03. Psychopharmacology

03.001 Cannabinoids compounds attenuate sensorimotor gating disruption induced by amphetamine in mice. Pedrazzi JFC[1], Issy AC[2], Gomes FV[3], Guimarães FS[3], Del Bel EA[2] [1]FMRP-USP – Neurociências e Ciências do Comportamento, [2]FORP-USP – Fisiologia, Morfologia e Patologia Básica, [3]FMRP-USP – Farmacologia

Introduction: Schizophrenia is a highly disabling disease, which could involve an imbalance in the dopaminergic neurotransmission and a glutamatergic hypofunction. The information processing appears to be deficient in schizophrenia. Prepulse inhibition (PPI), which measures the inhibition of a motor response by a weak sensory event, is considered particularly useful to understand the biology of information processing in schizophrenia patients. Drugs that facilitate dopaminergic neurotransmission such as amphetamine induce PPI disruption in human and rodents. Clinical and neurobiological findings suggest that the endocannabinoid system and cannabinoids may be implicated in the pathophysiology and treatment of schizophrenia. Cannabidiol (CBD), a non-psychotomimetic constituent of the *Cannabis sativa* plant, has also been reported to have potential as an antipsychotic. **Objective**: Our aim was to investigate if CBD pretreatment was able to prevent PPI disruption induced by amphetamine. Since one possible mechanism of CBD action is the facilitation of endocannabinoid mediated neurotransmission through anandamide, we tested the effects of an anandamide hydrolysis inhibitor (URB597) in the amphetamine induced PPI disruption. **Methods**: Male Swiss mice were treated with systemic or intra-accumbens CBD or systemic URB597 prior to amphetamine and were exposed to PPI test. 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. The Institution´s Animal Ethics Committe approved house conditions and experimental procedures (process number: 051/2012). **Results**: Amphetamine (10 mg/kg) disrupted PPI while CBD (15–60 mg/kg) or URB597 (0.1–1 mg/kg) administered alone had no effect. Systemic pretreatment with CBD (30 and 60 mg/kg) attenuated the disruptive effects of amphetamine in the PPI test at 85 and 80 prepulse intensities. Intra-accumbens CBD (60 nmol) microinjection attenuated the disruptive effect of systemic amphetamine (10 mg/kg) treatment in the PPI response at 90 dB prepulse intensity. The pretreatment with URB597 dose-dependently (0.3 mg/kg at all prepulse intensities and 1 mg/kg at 90 dB intensity) attenuated PPI impairment induced by amphetamine. **Conclusion**: These results corroborate findings indicating that CBD induces antipsychotic like effects. In addition, they pointed to the nucleus accumbens as a possible site of these effects. Corroborating the hypothesis that anandamide has a role in the antipsychotic-like effects of CBD, we provide the first demonstration that URB597, a known potent and selective inhibitor of the enzyme which catalyzes the hydrolysis of anandamide, has similar effects to CBD in the PPI test. **Financial support.** CAPES, CNPq, FAPESP.

03.002 Paroxetine potentiates antinociceptive process induced by chemical stimulation of ventrolateral periaqueductal gray matter. Biagioni AF, Santos GHR, Coimbra NC FMRP-USP – Farmacologia

