02. Neuropharmacology

02.001 Neuropharmacological effects of lipoic acid and ubiquinone on the mRNA level of Interleukin-1B and acetylcholinesterase activity in rat hippocampus after seizures. Oliveira GALD[1], Santos PS[1], Pires LF[2], Freitas RM[1] [1]UFPI – Pharmaceutical Sciences, [2]UFPI – Pharmacology

Introduction: Antioxidant compounds have significant effects against seizures. Studies showed that pilocarpine increases lipoperoxidation in hippocampus, suggesting the involvement of free radicals in cerebral injury induced convulsions, in which it is often active interleukin-1β(IL-1β). Lipoic acid(LA)and ubiquinone(UQ), whose deficiency can occur in epilepsy, exert physiological effects by reactive oxygen species. Thus, the purpose of this study was to determine the neuroprotective effects of LA and UQ on the mRNA expression of IL-1β and activity of acetylcholinesterase (AChE) in hippocampus in rats, which is known to be found, reduced in pilocarpine-induced seizures. **Methods:** there were groups formed of male Wistar rats which 400mg/kg pilocarpine(P400) was administered, serving as comparison to LA 10mg/kg(LA10); LA 20 mg/kg(LA20); UQ 20mg/kg(UQ20) or UQ 40mg/kg(UQ40), 30 min before P400 administration (all i.p.). After euthanasia, hippocampi were removed for determinate IL-1β mRNA level and AChE activity. Approved by the Ethics Committee for Animal Experimentation (# 013/2009). Results were compared using ANOVA and Student–Newman–Keuls post hoc test. **Results:** LA10+P400 decreased by 80%[P<.0001] the percentage of animals seized, increased survival by 50%[P<.0001] and increased by 156%[P<.0001] the latency for the first seizure, all compared with P400. LA20+P400 decreased percentage of seizures (100%)[P<.0001], increased survival by 70%[P<.0001] compared with P400 and LA10+P400, and latency to the first seizure by 245%[P<.0001] compared with P400 and LA10+P400. UQ20+P400 decreased animal seizures(90%), increased survival(60%) and the latency of the first seizure as longer(219%) [P<.0001, all compared with P400]. UQ40+P400 decreased percentage of seizures by 100%, increased survival(70%)[P<.0001] compared with P400 and increased(385%) latency to first seizure[P<.0001] compared with P400 and UQ20+P400. Hippocampal AChE activity of P400 was decreased(65%)[P<.0001 compared to control]. LA10+P400 increased of 197% AChE activity in hippocampus compared with P400 [P<.05]. LA20+P400 increased AChE activity(219%)in hippocampus compared with P400[P<.0001]. UQ20+P400 increased hipocampal AChE activity(121%)when compared with P400[P<.05]. UQ40+P400 increased hipocampal AChE activity(333%) compared with P400 [P<.05], and increased(35%)AChE activity compared with UQ20+P400[P<.05]. P400 increased IL-1B/GAPDH ratio when compared with control[P<.05], LA10/20+P400 and UQ20/40+P400 groups decreased IL-1B/GAPDH compared with P400[P<.05]. **Discussion:** LA and UQ appear to act in a more complex way than simply sweeping free radicals, since, according to our observations, they showed stimulatory action of AChE activity in models of epileptic mouse, as well as inhibitory effect on the expression mRNA of IL-1β, which may be speculated that these compounds prevent the damage caused by excitotoxicity through blockade of the oxidative stress induced by the inflammatory cascade. **Financial agencies**: CNPq and FAPEPI.

02.002 The microinjection of L-proline but not of D-proline into the paraventricular nucleus evokes cardiovascular responses in unanesthetized rats. Lopes Azevedo S, Busnardo C, Corrêa FMA USP – Farmacologia

