02. Neuropharmacology

02.001 Altered [^{3}H]-GABA release stimulated by Nicotinic Acetylcholine Receptor (nAChR) activation in cerebellar synaptosomes of dystrophic (mdx) mice. Silva JDP[1], Frangiotti MIB[1], Nogueira FM[1], Stilhano RS[2], Sinigaglia-Coimbra R[3], Ko GM[4], Han SW[2], Souccar C[1] [1]Unifesp-EPM – Pharmacology, [2]Unifesp-EPM – Biophysics, [3]Unifesp-EPM – Centro de Microscopia Eletrônica, [4]Unifesp-EPM – Laboratory of Animal Experimentation

Introduction: Dystrophin is a cytoskeletal protein expressed in striated muscles and at post-synaptic densities of neuronal synapses. A lack of dystrophin expression leads to a severe and irreversible muscle wasting known as Duchenne muscle dystrophy (DMD). Cognitive deficits have been reported in 30% of patients with DMD, but the role of dystrophin in the central nervous system (CNS) is still unclear. We have previously reported significant changes in the concentrations of α7- and β2-containing nAChRs subtypes, and in the nAChR-evoked [^{3}H]-ACh release in hippocampal preparations of mdx mice (Parames et al., Neuroscience 269:173, 2014). Reduction in the number and size of γ-aminobutyric acid receptor ($GABA_AR$) clusters have been also described in the hippocampus and cerebellum of mdx mice (Hendriksen et al*., Neurosci Biobehav Rev*. 51:255, 2015). These observations suggest a role of dystrophin in synaptic function. **Aims**: To evaluate the influence of dystrophin on nAChR–evoked [^{3}H]-GABA release in synaptosomes from brain regions that normally present a high concentration of dystrophin, of control and mdx mice. **Methods**: Crude synaptosomes were extracted from the cortex (CTX), hippocampus (HPC) and cerebellum (CBL) of male littermate control and mdx mice (4-months old). Synaptosomes were preloaded with [^{3}H]-GABA (40 nM) in the presence of aminooxyacetic acid (AOAA, 200 μM), and samples were superfused with Krebs buffer containing 0.1 μM atropine and AOAA, at 37°C. The release of [^{3}H]-GABA was stimulated by superfusion of nicotine (Nic) or K^+ depolarization. The amount of tritium released was expressed as percentage of the total synaptosomal radioactivity (fractional release, Fn). **Results**: Transmission electron micrographs did not reveal significant morphological or structural differences between control and mdx synaptosomes. Superfusion with Nic (1-10 μM) or KCl (9-15 mM) produced a concentration- and Ca^{2+}-dependent [^{3}H]-GABA release in control and mdx preparations. In CTX and HPC synaptosomes, the release of [^{3}H]-GABA induced by 10 μM Nic did not differ between control and mdx mice. In CBL samples, the nAChR-evoked [^{3}H]-GABA release was decreased by 47% in mdx compared to control values (Fn = 0.291 ± 0.062%, n=14). [^{3}H]-GABA release evoked by 9 mM K^+ in CTX, HPC and CBL synaptosomes did not differ between control and mdx groups. In both control and mdx synaptosomes, nicotine-induced [^{3}H]-GABA release was blocked by methyllycaconitine (10 nM) and dihydro-β-erythroidine (1-10μM), antagonists of α7- and β2-containing nAChR subunits, respectively. **Conclusions**: The reduced [^{3}H]-GABA release evoked by nAChR activation observed in mdx cerebellar synaptosomes is compatible with the increased GABA content determined in the same brain region (see poster by Frangiotti MIB et al.), and may reflect a compensatory mechanism for the reported decrease in $GABA_AR$ clustering. The results favor a possible role of dystrophin in GABAergic synapses, and may contribute to the incidence of cognitive deficits described in mdx mice and DMD patients. **Financial Support**: FAPESP, CAPES and CNPq. Animal Investigation Ethics Committee Protocol N° 1178/10.

02.002 Quantitative changes of amino acid transmitters in the brain of dystrophin-deficient (mdx) mice. Frangiotti MIB[1], Silva JDP[1], Castro Neto EF[2], Sousa PVV[2], Naffah-Mazzacoratti MG[3], Souccar C[1] [1]Unifesp-EPM – Pharmacology, [2]Unifesp-EPM Neurology and Neurosurgery, [3]Unifesp-EPM – Biochemistry

Introduction: Dystrophin is a cytoskeletal protein necessary for stabilization of the sarcolema during muscle contraction. Lack of dystrophin caused by mutations of the related gene results in a progressive and irreversible muscle degeneration known as Duchenne muscular dystrophy (DMD). Dystrophin is also found at post-synaptic densities of neuronal synapses, but its role in the central nervous system (CNS) is still unknown. Dystrophin absence in the mdx mouse reduced the size and number of clusters of $GABA_A$ receptors in the cerebellum and hippocampus.The frequency of miniature inhibitory postsynaptic currents (mIPSCs) was also decreased in cerebellar Purkinje cells, and increased in hippocampal CA1 pyramidal cells of mdx mice (Pilgram et al., Mol. Neurobiol. 41:1,2010). These observations suggest a role of dystrophin in synaptic function in the CNS. **Aims**: To evaluate the influence of dystrophin on the concentrations of excitatory and inhibitory amino acid transmitters in brain regions that express high density of dystrophin from control and mdx mice. **Method:** The cerebral cortex (CTX), hippocampus (HPC) and cerebellum (CBL) were isolated from 4- and 12-months old male control and mdx mice. Homogenates of each brain region were used to determine the concentrations of aspartate (Asp), glutamate (Glu), γ-aminobutyric acid (GABA) and glycine (Gly), using high performance liquid chromatography with fluorometric detection (Cavalheiro et al., Epilepsia 130:1043, 1994). The results from control and mdx groups were compared using the Student´s “t” test, and they were considered different at $p<0.05$. Results: In the 4-months old groups, the concentrations of Gly and GABA in CTX were decreased in mdx mice by 20% and 14% of control values (1.18 ± 0.04 and 1.32 ± 0.06 nmol/mg, respectively; means ± sem, n=8). No changes were detected in the amino acid content of HPC between control and mdx mice. However, the concentrations of Asp, Glu and GABA in CBL were increased by 35% to 45% in mdx mice compared to control values. In the 12-months old groups, the concentration of GABA in the mdx CTX was increased by 14% compared to age-matched controls (1.25 ± 0.04 nmol/mg, respectively). In contrast, the concentrations of Gly and GABA were reduced in the HPC by 30% and 18%, respectively, while that of Glu was decreased by 8% in CBL of mdx mice compared to control values. **Conclusions**: The results show that the amino acid transmitter contents in the cerebellum were the most affected by dystrophin deficiency in 4-months old mdx mice. Our data are consistent with the reported decrease in the frequency of spontaneous mIPSCs, and the reduction in nicotinic receptor-evoked [^{3}H]-GABA release in CBL synaptosomes from mdx mice (see poster by Silva JDP et al.). These observations may be related to disorganization of the neuronal cell membranes caused by dystrophin deficiency, resulting in presynaptic alterations. Such alterations may affect the synaptic function and contribute to the cognitive impairments and behavioral disorders described in mdx mice and patients with DMD. **Financial Support**: CAPES, CNPq, FAPESP, MCT-INNT Animal Investigation Ethics Committee - CEUA Nº 9049101316.

