Setor 02. Neurofarmacologia/Neuropharmacology

02.001

The pancuronium- and cisatracurium-induced fade depend on activations of facilitatory-M_1, inhibitory-M_2 and inhibitory-A_1 receptors on the motor nerve. Bornia ECS[1], Bando E.[2], Machinski Jr, M[2], Pereira MW[3], Santos IL[1], Alves-do-Prado W[1] [1]UEM - Farmácia e Farmacologia, [2]UEM - Análises Clínicas, [3]UEM - Ciências Biológicas

Introduction: Fade is a poorly sustained contraction that follows a fast muscular contraction of high amplitude when the motor nerve is electrically stimulated with high frequencies (Bowman WC. Anesth Analg.; (59):935, 1980). Although fade induced by d-tubocurarine (d-TC), hexamethonium (HEX) or neostigmine is antagonized by atropine, the distinct contribution of the presynaptic facilitatory-M_1 and inhibitory-M_2 receptors on fades produced by such agents is still to be evaluated. Since some antinicotinic (pancuronium (PANC) and cisatracurium, (CIS)) agents used in clinical practice exhibit anticholinesterase (PANC) and/or antimuscarinic activities (PANC or CIS) that could modify the cholinergic autoregulation of the motor nerve, the effects of blockage of M_1 presynaptic receptors by pirenzepine (PZP) and the effects of blockage of M_2 presynaptic receptors by methoctramine (MET) on the fade induced by d-TC, HEX, PANC and CIS were comparatively investigated in the phrenic nerve diaphragm muscle preparations of rats. As adenosine buildup from ATP catabolism activates presynaptic inhibitory A_1 adenosinergic receptors when the presynaptic M_1 receptors are fully operative, the effects of blockage of A_1 receptors by DPCPX (8-cyclopentyl-1,3-dipropylxanthine) were also investigated when the fade produced by antinicotinic agents involved the activation of presynaptic M_1 receptors. **Methods:** The Ethics Committee for Experimental Studies of the State University of Maringa approved the procedures (043-2007). The phrenic nerve diaphragm muscle preparations were isolated from Wistar rats and assembled according Bülbring (Bülbring E. Br J Pharmacol.; (1): 38,1946). The muscle was indirectly stimulated with tetanizing pulses at intervals of 15 minutes. The tension (B) recorded on end of the tetanizing stimulus (after 10 sec) was taken as a reason (R) of that (A) obtained at the beginning (R=B/A). The frequency capable to produce sustained muscle contraction (R=1.0) was determined (75±3.3Hz). The smallest concentration of each antinicotinic agent capable producing approximately 25% fade (reduction in R value) after 16 minutes of its administration, was researched. R values obtained in the presence of drugs were taken as a percentage of that obtained in the drug-free Krebs buffer. In studies with combinations of drugs, pirenzepine, methoctramine or DPCPX was given 15 minutes before addition of neuromuscular blockers in the bath. Data were compared with ANOVA followed by Bonferroni test ($P < 0.05$). **Results and Discussion:** PZP (10nM) or MET (1mM) alone did not produce the fade. MET attenuated the fade induced by HEX (413mM), d-TC (55nM), PANC (0.32mM), or CIS (0.32mM). PZP attenuated fades produced by only PANC and CIS. DPCPX (2.5nM) alone did not produce fade, but improved the PANC and CIS- induced fade. CIS showed antiacetylcholinesterase (anti-AchE) activity (in plasma= 14.2±1.6%; in erythrocytes= 17.2±2.6%) as PANC. **Conclusion:** Fades produced by antinicotinic agents depend on activation of the presynaptic M_2 receptors, but those induced by agents that exhibit anti-AchE activity seem also to depend on activations of M_1 and A_1 receptors on motor nerve. Research supported by Araucaria Foundation, FADEC-UEM and Cesumar.

02.002

LASSBio-767: a semi-synthetic Alzheimer's disease drug prototype combining anticholinesterase and muscarinic M3 inhibitory activities. Pimentel LSB[1], Fraga CAM[2], Barreiro EJ[2], Bolzani V[3], Castro NG[1] [1]ICB-UFRJ - Farmacologia Molecular, [2]LASSBio-UFRJ - Farmácia, [3]NuBBe-UNESP-Araraquara - Química Orgânica

Introduction: Central cholinergic hypofunction is a hallmark of the initial stages of Alzheimer's disease and is the target of current therapy with anticholinesterase drugs. LASSBio-767 was recently described as a semi-synthetic cholinesterase inhibitor derived from natural piperidine alkaloids obtained from the flowers of *Senna spectabilis* (Fabaceae) (Castro et al. *Eur. J. Pharmacol.,* 580:339, 2008). The product is in fact a mixture of two closely related compounds, (−)-3-*O*-acetyl-spectaline and (−)-3-*O*-acetyl-cassine, which are homologues differing only by two CH2 units in the alkyl chain attached to C6 of the piperidine ring. The product is orally effective and presents a remarkable balance between central anticholinesterase activity, shown by reversal of scopolamine-induced memory deficits, and low peripheral toxicity in rodents. In particular, salivation, lacrimation and diarrhea are very mild even at doses ten times above those effective in the memory tests. Considering that peripheral cholinergic adverse effects are common to all the anticholinesterase drugs currently in use for Alzheimer's disease, we decided to investigate other potential targets of LASSBio-767 that might explain its unique profile. We tested LASSBio-767 in different assays of peripheral cholinergic function and the most relevant results are described herein. **Methods:** Human colonic epithelial adenocarcinoma HT-29 cells natively expressing the type 3 muscarinic receptor (M3) were plated onto glass coverslips. Cells were loaded with the calcium indicator fura-2 AM and the coverslips were mounted in a small-volume perfusion chamber. Fluorescence intensity was measured by a photodiode-based dual-excitation optical system on an upright microscope with a 40x water immersion objective. Compounds were applied in 30-second pulses by switching between perfusing solutions. For inhibition tests, the antagonist was applied alone for 30 s before switching to the solution that also contained the agonist. **Results and Discussion:** The HT-29 cells showed robust transient increases in intracellular calcium concentration when stimulated by carbachol. These responses were completely prevented by atropine 1 microM. Neither LASSBio-767 nor pure (−)-3-*O*-acetyl-cassine induced calcium changes when applied at 100 microM. However, both compounds inhibited the calcium responses induced by carbachol, with 50% inhibition between 1 and 10 microM. The percent inhibition by either compound at 10 microM was smaller with 500 microM than with 100 microM carbachol, suggesting a competitive antagonism. Thus, LASSBio-767 and (−)-3-*O*-acetyl-cassine are muscarinic antagonists at the M3 receptor. Their antimuscarinic activity is in the same concentration range as that for brain cholinesterase inhibition, indicating that it might be relevant to the behavioral effects in vivo. The additional M3 antagonism can explain the lack of hypersecretory signs in rodents exposed to LASSBio-767 and seems ideally suited to minimize gastrointestinal intolerance in the setting of Alzheimer's disease therapy. Support: Finep (Ações Transversais) and Apsen Farmacêutica.

02.003

Apamin reduces the neuromuscular transmission activating the inhibitory M_2 receptors on the motor nerve terminal. Silva LFCM[1], Ramos ERP[1], Ambiel CR[2], Santos IL[1], Alves-do-Prado W[1], Correia-de-Sá P[3] [1]UEM - Farmacologia, [2]UEM - Ciências Morfofisiológicas, [3]Universidade do Porto - Farmacologia

Introduction: To clarify the origin of the beneficial effect of apamin in patients suffering from myotonic diseases, the effects of apamin were compared with those produced by 4-aminopyridine in the phrenic nerve diaphragm muscle preparation of healthy rats indirectly stimulated at 100 Hz. **Methods:** The Ethics Committee for Experimental Studies of the State University of Maringa approved the procedures (043-2007). The hemidiaphragm preparation was connected to a force displacement transducer (Grass FT 03, USA) to record muscular contractions on a Chart Software (Powerlab AD Instruments, Australia). The ideal length of the muscle was determined as being that one that produced greater amplitude of muscular contraction when 0.2 Hz was applied on phrenic nerve. Standard rate of stimulation was 0.2 Hz, but 100 Hz was applied to the nerve for 10 s at 10- min intervals. The fade of neuromuscular transmission was measured as the ratio (R) between the tension produced at the end of tetanic stimulation (B) and that recorded at the beginning (A, maximal tetanic tension) (R= B/A). The effect of apamin on amplitude of twitch induced by retrograde injection of acetylcholine (1.0 µg/50µL) into the thoracic inferior vein cava was also assayed. Data were submitted to ANOVA, followed by Bonferroni test at $P<0.05$ significance level. **Results**: Apamin (25 to 200 nM) and 4-aminopyridine (1.0 mM) worsened the fade produced by 100 Hz without changing the maximal tetanic tension. The effect produced by such agents did not appear when the preparations were directly stimulated. In contrast with 4-aminopyridine , apamin (25 nM) reduced the amplitude of muscle contraction when the preparations were indirectly stimulated at 0.2 Hz, but such effect of apamin was not followed by any variation in magnitude of twitch induced by retrograde injection of acetylcholine (1 mg x 50 mL^{-1}). The inhibitory effects of apamin at 0.2 or 100 Hz were impaired by methoctramine (0.3 µM), but pirenzepine (10 nM) only changed (worsened) the effect of apamin at 100 Hz. Methoctramine improved the 525 mM acetylcholine- and 200 mM hexamethonium-induced fades, but pirenzepine did not change the fades produced by such agent. Hexamethonium worsened the apamin-induced fade. **Discussion and conclusion**: Data indicate that the presynaptic inhibitory effect of apamin on neuromuscular transmission is not determined by interactions of drug with nicotinic receptors, and neither depends on blockage of potassium channels producing a previous increment on acetylcholine release from motor nerve, thereby activating the presynaptic inhibitory M2 receptors. However, data suggest that the effect produced by apamin on the neuromuscular transmission depends on a direct activation of the inhibitory M_2 muscarinic receptors on the motor nerve terminal by apamin. In such mechanism could be the origin of the beneficial effect of apamin in patients suffering from myotonic diseases. Research supported by FADEC-UEM and CESUMAR.

02.004

The antinicotinic agent-induced R_{TOF} fade depends on activations of M1 and M2 receptors on the motor nerve terminal by acetylcholine. Pereira MW[1], Bornia ECS[2], Ambiel CR[3], Santos IL[2], Alves-do-Prado W[2] [1]UEM - Ciências Biológicas, [2]UEM - Farmácia e Farmacologia, [3]UEM - Ciências Morfofisiológicas

Introduction: Train-of-Four (TOF) is a moderate frequency of stimulation (2.0 Hz) which, applied on motor nerve during 2s, may be useful to obtain information on neuromuscular transmission. TOF ratio (R_{tof}) is defined as the quotient between muscular tension produced by the 4th stimulus (T4) and the tension obtained by the 1st (T1) stimulus of train (R_{tof}= T4/T1). Decrease in R_{tof} value (T4<T1) is TOF fade. TOF fade starts on the motor nerve terminal and may be followed by a decrease in T1 values, if the drug is also blocking the nicotinic receptors on the end plate (Nm). Although TOF fade, triggered by antinicotinic agents, may have been caused by the blockage of the facilitatory presynaptic nicotinic receptors (Nn), the roles of the presynaptic facilitatory M1 or of the inhibitory M2 muscarinic receptors on the TOF fade, induced by antinicotinic agents, have not been investigated. Since some antinicotinic agents (pancuronium, cisatracurium) are able to exhibit other properties (antimuscarinic, anticholineterasic) than the antinicotinic ones, the effects produced by hexamethonium (HEXA), d-tubocurarine (d-TB), pancuronium (PANC) or cisatracurium on the TOF fade have been investigated in the phrenic nerve diaphragm muscle preparations of rats previously treated with pirenzepine (PZP) (selective blocker of M1 receptors) or methoctramine (MTC) (selective blocker of M2 receptors). **Methods:** The Ethics Committee for Experimental Studies of the State University of Maringá approved the procedures (043-2007). The neuromuscular preparations of Wistar rats were assembled according to Bülbring (Bülbring E. *Br J Pharmacol*.; (1):38, 1946).TOF stimuli were applied at 15 sec intervals during 4 min. The muscle was connected to a force displacement transducer (Grass FT 03 connected to Chart Software Power Lab Instruments). The lowest concentrations of HEXA, d-TB, PANC or CIS able to produce 25% TOF fade 3 min after addition in the bath were determined separately and administered 18 min after 10 nM PZP or 1.0 µM MTC. Data were compared with ANOVA followed by Bonferroni test ($p<0.05$). **Results and Discussion:** The lowest concentration was 5.47mM for HEXA (0.74±0.033 n=5), 3.0µM for PANC (0.75±0.010 n=4), 2.2µM for CIS (0.76±0.014 n=5), and 1.1nM for d-TB (0.75±0.013 n=5). HEXA-induced R_{tof} fade had a presynaptic origin, as it was not followed by changes in T1 values. However, R_{tof} fade by d-TB, PANC or CIS seemed to be also dependent on interactions of these agents with Nm receptors. PZP 10nM (n=6) or MTC 1uM (n=6) did not modify R_{tof} value. Although PZP attenuated R_{tof} fade by PANC (0.75±0.042 n=4) or CIS (0.76±0.039 n=4), it completely antagonized the R_{tof} fade by d-TB (0.91±0.024 n=4) or HEXA (0.93±0.013 n=4). MTC antagonized R_{tof} fade by PANC (0.87±0.019 n=6), CIS (0.88±0.042 n=5), d-TB (0.83±0.014 n=4) and HEXA (0.92±0.024 n=4). Data show that Rtof fades produced by antinicotinics agents investigated in current study depend on activations of M1 and M2 on motor nerve by acetylcholine, even though those produced by pancuronium or cisatracurim seem to be affected by the molecules' anticholinesterasic properties. Funding: Fundação Araucária, UEM (Fadec) and CESUMAR.

02.005

Activation of the serotonergic 5-HT_{1A} receptor in the paraventricular nucleus of the hypothalamus inhibits water intake and increases urinary excretion in water-deprived rats. Villa, PS[1], Camargo GMPA[2], Camargo LAA[1], Saad WA[3] [1]UNESP - Fisiologia, [2]UNESP - Análises Clínicas, [3]FOAr-UNESP - Fisiologia e Patologia

Introduction: The paraventricular nucleus (PVN) may be considered as a dynamic mosaic of chemically-specified subgroups of neurons. 5-HT_{1A} is one of the prime receptors identified and there is expressed throughout all magnocellular regions of the PVN. Several reports have demonstrated that a subpopulation of the magnocellular neurons expressing 5-HT_{1A} receptors are oxytocin (OT) neurons and activation of 5-HT_{1A} receptors in the PVN increases the plasma OT. Increasing evidence shows that OT inhibits water intake and increases urinary excretion in rats. The aim of this study was to investigate the role of serotonergic 5-HT_{1A} receptors in the lateral-medial posterior magnocellular region of the PVN in the water intake and diuresis induced by 24h of water deprivation. **Methods:** Rats were anesthetized with ketamine (80mg/kg bw) combined with xylazine (7mg/kg bw) and placed in a stereotaxic instrument. Stainless steel cannulae 0.6mm (o.d.), 0.33mm (i.d.) were implanted bilaterally just above the PVN, using the bregma as reference point (Process CEEA/FOAr n^{o} 08/2004). **Results:** 5-HT injections in the PVN reduced water intake and increased urinary excretion. 8-OH-DPAT (a 5HT_{1A} agonist) injections blocked the water intake and increased urinary output in all the periods of the observation. pMPPF (a 5-HT_{1A} antagonist) injected bilaterally before the 8-OH-DPAT blocked its inhibitory effect on water intake and its diuretic effect. **Discussion:** Dehydration is a potent physiological stimulus not only for AVP secretion, but also for OT secretion (Summy-Long JY, Brain Res; 309:362, 1984). The involvement of distinct serotonergic receptors in the mediation of the stress-induced AVP and OT responses is found to differ. Blockade of 5-HT_{1A} receptors inhibited restraint stress-induced OT, but not AVP secretion (Jorgensen H, *Eur J Endocrinol*; 147:815, 2002). Thus, it was observed that the 5-HT_{1A} agonist 8-OH-DPAT, which had no effect on AVP secretion, stimulated OT secretion (Bagdy G, *Eur J Pharmacol*; 210:285, 1992). It was concluded that 5-HT-induced AVP secretion is primarily mediated *via* 5-HT_{2C}, 5-HT_4 and 5-HT_7 receptors; 5-HT_{2A}, 5-HT_3 and 5-HT_{5A} receptors seem of minor importance. Induction of OT secretion by 5-HT involves 5-HT_{1A}, 5-HT_{2C} and 5-HT_4 receptors; in addition, 5-HT_{1B}, 5-HT_{3A} and 5-HT_7 receptors seem to be involved, whereas 5-HT_{2A} and 5-HT_3 receptors seem less important (Jorgensen H, *J Neuroendocrinol*; 15:242, 2003). Thus, it is reasonable to suggest that the stimulation of PVN 5-HT_{1A} receptors somehow disrupts the functional integrity of mechanisms normally triggered by PVN OT, which are essential to the expression of drinking behavior and diuresis during hypovolemia. **Acknowledgments:** Research supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisa (CNPq).

02.006

Vasopressin and angiotensin receptors of the medial septal area in the control of mean arterial pressure induced by vasopressin. Camargo GMPA[1], Saad WA[2], Camargo LAA[3] [1]UNESP - Análises Clínicas, [2]FOAr-UNESP - Fisiologia e Patologia, [3]UNESP - Fisiologia

Introduction: Brain arginine[8]-vasopressin (AVP), through the V_{1a}-and V_2 - receptors, is essential for the maintenance of mean arterial pressure (MAP). Central AVP interacts with the components of the renin-angiotensin system (RAS), which participate of the MAP regulation. This study was intended to determine the effects of Phenilac[1], D-Tyr(Me)[2], Arg[6,8],Lys-NH_2[9])-AVP (V_{1a} antagonist), [D($CH2)_5$[1], D-Ile[4], Arg[8], Ala-NH_2[9]]-AVP (V_2 antagonist), N-4,5-dihydro-2-methylimidazol [4-5-d] [1] benzopin -6(1H)-yl) carbonyl) phenyl)-(1,1"-biphenyl)-2-carboxanide (conivaptan, YM087, V_{1a}/V_2-antagonist) and AVP selective antagonists and AT_1 and AT_2 – angiotensin II (ANG II), losartan and CGP42112A selective antagonists of the AT_1 and AT_2 ANG II receptor, respectively on the MAP induced by AVP injected into the medial septal area (MSA) of the brain. **Methods:** Male Holtzman rats with stainless steel cannulae implanted in to the MSA were used in experiments. Direct MAP was recorded in conscious rats (Process CEEA/FOAr n^o 18/2004). **Results:** AVP administration into the MSA caused a prompt and potent pressor response in a dose-dependent fashion. Pretreatment with the V_{1a} and V_2 antagonists reduced, whereas previous injection of V_{1a}/V_2antagonist induced a decrease on the MAP that remained below the baseline. Both AT_1 and AT_2 antagonists elicited a decrease, while simultaneous injections of two antagonists were more effective in decreasing the MAP induced by AVP. **Discussion:** AVP exerts a variety of biological effects such as vascular resistance control through V_{1a}-receptors and regulation of water excretion through V_2-receptors. It is generally assumed that AVP is involved in several conditions such as heart failure, hyponatremia and hypertension, *via*, the V_{1a} -and V_2-receptors (Naitoh M, Am J Physiol; 267**:**2245, 1994). The development of nonpeptide AVP antagonists seems essential to the elucidation of the physiological and physiopathological roles of AVP. Combined V_{1A}/V_2-receptor antagonism resulted in reductions in systemic vascular resistance with increased cardiac output, as well as a substantial increase in urine output. Investigations with YM087 in receptor assay systems and animal models suggested substantial antagonism of both V_{1A}-and V_2-receptors. Udelson, (*Circulation*; 104:2417, 2001), demonstrated favorable short-term hemodynamic and renal effects of combined V_{1A}- and V_2-AVP receptor antagonism, as well as the lack of relation of these effects to baseline markers of AVP activation. It has been reported (Lenkei Z, Front *Neuroendocrinol*; 18:383, 1997) that body RAS is present in the CNS. Previous studies (Häuser W, *Eur J Pharmacol*; 348:101, 1998) showed the existence of both AT_1-and AT_2 -receptors in the CNS. In the brain, the ANG II receptors are involved in the pressor responses and AVP release (Shelat SG, *Regul Pept*; 73:03, 1998). The present results indicate that is a synergism between the V_{1a}-and V_2-AVP and AT_1 and AT_2-ANG II receptors into the MSA in the regulation of MAP. **Acknowledgments:** Research supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisa (CNPq).

02.007

Caffeine prevents cognitive and motor impairments in the intranasal MPTP rat model of Parkinson's disease. Wopereis S, Rial D, Moreira ELG, Prediger RD UFSC - Farmacologia

Introduction: Although Parkinson's disease (PD) is classically considered to be a motor system disease, cognitive impairments can be observed even during the early phases of PD. An increasing number of epidemiological studies have indicated that environmental toxins and coffee consumption can be associated with, respectively, higher and lower risks of future development of PD. Therefore, the aim of the present study was to evaluate the potential of caffeine to prevent the cognitive and motor deficits in rats infused with a single intranasal (i.n.) administration of the proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a valid animal model PD described by our group. **Methods:** Male Wistar rats (n=11-13 per group, 5-6 months-old, 350-400 g) were pre-treated by intraperitoneal (i.p.) route with vehicle (saline) or caffeine (10 mg/kg) once daily during 5 consecutive days. One hour after the last injection of caffeine, the animals were infused intranasally with a single bilateral dose of MPTP (1 mg/nostril). Animals were evaluated in cognitive (social recognition) and locomotor (open-field and activity chambers) tests 7 to 30 days after i.n. MPTP infusion. CEUA-UFSC: PP00111. **Results:** In the social recognition task, MPTP-treated rats spent as much time investigating the juvenile rat during the second encounter as they did in the first exposure, reflecting a clear impairment of the juvenile's recognition ability. More importantly, caffeine administration successfully prevented these cognitive deficits induced by i.n. MPTP since the animals behaved in a similar way to control-treated rats, i.e. they were able to recognize the juvenile after an interval of 30 min (investigation time (s): cont:46.9±7.9; caf:46.5±4.0; MPTP:61.4±6.2; caf+MPTP:42.7±6.7). At this time, no significant alterations on the locomotor activity were observed in the open-field. In contrast, a marked reduction in the spontaneous movements of the MPTP-treated rats was observed in activity chambers at 30 days after i.n. MPTP infusion. Once again, our findings indicated that caffeine was able to prevent these motor impairments induced by MPTP (crossings: cont:138.7±11.6; caf:122.2±13.4; MPTP:86.1±17.9 caf+MPTP:120.4±12.9). **Discussion:** The data of the present study reinforces and extends previous literature indicating the potential benefits associated to caffeine consumption in the prevention of cognitive and motor symptoms in PD. **Financial Support:** CNPq, FAPESC, Brazil.

02.008

Evaluation of pre- and post-treatment with LASSBio 881 in the reduction of infarct volume in mice submitted to focal cerebral ischemia. Balassiano N[1], Elias MJG[2], Mendonça Tributino JL[2], Fraga CAM[3], Miranda ALP[3], Castro NG[1] [1]ICB-UFRJ - Farmacologia Molecular, [2]UFRJ - Farmacologia Básica e Clínica, [3]LASSBio-UFRJ - Farmácia

Introduction: Cerebrovascular diseases are the leading cause of death in Brazil and Rio de Janeiro has the second highest specific mortality rate from stroke in the country. Due to the enormous disability and mortality caused by stroke worldwide, there is an intensive search for substances that could save the brain tissue from the ischemic damage. The substance LASSBio 881 is an aromatic N-acylhydrazone previously shown to present anti-inflammatory and central analgesic effects after oral administration, and low acute toxicity (Duarte et al., Bioorg. Med. Chem. 15:2421, 2007). Besides, it can modulate cannabinoid receptors, inhibit phosphodiesterases and cyclooxygenases and act as an antioxidant. These multi-target effects could be useful for neuroprotection in the stroke penumbra. Therefore, our main goal is to investigate the effect of LASSBio 881 in a mice model of focal cerebral ischemia. **Methods:** Male Swiss mice weighing 25-35 grams were submitted to a surgical procedure of permanent focal cerebral ischemia by middle cerebral artery electrocoagulation (MCAE). All experimental procedures were approved by the Ethics Committee on Animals Research of CCS/UFRJ, protocol DFBICB 029. Animals were observed in the immediate postoperative period, and a score from 0 to 4 was attributed to each one, according to neurological deficit. Animals with a 0 score were excluded from analysis. Pre-treated animals received one dose of LASSBio 881 (132 mg/kg, i.p.) one hour before MCAE. Post-treated animals received four i.p. doses of LASSBio 881 (1, 8, 20 and 32 h after MCAE), the first 100 mg/kg and the others 50 mg/kg. Control groups received vehicle in the same schedules. Infarct volume was measured 48 h after surgery, when the animals were anesthetized with ether and then euthanized by cervical displacement. Five 2-mm coronal sections of the brain were made and then stained with 2,3,5-triphenyltetrazolium chloride (TTC). Digital images of the stained sections were obtained with a table scanner. Infarct area in each section was measured, multiplied by 2 (thickness) and then added, giving the total infarct volume (mm^3). **Results** and Discussion: Control pre-treated animals had an infarct volume of 19.2 ± 2.6 mm^3 (n=21; mean ± s.e.m.), compared with 13.2 ± 1.5 mm^3 (n=17) for those receiving LASSBio 881 ($p<0.05$). Control post-treated animals had an infarct volume of 25.7 ± 4.1 mm^3 (n=19). Animals post-treated with LASSBio 881, however, had an infarct volume of 6.1 ± 1.6 mm^3 (n=5). Thus, pre-treatment with LASSBio 881 led to a reduction of 30.7% in the infarct volume, disclosing a significant neuroprotective effect. The post-treatment data, although preliminary, also show a trend towards a beneficial neuroprotective effect of LASSBio 881, when evaluated by the reduction in the infarct volume after focal cerebral ischemia in mice. Acknowledgements: This work was supported by FAPERJ and Programa Nacional de Núcleos de Excelência (PRONEX).

02.009

Functional effects of B1 and B2 receptors antagonists in the bladder overactivity induced by chronic spinal cord injury in rats. Forner S[1], Andrade EL[1], Martini AC[1], Medeiros R[1], Koepp J[2], Calixto JB[1] [1]UFSC - Farmacologia, [2]École Nationale Superiéure des Mines de Saint Etienne - Farmacologia

Objectives: Recently our group shown that the bladder overactivity (BO) induced by spinal cord injury (SCI) is characterized by potencialization of bradykinin (BK)-induced contraction and contractile response to B1 receptor agonist, des-Arg^9-BK in strips of urinary bladder collected 2, 7, 14 and 28 days after SCI (Neurolatam, 2008). Now, this study evaluated the effects of specific kinins antagonism as well as B1 and B2 receptors expression and the pro-inflammatory cytokines levels in the rat urinary bladder after chronic SCI. **Methods and Results**: All procedures were approved by our Institutional Ethics Committee (number 016/CEUA/PRPe/2008. Procedure PP00158). Adult male Wistar rats weighing 270-300 g were anesthetized and the spinal cord was injured at 10th thoracic vertebrae with a Fogarty 2F catheter to induce complete paraplegia. Twenty eight days after surgery, the urinary bladder contractility was assessed *in vitro*. In addition, western blot and ELISA analysis were carried out. In urinary bladder strips from sham-operated animals, pre-incubation with the selective B2 receptor antagonist, HOE-140 (30 nM) abolished the BK (0.0001-10 μM)-induced contractile response, while in chronic SCI animals, the BK-induced contractile response was reduced in 90 ± 2.4%. In contrast, contractile response induced by des-Arg^9-BK (DABK, 0.0001-10 μM) was not significantly reduced by the selective B1 receptor antagonist, des-Arg-Leu^8-BK (30 μM). Interestingly, when compared with naïve and sham-operated groups, SCI group showed a pronounced alteration in the basal activity of the urinary bladder characterized by enhancement in frequency and amplitude of discharge[E1], which were normalized by selective B2, but not B1 receptor antagonist. Finally, western blot analysis of urinary bladders in 2, 7, 14 and 28 days after surgery indicated that B1 and B2 receptors expressions were, respectively, 191% and 110% higher on the 7th day after SCI in comparison to the respective sham-operated groups. Sham-operated animals presented a constitutive expression of B2 and a slight B1 receptor expression in the urinary bladder. Moreover, the pro-inflammatory cytokines levels, IL-1β and IL-6 were, respectively, 60 and 14 times higher in the 2nd, but not in the 7th, 14th and 28th days after SCI when compared to sham-operated animals. **Conclusion:** The results show that chronic SCI induces changes in the basal activity of urinary bladder and enhancement in the BK-induced contractile response, which was significantly inhibited by the B2 receptor antagonist. In addition, the contractile response to B1 receptor agonist (des-Arg^9-BK) was not significantly inhibited by selective B1 receptor antagonist. Moreover, it was observed an increase in the pro-inflammatory cytokines levels during the first days after the SCI and the subsequent up-regulation of B1 and B2 receptor, suggesting a possible interaction between cytokines and regulation of B1 receptor expression.