Introduction: L-Proline (L-Pro) shares a number of properties with recognized neurotransmitters and thus it has been suggested to play a role in the synaptic transmission. Central effects of L-Pro on the cardiovascular system were first described in bulbar structures, but there are no reports on forebrain structures, such as the paraventricular nucleus (PVN) of the hypothalamus. We attempted to verify if cardiovascular effects of the microinjection of L-Pro into the PVN, in unanesthetized rats, are stereospecific with respect to its optical isomer D-Proline (D-Pro). **Methods**: Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (protocol-018/2010). We used Wistar rats weighing between 250-280 g. Guide cannulas were implanted into the PVN using a stereotaxic apparatus. After two days, animals were anesthetized with tribromoethanol and a polyethylene catheter was implanted into the femoral artery for mean arterial pressure (MAP) and heart rate (HR) recordings. **Results:** The microinjection of a dose of L-Pro (0.033 µmol/100nL) into the PVN of normotensive rats (basal MAP= 96.9 ± 4.2 mmHg; HR= 370.2 ± 5.5 bpm, n= 6) caused a significant pressor response (ΔMAP= 28.0 ± 2.3 mmHg, t= 6.7, P < 0.01, paired Student's t-test, n= 6) and heart rate decrease (ΔHR= -40.6 ± 5.8 bpm, t= 8.8, P < 0.001, paired Student's t-test, n= 6). While the microinjection of a 3 fold higher dose of D-Pro (0.10 µmol/100nL), a chiral amino acid of L-Pro, into the PVN (basal MAP= 96.0 ± 4.2 mmHg; HR= 364.3 ± 7.5 bpm, n= 6) did not cause significant changes in the blood pressure (ΔMAP= 1.3 ± 2.1 mmHg) or the heart rate (ΔHR= - 2.2 ± 4.3 bpm). A two way ANOVA followed by the Bonferroni's test indicated significant effects of L-Pro on the MAP and HR (MAP: $F_{1,200}$ = 308.0, P < 0.0001; HR: $F_{1,200}$= 33.1, P < 0.0001); a significant effect over time (MAP: $F_{19,200}$= 12.4, P < 0.0001; HR: $F_{19,200}$= 7.8, P < 0.0001) and an interaction between treatment and time (MAP: $F_{19,200}$= 10.9 P < 0.0001; HR: $F_{19,200}$= 5.5, P < 0.0001) when compared with the D-Pro. **Conclusion**: These results indicate that the cardiovascular effects of L-Pro are stereospecific with respect to its optical isomer D-Pro. **Financial support**: FAPESP 2010/11303-6.

02.003 Effects of cannabidiol administration into the ventral medial prefrontal cortex of rats submitted to the forced swimming test. Sartim AG[1], Guimarães FS[2], Joca SRL[1] [1]FCFRP-USP, [2]FMRP-USP – Farmacologia

Introduction: Cannabidiol (CBD) is the main non-psychotomimetic constituent of *Cannabis sativa.* Systemic treatment with CBD induces antidepressant-like effects in the forced swimming test, an effect that can be blocked by pretreatment with 5-HT1A antagonists. However, it is not yet known which brain regions are involved in mediating CBD-induced antidepressant-like effects. Preclinical and clinical studies have suggested that the ventral medial prefrontal cortex (vMPFC) is a limbic region with a central role in the neurobiology of depression. Therefore, the aim of this study was investigate the effects induced by CBD administration into the vMPFC of rats submitted to the forced swimming test (FST), an animal model predictive of antidepressant effects. The participation of 5-HT1A receptors in CBD-induced effects was also investigated by means of pretreatment with WAY100635, a 5-HT1A selective antagonist. **Methods**: Male Wistar rats with cannulae bilaterally implanted into the vCPFM received CBD (10, 30, 60 nmol/0,2µl) or vehicle and were submitted to the forced swimming test or to an open field test, 10 min later. Independent groups of animals received intra-vMPFC microinjections of the 5-HT1A agonist, 8-OH-DPAT (5, 10nmol/0,2µl), and were submitted to the same behavioral tests. Finally, additional groups were pretreated with WAY100635 (10, 30 nmol/0,2µl), followed 5 min later, by 8-OH-DPAT (10 nmol/0,2µL) or CBD (30 nmol/0,2µl), and were tested in the FST or the open field test. The experimental protocols were approved by the local Ethical Committee of University of Sao Paulo- Ribeirão Preto (protocol number 11.1.459.537). **Results:** CBD treatment significantly reduced the immobility time in the FST ($F_{3.41}=6.4$, $P<0.05$), at all doses tested (Duncan, $P<0.05$). Similarly, 8-OH-DPAT (10 nmol/0,2µl) treatment reduced the immobility time in the FST ($F_{2.16}=3.59$, $p=0.05$). WAY100635 treatment by itself did not change the immobility time, but blocked the 8-OH-DPAT ($F_{5.24}=5.81$,$P<0.01$) and CBD ($F_{3,27}=8.93$, $P<0.01$) induced effects in the FST. None of the treatments induced significant effects in the open field test. **Discussion**: These results suggest that CBD administration into the vMPFC induces antidepressant-like effects by means of 5-HT 1A receptor activation. **Acknowledgements**: The authors thank THC-Pharma for the kind supply of CBD and FAPESP for **Financial support**.