02.003 Montelukast Enhances the anticonvulsant effect of phenobarbital on PTZ-induced seizure in mice: an isobolographic analysis. Jesse AC, Fleck J, Marafiga JR, Temp FR, Mello CF UFSM – Fisiologia e Farmacologia

Introduction: Accumulating evidence suggests a role for inflammatory mediators in seizures. Arachidonic acid metabolites, such as leukotrienes, increase in the brain during kainate-induced seizures. Montelukast, a CysLT1 receptor inverse agonist and 1,2,3,4-tetrahydroisoquinoline regarded as a LTD4 synthetic pathway inhibitor, dose-dependently suppress the development of kindled seizures, as well as pilocarpine-induced spontaneous recurrent seizures. Interestingly, it has been recently shown that while LTD_4 facilitates, montelukast (a $CysLT_1$ inverse agonist), pranlukast (a CysLT1 antagonist) and Bay-u9773 (a dual $CysLT_1$/$CysLT_2$ antagonist) decrease PTZ-induced seizures and BBB permeability disruption. AIMS: In the current investigation we determined, by isobolographic analysis, whether the combination of montelukast with a classic anticonvulsant, phenobarbital, results in sub-additive, additive or supra-additive anticonvulsant effects. **Methods**: Adult female Swiss mice were used and obtained from the Animal House of the Federal University of Santa Maria. The estimated ED_{50} for each drug was the basis for the fixed ratio combination of phenobarbital and montelukast used to determine synergism. Isobolographic analysis of interactions between phenobarbital and montelukast was performed according to Tallarida (2000). This line of additivity has Cartesian coordinates that represent all possible combinations of drugs in equieffective doses and represents the theoretical isobole for an additive effect. From these values, the theoretical additive ED_{50} ($ED_{50\ add}$) was calculated and determined whether it differed from the experimentally determined ED_{50} ($ED_{50\ mix}$) of co-administered drugs. **Results** AND **Conclusions** Isobolographic analysis of the combination of phenobarbital with montelukast at the fixed-ratio of 1:1 resulted in a supra-additive (synergistic) effect of these compounds on PTZ-induced tonic–clonic seizures in mice. The experimentally derived $ED_{50\ mix}$ value for a fixed-ratio combination of montelukast plus phenobarbital was 0.06 ± 0.02 µmol, whereas the additively calculated $ED_{50\ add}$ value was 0.49 ± 0.03 µmol. The calculated interaction index was 0.12, indicating a synergistic interaction. The demonstration of a strong synergism between montelukast and phenobarbital is particularly relevant because both drugs are already used in the clinics, foreseeing an immediate translational application for epileptic patients who have drug-resistant seizures. **Financial Support and Acknowledgments**: CAPES, CNPq, E PPGF/UFSM. The protocols followed the official Government Ethics Guidelines and were approved by the university ethics Committee (authorization number: 084/2013). Lenz QF. Neuroscience v. 277, p.859, 2014. Rehni AK. Prostaglandins Leukot Essent Fatty Acids v. 85, v. 97, 2011. Tallarida RJ. Pain v. 98, p. 163, 2002. Vezzani A. Exp Neurol v. 244, p.11, 2013.

02.004 Selective blockade of EP1 and EP3 receptors attenuate pentylenetetrazole-induced seizures in mice. Marafiga JR[1], Reschke CR[1], Jesse AC[1], Masson CJ[1], Lenz QF[1], Mello CF[1] – [1]UFSM – Farmacologia e Fisiologia

Introduction: Epilepsy affects about 1% of the general population and is defined by as an enduring predisposition to generate epileptic seizures and by neurobiologic, cognitive, psychological, and social consequences of this condition. Accumulating experimental and clinical evidence suggests that inflammatory pathways contribute to the development of seizures in various forms of epilepsy. Proinflammatory cytokines, complement factors and prostaglandins contribute to seizures in experimental models, but the role of PGE2 receptors in seizures remain unclear. In the current study we investigated whether E-type prostanoid receptor 1 (EP1) and EP3 play a role in PTZ-induced seizures. **Methods:** The effect of EP ligands on PTZ-induced seizures was assessed 5-7 days after surgery. ONO-8713 (an EP1 antagonist), ONO-DI-004 (an EP1 agonist), ONO-AE3-240 (an EP3 antagonist), and ONO-AE-248 (an EP3 agonist), were generously donated by Ono Pharmaceutical Co. (Osaka, Japan). Mice were habituated for at least 10 minutes, and after this period, EP1 and EP3 agonist/antagonist (10 µg/kg), or their respective vehicle (1% DMSO in saline) were administrated subcutaneously (s.c.). Animals were injected with PTZ (60 mg/kg, i.p.) 30 minutes after antagonist/agonist administration and followed up for 30 min after PTZ administration for the appearance of seizures, by electrographic and behavioral **Methods**. A separated set of animals was investigated if the EP1 and EP3 agonists prevent the anticonvulsant actions of EP1 and EP3 antagonists by injecting a non-effective dose of EP1 (3 µg/kg, s.c.), EP3 (3 µg/kg, s.c.) agonists or their respective vehicles, followed by EP1 (10 µg/kg, s.c.), EP3 (10 µg/kg, s.c.) antagonists or their respective vehicles, 30 and 15 min before PTZ (60 mg/kg, i.p.) injection, respectively. Latency to myoclonic jerks and to tonic-clonic seizures were recorded. EEG signals were amplified, filtered (0.1 to 70.0 Hz, bandpass; 60 Hz Notch), digitalized (sampling rate 256 Hz) and stored in a PC for off-line analysis. **Statistical analysis:** Latencies to myoclonic jerks and to tonic-clonic seizures were analyzed by Kruskall-Wallis, followed by nonparametric Dunn's multiple comparison test, when indicated. Data are presented as median and interquartile ranges. Total time spent in seizures, mean amplitude of EEG recordings were analyzed by one or two-way ANOVA followed by Bonferroni's test, depending on the experimental design. Data are expressed as mean+S.E.M. A probability of $P<0.05$ was considered significant, and H and F values are shown only if $P<0.05$. **Results:** Systemic administration of EP1 and EP3 antagonists attenuated seizures induced by a full convulsant PTZ dose (60 mg/kg, i.p.). Accordingly, the respective EP1 and EP3 agonists not only prevented the anticonvulsant effect of EP1 and EP3 antagonists, but also facilitated seizures appearance in animals injected with a subconvulsant dose of PTZ (30 mg/kg, i.p.). **Conclusions:** The protective effect of the EP1 and EP3 antagonists were prevented by the respective agonists at doses that had no *per se* effect on PTZ-induced seizures, indicating its specificity and further indicating a role for both EP1 and EP3 receptors in this seizure model. In this respect, early evidence that EP1 and EP3 receptors are involved in PTZ-induced seizures came from an experiment that has shown that the protective effect of EP1 and EP3 antagonists against PTZ-induced seizures was prevented by the nonspecific EP agonist PGE_2.