02.010

Rimonabant repeated treatment inhibits behavioral sensitization previously developed to ethanol in mice. Baldaia MA[1], Santos R.[1], Hollais AW[1], Oliveira-Lima AJ[2], Wuo-Silva R[3], Araújo BA[1], Talhati F[4], Fernandes HA[3], Marinho EAV[2], Frussa-Filho R[3] [1]UBC - Farmácia, [2]UBC/UNIFESP - Ciências da Saúde/Farmacologia, [3]UNIFESP-Farmacologia, [4]UBC - Ciências da Saúde

Introduction: Drugs of abuse including ethanol, increase dopamine levels in the nucleus accumbens producing, in rodents, locomotor stimulation, a behavior which is sensitized after repeated administration. This sensitization has been proposed to share neuronal mechanisms with drug craving. The cannabinoid system can regulate the dopamine levels directly in the Nacc or through the VTA. Rimonabant is an antagonist cannabinoid to CB1 receptors. The aim of this study was to investigate if repeated treatment with the rimonabant is able to inhibit previously developed locomotor sensitization to ethanol in mice. The role of environmental context in the behavioral sensitization phenomenon was also investigated (Ethic committee number: 470/07). **Methods:** In the first experiment, Swiss mice received intraperitoneally (ip) 1.8g/kg of ethanol or saline every other day for 15 days (8 injections). Five minutes after each injection, the animals were exposed to an open field (OF) for 10 min. Locomotor activity (LA) was measured on the 1st and 15th days. On the 17th day animals began to be treated with vehicle or rimonabant (1 or 10mg/kg) i.p. during 8 consecutive days and 30 min after each injection they were placed in the OF for 10 min (LA quantified on the 17th and 24th days). On the 30th day all the animals were challenged i.p with 1.8g/kg of ethanol (LA was quantified for 10 min in the OF). Therefore, the following groups (n=11) were formed: Saline-Vehicle-Ethanol (Sal-Veh); Saline-Rimonabant1-Ethanol (Sal-Rim1); Saline-Rimonabant10-Ethanol (Sal-Rim10); Ethanol-Vehicle-Ethanol (Eth-Veh); Ethanol-Rimonabant1-Ethanol (Eth-Rim1); Ethanol-Rimonabant10-Ethanol (Eth-Rim10). In the second experiment the same protocol was used except that mice received vehicle or rimonabant in their home cages. **Results:** In the first experiment, the first ethanol injection produced a significant increase in LA of the ethanol groups (Eth-Veh: 416±31; Eth-Rim1: 425±35; Eth-Rim10: 428±37), compared to saline groups (Sal-Veh: 75±7; Sal-Rim1: 90±6; Sal-Rim10: 78±6) ($p<0,05$). This locomotor stimulant effect of ethanol was not potentiated with repeated administration not revealing through this within-subject design the development of behavioral sensitization to ethanol. During the treatment, rimonabant did not modify LA of either saline- or ethanol-previously treated animals. However, after ethanol challenge injection (day 30), two-way ANOVA revealed significant effects of ethanol previous treatment, rimonabant previous treatment as well as ethanol x rimonabant interaction. Indeed, ethanol previous treatment potentiated the hyperlocomotion induced by ethanol challenge injection (Sal-Veh: 406±42; Eth-Vehl: 666±72), demonstrating the behavioral sensitization phenomenon through the between-subject design. This behavioral sensitization to ethanol was inhibited by rimonabant previous treatment (Eth-Rim1: 472±33; Eth-Rim10: 419±47). However, rimonabant previous treatment did not modify ethanol challenge-induced hiperlocomotion in saline pre-treated mice (Sal-Veh: 406±42; Sal-Rim1: 403±32; Sal-Rim10: 423±41). Concerning experiment 2, ethanol-induced behavioral sensitization was also demonstrated by the between subject design but it was not inhibited by home-cage rimonabant previous treatment. **Conclusion:** rimonabant treatment was able to inhibit locomotor sensitization previously developed to ethanol treatment, but this inhibition was dependent on the enviromental context in which it is administered. Financial support-CNPQ Ethics committe: 0470/07

02.011

Rimonabant repeated treatment inhibits behavioral sensitization previously developed to morphine in mice. Santos R.[1], Baldaia MA[1], Hollais AW[1], Oliveira-Lima AJ[2], Wuo-Silva R[3], Fernandes HA[3], Araújo BA[1], Talhati F[4], Marinho EAV[2], Frussa-Filho R[3] [1]UBC - Farmácia, [2]UBC/UNIFESP - Ciências da Saúde/Farmacologia, [3]UNIFESP - Farmacologia, [4]UBC - Biologia

Introduction: Drugs of abuse including morphine, increase dopamine levels in the nucleus accumbens producing, in rodents, locomotor stimulation, a behavior which is sensitized after repeated administration. This sensitization has been proposed to share neuronal mechanisms with drug craving. The aim of this study was to investigate if repeated treatment with the rimonabant is able to inhibit previously developed behavioral sensitization to morphine in mice. The role of environmental context in the behavioral sensitization phenomenon was also investigated (Ethic committee number: 470/07). **Methods:** In the first experiment, Swiss mice received intraperitoneally (ip) 20mg/kg morphine or saline every other day for 15 days (8 injections). Thirty minutes after each injection the animals were exposed to an open field (OF) for 10 min. Locomotor activity (LA) was measured on the 1st and 15th days. On the 17th day animals began to be treated with vehicle or rimonabant (1 or 10mg/kg) i.p. during 8 consecutive days and 30 min after each injection were placed in the OF for 10 min (LA quantified on the 17th and 24th days). On the 30th day all the animals were challenged i.p with 20mg/kg morphine and their LA was quantified for 10 min in the OF. Therefore, the following groups (n=14) were formed: Saline-Vehicle-Morphine (Sal-Veh); Saline-Rimonabant1-Morphine (Sal-Rim1); Saline-Rimonabant10-Morphine (Sal-Rim10); Morphine-Vehicle-Morphine (Mor-Veh); Morphine-Rimonabant1-Morphine (Mor-Rim1); Morphine-Rimonabant10-Morphine (Mor-Rim10). In the second experiment the same protocol was used except that mice received vehicle or rimonabant in their home cages. **Results:** In the first experiment, the first morphine injection produced a significant increase in LA of the morphine groups (Mor-Veh: 282±30; Mor-Rim1: 284±63 and Mor-Rim10: 277±53), compared to saline groups (Sal-Veh: 98±12; Sal-Rim1: 97±11; Sal-Rim10: 78±9) ($p<0,05$). This locomotor stimulant effect of morphine was potentiated with repeated administration (for ex: Mor-Veh group was 559±78 on the 15th day), revealing the development of behavioral sensitization to morphine. During the treatment, rimonabant did not modify LA of either saline- or morphine-previously treated animals. However, after morphine challenge injection (day 30), two-way ANOVA revealed significant effects of morphine previous treatment, rimonabant previous treatment as well as morphinexrimonabant interaction. Indeed, morphine previous treatment potentiated the hyperlocomotion induced by morphine challenge injection (Sal-Veh: 318±52; Mor-Veh: 847±66), once again demonstrating the behavioral sensitization phenomenon. This behavioral sensitization to morphine was inhibited by rimonabant previous treatment (Mor-Rim1: 526±70; Mor-Rim10: 515±64). However, rimonabant previous treatment did not modify morphine-challenge induced hiperlocomotion in saline pre-treated mice (Sal-Veh: 318±52; Sal-Rim1: 349±56; Sal-Rim10: 349±48). Concerning experiment 2, morphine-induced behavioral sensitization was also demonstrated but it was not inhibited by home-cage rimonabant previous treatment. **Conclusion:** rimonabant treatment was able to inhibit locomotor sensitization previously developed to morphine treatment, but this inhibition was dependent on the enviromental context in which it was administered. Financial support: CNPQ Ethics committee: 0470/07

02.012

Nitric oxide production is decreased in the striatum of rats with L-DOPA-induced dyskinesia. Romano, ACD[1], Padovan FEN[1], Ferreira FR[2], Del Bel EA[1] [1]FORP-USP - Morfologia, Estomatologia e Fisiologia, [2]FMRP-USP – Farmacologia

Introduction: Previous results from our group demonstrated that nitric oxide synthase inhibition decreases dyskinesias induced by chronic administration of L-DOPA in rats with unilateral 6-hydroxydopamine lesion. Considering that NO oxidizes to the stable metabolites nitrite and nitrate measurement of these metabolites has been regarded as one of the most suitable and practical method to assess NO synthesis *in vivo.* **Methods:** In this study groups of parkinsonian and dyskinetic *Wistar* rats had their brain removed to measure tissue levels of nitric oxide metabolites NO(x)(-) (nitrite plus nitrate) in the striatum using the Griess reaction. Chronic L-DOPA treatment (30mg/kgX gavagen X 21 days) induced dyskinesia changes. **Results:** 7-Nitroindazole (10 mg/kg; 7-NI) a selective to neuronal nitric oxide synthase inhibitor given 30 min before L-DOPA attenuated dyskinesia (*t-test*, *p<0,05)*. NOx levels increased in the striatum ipsi and contralateral to lesion (*p<0,05*). 7-nitroindazole pretreatment reduced NOx in the striatum ipsilateral to lesion *(ANOVA, $F_{1,18}$=4,58; p<0,05).* L-DOPA chronic treatment *per se* reduced the levels of NOx in the striatum ipsilateral to lesion (*ANOVA, $F_{1,28}$=4,41; p<0,05)*. 7-nitroindazole pretreatment to dyskinetic rats induced no further NOx reduction *(t-test, p>0,05).* **Discussion:** NO(x)(-) levels in the brain largely reflects the metabolites of neuronally, endothelially and inducibly derived NO. The finding that exposure to 7-NI did not significantly lowered the levels of NO(x)(-) in the contralateral to lesion striatum was contrary to what was anticipated. Why this effect occurred is not immediately clear but experiments are in progress with increased 7-nitroindazole concentration. **Supported:** FAPESP, CNPQ **N° animal ethics committee:** 052/2007

02.013

Object location memory in mice: pharmacological validation and further evidence of hippocampal CA1 participation. Nakamura CA, Assini FL, Duzzioni M, Takahashi R
UFSC - Farmacologia

Introduction: Object location task (OLT) has been proposed as a model of spatial memory task. In mice, there are no studies evaluating the consequences of pharmacological modulation of NMDA receptors or examining the effects of hippocampal participation in the OLT. Also there are few studies investigating the cholinergic muscarinic participation in this task. The aim of this study was to further validate the usefulness of OLT to evaluate drugs and/or procedures that have either amnesic or positive effects on the spatial memory in mice. **Methods:** In a former set of experiments we evaluated the time course of object location memory expression and in a later protocol we evaluated the pharmacological validity of OLT in mice using glutamatergic and cholinergic-related drugs. The OLT consisted of training phase (3 min), a delay (30, 90, 180 or 360 min) and test phase (3 min). Two identical objects were placed in the corner of the open field and the mice explored it freely, after delay one of the objects was in a new location. The time spent exploring the objects in new (novel) and old (familiar) location (test phase) and the locomotor activity (training phase) were analyzed. Adult male Swiss albino mice were treated with MK-801, d-cycloserine, scopolamine, tacrine and lidocaine to evaluate the exploration behavior. All experiments were approved by UFSC - Ethical Committee (PP280). **Results:** In the time course protocol, animals trained and then tested after 90 min, or 180 min have shown a location index significantly increased from chance performance ($t= -3.96$ $p= 0.003$; $t= -4.02$ $p= 0.003$). After 30 min ($t= 0.59$ $p= 0.57$) and 360 min ($t= -0.8$ $p= 0.43$), the mice were not able to identify the spatial alteration in the OF. The NMDA-receptor antagonist (MK-801 0.1 or 0.05 mg/kg, i.p.) and cholinergic one (scopolamine 0.25; 0.5 or 1.0 mg/kg, i.p.) significant decreased the object location index ($F(3,32)= 9.40$ $p<0.0001$; $F(3,28) = 13.98$ $p < 0.0001$). On the other hand, the NMDA-receptor agonist (d-cycloserine 20.0 mg/kg, i.p.) and acetylcholinesterase inhibitor (tacrine 1.5 mg/kg or 3.0 mg/kg, i.p.) showed a significantly higher object location index than control animals ($F(3,33)= 4.40$ $p<0.01$; $F(3,28)= 3.34$ $p<0.05$). Lidocaine 4% infusion into the hippocampal CA1 region decreased the object location index when compared to the PBS group ($t= 2.21$ $p= 0.03$). None of the treatments altered significantly the locomotor activity of the mice evaluated in the open field during the 3 minutes of training. **Discussion:** Short (30 min) or longer (360 min) intertrial intervals were not characterized by a higher location index of memory in this task, suggesting that there was a window of time for the expression of object location memory. The blockade of NMDA and muscarinic receptors had an amnesic effect. Moreover, activation of these receptors improved the object location index and the infusion of lidocaine inactivated hippocampal CA1 region. In conclusion, this study shows that OLT is a useful one-trial learning and memory animal model that could be used to evaluate the effects of drugs and/or procedures associated with spatial learning in mice. **Financial support:** CNPq

02.014

Sildenafil prevents mortality and hippocampal neurodegeneration after 4VO/ACi model of chronic cerebral hypoperfusion. Romanini CV[1], Schiavon AP[1], Mori MA[2], Ferreira EDF[1], Oliveira RMMW[1], Milani H[1] [1]UEM - Farmácia e Farmacologia, [2]UEM - Ciências Biológicas

Introduction: Several animal models of chronic cerebral hypoperfusion (HCC) present limitations such as considerable variation in the behavioral deficits and neurophatological changes, which have important implications if the effects of drugs that protect against neurodegeneration must be assessed [1]. For this reason, establish an animal model of HCC that provide consistent and reproducible behavioral deficits and neuronal lesions is very important. The present study aimed to characterize the 4-VO/ACI model (four vessels occlusion, internal carotid arteries) in young rats, induced by ligation of vessels using different stages (2 or 3) and interstage oclclusion interval (1 to 7 days). We also aimed to evaluate the effect of repetitive treatment with sildenafil, a phosphodiesterase inhibitor, since it has been shown that this drug modulates the NO/GMPc pathway promoting regulation of blood flow [2]. For this purpose the rate of animal mortality and the hippocampal damage after 4-VO/ACI were analised. **Methods:** Male Wistar rats (280-320) were used. The vertebral arteries (VAs) plus the internal carotid arteries (ICAs) were progressively and permanently occluded, following different experimental sequences (2-VAs + ICAright → ICAleft or 2-VAs → ICAright → ICAleft) with interstage intervals ranging from 1 to 7 days. The treatment with sildenafil at doses of 0.75, 1.5 or 3.0 mg/Kg daily for 7 consecutive days started immediately after surgery. 30 days after the end of surgery the animals were deeply anesthetized, decapitated and the brain processed in paraffin for histological assessment. The number of intact–appearing pyramidal cells was counted in different hippocampal subfields (CA1, CA2, CA3 and CA4), stained with Celestine blue-acid fuchsin or Fluoro-Jade C. The experimental procedures were approved by the Ethics Committee on Animal \Experimentation of the State University of Maringá, Paraná, Brazil (CEEA 042/2007). **Results and Discussion:** This study showed that: i) the increase of the interstage occlusion intervals in the 4-VO/ACI model increased the rate of survival of rats; ii) when 3-stage rather than 2-stage 4-VO/ACI was used the rate of survival was also increased; iii) sildenafil (0.75 – 3.0 mg/Kg) prevented mortality when animals were subjected to 2-stage 4-VO/ACI with interstage intervals ranging from 3 ou 4 days between occlusions; iv) 4-VO/ACI caused reduction in the number of normal appearing pyramidal cells in all hippocampal subfields (CA1, CA2, CA3 and CA4) and v) sildenafil 3.0 mg/Kg prevented 4-VO/ACI-induced hippocampal damage in subfield CA1. Sildenafil reduces neurological deficits and promotes the functional recovery after 4-VO/ACI, probably because this drug leads to a significantly increased cerebral blood flow level [3]. However, future studies are required to investigate action mechanisms of sildenafil on survival rats and neurodegeneration of rats submitted to 4-VO/ACI model. **References:** [1] Barros et al., *Behav. Brain Res*. 197: 378-387, 2009; [2] Li et al., *Brain Res*. 1132: 185-192, 2007; [3] Puzzo et al., *Neuropsychiatr Dis Treat.* 4: 371-387, 2008. This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Universidade Estadual de Maringá (UEM).

02.015

Long-term treatment with fish oil prevents memory impairments but not hippocampal damage in rats subjected to transited, global cerebral ischemia. Mori MA[1], Ferreira EDF[2], Romanini CV[2], Schiavon AP[2], Milani H[2], Oliveira RMMW[2] [1]UEM - Ciências Biológicas, [2]UEM - Farmácia e Farmacologia

Introduction: Cerebral ischemia leads to neurodegeneration and cognitive impairment. Fish oil (FO) constitutes a rich dietary source of ω-3 polyunsaturated fatty acids especially docosahexaenoic acid (DHA). The objective of the present study was to investigate whether long-term treatment with commercial, high concentration DHA-containing FO could be effective in alleviating both the cognitive and neurodegenerative deficits caused by transient, global cerebral ischemia (TGCI) in rats. **Methods:** Naive rats were trained for 10 days in an 8-arm radial maze task and then subjected to TGCI for 15 minutes (4-VO model) 3 days later (day 13). Retention of the previously acquired cognition (ie, memory) was assessed weekly on days 20, 27, 34, 41, 48, and 55 and measured by 3 behavioral parameters as follows: (i) latency to find the goal box, (ii) number of reference memory errors, and (iii) number of working memory errors. The extent of pyramidal cell death in the hippocampus was examined at the end of the behavioral analysis on day 43. Fish oil (300 mg/kg DHA, gavage) administration occurred once daily beginning 3 days before TGCI (the last day of training) and continued until day 41. The experimental procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, Paraná (CEEA nº 015/2008.). **Results and Discussion:** Transient, global cerebral ischemia markedly disrupted memory performance measured by all 3 parameters ($P < .0001$ vs sham). This amnesic effect of ischemia persisted until the end of the behavioral analysis. Treatment with FO progressively reversed the TGCI-induced retention deficit until rats achieved control levels. This protective effect of FO on learning/memory function was clearly observed after both daily and cumulative data analysis ($P < .001$-0.01 vs vehicle). Such memory improvements remained statistically significant, even after cessation of FO treatment, indicating a sustained effect of FO. In contrast, FO failed to prevent ischemia-induced hippocampal damage in areas CA1, CA2, or CA4. Therefore, the present findings suggest that long-term FO treatment is able to facilitate functional recovery after ischemic brain damage, an effect that was distinct from hippocampal damage. This study was supported by the Conselho Nacional de Pesquisa (CNPq), Fundação Araucária, and Universidade Estadual de Maringá (UEM). **References:** [1] Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 2002;56:365-79; [2] CrawfordMA,Golfetto I,Ghebremeskel K,MinY,MoodleyT, Poston L, et al. The potential role for arachidonic and docosahexaenoic acids in protection against some central nervous system injuries in preterm infants. *Lipids* 2003;38:303-15; [3] Okada M, Amamoto T, Tomonaga M, Kawachi A, Yazawa K, Mine K, et al. The chronic administration of docosahexaenoic acid reduces the spatial congnitive deficit following transient forebrain ischemia in rats. *Neuroscience* 1996;71:17-25.

02.016

Chronic imipramine treatment enhances ischemia-induced hippocampal cell proliferation but fails in recovering spatial memory impairment in rats. Romanini CV[1], Schiavon AP[1], Mori MA[2], Ferreira EDF[1], Oliveira RMMW[1], Milani H[1] [1]UEM - Farmácia e Farmacologia, [2]UEM - Ciências Biológicas

Introduction: Transient global cerebral ischemia (TGCI) as well as chronic antidepressant treatment stimulate the proliferation of precursor cells in the dentate gyrus (DG) of the hippocampus (Malberg et al., 2000; Kawai et al., 2004). This study was aimed to determine whether imipramine further promotes the neurogenesis induced by TGCI in the rat dentate gyrus. The 8-arm aversive radial maze (AvRM) was used to address the effects of imipramine on ischemia-induced memory impairment (Fernandes et al., 2008). **Methods:** Rats were trained for 12 days in the AvRM and subjected to TGCI one day later. After that, the animals were administered imipramine (20 mg/kg, *i.p.*) or saline (1 ml/kg, *i.p.*) during 14 days. 5-bromo-2´-deoxyuridine-5´-monophosphate (BrdU, 200 mg/kg, i.p.) was injected 24 h after the last imipramine or saline injection to label proliferating cells. Retention of cognition was assessed weekly on days 16, 23 and 30 post-ischemia and measured by i) the latency to find the goal box, ii) the number of reference memory errors, and c) the number of working memory errors. Cell proliferation was examined 24 h after the last BrdU and neurogenesis was evaluated 15 and 31 days after ischemia by DCX-immunohistochemistry. The experimental procedures performed adhere to the ethical principles set down by the Brazilian College of Animal Experimentation (COBEA), and approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 066/2005), Paraná, Brazil. **Results and discussions:** The rate of cell proliferation increases 7 days after TGCI but returns to basal levels after 15 days. Concomitantly, there was an increase in the number of new neurons in the DG 15 days after ischemia. Imipramine chronic treatment increased the expression of BrdU and DCX-labeled cells in DG of ischemic rats, although it produced no effect on ischemia-induced memory disruption in the AvRM task. Similar to this study, in the Windle's (Windle and Corbett, 2005) study the pharmacological treatment was given for 14 days post-training. Similarly, fluoxetine administered for 14 days after stroke also failed to prevent stroke-induced both cognitive and sensorimotor deficits (Li et al., 2009). However, when fluoxetine was given for 28 days post-ischemia, the stroke-induced learning and memory impairment was reduced, and the rate of survival of newly generated neurons was increased (Li et al., 2009). Taken together, these data suggested that the duration of treatment with antidepressant drugs may be crucial for neurogensis-based functional recovery after ischemic brain injury. **Conclusion:** In conclusion, the present study shows that imipramine can promote the neurogenic response to cerebral ischemia, an effect that was not accompanied by recovery of cognitive function. Additional studies are needed to evaluate whether imipramine treatment for longer time than that used presently would be able to improve both acquisition (learning) and retention (memory) performance after TGCI, and its relationship with neurogenesis. This study was supported by the Conselho Nacional de Pesquisa (CNPq), Fundação Araucária, and Universidade Estadual de Maringá (UEM).

02.017

Further studies on the development of permanent, 3-stage, 4-vessel occlusion as a model of chronic and progressive cerebral hypoperfusion in rats. Ferreira EDF[1], Romanini CV[1], Mori MA[3], Schiavon AP[1], Oliveira RMMW[1], Milani H[1] [1]UEM - Farmácia e Farmacologia, [2]UEM - Ciências Biológicas

Introduction: Chronic cerebral hipoperfusion (CCH) may be causally related to age-associated dementia. Recently we started investigations on the development of an animal model of CCH based on bilateral occlusion of the vertebral arteries (VA) followed by stepwise ligation of the internal carotid arteries (ICA), with a 7-day interval between the occlusion (IBO) stages (4-VO/ICA model). Under these conditions, 4-VO/ICA did not cause neither hippocampal damage nor spatial learning/memory deficit measured 30 days after 4-VO/ICA. Now, other studies have been undertaken to determine the conditions under which 4-VO/ICA could be effective to cause neurohistological and cognitive outcomes. Here we are evaluating whether chronic 4-VO/ICA with an IBO shorter 7 days and chronicity larger than 30 days could be effective to cause both neuronal damage and cognitive impairment. **Method:** Rats were subjected to 4-VO/ICA according to the occlusion sequence AV®ICA®ICA. In a first experiment, an IBO of 7 days was used. Forty days later the spatial learning performance was measured in the aversive radial maze. In a second experiment, an IBO of 5, 4 or 3 days were used, and behavior was assessed 90 days later. At the end of behavioral testing the brains were examined histologically for damage to the hippocampus. The histomorphological integrity of the retina was also examined. This study was approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, Paraná (CEEA 045/2009). **Results and discussion:** Chronic 4-VO/ICA with a 7-day IBO, and a chronicity of 40-day did not cause neither hippocampal damage ($P > 0.05$) nor learning impairment (ANOVA, $P > 0.05$). Both sham and 4-VO/ICA groups learned the task very well across time ($P < 0.0001$). The morphology of retina was preserved after chronic 4-VO/ICA. In contrast, profound hyppocampal cell death, mainly in CA2, CA3 and CA4 was caused by 4-VO/ICA with an IBO of 3 or 4 days ($P < 0.001 – 0.05$). In the 5-day IBO group, neuronal death was restricted to the CA3 hippocampal sector. Cognitively, the groups with an IBO of 3 and 4 days took more time (latency) to accomplish the task, and committed more reference and working memory errors when compared to sham and 5-day IBO groups. These effects, however, did not reach statistical significance, probably because the small sample size. The data demonstrate that 4-VO/ICA with a 3- to 4-day IBO and chronicit of 90 days is able to cause profound and consistent hippocampal neurodegeneration. A clear tendency to cognitive impairment was also expressed, despite the lack of statistical significancy. Other individuals will be added to statistic, includinga group with a IBO of 2 days. Until the present, our data suggest that chronic, stepwise 4-VO/ICA procedure may be a reliable animal model of chronic cerebral hypoperfusion, with the advantage of preserving the neurohistological integrity of the retina. Further studies will be needed to investigate how the combination of IBO, chronicity of 4-VO and the age of animal should be used in order to optimize de 4-VO/ICA model of chronic, progressive cerebral hypoperfusion. Supportted by CNPq, Fundação Aráucária and UEM.

02.018

Modulatory action of glutamate on the catecholaminergic system in brainstem cell cultures from newborn rats. Silva SM[1], Carrettiero DC[2], Fior-Chadi DR[1] [1]IB-USP Fisiologia, [2]UFABC - Ciências Naturais e Humanas

Introduction: Glutamate (Glu) is the main excitatory neurotransmitter in the central nervous system. It is known to be a key neurotransmitter for the neural control of blood pressure in the nucleus of the solitary tract. The catecholamines are also involved with central cardiovascular regulation in medulla oblongata. Several studies in the literature reported an interaction between catecholamines and Glu, but this is not well addressed in medulla oblongata. Thus, the objective of the present study was to evaluate the modulation of the catecholaminergic system by glutamate in primary cell cultures from the medulla oblongata. **Methods:** All the procedures and protocols were performed in accordance with the Institutional Guidelines for Animal Experimentation (CEA/IB-USP: 084/2008). Cell culture was prepared from the medulla oblongata of newborn rats (n=30). Cells were enzymatically and mechanically dissociated, and plated in culture dishes pre-treated with Poly-D-Lisine and maintained for seven days in appropriated culture medium. Cells were characterized by immunohistochemical techniques, employing fluorescent antibodies against neurons and astrocytes. Cultures were treated with different concentrations of Glu and protein and mRNA levels of the α2 adrenoceptors (α2a) were analyzed 24 hours after treatment by Western Blotting and Real-Time PCR, respectively. All data were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni post test. **Results:** Results were expressed as percentage of control ± standard deviation. The percentage of neurons in culture was 15.6 ±2.1. The level of α2a protein in the cells was reduced by Glu treatment, in a dose-dependent manner (control: 100; 0,1uM 88.3 ± 3.8; 1uM: 62.5 ± 8.6; 10uM: 49.9 ± 5.5; 100uM: 72.4 ± 24.2). The level of α2a mRNA was also reduced in cultures treated with 10uM of Glu, after 12 and 24 hours (control: 1 ±0; 12h: 0.5 ± 0,2; 24h: 0,5 ± 0,1). α2a mRNA and protein expression are modulated by the treatment with Glu ($p<0.05$). **Discussion:** We showed that glutamate is able to modulate α2 adrenoceptor at the protein and mRNA level in cells from the medulla oblongata of newborn rats. We suggest that this modulation might be important to the neural mechanisms of blood pressure control. This study was supported by grants from FAPESP and CNPq.

02.019

Effect of reparixin on neutrophil recruitment in a mice model of middle cerebral artery occlusion. Sousa LFC[1], Candico, LCM[1], Valadão DF[2], Santos AG[2], Rodrigues DH[1], Teixeira MM[1], Teixeira AL[3], Coelho FM[1] [1]UFMG - Bioquímica e Imunologia, [2]UFMG - Microbiologia, [3]UFMG - Clínica Médica

Introduction: The early inflammatory response to stroke involves cerebral influx of neutrophils. It is disputable whether the modulation of this process is associated with less cerebral damage and, hence, a better neurological prognosis. In the present work, we investigated the effects of Reparixin, a noncompetitive allosteric blocker of the CXCR2 chemokine receptor, in a mice model of middle cerebral artery occlusion (MCAO). **Methods:** Eight-to-10–week-old male C57BL/6J (wildtype) mice were anesthetized and transient focal cerebral ischemia was induced by MCAO using an intraluminal filament method. The monofilament was removed after 90 minutes of occlusion. In the sham group, these arteries were dissected but no filament was inserted. The functional consequences of this cerebral ischemia/reperfusion (I/R) injury were evaluated after 24 hours by using a 5-point motor deficit score and the SHIRPA behavioral screen battery. The extent of neutrophil accumulation in the mouse tissue was measured by assaying MPO activity. The concentrations of chemokines CXCL1/CXCL2 and cytokine (IL-1b) were measured in the brain tissue using a commercially available enzyme-linked immunosorbent assay (ELISA). Treatment with Reparixin (30mg/kg) was given 1 hour previously to the ischemia. All experiments received prior approval from the UFMG ethics committee (certificate 166/2006). Data are expressed as means ± SEM and statistical analysis was performed using one-away ANOVA or t-student test ($p < 0.05$). **Results:** MCAO model induced an increase in the cerebral levels of CXCL1/CXCL2 at different time points. The peak of this chemokine concentration was at 24 h after ischemia (0.42±0.03 sham group and 0.56±0.02 I/R group (pg per 100mg of cerebral tissue), $p<0.05$). Because of this chemokine profile, Reparixin treatment was evaluated 24 h after ischemia. Reparixin treatment decreased the neutrophil recruitment to inflammated tissues (0.007±0.005 sham group, 0.219±0.176 I/R group and 0.072±0.011 on Reparixin Neutrophils (Relative Units), **$p<0.05$**). The neurological deficits assessed by SHIRPA (683.5±17.5 sham group, 462.4±186.4 I/R group and 570.3±95.5 on Reparixin (Motor Behavior), $p=0.1$) and 5 point motor deficit score (0±0 sham group, 1.3±1.2 I/R group and 2.4 I/R group and 1.3 on Reparixin) tended to be lower in Reparixin treated group. Also, Reparixin treatment lead to decrease of IL-1b (0,0196±0,007 sham group, 0.031±0.014 I/R group and 0,017±0,004 on reparixin group (pg per 100mg of cerebral tissue), **$p<0.05$**). **Conclusion:** The severity of the inflammatory response, as indicated by neutrophil recruitment and cytokine levels was reduced in mice treated with CXCR2 antagonist, Reparaxin. This result suggests that neutrophil influx into the brain following I/R may contribute to enhance cerebral damage and, hence, neurological sequels following stroke.

02.020

Ratos de alta e de baixa atividade no labirinto em cruz elevado diferem na atividade da K^+-*p*-nitrofenilfosfatase (K^+-*p*-NFFase) do hipocampo. Carvalho JGB, Benedicto MAC
UNIFESP - Psicobiologia

Introdução: O Labirinto em cruz elevado (LCE) é um dos modelos mais utilizados para a avaliação de ansiedade em animais de laboratório (Psychopharmacology, 112: 13-20, 1993). No rato, o comportamento de ansiedade é avaliado pelo medo apresentado em explorar os braços abertos do labirinto, por ser para este animal, um local aversivo e sem proteção (Naunyn-Schmiedeberg's Arch. Pharmacol., 327:1-5, 1984). Vários trabalhos têm mostrado que o hipocampo tem funções distintas nos processos emocionais, com um papel relevante no comportamento de medo/ansiedade (Behav. Neurosc., 118: 63-78, 2004). A Na^+-K^+/ATPase é responsável pela manutenção da excitabilidade neuronal (*Prog. Neurobiol.* 43: 37-71, 1994). Com base nessas informações, o objetivo deste trabalho foi verificar se ratos que diferem no comportamento de medo inato, avaliados pelo teste do LCE, apresentam diferenças na atividade da K^+-*p*-NFFase, que consiste na reação de defosforilação da Na^+-K^+/ATPase fosforilada, no hipocampo. **Métodos:** Ratos machos, adultos, da linhagem Wistar, de 3 meses de idade (N = 86) foram submetidos a uma única exposição ao LCE, por 5 minutos, para obtenção de 2 subgrupos de animais com características distintas: ratos de alta atividade (A.A) exploratória nos braços abertos do aparelho, e ratos de baixa atividade (B.A) exploratória nestes braços , tendo como parâmetros de avaliação, o número de setores cruzados (média ± desvio padrão: A.A = 32,7±7,8; B.A = 0,9±0,8, p =0,000071, Mann-whitney) e o tempo total de permanência nos braços abertos (A.A =101,6±30,6; B.A =6,1±4,8, p = 0,00007, Mann-whitney) (Behav. Brain Res., 39: 63-71, 1990). Adotando-se como critério de classificação, dados constantes acima ou abaixo da média mais ou menos 1 desvio padrão, foram selecionados 11 ratos A.A e 11 B.A, sendo os demais animais descartados. Os animais selecionados como A.A ou B.A foram reagrupados, pesados, e deixados em descanso por um período de aproximadamente 30 dias. Decorrido este período, estes dois grupos de ratos foram decapitados, sendo retirado o hipocampo. A atividade da enzima K^+-*p*-NFFase foi determinada em homogenatos ricos em terminais nervosos (*Brain Research*, 1058: 178-182, 2005). **Resultados:** A análise estatística mostrou haver uma diferença da atividade da K^+-*p*-NFFase do hipocampo entre os grupos de ratos A.A (N=10, média±EPM = 158,8±6,6 nmol de *p*-nitrofenol/mg proteína/min) e B.A (N=10 =135,0±4,1, p = 0,007, teste t de Student). **Discussão:** Os resultados obtidos sugerem o envolvimento da enzima Na+/K+-ATPase hipocampal na ansiedade. É possível que uma menor atividade da enzima no hipocampo dos ratos B.A, leve a uma repolarização neuronal mais lenta, alterando a excitabilidade hipocampal. **Apoio financeiro:** AFIP**. CEP** 1023/06**.**

02.021

Pharmacological activity of *Ruta graveolens* L.: disagreement between ethnopharmacological information and experimental data. Kohn DO, Costa CARA, Costa M IBB-UNESP - Farmacologia

Introduction: *Ruta graveolens* L., commonly known as rue (arruda in Brazil), is often reported as anxiolytic and anticonvulsant in ethnopharmacological studies. These possible actions, however, have not yet been experimentally demonstrated. The aim of this study is to analyze the effectiveness of preparations obtained from its fresh leaves: crude hidroalcoholic extract (CE) and its aqueous (AF) and hexanic fractions (HF). The experimental evaluation was made using an obsessive-compulsive disorder (marble burying test – MB – Ethical Committee License Number 009/06-CEEA) and a generalized absence seizure (pentylenetetrazol induced seizures test – PTZ – Ethical Committee License Number 010/06-CEEA) models. **Methods:** Experimental procedures were carried out using male Swiss mice (about 45 days old, n=6-8) treated 30 minutes before the beginning of each test with three different doses (100, 300 or 500mg/kg, orally given) of CE, AF or HF. In the MB test each pre-selected mice was placed in a covered cage with 25 glass marbles distributed on a 5-cm layer of sawdust. After 30 minutes, they were removed and the number of marbles totally hidden by sawdust was counted. In the PTZ test the latency for the first seizure and the number of seizures were counted up to 30 minutes after subcutaneous administration of PTZ (85mg/kg). Results [median(Q1-Q3)] were compared by Kruskal-Wallis Test, followed by Mann-Whitney Test, when necessary and differences were considered significant when $p \leq 0,05$. **Results:** None of the extracts showed protective effect in PTZ test. In the MB test, doses of 500mg/kg of AF [18(16-18)] and HF[19(16-20)] reduced the number of hidden marbles when compared to their control groups: saline[25(25-25)] and Tween 80® 2%[24(22-25)], respectively. **Discussion:** According to these results, fractions obtained from the hidroalcoholic extract were active in a procedure that evaluates a kind of anxiolytic disorder which is clinically treated with imipramine (among other antidepressive drugs). This profile of action has no relation with the inhibitory activity of GABA receptors, which can be altered by some drugs effective in PTZ model. Thus, we can suppose that different constituents present in fractions from CE are involved in different biological activities. This supposing could be tested in the continuity of this study, by identifying the chemical constitution of fractions. Financial Support: FAPESP (nº 06/07195-8)

02.022

Overexpression of cellular prion protein (PrP^C) in transgenic mice confers neuroprotection against intracerebroventricular infusion of beta-amyloid peptide $A\beta_{1-40}$.