02.004 The role of kinin B2 receptor on amyloid-ß– induced neuroinflammation in vivo: **Evidence for the modulation of PKC and MAPK pathways**. Bicca MA, Loch-Neckel G, Figueiredo CP, Costa R, Calixto JB UFSC – Farmacologia

Introduction: Recent evidence suggests that the (amiloid-β) Aβ peptide, particularly soluble oligomers, has a central role in Alzheimer's disease (AD) (FERREIRA S.T., Neurobiol Learn Mem, 96, 4:529, 2011) and early studies have reported the involvement of the kallikrein-kinin system in the pathophysiology of AD in both humans and experimental models (VIEL T.A., Curr Alzheimer Res, 8, 1:59, 2011). The aim of this study was to investigate the mechanisms underlying the role of the kinin B_2 receptor in the Aβ-induced neuroinflammation in mice. **Methods**: Experimental procedures were carried out using male *Swiss* mice treated with the selective kinin B_2 receptor antagonist, HOE 140 (50 ρmol/site, i.c.v.), given 2 h prior, or 24 h after the i.c.v. injection of $Aβ_{1-40}$ peptide (400 ρmol/site), mainly soluble oligomers, previously identified by both HPLC and microscopy electron transmission analyses. After 14 days, the animals were subjected to behavioral tests and on day 15 their brains were removed to perform Western blot analysis. The same treatment protocol was used to carry out tissue collections, at specific time points (6h, 24h or 8 days) for immunohistochemical analysis. These time point were chosen based on the maximum expression level of each protein. All procedures were approved by the ethical committee for the use of animals from UFSC (protocol number: PP00625). **Results:** The pretreatment (2 h prior) of animals with HOE 140 prevented the cognitive impairment induced by $Aβ_{1-40}$ peptide when assessed using object recognition task. Conversely the postreatment schedule (24h after $Aβ_{1-40}$ peptide) did not result in any significant effect. The administration of HOE 140 prior $Aβ_{1-40}$ peptide prevented Aβ-induced synaptic damage as assessed by the evaluation of synaptic proteins expression (synaptophysin and PSD-95). Furthermore, i.c.v injection of $Aβ_{1-40}$ peptide increased B_2 receptor expression in the mice hippocampi after 24 h and 15 days, an action that was prevented by HOE 140. Also, the $Aβ_{1-40}$-induced inflammatory process was evidenced by increased of both microglial activation (1 day) and the increased expression of COX-2 (1 day), iNOS and nNOS (both 8 days) in the mice hippocampi after $Aβ_{1-40}$ administration as evaluated by immunohistochemistry (at specified time-points) and western blot (15 days) after $Aβ_{1-40}$ injection. All these alterations were prevented by the pretreatment of animals with HOE 140. The neuroinflammatory effect caused by $Aβ_{1-40}$ seems to be mediated by activation of protein kinase C (PKC) (δ and ε isoforms), and by Mitogen Activated Protein Kinase (MAPKs), namely p-38/MAPK, JNK and by transcription factors NFκB and c-Jun as it injection of $Aβ_{1-40}$ caused significant increase in the expression/activation of this pathways. Importantly, these events seem to be modulated by activation of B_2 receptor, since blocking this receptor with HOE 140 prevented all these changes. **Discussion**: Taken together, the present data provided consistent evidence that the Aβ-induced neuroinflammation is greatly mediated by activation of kinin B_2 receptor and the signaling pathways activated by it, such as PKC, MAPKs and their effectors. Research support: **Financial support** was provided by FAPESC, CNPq and CAPES.

02.005 Noradrenergic and serotonergic neurotransmitions of the ventral medial prefrontal cortex modulate food intake in rats. Stanquini LA[1], Joca SRL[2], Scopinho AA[1] [1]FMRP-USP – Farmacologia, [2]FCFRP-USP – Física e Química

Introduction: The regulation of food intake is a complex interplay between the central nervous system (CNS) and the activity of several organs involved in energy homeostasis. The monoamines like serotonin and noradrenaline are recognized to play an important role in appetite and food intake regulation. Besides the hypothalamus, recognized as the center of this regulation, other structures are involved, especially limbic regions, such as the ventral medial prefrontal cortex (vmPFC). Therefore, the aim of this study was investigate the effects induced by fluoxetine (FLX, selective serotonin reuptake inhibitor) and reboxetin (RBX, selective noradrenaline reuptake inhibitor) microinjection into the vmPFC of fed and fasted rats submitted to a model of food intake. **Methods**: In the first day of experiment, male Wistar rats weighing 230 e 270g with guide cannulas aimed at the vmPFC were placed individually in plastic cages (test cage) inside a soundproof room. After thirty minutes of environmental adaptation, fed rats received a microinjection (FLX: 0.01, 1, 3 and 10 nmol/100nL; or RBX: 0.01, 1, 4 and 10 nmol/100nL) and they were submitted to the food intake test where a petri dish with previously weighed food pellets was placed in the test cage. The food intake test lasted for 1h, and the petri dish was reweighed to calculate food intake. After this test, the animals stayed without food for at least 18 hours (fasted condition) and, in the next day, all the procedures were repeated. **Results:** The amount of food ingested by fasted animals was significantly higher than fed animals ($F_{1,90}$=64.8, $P < 0.0001$). Moreover, FLX (at the doses of 1, 3 and 10 nmol) reduced food intake in fasted animals when compared with vehicle-treated group ($F4_{,90}$=12.1, $P < 0.001$; Dunnets, $P < 0.05$). A significant positive correlation (r2=0,97) between FLX doses and reduction in food consumption by fasted animals was observed. In fed animals, no significant effect of treatment was observed ($F_{4,42}$= 0.32, $P>0.05$). Regarding the experiment with RBX, the amount of food ingested by fasted animals was significantly higher than fed animals ($F_{1,73}$=95.17, $P<0.0001$). Moreover, RBX microinjection into the vmPFC significantly reduced food intake in fasted animals when compared with vehicle-treated group ($F_{4,73}$=12.72, $P<0.001$) at the doses of 1, 4 and 10 nmol (Dunnets, $P < 0.05$). A significant positive correlation between the doses and reduction in food consumption by fasted animals (r2= 0,99). In fed animals, no significant effect of treatment was observed ($F_{4,36}$= 0.4, $P>0.05$). **Conclusion**s: Our results suggest that the noradrenergic and serotoninergic neurotransmissions of the vmPFC are involved in the central control of food intake and appetite. **Financial support**: FAPESP, CNPq, FAEPA and CAPES.

