measure and frequently result in ambiguity in interpreting the data as being indicative of anxiety-like states or of non-specific effects of ethanol withdrawal on locomotion. The objective of the study was to evaluate behavioral changes induced by ethanol withdrawal in mice using the OF and the LDB tests. **Material and methods:** Male Swiss mice (25-30 g) received i.p. of saline or ethanol (2 g/kg) daily for 10 days. Seven, 21 or 35 h after ethanol withdrawal, each animal was individually placed in the OF (5 min) where it was evaluated for the time spent, the number of entries in the periphery and in the central area and the travelled distance. Subsequently, the animals were subjected to the LDB test (5 min), where they were evaluated for the time spent in the light side and the number of crossings between both sides of the box. All procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 031/2010). Data were expressed as mean ± S.E.M. and analyzed by one-way ANOVA followed by the Newman Keuls *post hoc* test. **Results:** Twenty one h after ethanol withdrawal, a significant decrease was detected in the time spent ($F_{5,67}$=3.94, P<0.01; saline=20.52±2.22; 21 h ethanol withdrawal=10.78±1.73) and in the number of entries ($F_{5,67}$=3.34, P<0.01; saline=12.64±1.52; 21 h ethanol withdrawal=7.31±1.02) in the central area of the OF. No significant difference was observed in the travelled distance in the OF at 21 h following ethanol withdrawal ($F_{5,67=}$1.38, P=0.24) in comparison to control group. A significant decrease was observed in the number of crossings in the LDB 21 h after ethanol withdrawal when compared with controls ($F_{5,67}$=9.62, P<0.001; saline= 10.79±1.25; 21 h ethanol withdrawal=6.16±1.44). There was no significant difference in any parameter analyzed in the OF or LDB tests 7 or 35 h after ethanol withdrawal when compared to controls (P>0.05). **Discussion:** Anxiogenic-like effects were detected at 21 h but not after 7 h or 35 h of ethanol withdrawal in mice, indicating that this period should be an opportune period to test pharmacological interventions aimed to decrease ethanol withdrawal-induced anxiety.

03.014 Absence of IL-33 receptor alters behavioral responses to antidepressant and anxiolytic drugs in mice: **Involvement of hippocampal inflammation**. Lisboa SF[1], Montezuma K[2], Biojone C[2], Cunha FQ[1], Guimarães FS[1], Liew FW[3], Verri Junior WA[4], Joca SRL[2] [1]FMRP-USP – Farmacologia, [2]FCFRP-USP – Farmacologia, [3]University of Glasgow – Immunology, [4]UEL – Patologia