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Introduction: Cellular prion protein (PrP^C) is a neuronal anchored glycoprotein that associates with multi-molecular membrane complexes, which mediates a variety of functions as synaptic plasticity, cognition and protection against oxidative stress. Recent studies demonstrated that mice lacking PrP^C disclose increased amyloidogenic process and decreased resistance to age-related locomotor and cognitive deficits. Considering the neurotoxic properties of $A\beta_{1-40}$ peptide, the aim of the present study was to determine the role of PrP^C in behavioral, neurochemical and neural viability alterations induced by central infusion of $A\beta_{1-40}$. **Methods:** 3 months-old male mice with different levels of PrP^C expression, control group ($Prnp^{+/+}$), PrP^C-knockout ($Prnp^{0/0}$) and PrP^C-overexpression mice (Tg-20) received a single i.c.v. infusion of $A\beta_{1-40}$ peptide (400pmol) or similar PBS volume. Animals were evaluated in locomotor (open field) and cognitive tasks (Morris water maze), 14 days after $A\beta_{1-40}$ infusion. Subsequently, neural tissue was collected for assay of viability (MTT and Propide Iodide in hippocampus) and acetylcholinesterase activity (cortex, hippocampus and cerebellum). CEUA-UFSC: PP00111. **Results and Discussion:** No differences were observed in locomotor activity among strains or treatment groups. In Morris water maze $Prnp^{+/+}$ $A\beta_{1-40}$ treated mice performed poorly when compared to PBS treated group, indicating a disruption in spatial memory both in training and test sessions (short and long-term memory, respectively). $Prnp^{0/0}$ mice despising the treatment showed not a satisfactory learning curve indicating that the genetic ablation of PrP^C induces cognitive deficits. Conversely, Tg-20 treated groups (PBS or $A\beta_{1-40}$) presented similar cognitive performance in training and test sessions inferring that the overexpression of PrP^C is able to counteract the $A\beta_{1-40}$–induced mnemonic disruption. These cognitive findings were in agreement with the MTT assay showing that treatment with $A\beta_{1-40}$ peptide diminished the neural viability in both $Prnp^{+/+}$ and $Prnp^{0/0}$ mice, but it did not in the Tg-20 animals (PBS or $A\beta_{1-40}$). In propide iodide (PI) assay, the $Prnp^{0/0}$ group showed significant increased PI captation when compared to $Prnp^{+/+}$ group. Otherwise Tg-20 mice presented reduced membrane permeability, suggesting lower levels of hippocampal cell death in comparison to $Prnp^{+/+}$ and $Prnp^{0/0}$ groups. Similar to the MTT assay, in PI captation, the i.c.v. infusion of the $A\beta_{1-40}$ peptide significantly reduced the neural viability in $Prnp^{+/+}$ and $Prnp^{0/0}$, but not in Tg-20 group of animals. We observed a significant reduction in the cortical acetylcholinesterase activity in Tg-20 mice treated with $A\beta_{1-40}$. Our results indicate a neuroprotective role for PrP^C-overexpression against $A\beta_{1-40}$ toxic events where increased availability of acetylcholine in synaptic cleft may be a compensating mechanism. **Financial support**: CAPES, CNPq and FAPESC.

02.023

Adenosine deaminase mRNA is differentially modulated by nicotine on medulla oblongata cells of normotensive and spontaneously hypertensive rats. Matsumoto JPP[1], Ferrari MFR[2], Fior-Chadi DR[1] [1]IB-USP - Fisiologia, [2]FMUSP - Neurologia

Introduction: Adenosine levels in the nucleus tractus solitarii (NTS) and other brain areas can increase during periods of physiological stress. Adenosine deaminase (ADA) is a key enzyme involved in the metabolism of adenosine intra and extracellularly. Variations in its activity reflect directly in adenosine homeostasis which might be involved in several physiological disorders. Nicotine acts in several brain areas including the NTS and modulate mRNA expression of intracellular components, including enzymes. The goal of this study was to analyze the effects of nicotine exposure on adenosine deaminase mRNA expression in cultured medulla oblongata cells of spontaneously hypertensive (SHR) and normotensive rats (WKY). **Methods:** All the procedures and protocols were performed in accordance with the Institutional Guidelines for Animal Experimentation (CEA/IB-USP protocol number 065/2008). Cell cultures were made using the medulla oblongata of one-day old WKY and SHR (n=30). Viable cells were plated and subjected to dose response and time course experiments. Cells were treated with 1, 10 and 100μM of nicotine for 24 hours in dose-response experiments. For time-course experiments cells were treated with 10μM of nicotine for 4, 12, 24 and 48 hours. Cells were washed after the treatment and total RNA was extracted and Real Time RT-PCR was carried out for quantitative analysis of adenosine deaminase mRNA expression. All data were evaluated by two-way analysis of variance following by Boferroni post-test. **Results:** Results were expressed as fold-change ± standard deviation. Cells from the SHR (1.42±0.8) express more ADA mRNA as compared to WKY (1.00±0.1) at basal condition. In WKY cells ADA mRNA expression was increased only at 4hrs (1.63±0.6) after nicotine treatment. On the other hand, nicotine induced a time-dependent increase in ADA mRNA expression in SHR cells showing the peak value after 48 hrs (2.88±0.3) as compared to it respective control (1.39±0.3). Nicotine induced a dose-dependent increase in ADA mRNA expression reaching the peak response with 100μM in the WKY (1.42±0.7) and the SHR (2.05±0.5) compared to their respective controls (WKY1.00±0.1; SHR1.39±0.8). ADA mRNA expression is modulated by the strain and treatment alone, as well by the interaction between strain and treatment ($p<0.05$). **Discussion:** We demonstrated that nicotine is able to differently modulate adenosine deaminase mRNA expression according to rat strains. This suggests that different mechanisms could be recruited in response to nicotine exposure. Nevertheless, the mechanism involved in this modulation needs further investigation. This study was supported by grants from FAPESP, CAPES and CNPq.

02.024

A relação entre o comportamento de *rearing* no campo aberto e a Na^+/K^+-ATPase no hipocampo. Alves R[1], Carvalho JGB, Benedicto MAC UNIFESP - Psicobiologia

Introdução: A Na^+/K^+-ATPase está envolvida em vários aspectos do funcionamento do Sistema Nervoso Central (SNC), dentre eles, na repolarização da membrana neuronal e captação/liberação de neurotransmissores. Por isso, diferenças individuais na atividade central da Na^+/K^+-ATPase podem resultar em diferenças no funcionamento do SNC que, consequentemente, podem levar a diferenças comportamentais. Dados de literatura mostram que a inibição da atividade da Na^+/K^+-ATPase induz mudanças no comportamento exploratório. Em trabalhos anteriores mostramos que ratos machos Wistar, selecionados no teste do Campo Aberto em subgrupos Baixo *Rearing* (BR) e Alto *Rearing* (AR) diferem na atividade da Na^+/K^+-ATPase no hipocampo (animais AR apresentam maior atividade da enzima). Existe uma correlação entre a ouabaína (inibidora da Na^+/K^+-ATPase) e a expressão das isoformas da Na^+/K^+-ATPase (α1, α2 e α3). O objetivo desse trabalho foi verificar se subgrupos selecionados no teste do Campo Aberto de acordo com o número de "rearing" diferem na ligação da [^{3}H]-Oubaína ao sítio alostérico para ouabaína no hipocampo, indicativo de diferença na expressão das isoformas da Na^+/K^+-ATPase. **Métodos:** Ratos Wistar, machos, 3 meses de idade, foram submetidos a uma sessão de 3 min no teste do Campo Aberto. De acordo com o número de "rearing" foram selecionados dois subgrupos: BR (14,3±0,63; n=29; média±EPM) e AR (34,8±0,85; n=27; média±EPM). Vinte dias após a seleção, os ratos foram sacrificados e o hipocampo foi dissecado. Para obter a fração mitocondrial, o homogenato foi preparado em sacarose 0.32M gelada. A curva de saturação da ligação da [^{3}H]-Ouabaína para o sítio de alta afinidade da isoforma α1 foi obtida por meio de 8 concentrações do ligante (6-296 nM). Outro experimento de ligação da [^{3}H]-Ouabaína na concentração de 1200 nM do ligante foi realizado para determinar a ligação nas subunidades de baixa afinidade (α2 e α3). A curva de saturação foi obtida pelo Prizma (San Diego, USA), para a determinação de Bmax e Kd. O teste t de Student foi realizado para verificar possíveis diferenças entre os subgrupos. O nível de significância para todas as comparações foi previamente estabelecido em $p \leq 0,05$, bicaudal. **Resultados:** Os resultados obtidos mostraram uma diferença estatisticamente significativa entre os subgrupos BR e AR na ligação da [^{3}H]-Ouabaína na concentração de 1200 nM (BR: 59,2±1,7 pmol/mg protein; n=8; mean±SEM; e AR: 65,8,0±1,0, n=8; $t_{(df=14)}=3,28$, p=0,005). Não houve diferença estatisticamente significativa entre os subgrupos no Bmax (BR: 75,2±4,7 pmol/mg protein; n=8; mean±SEM; e AR: 86,6±10,7; n=6; test $t_{(df=12)}=1,06$, p=0,31) e Kd (BR:51,3±4,8 nM; n=8; mean±SEM; e AR: 51,9±6,7; n=6; teste $t_{(df=12)}=0,08$, p=0,94) obtidos a partir da curva de saturação. **Discussão:** Esses resultados indicam que os subgrupos BR e AR diferem na concentração da Na^+/K^+-ATPase contendo a isoforma α1 no hipocampo, sugerindo que diferenças na atividade da Na^+/K^+-ATPase no hipocampo está envolvida na expressão do comportamento de *rearing*. **CEP** nº 1023/06. **Apoio financeiro:** FAPESP e AFIP

02.025

Evaluation of behavioral and pharmacological effects of *Hedyosmum brasiliense* and isolated sesquiterpene lactones in rodents. Souza MM[1], Zettermann LT[1], Bitencourt, DR[1], Mora TC[2], Oliveira, FL[3], Tolardo R[4] [1]UNIVALI Farmácia, [2]NIQFar-UNIVALI, [3]UNIVALI - Ciências Farmacêuticas, [4]UNIVALI - Farmacologia

Introduction *Hedyosmum brasiliense* is an essentially Brazilian species largely found in Atlantic Forest. It is popularly known as “cidrão” and in folk medicine this aromatic species is widely used as calmative/tranquilizer and for the treatment of sleep disorders. In the present study is examined the anxiolytic, antidepressant and hypnotic effect the ethanol extract of fresh leaves of *Hedyosmum brasiliense* Miq. (Chloranthaceae) (HB) and the antidepressant effect of the isolated sesquiterpene lactones podoandin and 13-hydroxy-8,9-dehydroshizukanolide. **Materials, methods and Results***:* The animals (rats, 250-300g / mice, 25-30g) were obtained from the Central Biotério UNIVALI and the experimental protocols were submitted to the ethics committee CEP-UNIVALI and approved (CEP-436/06). The effects of HB are demonstrated by the open field, elevated plus-maze, forced swimming, pentobarbital-induced sleeping time, PTZ- induced seizures tests and inhibitory avoidance tests. HB did not showed a protective effect against PTZ induced convulsions. In the Plus Maze Test, HB exhibited an anxiolytic effect through the effective enhancing of the frequency and time spent in the open arms (75 and 54.76%, respectively) as compared to controls, on the contrary, the time of permanence and the number of entrances in the closed arms were decreased (47.16 and in and 34.26%, respectively. All these effects were also completely reversed by pre-treatment with flumazenil (2.5 mg/kg, i.p./an antagonist of benzodiazepine receptors) similarly to those observed with diazepam used as a positive standard. In this test, the effect anxiolytic of HB also was totally blocked by reserpine (2.0 mg/kg, i.p.) pretreatment a drug known to induce depletion of biogenic amines. In the forced swimming test, the treatment of EB given acute and chronically, produced a decreased the immobility time (54.73%,88.23 %respectively) similarly to that of imipramine (10 mg/kg, i.p), the positive control. For the two isolated compounds (podoandin and 13-hidroxi-8,9-dihydroshizukanoide) tested in a single dose (10 mg/kg, i.p.), the antidepressant effect was observed only with the compound podoandin, that also decreased immobility time (56.66%). HB also produced decreased significantly the latency sleep time in 35.71 %, and on the contrary ,the treatment with HB) produced a increased of total time of sleep 82.59 and 100% suggesting a potentiation of the pentobarbital effect. EB did not interfere in memory consolidation. The results suggest that HB presents psychopharmacological activities, including anxiolytic, antidepressant, hypnotic and anticonvulsive effects. **Financial support:** CNPq - FAPESC

02.026

Effects of clonazepam (CNZP) on the acetyl cholinesterase activity in the pilocarpine-induced seizures in mice. Linhares MI[1], Oliveira, AA[1], Carvalho, AMR, Moura BA, Rios ERV, Venâncio ET, Sousa FCF, Fonteles MMF UFC - Fisiologia e Farmacologia

Introduction: The CNZP effects on epilepsy treatment have been demonstrated as being bigger than other benzodiazepines. By the way, antagonists of acetyl cholinesterase (AChE) present excitatory effects similar to colinergic agents, such as pilocarpine. The reduction on acetylcholine metabolism by AChE inhibition facilitates the installation of epileptic activity by the increase in endogenous acetylcholine (Imperato et al., 1998). The aim of the present study was to investigate the influence of the AChE activity on the antiepileptic effects of CNZP. **Methods:** Swiss adult male mice (25-35g) were divided into four groups, with 10 animals each, and received CNZP (0,5mg/kg) or saline solution (0,9%), i.p., alone or 60 min before pilocarpine administration, 400mg/kg, s.c. (P400). AChE activity was determined in hippocampus and striatum according to Ellman et al.,1961. **Results:** The following results were obtained: a) Hippocampus: Control = 31,73 $\pm$ 3,38; CNZP = 47,65 $\pm$ 4,61; P400 = 17,05 $\pm$ 3,13; CNZP + P400 = 83,12 $\pm$ 9,13. b) Striatum: Control = 104,20 $\pm$ 13,72; CNZP = 104,10 $\pm$ 15,81; P400 = 113,20 $\pm$ 7,94; CNZP + P400 = 90,01 $\pm$ 8,48. Specific activity was expressed in nanomoles of hydrolyzed ATC by mg of protein by minute (nmoles/mg of protein/min). **Conclusion:** The obtained data showed an increase of the AChE activity induced by CNZP previous administration on the hippocampus. It contributes to explain the antiepileptic effects of CNZP in the present animal seizure model. No significant alteration was observed on striatum. Supported by: CNPq and PIBIC/CNPq/UFC

02.027

Influence of melatonin on the acetyl cholinesterase activity in mice hippocampus and striatum submitted to seizures induced by pilocarpine. Linhares MI, Oliveira, AA, Carvalho, AMR, Moura BA, Rios ERV, Venâncio ET, Sousa FCF, Fonteles MMF UFC - Fisiologia e Farmacologia

Introduction: Melatonin (MEL) seems to play an important role in many behavioral processes including those related to anxiolytic, sedative, hypnotic and anticonvulsant properties. Seizures elicited by pilocarpine, a muscarinic cholinergic agonist, have been proposed as an animal model resembling some aspects of human temporal lobe epilepsy (Koop et al., 2000). The reduction on acetylcholine metabolism by acetyl cholinesterase (AChE) inhibition facilitates epileptic activity installation by the increase in endogenous acetylcholine. This study aimed to investigate the effects of MEL on the AChE activity in mice hippocampus and striatum. **Methods:** Swiss adult male mice (25-35g) were divided into four groups, with 10 animals each, and received MEL (5mg/kg) or saline solution (0,9%), i.p., alone or 60 min before pilocarpine administration (400mg/kg, s.c., P400). AChE activity was determined in hippocampus and striatum according to Ellman et al., 1961. **Results:** The following results were obtained: a) Hippocampus: Control = 31,73 $\pm$ 3,38; MEL = 46,17 $\pm$ 4,83; P400 = 17,05 $\pm$ 3,13; MEL + P400 = 46,95 $\pm$ 6,73. b) Striatum: Control = 104,20 $\pm$ 13,72 ; MEL = 177,80 $\pm$ 37,08; P400 = 113,2 $\pm$7,941; MEL+P400= 133,6 $\pm$ 13,87. Specific activity was expressed in nanomoles of hydrolyzed ATC by mg of protein by minute (nmoles/mg of protein/min). **Conclusion:** Our findings demonstrated a significant increment on AChE activity in the hippocampus after MEL administration, and a tendency to an increment in the striatum. It suggests a contribution of melatonin in protection events against the seizures induced by pilocarpine in mice. Supported by: CNPq and PIBIC/CNPq/UFC

02.028

Estrógeno minimiza morte celular causada por H_2O_2 em células de glioma de rato.
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Introdução: Várias evidências sugerem que a glia, além das funções de nutrição e sustentação, desempenha uma atividade importante na sinalização neuronal e na resposta inflamatória no sistema nervoso central. A presença de uma resposta inflamatória crônica está associada a uma série de doenças degenerativas relacionadas com o envelhecimento, tais como Parkinson e Alzheimer. Já se sabe que os receptores nucleares clássicos de estrógeno α e β (ER-α e ER-β) ficam retidos no citoplasma, de maneira inativada e que, na presença de estrógeno (E2), migram para o núcleo e funcionam como fatores de transcrição, modulando a transcrição de vários genes. Além deles, sugere-se que os receptores de membrana para E2, como o GPR30, seriam responsáveis pela sinalização aparentemente não-genômica e rápida deste hormônio. Ambos os tipos de receptores são muito importantes para as manifestações dos efeitos chave do E2 no encéfalo, tais como neuroproteção, plasticidade e controle da homeostase, uma vez que esses sinais modificam proteínas. O objetivo do nosso trabalho é analisar a participação dos receptores de estrógeno (ER-α, ER-β e GPR30) na proteção desencadeada por esse hormônio em linhagem de células C6 de glioma de rato, frente a um estímulo tóxico, o peróxido de hidrogênio (H_2O_2). **Material e Métodos:** Células C6 foram mantidas em meio de cultura DMEM sem vermelho de fenol suplementado com 10% de soro bovino fetal (SBF), 100 unidades/ml penicilina e estreptomicina. As células ($5x10^3$) foram pré-tratadas com 17-β-estradiol (E2) por 2 horas em várias concentrações (0,01µM, 0,1µM e 1µM), e depois incubadas com H_2O_2 (100 µM) por mais 24h na presença ou ausência de E2. Após esse tempo, foi coletado 50µL do meio, com o qual a morte celular foi avaliada pela presença da Lactato desidrogenase (LDH), que é uma enzima que é liberada para o meio extracelular na lise da célula. Ensaios de PCR, western blot e imunofluorescencia confirmaram a presença dos receptores de estrógeno ER-α e ER-β em células C6 e sua ativação e migração para o núcleo na presença de E2. Nossos resultados também confirmaram que a H_2O_2 na concentração de 100µM (0,23 ± 0,006 µU/µL) induz morte celular ($P<0,001$ n=6 vs controle 0,19 ± 0,008 µU/µL, n = 6) em células C6. O pré-tratamento com E2 tanto por 2 horas quanto por 24 horas diminui a toxicidade induzida por H_2O_2 de maneira dose-dependente ($P<0,05$ n=3 vs controle). E esse efeito protetor ocorre mesmo na ausência de E2 durante a incubação com H_2O_2. Nossos resultados confirmaram a presença dos ER-α e ER-β nas células C6 e que esses são funcionais, pois migram para o núcleo na presença de E2. Também notamos que as células respondem ao tratamento com E2 por 2 horas, desenvolvendo proteção frente a H_2O_2, caracterizando um efeito rápido de resposta possivelmente associado aos receptores de membrana. **Conclusão:** O E2 protege células C6 em modelo de morte celular induzido por H_2O_2, sugerindo uma participação desse hormônio e seus receptores em uma possível via farmacológica de prevenção da propagação de estímulos lesivos às células da glia. Apoio financeiro: FAPESP, CNPq e Procontes-USP

02.029

Efeitos da restrição calórica sobre a Na,K-ATPase na vigência de um estímulo inflamatório no cerebelo de ratos jovens e idosos. Vasconcelos AR, Sá Lima L, Yshii LM, Scavone C, Kawamoto EM ICB-USP - Farmacologia

Introdução: A restrição calórica sem desnutrição pode apresentar muitos efeitos benéficos ao expor os organismos a um estresse nutricional moderado, que não apenas estimula as proteínas de estresse, mas também os mecanismos de defesa do organismo, tornando a célula ou o organismo mais resistente a estímulos tóxicos. A enzima Na^+,K^+-ATPase (NAKA) é uma proteína de membrana que, através da utilização da energia proveniente da hidrólise de uma molécula de ATP, mantém as concentrações do meio intracelular de K^+ elevadas e as de Na^+ baixas. A enzima é uma proteína tetramérica constituída por duas subunidades α e duas subunidades β. A subunidade α apresenta três isoformas, que podem ser classificadas em dois tipos tomando-se como base a sua localização predominante: Isoforma Comum ($i\alpha_1$) e Isoforma Cerebral ($i\alpha_2$ e $i\alpha_3$). O glicosídeo ouabaína é um inibidor específico da NAKA. O seu sítio de ação está localizado na subunidade α. As $i\alpha_2$ e $i\alpha_3$ são sensíveis a concentrações de ouabaína 1000 vezes menores (3 μM) do que as necessárias para inibir a $i\alpha_1$ (3 mM). Portanto, através da medida de atividade da ATPase total na ausência e na presença de ouabaína (3 μM e 3 mM) é possível determinar as atividades das $i\alpha_1$ e $i\alpha_{2/3}$ da NAKA. Neste projeto nós comparamos os efeitos do LPS sobre a atividade das $i\alpha_1$ e $i\alpha_{2/3}$ da NAKA no cerebelo de ratos jovens e idosos submetidos à restrição calórica. **Métodos:** Foram administradas doses intravenosas de salina estéril ou de LPS dissolvido em salina (1mg/kg) em ratos machos Wistar jovens (4 meses) e idosos (24 meses) do grupo controle ou submetidos ao protocolo da dieta intermitente por 30 dias (dias alternos com e sem ração) (Protocolo Ética ICB/USP – nº 89, fls. 60 do livro 02, 15/10/2008). Após 2 horas, os ratos foram sacrificados e seus cerebelos foram retirados. As amostras de tecido foram homogeneizadas e utilizadas para medir a atividade da enzima NAKA. O método adotado na determinação da atividade da enzima se baseia na quantificação de moléculas de fosfato (Pi) livres provenientes da hidrólise de ATP por espectrofotometria, após a reação de complexação com molibdato de amônio, formando um cromóforo cuja absorbância é determinada através da leitura em comprimento de onda de 700 nm. **Resultados:** A atividade da $i\alpha_1$ da NAKA diminuiu nos ratos jovens que receberam as injeções de LPS em relação ao grupo controle, enquanto que observamos um aumento na atividade da $i\alpha_1$ nos ratos submetidos à dieta. Nestes ratos o LPS não induziu uma redução da atividade da $i\alpha_1$. Com relação as $i\alpha_{2,3}$ podemos notar que o LPS induziu um aumento na atividade enzimática no grupo submetido à restrição calórica. O mesmo resultado foi obtido nos animais idosos em relação as $i\alpha_1$ e $i\alpha_{2,3}$ onde os animais submetidos à restrição apresentaram aumento da atividade frente ao estímulo inflamatório. **Discussão:** Os resultados sugerem que os animais idosos perdem a capacidade de modulação da NAKA frente a um estímulo inflamatório, e que esta resposta adaptativa foi restabelecida nos ratos submetidos à restrição calórica. Neste sentido, a restrição calórica parece atuar como um agente neuroprotetor na medida em que restabelece o poder de modulação da NAKA frente a um estímulo inflamatório. **Apoio financeiro:** FAPESP, CNPq.

02.030

Antidepressant-like effect of carvacrol (5-Isopropyl-2-methylphenol) in mice. Citó MCO[1], Félix FHC[1], Moura BA[1], De Sousa DP[2], Macedo DS[1], Sousa FCF[1] [1]UFC - Fisiologia e Farmacologia, [2]UFS – Fisiologia

Many studies have demonstrated that plant derived essential oils exhibit a variety central activities properties. According to these informations, herbal medicine can be used as an alternative treatment to depression with less side-effects. Carvacrol (5-Isopropyl-2-methylphenol) is a monoterpenic phenol present in the essential oil of many plants. It is the major component of the essential oil fraction of oregano and thyme. Considering the antidepressant potential of plant derived essential oils and the importance of natural products as sources of new drugs the present experiments were designed to investigate the effect of carvacrol on animal models of antidepressant activity. This work was approved by Committee on Ethics in Animal Research (CEPA), number 15/09. In the present work, the effects of carvacrol (cvc) were studied in the tail suspension test (Steru et al 1985) and the forced swimming test (Porsolt et al, 1977). Carvacrol (cvc) was administered orally at single doses of 12.5; 25 and 50 mg/kg while imipramine 10 mg/kg, imipramine 30 mg/kg and fluoxetine 35 mg/kg were used as standard drugs and PCPA (100 mg/kg) was used to investigate the involvement of the serotonergic system in the anti-immobility effect of carvacrol in the forced swimming test. The results are presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Student-Newman-Keuls´s *post hoc* test. Results were considered significant at $P<0.05$. Results showed a significant reduction on the immobility time on forced swimming test [control: 98.88±4.987 (8); CVC 12.5 mg/kg 44.50±7,607 (8); CVC 25 mg/kg 12.70±1.630 (8); CVC 50 mg/kg 17,74±3.587] as compared to control. Imipramine 10 mg/kg also decreased the immobility time [IMI 16.08±1.603 (8)] as compared to control. Similar to those results observed in forced swimming test, in tail suspension test carvacrol (12.5, 25 and 50 mg/kg) and imipramine 30 mg/kg significantly decreased the immobility time in animals as compared to the control group [control: 90.86±8.678 (10); CVC-12.5: 50.64±1.899 (9); CVC-25: 36.21±4.538 (8); CVC-50: 36.64+4.786 (7); IMP-30: 17.57±1.948 (7)]. Results showed that the pretreatment of mice with PCPA (100 mg/kg) did not affect the antidepressant-like effect of carvacrol (25 mg/kg), but completely blocked the decrease in the immobility time elicited by fluoxetine (35 mg/kg) as compared to control group [control: 75.50±5.622 (8); CVC 25: 48.48±3.079 (7); FLU-35: 43.02±3.778 (10); PCPA: 107.1±7.583 (10); CVC-25+PCPA: 52.57±4.368(10); FLU-35+PCPA: 111.7±7.978(10)]. The results suggest that acute treatment with carvacrol at doses of 12.5; 25 and 50 mg/kg presents antidepressant-like effect and that this effect in not involved with serotonergic transmission. Financial Support: (CNPq). Porsolt et al, *Eur J Pharmacol*, 47:399, 1978; Steru et al, *Psychopharmacol* 85:367, 1985; Sousa et al. *Pharmacol, Biochem and Behav*, 78: 27, 2004. Gomes et al. *Journal of Ethnopharmacology* 120: 209, 2008.