02.006 Montelukast decrease pentylenetetrazol-induced seizures. Jesse AC, Lenz Q, Mello CF UFSM – Fisiologia e Farmacologia

Introduction: Clinical and experimental evidence suggests that inflammation plays an important role in the pathophysiology of epilepsy. In line with this view, selected pro-inflammatory arachidonic acid derivatives have been reported to facilitate seizures. The aim of this study was to examine whether montelukast, antagonist of cysteinyl leukotriene receptors (CysLTR1) decrease pentylenetetrazol – induced seizures and whether this effect can be reversed by use of agonist LTD4. **Methods**: The protocols followed the official Government Ethics Guidelines and were approved by the university ethics Committee (process: 23081.014781/2010-42). Adult male Swiss mice were stereotaxically implanted with a cannula into the right lateral ventricle, and two electrodes were placed over the parietal cortex along with a ground lead positioned over the nasal sinus for EEG recording. The experiments were performed 7 days after surgery. The effects of montelukast (0.03 or 0.3 μmol/μL, i.c.v.). in the presence or absence of the agonist LTD4 (0.2, 2 or 6 pmol/μL, i.c.v.), on PTZ (1.8 μmol/2μL, i.c.v.)-induced seizures was measured. The animals were placed in acrylic boxes and habituated for 20 min before the EEG recording. After this period, the electrode was connected to the digital electroencephalograph. The baseline EEG activity was recorded for 10 minutes. The animals were then injected with the antagonist, antagonist plus agonist or vehicle, 30 min before the administration of PTZ. After the injection of PTZ, the animals were monitored for additional 30 min for the appearance of seizures, by electrographic and behavioral **Methods**. The latency to and number of myoclonic jerk episodes and generalized tonic-clonic seizures were measured, as well as EEG amplitude. **Results:** Montelukast (0.03 and 0.3 μmol) increased the latency to both PTZ-induced myoclonic jerks [$H(2)=11.26$; $p < 0.05$] and generalized seizures [$H(2)=11.57$; $p < 0.01$]. Montelukast decreased the mean amplitude of EEG recordings during seizures [$F(2,15)=5.8$; $p < 0.05$]. LTD4 (0.2 pmol) reverted the anticonvulsant effect of montelukast (0.3 μmol) measured as myoclonic [$H(3)=15.41$; $p < 0.0$] and generalized tonic-clonic seizure [$H(3)=13.7$; $p < 005$]. **Discussion**: Montelukast increases the latency to seizures and decrease the mean amplitude of seizure-related encephalographic recordings. These findings suggest a facilitatory role of CysLTR1 in PTZ-induced seizures. **Sources of research support and Acknowledgements**: CAPES, CNPq, FAPERGS, PRPGP/UFSM, PIBIC/UFSM. The protocols followed the official Government Ethics Guidelines and were approved by the university ethics Committee (process: 23081.014781/2010-42). **References**: Cavalheiro, E. A., M. J. Fernandes, et al. (1992). Neurochemical changes in the hippocampus of rats with spontaneous recurrent seizures. *Epilepsy Res Suppl* 9: 239-247; discussion 247-238. Denzlinger, C. (1996). Biology and pathophysiology of leukotrienes. *Crit Rev Oncol Hematol* 23(3): 167-223. Vezzani, A. (2005). Inflammation and epilepsy. *Epilepsy Curr* 5(1): 1-6.