Introduction: Paroxetine, a selective serotonergic inhibitor reuptake (SSIR), is a first-line treatment for panic disorder. However, that SSIR is not effective in the treatment of paresthesia, a common tingling or numbness symptom during panic attacks. Besides that, paroxetine treatment can even intensify the paresthesia symptoms [1]. **Aim**: In order to evaluate the role of paroxetine in an animal model of panic attacks, it was evaluated the effect of chronic treatment with paroxetine at 10 mg/kg in the defensive behavioural response characterised by defensive immobility and antinociception induced by chemical stimulation of ventrolateral column of periaqueductal gray matter (vlPAG). **Methods**: Male Wistar rats (n=4 or 6) were chronically pre-treated with intraperitoneal (i.p.) injections of paroxetine hydrochloride (HCl) at 10 mg/kg or vehicle (physiological saline + 2% tween 80). After 14 days, anesthesiated rats were implanted with guide-cannula directed to vlPAG. Seven days later, 0.6 nmol of NMDA or physiological saline (0.2 µL) was microinjected into the vlPAG. Each rat was placed in the open-field test arena where the defensive immobility behaviour was recorded during 5 minutes. Posteriorly, tail-flick latencies were measured for 30 minutes. **Results**: Two-way ANOVA followed by Newman-Keuls's post hoc test showed that microinjection of NMDA into vlPAG induced an increase in the frequency (central treatment effect, $F_{(1,16)}$=258,1; P<0.001) and duration (central treatment effect, $F_{(1,16)}$=782,2; P<0.001) of defensive immobility. However, there was no significant effect caused by the systemic treatment with paroxetine, neither interaction between central and systemic treatments (P>0.05 in both cases) considering the defensive immobility behaviour. Regarding the antinociceptive process, repeated measures ANOVA followed by Duncan´s post hoc test showed that vlPAG treatment with NMDA increased the nociceptive threshold ($F_{(3,16)}$=67.74; P<0.001). Moreover, statistical analyses showed that i.p. treatment with paroxetine potentiated the antinociceptive phenomenon ($F_{(3,16)}$=67.74; P<0.001). **Conclusion**: The present results showed that the chronic treatment with paroxetine potentiates the antinociceptive process elicited by microinjection of NMDA in the vlPAG. Although the peripheral treatment with paroxetine showed no effect on defensive immobility response, it is well stablished that the SSIR can decrease the expression of panic-like defensive responses in other animal models of panic attacks [2]. Thus, these findings suggest that the serotonergic systems might mediate the sensory pathways related to panic-like behavioural response, similar to that reported by patients diagnosed with panic syndrome and treated with paroxetine. In this sense, additional studies have been performed in our laboratory in order to validate an animal model of paresthesia. **Reference**: [1] de Graaf, L. J. Clin. Psychiatry. 64(8): 969, 2003. [2] Zangrossi H Jr. Neurosci Biobehav Rev. 46 (3): 397, 2014. **Financial Support** This work was supported by FAPESP (2014/10742-7). Ethical Committee approval (protocol number 96/2014).

03.003 Antidepressant-like effects of Nociceptin/Orphanin FQ receptor antagonists in the learned helplessness model in mice. Holanda VAD, Asth L, Medeiros IU, Guerrini R, Calo' G, Gavioli EC UFRN

Introduction: The heptadecapeptide nociceptin/orphanin FQ (N/OFQ) is the endogenous ligand of the Gi protein-coupled receptor named N/OFQ peptide receptor (NOP). Pharmacological and genetic evidence suggests that the blockade of the NOP receptor elicits antidepressant effects. The learned helplessness model (LH) employs uncontrollable and unpredictable electric footshocks as a stressor to induce a learned helplessness state. During this state, animals display deficits in avoidance behavior of the electrified chamber, which is reversed by antidepressants. The LH is a well-validated model of depression compared with behavioral despair tests that are currently most used in the screening of new antidepressants. **Aims**: The present study aims to evaluate the action of NOP receptor antagonists in helplessness mice. **Methods**: Male Swiss mice were subjected to the three steps of the LH protocol (i.e., (1) induction, (2) screening, and (3) test). Only animals that developed the helplessness behavior were subjected to the test session. During the test session, animals were placed at the electrified chamber and the latency to escape from this chamber after the footshock (assessed by the area under the curve between latency to escape and trials), and the frequency of failures (i.e. when the mouse does not escape from the electroshock chamber) were recorded. The effect of the following treatments administered before the test session was evaluated: nortriptyline (30 mg/kg, ip, 60 min), a tricyclic antidepressant, SB-612111 (1 and 10 mg/kg, ip, 30 min) and UFP-101 (1 and 10 nmol, icv, 5 min), a non-peptide and a peptide NOP antagonist, respectively. To rule out possible biases, the effect of NOP antagonists on locomotion and cognition were also assessed. **Results and Conclusions**: In helplessness mice, nortriptyline, UFP-101 (10 nmol) and SB-612111 (10 mg/kg) significantly reduced the latency to escape from the electrified chamber assessed during 30 trials (Control: 628±14, nortriptyline: 236±25*, UFP 1 nmol: 612±8, UFP 10 nmol: 314±19*, SB 1 mg/kg: 623±21, SB 10 mg/kg: 308±14*; n=6-9, ANOVA, Tukey´s test, *p<0.05) and decreased the frequency of failures from the electrified chamber (Control: 25.7±0.7, nortriptyline: 4.5±0.9*, UFP 1 nmol: 26.0±0.6, UFP 10 nmol: 5.6±0.8*, SB 1 mg/kg: 24.6±1.0, SB 10 mg/kg: 7.2±0.8*; n=6-9, ANOVA, Tukey´s test, *p<0.05). No significant deficits in locomotion and cognition were detected in mice treated with NOP antagonists. In conclusion, acute treatment with NOP antagonists promotes an antidepressant-like effect in mice subjected to the learned helplessness model. **Financial Support**: CNPq and UFRN; CEUA (License# 063/2013). **Keywords**: antidepressants; NOP receptor antagonist; learned helplessness; mouse; animal models.