02.031

Anxiolytic-like effect of carvacrol (5-isopropyl-2-methylphenol) in mice. Silva FCC[1], Félix FHC[1], Citó MCO[1], Santos LKX[1], De Sousa DP[2], Sousa FCF[1] [1]UFC - Fisiologia e Farmacologia, [2]UFS - Fisiologia

Introduction: Many studies have demonstrated that plant derived essential oils exhibit a variety central activities properties. Several of these described effects are frequently attributed to monoterpenes, whose are the major chemical components of those essential oils. Carvacrol (5-Isopropyl-2-methylphenol) is a monoterpenic phenol present in the essential oil of Labitae. Since there are no studies in the literature on the central actions of carvacrol, in the present study we performed a pharmacological investigation of carvacrol on anxiety. **Methodology:** This work was approved by Committee on Ethics in Animal Research, number 15/09. In the present work, the effects of carvacrol (CVC) were studied in two behavior animal models: elevated plus maze (EPM), described by Lister (1987) and barbiturate-induced sleeping time, described by Wambebe (1985) tests in mice. CVC was administered orally at single doses of 12.5; 25 and 50 mg/kg while diazepam 1 mg/kg was used as standard drug and flumazenil (2.5 mg/kg) was used to elucidate the possible mechanism by which CVC is actuating in the antianxiety investigation on EPM. The results are presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Student-Newman-Keuls´s *post hoc* test. Results were considered significant at $p<0.05$. **Results:** In the EPM groups treated with CVC (12.5; 25 and 50mg/kg) and diazepam (1mg/kg) significantly increased NEOA compared to control group [control: 4.214±0.4591 (10); CVC-12.5: 7.071±0.7222 (10); CVC-25: 9.357±0.9412 (10); CVC-50: 7.786±0.8064 (10); DPZ-1: 10.46±0.6466 (10)]. The studied doses of CVC and diazepam increased all other parameters analyzed, PEOA, TPOA and PTOA compared to respective controls: PEOA [control: 30.68±2.962 (10); CVC-12.5: 44.29±3.402 (10); CVC-25: 46.23±3.238; CVC-50: 43.37+3.101 (10); DZP-1: 63.56±2.594 (10)]; TPOA [control: 52.00±7.760 (10); CVC-12.5: 86.13±8.486 (10); CVC-25: 99.31±9.614 (10); CVC-50: 87.74±8.413 (10); DZP-1: 168.7±5.366 (10)]; PTOA [control: 23.61±3.201 (10); CVC-12.5: 39.09±3.882 (10); CVC-25: 49.14±3.990 (10); CVC-50: 44.00±3.647 (10); DZP-1: 61.27±2.974 (10)]. Results showed that the pretreatment of mice with flumazenil (2.5 mg/kg) blocked the anxiolytic effect of CVC (25 mg/kg) and diazepam (1 mg/kg) in the EPM test and did not altered significantly all the parameters analyzed, NEOA, PEOA, TPOA and PTOA compared to respective controls. In the barbiturate-induced sleeping time, CVC did not alter the parameters sleeping latency and sleeping time as compared to control group. Animals treated with diazepam (1mg/kg) presented a decrease in the sleep latency [control: 247.2±15.62 (9); DZP-1: 177.7±4.042 (10)] and prolongation of pentobarbital-induced sleeping time [control: 1441±272.8 (8); DZP-1: 4118±192.8 (10)]. **Discussion:** The results suggest that acute treatment with CVC at doses of 12.5; 25 and 50 mg/kg presents anxiolytic-like effects and that this effect is involved with gabaergic transmission as demonstrated in the EPM test. It's important that CVC at the three studied doses did not present sedative properties as demonstrated in sodium pentobarbital sleeping test. **References:** Lister RG. *Psychopharmacology* 92: 180, 1987; Wambebe C. *Braz J Pharmacol* 84: 185, 1985. **Financial Support**: CNPq

02.032

Social isolation of young adult rats induces an increase in the expression of AMPA glutamate receptors in hippocampus. Tonso VM[1], Sinhorini ERA[2], Pereira MTR[1], Limonte FH[2], Iyomasa MM[3], Rosa MLNM[1] [1]FAMECA – Bioquímica, [2]FAMECA-FIPA - Bioquímica, [3]FORP-USP - Morfologia, Estomatologia e Fisiologia

Introduction: Glutamate is involved in several physiological functions and pathological processes of the brain, by interacting with distinct receptors. Studies using *postmortem* human brains with depression and animal models have shown that glutamate is involved in this disorder. The social isolation is a chronic affective stress largely used as experimental model of psychiatric disorders like depression. The aim of this study was to investigate the changes in GluR1 and GluR2 expression induced by social isolation of young adult rats in the hippocampal formation. **Methods:** Two groups of male Wistar rats (140g, n=6/each) were used. They were housed in a temperature-controlled room (23ºC), on a 12:12-h light:dark cycle, with free access to food and water. The rats were allocated randomly to one of two conditions: 1) grouped, housed 4 per cage and handled 3 times a week; 2) isolated, housed individually and handled once a week. After 10 weeks the animals were deeply anaesthetized, perfused and their brains removed (project approved on 3th July 2008 by CEUA-Faculty of Medicine of Catanduva, certificate number: 01/08). 40-mm sections were used for immunohistochemistry: anti GluR1 (Chemicon), anti GluR2 (Invitrogen), secondary antibody (Dako), ABC Kit (Vectastain), DAB (Sigma). Using a light microscope Axioskop 40 with AxioCam ICc3 and AxioVision Release 4.6.3 04-2007, Zeiss, the immunopositive cells were counted by 2 examiners independently, in 3 sections/rat and bilaterally in hippocampus, amygdala and entorhinal cortex. Data were compared by Student *t*-test ($p<0.05$). **Results**: Social isolation induced a significant increase in GluR1- immunopositive cells in CA3 (90%, $p=0.004$) and CA1 (270%, $p=0.001$) of the hippocampus. However, no change was found in the hillus of dentate gyrus ($p>0.05$). In contrast to hippocampus, a significant decrease in GluR1- immunopositive cells was induced by isolation in lateral (18.3%, $p=0.015$) but not in basolateral amygdala. No alterations were found in both GluR1- and GluR2- immunopositive cells in entorhinal cortex when isolated were compared to controls animals. Isolation has also induced no changes in GluR2-immunopositive cells in the hillus of hippocampus. GluR2 - immunopositive cells were not detected in CA3 and CA1 of the hippocampus and in the lateral and basolateral amygdala. **Discussion**: Social isolation of adult rats induces an increase in GluR1 expression in hippocampus suggesting that glutamatergic neurotransmission through this receptors may be involved on the brain mechanisms altered in psychiatric disorders like depression**.** **Financial Support**: FAPESP (05/01501-7) and Padre Albino Foundation.

02.033

Rats submitted to affective stress in different periods of brain development show distinct patterns of exploration of a new object. Sinhorini ERA[1], Tonso VM[1], Limonte FH[1], Pereira MTR[1], Iyomasa MM[2], Rosa MLNM[1] [1]FIPA-FAMECA - Bioquímica, [2]FORP-USP - Morfologia, Estomatologia e Fisiologia

Introduction: Affective stress induces several behavioral responses. The nature and severity of these effects depend on the age at isolation which can be associated to neuronal plasticity. This study aimed at evaluating if chronic isolation in different periods of brain development affects the recognition memory and the exploration of a new object. **Methods:** In the social isolation four groups of rats (n=12/each) were used. In two groups the pups remained with their mothers (6/mother) until weaning (21 days) when they were allocated randomly to one of two conditions: 1) grouped, housed 4/cage and handled 3 times a week; 2) isolated, housed individually and handled once a week. In the other two groups, the rats (140g) were allocated in the same conditions. Behavioral tests began after ten weeks. In the maternal separation the pups (n=10) underwent a daily-3h separation from their mothers from PND1 to weaning (PND21) and the controls (n=11) were left undisturbed. Following weaning the animals were housed in groups of 4 for 5 weeks before testing. On the first day rats were submitted to a habituation session on the training arena for 5-min. On the following day, rats were given 5-min training trial in which they were exposed to two identical objects (A1 and A2). On the short-term memory (STM) testing trial (90 min after training), rats were allowed to explore two objects: a familiar one (A) and a different one (B) for 5-min. On the long-term memory (LTM) testing trial (24 hours after training), rats were allowed to explore two objects: (A) and a third different one (C) for 5-min. Exploration were measured by recording the time spent exploring the objects. A recognition index was expressed by the ratio $T_B/(T_A+T_B)$ or $T_C/(T_A+T_C)$. Groups compared by Student *t*-tests ($p<0.05$) (project approved on 3th July 2008 by CEUA-Faculty of Medicine of Catanduva, certificate number: 01/08). **Results:** No change in the index of recognition was induced by any condition of isolation in the training, STM or LTM sessions. However, isolation from weaning induced a significant decrease (64%, p=0.001) in the total exploration of the objects 24 hours after training while maternal separation induced a significant increase (33%, p=0.01). Isolation of adult rats induced no alterations in this behavior. **Discussion:** The results suggest that rats reared under isolation from weaning do not remain motivated in the exploration of objects, according to emotional features of schizophrenia. In contrast, the neuronal plasticity seems to reverse the possible effects of the maternal separation in this behavior. **Financial support:** FAPESP (05/01501-7) and Padre Albino Foundation.

02.034

Protective effect of folic acid on dexamethasone-induced death cellular in SH-SY5Y cell. Budni J[1], Romero A[2], Molz S[1], Egea J[2], Saavedra M de[2], Del Barrio L[2], Tasca CI[1], Rodrigues ALS[1], López M[2] [1]UFSC - Bioquímica, [2]UAM - Farmacologia

Introduction: Folic acid (FA) is a B-vitamin that is essential for cell replication. FA is a major determinant of one-carbon metabolism, in which S-adenosylmethionine formed donates methyl groups that are crucial for neurological function. Many functions for FA have been reported, including neuroprotective and antidepressant properties (Mattson et al., *Neuroscience* 26: 137, 2003; Iskandal et al., *Ann. Neurol.* 56: 221, 2004; Brocardo et al., *Neuropharmacology* 54: 464, 2008). Hence, the purpose of this study was to investigate the protective effect of FA on dexamethasone-induced cellular death in SH-SY5Y cells and the possible signaling pathway involved. Dexamethasone has been reported to cause cellular death in cerebellar and hippocampal neurons (Zhu et al., *Neuroscience* 141: 2019, 2006; Jacobs et al., *Brain Res.* 1070: 116, 2006). **Methods:** SHSY5Y neuroblastoma cells (commercially available) were cultured in DMEM (containing 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin) in a humidified air/5% CO2 chamber at 37°C. The cells were seeded into 96-well (100.000 cells/well) culture plates and 48 h after, cells were washed and treated with dexamethasone (diluted in serum-free medium) at the concentrations 0.05, 0.1, 0.5, and 1 mM for 48 h to induce cellular death. SHSY5Y cells were treated with FA (0.001, 0.01, 0.1, 0.3, 0.5 and 1 mM, diluted in serum-free medium) for 72 h, 24h before dexamethasone incubation. Additionally, 1 h before incubation with folic acid, the inhibitors of the following kinases were added: PI3K (LY 294002, 10µM), MEK ½ (PD 98059, 10µM), PKC (chelerythrine, 0.1 µM), CAMK II (KN-93, 1µM), PKA (H-89, 2µM) as a tool to investigate the mechanism underlying the neuroprotective effect of folic acid against dexamethasone-induced cellular death. The viability of cells was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). **Results and Discussion:** The exposure of SH-SY5Y cells to dexamethasone (1mM) caused a significant dose-dependent decrease in cellular viability. The treatment of these cells with FA (300 µM) reversed the dexamethasone effect. The administration of the inhibitors LY 294002, KN-93 and H-89, but not PD 98059 and chelerythrine, was able to reverse the protective effect of FA on dexamethasone-induced injury. FA and the inhibitors per se did not produce an alteration in cellular viability. Together, these results demonstrate that FA protects human neuroblastoma cells against the dexamethasone-induced damage and suggest that this protective effect may be mediated by a signaling pathway modulation that involves PI3K, CAMK II and PKA. **Financial support:** CAPES, UFSC, Instituto Teófilo Hernando (UAM - Universidad Autónoma de Madrid).

02.035

Efeitos do estrógeno na potencialização da sinalização inflamatória induzida pelo estresse crônico imprevisível no sistema nervoso central *in vivo.* Sá Lima L[1], Porto CS[2], Scavone C[1], Munhoz CD[1] [1]ICB-USP - Farmacologia, [2]UNIFESP - Farmacologia

Introdução: O interesse científico pelo estresse é bem antigo e seu estudo vem crescendo desde que evidências começaram a associar o estresse à ocorrência de doenças cardiovasculares, imunológicas e alguns tipos de câncer. Também tem se demonstrado a associação entre estresse e doenças neuropsiquiátricas, neurológicas e neurodegenerativas. Estudos realizados pelo nosso grupo demonstraram que, o estresse crônico imprevisível (EI) potencializa a ativação do fator de transcrição NF-kappaB (NFKB) induzidos por LPS (lipopolissacarídeo), em córtex frontal e hipocampo de ratos, via ativação dos receptores de glicocorticóides. Evidências experimentais e clínicas indicam que o estrógeno, hoje amplamente utilizado na terapia de reposição hormonal em mulheres na pós-menopausa, reverte danos cognitivos causados por estresse e previne doenças neurodegenerativas. Além disso, estrógeno parece ter uma ação antiinflamatória, uma vez que este hormônio pode inibir a ativação de microglia, aumentar a expressão de genes antioxidantes e ainda, modular alguns parâmetros da resposta inflamatória, como por exemplo, a ativação do NFKB. **Objetivos:** Estudar a ação do estrógeno nos efeitos induzidos por estresse imprevisível associado a um estímulo inflamatório agudo (administração de LPS), em algumas regiões do encéfalo de ratas Wistar. (Protocolo de Ética em experimentação Animal ICB/USP nº102 aprovado em 19/09/06). **Métodos:** Definimos o tempo de tratamento do LPS (1,2,4 ou 6hs) através do Ensaio de Gel de Retardo. Uma vez definido este parâmetro, analisamos se a fase do ciclo estral (através de lavado vaginal), e consequentemente da concentração plasmática de estrógeno, poderiam influenciar esta resposta. Sendo assim, ratas em diferentes fases do ciclo foram injetadas com LPS (1 mg/kg; i.v) e 1hora após a injeção, os animais foram sacrificados e o padrão de ativação do NFKB foi avaliado em diversas regiões do encéfalo dessas ratas. Além disso, avaliamos também os efeitos do estresse crônico imprevisível (14dias) na ativação do NFKB pelo LPS, com o objetivo de avaliar se as ratas apresentavam o mesmo padrão de resposta encontrado nos nossos estudos realizados em ratos (Munhoz et al., *J Neurosci*. 2006). **Resultados:** Os resultados obtidos mostraram que o LPS (1 mg/kg; 1h i.v) ativou o NFKB no cerebelo, córtex frontal, hipotálamo, hipocampo e estriado das ratas e a fase do ciclo estral não influenciou na ativação deste fator induzida por LPS. Já em relação ao estresse crônico imprevisível, notamos diferenças importantes no padrão de resposta das ratas. A exposição crônica ao estresse foi capaz de potencializar a ativação do NFKB (LPS=79%; EI=44%; LPS+EI=122% vs controle) induzida por LPS apenas no estriado, região onde também observamos um maior aumento da ativação deste fator de transcrição pelo LPS. **Conclusão:** 1) LPS (1 mg/kg; i.v) induz ativação do NFKB com pico de atividade no intervalo de 1 hora no hipocampo, córtex frontal, estriado, hipotálamo e cerebelo de ratas; 2) A ativação do NFKB por LPS no SNC independe da fase do ciclo estral; 3) O estresse crônico imprevisível aumentou a ativação do NFKB apenas no estriado das ratas quando comparado com as outras estruturas encefálicas estudadas,e potencializou, nesta mesma estrutura, os efeitos do LPS na ativação do NFKB. **Apoio Financeiro:** FAPESP, CNPq e *Procontes-USP

02.036

Relação entre limiar de convulsão clônica induzida por agonista inverso benzodiazepínico e enzima NA^+/K^+-ATPase em regiões encefálicas de ratos. Conto MB, Carvalho JGB, Benedicto MAC UNIFESP - Psicobiologia

Introdução: O complexo $GABA_A$/sítio benzodiazepínico e a enzima Na^+/K^+-ATPase são entidades moleculares relacionadas com o controle da excitabilidade encefálica, e convulsões motoras são desencadeadas pela inibição do receptor $GABA_A$ (Braestrup, Science 216,1241,1982) e da enzima (Bignami, Nature,209,413,1966) no encéfalo. As isoenzimas de alta afinidade pelo glicosídeo ouabaína são predominantemente ativas em situações de alta atividade neuronal (Blanco, *Am.J.Physiol*., 275, F633, 1998), tal como na atividade epileptiforme. O objetivo do presente estudo foi verificar se ratos que diferem no limiar de convulsão clônica induzida pelo DMCM, um agonista inverso benzodiazepínico, apresentam diferenças na atividade da Na^+/K^+-ATPase e na ligação de [^{3}H]-ouabaína às isoenzimas de alta afinidade em diferentes regiões encefálicas. **Métodos:** Ratos Wistar, machos, adultos foram administrados com duas injeções intraperitoneais de DC_{50} de DMCM (intervalo de uma semana entre ambas). Os ratos que apresentaram convulsão clônica em ambas as administrações constituíram o grupo SC (susceptível às convulsões) e aqueles que não apresentaram nenhum indício de alterações motoras constituíram o grupo NSC (não-susceptível às convulsões). Vinte e cinco dias após a segunda injeção, os indivíduos selecionados foram sacrificados, e as seguintes regiões foram dissecadas: hipocampo, tronco encefálico, córtex frontal, amígdala + córtex límbico e estriado. Alíquotas dos homogenatos destas estruturas foram utilizadas para determinar a atividade da K^+-paranitrofenilfosfatase (defosforilação K^+-dependente da Na^+/K^+-ATPase) e a ligação de [^{3}H]-ouabaína (40 nM). Nas regiões que apresentaram diferenças estatísticas entre os grupos na ligação da [^{3}H]-ouabaína, foram realizadas curvas de competição homólogas para determinar se as mesmas se devem a densidade máxima (B_{MAX}) ou a afinidade de ligação à ouabaína (K_D). **Resultados:** Não foram encontradas diferenças significativas na atividade da K^+-paranitrofenilfosfatase entre os grupos nas regiões encefálicas analisadas. Contudo, foram detectadas diferenças na ligação de [^{3}H]-ouabaína nas seguintes regiões: tronco encefálico (SC: 33,17 ± 0,8 pmol/mg de proteína, média ± EPM; grupo NSC: 35,5 ± 0,51; p=0,02); córtex frontal (SC: 31,75 ± 0,7; NSC: 34,01 ± 0,51; p=0,02) e hipocampo (SC: 31,52 ± 1,39; NSC: 34,73 ± 0,55; p=0,05). As curvas de competição homólogas indicaram que estas diferenças são decorrentes do B_{MAX} no tronco (SC: 51,85 ± 2,25 pmol/mg de proteína, média ± EPM; NSC: 58,53 ± 1,37; p=0,012) e no córtex frontal (SC: 36,8 ± 1,4 pmol/mg de proteína, média ± EPM; NSC: 40,9 ± 1,4; p=0,04), enquanto que no hipocampo esta diferença se deve ao K_D (SC: 12,25 ± 2,4 nM, média ± EPM; NSC: 5,7 ± 1,7; p=0,03). **Discussão:** Não obstante a ausência de diferenças na atividade da K^+-paranitrofenilfosfatase entre os grupos, os ensaios de ligação da [^{3}H]-ouabaína indicaram diferenças nas isoenzimas de alta afinidade no tronco encefálico, no córtex frontal e no hipocampo entre os grupos. É possível que um menor funcionamento destas isoenzimas por parte do Grupo SC leve a uma repolarização neuronal mais lenta, diminuindo o limiar de deflagração do potencial de ação, e levando, portanto, a uma maior sensibilidade à convulsão induzida pelo DMCM. **Apoio Financeiro:** Capes e AFIP. **CEP:** 1058/06.

02.037

Stress-induced exploratory deficit in an elevated plus maze is prevented by NMDA administered into the median raphe nucleus. Gonzaga NA[1], Padovan CM[2] [1]FFCLRP-USP - Psicobiologia, [2]FFCLRP-USP - Psicologia e Educação

Introduction: Exposure to uncontrollable stressors leads to behavioral and neurochemical changes, which has been associated to mal functioning of the Median Raphe Nucleus (MnRN)-Dorsal Hippocampus (DH) serotoninergic pathway. These deficits can be attenuated by intra-hippocampal injections of NMDA antagonists or 5-HT1a agonists. Activation of MnRN glutamatergic NMDA receptors (NMDAr) increases serotonin release in both MnRN and DH. We previously showed that MnRN injections of NMDA (NMDAr agonist) immediately after forced restraint attenuated the restraint-induced exploratory deficit of an elevated plus maze (EPM). This effect could not be blocked by previous treatment with AP7 (2-amino-7-phosphonoheptanoic acid, an NMDAr antagonist). Therefore, the aim of this work was to investigate whether activation of MnRN NMDAr before exposure to restraint stress could prevent its behavioral effects. This project is approved by the Animal Ethics Committee of USP Ribeirão Preto (protocol 06.1.1131.53.0). **Methods:** Rats with cannulas aimed to the MnRN received two intracerebral injections (0.2 µl each) of Saline (SAL), AP7 (3nmols) and/or NMDA (1nmol) (5 min interval), administered as follows SAL+SAL, SAL+NMDA, AP7+SAL and AP7+NMDA. Immediately after injections, animals were restrained for two hours and tested in the elevated plus maze (EPM) 24 hours later. In control experiment, animals received intra-MnRN treatments 24 hours before test in the EPM, but were not exposed to restraint stress. Number of enclosed arm entries (EAE) and percentages of entries (%EO) and time spent (%TO) in the open arms were registered and analyzed by Oneway ANOVA followed by Duncan test, considering $p<0.05$ for significance. Means±SEM are represented. **Results:** In non stressed rats no differences were observed between groups for EAE ($F_{3,43}=1.72$; $p>0,05$; SAL+SAL=12.1±1.37; n=10; SAL+NMDA=13.58±1.13; n=12; AP7+SAL=12.90±1.05; n=11; AP7+NMDA=9.63±1.69; n=11), %EO ($F_{3,43}=0.33$; $p>0.05$; SAL+SAL=28.8±5.1; SAL+NMDA=32.5±2.3; AP7+SAL=27.5±2.2; AP7+NMDA=30.1±4.8) or %TO ($F_{3,43}=0.93$;$p>0.05$;SAL+SAL=21.1±4.2; SAL+NMDA=27.5±2.9; AP7+Sal=18.9±1.24; AP7+NMDA=21.7±5.9). In stressed rats no differences were observed for EAE ($F_{3,20}=2.71$; $p>0.05$) after treatment with NMDA (SAL+NMDA=12.33±1.26; n=6) or AP7 (AP7+SAL=11.14±0.96; n=7) when compared to control (SAL+SAL=10.4±1.63; n=5). Treatment with NMDA and AP-7 tended to decreased EAE (AP7+NMDA=7.50±1.28; n=6) when compared to SAL+SAL, but it did not reach significance level ($0.10>p>0.05$). When considering %EO, no differences were observed between control (SAL+SAL=19.78±6.23) and treated groups (SAL+NMDA=30.67±3.16; AP7+SAL=24.65±4.84; AP7+NMDA=20.92±3.88) ($F_{3,20}=1.10$, $p>0.05$).On the other hand, %TO was increased after treatment with NMDA (SAL+NMDA=22.18±4.28) when compared to control (SAL+SAL=10.95±3.09) ($F_{3,20}=3.17$; $p<0,05$). AP7 (AP7+SAL=9.18±4.28) and AP7+NMDA (AP7+NMDA=8.31±2.28) treatments were not different from Saline treated animals. **Discussion:** Our results show that activation of NMDA receptors before stress prevents the restraint-induced decrease in exploratory activity of an EPM. These results suggest that the MnRN glutamatergic NMDA-mediated neurotransmission is involved in the behavioral effects of stress. **Affiliation:** SBFTE. **Financial Support:** CAPES, CNPq and FAPESP.

02.038

A putative protective role of melatonin on cerebellar granule cells challenged by lipopolyssaccharide. Franco DG, Ferreira ZS, Markus RP IB-USP - Fisiologia

Introduction: Central nervous system is a privileged organ regarding innate immune response, as it has a damped or even inverse response to pathogen associated molecules patterns, which triggers inflammatory response. Lipopolyssaccharide (LPS), from Gram-negative bacteria outer membrane, induces bacterial meningitis and septic shock. The effect of LPS is mediated by nuclear factor kappa B (NFkB) translocation, a pivotal pathway in innate immune response that leads to the transcription of several genes as the inducible nitric oxide synthase (iNOS). Melatonin (MEL), the main product of the pineal gland, synthesized also by activated immune competent cells, blocks NFkB nuclear translocation in macrophages (Gilad E. FASEB J. 12:685-93, 1998) and endothelial cells (Tamura EK. J Pineal Res. 46:278-74, 2009). As a consequence, MEL inhibits NO synthesis catalyzed by iNOS. It is interesting to note that MEL also inhibits constitutive NOS (cNOS), as shown in cerebellar homogenate (Pozo D. J Cell Biochem. 65:430-42, 1997) and endothelial cells (Tamura EK. J Pineal Res. 41:267-74, 2006). The aim was to determine the action of MEL on cultured cerebellar granule cell challenged by LPS, regarding the NFkB translocation and NO production. **Methods:** Rat cerebellar granule cell cultures (Wistar, 7-8 days-old) were cultured for 8 days in DMEM. At the $7^{th}/8^{th}$ day the cells were incubated with LPS (100 ng/mL, 2-21h) or LPS + MEL (100nM). The nuclear translocation of NFκB was assayed by EMSA on nuclear extracts of granule cells and the identification of its subunits by supershift with specific antibodies. NO production was measured by confocal microscopy on cells loaded with DAR 4M-AM (30 min, room temperature). Comparison of two or more means was done by student “t” test or ANOVA, respectively. Ethical Committee (CEA-IB protocol 046/2007). **Results:** Constitutive activity of NFkB/DNA binding activity was found in nuclear extracts of cerebellar granule cells. Supershift analysis indicated that p50-p65 heterodimers and p50-p50 homodimers were present. LPS (12h)-treatment was able to activate NFkB binding activity in the cells. Both DNA/protein complexes formed by p50-p65 and p50/p50 were altered by the treatment (2.6 and 1.2 fold over basal levels, respectively). This effect was inhibited by MEL ($p<0.05$) returning to the initial basal levels observed. On NO production, maximal LPS effect observed was at 2 h (13.2 ± 3.5 fold, n=28 cells, $p<0.05$). Then, NO content presented an exponential reduction at 3, 12 and 21h attaining a value 2.5 fold over basal levels after 21h. MEL impaired LPS-induced NO production at all times recorded. **Discussion:** We show that MEL inhibits the NFkB nuclear translocation and NO production on cultured cerebellar granule cells challenged by LPS. It is known that heterodimmer p50-p65 has a transactivation domain responsible for transcription of a package of genes involved in the innate immune response, which hasn’t the homodimer p50-p50. The fact that the p50-p50 is also activated it´s an interesting information indicating that LPS inhibits the transcription of some genes in this kind of culture. In summary, our results show that LPS activates NFkB and NO production in cerebellar granule cells, being both effects abolished by melatonin. These results strongly suggest a neuroprotective effect of MEL against inflammatory infections. **Supported by** FAPESP, CNPq, CAPES.

02.039

Investigation of depression associated to animal models of Parkinson's disease. Santiago RM[1], Barbiero J[1], Vital MABF[1] [1]UFPR - Farmacologia

Introduction: Parkinson´s disease is a neurodegenerative disease, whose symptoms include bradykinesia, tremor at rest and postural instability associated with depression. The prevalence of depression in these patients is between 40% to 70%, with an incidence of 1.86% per annum and a cumulative risk of 8.5% over life. The current study investigated and compared depressive symptoms among the animal models of Parkinson's disease induced by MPTP, 6OHDA or LPS. **Methods:** Male Wistar rats from the animal house of Federal University of the Parana were used. The protocols were approved by the Ethics Committee in Animal Experimentation (CEEA) of UFPR (number 0144). The rats were infused bilaterally in the *substantia nigra* with MPTP, 6-OHDA or LPS. The open field test was used to evaluate motor behavior, and for the evaluation of depression the forced swimming test was performed. The animals were randomly divided into 5 groups, control, SHAM, MPTP, 6-OHDA and LPS. The evaluation of the locomotor activity used the test of open field, an apparatus consisted of a rectangular box (40×50×63 cm) whose floor was divided into 20 (10×10 cm) small rectangles. The animals were gently placed in the right corner of the open field and were allowed to freely explore the area for 5 min, and then the following aspects were determined: locomotion frequency, rearing frequencies and immobility. This procedure was performed 1, 7, 14 and 21 days after the stereotaxic surgery. The forced swimming test consisted in putting the animal in a container with water (30 cm and 20 ºC) for 15 minutes and, after 24 hours, the animal was re-exposed to the test for 5 minutes, when the swimming time, escalation and the animal immobility were evaluated. This proceeding was carried out 22 after the stereotaxic surgery. The results were expressed by mean ± S.E.M, using ANOVA followed by Tukey test, $p < 0.05$. **Results:** In the open field the groups MPTP and 6-OHDA presented a decrease in locomotion and rearing frequencies 1 day after surgery in comparison to control and SHAM groups ($p < 0.05$). In the days 7, 14, 21 the groups did not demonstrate significant differences in all the evaluated parameters. The results of forced swimming test indicated that the 6-OHDA rats exhibited reduction in the time of swimming and increased the time of immobility regarding the groups control and SHAM ($p < 0.05$). **Discussion:** In accordance with the obtained results MPTP and 6OHDA animals presented hypolocomotion 24 h after the surgery. Moreover, 6OHDA caused depressive signs in rats evaluated in the forced swimming test 22 days after the surgery. In conclusion, 6OHDA was the best model to study depression associated to Parkinsonism. Sponsored: CNPq, REUNI

02.040

Investigation of neurochemical alteration caused for different periods of withdrawal after treatment subchronic of cocaine in rats. Citó MCO, Silva FCC, Silva MIG, Santos LKX, Moura BA, Aquino Neto MR, Sousa FCF UFC - Fisiologia e Farmacologia

Introduction: Catalase is an intracellular antioxidant enzyme that catalyzes the reaction of hydrogen peroxide to water and molecular oxygen, and is particularly enriched in erythrocytes, where it metabolizes 90% of the hydrogen peroxide. Due to its poor expression in the brain, catalase has been considered a secondary enzyme in controlling free radical-induced damage in this organ. Oxidative stress is involved in the pathogenesis of cocaine-induced neuropathy in humans. In the present study, we aimed to determine the effects of cocaine in the catalase after withdrawal cocaine 24 hours, 7 days and 21 days. **Methods:** Rats wistar males weighing 180-200g at the start of the experiment, were individually housed in a temperature and humidity-controlled environment on a reversed light/dark cycle. The experiments carried through they are in accordance with the guide of cares and uses of animals of laboratory of the Department of Health and Human Services of the United States of America (U.S.A.), approved for the Committee of Ethics in Animal Research, protocol number 16/09, of the Federal University of Ceará (UFC). Cocaine was dissolved in water bidistilled and administered intraperitoneally (i.p) in a final volume of 1,0 mL for each 100 g of weight of animal. Animals were given cocaine (20 mg/kg) during 07 consecutive days. Later the activity of catalase in prefrontal cortex was determined daily to evaluate the participation on stress oxidative. **Results:** The results showed that 24 hours of withdrawal of cocaine (1.41±0.11), as well as in the abstinence of 7 days (1,92±0,37) there was a statistically significant reduction of catalase activity in relation to control (4.95±0.86). However, after 21 days of abstinence (4.67±0.87) did not have any significance of catalase activity. **Discussion:** The oxidative stress can be associate with neurological deseases including the Alzheimer illnesses, epilepsies, among others (Beyer et al., 1998). Recent studies of our laboratory (Macêdo et al., 2005) and of other groups (Sharan et al., 2003) have demonstrated the participation oxidative stress in the mechanism of action of the cocaine, that can result in neurodegeneration. Dietrich et al (2005) also demonstrated that the cocaine was capable to increase the production of the EROs in cortex frontal and striatum of rats treated to acute and repeated form. **Conclusion:** As the result showed we observed that withdrawal of cocaine of 24 hours and 7 days can be associated with oxidative stress, because was reduction of catalase enzyme activity. **References:** Beyer et al, Biochem. *Pharmacol*., 56: 1265, 1998; Dietrich et al, *Neuropharmacology*, 48: 965, 2005; Macêdo et al, *Neurosci Lett***,** 387:1, 2005. Financial support: CAPES, Funcap.