02.005 Pharmacological evaluation of new aldehyde dehydrogenase-2 Inhibitors as candidates for the treatment of cocaine addiction. Silva RR[1], de Oliveira CR[1], Costa PRR[2], Cunha TTS[3], Fraga CAM[3], Noël F[1] [1]ICB-UFRJ, [2]IPPN-UFRJ, [3]UFRJ – Farmacologia e Química Medicinal

Introduction: The absence of an effective treatment for cocaine addiction, the widespread use of this drug and the morbidity related to its use make it necessary to develop a drug therapy for addiction. Among the different approaches studied, inhibition of aldehyde dehydrogenase-2 (ALDH-2) is attractive since a specific inhibitor of this enzyme (CVT-10216) suppressed cocaine seeking in rats (Yao; Nat Med 16:1024, 2010) by blocking the conversion of 3,4-dihydroxyphenylacetaldehyde (DOPAL), a dopamine metabolite, to 3,4-dihydroxyphenyl-acetic acid (DOPAC). The increased level of DOPAL increases the production of tetrahydropapaverolin (THP), a potent inhibitor of phosphorylated (activated) tyrosine hydroxylase (TH), reducing the synthesis of dopamine and thus, blunting the rewarding effects of cocaine. Daidzin, a natural isoflavonoid that guided the synthesis of CVT-10216 (Keung; Proc Natl Acad Sci U S A 90:1247, 1993), was chosen as the prototype of new compounds designed as selective inhibitors of ALDH-2. Aims: To screen different synthetic compounds selected by virtual docking (daidzin binding site at the human ALDH-2) for their capacity to inhibit the enzymatic activity of ALDH-2 in order to guide the synthesis of more potent drug candidates. **Methods**: We use a mitochondrial preparation of rat liver as a source of ALDH-2 and the ALDH-2 assay kit (abcam) to measure the enzyme activity. The enzyme is captured within the microplate wells (coated with antibodies specific for the ALDH-2 isoform), allowing the removal of all other enzymes, including other aldehyde dehydrogenases. The activity of ALDH-2 is determined by monitoring the NADH production in the following reaction: acetaldehyde (25 mM) + NAD (1mM) → acid + NADH. The generation of NADH is coupled to reduction of a dye whose concentration can be monitored at 450 nm over time. The initial velocity is measured in the presence and absence of inhibitors. Results and **Conclusions**: the assay was validated by constructing a concentration-effect curve for daidzin and obtention of a IC50 value (3.8 µM) in good accordance with the literature for these experimental conditions. At the screening concentration of 10 µM, daidzin was able to inhibit 80 % (n=6) of the control ALDH2 activity. Among the 19 compounds evaluated, LBQ308, LQB394, LQB397, LQB308 and CIVI had good activity at the concentration of 10 µM (54, 52, 41, 33 and 32% inhibition of the control, respectively). In the present study, we identified new compounds with significant inhibition of ALDH-2, allowing to start a structure-activity study. The inhibition curves are now being performed for determination of the IC50 values of each substance. **Financial Support**: FAPERJ and CAPES. The protocol was approved by the Ethics Committee of UFRJ (CAAE-0029.0.197.000-05 and DFBC/ICB011).

02.006 Celecoxib decreases proinflammatory cytokines in the hippocampus and cerebral cortex after pentylenetetrazole (PTZ)-induced seizures in mice. Temp FR[1], Marafiga JR[1], Jesse AC[1], Milanesi LH[1], Hessel AT[1], Rambo LM[1], Mello CF[1]
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Introduction: Epilepsy, the most common chronic neurologic disease worldwide, is defined as the sporadic occurrence of spontaneous recurrent seizures (1). Epileptic seizures increase key inflammatory mediators, such as cytokines, which in turn cause secondary damage to the brain and increase the likelihood of recurrent seizures (2). Prostaglandins (PGs) are well-known inflammatory mediators in the brain which biosynthesis increases following seizures (3), initially by cytokine-independent and, after microgial and astrocytic activation, by cytokine-dependent mechanisms (4). Therefore, the sequence cytokine-prostaglandin production that has consolidated in the literature as a logical sequence for the peripheral inflammatory response, may not occur exactly in the same way in the central nervous system. As a consequence, it is possible that COX-2 derived prostaglandins modulate seizure-induced cytokine production and release in the central nervous system. In this study we investigated whether the subchronic administration of the COX-2 inhibitor celecoxib alters cytokine levels in hippocampus and cerebral cortex of mice subjected to PTZ-induced seizures. **Methods:** Adult male Swiss mice received subchronic administration of vehicle (0.1% carboxymethylcellulose plus 5% Tween 80, p.o.) or celecoxib (0.2, 2 or 20 mg/kg, p.o.), daily for 14 successive days. On the 15th day mice were challenged with pentylenetetrazol (PTZ, 50 mg/kg, i.p.). After PTZ administration animals were monitored for 20 minutes for the appearance of myoclonic jerks and generalized tonic-clonic seizures. The number of seizure episodes, total time spent seizing and Racine's scale score were recorded. After behavioral analysis animals were euthanized and temporal cerebral cortex and hippocampi were dissected and homogenized according to manufacturer's protocol for posterior analysis of interleukins (IL-1β, TNF-α, INF-□, IL-6 and IL-10) by ELISA. **Results**: Subchronic administration of celecoxib significantly decreased the latency to PTZ-induced tonic-clonic seizures [$H(3)=8.73$; $p<0.05$]. However, celecoxib did not alter the latency to PTZ-induced myoclonic jerks, number of seizure episodes, total time spent seizing and Racine scale. Furthermore, the increase in IL-1β [$F(1,11)=5.79$; $p<0.05$], TNF-α [$F(1,11)=7.18$; $p<0.05$] and INF-β [$F(1,11)=5.19$; $p<0.05$] levels in cerebral cortex and hippocampi induced by PTZ was reverted by subchronic celecoxib treatment. PTZ administration increased IL-6 and and decreased IL-10 levels in cerebral cortex and subchronic celecoxib treatment prevented such alterations. On the other hand, no changes were observed in IL-6 and IL-10 levels in the hippocampus. **Discussion:** Our results suggest that though celecoxib decreased cerebral levels of pro-convulsant cytokines, it did not decrease seizures. Instead, subchronic celecoxib decreased latency to PTZ-induced tonic-clonic seizures, indicating that persistent inhibition COX-2 may not be a suitable therapeutic strategy for epilepsy. More studies are needed to elucidate the mechanisms involved in the pro-convulsant effects of celecoxib. The protocols followed the official Government Ethics Guidelines and were approved by the University Ethics Committee (N°024/2014). **References:** 1. Fisher et al. Epilepsia. 55:492 (2014); 2. Vezzani and Granada, Epilepsia. 46:1724 (2005); 3. Kaushik et al. Exp Neurol. 257:157 (2014); 4. Choi et al. Trends Pharmacol Sci. 30:174 (2009).

02.007 The role of dorsal medial prefrontal cortex in context-induced alcohol-seeking in rats. Palombo P[1], Bianchi PC[1], Leão RM[1], Oliveira PEC[1], Planeta CS[1], Cruz FC[2] [1]Unesp-Araraquara – Princípios Ativos Naturais e Toxicologia, [2]IFSC-USP

Abstract: Background: In human addicts, relapse is often precipitated by re-exposure to environmental contexts that were previously associated with drug use. Specific patterns of sparsely distributed neurons, called neuronal ensembles, have been hypothesized to encode learned associations between drug-associated contexts and drug effects. **Methods**: Male and Female Long Evans rats were trained to self-administer ethanol 10% (1h/day for 14 days). Drug infusions were paired with a discrete tone-light cue. Subsequently, lever responding was extinguished over 10 days in the presence of the discrete cue in a non-ethanol context with different sensory features than the drug self-administration context. Rats were then re-exposed to the ethanol-associated context (or the non-drug extinction context as the control condition) and lever pressing was assessed under the same extinction conditions for 60 min as a measure of ethanol seeking. Neuronal ensembles in dorsal medial prefrontal cortex that were activated during context-induced reinstatement were identified using Fos immunohistochemistry. We also determined the proportion of dorsal medium prefrontal cortex (dmPFC) neurons expressing Fos during the reinstatement test by double-labeling Fos and the neuron-specific protein marker (NeuN). Results: Reexposure to the ethanol-associated context reinstated alcohol seeking (active lever presses: 16.61 ± 2.59, extinction context and 34.78 ± 6.32, training context; n=14 per group) and increased expression of the neural activity marker Fos in the dorsal medial prefrontal cortex (fos/mm^2 - extinction context: 38.9 ± 6,6 n=6 and training context: 60.6 ± 9.3; n=5). Double-labeling for Fos and NeuN indicated that in the dmPFC, only a small proportion of neurons were activated during context-induced ethanol seeking (5.6 ± 0.86% in the extinction context and 6,4 ± 0.4% in the training context). **Conclusions**: Our results showed context-induced alcohol seeking correlated with activation of dmPFC. **Keywords**: self-administration, dependence, ethanol. **Financial Support**: FAPESP 2103 / 24986-2 and CAPES. Ethics Committee: 2015/01