03.004 Intra-dorsal periaqueductal gray injection of noradrenaline induces anxiolytic-like effect in the elevated T maze. Carvalho JJV[1], Souza DO[2], Martins JM[1], de Bortoli VC[1,2] [1]UFES – Bioquímica e Farmacologia, [2]UFES – Ciências Farmacêuticas

Introduction: The electrical or chemical stimulation of the dorsal periaqueductal gray matter (DPAG) induces defensive reactions, such as freezing and escape behaviors that have been related to panic attacks in humans. The injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT or the preferential 5-HT_{2A} agonist DOI into this area inhibits the escape reaction evoked by local electrical stimulation. Furthermore, chronic treatment with antipanic drugs such as imipramine and fluoxetine facilitates the anti-escape effect caused by the intra-DPAG injection of these 5-HT agonists. Regarding to noradrenaline, has been suggested an anxiolytic effect evidenced by the clinical efficacy of treating anxiety disorders with tricyclic antidepressants and monoamine oxidase inhibitors, which act, in part, by facilitating noradrenaline effects. Despite the presence of noradrenaline in the nerve terminals of the periaqueductal gray matter (PAG), there are few studies investigating its possible local participation in defensive responses. Thus, in the present study we used the elevated T maze (ETM), an animal model that also associates the escape response to panic attack. This test also allows the measurement of inhibitory avoidance, which has been related to generalized anxiety disorder. **Aims**: Analyze the participation of noradrenergic neurotransmission of the dorsal periaqueductal gray matter in the mediation of defensive behaviors related to anxiety and panic in the elevated T maze. **Methods**: A guide cannula was implanted in the DPAG of adult male Wistar rats (220-250 g). Five to seven days after surgery the rats were pre-exposed to an open arm of the ETM and 24 hours later tested to evaluate inhibitory avoidance and escape behavior. Different doses of the noradrenaline (10, 30 or 60 nmol/0.1 µL) or saline were injected into the DPAG 30 seconds before the test in the ETM. Immediately after the ETM test, the animal was placed in the open field for the observation of locomotor activity for 5 minutes. To evaluate the avoidance and escape data in the ETM was performed a repeated measures analysis of variance (Anova) followed by Tukey post hoc test. The data from the open field test were analysed by one-way Anova. A value of $p < 0.05$ was considered significant. **Results and Conclusions**: The results showed that intra-DPAG injection of noradrenaline (60 nmol) impaired inhibitory avoidance acquisition in the ETM [(mean ± SEM, seconds): saline = 153.00 ± 41.20; noradrenaline 60 nmol = 36.92 ± 9.62]. No significant effects of treatment with noradrenaline on escape latencies in the ETM or the number of lines crossed in the open field were found. Our results indicate that noradrenaline in the DPAG participates of inhibitory avoidance response in the ETM, suggesting an anxiolytic-like effect. **Financial Support**: FAPES and CNPq (Process Number 53672313/11). Commission ethical protocol number 046/2014, CEUA-UFES.