02.007 Systemic administration of different doses of antioxidant agent attenuates the increased conditioned emotional response induced by restraint-stress. Fedoce AG[1], Lisboa SF[1], Souza-Pinto NC[2], Resstel LBM[1] [1]FMRP-USP – Farmacologia, [2]IQ-USP – Bioquímica

Introduction: The stress is a risk factor for the development of affective disorders, such as anxiety and depression. The organism response to stress leads to neurochemical and behavioral changes that may be associated with oxidative stress. Recent evidences suggest that anxiolytic drugs may have antioxidant properties. The hypothesis of the present study was that systemic administration of 4-hydroxy-2, 2, 6, 6-tetramethylpiperidine 1-oxyl oxil (Tempol), a superoxide dismutase (SOD) mimetic, reduces the consequence of a stress situation evaluating the contextual conditioned emotional responses. **Methods**. Male Wistar rats (230-270 g) were divided in two groups: control or restrained for 3 h (acute stress). These animals received Tempol (5 or 20 mg/kg, i.p.) 30 minutes before the restraint-stress (RS). Twenty four hours later animals were submitted to the contextual fear conditioning (3 electrical foot shocks, 0.8 mA, 2 s). After this, a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. 24h later the animals were re-exposed to the chamber, but without shock presentation, and during this period the freezing behavior and the cardiovascular activity, mean arterial pressure (MAP) and heart rate (HR), were continuously acquired. **Results:** The RS increased freezing behavior during re-exposition to the aversive chamber (non-stressed vehicle: 55 ± 1.5 % vs stressed vehicle: 86 ± 2.7%; $F_{1,29}$=6.4, P<0.02). In addition, there was significant effect the Tempol ($F_{2,29}$=100.7, P<0.001) which was only observed on stressed animals ($F_{2,29}$=74.4, P<0.001). Both doses of Tempol attenuated freezing behavior in stressed rats (veh: 86.4±2.7%; 5 mg/kg: 29.2 ± 1.1%; 20 mg/Kg: 22.5 ± 3.4%; $F_{2,14}$=171.4, P<0.0001, n=5-6/group), but not in non-stressed (veh: 55.3±1.5%, 5 mg/Kg: 48.7±3.1%, 20 mg/Kg: 52.2 ± 3.7%; P>0.05, n=2-6/group). The RS also increased the cardiovascular response during re-exposure to the chamber (MAP: $F_{1,150}$=124, P<0.0001 and HR: $F_{1,150}$=102, P<0.0001, n=5-6/group). In addition, treatment with both dose of tempol attenuated this effect (5 mg/Kg - MAP: $F_{1,135}$=118, P<0.0001 and HR: $F_{1,135}$=116, P<0.0001 and 20 mg/Kg - MAP: $F_{1,150}$=154, P<0.0001 and HR: $F_{1,135}$=192, P<0.0001, n=5-6/group). **Conclusion**: The present results showed that the increase of behavioural and cardiovascular responses during re-exposure to an aversive context observed in stressed animals is attenuated by systemic administration of an antioxidant agent before the stress session. Our findings suggest that acute stressors could sensitize the animal to a subsequent aversive condition by increase oxidative state. **Financial support**: CAPES, CNPq, FAEPA, FAPESP.

02.008 Stimulatory-M_1, inhibitory-M_2, inhibitory-A_1 and stimulatory-A_{2A} presynaptic receptors play keys roles in the facilitatory effect caused by methylprednisolone in neuromuscular transmission. Ambiel CR[1], Ramos EP[2], Dal Belo CA[3], Corrado AP[4], Correia-de-Sá P[5], Alves-do-Prado W[2] [1]UEM – Ciências Fisiológicas, [2]UEM – Farmacologia e Terapêutica, [3]UNIPAMPA, [4]FMRP-USP – Farmacologia, [5]ICBAS-UP