02.008 Protocols to study modulation of long-term excitatory synaptic plasticity in hippocampal slices. Paiva KV[1], Santana PHDAS[2], Castro NG[2] [1]UFRJ – Farmácia, [2]UFRJ

Long term potentiation (LTP) is required for long duration memory development and storage in the brain. In this process, synaptic transformations happen, intensifying the excitatory transmission by glutamate. Through different high frequency electric stimulus protocols, LTP can be induced in vitro, in hippocampal slices. To investigate a possible LTP facilitation by new pharmacological agents it's necessary to establish a stimulus of controlled intensity, with low probability to induce a consistent LTP. This project's purpose was to develop a protocol with low stimulation intensity, appropriate to evaluate a possible increase of probability of LTP induction/maintenance by facilitator substances. We used transverse hippocampus slices of male Wistar rats (49 weeks), bathed in artificial cerebrospinal fluid and a bipolar electrode to stimulate Schaffer collateral axons. Field excitatory postsynaptic potentials (fEPSP) were registered on dendrites of pyramidal cells of the CA1 region, on stratum radiatum. With single submaximal stimuli at 0.05Hz, we have recorded stable fEPSPs of 0.2-0.5mV in most of the slices and the initial slope was used to evaluate response intensity. For the induction of full LTP, we have tested high frequency stimulation (HFS) and theta burst stimulation (TBS). HFS consisted of 4 bursts of 100 pulses at 100 Hz spaced by 20 s (4HFS) and TBS consisted of 10 bursts of 4 pulses at 100 Hz spaced by 200 ms. To assess the presynaptic function, we measured paired pulse facilitation (PPF), adding a pulse 40 ms after the first pulse. We tested different weak or graded stimulation protocols: 1 or 2 bursts of HFS (1HFS, 2HFS) and bursts of 12, 25 and 50 pulses at 100 Hz, fired on the same slice. After the facilitation protocols on the same slice, we fired the 4HFS protocol, to confirm the viability of the tissue. In the initial experiments of induction of full LTP, the 4HFS protocol was more effective than TBS. The amplitude of the fEPSP 30 min after 4HFS increased significantly by 10 to 120 % ($p < 0.05$) with an average increase of 49 ± 17 % (SEM, n = 6). During LTP, there was a reduction in the ratio of paired pulses by 20% ($p < 0.05$), demonstrating the involvement of presynaptic modulation. LTP of more than 30% was also observed with 1HFS and 2HFS protocols. The protocols of 12, 25 and 50-pulse bursts applied in sequence resulted in gradual increments in the intensity of fEPSP, by 26%, 69% and 112%, respectively (n = 2). Therefore, protocols with bursts of 12 and 25 pulses caused partial LTP and seem suitable for evaluation of substances potentially facilitating transmission in the Schaffer-CA1 synapses. Authors thank CNPq and FAPERJ for the **Financial Support**. All procedures involving animals followed the recommendations of the Guide for the Care and Use of Animals NIH-US, with specific protocols approved by CEUA-CCS (DFBCICB039).

02.009 Proteinase Activated receptor-4 agonist elicits TRP-mediated *in vitro* and *in vivo* responses. Patricio ES[1], Costa R[1,2], Figueiredo CP[1,2], Gers-Barlag K[3], Bicca MA[1], Manjavachi MN[1], Segat GC[1], Gentry C[3], Luiz AP[1], Fernandes ES[4], Cunha TM[5], Bevan S[3], Calixto JB[1] [1]UFSC – Farmacologia, [2]UFRJ – Farmácia, [3]King's College – Wolfson Centre for Age Related Diseases, [4]Ceuma – Biologia Parasitária, [5]FMRP-USP – Farmacologia

Introduction: Chronic itch is a common symptom of dermatological and systemic diseases. This condition lowers patients' quality of life and is still difficult to treat with conventional therapy. A role for proteinase-activated receptor-4 (PAR-4) was recently suggested in itch sensation. AIMS: Here, we sought to investigate the mechanisms underlying the pruriceptive actions of the selective PAR-4 agonist AYPGKF-NH_2 (AYP) in mice. **Methods**: To perform the in vivo studies, we used female CD1 mice (8-10 weeks) and TRPV1$^{-/-}$, TRPA1$^{-/-}$ and WT female C57BL/6J mice (8-12 weeks). Animals received different treatments before the AYP injection (n=6-8 animals per group). Immediately after AYP injection (200 nmol/site, i.d.) the scratching behavior was evaluated for 30 minutes and quantified as the number of scratches made with the mouse hindpaws near the injected site. Skin and DRG of naïve CD1 mice were used to perform immunohistochemistry and DRG neurons of TRPV1$^{-/-}$, TRPA1$^{-/-}$ and WT C57BL/6J mice were used to perform intracellular [Ca^{2+}] measurements (ethics committee: 23080.024591/2010-43/CEUA/UFSC). Results were analyzed by One-way ANOVA + Bonferroni's post-test or Student's *t* test, and values of $p<0.05$ were considered significant. **Results**: Dorsal intradermal (i.d.) administration of AYP elicited intense scratching behavior in mice, which was prevented by the selective PAR-4 antagonist (pepducin P4pal-10). PAR-4 was found to be co-expressed in 32% of tryptase-positive skin mast cells and AYP caused a 2-fold increase in mast cell degranulation. However, neither the treatment with cromolyn nor the deficiency of mast cells (WBB6F1-Kit$^{W/Wv}$ mice) were able to affect AYP-induced itch. PAR-4 was also found on 87% of gastrin releasing peptide (GRP)-positive neurons (pruriceptive fibers), and AYP-induced itch was reduced by the selective GRP receptor antagonist RC-3095. In addition, AYP evoked calcium influx in ~1.5% of cultured DRG neurons. Of all AYP-sensitive neurons, 54.2% were responsive to both capsaicin (TRPV1 agonist) and allyl isothiocyanate (AITC; TRPA1 agonist), while 31% and 11.6% were responsive to only capsaicin or AITC, respectively. Importantly, AYP-induced itch was reduced by treatment with either the selective TRPV1 (SB366791), TRPA1 (HC-030031) or NK1 (FK888) receptor antagonists. However, genetic loss of TRPV1, but not of TRPA1, diminished AYP-induced calcium influx in DRG neurons and the scratching behavior in mice. **Conclusions**: These findings provide evidence that PAR-4 activation by AYP causes pruriceptive itch in mice via a TRPV1/TRPA1 dependent mechanism. **Financial Support**: CNPq, CAPES, FAPESC.