Goals: Stress is known to activate the immune system and to increase the release of proinflammatory cytokines, which can trigger and sensitize neural activity in limbic regions of the brain. Although this mechanism has been related to the development of psychiatric disorders, the precise involvement of many cytokines, particularly of IL-33 and its receptor, are still unknown. Therefore, the aim of this study was to investigate the behavioral phenotype of IL-33 receptor (ST2) knockout (KO) mice submitted to animal models of the psychiatric disorders anxiety and depression. In addition, their response to classical anxiolytic and antidepressant drugs was tested. **Methods:** ST2KO and their wild type (WT) littermates were submitted to the elevated plus-maze (EPM) and forced swimming test (FST), predictive of anxiolytic and antidepressant-like effects, respectively. They were also tested for locomotor activity in the open field (OF). The animals submitted to the EPM received systemically injection of different doses of diazepam (DZP), while the animals submitted to the FST received the selective serotonin (5-HT) reuptake inhibitor fluoxetine (FLX) or the noradrenaline/5-HT reuptake inhibitor imipramine (IMI). The levels of IL-1β, IL-18 and IL-33 in hippocampus (HIP) of WT and KO mice were evaluated by enzymatic immunoassay (ELISA). The local ethic committee approved the experimental procedure (CEUA 10.1.135.53.6) **Results:** In the EPM, although all the doses of DZP tested induced anxiolytic-like effect in the WT mice (%time in the open arms: veh=17.8±4.5; 0.5mg/Kg=35.6±6.6; 1 mg/Kg=44.6±3.9; 2.5mg/Kg=55±9.2, respectively; n=5-11/group), only the higher dose induced anxiolytic-like effects in KO mice (veh=23.8±3.9; 2.5 mg/Kg=64±6.9; $P<0.05$). In the FST, FLX (20 mg/Kg) induced antidepressant-like behaviour in the WT mice (Immobility time-seconds; veh=142±9.2; FLX=97±9; $P<0.05$), but not in the KO ($p>0.05$). IMI, on the other hand, significantly attenuated immobility behaviour both in the WT and KO mice in the dose of 30 mg/Kg (WT veh=156.4±9.8; WT IMI=111.7±13.9; KO veh=148.1±11.2; KO IMI=105.1±6; n=7-10/group). In the OF, there was no effect of genotype on locomotion ($P>0.05$). KO mice presented increased levels (pg/µg total protein) of IL-1β (WT=$2x10^{-3}$±$6x10^{-4}$; KO=$7x10^{-3}$±$5x10^{-4}$), IL-18 (WT=$9x10^{-3}$±$9x10^{-4}$; KO=10^{-2}±$2x10^{-3}$) and IL-33 (WT=$4x10^{-3}$±$6x10^{-4}$; KO=$6x10^{-3}$±$9x10^{-4}$) in the HIP (n=5-6/group; Student t test, $p<0.05$). **Discussion and Conclusions:** These results suggest that the absence of ST2 receptor does not modulate anxiety- or depressive-like behaviours in normal conditions, since there was no difference between animals receiving vehicle, but this genotype could confer drug resistance to anxiolytic and to antidepressant drugs with serotoninergic mechanisms. This may occurs by an overcompensation of inflammatory mediators of the same family in the HIP, which supports the inflammatory hypothesis of psychiatric disorders. In addition, these results suggest that during inflammatory conditions, it could be necessary to adjust the doses of psychotropic drugs to produce the same clinical effect. **Financial Support**: CNPq, CAPES, FAEPA.

03.015 Antidepressant-like effect of melatonin in a rotenone-induced Parkinson's disease model. Bassani TB, Gradowski RW, Zaminelli T, Barbiero JK, Santiago RM, Boschen SL, Vital MABF UFPR – Pharmacology Department

Introduction: Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta. The loss of nigral neurons leads to reduced striatal dopamine content and classical motor symptoms. Besides, patients also present non motor symptoms such as depression (Long-Smith et al. 2009). Neurotoxin-based models of PD, as rotenone, have been used to understand the pathophysiology of disease and to develop new therapeutic strategies. Systemic exposure of rats to rotenone reproduces many pathological features of PD (Bové et al. 2012). As many studies report the antidepressant-like effect of melatonin in animal models, we sought to investigate its antidepressant-like effect in a rat model of PD induced by rotenone exposure (Morais et al. 2012). **Methods:** Male Wistar rats were randomly distributed in 2 groups, control and rotenone. Intraperitoneal injections of rotenone (2.5 mg/kg) or vehicle (sunflower oil) were administered for 10 days. After, the rats were redistributed in 4 groups: control, rotenone, melatonin and rotenone+melatonin (rot+mel). Intraperitoneal injections of melatonin (10 mg/kg) or vehicle (saline with 5% propilenoglycol) were given for 28 days. The open field test (OFT) was performed at days 1 and 28 and the forced swim test (FST) at day 29 after the rotenone exposure. The striatal dopamine (DA), hippocampal Noradrenaline (NA) and Serotonine (5-HT) levels and its metabolites were measured after the behavioral tests through high performance liquid chromatography (HPLC). OFT data were analyzed by two-way ANOVA followed by Bonferroni´s test and the FST and HPLC data by one-way ANOVA followed by Newman-Keuls test. All procedures were approved by the Ethical Committee of Animal Experiment of Federal University of Paraná (protocol #579). **Results and discussion:** Our data indicate that rotenone caused hypolocomotion in the first OFT because it significantly reduced locomotion ($p<0.01$) and rearing frequencies ($p<0.01$) and elevated immobility time ($p<0.01$) when compared to the control group. At the last OFT, there were no differences among the groups. Rotenone was able to induce depressive-like behavior, since it significantly increased immobility time ($p<0.01$) in the FST compared to the control group. However, rot+mel group showed immobility time similar to control values, indicating that melatonin prevented the appearance of this depressive-like behavior. Regarding HPLC data, rotenone significantly decreased the striatal DA ($P<0.05$) compared to the control, but not affected its metabolites. Melatonin treatment restored the rotenone-induced dopamine decrease in striatum However, in the hippocampus, rotenone did not alter NA or 5-HT concentrations or its metabolites. In conclusion, melatonin presents antidepressant-like effect in the rotenone model of PD probably related to the dopaminergic system. **Sponsored by:** CAPES, CNPq, Fundação Araucária. **References:** Bové J, et al. Neuroscience, v.211, p.51, 2012. Long-Smith CM, et al. Prog Neurobiol, v.89, p.277, 2009. Morais LH, et al. Pharmacol Rep, v.64, p.1081, 2012.