03.005 Does Standard treatment for organophosphorus pesticides poisoning affects depressive like-behavior induced by chlorpyrifos in rats? Siqueira AA[1], Marques GLM[1], Minassa VS[2], Sampaio KN[1], Beijamini V[1,3] [1]UFES – Ciências Farmacêuticas, [2]UFES – Ciências Farmacêuticas, [3]UFES – Bioquímica e Farmacologia

Introduction: Organophosphorus pesticides (OPs) are widely used in agriculture worldwide. Chlorpyrifos (CPF), a largely used OP, induces toxicity by inhibiting cholinesterase (ChE) activity in the central and peripheral nervous systems. Clinical studies have shown that chronic exposure to OPs at low doses can induce affective disorders such as depression. Preclinical trials have also shown that even acute exposure can trigger behavioral changes related to psychiatric disorders. Standard treatment of OP intoxication involves the use of atropine (ATR), which acts antagonizing the effects of cholinergic syndrome by blocking the muscarinic receptors and the use of an oxime, such as pralidoxime (2-PAM), to reactivate inhibited ChE. Recently, our research group observed that intoxication by CPF 20mg/kg induced a depressive-like behavior in rats submitted to Forced Swimming Test (FST) with no changes on locomotor activity in the Open Field Test. **Aim**: The aim of this study was to assess if treatment with ATR and/or 2-PAM would prevent depressive-like behavior induced by acute CPF poisoning in rats tested in the FST. **Methods**: Male Wistar rats were divided into 8 groups and were individually forced to swim inside of cylinders containing 30 cm of water at 22ºC for 15 minutes (min) (pre-test session on FST). Immediately after, CPF 20mg/kg or saline (SAL) were delivered intraperitoneally; One hour later, rats received either ATR (10mg/kg, i.p.), 2-PAM (40mg/kg, i.p.), ATR plus 2-PAM or SAL only. Twenty-four hours and 30 days after CPF or SAL injection, animals were replaced into cylinders during a 5 min test and re-test session on FST, respectively, in order to measure the immobility time. Plasma ChE activity was measured by colorimetric assay in blood samples collected after decapitation 24 hours and 30 days after CPF injection. Data were analyzed by repeated measures ANOVA (time and treatment as variables), one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$) or independent t-test. **Results**: The analysis by repeated measures ANOVA showed the effect of time ($F(1,72)=25,64$; $p<0.001$) and the effect of treatment ($F(1,72)=2,715$; $p=0.015$), with a trend of interaction between time and treatment ($F(7,72)=2,025$; $p=0.063$). CPF enhanced immobility time at 24 hours ($F(7,72)=3,85$;$p=0.001$), but not at 30 days ($F(7,72)=1,575$;$p=0,157$) compared with SAL group. Treatment with ATR attenuated the increase of immobility time induced by CPF. 2-PAM or ATR plus 2-PAM treatment had no effect on immobility behavior of animals intoxicated with CPF. Plasma ChE activity was significantly lower in CPF group 24 hours post-injection vs. SAL group (t-test; $p<0.001$), but not 30 days later. Conclusions: The results showed that CPF decreased plasma ChE activity and induced depressive-like behavior. Besides that, only treatment with ATR attenuated the behavioral change induced by CPF. Further study is in progress to evaluate cerebral ChE activity and to identify which brain regions are involved with this effect. Financial Support: FAPES Project approval nº. 047/2014 on Ethical Committee in Animal Experimentation (CEUA-UFES)

03.006 Rapid and sustained anticompulsive effect of ketamine in mice submitted to the marble burying test. Tosta CL, Silote GP[1], Souza MM[2], Soares FRC[1], Joca SRL[3], Beijamini V[4,5] [1]UFES – Bioquímica e Farmacologia, [2]UFES – Ciências Farmacêuticas, [3]FCFRP-USP, [4]UFES – Bioquímica e Farmacologia, [5]UFES – Ciências da Saúde