02.010 Evaluation of the protective effect of Simvastatin nanocapsules on seizures induced by quinolinic acid in rats. Guerino CB[1], Alves BC[2], Thumé L[3], Cardoso PA[4], Cardoso MM[4], Boeck CR[1] [1]Unifra – Nanociências, [2]UFRGS – Bioquímica e Farmacologia, [3]Unifra – Acadêmico

Introduction: Statins are cholesterol-lowering agents due to the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Recent studies have shown statins possess pleiotropic effects, such as anti-epileptic effect in rodents. However, low levels of simvastatin cross the blood brain barrier and side effects associated to these drugs are a problem for their use in diseases affecting the central nervous system. Quinolinic acid (QA) is an endogenous analog of glutamate involved in the etiology of epilepsy and related to disturbances on glutamate release and uptake. The nanoparticles have become an important focus of therapeutic research on brain because they are an especially effective form of drug delivery. Considering the therapeutic potentials of nanocapsules, the aim of study is evaluate the protective effect of free form (SF) or nanoencapsulated (SN) simvastatin on QA-induced seizures in rats. Materials and **Methods**: SN suspensions were prepared by interfacial deposition of polymer at 1 mg/mL. Male adult Wistar rats (250-300 g) were pretreated orally during 21 days with control formulation (drug-unloaded nanocapsules, NCF), SF or SN at 1 mg/kg/day. Naïve rats were used as general control. All procedures were approved by ethical local Committed (CEUA/prot. 002/2014). After pretreatment, rats were infused with 4 µL of 239.2 nmol QA at right lateral brain ventricle and observed for behavioral changes. Twenty four hours after seizures, rats were evaluated for balance and memory. Results: NCF showed pH 6.75 ± 0.19, particle diameter of 213.91 ± 19.96 nm, polydispersity index of 0.167 ± 0.05 and zeta-potential values were -16,28 ± 7,90. SN had pH 6.68 ± 0.25, particle diameter of 215.25 ± 30.58 nm, polydispersity index of 0.151 ± 0.07 and zeta potential values were -16.13 ± 8.19 mV. The pretreatment with SN or SF did not change the seizures or number of death following QA infusion. The rats treated with NCF + AQ displayed memory impairment in the inhibitory avoidance task and pretreatment with NS or SL did not prevent this effect. In the group NCF + QA the number of rats with fall in the horizontally elevated beam was higher than Naïve group, effect prevented by SN and SF. **Conclusion**: Altogether, the results exhibit that free form or nanoencapsulated simvastatin at the dose 1 mg/kg/day administered for 21 days prevented balance skill learning outcome following QA in rats, but had no effects on seizures and memory deficits. Probably lower efficiency was achieved by delivering simvastatin within nanocapsules. Biodistribution and pharmacokinetic studies should be performed to define the action of simvastatin-loaded lipid-core nanocapsule. **Acknowledgements**: Research supported by FAPERGS and CAPES.

02.011 Effects caused by the CB1 inverse agonist rimonabant in a pharmacologic animal model of schizophrenia. Nazareth NJ, Marques AM, Neves GA ICB-UFRJ – Farmacologia Molecular

Introduction: Schizophrenia is a highly disabling disease which makes patients unable to distinguish what is real from his delusions. This kind of disturb is characterized by clinical symptoms that are divided into three subgroups: positives symptoms, negative symptoms and cognitive impairments. The first subgroup includes delusions, hallucinations, disorganized thoughts and restlessness. The second one refers to social isolation, lack of motivational stimulus and anhedonia, and the last one comprises loss of memory and impaired attention. Several studies try to explain the neurochemical basis of schizophrenia through an imbalance in the glutamatergic and dopaminergic systems. However, the endocannabinoid system seems to be involved in this disease, since endogenous ligands levels are increased in schizophrenia patients and CB1 receptor density in patients' brain is altered when compared to control individuals. Since this system can regulate several neural networks, directly or indirectly, it is important to investigate the effects of compounds acting at CB1 receptors to understand their role in schizophrenia symptoms development and as a new therapeutic approach. Aims: This work intends to investigate the effects of the CB1 inverse agonist rimonabant in a pharmacological animal model of schizophrenia. **Methods**: Adult male Swiss mice were obtained from Centro de Criação de Animais de Laboratório (CECAL/Fiocruz) breeding colony. Groups of mice received intraperitoneal injections of rimonabant in the following doses: 0.3; 1.0 e 3.0 mg/kg. Fifteen minutes after, they were treated with MK-801 (0.3 mg/kg i.p.). Control groups received saline (NaCl 0.9%). Thirty minutes after the second injection, the Y-maze task was conducted to assess animals working memory, measured as the percentage of spontaneous alternations (consecutive entries in the three arms of the apparatus). As an experimental control, the total numbers of arm entries in the maze was recorded. Data analysis was performed using ANOVA with Tukey post-hoc test. Results: Rimonabant treatment didn't change the total number of arm entries in the Y-maze. MK-801 induced a decrease in total arms entries and the pretreatment with rimonabant did not alter this motor effect. When the percentage of spontaneous alternations was evaluated, it was possible to see that rimonabant at 3.0 mg/kg induced an impairment in animals working memory since it reduced the percentage of alternations (58.2 ± 2.2 % *versus* 71.8 ± 2.4 % from the control group, $P < 0.01$). As expected, animals treated with MK-801 showed a significant reduction in this parameter (42.6 ± 4.4%, $P < 0.001$ *versus* control group) which was partly blocked by rimonbant 1.0 mg/kg (55.6 ± 3.4 %, $P < 0.05$ *versus* MK treated group). Conclusion: According to the results, rimonabant seems to present a dual effect on mice working memory. Future experiments are being conducted in order to understand a larger picture of the effects caused by the compound under study. **Financial Support**: FAPERJ, PIBIC/UFRJ. Approved by ethical committee on animal use (CEUA/ UFRJ), number DFBCICB045.

02.012 Effect of ketamine on the improvement of depressive-like behavior and memory loss in animal model of Parkinson's disease induced by 6-OHDA.

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Parkinson Disease (PD) is characterized by progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) and the most common signals are motor impairments, but most patients also present depression and cognitive impairment. Ketamine, a NMDA antagonist, has showed efficacy in depressive patients and in animal models of depression. Thus, the present study evaluated the action of ketamine on depressive-like behaviour and memory impairment in an animal model of PD (nigral lesion by 6-OHDA). Male Wistar rats received bilateral intranigral 6-OHDA infusion. Depressive-like behaviours were evaluated by sucrose preference test (SPT) and modified forced swimming test (mFST). Social memory was evaluated by social recognition test, with 30 min interval between trials. SPT was performed weekly for three weeks to confirm a stable depressive-like behaviour (before drug treatment) and then weekly during 3 week of drug treatment. SRT and mFST was evaluated after 2 weeks of treatment (42 and 43 days after the surgery, respectively). Open-field (7, 28 and 42 days after surgery) was used to measure locomotor activity. Drug treatments were: ketamine (5, 10 and 15mg/kg, ip, once a week) and imipramine (20mg/kg, ip, daily). The rats were divided into 6-OHDA plus vehicle, imipramine or ketamine (3 doses) and SHAM plus the same treatments, totaling 10 groups. This protocol was approved by institutional ethical board (CEUA #786). After surgery and before drug treatment rats treated with 6-OHDA showed depressive-like behaviour (anhedonia, represented by decrease in sucrose preference), without any change in locomotor activity, replicating previous findings. Drug treatment increased sucrose preference when compared to the 6-OHDA+vehicle, indicating an antidepressant-like effect. The same effect was seen in the mFST: 6-OHDA increased immobility compared to SHAM rats and ketamine and imipramine reversed this depressive-like behaviour. This anti-immobility effect of ketamine at lower doses was associated with increase in swimming behaviour (suggesting a serotonergic effect) while the higher dose of ketamine increased swimming and climbing, indicating noradrenergic/dopaminergic and serotonergic actions. Moreover, 6-OHDA also decreased social recognition and all drug treatments reversed this impairment. In conclusion, ketamine appears a suitable treatment for affective and cognitive impairments in patients with Parkinson 's disease.