02.041

The plus-maze discriminative avoidance task: an animal model able to evaluate learning/memory relationship. Fernandes HA[1], Wou-Silva, R.[2], Patti CL[2], Bittencourt, L. R. A.[1], Tufik S[1], Frussa-Filho, R[2] [1]UNIFESP - Psicobiologia, [2]UNIFESP - Farmacologia

Introduction: The plus-maze discriminative avoidance task (PM-DAT) is an animal model that evaluates, in a concomitant but independent fashion, learning, memory, anxiety and motor function in rodents. In the training session of this paradigm, every time the animals enter the aversive enclosed arm (Av), they receive aversive stimuli which are composed by a 100-W light and a frontal cold-air blow produced by a 700-W hair drier placed above the end of the Av. In this way, learning is evaluated by the comparison of time spent in both enclosed [aversive vs. non-aversive (NAv)] arms throughout a 10-min training session while memory is also evaluated by this parameter and by the percent time spent in the Av during a later 3-min test session performed in the absence of the aversive stimuli. Thus compared to classical animal models of memory such as the inhibitory avoidance task, the PM-DAT has the great advantage of evaluation learning and memory (and not only memory). In this scenario, the aim of the present study was to verify if there would be a time dependent relationship between learning and memory in the PM-DAT model. Thus, we investigate if distinct training durations would modulate the retention of the discriminative task. **Methods:** Swiss male mice, 3-month-old, received a saline injection and 30 min later were submitted to the training session of the PM-DAT with duration of 1 (1M), 3 (3M), 5 (5M) or 10 (10M) minutes. Eleven days after this session, animals were submitted to the test session. All experimental procedures were approved by the Ethical Committee of UNIFESP (#1162/08). **Results:** During the training session, all groups spent more time (seconds) in the non-aversive enclosed arm when compared to the aversive one (Av: 1M: 1.9±0.4; 3M: 6.2±1.1; 5M: 10.1±2.2; 10M: 15.4±3.7 and NAv: 1M: 18.3±2.4; 3M: 100.7±4.8; 5M: 159.6±9.9; 10M: 363.1±34.6). In the test session, all groups presented memory retention since they spent more time (seconds) in the NAv (Av: 1M: 19.2±4.3; 3M: 14.0±3.2; 5M: 8.8±2.7; 10M: 4.8±1.4 and NAv: 1M: 90.9±11.4; 3M: 101.2±9.7; 5M: 120.1±10.0; 10M: 146.4±5.4). Additionally, the 5M and 10M groups presented a decreased percent time in the Av when compared to 1M group (1M: 18.7±3.8; 3M: 13.7±3.4; 5M: 7.7±2.2; 10M: 3.3±1.1). **Discussion and Conclusion:** Taken together, our data revealed that although a single 1-min training session was effective in producing retention of the discriminative task, a 10-min training session was more effective in diminishing the time spent in the Av during the test session. These results demonstrate a time dependent relationship between learning and memory in the PM-DAT model. **Financial Support:** CNPq, FAPESP, CAPES, AFIP and CEPID.

02.042

Hypolocomotion and depressive behavior in rotenone prolonged treated rats. Morais LH, Takahashi TT, Lima MMS, Ariza D, Vital MABF UFPR - Farmacologia

Introduction: Mitochondrial dysfunction and environmental exposures to pesticides is being suggested to explain the progressive degeneration on Parkinson´s disease (PD). In this context, Rotenone, a classical complex I inhibitor and commonly used as pesticide, is being proposed as a good animal model of PD. Nevertheless, behavioral consequences of a prolonged exposition of this toxin in a low-dose are yet to be fully considered. This way, the goal of this study was to investigate the effects of prolonged exposition of rotenone in motor parameters and depression in rats by using the open field test and forced swimming test (FST). **Methods:** In this study 24 Wistar rats were distributed in two groups, control group (n=10), and rotenone group (n=14). The rotenone group received 2,5 mg/kg of drug dissolved in sunflower oil by intraperitoneal injection up to ten days. The control group received sunflower oil by intraperitoneal injection at the same period. Motor parameters were observed 24 hours, 7, 14 and 21 days after the first administration of rotenone. In addition, FST was performed 22 days after rotenone. The protocols were approved by the Ethics Committee in Animal Experimentation (CEEA) of UFPR (number 0144). **Results and Discussion:** The data was analyzed by ANOVA followed by Tukey post-hoc test (results are presented as mean±SEM). The rotenone group presented a significant decrease ($p<0.05$) in the locomotion frequency (62.6±5.17) compared to the control group (78.3±7.53) 24 hours and 7 days for the rotenone (35.4±4.72) and the control group (66.7±9.11), respectively. Moreover, the results indicated an increased immobility time, at the time-point 7 days, for the rotenone group (102±15.22) compared to the control group (31.4±7.8). On 14^{th} and 21^{th} days there were not significant differences between groups observed on the open field test, then the depressant behaviors could be measured by FST whitout motor bias. Rotenone significantly reduced ($p<0.05$) swimming time (33.6±6.8) in comparison to the control group (79.5±17.4). Otherwise, rotenone significantly increased immobility ($p<0.05$) in the rotenone group (190.2±16.2) when compared to the control group (118.2±19.1). These results demonstrated that rotenone caused Parkinsonian signs such as specific motor deficits and depressive-like behavior. The core of evidence herein presented suggests a possible animal model of PD inflicted by the current protocol adopted. Further neurochemical studies will help us to understand how rotenone causes these behavioral disruptions. Sponsorship: CAPES, CNPq.

02.043

Pharmacological evaluation *in vitro* of new substances derived from cardanol with potential application in Alzheimer's disease. Vieira KST[1], Gambôa NF[1], Areas TFMA[1], de Paula AAN[2], Martins JBL[3], dos Santos ML[3], Nascente LC[4], Romeiro LAS[4], Gargano R[2], Castro NG[1] [1]ICB-UFRJ - Farmacologia Molecular, [2]UnB - Física, [3]UnB - Química, [4]UCB - Gerontologia

Introduction: The prevalence of Alzheimer's disease (AD) increases gradually with age and the proportion of elderly in the Brazilian population is increasing rapidly, therefore studies about that condition and its treatment are important. In the search of new drugs for AD, we have studied new potential acetylcholinesterase (AChE) inhibitors designed from cardanol, a non-isoprenoid phenolic lipid of the cashew (*Anacardium occidentale*) nut-shell liquid. This choice was determined based on the structural similarity to LASSBio-767, a derivative of (-)-spectaline and a promising drug candidate, already characterized as an AChE inhibitor (AChEI) with central action (Castro et al., *Eur. J. Pharmacol*., 580:339, 2008). The proposed phenolic lipid derivatives should have electronic, structural and hydrophobic characteristics relevant to the molecular recognition by AChE and pharmacophoric similarities with both spectaline and the AD drug rivastigmine. **Methods:** We have first sought molecular characteristics relevant for AChE inhibition by the comparison of electronic properties of the proposed phenolic lipids with those of rivastigmine, using semi-empirical and RHF theoretical chemistry methods. A subset of relevant properties was identified by principal component analysis and was used to select the structures with greater similarity to rivastigmine. The molecular pattern used involves the hybridization between the primary pharmacophoric subunit of rivastigmine and the secondary subunit of an alkyl side chain of spectaline. The prediction of the theoretical model was evaluated experimentally for the selected derivatives of cardanol, which were synthesized and tested for inhibitory activity of AChE. Fourteen compounds were assayed (at 100 microM) for inhibition of *Electrophorus electricus* AChE by Ellman´s method, adapted to a microplate reader. Subsequently, we have obtained concentration-response curves and determined the IC50 of those compounds showing more than 50% of inhibition in the initial screening. **Results and Discussion:** We investigated substitutions of the hydrogen from the phenolic group of cardanol by methyl, acetyl and N,N-dimethylcarbamoyl, as well as substitutions at the benzylic carbon from the side chain by N,N-dimethylamine, N,N-diethylamine, pyrrolidine, piperidine and N,N-methylbenzylamine (de Paula et al., *Eur. J. Med. Chem.,* 2009, doi:10.1016/j.ejmech.2009.03.045). The theoretical calculations indicated that the structures with a phenolic N,N-dimethylcarbamoyl together with N,N-dimethylamine and pyrrolidine substituents in the alkyl chain were better correlated with rivastigmine. Among the compounds synthesized, these two indeed showed the highest AChEI activity, with IC50s of 50.0 microM (40.2 to 62.2 microM, 95% c.i.) and 84.3 microM (74.5 to 95.4 microM), respectively. Therefore, the theoretical model proved to be adequate to help designing and proposing the synthesis of new candidate AChEIs derived from cardanol. **Support:** CNPq and Finatec.

02.044

Caffeine protection against behavioral impairment caused by sepsis. Barbosa WL[1], Borges DM[1], Fernandes DV[1], Souza RB[1], Araújo JR[1], Doroteu C[1], Carneiro FP[1], Lucena GMRS[2], Sousa JB[1], Ferreira VMM[2] - [1]UnB - Medicina, [2]UnB - Ciências da Saúde

Introduction: Caffeine is the most widely consumed psychoactive drug, and acts as an adenosine receptor antagonist at non-toxic doses. Our interest was to investigate caffeine effect at sepsis behavioral impairments (anxiety and memory) especially because it is a common daily intake beverage. **Methods:** Male Wistar rats (n= 8 animals/treatment), 2.5 month-old, 250 g, were obtained from the Animal Facility, in accordance with the recommendation of the ethical committee for animal care (UnB doc 33880/2009). All experiments were carried out at the Pathology Laboratory/Faculty of Medicine. The animals were anesthetized i.p., using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), to allow exposure and puncture of the cecum, which was then squeezed to extrude a small amount of feces from the perforation site, and, then, was later placed back into the peritoneal cavity. All animals were returned to their cages after administration of ceftriaxone (30 mg/kg) + clindamicine (25 mg/kg). Caffeine (10 mg/kg) was administered by gavage for one week, after sepsis induction. On the last day, one hour after caffeine administration, the animals were submitted to elevated plus-maze, social recognition and step-down inhibitory avoidance tests. **Results**: Experimental results showed that caffeine administered for one week after sepsis induction, increased the percentage of open arm entries (41.53±3.38) in the elevated plus-maze test, when compared to the sham-operated group (20.78±2.45). Caffeine improved the short-term social memory when the same juvenile rat was re-exposed after a delay period of 30 min (127.1±15.9 versus 143.1±8.9, $p<0.05$, one-way ANOVA, Tukey's test). In addition, at this time, the animals from the sham-operated, sepsis and sepsis plus caffeine groups were not able to recognize the juvenile rat, since no significant reduction in the investigation time was observed during the second encounter ($p>0.05$). Caffeine also facilitated the step-down inhibitory avoidance long-term memory 24 h ($H(3, N=32)=13.30$; $p<0.004$) evaluated after training (Kruskal-Wallis). However, the sepsis group showed a reduction at the step-down latencies during the acquisition of short- and long-term memory when compared to the respective control sham-operated group ($p<0.05$) (Mann-Whitney test). This effect of cognitive deficit was reverted by treatment with caffeine in the sepsis plus caffeine group ($p<0.05$). **Discussion:** Our results demonstrated that caffeine (at the present dose), administered after sepsis induction, produced behavioral changes and improved the neurocognitive deficits of sepsis-surviving animals. Since one of the main pharmacological mechanisms of caffeine is blockage of adenosine receptors, our results suggest that these receptors may be involved in the behavioral and cognitive changes observed in sepsis. **Acknowledgements:** Pathology Laboratory, Faculty of Medicine/UnB, for financial and technical support.

02.045

Effects of nicotine at behavioral responses of sepsis-surviving rats. Queiroz AJR[1], Martins NT[1], Ayres GMC[1], Silva FM[1], Silveira JPB[1], Nóbrega YKM[1], Carneiro FP[1], Sousa JB[1], Lucena GMRS[2], Ferreira VMM[2] [1]UnB - Medicina , [2]UnB - Ciências da Saúde/Ciências Farmacêuticas

Introduction: Nicotine affects many physiological functions of the central nervous system by modulating the activity of neurons populations that contribute to emotional behavior, learning and memory. On the other hand, there is much information about the behavior dysfunctions in sepsis survivors, humans and experimental animals. Despite this information, our main goal is to verify the effect of the subchronic treatment with nicotine in the behavioral alterations of rats caused by sepsis. **Methods:** Male Wistar rats (08 animals/treatment), 2.5 month-old, 250 g, were obtained from the Animal Facility, in accordance with the recommendation of ethical committee for animal care (UnB doc 33880/2009). All experiments were carried out at the Pathology Laboratory/Faculty of Medicine. The animals were anesthetized i.p. using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), to allow exposure of the cecum, which was then squeezed to extrude a small amount of feces from the perforation site that was later placed back into the peritoneal cavity. All animals returned to their cages, after administration of ceftriaxone (30 mg/kg) + clindamicine (25 mg/kg). Nicotine (0.01 mg/kg, s.c.) was administered for one week, before and after sepsis induction. On the last day, 30 minutes after nicotine administration, the animals were submitted to the elevated plus-maze and step-down inhibitory avoidance tests. **Results**: The experimental results showed that nicotine sepsis induction increased the percentage of open arm time [$F_{(3,30)}$=4.615] in the elevated plus-maze test, when compared to the sepsis group (ANOVA, Newman-Keuls' test). Treatment with nicotine also improved the animals' latencies during the acquisition of short- (H(3, N=32=20.4603; p=0.0001) or long-term memory (H(3, N=32=16.6127; p=0.0009) when compared to the sham-operated group in the step-down inhibitory task (Kruskal-Wallis test). The Mann-Whitney test indicated that this treatment, performed 30 minutes before the training session, significantly increased the animal's latencies during of the short- (performed 1.5 h after the training session) or long-term memory (24 h after the training session) when compared to sham-operated group (p<0.05). The test also indicated that the sepsis group significantly decreased the animal's latencies during the short- and long-term memory (p<0.05). **Discussion**: The results suggest that the increased cholinergic stimulation by nicotine is involved with improvement in memory acquisition and anxiety-like behavior of sepsis-surviving rats. However, pharmacological alternatives that are more specific for cholinergic receptors are necessary to confirm this hypothesis. **Acknowledgements:** Pathology Laboratory, Faculty of Medicine/ UnB, for financial and technical support.

02.046

Modulation and functional participation of the renin-angiotensin system in a rat model of epilepsy. Pereira MGAG[1], Becari C[2], Oliveira JAC[3], Salgado MCO[2], Garcia-Cairasco N[3], Costa-Neto CM[1] [1]FMRP-USP - Biochemistry and Immunology, [2]FMRP-USP - Pharmacology, [3]FMRP-USP - Physiology

Introduction: The renin-angiotensin system (RAS) is classically involved in blood pressure regulation and water-electrolyte balance; in the central nervous system it has been mostly associated with homeostatic processes, such as thirst, hormone secretion and body temperature regulation. More recently, brain RAS has been associated with other physiological events, such as learning and memory, and also with pathological situations such as Alzheimer's disease and epilepsy. In our study we analyzed the profile of expression and function of RAS components in a rat model of epilepsy. **Methods:** Using a rat model of genetic epilepsy named Wistar Audiogenic Rats (WAR) we examined the expression of components of RAS, namely angiotensin I converting enzyme (ACE) and angiotensin type 1 receptor (AT_1 receptor) transcripts in WAR and Wistar rats by semi-quantitative RT-PCR. Subsequently, the WAR and Wistar animals were chronically treated by gavage with drugs broadly used in anti-hypertensive therapies: an inhibitor for ACE (Enalapril, 10 mg/kg/day) or the antagonist of the AT_1 receptor (Losartan, 50mg/Kg/day). ACE activity and the efficiency of ACE inhibition were confirmed by enzymatic assay using Hip-His-Leu as the substrate in hippocampus homogenates and plasma. All experiments were conducted in accordance with the local Animal Care and Use Committee (protocol FMRP-USP 200/2005). **Results and Discussion**: Our results demonstrated that ACE mRNA in the hippocampus is upregulated about 11-fold in naïve WARs when compared to naïve Wistar animals, corroborating with the increased ACE activity in naïve WARs. In the WARs after multiple seizures ACE and AT_1 mRNA levels were upregulated in the hippocampus (2.5- and 8-fold, respectively) when compared to naïve WAR. The treatment with Enalapril and Losartan led to a significant impairment of seizures in WAR animals, whereas Wistar rats presented no alterations under the same conditions. The results demonstrated that the functional blockage of RAS, by usage of either an enzymatic inhibitor or a receptor antagonist, was able to pronouncedly impair the triggering and maintenance of epileptic seizures. Furthermore, these data support a potential new application for centrally acting drugs that target RAS which have the benefit of lower side-effects than classical anticonvulsants, and may represent an opportunity to a large percentage of epileptic patients who are non-responsive to the classical therapies. Supported by FAPESP, CNPq, FAEPA and CAPES.

02.047

Influência do sistema endocanabinóide nas alterações comportamentais durante o estado doentio induzido por lipopolissacarídeo. Stivanin TS, Giusti-Paiva A UNIFAL - Ciências Biomédicas

Introdução: Lipopolisacarídeo (LPS) é uma parede celular componente de bactérias gram-negativas reconhecida pelo sistema imune dos vertebrados como um patógeno. Em animais experimentais, injeções periféricas de LPS, resultam em uma infecção e ativam o sistema imune produzindo inflamação, a qual produz efeitos neuroendócrinos e comportamentais caracterizando o comportamento doentio (*sickness behaviour*). Espera-se que a manipulação do sistema endocanabinóide possa modificar as avaliações de comportamentais do animal durante o estado doentio induzido pelo LPS. Desta forma, neste trabalho avaliamos a participação dos endocanabinóides nas alterações comportamentais durante o estado doentio induzido por LPS. **Métodos:** Foram utilizados camundongos machos pesando entre 25 e 30 g. No dia do experimento, os animais foram pré-tratados com veículo ou AM251 (antagonistas do receptor CB1 para endocanabinóides) nas doses de 1 e 3 mg/kg (n=6-8 por grupo) e trinta minutos após os camundongos foram tratados com veículo (NaCl 0,9%) ou lipopolissacarídeo de E. coli (200 µg/kg). Duas horas após, os animais foram submetidos aos testes de campo aberto, nado forçado ou foi avaliado a ingestão de alimentos. No teste de campo aberto, os camundongos foram colocados no centro da arena, e foi avaliado o número de linhas cruzadas pelos camundongos e o número de vezes que o animal se posicionou sobre as patas traseiras, durante 5 minutos, para avaliação da atividade motora espontânea. O teste de nado forçado foi realizado em cilindro de 10 cm de diâmetro, contendo água a 25 ±1ºC por 6 min, e foi avaliado o tempo de imobilidade durante os últimos 4 minutos. A avaliação da ingestão de alimentos consistiu na mensuração do consumo de ração durante 24 horas após administração de LPS. **Resultados e Discussão:** No campo aberto foi observada uma redução do número total de linhas cruzadas após a administração de LPS (83,3±5,4; $p<0,05$) quando comparado com o grupo controle (122,9±7,2). O pré-tratamento com AM251 nas doses de 1 e 3 mg/kg acentuaram o efeito do LPS (51,0±5,3 e 54,4±7,5 respectivamente; $p<0,05$), sem provocar alterações nos animais tratados com salina (102,8±2,3 e 116,5±14,9 cruzamentos). Da mesma forma, o LPS reduziu o número de vezes que o animal ficou sobre as patas traseiras (22,2±3,7 vezes; $p<0,05$) quando comparado com o grupo controle (38,0±4,8 vezes) e esta redução foi acentuada pelo AM251 nas doses de 1 e 3 mg/kg (8,16±1,7 e 10,6±2,6 vezes, respectivamente; $p<0,05$). No nado forçado o LPS provocou um aumento do tempo de imobilidade de 65,3±14,6 s para 130±13,5 s; $p<0,05$). O pré-tratamento com AM251 acentuo este efeito do LPS (170±8,7 e 179±6,7 s; respectivamente 1 e 3 mg/kg de AM251$p<0,05$), sem provocar alterações nos animais controle. O LPS ainda provocou uma redução no consumo de ração de 2,91±0,10 para 2,10±0,12 g/10 g de camundongo ($P<0,05$). Os pré-tratamentos com AM251 acentuou este efeito do LPS (1,35±0,27 e 1,21±0,25 g/10g; $p<0,05$), sem provocar alterações nos animais tratados com salina. Assim evidenciamos que bloqueio do sistema endocanabióide acentua o comportamento doentio durante a sepse induzida por LPS. Todos os protocolos experimentais foram aprovados pelo Comitê de ética da UNIFAL-MG para o uso de animais (protocolo 180/2008). Apoio Financeiro: CNPq, FAPEMIG

02.048

Behavioral evaluation in mice withdrawn from repeated ketamine treatment and exposed to the forced swimming and tail suspension tests. Silva FCC, Citó MCO, Silva MIG, Santos LKX, Aquino Neto MR, Moura BA, Lima ST, Sousa FCF UFC - Fisiologia e Farmacologia

Introduction: Ketamine (ket) has been used clinically as a dissociative anesthetic since the 1960s and is regarded as a noncompetitive glutamate N-methyl-D-aspartic acid (NMDA) receptor antagonist (MARTIN, 1985). Currently, it was observed frequently that its use does not restrict only to the practical clinic or research, but been used as drug of abuse for the young in parties as a powerful hallucinogen. However, the unwanted effects that could appear when used abusively were not studied yet (FREESE, 2002). The aim of study is to investigate if the acute administration of ket promotes antidepressive-like effect, as showed the literature, and if this effect persists after 4 and 7 days of treatment with 24 hours of abstinence. **Metodology:** This work was approved by Committee on Ethics in Animal Research, number 41/09. Male Swiss mice (30 g) were injected intraperitoneally with ket (20 mg/kg) or its vehicle and tested in the forced swimming test (FST) or the tail suspension test (TST), the evaluated parameter was the immobility time in seconds. In the next series of experiments, mice were treated acutely and subchronic (4 and 7 days of treatment). Animals in the subchronic treatment were exposed to 24 hours of withdrawal before the experiments. Statistical analyses were performed using Graph Pad Prism 5.03 for Windows and statistical significance was calculated using one-way analyses of variance (ANOVAs) followed by Newman-Keuls as *post hoc* test. The results are presented as mean ± S.E.M. Results were considered significant at $p<0.05$. **Results:** We report antidepressant-like effect after single administration of ket as compared to control group in FST (control: 115.7±11.7, ket 20mg: 73.3±6.4 $p< 0.01$) and TST (control:118.8±3.6, ket 20 mg: 89.0±12.0 $p< 0.05$). The 4 days treatment group also showed an antidepressant-like effect in FST (control: 138.1±9.5, ket 20 mg: 86.1±18.1 $p< 0.05$) and TST (control: 117.0±7.4, ket 20mg: 75.7±14.0 $p< 0.05$), although the 7 days treatment had showed a depressive-like effect in FST (control: 67.0 ± 6.3, ket 20 mg: 109.0 ± 14.6 $p< 0.05$) and TST (control:67.8± 5.0, ket 20 mg: 115.5± 3.5). **Discussion:** Acute administration of ket showed a reduction in the immobility time in both experiments FST and TST, indicating an antidepressive-like effect, as previous studies showed (BERMAN, 2000). Grasing & Ghosh have reported similar increase of immobility duration in the FST during withdrawal from long-term amphetamine treatment. Similarly to these results we verified that 4 days of treatment with ket reduced the immobility time in FST and TST as compared to control, and with a longer treatment with ket (7 days) increased the immobility time in the FST and TST during withdrawal from long-term Ket treatment. **References:** 1. Martin, D. *Neuropharmacology*, v.24, p.999–1003, 1985; 2. Freese, T.E. *J Subst Abuse Treat*, v.23, p.151-6, 2002; 3. Berman, R.M. *Biol Psychiatry*, v.47, p.351-4, 2000; 4. Grasing, K. *Neuropharmacology*, v.37, p.1007-1017, 1998. **Financial Support:** CAPES, Funcap, CNPq.

02.049

Participação dos receptores B1 e B2 para cininas na perda de memória após a infusão crônica do peptídeo beta-amilóide 1-40 humano (BA), em camundongos. Amaral FA[1], Lemos MTR[1], Dong KE[1], Bittencourt MFQP[1], Caetano AL[1], Pesquero JB[2], Viel TA[3], Buck HS[1] [1]FCMSCSP - Ciências Fisiológicas, [2]UNIFESP - Biofísica, [3]EACH-USP

Introdução: A infusão crônica do BA no ventrículo lateral (VL) de ratos leva à neurodegeneração, aumento da densidade dos receptores cininérgicos em áreas corticais e hipocampais, depósitos de BA e perda de memória. A injeção aguda de BA no VL causa déficit de aprendizagem em camundongos e foi abolida em animais com deleção do gene para os receptores B1 (koB1) ou B2 (koB2). Considerando que o tecido cerebral e a ativação dos receptores cininérgicos podem ocorrer de forma diferente à aplicação aguda ou crônica de concentrações semelhantes de BA, esse estudo avaliou a participação dos receptores B1 ou B2 no déficit de memória após a infusão crônica de BA. **Métodos e Resultados:** Foram utilizados camundongos machos das linhagens C57Bl/6J (wt, controle genético), koB1 ou koB2 (12 semanas de idade), previamente treinados em esquiva ativa de duas vias e que apresentaram respostas condicionadas (RCs, % de 50 testes) entre 30 e 70 % 24 horas após a sessão de treino. Os camundongos que apresentaram esse índice de memória receberam a infusão crônica de BA (460 pmol, 0.12 µL/h, 28 dias, n=8) ou veículo (Controle, n=8) no VL, através de uma cânula guia de inox conectada por um tubo de vinil a uma mini bomba osmótica implantada subcutaneamente na nuca do animal. As comparações estatísticas foram realizadas por análise de variância de duas vias, seguida pelo pós-teste de Bonferroni. No teste antes da cirurgia (T1) não observamos diferenças nas RCs entre os controles (wt=59,71±6,75%; koB1=46,67±4,05%; koB2=64,4±5,77%) e os BA (wt=66±3,05%; koB1=66,8±8,23%; koB2=58,75±5,95%). Os animais foram testados sete e 35 dias (T7 e T35) após o início da infusão. Não foram observadas alterações significantes nas RCs dos grupos Controles nos testes sete e 35 em comparação ao T1. No T7 somente o grupo BA-koB2 apresentou diminuição significante das RCs (41,0±8,64%), quando comparado com o grupo Controle-koB2 (72,8±2,24; $P<0,05$) e com o T1 ($P<0,05$). No T35 foi observada a diminuição significante das RCs nos grupos BA-wt (40,67±3,33%; $P<0,05$) e BA-koB2 (41,25±10,72%; $P<0,05$), em comparação aos seus respectivos grupos controles. O grupo BA-koB1 não apresentou diminuição das RCs ao longo do período de infusão (T35=64,0±13,96%) quando comparado ao grupo Controle e ao T1. **Conclusões**: Nós sugerimos que na infusão crônica de BA o receptor B1 pode ter uma função no processo neurodegenerativo ocasionado pela BA. Por outro lado, considerando o déficit de memória prematuro dos animais koB2 na presença de BA indica que o receptor B2 possa atuar como um fator neuroprotetor. **Fuporte Financeiro:** FAPESP, CNPq, CAPES. Protocolo nº 155 do CEEA-FCMSCSP.

02.050

Antidepressant-like effect of carvacrol (5-Isopropyl-2-methylphenol) in mice: evidence for the involvement of the dopaminergic system. Cavalcante GIT[1], Félix FHC[1], Moura BA[1], De Sousa DP[2], Vasconcelos SMM[1], Sousa FCF[1], Fonteles MMF[1] [1]UFC - Fisiologia e Farmacologia, [2]UFS - Fisiologia

Introduction: Depressive disorders are among the most common diseases in humans. The use of tricyclic antidepressants and monoamine oxidase inhibitors are associated with many side-effects and have limited safety in overdose. Since ancient times, natural products have consistently been an important source of therapeutic agents. According to these informations, herbal medicine can be used as an alternative treatment to depression with less side-effects. Carvacrol (5-Isopropyl-2-methylphenol) is a monoterpenic phenol present in the essential oil of Labitae including Origanum, Satureja, Thymbra, Thymus, and Corydothymus. The objective of this present work is to investigate the possible mechanism by which carvacrol is actuating in the antidepressant effect on forced swimming test (Porsolt et al, 1977). **Methods:** This work was approved by Committee on Ethics in Animal Research (CEPA), number 15/09. Carvacrol (cvc) was administered orally at single doses of 25 mg/kg while bupropion 30 mg/kg, prazosin (1 mg/kg), yohimbine (1 mg/kg), SCH23390 (0.05 mg/kg) and sulpiride (50 mg/kg), were administered i.p. and used to investigate the involvement of the noradrenergic and dopaminergic systems in the anti-immobility effect in seconds of carvacrol in the forced swimming test. **Results and discussion:** The results are presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Student–Newman–Keuls´s *post hoc* test. Results were considered significant at $P<0.05$. Results showed that the pretreatment of mice with prazosin (1 mg/kg) and yohimbine (1 mg/kg), did not affect the antidepressant-like effect of carvacrol (25 mg/kg) as compared to control group [control: 98.88±4.987 (8); CVC 25: 12.70±1.630 (8); PRZ-1: 106.3±4.770 (9); YOIM-1: 109.8±4.329 (9); CVC-25+PRZ-1: 18.53±4.871(8); CVC25+YOIM1: 25.83±5.581 (8) (sec)]. On the other hand, the pretreatment of mice with SCH23390 (0.05 mg/kg) and sulpiride (50 mg/kg) completely blocked the decrease in the immobility time elicited by carvacrol (25 mg/kg) and bupropion (30 mg/kg), as compared to control group [control: 85.24±10.47 (8); CVC 25: 31.10±3.657 (7); SULP-50: 82.95±11.86 (8); SCH-0.05: 86.06±12.21 (7); BUP-30: 35.29±8.294 (7); CVC-25+SULP-50: 133.5±13.71 (10); CVC-25+SCH-0.05: 90.49±11.91 (7); BUP-30+SULP-50: 102.4±5.137 (8); BUP-30+SCH-0.05: 101.0±5.220 (sec)]. The results suggest that the antidepressant-like effect of oral administration of carvacrol at 25 mg/kg is not involved with noradrenergic transmission but is involved with dopaminergic transmission as demonstrated in the forced swimming test. Financial Support: CNPq. **References**: Porsolt et al, *Eur J Pharmacol*, 47:399, 1978; Ultee et al, Appl and *Environ Microbiol*, 65: 6406, 1999; Abu-Lafi et al, *Bioresource Technology*, 99: 3914, 2008; Machado et al, *Eur J Pharmacol*, 10: 587, 2008.