Introduction: Ketamine (KET), a non-competitive antagonist of glutamatergic N-methyl-D-aspartate (NMDA) receptors, has rapid and sustained antidepressant effect in preclinical and clinical studies, possibly related to activation of α-amino-3-hydroxy-5-methyl-4-isoazolepropiônico (AMPA) receptors, as well as to inhibition of protein glycogen synthase kinase-3 (GSK-3). Some NMDA antagonists induce anticompulsive effect on the marble-burying test (MBT), a potential model of obsessive-compulsive disorder (OCD). Besides that, KET seems to be efficient in patients with OCD. **Aims**: (1) test the hypothesis that KET induces acute and persistent anticompulsive effect in the MBT after a single administration in mice; (2) evaluate whether this effect of KET would be mediated by inhibition of GSK-3 using an association with lithium (LITH), an inhibitor of this protein, and (3) evaluate whether this effect of KET would be blocked by pretreatment with NBQX, an AMPA receptor antagonist. **Methods**: Adult male Swiss mice were treated with saline 0.9 %, (SAL), diazepam (DZP 2mg/kg, positive control), KET (3, 10 and 30mg/kg), LITH (10 and 30mg/kg), and NBQX (1mg/kg) once intraperitoneally (ip) on the first day of protocol. Forty min after injections, mice were placed in an open field (OF) to assess total distance moved. Immediately after the OF test, the animals were submitted to MBT, that was repeated 24, 72h and 7 days after drug injection. The number of buried marbles was registered in the MBT. The experimental groups were: experiment 1) SAL or DZP or KET 3, 10 and 30mg/kg (n=9–16/group); experiment 2) SAL+SAL; SAL+DZP, SAL+KET 3mg/kg; LITH 10 mg/kg+SAL, LITH 30mg/kg+SAL, LITH 10mg/kg+KET 3mg/kg, LITH 30mg/kg+KET 3mg/kg (n=11–12/group); experiment 3) SAL+SAL; SAL+DZP, SAL+KET 10mg/kg; NBQX+SAL, NBQX+KET 10mg/kg (n=6–9/group). MBT data were analyzed by a repetead measure ANOVA followed by one way ANOVA plus Dunnett post hoc test. OF data were analyzed by one way ANOVA followed by Dunnett test. The values were considered significant when $p<0.05$. **Results**: Experiment 1: KET 10mg/kg significantly decreased the number of buried marbles acutely (KET 4.7±1.1, SAL 9.3±0.5, $p=0.002$), 24h (KET 6.8±0.6, SAL 10.3±0.4, $p=0.012$), 72h (KET 5.1±1.0, SAL 8.4±0.6, $p=0.043$), and 7 days (KET 6.0±1.0, SAL 9.6±0.5, $p=0.003$) after injection compared to control group, without changing locomotor activity ($p>0.05$). Experiment 2: The association of a subeffective dose of KET (3mg/kg) and LITH (10 or 30 mg/kg) did not change behavior of animals in the MBT ($p>0.05$). Experiment 3: Pretreatment with NBQX attenuated the decrease in number of buried marbles induced by KET acutely (SAL+SAL 9.6±0.7, SAL+KET 4.5±1.5, NBQX+SAL 11.4±0.2, NBQX+KET 8.0±1.4, $p<0.001$) without changing locomotor activity. **Conclusions**: KET induced a rapid and sustained anticompulsive effect in mice submitted to the MBT. This effect seems to depend partly on activation of AMPA receptors, but not on inhibition of GSK-3. Project approval no. 055/2012 on Ethical Committee in Animal Experimentation (CEUA-UFES).

03.007 Thimet oligopeptidae (EP24.15) knockout mice show depressive behavior.
Reckziegel P, Franco RD, Ferro ES USP – Farmacologia