03.016 Antimanic-like effects of PKC inhibitor myricitrin in animal models. Pereira M[1], Siba IP[1], Pizzolatti MG[2], Santos ARS[3], Ruani AP[2], Andreatini R[1] [1]UFPR – Dept of Pharmacology, [2]UFSC – Chemistry, [3]UFSC – Physiology

Introduction: Mania has been associated with protein kinase C (PKC) changes and PKC inhibitors have showed antimanic effects both in animals and in clinical studies. For example, lithium inhibits PKC alpha and epsilon. Myricitrin, a flavonoid that also inhibits PKC alpha and epsilon, shows anxiolytic-like and antinociceptive actions. The aim of this work was to evaluate the antimanic-like activity of myricitrin in animal models of manic-like behavior. **Methods:** Swiss albino male mice (~30 g) were used and its locomotor activity was measured in an automated locomotion-box equipped with three photocells. The number of beam interruptions was cumulatively recorded for 20 min. The animal models of mania used were: (a) amphetamine-induced hyperlocomotion; (b) sleep deprivation induced hyperlocomotion and insomnia. In amphetamine-induced hyperlocomotion, the mice were habituated with locomotion-box (2 sessions) and 24h after the last habituation session they were treated with test drug; 20 min after they received amphetamine (AMP, 3 mg/kg, sc) or saline and 15 min after they were put into the locomotion-box. The test drugs were: myricitrin (MYR 10 and 30 mg/kg), lithium (LIT 100 mg/kg, the positive control), tamoxifen (TAM 1 mg/kg, a PKC inhibitor) and vehicle (VEH). A different group of mice was tested in sleep deprivation (SD)-induced hyperlocomotion: after the first exposure to the locomotion-box, the mice were treated with test drugs (as described above) and then they were submitted to 24h of sleep deprivation (multiple platform method); then, they were treated again with the test drugs and after 30 min they were tested in locomotion-box. The sleep latency (time spent by mice submitted to SD to sleep after the locomotion test) was also recorded. This study was approved by Institutional Ethics Committee for Animal Experiments (number 385). **Results:** Amphetamine and sleep deprivation induced hyperlocomotion (VEH 186±13 vs AMP 491±17; control 250±13 vs SD 452±15**).** As expected, lithium and tamoxifen blocked the increase in locomotor activity induced by amphetamine (LIT: 193±13; TAM: 208±17) and sleep deprivation (LIT: 292±11; TAM: 194±12) without any impairment in spontaneous activity (LIT: 200±19; TAM: 166±20). Myricitrin 10 and 30 mg/kg also presented these effects (amphetamine model: MYR 10 192±19, MYR 30 161±18; sleep deprivation model: MYR 10 223±12) at dose that did not impair locomotor activity (amphetamine model: MYR 10 121±19, MYR 30 182±19; sleep deprivation model: MYR 10 234±18). However, only lithium and myricitrin were able to reduce the sleep latency (LIT: 30±3; MYR 10: 15±3; TAM: 61±4; VEH: 57±4). All significant comparisons showed $p<0.01$ (ANOVA followed by Newman-Keuls test). **Discussion:** Tamoxifen and lithium results validated the procedures and support the proposal that PKC inhibition has antimanic effect. Thus, myricitrin results indicated an antimanic-like effect. Interestingly, as lithium, myricitrin reduced the latency to sleep after SD, while tamoxifen did not that may indicates a slight different mechanism of action of these drugs. Therefore, myrictrin may be a potential drug treatment for the mania. Financial support: CAPES, CNPq, Araucária Foundation and FAPESC