Aim: To investigate the effects of blockage of cholinergic (Nn, M_1, M_2), A_1 and A_{2A} adenosine presynaptic receptors in the facilitatory effect caused by methylprednisolone in the rat phrenic nerve-diaphragm preparations (RPND) indirectly stimulated at 50 Hz. **Methods** and **Results:** The experimental procedures were approved by The Ethics Committee Animal Experimentation – State University of Maringa (license number 073/2012). The RPND were mounted as described elsewhere (BÜLBRING, Brit. J. Pharmacol. 1: 38, 1946). The muscle tensions registered at the end (B) and at the beginning (A) of high-frequency (50 Hz, 5 sec) elicited by electric indirect stimulation was taken as ratio (R=B/A). R-values were measured followed a previous 35 min methylprednisolone incubation, in absence or presence of M_1 (10 nM, pirenzepine), M_2 (0.5 µM, methoctramine), Nn (413 µM, hexamethonium), A_1 (5.0 nM, DPCPX) and A_{2A} (10 nM ZM241385) receptors blockers. Studies with addition of hemicholinium (1.0 µM), adenosine deaminase (ADA, 0.5 U/mL) or choline (1.0 µM) in the bath were also performed. Methylprednisolone (0.3 mM) increased R-values (16.8±3.0%; n=4) when RPND were indirectly stimulated, but did not change R-values, when the preparations were previously paralysed by D-tubocurarine and directly stimulated (n=4). The facilitatory effect caused by methylprednisolone was incremented by pirenzepine (40.9±5.4; n=4), methoctramine (31.26±2.7; n=4) and by ZM 241 385 (29.19±2.0; n=4), but it was not affected (P>0.05) by hexamethonium (16.99±1.36; n=3), or by DPCPX (12.96±2.6; n=4). Simultaneous blockade of the muscarinic M_1 and M_2 receptors by atropine (0.2 µM) produced an intense (P> 0.001) increase (49.13± 5.8%;n= 4) in facilitatory effect of methylprednisolone. ADA increases (55.01±8.6; n=4) the facilitatory effect of methylprednisolone on neuromuscular transmission, and this effect was not different (P> 0.05) of that recorded with the association of corticosteroid with atropine. Previous block of choline uptaker by hemicholinium prevented (0.7±11.9; n=6) the facilitatory effect of methylprednisolone, but the reversing sequence of drugs administrations did not modify (22.15±5.2%; n=3) the facilitatory effect of the corticoid. The facilitatory effect (27.8±4.0%; n=5) of choline recorded at 50 Hz was incremented (44.97±7.6; n=3) by methylprednisolone. **Conclusion**: The effect caused by methylprednisolone in neuromuscular transmission is strongly influenced by activations of the M_1, M_2, A_1 and A_{2A} presynaptic receptors at 50 Hz. Since the combined administration of methylprednisolone with atropine seemed to be that one which would determine greater gains for clinical neuromuscular transmission of patients with myasthenia gravis, caution is necessary to utilization of such combination of drugs, as the patients with myasthenia gravis have the activities of the M_1 receptor on motor nerve terminal compensatory increased (Takamori, *Eur. J. Neurol*., 14: 1230, 2007). Sources of research support: Fundação Araucária.

02.009 Effect of the dorsolateral periaqueductal gray CB1 cannabinoid receptor antagonism in the expression of contextual fear conditioning. Uliana DLM[1], Hott SC[2], Lisboa SF[2], Resstel LBM[2] [1]USP – Farmacologia, [2]FMRP-USP – Farmacologia

Introduction: The periaquedutal grey (PAG) is a mesencephalic structure which has an important role in the expression of defensive response of fight or flight, immobility and autonomic response. This region is functionally divided into four longitudinal columns: dorsomedial, dorsolateral, lateral and ventrolateral. The portion of the dorsolateral PAG (dlPAG) is involved particularly with immobility, fight, tachycardia and expression of conditioned emotional response (CER), immobility and increased autonomic response, in the model of contextual fear conditioning (CFC). Cannabinoid receptor type 1 (CB1) are one of the targets of endocannabinoids. The CB1 receptor in the dlPAG is related to reduction of CER in CFC. The antagonism of this receptor in the dlPAG did not affect the CER expression in a protocol of high intensity shocks. Nevertheless, the blockade of this receptor in the medial prefrontal cortex using a lower intensity protocol, CER expression was increased, showing that this receptor tonically modulates this response. Thus, the aim of this work was to study if the dlPAG CB1 receptors tonically modulate the CER. **Methods**: Male Wistar rats (240-260g) with unilaterally implanted cannulae into the dlPAG were submitted to a protocol conditioning of low intensity for 10 min (3 footshocks, 0.850 mA, 2 s), forty-eight hours before the test session. Twenty four hours later, a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. In day of the test, the behavioral and autonomic mean arterial pressure (MAP) and heart rate (HR) responses to the context were measured in a 10 min test session. Microinjection of 0.1µL , of 0.1 nmol or 0.3 nmol of the CB1 receptor antagonist (AM251), or saline occurred 10 min before test. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 127/2011). **Results:** The dose of 0.1 nmol of the AM251 (n=9) had no effects on percentage of freezing (P>0,05) and cardiovascular response (MAP, P>0.05; HR, P>0.05). However, the dose of 0,3 nmol (n=9) increased the percentage of freezing ($F_{2,25}$=15,56, P<0.0001) followed by increased of both MAP ($F_{2,27}$ =4,703, P < 0,0185) and HR ($F_{2,27}$= 4,198, P<0,0268), when compared to vehicle group (n=10) in re-exposition of aversive context. **Discussion**: Our findings showed that the administration of CB1 antagonist at the dose of 0,1nmol did not increase the CER. However, the dose of 0.3 nmol increased CER expression, presenting as the effective dose. Thus, the present results suggest that the dlPAG CB1 receptors tonically modulate the CER in CFC. **Financial support**: CAPES, FAPESP, CNPq and FAEPA.