02.051

Alteração da densidade de receptores colinérgicos nicotínicos α7 em áreas relacionadas aos processos de memória após exercício físico crônico e moderado. Albuquerque MS[1], Baraldi T[1], Buck HS[2], Viel TA[1] [1]EACH-USP, [2]FCMSCSP - Ciências Fisiológicas

Introdução: Os processos de memória envolvem diversos circuitos neuronais que recrutam, principalmente, o córtex entorrinal, áreas hipocampais e amígdala. A formação da memória de longa duração ocorre a partir da gênese de potenciais de longa duração (LTP – *long term potentiation*) que são modulados em algumas áreas pelo sistema colinérgico. Em estudo anterior, mostramos que o exercício físico crônico e moderado, realizado em esteira ergométrica, aumentou significativamente a evocação da memória de ratos pouco-responsivos ao condicionamento clássico, avaliado em esquiva ativa. O objetivo desse trabalho foi avaliar a densidade de receptores colinérgicos nicotínicos (nAChR) α7 nas áreas cerebrais envolvidas com aprendizado, consolidação e evocação das memórias desses animais. **Métodos:** Ratos machos (3-4 meses) foram submetidos ao teste de memória em esquiva ativa e aqueles com baixo desempenho no teste (pouco-responsivos) foram selecionados. Logo após as avaliações de memória, os animais sedentários (n=3) e treinados (n=4), por 11 semanas em esteira ergométrica, foram anestesiados e os cérebros foram extraídos e congelados. Amostras histológicas (20μm) foram obtidas em criostato (-21ºC) e utilizadas em ensaios de radioautografia para os nAChR α7 utilizando o radioligante [^{125}I]-α-bungarotoxina (5nM, 90min, 25ºC). A ligação não-específica foi determinada usando 2μM da toxina não marcada. Os radioautogramas foram analisados em equipamento MCID de análise densitométrica digital. **Resultados:** Foram observadas marcações para esses receptores em diversas áreas cerebrais como: córtex orbital, núcleo *accumbens*, núcleos amigdalares, áreas hipocampais, talâmicas e hipotalâmicas tanto nos animais treinados quanto nos sedentários. Nos animais treinados, houve aumento significativo ($p>0,0001$) na densidade de nAChR α7 nas células piramidais da área CA1 do hipocampo (12,4 ± 0,6 fmols/mg de tecido) e na concha do núcleo *accumbens* (14,1 ± 0,8 fmols/mg de tecido) comparativamente às leituras obtidas das amostras dos animais sedentários (5,2 ± 0,3 fmols/mg de tecido e 9,1 ± 0,6 fmols/mg de tecido, respectivamente). **Discussão:** Sabe-se que o treinamento físico aumenta a produção de endorfinas e neurotrofinas como a neurotrofina derivada do cérebro (BDNF – *brain-derived neurotrophic factor*), que modula e estabiliza a expressão dos nAChR α7 pré-sinápticos. Nesse trabalho, os animais com déficit de memória apresentaram melhora considerável da memória após treinamento físico crônico e moderado. Além disso, houve aumento significativo de nAChR α7 nas células piramidais da área CA1 do hipocampo, onde ocorre LTP, o que sugere que esses receptores podem estar envolvidos com a melhora da memória de longa duração. A alteração de receptores no núcleo *accumbens* pode estar relacionada à modulação dopaminérgica que sabidamente ocorre com a prática de atividades físicas. **Aprovado pelo Comitê de Ética em Experimentação Animal da FCMSCSP:** Protocolo nº 175 **Apoio Financeiro:** FAPESP, PIBIC-CNPq/USP

02.052

Estimulação cognitiva e recuperação da memória ao longo do envelhecimento de camundongos: efeitos na memória espacial e emocional. Baraldi T[1], Albuquerque MS[1], Buck HS[2], Viel TA[1] [1]EACH-USP, [2]FCMSCSP - Ciências Fisiológicas

Introdução: Durante o processo de envelhecimento ocorrem muitas alterações nas funções fisiológicas. No sistema nervoso central o grau de declínio da memória depende da quantidade e da qualidade de estímulos recebidos ao longo da vida. O objetivo desse trabalho foi avaliar a recuperação da memória espacial e daquela gerada por estímulo aversivo durante o processo de envelhecimento de camundongos submetidos ou não à estimulação cognitiva. **Métodos:** Camundongos C_{57}Bl/6J (dois meses de idade) foram submetidos ao labirinto de Barnes por cinco minutos ao longo de cinco dias consecutivos para avaliação da memória espacial. Em cada observação foi registrada a latência para achar uma caixa de escape sob o labirinto. Em seguida, os animais foram submetidos ao equipamento de esquiva inibitória (2 seg, 0,5 Hz, máximo de 180 seg) para avaliar a memória gerada por estímulo aversivo. Após os testes, parte dos animais foi colocada em um ambiente com brinquedos, pontes e rodas (n = 10, "grupo estimulado"). Os objetos foram trocados a cada 2 dias. A outra parte dos animais foi deixada em caixas regulares, sem objetos (n = 10, "grupo controle"). Ao chegarem aos cinco meses de idade, os animais foram submetidos aos testes comportamentais novamente. **Resultados:** Quando os animais estavam com dois meses de idade, todos apresentaram uma redução significativa ($P < 0,05$) na latência para achar a caixa de escape no labirinto de Barnes cinco dias (195,1 ± 22,0 seg) após o início do teste (255,9 ± 13,4 seg), indicando que ocorreu o aprendizado. Após três meses no ambiente enriquecido, os camundongos estimulados apresentaram uma redução significativa ($P < 0,05$) nas latências para achar a caixa de escape, no primeiro e terceiro dias de observação (181,1 ± 30,4 seg e 143,3 ±27,7 seg), quando comparados com as observações feitas quando os animais tinham dois meses de idade (255,9 ± 13,4 seg e 222,5 ± 16,3 seg, respectivamente). Os animais do grupo controle não apresentaram diferenças nas latências em relação às performances observadas quando eram mais jovens. No teste de esquiva inibitória, aos dois meses de idade, todos os animais apresentaram aumento das latências registradas nas sessões de teste (180seg [180/180], $P < 0,001$) realizadas 24 horas após as sessões de treino (44seg [25/68]). Após três meses de observação ambos os grupos, controle (180 seg [180/180]) e estimulado (180 seg [143/180]), mostraram aumento significativo das latências ($P < 0.001$), quando comparados à sessão de treino realizada três meses antes. **Discussão:** A estimulação cognitiva foi efetiva em melhorar a memória espacial dos camundongos adultos, em comparação às observações realizadas quando os animais eram mais jovens e àquelas obtidas com os animais controles. No teste de esquiva inibitória não foi observada influência do estímulo cognitivo. Isso provavelmente ocorreu devido ao procedimento experimental uma vez que, nesse teste, o aprendizado envolve o condicionamento do medo como forte reforço da memória. **Aprovado pelo Comitê de Ética em Experimentação Animal da FCMSCSP:** Protocolo nº 173. **Apoio financeiro:** PIBIC-CNPq/USP, FAPESP

02.053

Cold stress is similar to the restraint stress in the modulation of the rat pineal gland. Couto-Moraes R[1], Monteiro AA[1], Ferreira ZS[1], Palermo-Neto J[2], Markus RP[1] [1]IB-USP - Chronopharmacology, [2]FMVZ-USP - Neuroimunomodulation

Introduction: Previous studies in our laboratory have demonstrated that the incubation of cultured rat pineal with the adrenal anti-inflammatory hormone, corticosterone, potentiates, while the pro-inflammatory cytokine TNF-alpha inhibits, Noradrenalin-induced N-acetylserotonin production, as they enhance or inhibit, respectively, the transcription of arylalkylamine N-acetyltransferase (see review by Markus *et al.*, Neuroimmunomodulation,14:126, 2007). In another study it was demonstrated that restraint stress in apparatus for 2h was able to modulate the rat pineal gland (COUTO-MORAES *et al.*, Ann NY Acad Sci, 1153:193, 2009). The cold exposure (4°C for 1 hour) increases the plasma corticosterone levels (PALMA *et al.*, Brain Res, 861:97, 2000), turning it an useful model of stress. Therefore, our aim was to test if cold stress could modulate the nocturnal N-acetylserotonin/melatonin surge as observed in restraint stress. **Methods:** Male Wistar rats (12:12 L/D cycle, aged 3-4 months) were used. In the 1st study, the rats were divided into 2 groups (8/group): *control* with social isolation in housed cage (CTRL), and *cold* in a refrigerator (5.0 ± 1.0 °C) (COLD), both for 0.5 or 2h. Thus, the pineal N-acetylserotonin (NAS) and melatonin (MEL) content (ng/pineal) was quantified by HPLC (5/group) after euthanasia at the ZT18-20. In the 2nd study, the rats were divided into 2 groups (3/group): *naïve* non experimentally manipulated euthanized at ZT11 (N Day) or at ZT14 (N Night), and *cold* (COLD 2.0) which were euthanized at 0, 10, 30, 60 and 120 min after stress. In this study, the plasma corticosterone (CORTICO) levels (ng/mL) were measured by RIA. In both studies, gastric ulcers were determined after euthanasia. The regression of the data were tested by two-way ANOVA. Results*:* Pineal NAS did not modify among groups. Pineal MEL increased 1.8 fold from COLD 0.5 (1.92±0.53) to COLD 2.0 (3.44±0.85), while no regression on time was detected for CTRL animals. CORTICO increased just after cold stress for 2h (0, 10 min) was significantly different from control, and the plasma levels regressed to naïve levels (148.8±32.5) following an exponential curve. ULCERS were not observed in any experimental group. Therefore, 2h of cold induced a mild stress. Discussion*:* The COLD was not enough to promote ULCERS, but activated the HPA axis, according to the increase in CORTICO levels. This increase in adrenal hormone imposes an enhancement in nocturnal MEL surge, confirming again the interaction between adrenal and pineal function observed in restraint stress. Restraint and cold stress are different kind of stress, being one psychological and the other physical stress, respectively (Sawchenko *et al.*, *Prog Brain Res*, 107:201, 1996). Therefore, the stress effect upon the pineal gland in rats is not dependent of the stress type what could be learned as effect of the enhancement in the plasma corticosterone levels and not by the neurocircuitry activation. This is supported by the corticosterone infusion directly upon the pineal gland in light phase or dark phase what increases the melatonin production (Fernandes *et al.*, *J Neuroendocrinol*, 21:90, 2008). **Financial Support:** CAPES, CNPq and FAPESP.

02.054

Antioxidant effects of different extracts from *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citrates.* Pereira RP[1], Fachinetto R[2], Prestes AS[1], Puntel RL[1], Da Silva GNS[2], Athayde ML[2], Bürger ME[1], Morel AF[1], Rocha JBT[1] [1]UFSM - Química, [2]UFSM - Farmácia Industrial

Introduction: Considering the important role of oxidative stress in the pathogenesis of several neurological diseases, and the growing evidence of the presence of compounds with antioxidant properties in the plant extracts, the aim of the present study was to investigate the *in vitro* antioxidant capacity of three plants used in Brazil to treat neurological disorders: *Melissa officinalis*, *Matricaria recutita* and *Cymbopogon citratus*. The *in vitro* antioxidant effect of phenolic compounds commonly found in plant extracts, namely, quercetin, gallic acid, quercitrin and rutin was also examined for comparative purposes. **Methods:** Cerebral lipid peroxidation (assessed by TBARS) was induced by iron sulfate (10 μM), sodium nitroprusside (5 μM) or 3-nitropropionic acid (2 mM). Free radical scavenger properties and the chemical composition of plant extracts were assessed by 10-10 Diphenyl-20 picrylhydrazyl (DPPH) method and by Thin Layer Chromatography (TLC), respectively. The protocol of study was reviewed and approved by the appropriate institutional review board from Guidelines of the Committee of UFSM (0089.0.243.000-07). Results and discussion: *M. officinalis* aqueous extract caused the highest decrease in TBARS production induced by all tested pro-oxidants. In the DPPH assay, *M. officinalis* presented also the best antioxidant effect, but, in this case, the antioxidant potencies were similar for the aqueous, methanolic and ethanolic extracts. Among the purified compounds, quercetin had the highest antioxidant activity followed by gallic acid, quercitrin and rutin. In this work, we have demonstrated that the plant extracts could protect against oxidative damage induced by various pro-oxidant agents that induce lipid peroxidation by different process. Thus, plant extracts could inhibit the generation of early chemical reactive species that subsequently initiate lipid peroxidation or, alternatively, they could block a common final pathway in the process of polyunsaturated fatty acids peroxidation. Our study indicates that *M. officinalis* could be considered an effective agent in the prevention of various neurological diseases associated with oxidative stress. The financial support by CAPES/SAUX/ PROAP, VITAE Fundation, CNPq, FAPERGS, ICTP and FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00 is gratefully acknowledged.

02.055

Effect of metadoxine *per se* and on the acute effect of ethanol in the locomotor activity in mice. Higa V.S.[1], Santos Renan[2], Baldaia, M. A.[2], Wuo-Silva R[3], Hollais AW[2], Araújo BA[2], Talhati F[2], Rosa LFD[1], Frussa-Filho R[1], Marinho EAV[4] [1]UBC - Farmácia, [2]UBC/UNIFESP - Farmácia/Farmacologia, [3]UNIFESP - Farmacologia, [4]UBC/UNIFESP - Ciências da Saúde/Farmacologia

Introduction: Metadoxine is an ion-pair between pyrrolidon carboxilate and pyridoxine with the two compounds linked in a single product by salification. In animal studies metadoxine increases plasma and urinary excretion of ethanol, inhibits the increased production of fatty acid esters in the liver during chronic alcohol intake and reduces oxidative stress. In the brain of rodents metadoxine increases the levels of GABA and acetylcholine in the frontoparietal cortex and of dopamine in the striatum. Drugs of abuse, including alcohol, increase dopamine levels in the nucleus accumbens producing, in rodents, locomotor stimulation, a behavior which is sensitized after repeated administration. This sensitization has been proposed to share neuronal mechanisms with drug craving. The aim was to investigate the effect of different doses of metadoxine *per se* on the locomotor activity (LA) as well as on the acute locomotor stimulant effect of the ethanol in mice (UBC Ethic committee: 017/2009). **Methods:** Swiss mice were habituated for 2 days receiving intraperitoneally (i.p.) saline being 30 min later exposed to the open field (OF) for 5 min. On the 3rd day the animals received an i.p. injection of saline (Sal) or 10, 30, 100, 200 or 400 mg/kg of metadoxine (Met) and 30 min after they were exposed to an OF for 5 min for LA quantification (1st session). Immediately after this 5 min of session, the animals received saline or ethanol (Eth) (1.8g/kg) and 5 min later were placed again in OF for LA quantification for 5 min (2nd session). Seven days later the animals were challenged with saline or ethanol (1.8g/kg) (3rd session). Thus the following groups were formed: Sal-Sal-Sal, Sal-Sal-Eth, Sal-Eth-Eth, Met10-Eth-Eth, Met30-Eth-Eth, Met100-Eth-Eth, Met200-Eth-Eth and Met400-Eth-Eth. **Results:** In the 1st session the administration of Sal or Met in all doses did not promote alterations in the LA of mice (ANOVA, $p<0.05$). The animals of the Sal-Eth-Eth group presented higher LA (165±19) than the animals of the Sal-Sal-Sal (47±13) and Sal-Sal-Eth (45±11). In the 2nd session the animals of the Met10-Eth-Eth and the Met30-Eth-Eth groups (137±23 and 141±11 respectively) did not present differences when compared to the Sal-Eth-Eth group. The groups pretreated with Met in the 100, 200 and 400 mg/kg doses presented a significant reduction in the LA (92±15, 79±19 and 99±8 respectively) when compared to the Sal-Eth-Eth group. In the 3rd session the Sal-Sal-Eth group, presented a higher locomotion (184±17) compared with the group treated with Sal-Sal-Sal (66±5). The Sal-Et-Et group presented a significantly higher LA (278±33) than the Sal-Sal-Eth group, suggesting the development of behavioral sensitization to ethanol. All the groups that were previously treated with Met and that received the challenge injection of Eth, presented a significant reduction in the LA (162±32, 186±30, 155±29, 188±35 and 179±41 to the 10, 30, 100, 200 and 400mg/kg doses of Met) when compared to the Sal-Eth-Eth presenting no statistical difference when compared to the Sal-Sal-Eth group. **Conclusion:** Metadoxine in the doses of 100, 200 and 400 mg/kg reduced the acute locomotor stimulant effect of ethanol. Metadoxine in all the doses utilized was efficient to avoid the expression of behavioral sensitization to ethanol. **Financial support**: Universidade Braz Cubas and Laboratórios Baldacci

02.056

Sodium fluoride-induced memory impairment is associated with changes in striatal monoaminergic levels. Pereira M[1], Dombrowski PA[1], Da Cunha C[1], Losso EM[2], Andreatini R[1] [1]UFPR - Farmacologia, [2]Universidade Positivo - Odontologia

Protocolo Com. ética: CEEA 088. Apoio financeiro: Fundação Araucária.

Purpose of the study: We have previously showed that chronic (30 days) NaF (sodium fluoride) intake (100 ppm) impaired open-field habituation and two-way active avoidance. Thus, in the present study we evaluated: (a) whether the NaF withdrawal 15 days reverts this impairment; (b) the impact of NaF intake and its withdrawal in monoamine levels (and their metabolites) in the hippocampus and striatum; (c) degree of teeth fluorosis. **Methods:** Young male Wistar rats (30 days old) were treated with: (a) 45 days of tap water (W+W) with 1.54ppm NaF; (b) initial 15 days with tap water following 30 days of 100 ppm NaF (W+NaF); (c) initial 30 days with 100 ppm NaF following 15 days of tap water (NaF+W). NaF was administered in drinking water. The rats were tested in the open-field and re-tested 24h later. Total ambulation (number of square crossed) was recorded during 2 min. Dental fluorosis was scored by visual inspection of central incisors. The data were analyzed by Chi-square test, Student t-test for paired samples or ANOVA followed by Newman-Keuls test. $p<0.05$ was considered statistically significant. **Results**: There was a decrease in the total ambulation (an indication of habituation and memory acquisition) in the W+W group ($p<0.05$; table 1). In opposite, both groups treated with NaF did not show decrease in open-field habituation. Dopamine, noradrenaline, serotonin and 5-HIAA levels in striatum increased significantly ($p<0.05$) in all NaF treated groups (W+W: 4826 ± 897 ; W+NaF: 10131 ± 2041; NaF+W: 12040 ± 1647; W+W: 66 ± 19 ; W+NaF: 136 ± 24; NaF+W: 147 ± 33; W+W: 742 ± 271 ; W+NaF: 1900 ± 587; NaF+W: 1972 ± 283; W+W: 863 ± 141; W+NaF: 1213 ± 181; NaF+W: 1266 ± 214). In the hippocampus, there was only a trend to increase noradrenaline and serotonin level ($p=0.06$). A trend to increase in fluorosis was seen only in the NaF+W group ($p=0.06$). During 100 ppm NaF solution administration, daily fluid intake lead NaF intake around 8 mg/kg/day. **Discussion**: These results indicated that NaF impairs habituation (a non-associative learning), and that short-term withdrawal did not revert this impairment. Moreover, this impairment is associated with an increase in DA, NA, 5-HT and 5-HIAA striatal levels and NA and 5-HT in hippocampus because habituation to an open field is proposed to be related to hippocampus and striatum, those structures may be the major candidate for target of the NaF effect. Thus, the present study suggests that the NaF-induced memory impairment may be long lasting and may be related to central monoaminergic alteration; and short-term NaF withdrawal cannot reverse the fluoride-induced effects. Based on this last finding, drugs that reduce monoaminergic neurotransmission may be useful to reverse this effect of NaF. **References**: (1) Chioca, L.R., Raupp, I.M., Da Cunha, C., Losso, E.M., Andreatini, R., 2008 Subchronic fluoride intake induces impairment in habituation and active avoidance tasks in rats. *Eur J Pharmacol* 579(1-3),196-201. (2) Spittle, B., 1994 Psycopharmacology of fluoride: a review. *Int Clin Psychopharmacol* 9, 79-82. (3) Wang, J., Ge, Y., Ning, H., Wang, S., 2004 Effects of high fluoride and low iodine on biochemical indexes of the brain and learning-memory of offspring rats. *Fluoride* 37, 201-8.

02.057

Anxiolytic-like effect of chamba (*Justicia pectoralis)* in mice*:* involvement of gaba/benzodiazepine receptor. Venâncio ET[1], Rocha NFM[1], Rios ERV[1], Feitosa ML[1], Linhares MI[1], Fonseca FN[2], Leal LKAM[2] Fonteles MMF[2] [1]UFC - Fisiologia e Farmacologia, [2]UFC - Farmácia

Introduction: Anxiety disorders are among the most common diseases in humans. The use of benzodiazepines is associated with many side-effects. Herbal medicines have consistently been an important source of therapeutic agents, including to the treatment of anxiety, probably with less side-effects. In this context, Chamba (*Justicia pectoralis*) is a plant widely used in popular medicine and its effect on central nervous system should be more investigated. This present work aimed to evaluate the possible mechanism taken by Chamba while actuating as anxiolytic on plus maze test. **Methods:** Chamba (CH) was administered orally at doses of 50, 100 and 200 mg/kg; diazepam, 1 mg/kg, was used as standard drug and flumazenil (FLU;10mg/kg) was used to investigate the involvement of the GABA/benzodiazepine receptor in the anxiolytic effect on the plus maze test. The parameters observed were: number of entries in the open arms (NEOA), time of permanence in the open arms (TPOA), percentage of entries and permanence in the open arms (PEOA, PTOA). The results were presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Student–Newman–Keuls´s *post hoc* test, with significance of $p<0.05$. This work was approved by Committee on Ethics in Animal Research, number 14/09. **Results and Discussion:** The treatment of mice with CH (100 and 200mg/kg) increased all parameters analyzed, NEOA, PEOA, TPOA and PTOA when compared with respective controls: NEOA [control: 3.76 ± 0.34 (10); CH100: 6.14 ± 0.67 (8); CH 200: 7.85 ± 0.79 (8); DZP-1: 10.75 ± 0.67 (8)]; PEOA [control: 33.35 ± 2.65 (10); CH 100: 48.44 ± 2.98 (8); CH 200: 49.76 ± 20.47 (8); DZP-1: 64.24 ± 3.03 (8)]; TPOA [control: 51.43 ± 4.79 (10); CH 100: 107.9 ± 7.85 (8); CH 200: 143.1 ± 10.32 (8); DZP-1: 157.3 ± 5.85 (8)] and PTOA [control: 19.81 ± 2.06 (10); CH 100: 41.81 ± 4.77 (8); CH 200: 49.92 ± 3.84 (8); DZP-1: 59.45 ± 3.26 (8)]. On the other hand, the pretreatment of mice with flumazenil decrease all the parameter observed in the plus maze test elicited by Chamba as compared to CH 200 group: NEOA [CH200: 7.85 ± 0.79 (8); CH200 + FLU:2,28±0,56 (7)]; PEOA [CH 200: 49.76 ± 20.47 (8); CH200 + FLU:27,99± 4,07(7)]; TPOA [CH200: 143.1 ± 10.32 (8); CH200 + FLU: 34,40±9,76(7)] and PTOA [CH 200: 49.92 ± 3.84 (8); CH200 + FLU: 22,76±4,88(7)]. Our findings suggest that the anxiolytic-like effect of Chamba is involved with GABA/ benzodiazepine receptor once it flumazenil reversed not only the diazepam effect but also the Chamba effect, indicating that both drugs might present a similar mechanism of action. **Financial Support**: CAPES and CNPq

02.058

Evaluation of effects of aqueous standardized extract of chamba (*Justicia pectoralis*) in depression models in mice. Siqueira RMP[1], Venâncio ET[1], Rocha NFM[1], Rios ERV[1], Linhares MI[1], Feitosa ML[1], Moura BA[1], Félix FHC[1], Fonseca FN[1], Sousa FCF[1], Leal LKAM[2], Fonteles MMF[2] [1]UFC - Fisiologia e Farmacologia, [2]UFC - Farmácia

Introduction: Depressive disorders are among the most common diseases in humans. The use of tricyclic antidepressants are associated with many side-effects. Since ancient times, natural products have consistently been an important source of therapeutic agents. According to these informations, herbal medicine can be used as an alternative treatment to depression with less side-effects. *Justicia pectoralis* var. stenophylla Leonard), from the Acanthaceae family, popularly named chamba in Brazil is a plant widely used in popular medicine and its effect on central nervous system should be more investigated. This present work aimed to investigate the effects of Chamba in depression models in mice as forced swimming and tail suspension tests (Porsolt et al, 1977; Steru et al. 1985). **Methods:** Chamba (CH) was administered orally at doses of 50, 100 and 200 mg/kg; imipramina (10 and 30 mg/Kg, ip) was used as standard drug in forced swimming and tail suspension tests, respectively. The parameter observed was**:** duration of immobility. Time unit: seconds. The results were presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Student–Newman–Keuls´s post hoc test, with significance of $p<0.05$. This work was approved by Committee on Ethics in Animal Research, number 14/09. **Results and Discussion:** The treatment of mice with CH (50, 100 and 200mg/kg) increased the parameter analyzed when compared with respective control. Forced swimming [cont (82,14±5,89), CH50 (104,6±5,54), CH100 (122,8±7,22), CH200 (141,8±8,05), IMI10 (31±4,47)]; Tail suspension [cont.(104,4±9,81), CH50 (159,7±12,36), CH100 (76,17±9,25), CH200 (117±7,24), IMI30 (20,38±2,23). Previous findings show that *Justicia pectoralis* had no alteration in locomotor activity so, our findings suggest that *Justicia pectoralis* (chamba) presented depressant effect on central nervous system.

02.059

Investigation of possible antidepressive-like effects of (-)-menthol in forced swimming and open field tests in mice. Vasconcelos PF[1], Silva MIG[1], Moura BA[1], Aquino Neto MR[1], Falcão do Amaral J[1], De Sousa DP[2], Macedo DS[1], Sousa FCF[1] [1]UFC - Fisiologia e Farmacologia, [2]UFS - Fisiologia

Introduction: It is well known that bupropion, an atypical antidepressant, at high doses, induces increased locomotor activity in animal models. However, at low doses, bupropion is able to continue producing antidepressant effects without affecting the locomotion of animals. Previous studies have reported the ability of (-)-menthol, a major constituent of peppermint oil, to promote ambulation in mice at high doses. Thus, the present study aimed to investigate whether the intraperitoneal administration of (-)-menthol, at low dose, would be able to induce similar effects to that promoted by bupropione, at same dose, in the Forced Swimming (FST) and Open Field Tests (OFT) in mice. **Methodology:** (-)-Menthol was emulsified with 0.2% Tween 80 (Sigma-USA) and dissolved in distilled water. Male *Swiss* mice (20-30g) received (-)-menthol (10 mg/kg, i.p.) or vehicle (saline 0.9%, i.p.) 30 minutes before the experiments, while buproprion (10 mg/kg, i.p.) was used as a positive standard. The work was approved by the CEPA, protocol number 078/07. **Results:** (-)-Menthol showed a significant decreased immobility time in the FST, as compared to the control group [Control: 95.00 ± 3.51 s (6); Ment 10: 65.83 ± 7.88 s (6); $p<0.01$]. Similar results were presented by buproprion [Bup: 51.00 ± 7.22 (4) s; $p<0.01$]. Also, similarly to bupropion, (-)-menthol did not presented any significant alteration in the OFT, as compared to control [Control: 61.20 ± 3.76 (5); Ment 10: 61.38 ± 3.17 (8); Bup:79.00 ± 8.14 (4)]. **Discussion:** FST is a classical behavioral animal model for detecting the antidepressant potential of drugs. The decreased immobility of animals in the FST induced by (-)-menthol and bupropione suggests antidepressive-like effects. However, FST is based in the animal motor response and drugs that increase general motor activity may provide false positive results in this test. Taking in account these considerations, we also decided to investigate the effects of (-)-menthol in the OFT, a recognized classical animal model used to evaluate autonomic effects of drugs and general activity of animals. Similarly to bupropion, used at low dose as positive control for antidepressant action, (-)-menthol did not induced any alteration on locomotor activity of animals in the OFT, suggesting absence of psychostimulants effects. **Conclusion:** Results suggest that (-)-menthol at low doses presents possible antidepressive-like effects. **Financial Support:** CAPES, CNPq

02.060

Effect of alterations on sleep-wake cycle and serotonin levels on food intake and body weight of wistar rats. Stefanello TF[1], Rosolem PS[2], Ramos ERP[3] [1]UEM - Bioquímica Clínica, [2]CESUMAR - Farmacologia, [3]UEM / CESUMAR - Farmacologia e Bioquímica Clínica

Introduction: The serotonin (5-HT) is related to the process of satiety during and after meals, which justifies the use of 5-HT reuptake inhibitors, such as fluoxetine (FLU) as anorectic agents (HALFORD, Drugs, v.67, p.27, 2007). The 5-HT also participates in the regulation of sleep-wake cycle (SWC) (GASPAR, Rev Ass Med Bras, v.44, p.239, 1998) and behavioral situations, such as stress, depression and anxiety (GRAEFF, Pharmacol Biochem Behav, v.54, p.129, 1996). Thus, it is possible that people with alterations on SWC may have alterations on eating behavior and weight gain. This work had as main objective to quantify and compare the food intake and weight gain in animals submitted or not to alterations on SWC and treated or not with FLU. **Methods:** Male Wistar rats were divided into four groups (n=9 each). In groups 1 and 2 the animals were subjected to a dark-light cycle of 12 hours and treated respectively with saline and FLU (5mg/kg). In groups 3 and 4 the pattern of light was constant (24 hours) and the animals also received, respectively, saline and FLU (5 mg/kg). FLU solution and saline were administered intraperitoneally for 30 days. The body weight and food intake were measured twice a week. At the end of the experiments, the anxiety state was evaluated using conventional and ethological measures of elevated plus-maze (EPM). Changes in metabolism were also evaluated by dosages of serum levels of glucose and triglycerides after 30 days of treatment. The results were described in a quantitative test and analyzed by One-Way ANOVA (non-parametric) followed by Bonferroni and level of significance $p<0.05$. This research was carried out in assent nº 37/06 of the *Comitê de Ética em Experimentação Animal* (CEEA) from Universidade Estadual de Londrina (UEL). **Results and Discussion:** The pattern of light and treatment with FLU didn't change the body weight of animals. However, animals with altered SWC and treated with FLU showed a significant reduction in food intake compared with group treated with FLU and normal SWC. This effect, probably, was not associated with the development of an anxious state, because the conventional and ethological measures of LCE did not differ between groups. Although the four groups have not shown differences in the values of fasting plasma glucose, there was significant reduction of serum triglycerides in the group treated with FLU and altered SWC. These results demonstrate that elevated levels of 5-HT by FLU in association with altered SWC reduces significantly the food intake. As the 5-HT is precursor of melatonin, a hormone released during dark phase of the SWC, it is possible that the constant light has reduced the production of melatonin and thus have increased the availability of 5-HT to exert its activity of satiety on hypothalamus (Simonneaux, Pharm Rev, v.55, p.325, 2003). The reduction of triglycerides levels showed a change in lipid metabolism, suggesting that prolongation of treatment and alterations on SWC could promote change on body weight of animals. These results suggest anorectic effect of fluoxetine might be influenced by alterations on SWC, probably due to increased levels of hypothalamic 5-HT. **Financial Support:** PROBIC / IC-CESUMAR.

02.061

Role of median raphe nucleus 5-HT1A receptors on the behavioral consequences of exposure to forced swim stress. Trovo, MC, Padovan CM FFCLRP-USP - Psicologia e Educação

Introduction: Exposure to chronic aversive stimuli leads to significant behavioral and neurochemical changes. Such alterations may be related to mal functioning of the Median Raphe Nucleus (MnRN)-Dorsal Hippocampus (DH) serotoninergic pathway, which involves 5-HT1a receptors (5-HT1aR). Previous data from our laboratory showed that intra-MnRN treatment with 8-OH-DPAT (DPAT), a 5-HT1aR agonist, after exposure to forced swim stress is able to attenuate stressor effects. These effects were blocked by previous treatment with WAY100635 (WAY), a 5-HT1aR antagonist. Therefore the aim or this work was to investigate whether intra-MnRN treatment with DPAT and/or WAY previous to forced swim stress could prevent its effects. All the procedures were approved by the local Animal Ethics Committee (CEUA) of the USP Ribeirão Preto (protocol 06.1.1131.53.0). **Methods:** Male wistar rats with cannulas aimed to the MnRN received two intracerebral injections (0.2 µL each) of Saline (Sal), DPAT (3nmols) and/or WAY (0.3nmoles) before being exposed to 15 minutes (min.) of forced swim. Groups were composed as follows: Sal+Sal, Sal+DPAT, WAY+Sal and WAY+DPAT. Test was performed 24 hours later during 5 min.. Latency (LAT) to the first episode of immobility and total time spent immobile (TTSI) were registered and analyzed by Oneway ANOVA followed by Duncan, considering $p<0.05$ for statistical significance. Data represent mean ± standard error of the mean (SEM) in seconds of animals which had their brain sites confirmed by histological analysis. **Results:** Treatment with WAY+DPAT (183.7±20.2; n=10) increased LAT ($F_{3,39}=8.6$) when compared to control group (Sal+Sal=76.58±13.86; n=13), while DPAT (Sal+DPAT=103,87±23,82;n=8) did not reach significance ($0.10<p<0.05$). WAY alone (Way+Sal=85.83±19.75;n=12) was not different from control group. Considering TTSI, intra-MnRN administration of DPAT and/or WAY (Sal+DPAT=76.8±8.8; WAY+Sal=90.5±10.5; WAY+DPAT= 51.1±11.6) were different from control group (Sal+Sal=133.1±5.6; $F_{3,39}=11.6$; $p<0.05$). **Discussion:** Behavioral consequences of exposure to forced swim stress were prevented by intra-MnRN treatment with DPAT and WAY. These results suggest that the MnRN may be under a serotoninergic tonic control which can be modulated by exposure to uncontrollable stress. **Affiliation:** SBFTE. **Financial Support:** CAPES, CNPq and FAPESP.