03.017 Role of IFN-alpha/beta receptors in the genesis of anxiety-like behaviors in mice. Cardoso BA, Teixeira AL, Campos AC FM-UFMG – Imunofarmacologia

Introduction: The involvement of cytokines in the pathogenesis of mood and anxiety disorders has been evaluated by several approaches. The present work aimed to evaluate the behavioral profile of mice knockout (K.O) for the cytokine IFN-α/β receptor in two animal models of anxiety: the marble burying and the elevated plus maze (EPM) tests. **Methods:** Male wild type (WT) SV129 or IFN-α/β receptor K.O. mice (9 weeks old) were divided in 2 independent experimental groups and submitted to the marble burying and EPM behavioral tests. Statistical analysis was performed by Student independent t-test. All experiment protocols were approved by the local ethics committee (protocol 104/209). **Results:** In the marble burying test, IFN-α/β receptor K.O. mice displayed less burying behaviors than the WT controls [WT: 12.7 ± 3.5 (n=3); K.O: 2.6 ± 0.9 (n=5); $p<0.01$]. No effects on the distance travelled were found. In the EPM test, K.O. mice exhibited decreased number of entries and time spent in the open arms when compared to the WT group [% entries, WT: 46,7 ± (n=3); K.O: 22,9 ± (n=5); $p<0.01$ and % time, WT: 58,7 ± (n=3); K.O: 16,4 ± (n=5); $p<0.01$). No significant differences in the entries in the closed arms were found between the two groups. **Discussion:** The results presented here suggest a role for the cytokine IFN- α/β receptors in the control of anxiety related behaviors. **Financial support:** CNPq.

03.018 Acute interactions of ayahuasca and antidepressants on apomorphine-induced hypothermia. Amaral WC, Mendes FR UFABC – Ciências Naturais e Humanas

Introduction: Ayahuasca tea is prepared by boiling the leaves of *Psychotria viridis* together with liana of *Banisteriopsis caapi.* Ayahuasca has hallucinogenic properties due to the presence of N-N-dimethyltryptamine (DMT) in leaves of *Psychotria viridis.* Association of the two species increases considerably the amount of DMT absorbed, since the beta-carbolines harmine and harmaline present in *Banisteriopsis caapi* are strongest inhibitors of monoamine oxidase (MAO) that metabolizes DMT in the intestinal lumen. Since these drugs usually alter the levels of monoamines, the aim of this study was to evaluate the possible interactions between the ayahuasca tea and three antidepressants: imipramine (tricyclic), sertraline (selective inhibitor of serotonin reuptake) and moclobemide (selective and reversible inhibitor of MAO-A), through test of apomorphine-induced hypothermia. The apomorphine-induced hypothermia is based mainly in dopaminergic D2 effects, producing inhibition of noradrenergic neurons on pre-optic area. Both noradrenergic and serotonergic neurons also contribute for thermoregulation.

Methods: Male mice (n=7-9 per group) from CEDEME / UNIFESP, 3-4 months were used. The animals were administered with ayahuasca (120 or 1200 mg/kg, vo), imipramine (0.5-5 mg/kg, ip), sertraline (1-20 mg/kg, ip) or moclobemide (5-40 mg/kg, ip) and after 30 minutes received apomorphine (10 mg/kg, ip). Control group (CTR) received water vo or saline ip before apomorphine administration. The temperature was registered before the drugs (basal), after 30 min of ayahuasca or antidepressant treatment (intermediate), and after 30 min of apomorphine (final temperature). To verify the effect of ayahuasca and antidepressants association, the tea was pre-administered to the animals 30 min before sub-effective doses of the antidepressants. The basal, intermediate and final temperature of the animals was registered as described earlier. The Committee of ethics in animal use of UFABC approved the protocol (#011/2011).