Introduction: Intracellular peptides are involved in several important cell functions. They are produced by the proteasome and by peptidases such as thimet oligopeptidase (EP24.15) and neurolysin (Nln). EP24.15 is an intracellular metallopeptidase involved in the metabolism of peptides, such as bradykinin, angiotensin and neurotensin. The inhibition of EP24.15 has been shown to change the intracellular amount of peptides, and may cause alterations in animal phenotype. Our group produced a colony of EP24.15 knockout mice (KO) and is interested in identifying the phenotype of these animals. **Aims**: The aim of the present study was characterizing the phenotype of KOs, focusing on depressive behaviors. **Methods**: This protocol used 3-4 month old mice (wild type CL57BL/6 or KO), weighing 24 ± 2 g, provided from an intern colony of our laboratory (group, n=8-14). All animals were genotyped for previously confirmation for EP24.15 gene deletion on KOs. Locomotor and exploratory activities were evaluated by the number of crossed lines or the number of rearings on open-field test (30 x 30 cm, divided in 9 squares) during 5 minutes. In addition, the latency time for first immobility event and the immobility time on forced swimming test were counted during 6 minutes. This protocol was submitted to the USP's Internal Ethical Committee and duly approved (license no. 043/2015). Data were analyzed by t-test ($p<0.05$). Results are expressed as mean ± SEM. **Results**: No significant differences were observed between the two groups with regards to either locomotor (WT = 90.8 ± 10, EP24.15 KO = 88.8 ± 7.5 ; $p=0.873$) or exploratory activity (WT = 34.4 ± 5.1, EP24.15 KO = 38.9 ± 2.5; $p=0.383$) in open-field test. However, in the forced swimming test, EP24.15 KO animals showed a reduced latency for appearance of the first immobility event (WT = 8.1 ± 2.3, EP24.15 KO = 3.5 ± 0.9 seconds; $p<0.05$) and an increased immobility time (WT = 145.9 ± 17.2, EP24.15 KO = 200.4 ± 14.1 seconds; $p<0.05$), indicating a depressive-like behavior in EP24.15 KO mice. **Conclusions**: Future peptidomic studies in EP24.15 KO mice may enable the identification of new peptides and genes differentially expressed and involved in this depressive process, facilitating the comprehension of depression pathophysiology and the search for new antidepressive therapies. **Financial support**: CNPq, FAPESP

03.008 50-kHz USV calls as a marker for mania in a sleep deprivation model. Wendler E[1], Dalla Vecchia D[1], Kanazawa LKS[1], de Souza CP[1], Hocayen PAS[1], Schwarting RKW[2], Andreatini R[1] [1]UFPR – Farmacologia, [2]Philipps-University of Marburg

Bipolar disorder (BD) is a severe, chronic and prevalent psychiatric disorder whose biology and pathophysiology is yet to be better elucidated. It is characterized by bouts of mania and depression with periods of euthymia between them. Rats deprived of paradoxical sleep don't immediately fall asleep, but show a brief period of hyperactivity, aggressive behaviour, stereotypy and hypersexuality that respond to lithium treatment and are similar to those observed in manic episodes of BD patients. Ultrasonic vocalizations (USV) of 50-kHz are emitted by rats in response to rewarding situations, such as mating and rough and tumble play and are a parameter that can be induced by amphetamine (AMP) administration. Hyperactivity and pressured speech are mania-like symptoms observed in humans under the influence of AMP and such parameters can be reproduced and observed in a rat model of mania induced by AMP administration, through analysis of activity and of 50-kHz USV. Since sleep deprivation, as a model of mania, can reproduce markers of affect similar to those of AMP administration, the aim of this study was to evaluate whether 50-kHz USV are a parameter of mania that can be induced by sleep deprivation. For such, rats underwent a period of paradoxical sleep deprivation in which animals are kept isolated on a small platform (7 cm diameter) surrounded by water for 24h. The animal is deprived of entering paradoxical sleep since muscle relaxation leads to falling into water. After this period, the animal is immediately led to a dimly-lit room with an acrylic box (40x40x40 cm) equipped with an ultrasonic microphone fixed centrally at 45 cm above the floor of the box so that 50-kHz calls and locomotion could be analyzed for 20 minutes. Rats that underwent a 24h period of sleep deprivation presented an enhancement in the 50-kHz USV rates when in comparison to non-SD animals. They also showed hyperactivity in the open field test which is another marker of mania. In conclusion, SD is able to reproduce several markers of affect observed in manic states induced by psychostimulants, 50-kHz calls being the main one in this study, and it seems to be a good alternative model with etiological similarities to mania. Animal Research Ethical Committee (CEUA-UFPR) number 854. **Financial support**: CAPES, CNPq

03.009 Initial phenotype characterization of thimet oligopeptidase (EP24.15) knockout mice. Franco RD[1], Castro LM[2], Reckziegel P[1], Camarini R[1], Ferro ES[1] [1]USP – Farmacologia, [2]Unesp – Biologia