02.062

Harmine increases creatine kinase activity in the prefrontal cortex of adult rats. Santos PM[1], Scaini G[1], Réus GZ[2], Quevedo J[2], Crippa JA[3], Hallak JEC[4], Streck EL[1] [1]UNESC - Fisiopatologia Experimental, [2]UNESC - Neurociências, [3]USP - Neuropsiquiatria e Psicologia Médica, [4]FMRP-USP - Neurologia, Psiquiatria e Psicologia Médica

Objectives: Harmine is a β-carboline alkaloid that inhibits monoamine reuptake systems. Findings point to an antidepressant effect of the compounds that increases the levels of monoamines after monoamine oxidase inhibition. Considering that creatine kinase (CK) is important for brain energy homeostasis and that harmine effects on brain metabolism are poorly known, we evaluated CK activity in the prefrontal cortex of adult rats following acute or chronic the harmine and imipramine. **Methods:** To this aim, adult Wistar rats (250g) were acutely treated with harmine (5, 10 and 15 mg/kg) and imipramine (10, 20 and 30 mg/kg). Creatine kinase activity was measured in prefrontal cortex homogenates pre-treated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 mg protein in a final volume of 100 mL. After 15 min of pre-incubation at 37 oC, the reaction was started by the addition of 0.3 mmol of ADP plus 0.08 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 mmol of p-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). Results were expressed as units/min x mg protein. This work has been approved by UNESC Ethics Committee under protocol number 325/2008. **Results:** Our results showed that acute administration of harmine increased CK activity in the prefrontal cortex (sal: 5.84±0.16; harm 5: 10.64±0.25*, harm 10: 12.52±0.10*; harm 15: 11.2±0.24*; $p<0.05$, n=5). Imipramine acute administration also increased the enzyme (sal: 5.84±0.16; imi 10: 12.9±0.15*; imi 20: 14.2±0.17*; imi 30: 11.7±0.33*; $p<0.05$, n=5). Chronic administration of harmine (sal: 4.32±0.14; harm 5: 6.49±0.12*, harm 10: 3.59±0.13; harm 15: 3.57±0.24; $p<0.05$, n=5) and imipramine (sal: 4.32±0.14; imi 10: 3.48±0.12; imi 20: 6.72±0.32*; imi 30: 3.55±0.60; $p<0.05$, n=5) increased CK activity in the prefrontal cortex. **Conclusion:** These findings support the hypothesis that harmine, which has been considered as a new drug for the treatment of depression, increased CK activity, an important enzyme involved in brain energy homeostasis. **Financial support:** CAPES, CNPq, FAPESC, UNESC.

02.063

Cannabidiol increases creatine kinase activity in rat brain. Scaini G[1], Santos PM[1], Valvassori SS[2], Crippa JA[3], Hallak JEC[4], Streck EL[1], Quevedo J[2] [1]UNESC - Fisiopatologia Experimental, [2]UNESC - Neurociências, [3]USP - Neuropsiquiatria e Psicologia Médica, [4]FMRP-USP - Neurologia, Psiquiatria e Psicologia Médica

Objectives: Cannabidiol (CBD), one of the major components of marijuana may exert antipsychotic and anticonvulsant effects. Nevertheless, the mechanism of action of this nonpsychoactive phytocannabinoids remain unknown. Creatine kinase (CK) is an important enzyme that plays a key role in energy metabolism of nervous tissue. Here, we evaluated CK activity in the brain of adult Wistar rats (250g) following acute or chronic administration of CBD. **Methods:** CBD was administered intraperitoneally in the acute (one injection) and chronic (14 days) protocols in the doses of 15, 30 or 60 mg/kg. Creatine kinase activity was measured in prefrontal cortex, hippocampus, striatum and cerebral cortex homogenates pre-treated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 mg protein in a final volume of 100 mL. After 15 min of pre-incubation at 37 oC, the reaction was started by the addition of 0.3 mmol of ADP plus 0.08 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 mmol of p-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). Results were expressed as units/min x mg protein. This work has been approved by UNESC Ethics Committee under protocol 031/2009. **Results:** Our results showed that CBD increased the activity of CK only in the hippocampus (sal: 1.47±0.13, CBD15: 1.63±0.41, CBD30: 1.97±0.49, CBD60: 3.14±0.32*; $p<0.05$, n=5) and cortex (sal: 1.22±0.28, CBD15: 1.27±0.25, CBD30: 1.55±0.2, CBD60: 2.76±0.06*; $p<0.05$, n=5) after acute treatment. The chronic administration increased CK activity in hippocampus (sal: 4.67±0.39, CBD15: 7.22±0.29*, CBD30: 6.85±0.44*, CBD60: 8.21±0.33*; $p<0.05$, n=5), striatum (sal: 5.45±0.15, CBD15: 7.64±0.31*, CBD30: 7.73±0.2*, CBD60: 8.08±0.79*; $p<0.05$, n=5) and cortex (sal: 5.02±0.35, CBD15: 5.68±0.42, CBD30: 7.76±0.33*, CBD60: 7.53±0.44*; $p<0.05$, n=5). **Conclusion:** In conclusion, since CBD increases rat brain CK activity, our data support the notion that one of the mechanisms of action of CBD may be modulating brain CK activity function.
Financial support: CAPES, CNPq, FAPESC, UNESC.

02.064

B-amyloid precursor proteins and peptide B-amyloid are decreased in hippocampus and amygdala of rats reared in isolation from weaning. de Souza RG[1], Kerbauy LN[1], Sestito RS[1], Trindade LB[1], Iyomasa MM[2], Rosa ML[3] [1]FAMECA - Bioquímica, [2]FORP-USP - Morfologia, Estomatologia e Fisiologia, [3]FAMECA - Ciências Biofisiológicas

Introduction: Isoforms of amyloid precursor protein (APP) and β-Amyloid play a range of role in several physiological and pathological processes in the brain. Isolation rearing of rats from weaning has been used as experimental model of affective disorders like schizophrenia. This study aimed at evaluating the changes induced by isolation rearing in the expression of APP_{695}, $APP_{751/770}$ and peptide β-Amyloid in the hippocampal formation of rats. **Methods:** Two groups of Wistar rats (n=6/each) were used. In both groups the pups remained with their mothers (6 pups per mother) until weaning (21 days - 40g) when they were allocated randomly as follow: 1) grouped, housed 3 per cage and handled 3 times a week; 2) isolated, housed individually and handled once a week for cleaning purpose. After 10 weeks all animals were deeply anaesthetized, perfused and their brains removed. 40-mm sections were used for immunohistochemistry: anti APP_{695} (Sigma), anti $APP_{751/770}$ (Sigma), anti β-Amyloid (Sigma), secondary antibody (Dako), ABC Kit (VectaStain), DAB (Sigma). Using a light microscope Axioskop 40 with AxioCam ICc3 and AxioVision Release 4.6.3 04-2007, Zeiss, the immunopositive cells were counted by 3 examiners independently, in 3 sections/rat and bilaterally in hippocampus, amygdala and entorhinal cortex. Data were compared by Student *t*-test ($p<0.05$). Results: Isolation rearing induced a significant decrease in APP_{695}-immunopositive cells (43%) only in the hillus of dentate gyrus ($p=0.001$). No difference was seen in any other hippocampal area, amygdaloid nuclei or entorhinal cortex in isolated compared to grouped rats. $APP_{751/770}$-immunopositive cells were noted only in CA2 area of the hippocampus, where a high reduction (38%) was induced by isolation rearing ($p>0.001$). Although a few number of β-Amyloid-immunopositive cells was found in all areas of the hippocampus, a dramatic reduction was induced by isolation rearing in all areas (100% in hillus, $p=0.001$; 64% in CA3, $p=0.002$ and 51% in CA1, $p=0.02$). A higher number of β-Amyloid-immunopositive cells was seen in basolateral and lateral amygdala compared to hippocampus. However, a 100% of reduction was induced by isolation rearing only in lateral amygdala ($p=0.001$). No change was induced by isolation rearing in $APP_{751/770}$ or β-Amyloid in entorhinal cortex. **Conclusions**: APP isoforms and β-Amyloid may be involved on the complex mechanisms triggered in the hippocampus and amygdala during the development of schizophrenia. Committee on Animal Research and Ethics: CEUA-USP/Ribeirão Preto: 05.1.769.53.0. Financial support: FAPESP (Processos: 05/01501-7; 06/53343-9; 06/53345-1; 06/53342-2; 06/53344-5).

02.065

Effect of acute and chronic treatment with fluphenazine on monoamines and their metabolites in rats: relationship with orofacial dyskinesia. Fachinetto, R[1], Pereira RP[1], Villarinho, JG[1], Bandinélli, R.[1], Bandinélli, D.[1], Moraes, TA[1], Santanna, GS[1], Dombrowski PA[2], Da Cunha C[2], Cabrini DA[2], Rocha JBT[1], Ferreira J[1] [1]UFSM - Química, [2]UFPR - Farmacologia

Introduction: Tardive dyskinesia (TD) is a serious side effect caused by long-term treatment with neuroleptic drugs. Despite of innumerous studies concerning about the pathophysiology of TD, the exact mechanisms involved in its development remains unclear. Thus, in the present study, we investigate the participation of monoamines on acute and chronic model of orofacial dyskinesia (OD) induced by fluphenazine in rats. **Methods:** Adult male rats were treated during 3 or 24 weeks with fluphenazine (25 mg/kg, i.m., once every 21 days) and vehicle (1 mL/kg, i.m., once every 21 days). OD (assessed by vacuous chewing movements, VCMs), the levels of monoamines and its metabolites and the monoaminoxidase (MAO) activity were quantified after 3 (acute) or 24 (chronic) weeks after beginning of treatment. . The protocol of study was reviewed and approved by the appropriate institutional review board from Guidelines of the Committee of UFSM (0089.0.243.000-07). **Results and discussion**: Fluphenazine treatment produced VCMs in part of treated rats (50% after 3 weeks and about 70% after 24 weeks). Thus, we separated the rats that developed (+VCM) or did not develop (-VCM) OD. Acutely, fluphenazine treatment caused a significant decrease in dopamine levels in +VCM rats, but did not modify dopamine metabolites levels. We have also detected a negative correlation between dopamine levels and VCM number. Chronically, fluphenazine treatment did not produce alterations in dopamine levels; however it caused a significant increase in dopamine metabolites levels, HVA and DOPAC. We have also detected a positive correlation between DOPAC levels and VCM number and between HVA levels and VCM number. On the other hand, we did not find any alterations in the levels of serotonin (5-HT), noradrenaline (NA) and their metabolites or in the activity of MAO. Our data suggest that an increase in dopamine metabolism could contribute to the maintenance (chronic treatment) of VCMs in rats. The financial support by CAPES/SAUX/ PROAP, VITAE Fundation, CNPq, FAPERGS, ICTP and FINEP research grant “Rede Instituto Brasileiro de Neurociência (IBN-Net)” # 01.06.0842-00 is gratefully acknowledged.

02.066

The effect of the maternal separation on fear and anxiety behaviors and on spatial memory. Pereira MTR[1], Limonte FH[2], Iyomasa MM[3], Rosa MLNM[2] [1]FIPA-FAMECA - Neurociência, [2]FIPA-FAMECA - Bioquímica, [3]FORP-USP - Morfologia, Estomatologia e Fisiologia

Early life stress seems to put an individual at a greater risk for many mental disorders, such as depression and posttraumatic stress disorder, which may affect the levels of fear and anxiety and also the functions of learning and memory. The aim of this study was to evaluate the levels of anxiety and spatial memory in rats submitted to maternal separation (MS). Pups (n=10) underwent a daily-3h separation from their mothers from PND1-21 and the controls (n=11) were left undisturbed. On PND21, they were housed (4/cage) for 5 weeks before being tested on the elevated plus-maze (EPM). Behavioral responses were scored for 5min: number of entries and time on the open or closed arms and number of stretched attend postures (SAPs). They were retested after 24 hours. Groups were compared by Student *t*-tests ($p<0.05$). Maternal separation induced a non significant increase in both number of entries and time in the open arms on the first test. However, a non significant reduction was induced by MS in both scores when the animals were re-exposed to EPM 24 hours later. The time in the open arms was only 1,8 - 2,0% of the total time in any arm. A significant decrease was induced by MS in the SAPs when the animals were re-exposed to EPM (46.8-51%, $p<0.05$). This stress also induced a reduction in the number of feces during habituation, STM and LTM sessions (68-92%, $p=0.01$). Chronic affective stress in early life may affect the spatial memory. However, this effect may be reversed by neuronal plasticity that occurs during the brain development. **Committee on Animal Research and Ethics:** 01/08. **Support:** FAPESP and FPA.

02.067

Um antigo método para uma nova função: o esmagamento do nervo ciático (*crush*) induz comportamento depressivo no teste da cauda. Nascimento FP[1], Martins DF[2], Mazzardo L[2], Macedo Junior, SJ[3], Cremonese, RP[4], Bento AF[1], Marcon R[1], Calixto JB[1], Budni J[5], Binfaré RW[5], Rodrigues ALS[5], Santos ARS[3] [1]UFSC - Farmacologia, [2]UFSC - Fisiologia, [3]UFSC - Ciências Fisiológicas, [4]ANGLO-Foz - Farmácia, [5]UFSC - Bioquímica

Introdução: A relação entre dor crônica e depressão é claramente reconhecida. Vários estudos clínicos randomizados têm sido publicados demonstrando a eficácia de antidepressivos para o tratamento deste tipo de dor. O objetivo deste estudo é avaliar um modelo clássico de dor neuropática, o esmagamento do nervo ciático (*crush*), como possível indutor de comportamento tipo depressivo e avaliar a participação de citocinas pró-inflamatórias neste fenômeno. **Métodos:** Foram utilizados camundongos machos Swiss, pesando entre 30-40 gramas. Os animais foram anestesiados com xilazina e quetamina pela via intramuscular, consequentemente tiveram o nervo ciático esmagado por uma pinça hemostática durante 30 segundos. Os animais foram divididos em 4 grupos, falso-operado e controle que foram tratados diariamente com solução fisiológica, e dois grupos tratados diariamente com fluoxetina pela via oral, nas doses de 20 e 30 mg/kg, respectivamente. Todos os animais foram submetidos à avaliação do limiar nociceptivo pelo método de von Frey (frequência de retirada) a cada 2 dias. Ao final do 14º dia os animais foram submetidos ao teste da cauda, um teste preditivo de depressão. Após isto, os animais foram sacrificados e realizou-se coletas de tecido do hipocampo e córtex cerebral para dosagem das citocinas pró-inflamatórias TNF-alfa e interleucina 1-beta pelo método de ELISA. Protocolo 23080.006747/2008-90 CEUA/UFSC. **Resultados:** O *crush* no nervo ciático induziu um aumento da resposta nociceptiva dos animais controle submetidos ao teste de von Frey a partir do sétimo dia após a cirurgia, sendo que nos dias 13 e 14, a frequência de resposta nociceptiva destes animais controle foi de 35 ± 3,45% e 39 ± 4,76%, respectivamente (Média ± EPM). A administração de fluoxetina na dose de 30 mg/kg foi capaz de reduzir a nocicepção para 17 ± 2,34% e para 21 ± 2,98%, uma redução de 55% ($p<0,05$) e 49% ($p<0,05$) no 13º e 14º dia após a cirurgia, respectivamente (ANOVA 2 vias). No teste da cauda, o *crush* induziu comportamento tipo depressivo, pois aumentou em 55% (média de 144,3 ± 4,9% segundos) a imobilidade no animal controle em relação ao animal falso-operado. A fluoxetina administrada em ambas as doses (20 e 30 mg/kg) reverteu o comportamento depressivo dos animais, reduzindo o tempo de imobilidade para 109 ± 11,1% e 114,2 ± 8,6% segundos, ou em 24% ($p<0,05$) e 21% ($p<0,05$), respectivamente (ANOVA 1 via). Finalmente, os animais controle apresentaram uma expressão de 101,4 ± 6,9% de pg/mg de proteína para TNF-alfa e de 167 ± 21,1% pg/mg para IL-1beta. Os animais que receberam tratamento diário de fluoxetina 20 mg/kg apresentaram uma redução da expressão de TNF-alfa em 48%, (53,1 ± 2,7% pg/mg) com $p<0,001$ e de IL-1beta em 38% (105,6 ± 5,7% pg/mg) com $p<0,05$ no hipocampo em relação aos animais controle (Teste T não pareado). Entretanto, quando a expressão das mesmas citocinas pró-inflamatórias foi avaliada no córtex cerebral, não se observou nenhuma diferença estatística entre os grupos avaliados. **Discussão:** O esmagamento do nervo ciático (*crush*) induziu dor crônica e comportamento tipo depressivo. Além disso, a fluoxetina produziu efeito antinociceptivo e antidepressivo nos modelos utilizados. Por fim, as citocinas pró-inflamatórias demonstraram ser importantes na indução e manutenção tanto da dor crônica como do comportamento depressivo. Conclui-se portanto que o *crush* pode ser um modelo útil para avaliações pré-clínicas de depressão associada à dor crônica, e auxiliar na pesquisa de tratamentos unificados para estas duas patologias altamente relacionadas. Apoio Financeiro - CNPq e CAPES

02.068

Antipsychotic-like effect of myricitrin in apomorphine-induced stereotypy in mice. Pereira M[1], Vital MABF[1], Santos ARS[2], Pizzolatti MG[3], Andreatini R[1] [1]UFPR - Farmacologia, [2]UFSC - Ciências Fisiológicas, [3]UFSC - Química

Purpose of the study: Protein kinase C (PKC) has been associated with the pathophysiology of bipolar disorder and it was inhibited by antimanic drugs (lithium and valprote). Thus, PKC would be an important target for new antimanic drugs. Myricitrin, a naturally occurring flavonoid in several plants (e.g. genus Eugenia), is a PKC inhibitor that has showed antinociceptive and anxiolytic-like effect in animal models. Since psychotic symptoms are frequently seen in manic patients, the aim of the present study was to evaluate the effect of myricitrin in the apomorphine-induced stereotypy in mice. **Methods:** Adult male mice were randomically allocated in 3 groups and treated with: vehicle, myricitrin 5 mg/kg or myricitrin 10 mg/kg (all ip). After 30 min one half of mice from each group was treated with saline and the other half was treated with apomorphine (1.0 mg/kg). Stereotypy was measured according the scale proposed by Chinen and Frussa-Filho (Chinen Neuropsychopharmacology, 21, 670, 1999) during 90 min at 10 min interval. Another group of mice were also randomically allocated in 3 groups treated with vehicle, myricitrin 5 mg/kg or myricitrin 10 mg/kg and after 30 min they were tested in automated activity chamber to evaluate locomotor activity (number of beam interruptions). The data were analyzed by one- or two-way ANOVA followed by Duncan test. Results: Vehicle + apomorphine (17.5 +/- 1.7) and myricitrin 5 + apomorphine (13.8 +/- 0.9) showed a significant increase in total stereotypy score (the sum of score in each time) compared to other groups (vehicle+vehicle: 4.7 +/- 0.6; myricitrin 5 + vehicle: 2.5 +/- 0.7; myricitrin 10 + vehicle: 7.2 +/- 0.6; myricitrin 10 + apomorphine: 6.3 +/- 0.9; mean +/- SEM). There is a significant difference between groups at 10, 20, 30, 40, 50 and 60 min after apomorphine. In these moments vehicle + apomorphine and myricitrin 5 + apomorphine exhibited significantly higher stereotypy scores than the other groups (all $p<0.5$), which did not differ among then. No significant difference among groups was found in locomotor activity. **Discussion**: Myricitrin dose-dependently blocked apomorphine-induced stereotypy. Since this effect was not due to locomotor impairment, it suggests that myricitrin may have antipsychotic-like effect. **References**: Hanh CG, Umapathy, Wang HY, Koneru R, Levinson DF, Friedman E. Lithium and valproic acid treatments reduce PKC activation and receptor-G protein coupling in platelets of bipolar manic patients. *J Psychiatr Res*. 2005; 39(4): 355-63. Ø Meotti FC, Luiz AP, Pizzolatti MG, Kassuya CA, Calixto JB, Santos AR. Analysis of the antimociceptive effect of the flavonoid myricitrin: evidence for a role of the L-arginine-nitric oxide and protein kinase C pathways. J Pharmarcol Exp. Ther. 2006; 316(2):789-96. Protocolo Com. ética: CEEA 232. Apoio financeiro: Fundação Araucária

02.069

Effects of acute and chronic treatment of memantine on brain creatine kinase activity.

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Objectives: A growing body of evidence has pointed to the NMDA receptor antagonists as a potential therapeutic target for the treatment of major depression. Considering that NMDA receptor antagonists effects on brain metabolism are poorly known and that creatine kinase (CK) plays an important role in cell energy homeostasis, we evaluated CK activity in brain of adult rats following acute or chronic the memantine and imipramine. **Methods:** Memantine (5, 10 and 20 mg/kg) and imipramine (10, 20 and 30 mg/kg) were acutely or chronically administered to adult male Wistar rats (300g). For acute administration, a single injection of memantine and imipramine was given to rats. For chronic administration, memantine and imipramine injections were given to adult rats for 14 days once a day. CK activity was measured in prefrontal cortex, hippocampus, striatum, cerebellum and cerebral cortex homogenates pre-treated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 mg protein in a final volume of 100 mL. After 15 min of pre-incubation at 37 oC, the reaction was started by the addition of 0.3 mmol of ADP plus 0.08 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 mmol of p-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). Results were expressed as units/min x mg protein. This work has been approved by UNESC Ethics Committee under protocol number 014/2008 and 059/2008. **Results and discussion:** Our results demonstrated that imipramine acute administration increased the enzyme in the prefrontal cortex (sal: 11.81±0.39; imi 10: 17.13±0.33*; imi 20: 12.30±0.44, imi 30: 11.38±0.70 $p<0.05$, n=5) and striatum (sal: 9.17±0.35; imi 10: 18.68±1.53*; imi 20: 5.95±0.64; imi 30: 29.66±0.54*; $p<0.05$, n=5). Memantine acute administration also increased CK activity in the prefrontal cortex (sal: 11.81±0.39; mem 5: 34.40±5.49*; mem 10: 9.23±0.80; mem 15: 4.35±0.99 $p<0.05$, n=5), hippocampus (sal: 6.15±0.81; mem 5: 19.14±0.95*; mem 10: 5.52±0.92; mem 15: 5.30±1.04 $p<0.05$, n=5) and striatum (sal: 9.17±0.35; mem 5: 20.40±6.26*; mem 10: 11.44±0.88; mem 15: 11.9±0.73 $p<0.05$, n=5), but 10 mg/kg memantine inhibited the enzyme in the prefrontal cortex (sal: 11.81±0.39; mem 10: 9.23±0.80* $p<0.05$, n=5). On the other hand, imipramine chronic administration increased CK activity in the striatum (sal: 3.96±0.90; imi 10: 3.61±0.88; imi 20: 2.70±0.61; imi 30: 6.30±1.38*; $p<0.05$, n=5) and prefrontal cortex (sal: 4.26±0.79; imi 10: 4.39±0.55; imi 20: 3.30±0.68; imi 30: 6.41±1.48*; $p<0.05$, n=5), the activities of CK were not affected in hippocampus (data not demonstrated). The chronic administration of memantine also increased CK activity in hippocampus (sal: 5.14±0.91; mem 5: 5.02±1.65; mem 10: 9.63±1.03*; mem 15: 6.33±1.11; $p<0.05$, n=5) and striatum (sal: 3.34±1.60; mem 5: 6.02±0.88*; mem 10: 3.86±1.00; mem 15: 5.08±0.40; $p<0.05$, n=5), prefrontal was not affected (data not demonstrated). These findings suggest that memantine, which has been considered as a new drug for the treatment of depression, increased CK activity, an important enzyme involved in brain energy homeostasis. **Financial support:** CAPES, CNPq, FAPESC, UNESC.

02.070

Brain creatine kinase activity in an animal model of mania induced by ouabain. Streck EL[1], Santos PM[1], Scaini G[1], Freitas TP[1], Valvassori SS[2], Rezin GT[1], Quevedo J[2]
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Objectives: Bipolar disorder (BD) is a common and severe mood disorder associated with higher rates of suicide and disability. The development of new animal models, and the investigation employing those available have extensively contributed to understand the pathophysiological mechanisms of BD. Intracerebroventricular (ICV) administration of ouabain, a specific Na^+,K^+- ATPase inhibitor, induced behavioral changes in rats, a putative animal model for BD. It has also been demonstrated that Na^+,K^+- ATPase is altered in psychiatric disorders, such as, BD. Creatine kinase (CK) is important for normal energy homeostasis by exerting several integrated functions. In the present work we evaluated CK activity in the striatum, prefrontal cortex and hippocampus of rats subjected to administration of ouabain. **Methods:** Adult male Wistar rats received a single injection of vehicle or ouabain (10^{-2} and 10^{-3} M). Locomotor activity was measured using the open field test. Creatine kinase activity was measured in brain homogenates pre-treated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 mg protein in a final volume of 100 mL. After 15 min of pre-incubation at 37 oC, the reaction was started by the addition of 0.3 mmol of ADP plus 0.08 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 mmol of p-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). Results were expressed as units/min x mg protein. This work has been approved by UNESC Ethics Committee under protocol number 032/2009. **Results and Discussion:** Our results showed that ouabain increased rat spontaneous locomotion immediately after injection (control: 15.6 ± 1.7; ouabain 10^{-3} M: 106.0 ± 3.2*; ouabain 10^{-2} M: 106.7 ± 2.6*; P<0.001) and the hyperlocomotion was still observed 7 days following a single ICV injection of ouabain (control 7.4 ± 0.6; ouabain 10^{-3} M: 82.5 ± 1.7*; ouabain 10^{-2} M: 80.9 ± 1.4*; P<0.001). CK activity in the striatum (control: 1.43 ± 0.71; ouabain 10^{-2} M: 0.84 ± 0.56*; ouabain 10^{-3} M: 0.9 ± .056*; p<0.05, n=5), hippocampus (control: 1.05 ± 0.6; ouabain 10^{-2} M: 0.49 ± 0.9*; ouabain 10^{-3} M: 0.57 ± 0.8*; p<0.05, n=5) and prefrontal cortex (control: 1.32 ± 0.9; ouabain 10^{-2} M: 0.34 ± 1.1*; ouabain 10^{-3} M: 0.6 ± 0.68*; p<0.05, n=5) was inhibited immediately after the injection of ouabain. However, in animals which CK activity was assessed 7 days following ouabain administration, no alterations were observed in the striatum (data not demonstrated). The inhibition in enzyme in the prefrontal cortex (control: 17.86 ± 1.86; ouabain 10^{-2} M: 12.5 ± 2.78*; ouabain 10^{-3} M: 9.85 ± 0.75*; p<0.05, n=5) and increased in the CK activity in hippocampus (control: 9.86 ± 0.56; ouabain 10^{-2} M: 8.83 ± 0.75; ouabain 10^{-3} M: 12.32 ± 0.75*; p<0.05, n=5) was observed. Evidence suggest that energy impairment is involved in BD and speculate that the reduction of brain metabolism may be probably related to the pathophysiology of this disease. **Financial support:** CAPES, CNPq, FAPESC, UNESC.

02.071

Behavioral and neural consequences of short-term nicotine administration after system inflammation in rats. Apolinário RT[1], Buenaga ML[1], Khouri Neto SY[1], Greggianin GF[1], Nóbrega YKM[1], Carneiro FP[1], Sousa JB[1], Lucena GMRS[2], Ferreira VMM[2] [1]UnB - Medicina, [2]UnB - Ciências da Saúde/Ciências Farmacêuticas

Introduction: Sepsis and its complications are the leading causes of morbidity and mortality in intensive care units. Septic encephalopathy represents a brain dysfunction due to systemic inflammatory response that can reflect behavioral consequences related to anxiety and memory deficits. Nicotine consumption in sepsis survivors can be used due its cognitive and anxiolytic properties. These actions have been shown to be mediated by different brain areas, which can be affected by sepsis. Nevertheless, the aim of this study is to verify the behavioral and neural consequences of nicotine after sepsis induction. **Methods:** Female Wistar rats (n= 8 animals/treatment), 2.5 month-old, 200 g, were obtained from the Animal Facility, in accordance with the recommendation of ethical committee for animal care (UnB doc 33880/2009). All experiments were carried out at the Pathology Laboratory/Faculty of Medicine. The animals were anesthetized i.p. using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), to allow exposure of the cecum, which was then squeezed to extrude a small amount of feces from the perforation site that was later placed back into the peritoneal cavity. All animals returned to their cages, after administration of ceftriaxone (30 mg/kg) + clindamicine (25 mg/kg). Nicotine (0.01 mg/kg, s.c.) was administered for one week, after sepsis induction. On the last day, before the experimental tests, the neural evaluations were determined by using a scale with different behaviors. The scored parameters included vocalizations, general tremor, motor task, tail-lifting tremor, avoidance, rigidity of axial muscles by palpitation, head tremor, bracing posture and grooming. After 30 min of the nicotine administration, the animals were submitted to the elevated plus-maze (EPM) and to the step-down inhibitory avoidance (SDIA) tests. **Results**: The experimental results showed a clear rigidity of axial muscles by palpitation in sepsis-surviving rats that was not changed by nicotine administration. ANOVA, followed by Tukey's test showed that nicotine administered after sepsis induction, increased the percentage of open arm entries (46.50±4.03) and open arm time (23.64±3.99) in the EPM test when compared to the sham-operated group (7.56±2.07). At SDIA, the Kruskal-Wallis' test did not revealed a significant effect of the treatment with nicotine on the step-down latencies during the short- (H(3, N=32)=14.19; p=0.0008) and long-term (H(3, N=32)=12.96; p=0.0015) acquisition test session. The Mann-Whitney test indicated that the sepsis group, significantly reduced the step-down latencies during the short- (p<0.05) and long-term (p<0.05) session (performed 1.5 h or 24 after the training session, respectively), when compared to the control group. **Discussion**: These results indicated that nicotine was able to improve some behavioral changes but, did not improve the cognitive deficits caused by sepsis. Taken together, they suggest the nicotine effects administered after sepsis induction is not able to interfere at cognitive dysfunction, even though it can modify anxiety-like behavior. **Acknowledgements:** Pathology Laboratory, Faculty of Medicine/ UnB, for financial and technical support.

02.072

Isolation rearing induces a decrease in the expression of AMPA glutamate receptors in hippocampal formation in rats. Sestito RS[1], Trindade LB[1], Kerbauy LN[1], de Souza RG[1], Iyomasa MM[2], Rosa ML[3] [1]FAMECA - Bioquímica, [2]FORP-USP - Morfologia, Estomatologia e Fisiologia, [3]FAMECA - Ciências Biofisiológicas

Introduction: Reduced glutamatergic signaling may contribute to cognitive dysfunction in schizophrenia. Glutamatergic synapses might be the site of primary abnormalities in this disorder with the dopaminergic changes being secondary to altered glutamatergic transmission. Isolation rearing of rats from weaning has been used as experimental model of affective disorders like schizophrenia. This study aimed at evaluating the changes in GluR1 and GluR2 expression induced by isolation rearing in the hippocampal formation of rats. **Methods:** Two groups of Wistar rats (n=6/each) were used. In both groups the pups remained with their mothers (6 pups per mother) until weaning (21 days - 40g) when they were allocated randomly to one of two conditions: 1) grouped, housed 3 per cage and handled 3 times a week; 2) isolated, housed individually and handled once a week for cleaning purpose. After 10 weeks all animals were deeply anaesthetized, perfused and their brains removed. 40-mm sections were used for immunohistochemistry: anti GluR1 (Chemicon), anti GluR2 (Invitrogen), secondary antibody (Dako), ABC Kit (Vectastain), DAB (Sigma). Using a light microscope Axioskop 40 with AxioCam ICc3 and AxioVision Release 4.6.3 04-2007, Zeiss, the immunopositive cells were counted by 3 examiners independently, in 3 sections/rat and bilaterally in hippocampus, amygdala and entorhinal cortex. Data were compared by Student *t*-test ($p<0.05$). **Results**: Isolation rearing induced a significant decrease in GluR1- and GluR2- immunopositive cells in hippocampus. For GluR1 the reduction was 31% in the hillus ($p=0.02$) and 47% in CA3 ($p=0.002$). Although the number of GluR2- immunopositive cells in hippocampus was smaller, a higher reduction (57%) was induced by isolation rearing only in the hillus ($p=0.001$). Immunopositive cells were not seen in CA3 for GluR2 or in CA1 and CA2 for either GluR1 or 2 in grouped and isolated rats. It was found an almost significant decrease (18,5%) in GluR1-positive cells in basolateral ($p=0,066$) but not in lateral ($p=0.898$) amygdala in isolated reared rats. In contrast, GluR2-positive cells were not detected in any nucleus of the amygdala. No change was induced by isolation rearing in the expression of GluR1 and 2 in entorhinal cortex. **Conclusions**: Isolation rearing from weaning induces changes in AMPA glutamate receptors in hippocampus and amygdala similar to those reported for human brains with schizophrenia. Committee on Animal Research and Ethics: CEUA-USP/Ribeirão Preto**:** 05.1.769.53.0. **Financial Support**: FAPESP (05/01501-7; 06/53343-9; 06/53345-1; 06/53342-2; 06/53344-5).