Results: Data represent mean $\pm$ EPM. Ayahuasca alone at dose of 1200 mg/kg inhibited the hypothermia induced by apomorphine, as well the dose of 5 mg/kg of imipramine (CTR = 33.1 $\pm$ 0.3 vs AYA = 35.6 $\pm$ 0.8* ^{o}C; CTR = 33.8 $\pm$ 0.6 vs IMI = 36.7 $\pm$ 0.6* ^{o}C; *p<0.05 – Anova/Duncan). When the dose of 120 mg/kg (non-effective dose) was pre-administered before sub-effective doses of imipramine (1 mg/kg) or sertraline (5 mg/kg), the association initially produced decreased in the temperature of animals (Intermediate temperature: CTR = 36.9 $\pm$ 0.3 vs AYA+IMI = 34.8 $\pm$ 0.5 ^{o}C*; CTR = 37.0 $\pm$ 0.3 vs AYA+SER = 33.8 $\pm$ 0.4 ^{o}C*; *p<0.05 – Anova/Duncan), but inhibited the hypothermic effect of apomorphine (Final temperature: CTR = 32.7 $\pm$ 0.4 vs AYA+IMI = 35.6 $\pm$ 1.0 ^{o}C*; CTR = 32.5 $\pm$ 0.6 vs AYA+SER = 34.1 $\pm$ 0.6 ^{o}C*; *p<0.05 – Anova/Duncan). On the other hand, the pre-administration of ayahuasca before moclobemide (5 mg/kg) did not prevent the apomorphine-induced hypothermia. **Discussion:** The results indicated interaction between inactive doses of ayahuasca and the antidepressants imipramine and sertraline. The MAO inhibition by beta-carbolines would contribute to increase the noradrenaline and serotonin levels in animals treated with imipramine and sertraline, respectively. **Financial support:** UFABC; AFIP; CAPES.

03.019 Fluoxetine exposure during pregnancy and lactation induces anxiogenic-like effect on adult rat offspring. Estrada VB[1], Silva AS[1], Gomes MV[2], Moreira EG[1], Pelosi GG[1]
[1]UEL – Ciências Fisiológicas, [2]Unopar – Ciências da Saúde

Introduction and goals: Fluoxetine (FLX) is an antidepressant of the selective serotonin reuptake inhibitor class that has largely been used to treat anxiety and depressive disorders in pregnant women. Evidences indicate that FLX cross placenta and is excreted in milk [1], nevertheless little is known about its impacts on the initial stage of neurodevelopment and on the etiology of late onset diseases [2]. In this context, we aimed in the present study to evaluate the effect of the maternal exposure to FLX during pregnancy and lactation on behavioral anxiety of adult rat offspring. **Methods:** All experimental protocols were approved by the State University of Londrina Ethics Committee for Animal Research (CEUA 17142/2011). Male and female Wistar rats (200-240G) were mated overnight, and gestational day (GD0) was determined through vaginal smear, by the presence of spermatozoids and estrous phase. Dams were received daily by gavage tap water (CON) or FLX (5 mg/kg, Daforin oral solution, EMS Laboratory, Brazil) from GD 0 through postnatal day 21 (PND 21). Animals (PND68) were paired in home cage 10 days before social interaction test on open field apparatus. The time of social interaction between animals [3] was analyzed by non-parametric test (Mann-Whitney, $p<0,05$). Data were presented as median± minimum/maximum values. **Results:** The exposure to FLX during pregnancy and lactation reduced interaction social time between animals (25±7/36 seconds, n=8) compared to control group (47±37/63 seconds, n=8; $p = 0,0002$]. **Conclusion:** Our data suggest that exposure to FLX causes anxiogenic-like effect on adult rat offspring. **References:**1. Sinclair et aL. Soc. Reprod. Fertil. Suppl. 64: 425, 2007.2.Hendrick et al. Biol Psychiatry, 50: 775, 2001. 3. File. Br J Pharmacol, 62: 19, 1978.**Financial support:** CAPES, Fundação Araucária, FUNADESP.