Introduction: Our group has shown that proteasome and intracellular peptidases function in concert to release functional peptides within cells. These peptides were coined "intracellular peptides", and suggested to modulate signal transduction of either G-protein coupled or tyrosine kinase receptors affecting protein-protein interactions. In order to further investigate the role of peptidases and intracellular peptides we have now generated knockout mice (KO) for thimet oligopeptidase (EP24.15). **Aims**: The present study aimed to characterize possible differences in the phenotype of EP24.15 KO compared to wild type CL57BL/6 (WT) mice. These initial studies focused on semi-quantitative analysis of intracellular peptide levels in hippocampus. **Methods**: The study was conducted using male 3-4 month old wild type CL57BL/6 (WT) or EP24.15 KO mice, weighing 24 ± 2 g and raised in our Department's internal colony (group, n=8-14). All EP24.15 KO mice were genotyped beforehand. For the peptidomic studies, animals were euthanized and peptide extracts were prepared from either WT or KO hippocampus. Peptides (2 µg) were labeled by demethylation of amine groups with formaldehyde (light form) or deuterated formaldehyde (heavy form) in the presence of cyanoborohydride, to allow semi-quantitative determination of peptide levels between WT and KO mice by mass spectrometry. This protocol was submitted to the USP's Internal Ethical Committee and duly approved (license no. 043/2015). **Results**: Peptidomic analyses identified 281 intracellular peptides in the hippocampus of both WT and KO mice, and semi-quantitative analyses are under investigation. Among the proteins identified that give raise to intracellular peptides in hippocampus are synapsin-1, peptidyl-prolyl cis-trans isomerase and neurogranin, which are known to play a role in long-term potentiation, spatial learning and hippocampal plasticity. **Conclusions**: The phenotype characterization of EP24.15 KO mice should be helpful to define the functional significance of this oligopeptidase in peptide metabolism and animal behavior. Here, peptidomic studies identified several intracellular peptides in the hippocampus of both WT and KO mice. These exciting new findings suggest a novel level of complexity in neuronal signaling that could contribute to animal behavior, because intracellular peptides are shown to modulate signal transduction and protein-protein interaction within cells. Behavioral experiments are currently underway to compare the performance of wild-type and EP24.15 KO mice in object recognition and passive avoidance tests. Further semi-quantitative analysis, alongside the results of experiments evaluating the effect of EP24.15 KO on memory and learning behavior, should allow the identification of the intracellular peptides involved in these processes. **Financial support**: CNPq, FAPESP.

03.010 Cocaine oral self-administration and GABA-A receptor subunits in a rat model of ADHD. Umpierrez L[1], Gonçales T[1], Kimura K[1], Costa P[1], de Souza MF, Barros HMT[4] [1]DFC-UFCSPA, [2]UFCSPA – Farmacociências

Introduction: Cocaine abuse is more evident in Attention Deficit Hyperactivity Disorder (ADHD) patients. Both disorders are related to altered functions in the dopaminergic and may also be due to brain GABA pathways dysfunctions. Females and males show behavioral differences in both disorders. Our objective was to evaluate cocaine self-administration in ADHD male and female rats. **Methods**: Male and female Wistar rats were randomized to 10µL intrathecal 6-OHDA (100 µg free base) or SHAM solution (10 µL-0.1% ascorbic acid) when 4 days old. When 45 days old rats were trained for operant oral self-administration and thereafter were evaluated for oral cocaine self-administration during 27 days (0, 0.2, 0.3, 0.4 mg/ml cocaine). Cocaine solutions were prepared with Cocaine Hydrochloride (Merck, Germany). The prefrontal cortex was used in Real time Quantitative PCR to quantify mRNA expression of $GABA_A$ subunits (α1,α2,γ2). This protocol was approved by the Ethics Committee for animal Use of UFCSPA (13-122/2013). **Results**: The 6-OHDA-lesioned animals presented less active lever press than the non-lesioned animals considering cumulative responses. As expected, M/6-SHAM decreased lever pressing with the increased concentration of cocaine offered; this classical paradigm was not observed in F/SHAM, M/6-OHDA or F/6-OHDA. The daily dose of cocaine was significantly higher for F/SHAM, followed by M/SHAM and by 6-OHDA lesioned animals. Overall, there was an increase in the dose of cocaine self-administered with time. There was a higher increase in daily cocaine dose self-administered by F/SHAM, in comparison to M/SHAM. The 6-OHDA-lesioned animals presented the lower cocaine self-administration. Female rats, irrespective of previous dopaminergic lesion, showed higher mRNA expression of γ2-$GABA_A$ than males. M/OHDA presented lower mRNA expression of γ2 and α1-$GABA_A$ and higher α2-$GABA_A$ in comparison to the other groups. **Conclusion**: 6-OHDA-lesioned animals show less cocaine self- administration and it is possible that the increased drug consumption seen in ADHD patients does not relate to neurobiological changes that increase susceptibility to co-morbid drug abuse behaviors.