02.010 Blockage of N-type voltage-gated calcium channels, but not P/Q-type, inhibits trypsin-evoked scratching behavior in mice. Maciel IS[1], Azevedo MV[2], Morrone FB[2], Souza HA[3], Gomez MV[4], Campos MM[5] [1]PUCRS – Farmacologia, [2]LAFAP-PUCRS – Pharmacy, [3]UFMG – Neuroscience, [4]UFMG – Neuroscience, [5]PUCRS – Toxicology and Pharmacology

Introduction: Itching or pruritus is a common symptom present in most skin diseases, and also in other pathological states, such as cancer, infection and metabolic disorders (Metz, M et al; *Vet Dermatol* 22(2): 121, 2011). The involvement of voltage-gated calcium channels (VGCC) has been recently described in the mouse model of ozaxolane-induced pruritus (Tsukumo *et al*; *J Pharmacol Sci* 115(1): 27, 2011). This work was aimed to assess the effects of selective blockage of N- and P/Q-type VGCC in scratching behavior induced by trypsin in mice, by using animal-derived toxins. **Methods**: Male Swiss mice were used (8 per group, 25 - 30g). Two days before the experiments, the hair at the back of the mouse neck and lumbar region was shaved. On the day of experiments, the animals were individually placed into glass cylinders 20-cm in diameter, for 30 min, in order to acclimatize them to the experimental environment. To induce scratching behavior, the animals received an intradermal (i.d.) injection of trypsin (50 µl, containing 200µg/site) in the neck region and were observed for 40 min. The number of scratches with forepaws and hindpaws close to the injected site was registered. Scratching behind the ears, but not on the face, was also counted. At least two mice (control and treated) were observed simultaneously in each experimental session. One hour before trypsin injection, mice received an intrathecal (i.t) injection of the selective N-type VGCC inhibitors MVIIA (0,3; 1 and 10 ρmol/site) and PhTx3.6 (10; 50; 100; 300 ρmol/site); or the selective P/Q-type blockers MVIIC (50; 100; 200 ρmol/site) and PhTx3.3 (50; 100; 300 ρmol/site), respectively. All the experimental protocols were approved by the local Ethics Committee (09/00101, PUCRS). **Results:** The treatment with MVIIA was able to significantly prevent the scratches induced by trypsin at the doses 1 and 10 ρmol/site (58 ± 6% and 66 ± 7%, respectively), but not at 0.3 ρmol/site. Similarly, the treatment with the toxin PhTx3.6 reduced the scratching behavior induced by trypsin at the doses of 50, 100 and 300 ρmol/site (54 ± 14%; 49 ± 7% e 49 ± 6%, respectively), although the dose of 10 ρmol/site did not affect trypsin-induced responses. On the other hand, the treatment with MVIIC (50; 100; 200 ρmol/site) and PhTx3.3 (50; 100; 300 ρmol/site) completely failed to prevent the scratching behavior induced by trypsin. **Discussion**: The present study demonstrates, for the first time, the relevance of N-type VGCC, but not P/Q-type, in signaling pathways underlying the scratching behavior induced by trypsin. Our results suggest new mechanisms implicated in itching transmission. Additional studies are under development to further characterize the mechanisms involved in this response. **Financial support**: PRPPG/PUCRS, CAPES-AUX-PE Toxinologia, CNPq and FINEP/PUCRSINFRA #01.11.0014-00.

02.011 Effects of phenobarbital on bone repair and biomechanics in rats. Ferreira LVC[1], Marchi KC[2], de Ávila MA[1], Pereira VA[1], Camilli JA[3], Soares EA[1] [1]Unifenas – Farmacologia e Cirurgia Experimental, [2]FMRP-USP – Farmacologia, [3]IB-Unicamp – Anatomia