03.020 Effect of *Dioclea violacea* M. (Aqueous Extract) on general activity observed in the open-field arena and its dyskinetics movements after haloperidol acute treatment in rats. Mariani MP[1], Gemignani S[2], Pedroso-Mariani SR[2] [1]FM-PUCCamp – Pharmacology, [2]FMJ – Pharmacology

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. Ministério da Agricultura, RJ-BR, 1:76, 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 aim of this work was to study the action of acute administration of aqueous extract of DVM after acute haloperidol pre-treatment (Halo) on the general activity observed in rats in the open-field arena on frequency of locomotion (FL) and the effects on jaw movements (JM) and protrusion of the tongue (PT). **Methods:** (EAEC-FMJ number: 165/09) male Wistar Rats (N= 20), weighing 280 g on average, were divided into 4 groups. The animals in the control group (C) were injected with saline (1ml/kg, i.p.). The DVM group was treated with a suspension of DVM seed powder, this was prepared in the form of infusion and injected with a (36mg/kg, i.p.). The Halo group was injected with a (3mg/Kg, i.p.) and the fourth group (Halo+DVM) was Halo twenty minutes before the administration of DVM (in the same doses for the individual groups mentioned). After twenty minutes it was recorded the activity of these animals in the open-field arena, during 5 minutes (frequency of locomotion).Then, the animals were isolated in metal cages for observation for ten minutes and the frequencies of JM and PT were recorded (Neiswander, J. L. et al. , Psycopharmacol,116:79,1994). 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 al. Biol.Psycoat.,18:1181, 1983). **Results:** Data are presented as mean ± standard deviation and have been analyzed by ANOVA. The frequency of JM in rats administered with DVM (78.60 ± 17.57) and with Halo (33.40 ± 9.79) had significant increase when compared to the control animals (6.80 ± 2.28) ($p<0.05$ Tukey-Kramer Test). However, DVM + Halo (7.0 ± 2.24) group presented JM similar to control. The Frequency of Locomotion (FL) in the open-field arena obtained using Halo (2.20 ± 1.10) and DVM + Halo (4.20 ± 1.64) presented significant decrease when compared to the control (28.80 ± 11.69). **Discussion:** The DVM (aqueous extract) promotes increased jaw movement. The same effect was observed in the treatment with Halo. However, the interaction between Halo and DVM has not changed JM. One possible explanation for these results is a change in the percentage of D2 receptor occupancy, with this change was not induced dyskinesia. The results of the frequency of locomotion for the group (DVM + Halo) indicate that the same changes in the percentage of D2 receptor occupancy were not sufficient to modify the decrease in motor activity haloperidol-induced. **Financial Support:** FMJ.

03.021 Beneficial effect of the antioxidant vitamin e on behaviors related to anxiety in normoglycemic and diabetic rats. Andrade EG, Souza CP, Rodrigues AB, Cunha JM, Zanoveli JM UFPR – Farmacologia