02.013 Characterization of a model of neuronal PTEN haploinsufficiency: Memory- and metabolism-associated effects. Cabral-Costa JV[1], Andreotti DZ[1], Mattson MP[2], Camandola S[2], Scavone C[1], Kawamoto EM[1] [1]USP – Farmacologia, [2]NIA-NIH

Introduction: First described as a tumor suppressor, phosphatase and tensin homolog deleted on chromosome 10 (PTEN) has been more recently associated with other important processes in the central nervous system, such as neurogenesis and synaptic plasticity, due to its key modulatory role on cellular proliferation, migration and survival. Such influence over these cellular processes culminates in an embryological lethality on a PTEN complete knockout context. Therefore, conditional knockouts (*e.g.*, through the Cre-lox system) are interesting approaches that overcome the lethality limitation, in addition to allow to study site- and time-specific functions of PTEN. AIMS: This study aimed to establish and validate the model of neuronal Pten conditional deletion driven by the promoter of the neuron specific enolase (NSE) gene (NSE-PTEN$^{+/-}$) and to characterize its effects on memory and metabolism. **Methods**: Male mice of 3-4 months from the NSE-PTEN$^{+/-}$ lineage were assessed for metabolic parameters (food consumption, body and brain weight, glycaemia and glucose tolerance) as well as for behavioral changes (memory and anxiety). Student t-test was used for statistical analysis, with significance considered for $p \leq 0.05$. **Results**: NSE-PTEN$^{+/-}$ mice did not differ in glycaemia, glucose tolerance or in body weight. However, mutant mice showed an increased ratio of food intake per body weight. No significance was found in anxiety-associated parameters in the open field test. In addition, NSE-PTEN$^{+/-}$ mice performed similarly in the learning curve of the Morris water maze in comparison with their wild-type littermates. However, NSE-PTEN$^{+/-}$ mice spent significantly less time on the target quadrant on the first probe test (4h) and showed decreased latency in the inhibitory avoidance test. Moreover, we confirmed the expected total and cortical macrocephaly on this model. **Conclusions**: Through this study we were able to validate the NSE-PTEN$^{+/-}$ mouse model in our laboratory, observing some effects which corroborate data already published by other groups using this or similar models. Furthermore, the effect of this deletion over the ratio of food intake per body weight constitutes a potential indicator of a metabolic consequence of neuronal PTEN haploinsufficiency. However, as the studied metabolic indicators (glycaemia and glucose tolerance) were similar in both mutant and wild-type mice, it is still necessary to further assess this topic through other approaches in order to confirm our findings. **Financial Support**: This study was supported by CAPES and FAPESP. This study was approved by the institute's Animal Research Ethical Committee and followed the required guidelines for animal manipulation (Protocol number CEUA/ICB-USP 167/11).

02.014 Morphine impairs the persistence of memory via a cAMP/PKA-dependent pathway. Milanesi LH, Porto GP, Signor C, Funck VR, Rubin MA, Mello CF UFSM – Fisiologia e Farmacologia

Introduction: Long-term memories are those that last several hours, days, weeks, or even longer periods. The formation of these memories requires an early consolidation process which extends up to 6 h posttraining(1; 2). A significant body of evidence has indicated that during this period, memories can be negatively modulated by opioids (3; 4).Previous studies have shown that morphine, when injected 12 hours post-training, impairs the persistence of fear conditioning memory (5). It has been suggested that ERK and cAMP/PKA pathways are activated in the hippocampus around 12 hours post-training (6, 7). Accordingly, blocking ERK activation in the hippocampus by injecting the MEK inhibitor U0126 impairs memory persistence and blocks the positive modulatory effects of BDNF (7). Furthermore, Rossato and colleagues (6) have shown that while the intra-CA1 infusion of PKA inhibitor PKI hampers memory persistence, the infusion of the PKA activator 8-Br-cAMP enhances it, evidencing a role for PKA in the maintenance of long term memory. Morphine is an opioid that impairs the persistence of memory. Although PKA signaling has been implicated in memory persistence, no study has investigated whether morphine alters persistence of long-term memory by interfering withcAMP/PKA signaling. **Aims:** Therefore, in the present study we investigated whether drugs that alter cAMP/PKA signaling modify the deleterious effect of morphine in the persistence of memory. **Methods:** Adult rats were training in contextual fear conditioning task and 11.5 hours post-training were injected with the adenylyl cyclase activator forskolin (0.13 mg/mL) or with the PKAactivator 8-Br-cAMP (7.5 mg/mL), via intrahippocampal, followed by morphine (10 mg/kg, i.p.) 30 min later. The testing session was performed 7 days after training and during the test the number of observations scored as freezing was recorded. **Results:** The intrahippocampal administration of forskolin (0.13 mg/mL), or 8-Br-cAMP (7.5 mg/mL), did not alter freezing to context in animals tested seven days after training but prevented morphine-induced decrease of contextual freezing ([$F(1,47)=6.6$, $p=0.01$] and [$F(1,20)=7.9$, $p=0.01$], respectively). **Conclusions:** This study shows that the intrahippocampal administration of forskolin and 8-Br-cAMP prevent the impairing effect of morphine on memory persistence, suggesting the involvement of the cAMP/PKA pathway in the memory persistence deficit induced by morphine.

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Financial Support: CNPq, CAPES. All experimental protocols were approved by the institutional animal care and use ethics committee (015/2013).

02.015 Chronic ouabain counteracted the effects of chronic unpredictable stress in the HPA axis and CREB signaling. Leite JA, Orellana AMM, Kinoshita PF, de Sá Lima L, Andreotti DZ, Kawamoto EM, Munhoz CD, Scavone C ICB-USP – Farmacologia

Ouabain (OUA), a potent inhibitor of the Na,K-ATPase pump, was identified as an endogenous hormone produced in hypothalamus and adrenal gland. This cardiac glycoside binds to Na,K-ATPase and it can activate signaling pathways in concentrations that is not linked to the common effect of the pump inhibition. It has been demonstrated the involvement of the OUA in the acute stress response, where physical exercise is capable of increasing OUA levels in rats, dogs and humans minutes after the onset of physical activity. The central effectors of the stress response are the corticotrophin releasing hormone (CRH), which stimulates the secretion of adrenocorticotropic hormone (ACTH), and this one acts on the adrenal cortex release glucocorticoid (GC) hormones. GCs in turn act back on the hypothalamus and pituitary in a negative feedback cycle to suppress CRH and ACTH production. Chronic unpredictable stress (CUS) activation has been shown to affect health, making the individual more susceptible to infections, tumors, hypertension, heart attack, stroke, autoimmunity and psychopathology. Furthermore, animal models of CUS memory impairment associated with atrophy of neuron in hippocampus and frontal cortex showed down-regulation BDNF expression. The present work investigated the effects of OUA on CUS induced changes in HPA axis and cyclic AMP response element-binding protein (CREB) expression in the hippocampus, frontal cortex and hypothalamus. Adult male rats were subjected to CUS protocol for 14 days and pre-treated intraperitoneally (i.p.) with Ouabain (1.8 mg/kg) (every other day). Serum CORT (corticosterone, the principal form of GCs in rodents) and ACTH were measured using ELISA kit. Electrophoretic mobility shift assay (EMSA) was used to evaluate CREB activity in brain tissues. Our results showed that CUS induced an increase in serum CORT and ACTH levels and chronic treatment with OUA (1.8mg/kg) counteracted the CUS effect by reducing both CORT (39%) and ACTH (53%). In addition, we found that CUS reduced CREB activity and OUA reverted it only in the pre-frontal cortex. Taken together our results indicate that OUA modulates HPA axis and CREB activities in the pre-frontal cortex. Further studies are necessary for elucidation a putative physiological role of this hormone in chronic stress response. **Financial Support**: FAPESP, CNPq. All procedures were approved by the Biomedical College of Animal Experimentation and by the Ethical Committee for Animal Research of the Biomedical Sciences Institute of the University of São Paulo (n.52; fls 19; book 03).