02.073

Evaluation of effects of aqueous standardized extract of chamba (*Justicia pectoralis*) in monoamines levels in the estriatum of mice. Venâncio ET[1], Rocha NFM[1], Rios ERV[1], Feitosa ML[1], Linhares MI[1], Cavalcante GIT[1], Moura BA[1], Fonseca FN[2], Leal LKAM[2], Fonteles MMF[2] [1]UFC - Fisiologia e Farmacologia, [2]UFC - Farmácia

Introduction: Depressive disorders are among the most common diseases in humans. Neurobiological evidences in animal in human have indicated the role of monoaminergic systems in the pathophysiology of mental depression. *Justicia pectoralis* var. stenophylla Leonard), from the Acanthaceae family, popularly named chamba in Brazil is a plant widely used in popular medicine and present depressant effect on central nervous system. This present work aimed to investigate to involvement of monoaminergic systems in the depressant effect of Chamba in mice. **Methods:** The animals were divided in two groups (n=8), Chamba (CH) was administered orally at doses of 200 mg/kg and controls received vehicle (distilled water). One hour after the administration the mice were decapited and brains were removed. The monoamines levels (serotonin, dopamine and their metabolites) were measured. The results were presented as mean ± S.E.M. Data were analyzed by t test, with significance of $p<0.05$. This work was approved by Committee on Ethics in Animal Research, number 14/09. **Results and Discussion:** The treatment of mice with CH 200 mg/kg decreased the monoamines levels and their metabolites. DA [cont.(1821±147,5),CH200(832,9±97,49)], DOPAC [cont.(2658±226,7), CH200(1596±175,8)], 5-HT[cont.(991,5±133,8), CH200(603,4±77,28). Our findings suggest that *Justicia pectoralis* (chamba) decrease monoamines levels, confirming their depressant action on central nervous system. **Financial Support**: CAPES e CNPq

02.074

Investigation of behavioral disturbances in MOG_{35-55}–induced experimental autoimmune encephalomyelitis in mice. Rodrigues DH[1], Vilela MC[1], Queiroz NL[2], Teixeira AL[3] [1]ICB-UFMG, [2]UFMG - Parasitologia, [3]UFMG - Medicina

Introduction: Multiple sclerosis is a demyelinating disease of the central nervous system that affects young adults and results in sensory, motor and cognitive deficits. Experimental autoimmune encephalomyelitis (EAE) is considered an experimental model of multiple sclerosis. Only a few articles have studied cognitive and behavioral disturbances in EAE-induced mice. Therefore, in this work, we investigated behavioral features of the disease in MOG_{35-55} EAE induced C57BL/6 mice. **Method:** Female C57BL/6 mice ageing 8 – 12 weeks were house in cages and had food and water *ad libitum*. Animals were kept in the same room of the behavioral experiments for habituation. Also, experiments were performed between 8h and 16h during the day. The SHIRPA screen provides a behavioral and functional profile of mice using standardized methods (Lackner *et al*., 2006). A plastic cage of 70x30x30cm and other tools are used in the test battery. The scores of each test are summed and organized in five categories (reflex and sensory function, neuropsychiatric state, motor behavior, autonomous function, muscle tone and strength). Statistical analyses are performed comparing scores of the same categories between control and experimental groups. This study was approved by the protocol 129/2006 from CETEA/UFMG in November 22, 2006. **Results:** There was no significant difference in any of the SHIRPA categories: reflex and sensory function ($p=0.4$), neuropsychiatric state ($p=0.1$), motor behavior ($p=0.7$), autonomic function ($p=1.0$) and muscle tone and strength ($p=0.4$). **Discussion:** EAE mice did not seem to have altered behavior in comparison with control mice in several domains. There was a trend for reduced scores in the neuropsychiatric category (i.e. arousal, fear, passivity, irritability, aggression) at day 8 p.i. As typical motor EAE symptoms are present from day 11 p.i. onwards, it is possible that behavioral symptoms are subtle at day 8 p.i. Studies using more specific tests for neuropsychiatric states are warranted. **Financial support**: CAPES. Lackner, P. *Neuropathol Appl Neurobiol* v.32 p.177, 2009.

02.075

Evaluation of the possible pathways involved on the diacerin-mediated performance improvement at step down model in rats. Guginski G[1], Silva MD[2], Santos ARS[2] [1]UFSC - Farmacologia, [2]UFSC - Ciências Fisiológicas

Introduction: Diacerein is a novel symptomatic slow acting osteoarthritis drug that act mainly by inhibit inflammatory cytokines such IL-1β and TNF-α, and is used mainly by aged people (were arthritis is more prevalent). Some studies have already showed that cytokines may cause memory impairment (Lynch, 1998) and our group previous data demonstrate that Diacerein is able to improve the rat performance in the step down model. So the aim of this study was evaluate what pharmacological pathways are involved in this action. **Methods:** Were used Wistar male rats weighting between 180 – 220 g (CEUA: 23080.0011700/2005-03/UFSC). The step-down apparatus consists of a 50 cm × 25 cm × 25 cm Plexiglas box with a platform on the left end of a series of bronze bars that make the floor of the box. In the training session (day 1), animals were gently placed on the platform facing the left rear corner of the training box. When they stepped down and placed their four paws on the grid, the animals received a 0.4 mA 1 s scrambled foot shock and were immediately withdrawn from the training box. The animals were evaluated 1.5 h and 24 h after training to short (STM) and long term memory (LTM), respectively. The animals were pre treated with diacerein (100 mg/kg, 60 min before training), vehicle (10 µl/mg, 60 min before training) or the specific antagonist (MK – 801 (glutamatergic antagonist, 0.01 mg/kg, 30 min before training or receive a dose of diacerein), L-NAME (NOS inhibitor, 2 mg/kg, 30 min before training or receive a dose of diacerein) , atropine (unspecific cholinergic muscarinic antagonist, 1 mg/kg, 30 min before training or receive a dose of diacerein) or mecamylamine (α2β3 cholinergic nicotinic antagonist, 5 mg/kg, 30 min before training or receive a dose of diacerein). The data were expressed by the mean of the variation between the latency time during the test and during the training ± SEM, and were analyzed by one way ANOVA followed by Newman Keulls *post hoc* test. Results: Diacerein were able to improve the performance of the animals in the step down test both at STM (118.4 ± 27.11 s, $p \leq 0.01$) and LTM (79.25 ± 27.4 s, $p \leq 0.01$) when compared with the control group (24.9 ±13 s for STM and 11.6 ± 1.97 s for LTM). All the antagonists did not cause any per se effect both in STM and LTM, when compared with the control group. When evaluated at STM, the pre-treatment with L-NAME did not reverse the diacerein effect, but the pre treatment with MK-801 (8.2 ±3.1 s, $p \leq 0.01$), atropine (48.27±26.4, $p \leq 0.05$) and mecamylamine (49.4±20 s, $p \leq 0.05$) significantly reversed the diacerein effect. When evaluated at LTM, just L-NAME (33.4±13.1 s, $p \leq 0.05$) and atropine (10.6±3.1 s, $p \leq 0.001$) significantly reversed the diacerein effect, what did not occur with MK-801 and mecamylamine. Discussion: This data suggest that the glutamatergic and the cholinergic (both nicotinic and muscarinic) pathways are involved in the STM diacerein-mediated memory improvement while the L-arginine- NO and just the muscarinic cholinergic pathways seems to be involved with the LTM diacerein-mediated memory improvement. **Referências**: Lynch MA et al., *Prog Neurobiol,* 56, 571, 1998. Pavelka K et al*., Arthritis &Reumatism*, 56, 4055, 2007. **Apoio**: CAPES, CNPq, FAPESC

02.076

Evaluation of probenecid's neuroprotective effect on excitotoxic and ischemic neural lesion. Elias MJG, Castro NG ICB-UFRJ - Farmacologia Molecular

Introduction: Stroke is the second most prevalent cause of death in the world. In Brazil, it is responsible for 9% of all causes of death. After an ischemic incident, excess release of glutamate triggers a series of intracellular events leading to the increase of several substances, some of them contributing to cell death, others to cell survival. Many mediators involved in this process are under investigation, in order to decrease cell death in the area surrounding the infarct core known as the penumbra zone, where delayed cell death occurs. Probenecid (PB), an organic anion transporter blocker, organic anion transporter blocker, used in the treatment of gout and to increase penicillin's half-life is capable of inhibiting the transport of several substances, including some that are involved in the intracellular cascade due to ischemic insult. Some evidences show PB's action as adjuvant of neuroactive molecules, such as kynurenic acid, however little is known about its direct effect in CNS. Our goal is to investigate the therapeutic role of PB, *in vitro* and *in vivo* as a neuroprotective drug in cerebral ischemia and other neurodegenerative diseases, as well as the mechanism by which this protection would occur. **Methods and Results:** Glutamate-induced damage (GID) assay was performed in cortical cells from rat fetuses, with 14-18 d in culture. Late cellular damage was evaluated by the amount of lactate dehydrogenase (LDH) released 4 h after 5 min of glutamate 500 µM + d-serine 10 µM. PB showed neuroprotection concentration-dependently, reducing GID completely on the highest concentration used (3 mM) with IC_{50} of 0,37 mM, when applied full-time (before, during and after GID). The same experiment adding PB only 15 min after GID showed maximum protection of 75 ± 8.5% (mean ± SEM) at 3 mM, with IC_{50} of 0,85 mM. After these results *in vitro*, we decided to investigate the effect of PB in vivo, in a middle cerebral artery electrocauterization model (MCAO) in mice. All experimental procedures were approved by the Ethics Committee on Animals Research of CCS/UFRJ, protocol DFBICB 029. With one administration of 200 mg/kg i.p., 1 h after MCAO, the infarct sizes were 16.7 ± 5.3 mm^3 (n = 10) for the control group and 12.3 ± 4.1 mm^3 (n = 9) for the PB group. Then, we tried post-treatment with several administrations of PB (200, 100, 100 and 100 mg/kg; 1, 9, 21 and 33 h after MCAO). The infarct sizes were 25.7 ± 4.1 mm^3 (n = 20) for the control group and 17.3 ± 4.2 mm^3 (n = 19) for the PB group. Citotoxicity was evaluated trough PB exposure of cortical cell culture, with LDH measurement after 24 h incubation. At 10 mM (the highest concentration), PB induced 34.7 ± 3.8% LDH release (n = 3), when compared with the maximum release, achieved by lysis with Triton X-100. **Conclusion:** PB completely prevented the damage induced by glutamate in cortical cells when exposed full-time, at 3 mM, and still showed protection of 75% when added only after GID. The results in vivo indicate that PB might have a neuroprotective effect of its own, reducing infart volume by 33% when applied in multiple doses after MCAO. **Financial Support:** CAPES, FAPERJ, CNPq.

02.077

Participação dos receptores B1 e B2 para bradicinina na consolidação da memória durante o processo de envelhecimento em camundongos. Lemos MTR[1], Amaral FA[1], Bittencourt MFQP[1], Caetano AL[1], Dong KE[1], Pesquero JB[2], Viel TA[3], Buck HS[1] [1]FCMSCSP - Ciências Fisiológicas, [2]UNIFESP - Biofísica, [3]EACH-USP

Introdução: Várias evidências sugerem o envolvimento dos receptores cininérgicos com a neurodegeneração associada à doença de Alzheimer (DA), ocasionando perda de memória. Durante o envelhecimento natural também ocorre perda neuronal e de memória, mas de forma menos intensa daquela observada na DA. Considerando envolvimento dos receptores cininérgicos com a perda neuronal e de memória na DA, esse estudo avaliou o papel dos receptores cininérgicos na consolidação de memória durante o processo de envelhecimento. **Métodos:** Camundongos machos das linhagens C57Bl/6J (wt), *knock-out* B1 (koB1) ou B2 (koB2) nas idades de três (3me), seis (6me), doze (12me) e 18 (18me) meses de idade (n=10 por grupo) foram submetidos a um treino, reforço de aprendizagem 24 horas após o treino (*teste 1*) e sessão de teste final da memória sete dias após o *teste 1* (*test 2*), em equipamento de esquiva ativa de duas vias para avaliarmos a memória. Os resultados foram registrados em % de respostas condicionadas (RCs) em 50 testes. **Resultados:** Na sessão de treino foram obtidas RCs similares entre os animais de mesma idade nas diferentes linhagens. No entanto, uma diminuição significativa foi observada nas RCs durante o envelhecimento (3 meses: 8,8 ± 2,3%; 6 meses: 4,1 ± 0,6%; 12 meses: 2,2 ± 0,6%; 18 meses: 3,6 ± 0,6%, P<0,01), indicando redução no processo de aprendizado. No teste 1, os animais 3me-wt (33,1±10,4%), 3me-koB1 (27,3±7,3%), 3me-koB2 (34,4±7,9%), 6me-wt (26,8±4,2%), 6me-koB1 (50,0±4,6%) e 6me-koB2 (25,1±6,3%) apresentaram aumento significativo na retenção da memória (P < 0,05), assim como os animais 12me-wt (24,8±5,8 %) e 12me-koB1 (22,0±4,4%; P<0,01), quando comparados com a sessão de treino. Porém, 12me-koB2, assim como todos os animais de 18 meses, não mostraram aumento na retenção da memória. No teste 2, 3me-wt (54,2±4,1%), 3me-koB1 (55,7±8,2%), 6me-wt (37,3±4,7%), 6me-koB1 (72,0±3,8%), 12me-koB1 (42,3±6,7%) e 18me-koB1 (23,5±4,9%) mostraram melhora significativa da memória (P<0,05), quando comparados com as observações feitas no teste 1. Por outro lado, 12me-wt (32,8±5,3%), 3me-koB2 (48,2±9,8%), 6me-koB2 (37,3±7,6%), 12me-koB2 (21,3±12,2%) e 18me-koB2 (7,7±4,3%) não mostraram diferenças na retenção da memória. **Conclusões:** A partir desses resultados, nós sugerimos que durante o processo de envelhecimento o receptor B1 possa estar envolvido com a neurodegeneração e a perda da memória. Por outro lado, o declínio precoce da memória em animais koB2 indica que esse receptor seja importante na manutenção da memória dos animais, aparentemente atuando como um fator neuroprotetor. Também podemos sugerir que a linhagem koB2 possa ser utilizada como um modelo para estudos de novas substâncias com efeitos sobre os processos mnemônicos. Estudos estão em andamento para a determinação morfométrica do cérebro desses animais, assim como para determinarmos as concentrações de receptores B1 em koB2 e B2 em koB1. **Bibliografia:** Beach TG, Neurodegener Dis. 5(3-4), p.143, 2008. Viel et AL, Neurobiol Aging. 29(12), p.1805, 2008. **Aprovado pelo CEEA-FCMSCSP:** Protocolo nº: 155. **Apoio Financeiro:** FAPESP, CNPq, CAPES.

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02.078

Neuroinflammatory process and behavioral symptoms in an experimental model of hepatic encephalopathy: preliminary results. Miranda AS[1], Rodrigues DH[2], Vieira LB[3], Vilela MC[2], Lima CX[4], Amaral DCG[2], Teixeira MM[2], Guatimosim C[5], Helton JReis[3], Teixeira AL[6] - [1]UFMG - Infectologia e Medicina Tropical; [2]ICB-UFMG; [3]UFMG - Farmacologia; [4]UFMG - Fisiologia e Farmacologia; [5]UFMG - Morfologia; [6]UFMG - Medicina

Introduction: Hepatic Encephalopathy is a neuropsychiatry syndrome that results from an acute or chronic hepatic failure. This complex disease can cause a range of cognitive and behavioral symptoms which include altered arousal, psychomotor dysfunction, impaired memory and learning (Monfort. *Neurochem Int.* 55:106, 2009). In its most severe forms, it can lead to coma and death. The HE pathologic mechanism is not known. The release of systemic and brain inflammatory mediators has been implicated in the pathogenesis of HE. Therefore, the aim of this study was to investigate cognitive and behavioral symptoms, and neuroinflammatory process in a thioacetamide (TAA) - induced HE experimental model in C57BL/6 mice. **Method:** Eight to 12 week-old C57Bl/6 WT mice (20-25 g) were used in this study. The HE was induced by TAA administration. TAA was injected by the intraperitoneal route (i.p.) as a single dose of 600 mg/kg dissolved in a saline solution (NaCl 0,9%). Each animal received 300 µL of TAA. The control group received the same volume of saline. The cognitive and behavioral alterations were evaluated using a battery test called SHIRPA (Lackner*. Neuropathol Appl Neurobiol*. 32:177, 2006) The concentration of cytokines (TNF-α) and chemokines (IL-1β, Kc and MCP-1) were assed in the brain tissue at 52 hours after TAA induction, using the enzyme-linked immunosorbent assay (ELISA). This study was submitted to ethical approval by CETEA/UFMG, protocol number 114/09. **Results:** Hepatic Encephalopathy-induced animals presented altered neuropsychiatric state ($p<0.05$) and altered motor behavior ($p<0.01$) compared to controls at 48h p.i. Hepatic Encephalopathy-induced animals presented higher brain levels of TNF-a ($p<0.05$), Kc ($p<0.05$) and MCP-1 ($p<0.001$) compared to controls after 52h of Hepatic Encephalopathy induction. **Discussion:** Neuropsychiatric state and motor behavior changes in TAA-injected animals suggest a correlation of liver failure and changes in brain function, which characterizes the hepatic encephalopathy. These changes were correlated with an increase in cytokines and chemokines production in the brain. This suggests that an inflammatory response may be involved in the behavioral changes in Hepatic Encephalopathy-induced mice. **Financial support:** CAPES

02.079

Evaluation of the acquisition/retention and recall of spatial memory in different strains of mice. Pereira CAS[1], Zobiole NN[1], Santos AF[1], Batiston AP[2], Souza, LA[2], Schiaveto de Souza A[3] [1]UCDB - Biotecnologia, [2]DTA-UFMS, [3]FMRP-USP - Fisiologia

Introduction: Comparison of different mouse strains provides valuable information about the importance of genetic background in behavioral and pharmacodynamic phenotypes [1]. Strain differences to a variety of behaviors are not conclusive at the moment. **Aim:** The aim of the present study was to compare three mice strains (swiss, C57BL/6 and Balb/c) in relationship to spatial memory. **Material and Methods:** One outbred (Swiss) and two inbred (C57BL/6 and Balb/c) strains of mice were used in this study. Each group was composed by 8 animals weighting between 25-30g. Spatial memory was assessed on Morris water maze in 6 moments: 1-5 training days (3 trials/day) and test day (48h after the last training day). The escape latency (seconds) to reach the hidden platform was measured to during each trial. The experiments were carried out according to the Brazilian Society of Neuroscience and Behavior guidelines for care and use of Laboratory animals and the Ethics Committee of the Universidade Católica Dom Bosco approved all protocols (number 030/2007). **Results:** Balb/c mice did not show significant spatial memory learning in Morris water maze (repeated measures ANOVA, $p>0,39$ – day 1: 68.29±10.69; day 2: 62.13±6.63; day 3: 51.08±13.07; day 4: 52.75±9.22; day 5: 42.67±10.96; test: 48.13±6.75) but swiss and C57BL/6 mice did (repeated measures ANOVA, swiss – $p=0,006$ – day 1: 54.25±6.74; day 2: 34.87±7.80; day 3: 29.92±5.87; day 4: 26.79±4.39; day 5: 23.75±4.21; test: 23.46±4.94; C57BL/6 – $p<0,001$ – day 1: 36.5±5.38; day 2: 21.04±4.31; day 3: 19.91±4.31; day 4: 11.58±2.76; day 5: 11.96±2.10; test: 9.5±1.06). The spatial memory learning to C57BL/6 and swiss mice was better than to Balb-c (ANOVA, $p<0.05$; Tukey post hoc test – $p<0.05$; training days 1, 2, 4, 5 and test). **Conclusion:** The three mouse strains studied shown significant differences on spatial memory learning. Knowledge of these behavior differences is very important before to choose the best strain to specific research. Research Grants from: FUNDECT, UCDB, CAPES. References: 1. Sık, A. *Behav Brain Res,* 147, 49, 2003.

02.080

Study of spinal cord injury in rats by cryogeny: the neuroprotective effect of cannabidiol. Kwiatkoski M[1], Del Bel EA[2] [1]FMRP-USP - Fisiologia, [2]FORP-USP - Morfologia, Estomatologia e Fisiologia

Recently, the non-psychoactive constituent of cannabis cannabidiol (cbd) has been associated with neuroprotection[1]. As the spinal cord injury models present at to date are not well established[2], we evaluated in the present study the ability of the application of nitrogen liquid jet´s to induce spinal cord injury. Additionally, the effect of the cbd in promote neuroprotection was investigated in the rat spinal cord injury performed by the application of liquid nitrogen. Sensory-motor recovery was evaluated by Basso, Beattie and Bresnahan´s Scale (BBB-scale) and tail flick test. The longitudinal and radial extension of the cryogenic lesion was evaluated histologically in hematoxilin eosin and neuronal nuclear protein (Neu-N) spinal cord serial sections. Since the Fos-b expression has been related to inflammatory process in the spinal cord injuries we evaluated its expression in the ventral horn of the spinal cord. Male *Wistar* rats (280-300g) were divided into groups (n = 5-8 rats/group) Control (cont); laminectomy (Lam); lesion + vehicle (vehic- application of liquid nitrogen 05 seconds, segment thoracic 10, followed by i.p. injections of vehicle); lesion-cannabidiol 10mg (cbd 10- cryogenic lesion + cbd 10mg/kg); lesion-cannabidiol 20mg (cbd 20 -cryogenic lesion + treatment with cbd 20mg/kg). Immediately before and 3 hours after the surgical procedure the vehicle, cbd 10mg or 20mg were administrated. Followed this period those treatments were repeated once a day for 6 consecutive days. Functional recovery was assessed one day before the surgery and the first, third and seventh postoperative days (PO). The cryogenic lesion of spinal cord resulted in significant motor deficit ($P < 0.05$). Treatment with cannabidiol improved motor function, increasing the score in the BBB scale (vehic, cbd 10 and cbd 20 respectively: 1^{st} PO- 0 ± 0, 0 ± 0 and 0 ± 0; 3^{rd} PO- 1 ± 0.2; 1 ± 0.1; 2 ± 0.2; 7^{th} PO- 4 ± 0.3; 6 ± 0.4; 7 ± 0.4; $P < 0.05$). Moreover, there was a significant reduction in the extent of tissue injury ($P < 0.05$) and Fos-b expression in the ventral horn of the spinal cord seven days after injury ($P < 0.05$). Our data showed that spinal cord injury by the application of liquid nitrogen induce severe locomotor deficit in wistar rats, which constitutes a reproductive model of spinal cord injury with low variability. Additionally, the sub-chronic treatment with cannabidiol reduced both the longitudinal and radial extent of the injury improving the locomotor function. CETEA protocol n°-011/2007. Financial Support: CAPES, CNPq e FAPESP. References: 1. *J Neurol Sci*., v.233,n. 1-2, p. 21-25, 2005. 2. *Exp Neurol*, v. 139, n. 2, p. 244-256, 1996.

02.081

7-nitroindazole (7-NI) prevents the increase in the turnover of striatal dopamine (DA) and blocks dyskinesias in 6-OHDA-lesioned rats treated with L-DOPA. Padovan FEN, Szawka RE, Silva CA, Anselmo-Franci JA, Del Bel EA FORP-USP - Morfologia, Estomatologia e Fisiologia

We have recently described that nitric oxide (NO) synthase inhibition is able to reduce L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesias (LID) in experimental Parkinson [1]. This result suggests that controlling NO production may be useful in the prevention of dyskinesias. Because the mechanism of this effect is poorly understood, it is of interest to determine whether NO synthase inhibitor 7-NI would affect neurochemical responses. Therefore, we determined the effects of 7-NI in cerebral levels of catecholamines and indoleamines in 6-hydroxydopamine (6-OHDA)-lesioned rats with LID. Male Wistar rats with unilateral 6-OHDA lesion of the medial forebrain bundle or sham animals (n=5-7/group) were treated chronically (21 days) with L-DOPA (30mg/kg) to induce abnormal involuntary movements (AIMs) [2]. Comparisons between 6-OHDA-lesioned (dyskinetic) and sham L-DOPA-treated rats, receiving either saline or 7-NI, were then carried out with regard to striatal levels of DA, DOPAC, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) measured by HPLC analyses. Measurements were made separately in ipsi- and contralateral striatum. L-DOPA induced AIMs in all 6-OHDA lesioned rats, which was attenuated by 7-NI. 6-OHDA lesion induced a decrease in DA and DOPAC levels in the striatum ipsilateral to lesion (85.2 and 90%, respectively, $F_{1,19}=87.3$ $p<0.001$); 7-NI treatment decreased DOPAC in both striatum (28-30%). 7-NI treatment /per se/ increased DA level in sham-L-DOPA treated rats (42-60%). The content of 5-HT decreased in the ipsilateral striatum (28.2% of control); however 7-NI increased it in the contralateral one (24.5%). 5-HIAA decreased in the striatum ipsilateral to lesion (35.6%) and 7-NI treatment did not change lesion effect. DOPAC/DA ratio regarded as a measure of DA turnover was significantly higher (391%, $F_{1,19}=4.38$, $p=0.05$) in the ipsilateral striatum of dyskinetic rats. This effects was prevented by 7-NI. 5HIAA/5HT ratio increased in the striatum ipsilateral (119%) and did not change after 7-NI treatment. Treatment with 7-NI attenuated LID in animals with unilateral striatal 6-OHDA lesions. Dyskinetic animals show an increase in DA metabolism as expressed by increased DOPAC/DA. This increase in DA turnover could serve to maintain DA levels in the DA-depleted striatum and may account for the therapeutic benefit in L-DOPA. However, it may also be related to the dyskinesias induced by this drug. Interestingly, 7-NI, a preferential neuronal NO synthase, was able to prevent both this turnover increase and attenuate LID. These results suggest that NO production could be important for the occurrence of LID. Support: FAPESP, CNPq, CAPES. References: (1) *Neuroscience*. 2009 Mar 31;159(3):927-35. (2) *Eur J Neurosci*. 1998 Aug;10(8):2694-706. CETEA protocol n° 012/2007.

02.082

Exposure to caffeine enhances short and long-term memory in sepsis-surviving rats.

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Introduction: Severe infections often provoke various neurologic abnormalities, including long-term cognitive deficits. The underlying mechanisms of the changes in mental status and cognitive dysfunctions have not been clarified. Caffeine is a significant nutritional component which is also a potential drug of abuse, as it can be addictive. The estimation of caffeine consumption by people is difficult due to its presence in many beverages (coffee, teas), chocolate and medications. We were interested in investigating the effects of caffeine in memory impairments caused by sepsis, especially because it is a common beverage consumed daily. **Methods:** Male Wistar rats (n=8 animals/treatment), 2.5 month-old, 250 g, were obtained from the Animal Facility, in accordance with the recommendation of the ethical committee for animal care (UnB doc 33880/2009). All experiments were carried out at the Pathology Laboratory/Faculty of Medicine. The animals were anesthetized i.p., using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), to allow exposure of the cecum, which was then squeezed to extrude a small amount of feces from the perforation site, which was later placed back into the peritoneal cavity. All animals were returned to their cages after administration of ceftriaxone (30 mg/kg) + clindamicine (25 mg/kg). Caffeine (10 mg/kg, by gavage) was administered daily for one week, before and after sepsis induction. On the last day, one hour after caffeine administration, the animals were submitted to the step-down inhibitory avoidance test. **Results**: Kruskal-Wallis test revealed a significant effect of the caffeine treatment in the animal's latencies, during the short- (H(3, N=32)=23.55; p=0.0004) and long-term memory (H(3, N=32)=42.75; p=0.0001) of the retention test session. The Mann-Whitney test indicated that the sepsis group significantly decreased the animal's latencies during the short- (p<0.05) and long-term memory (p<0.05), performed 1.5 h or 24 after training session, respectively, when compared to the sham-operated group. It has also been suggested that caffeine improved the animal's latencies, when compared to the sepsis group (p<0.05). **Discussion**: The onset of encephalopathy often precedes the abnormalities in memory, suggesting that septic encephalopathy can be influenced by substances consumed daily. Nevertheless, associated conditions of these substances and the improvement of the behaviors make it difficult to understand the effects of sepsis on the brain. In this study the cognitive dysfunctions produced by sepsis in rats was blocked by caffeine suggesting that the adenosinergic receptors are responsible for the results here observed. Further research will be required to determine how this interaction alters the animal's memory in a specific point related to the septic encephalopathy.

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02.083

Effects of acute administration of fenproporex and amphetamine on brain creatine kinase activity. Scaini G[1], Santos PM[2], Rezin GT[1], Ferreira GK[1], Jeremias IC[1], Quevedo J[3], Streck EL[1] [1]UNESC - Fisiopatologia Experimental, [2]UNESC - Fisiopatologia, [3]UNESC - Ciências da Saúde

Objectives: Obesity is a chronic disease of multiple etiologies, including genetic, metabolic, environmental, social, and other factors. Pharmaceutical strategies in the treatment of obesity include drugs that regulate food intake, thermogenesis, fat absorption, or fat metabolism. Fenproporex is the second most commonly consumed amphetamine-based anorectic worldwide. Besides, fenproporex is rapidly converted in vivo into amphetamine. Studies suggest that amphetamine induces neurotoxicity through the production of free radicals and mitochondrial apoptotic pathway by cytochrome c release, accompanied by a decrease in mitochondrial potential. Creatine kinase (CK) is a crucial enzyme for brain energy homeostasis, and a decrease of its activity has been associated with cell death. In the present work we evaluated activity of CK in brain of rats submitted the acute administration of fenproporex. **Methods:** Young male Wistar rats received a single injection of fenproporex (6,25; 12,5 or 25 mg/kg i.p.) or saline. Creatine kinase activity was measured in prefrontal cortex, hippocampus, striatum, cerebellum and cerebral cortex homogenates pre-treated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO4 and approximately 0.4–1.2 mg protein in a final volume of 100 mL. After 15 min of pre-incubation at 37 oC, the reaction was started by the addition of 0.3 mmol of ADP plus 0.08 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 mmol of p-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). Results were expressed as units/min x mg protein. This work has been approved by UNESC Ethics Committee under protocol number 033/2009. **Results:** Our results showed that acute administration of fenproporex inhibited CK activity in prefrontal cortex (sal: 3.21±1.27; 6.25 mg/kg: 1.83±0.10*; 12,5 mg/kg: 2.13±0.30; 25 mg/kg: 2.05±0.62 $p<0.05$, n=5) and cerebellum (sal: 3.74±1.37; 6,25 mg/kg: 1.06±0,71*; 12,5 mg/kg: 1.44±0.60*; 25 mg/kg: 1.04±0.23*; $p<0.05$, n=5). On the other hand, the activities of CK were not affected in hippocampus, cortex cerebral and striatum (data not demonstrated). **Conclusion:** The present findings suggest that acute exposure to fenproporex inhibited CK activity, an enzyme involved in energy production in the brain of young rats. **Financial support:** CAPES, CNPq, FAPESC, UNESC.