Introduction: Diabetes is a chronic disease with multifaceted etiology. Evidences show that people with diabetes has higher prevalence to develop anxiety disorders. Given the high cost involving anxiety disorders treatment as well diabetes, this study aimed to investigate the potential anxiolytic effect of the antioxidant vitamin E. **Material and Methods:** Male *Wistar* rats (180-250g; n=8-10) were used in all protocols, which were previously approved by the UFPR's Committee on the Ethical Use of Animals (authorization #668). Animals were treated intraperitoneally with citrate buffer (10mM, pH 4.5, normoglycemic control group – NLG) or streptozotocin (50mg/kg, diluted in citrate buffer, diabetic group – DBT) and were submitted to a prolonged treatment during 28 days with Vitamin E (300mg/kg, p.o. - VITE) or corn oil (vehicle - VEH). In the 28° day, the animals were submitted to Elevated T-Maze (ETM) test, in which the response of inhibitory avoidance (Avoid) was evaluated (three latencies to exit of the closed arm – baseline, Avoid 1 and Avoid 2). Immediately after ETM test, the animals were submitted on Open Field (OF) to evaluate the locomotor activity. Two days after, the animals were submitted to contextual fear conditioning test, that consisted of a conditioning session (footshock - 1.5mA, 1s duration - day 1) and three more test sessions (without shock) on subsequent days (S1, S2 and S3). The time spent in conditioned freezing was evaluated in each session, being the behavior assessed in S1 more related to anxiety and the behavior assessed during S2 and S3 with the aversive memory extinction. **Results:** When compared to NGL animals, DBT rats showed a more pronounced anxiety-like response by facilitating the Avoid 1 and 2 [Avoid 1/Avoid 2 (mean ± SEM, seconds): VEH/VEH = 31.4 ± 5.0/195.5 ± 8.2; STZ/VEH = 90.1 ± 27.4/236.1 ± 26.1], as well by increasing the conditioned freezing behavior during S1, S2, S3 [freezing response (mean ± SEM, seconds): S1 - VEH/VEH = 308.5 ± 24.5; STZ/VEH = 413.7 ± 28.1; S2 – VEH/VEH = 229.4 ± 22.1; STZ/VEH = 325.8 ± 51.3; S3 – VEH/VEH = 151.5 ± 20.9; STZ/VEH = 254.9 ± 41.7]. Vitamin E treatment induced an anxiolytic-like effect in NGL and DBT animals by reducing latency of Avoid 1 and 2 [Avoid1/Avoid 2: VEH/VITE = 8.8 ± 1.5/121.1 ± 24.5; STZ/VITE = 17.4 ± 2.9/92.4 ± 26.3]. Accordingly, this anxiolytic-like effect was also observed in NGL and DBT animals exposed to contextual fear conditioning test by reduction in the time of conditioned freezing during S1 [VEH/VITE = 202.9 ± 0.6; STZ/VITE = 336.6 ± 24.2]. Interestingly, during S2 and S3 Vitamin E treatment facilitated the extinction of aversive memory only in NGL animals [S2: VEH/VITE = 98.4 ± 12.0; S3 = 62.8 ± 10.9]. **Discussion:** Our data showed that Vitamin E treatment induced an anxiolytic-like effect in both NGL and DBT animals. Moreover, a beneficial effect of the treatment was also observed in the extinction of aversive memory from NGL animals, but not in DBT animals. Based in our findings and the previous evidences showing a neuroprotective effect of Vitamin E treatment, an alternative in the treatment of diabetic or normoglycemic patients suffering of anxiety disorders can be by supplementation with Vitamin E. **Financial Support:** CAPES, CNPq, Brazil.

03.022 Serotonin regulation of anxiety-related defensive responses in the prelimbic cortex of rats. Yamashita PSM, Zangrossi Jr H FMRP-USP – Farmacologia

The dorsal raphe nucleus (DRN), the main source of serotonergic (5-HT) projections to limbic areas involved in the regulation/genesis of anxiety and fear, is densely innervated by the medial prefrontal cortex. The glutamatergic projection linking the medial prefrontal cortex to the DRN is shown to indirectly inhibit 5-HT cell firing, via activation of local GABAergic interneurons. It has been also shown that 5-HT receptors in the medial prefrontal cortex can exert a post-synaptic control on the activity of 5-HT neurons in the DRN. Electrophysiological studies revealed that local application of 5-HT1A receptor agonists in the medial prefrontal cortex decreases 5-HT cell firing in the DRN [1]. In the present study, we investigated the behavioral effects caused by the activation of 5-HT1A receptors in the prelimbic cortex (PL) of animals exposed to the elevated T-maze (ETM). This animal model, derived from the elevated plus-maze, allows the measurement, in the same rat, of an anxiety- and a panic-related defensive response, respectively, inhibitory avoidance and escape. Male Wistar rats weighing 270-290 g were anesthetized and fixed in a stereotaxic frame. Guides cannulae were bilaterally implanted in the forebrain aimed at the PL. Seven days after surgery, the animals were injected (0.2μL) in the PL with 5-HT (0, 10, 20, 40 nmol), the 5-HT1A agonist 8-OHDPAT (0, 2, 4, 8 nmol), the 5-HT2 agonist DOI (0, 4, 8, 16 nmol) or the 5-HT2C agonist MK-212 (0, 0.05, 0.5, 5 nmol) and tested in the ETM. The test apparatus consists of three elevated arms – one enclosed and two open. The test in the ETM was initiated by measurement of inhibitory avoidance acquisition. To this end, each animal was placed at the distal end of the enclosed arm of the ETM. The time taken by the rat to leave this arm with all four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and 2) at 30-s intervals. Following avoidance training (30 s), rats were placed at the end of the same previously experienced open arm and the latency to leave this arm with four paws was recorded for 3 consecutive times (escape 1, 2 and 3) with 30-s intertribal intervals. A cut off time of 300 s was established for the avoidance and escape latencies. Immediately after being tested in the ETM, each animal was placed for 5 min in the open field for the evaluation of locomotor activity. 5-HT (40 nmol) and 8-OHDPAT (4, 8, 16 nmol) treatments impaired inhibitory avoidance acquisition, indicating an anxiolytic effect, without affecting escape expression. The drug did not change the total distance traveled in the open field test, suggesting that the result observed was not due to non-specific alterations in motor function. Neither DOI nor MK-212 interfered with the defensive responses measured in the ETM. Our results are compatible with the view that inhibition of the firing of 5-HT neurons in the DRN causes anxiolytic effects. Intra-PL injection of 5-HT selectively interferes with defensive behaviors associated with anxiety, but not panic. This effect is mediated by 5-HT1A receptors of the PL. [1] Sharp, T. In: Handbook of the behavioral neurobiology of serotonin. Elsevier, p 233, 2010. Protocolo para uso de animais em experimentação número 085/2010 (COBEA). Financial support: Fapesp, CNPq, Capes.