02.016 Effect of acute and subcronic nimesulide treatment on pentylenetetrazol (PTZ)-induced seizures in mice. Köche EM[1], Temp FR[1], Marafiga JR[1], Jesse AC[1], Hessel AT[1], Milanesi LH[1], Rambo LM[1], Mello CF[2] – [1]UFSM – Farmacologia e Fisiologia, [2]UFSM – Fisiologia e Farmacologia

Introduction: Cyclooxygenase-2 (COX-2) inhibitors reduce prostaglandins (PGs) synthesis and play a significant role in inflammation and fever (1). In the past decades research has been focused on the use of COX-2 inhibitors and inflammatory process in central nervous system (2,3,4). However, there is a discrepancy regarding the effect of acute or chronic COX-2 inhibitors treatment in seizures. Some studies have shown that both acute or chronic COX-2 inhibition decreases seizure, while others have reported that it may facilitate convulsive episodes (3,4,5,6). Thus, considering the current divergence regarding the pro- and anticonvulsant effect of COX-2 inhibitors, and the use of different drugs and treatment regimens (3,4), the aim of the current study was to investigate whether acute and chronic of nimesulide treatment alter seizures in mice. **Methods:** Adult male Swiss mice were used. Vehicle (0.1% carboxymethylcellulose (CMC) plus 5% Tween 80, p.o.) or nimesulide (0.2, 2 or 20 mg/kg, p.o.) were administered 60 minutes before administration of convulsant agent PTZ (50 mg/kg, i.p.) in acute experiments. Mice received subchronic administration of vehicle (0.1% CMC plus 5% Tween 80, p.o.) or nimesulide (0.2, 2 or 20 mg/kg, p.o.), daily on successive days for 14 days. On the 15th day mice were challenged with PTZ (50 mg/kg, i.p.) injection. After PTZ administration animals were behaviorally monitored by 20 minutes for the latency to myoclonic and generalized tonic-clonic seizures, number of seizure episodes, total time spent seizing and Racine's scale score. **Results**: Acute administration of nimesulide significantly and dose-dependently decreased PTZ-induced myoclonic jerks [$H(3)=11.63$; $p<0.05$], generalized tonic-clonic seizures [$H(3)=9.44$; $p<0.05$] and number of seizure episodes [$F(3,28)=4.2$; $p<0.05$]. The subchronic administration of nimesulide significantly increased the latency to PTZ-induced generalized tonic-clonic seizures [$H(3)=8.73$; $p<0.05$]. **Discussion:** In the present study the pretreatment with nimesulide significantly attenuated PTZ-induced seizure in three different protocols. The results strongly suggest the possible role of COX-2 enzymes in the pathophysiology of epilepsy and the use of COX-inhibitors should be further investigated as a potential therapeutic approach in neurodegenerative diseases with an inflammatory component. **The protocols were approved by the University Ethics Committee (N°024/2014).** 1.Auriel et al. Handb Clin Neurol 119:577 (2014); 2.Hewett et al. J Pharmacol Exp Ther 319:1219 (2006); 3.Oliveira et al. Epilepsy Res. 79:14 (2008); 4.Toscano et al. Brain Res Bull 75:598 (2008); 5.Chung et al. Exp Neurol 249:95 (2013); 6.Salvadori et al. Epilepsia 53:189 (2012)

02.017 Anxiogenic-like effect of a single subconvulsant dose of pilocarpine in Swiss mice depends on the gender. Barbosa MN[1], Silva NKGT[1], Santos JA, Silva BL, Gavioli EC, Duarte FS, de Lima TCM, Duzzioni M UFAL – Ciências Biológicas e da Saúde

Introduction: A single subconvulsant dose of pilocarpine (PILO, a non-selective muscarinic cholinergic agonist) produces long-lasting anxiogenic-like effects in male Wistar rats, as reported from our group [Duarte, Psychopharmacology (Berl), 227, 209, 2013). However, it is unknown if those effects could be extended to other rodent species, especially in mice. **Aims:** To investigate the effects of systemic administration of a single subconvulsant dose of PILO on anxiety behavior of male and female mice. **Methods:**Adult male and female Swiss mice were pretreated with scopolamine methyl bromide (1mg/kg, sc), and after 30 min with saline (SAL, ip) or PILO (75 mg/kg, ip). After 24 h, the animals were submitted to the elevated plus maze (EPM) and open field tests (OF). Results were expressed as mean±SEM and compared by the Student's *t*-test (GraphPad Prism5®). A level of $P < 0.05$ was defined as significant. Different doses of PILO were evaluated (25, 50, 75, 100 and 150 mg/kg), but only the highest non-convulsant dose of PILO was tested for anxiety and locomotion. Racine's scale was used to discern convulsive or not convulsive behavior. **Results and Conclusions:** Administration of PILO (N=8) significantly reduced the frequency of entries into the open arms (40.26 ± 09.03 *vs* 25.99 ± 3.84, $P = 0.01$), and increased the number of protected stretch-attend postures (04.02 ± 6.62 *vs* 15.88 ± 2.03 $P = 0.00$), when compared to the SAL group (N=8), in male mice evaluated in the EPM. Other behaviors in the EPM (frequency of time spent into open arms, frequency of entries into enclosed arms, unprotected head-dipping and rearing) did not differ between groups. In the OF, no significant differences were observed in the number of crossings and rearing between PILO (N=8) and SAL (N=8) groups ($P > 0.05$), ruling out any nonspecific motor effect. In female mice, administration of PILO did not affect behavioural responses in the EPM and OF tests. In conclusion, these results showed that the administration of a single subconvulsant dose of PILO produces an anxiogenic-like effect only in male mice in the EPM as previous report to rats; thus this effect seems to be gender depended. All data together support the involvement of the cholinergic system in the modulation of defensive behaviors. **Financial Support:** CNPq. Animal Research Ethical Committee (17/2014-UFAL).

02.018 Quercetin did not reverse methylphenidate-induced hyperlocomotion, an animal model of mania. Kanazawa LKS, de Mélo ML, Beirão Júnior PS, Barcaro IMR, Andreatini R UFPR – Farmacologia

Introduction: The manic episode of the Bipolar Disorder is associated with an increased activity of the protein kinase C (PKC), which can, therefore, constitute a potential therapeutic target in the stabilization of this psychiatric disorder. Moreover, mania has been also associated to oxidative stress. Quercetin is a bioflavonoid with known antioxidant properties and inhibitory activity over PKC. AIMS: The objective of this study was to evaluate the effects of acute quercetin administration in mice submitted to an animal model of mania. **Methods**: The methylphenidate-induced hyperlocomotion was used as animal model of mania. Male Swiss mice were treated with lithium (100 mg/kg i.p.), quercetin (2.5; 5; 10 or 40 mg/kg i.p) or vehicle (saline i.p.) 15 minutes before saline or methylphenidate (5 mg/kg s.c.) administration. Locomotor activity was measured for 20 minutes in the locomotor activity cage 20 minutes after methylphenidate administration. The results were analyzed by one-way ANOVA followed by Newman-Keuls post hoc test. **Results**: There was an increase in the locomotor activity in the group treated with vehicle + methylphenidate, when compared to the group vehicle + vehicle ($p < 0,05$). The acute administration of quercetin was not capable of blocking the methylphenidate-induced hyperlocomotion ($p > 0,05$ for all doses). Lithium, however, was effective in blocking the hyperlocomotion ($p < 0,05$). None of the drugs tested in the study decreased the spontaneous locomotor activity of the animals. **Conclusions**: The acute administration of quercetin did not show antimanic-like effect in the methylphenidate-induced hyperlocomotion model, despite the fact that this substance has inhibitory activity over PKC and antioxidant action, which could be related to a control of the manic episode. The positive effect of lithium validated the procedure used. Studies involving the possible antimanic-like effect of chronic administration of quercetin in mice are already on course. **Financial Support**: CAPES, CNPq CEUA/BIO-UFPR; process approval number 733