03.011 Exposure to running wheels prevents the development of conditioned place preference induced by ethanol in mice: **The role of transcriptional factor CREB in specific brain tissues.** Contó MB[1], D' Almeida V[2], Camarini R[1] – [1]ICB-USP – Departamento de Farmacologia, [2]Unifesp – Psicobiologia

Introduction: The conditioned place preference is an animal model used to study the rewarding properties of psychotropic drugs, as well as the potential of pharmacological or environmental stimulation in diminishing their reinforcing effects. An environmental stimulation that possibly presents such potential is the running-wheel model, known for having intrinsic reinforcing properties to rodents. Alterations in the expression of transcriptional factors like CREB seem to play an important role in rewarding effects of drugs and are possibly related to the conditioning expression in the model of conditioned place preference. **Aims**: 1) Verify if exposition of mice to running wheels along the process of pavlovian conditioning to ethanol in the model of conditioned place preference induces an alteration in its reinforcing properties; 2) Evaluate possible differences in the protein expression of the transcriptional factor CREB in brain areas related to the rewarding effects of drugs of abuse. **Methods**: Naïve adult male Swiss mice, 3 months old, were housed in polycarbonate boxes (42 cm length x 28 cm width x 21,5 cm high), 4 mice/cage. The protocol of conditioned place preference by ethanol (1.8 g/kg, i.p.) was divided in habituation (H1), conditioning (D2-D9) and test (D10). After the habituation, the mice were assigned in cages (4 running wheels/cage), containing free running wheels (groups exercise) or locked running wheels (groups controls), in which they remained throughout the 10 days of the experiment, until the day of the test. After the test, the animals were sacrificed and prefrontal cortex and nucleus accumbens were dissected for realization of molecular experiments. This work was approved by our institution's Ethics Committee on Animal Research (Protocol 172/2013). **Results**: For the comparison of the behavioral parameter analyzed (Score) in the test, the two-way ANOVA for independent samples indicated significant differences in the interaction of factors (p=0,049). The post-hoc test of Newman-Keuls indicated significant differences between the Ethanol-Control group (381,6 ± 52,5; medium ±SEM) compared to the Saline-Control (77,6 ± 104,2; p=0,008), Saline-Exercise (74,5 ± 79,7; p=0,02) and Ethanol-Exercise (67,1 ± 70,4; p=0,031). Concerning the CREB expression, the two-ANOVA for independent samples indicated no interaction in prefrontal cortex, but in nucleus accumbens indicated significant differences for interaction of factors (p=0,03), and the post-hoc of Newman-Keuls indicated significant differences between Ethanol-Control group (140,4 ± 9,91; medium ±SEM) compared to Saline-Control (99,6 ± 7,4; p=0,03), Saline-Exercise (109,6 ± 8,2; p=0,03) and Ethanol-Exercise (105,2 ± 11,6; p=0,04). **Conclusion**: Our results demonstrated that the voluntary physical exercise of running wheels abolished the conditioned place preference induced by ethanol, possibly by desensitizing the brain circuits associated to the rewarding effect of ethanol. A higher expression of CREB in the nucleus accumbens in the Ethanol-Control group, as well as its normalized level in the ethanol-exercise group suggest the importance of this transcriptional factor in the rewarding effect of ethanol in this brain structure. **Financial support**: FAPESP