03.023 Pharmacologic manipulation of 5-HT1A receptors located in the dorsal sub-region of the dorsal raphe nucleus exerts opposed control on inhibitory avoidance and escape behaviors. Pobbe RLH[1], Spiacci Jr A[1], Zangrossi Jr H[1] – [1]FMRP-USP – Pharmacology

Introduction: A wealth of evidence indicates that the dorsal raphe nucleus (DR) is not a homogenous structure, but an aggregate of different subpopulations of serotonergic and non-serotonergic neurons that are morphologically and functionally distinct. In this study we assessed the effects of the pharmacologic manipulation of 5-HT1A receptors placed within the dorsal sub-region (DRd) of the DR on the behavior of rats submitted to the elevated T-maze. This model allows the measurement, in the same rat, of two subtypes of anxiety-related responses, inhibitory avoidance and escape. **Methods:** Male Wistar rats were tested in the elevated T-maze ten minutes after intra-DRd administration of different doses of the 5-HT1A receptor agonist 8-OH-DPAT (1.6, 3.2 or 6.4 nmol/50 nL), and of the 5-HT1A receptor antagonist WAY-100635 (0.185, 0.37 or 0.74 nmol/50 nL). **Results:** Intra-DRd injection of 8-OH-DPAT impaired inhibitory avoidance acquisition (SAL: 99.48 ± 34.81; DPAT 1.6: 14.07 ± 2.95; DPAT 3.2: 13.5 ± 2.71; DPAT 6.4: 13.55 ± 1.91), and facilitated escape response (SAL: 9.82 ± 0.64; DPAT 1.6: 5.78 ± 1.09; DPAT 3.2: 5.6 ± 0.69; DPAT 6.4: 4.75 ± 0.65). In addition, intra-DRd infusion of WAY-100635 facilitated inhibitory avoidance acquisition (SAL: 77.37 ± 30.83; WAY 0.185: 46.79 ± 10.17; WAY 0.37: 129.58 ± 32.14; WAY 0.74: 195.36 ± 39.53), and impaired escape (SAL: 8.17 ± 0.81; WAY 0.185: 11.34 ± 0.46; WAY 0.37: 14.4 ± 1.98; WAY 0.74: 16.91 ± 1.23). These results are not due to motor alterations since locomotor activity was not modified by DRd manipulations. **Discussion:** Overall, our results indicate that 5-HT1A receptors located in the DRd exert opposed control on inhibitory avoidance and escape behaviors. **Protocol Number:** 90/2013 (Animal Ethics Committee of the University of São Paulo). **Financial Support:** FAPESP (Process Number 2013/03065-6).