02.019 AT1 receptors in the prelimbic cortex modulate cardiovascular responses to acute restraint stress in rats. Brasil TFB, Fassini A, Corrêa FMA FMRP-USP – Farmacologia

Introduction: The ventral portion of of the medial prefrontal cortex (vMPFC) is a limbic structure, which is comprised of prelimbic (PL), infralimbic (IL) and dorsal peduncular (DP) cortices. The PL sends projections to structures involved in the control of cardiovascular responses. Electrical or chemical stimulation of the PL causes cardiovascular responses. Restraint stress (RS) is an acute unescapable and aversive situation, which evokes a sustained increase in blood pressure (BP) and heart rate (HR). Microinjection of a nonspecific synapses blocker into the PL increased HR responses to RS, but did not affect the BP response, suggesting that this area has an inhibitory influence on RS-evoked HR changes. However, the vMPFC neurotransmitters involved in this modulation have not been identified. Angiotensinergic peptides and their receptors are present in the PL. Moreover, the central angiotensinergic system is known to modulate cardiovascular responses during aversive situations. Hence, the objective of this study was to investigate the role of angiotensinergic receptors in the PL on the autonomic responses (BP and HR increase, and reduction in tail temperature) evoked by RS. **Method:** Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (nº 074/2012). Male Wistar rats (240-280g) were used. Guide cannulas were implanted bilaterally in the PL for injection of candesartan (AT1- selective antagonist in the doses of 0.1, 0.5 and 1nmol), or vehicle (artificial cerebrospinal fluid, ACSF, 100 nL). A polyethylene catheter was implanted into the femoral artery for BP and HR record, using a computerized acquisition system. Ten minutes after the microinjection of either candesartan or vehicle into the PL, rats were subjected to RS during one hour. **Results:** The bilateral microinjection of candesartan reduced the pressor response ($F_{3,108}$=4,412, p<0,05), but had no effect on the restraint-evoked tachychardia ($F_{3,114}$=0,7910, p>0,05) or the reduction in the tail temperature ($F_{3,114}$=0,3497, p>0,05) caused by RS. **Conclusion:** The present study demonstrates that AT1 receptors in PL modulate RS-evoked cardiovascular responses, suggesting a facilitatory role of this structure on the RS-evoked blood pressure increase. **Bibliographic References:** TAVARES, J Neurosci Res. 87(11):2601-7, 2009. KIRITSY-ROY, J Pharmacol Exp Ther. 239(3):814-22, 1986. **Financial Support:** CAPES and FAEPA.

02.021 Allopregnanolone effects on $GABA_A$ receptor subunits mRNA expression in the prefrontal cortex (PFC) of rats. Almeida FB[1], Agnes G[2], Nin MS[3,1], Barros HMT[1] [1]UFCSPA – Farmacociências, [2]UFCSPA – Biologia Molecular, [3]Centro Universitário Metodista do IPA

Allopregnanolone (ALLO) is a neurosteroid acting as a positive modulator in $GABA_A$ receptors ($GABA_A$-R). ALLO presents antidepressant-like effect in animals. Intra-hippocampus ALLO administration reduces immobile behavior in the FST and increases γ2 GABA(A) subunit mRNA was observed. The increase is higher in the right hemisphere than in the left hemisphere. However, the role of its interaction with specific $GABA_A$-R subunits in specific brain regions to the antidepressant effects needs further elucidation. Herein, we evaluated the effect of bilateral infusions (.75, 1.25, 2.5 microg/hemisphere) of ALLO into the PFC of male rats on the mRNA expression of γ2 and δ $GABA_A$-R subunits in the PFC of both hemispheres using real-time quantitative PCR technique (endogenous control genes: β-actin and GAPDH). This project was approved by the ethics committee for animal use of UFCSPA (number 13/137). There were no differences between hemispheres regarding the δ subunit (P = 0,971). ALLO 2.5 increased δ subunit mRNA expression when compared to controls (P = 0,001) and ALLO .75 (P = 0,004). The right hemisphere had a higher γ2 subunit mRNA expression than the left hemisphere in the controls (P = 0,007), and ALLO .75 (P = 0,011). The γ2 mRNA expression was increased in the left hemisphere by ALLO 2.5 when compared to control (P = 0,013) and to ALLO .75 (P = 0,007); no significant change was induced by ALLO in γ2 mRNA expression in the right hemisphere PFC. These results indicate that allopregnanolone in the high dose used increases the mRNA expression of δ and γ2 in the PFC and that the γ2 change is evident in the left PFC. These neurochemical changes reflect the effects of ALLO towards a hemispheric symmetry of the GABA A subunits that may be important in some affect behavioral effects diverse from the mood behaviors represented in the FST.

02.022 Evaluation of voluntary running effects in metabolism and neurogenesis in female mice during pregnancy and breast-feeding. Andreotti DZ, Cabral-Costa JV, de Sá Lima L, Kawamoto EM, Scavone C ICB-USP – Farmacologia

It has long been found in literature that voluntary running shows benefits in some brain functions, like neurogenesis increase in hippocampus, enhanced synaptic plasticity, and, as consequence, cognitive improvement. Studies show increased hormones and growth factors like Brain-derived neurotrophic factor (BDNF) in neurogenesis process. Besides, there are neurotransmitters evolved, as glutamate, especially in synaptic plasticity and cellular proliferation. After voluntary running sessions, there is an increase in expression of glutamatergic system genes, like NMDA receptor subunits. Thus, the present study aims to evaluate some biochemical effects related with neurogenesis and synaptic plasticity in females, submitted to voluntary running before and during pregnancy and breast-feeding period, just like metabolism related factors. Female C57BL/6J mice of 60 days were assessed for this assay. During 10 days they are conditioned individually in cages with or without a running wheel, depending on their experimental group (sedentary or runner). After this period, male mice were placed with each female for mating. Over all pregnancy and breast-feeding the females were kept in their respective cage. Preliminary results showed that voluntary running increased NR1receptor expression in runners' animals, in comparison with sedentary ones. The NR1 receptor is one of NMDA receptor subunit. Through another assay, runner females also showed an increase on a transcription regulator of BDNF: cAMP response element-binding protein. Synaptophysin, a synaptic vesicle protein, was performed in these females, however there was no difference in its expression among all groups. Measures of differences in body weight did not show difference between sedentary and runner females, as well as food consumption. These measures were done in two distinct periods: first ten days of experiment during a breast-feeding period. In conclusion, these data demonstrate a potential role for voluntary running in neurogenesis, setting up an alternative prevention and treatment for neurodegenerative disease, including during pregnancy and breast-feeding. **Financial Support**: This study was supported by University of São Paulo (USP) and was approved by the institute's Animal Research Ethical Committee and followed the required guidelines for animal manipulation (Protocol number CEUA/ICB-USP 114/14).