Introduction: Clinical and experimental observations highlight the noxious action of anticonvulsant therapy on bone tissue and also on this repair. Adults and children treated with anticonvulsants for a long time have reduction in bone mineral density and reduction of trabecular bone volume. Anticonvulsants alter calcium homeostasis and require the monitoring of the skeletal system of patients undergoing prolonged therapy with this drug. The evaluation of the effects that prolonged use of phenobarbital may present on the bone tissue becomes important when considering the distribution of medicament in the treatment of epilepsy. So, this study aimed to investigate the morphological effects of prolonged treatment with phenobarbital on bone formation and on bone biomechanics in rats. **Methods**: After approval by the Ethics Committee in Research of Universidade José do Rosário Vellano (UNIFENAS), Protocol nº 22A/2009, this study was conducted, respecting the Brazilian legislation for Experimental Animals, regulated by Federal Law nº 6.638/79. Were used ten male Wistar rats, 30 days old, weighing 85 ± 2g, which were divided into two groups: control (CT) and phenobarbital (FE). The FE group received daily doses of phenobarbital 0.035 ml / kg intramuscularly for 60 days. The CT group received the same dose of 0.9% saline solution. After 30 days, was introduced surgically a failure in the parietal bone that was kept open for verification of new bone formation. In the proximal epiphysis of the right tibia was performed another failure and implanted porous hydroxyapatite bioceramic (PHA). After surgery, we continued the protocols until the end of 60 days when the rats were euthanized, and the bones collected. Histomorphometric studies were performed to obtain the volume of new bone formation and mechanical strength testing was made to assess the maximum force required for complete rupture of this femurs. **Results:** The morphometric results showed that FE group had lower volume of new bone around the PHA implants (25.0$\pm$0.5) compared to CT group (37.0$\pm$3.7). The failure of parietal bone of CT animals was practically repaired (37.2$\pm$3.7), unlike the found in FE group (25.0$\pm$0.5), which still had osteogenic activity and clear distance between the borders of the bone failure. The bone of CT group had a higher force(N) (119.0$\pm$2.0), displacement(mm) (37.2$\pm$3.7) and stiffness(N/mm) (119.0$\pm$2.0) when compared with FE group (84.0$\pm$5.1; 25.0$\pm$0.5; 96.0$\pm$3.1, respectively). **Discussion**: Studies show that the use of anticonvulsant agents affects the bone tissue, hindering the process of bone repair and remodeling, causing alterations in the metabolism of vitamin D. It is known that this vitamin assists in maintenance of bone mass, mobilizes calcium from the bone into the circulation, participates in the maturation of collagen and stimulates the formation of osteocalcin, alkaline phosphatase and osteopontin. The risk of osteoporosis and osteopenia is increased by adult users phenobarbital, since the drug alters bone metabolism, decreases bone mineral density, interferes with bone repair after injury and makes bones less resistant. **Financial support**: CNPq

02.012 Montelukast prevents disruption of the blood-brain barrier (BBB) associated with PTZ-induced seizures. Marafiga JR, Jesse AC[1], Lenz Q[2], Mello CF[2]
[1]UFSM – Neurotoxicity and Psychopharmacology

Introduction: Growing clinical and experimental evidence suggests that disruption of the blood–brain barrier (BBB) is associated with various neurological disorders, including epilepsy. It has been shown that montelukast, (a CysLT1 receptor antagonist) presents anticonvulsant effect. Furthermore, it has been shown that montelukast prevents BBB disruption in a rat model of traumatic brain and focal cerebral ischaemia. Although there is convincing evidence supporting that blood-brain-barrier (BBB) dysfunction facilitates seizures, no study has investigated whether the anticonvulsant effect of montelukast is associated with its ability to maintain BBB integrity. In this study we investigated whether montelukast preserve BBB during PTZ-induced seizures and also whether this effect can be reversed by agonist LTD_4. **Methods**: The Adult male Swiss mice were stereotaxically implanted with a cannula into the right lateral ventricle. The experiments were performed 7 days after surgery. The animals were injected with 10 mg sodium fluorescein in 0. 1 ml sterile saline (i. p.). Fifteen minutes thereafter they were injected (i. c. v.) with montelukast (0. 03 or 0. 3 μmol/μL), LTD_4 (0. 2 or 2 pmol/μL) or montelukast (0. 3 μmol/μL) plus LTD_4 (0. 2 pmol/μL). Fifteen minutes after CysLT ligand injection, the mice were injected with PTZ (1. 8 μmol/2 μL, i. c. v.), and observed during 20 min for the appearance of seizures. At the end of the observation period, the animals were anaesthetized with ketamine/xylazine (50/5 mg/kg, i. p.) for ventricular blood collection (cardiac puncture). The blood was centrifuged and serum was stored at -70ºC until processing. The brain was perfused with 150 ml of 0. 9% NaCl, weighed, and homogenized in 1 ml sterile PBS, and then stored at -70ºC until processing. Fluorescence of brain and serum were measured in a fluorometer. The degree of BBB permeability was measured as the percentage (w/v) of sodium fluorescein in a gram of brain tissue per the amount of sodium fluorescein in a millilitre of serum,in both hemispheres. The protocols followed the official Government Ethics Guidelines and were approved by the university ethics Committee (process: 23081. 014781/2010-42). **Results:** Montelukast (0. 03 and 0. 3 μmol) prevented PTZ-induced BBB disruption [$F(11,47)=5.\ 901$; $p < 0.\ 05$] and LTD_4 facilitates this leakage [$F(11,48)=5.\ 12$; $p < 0.\ 01$]. The effect of montelukast (0. 3 μmol/μL) was not reversed by LTD_4 (0. 2 and 2 pmol) - three way ANOVA followed by *Bonferroni's test.* **Discussion**: Montelukast prevents and LTD4 facilitates BBB disruption. LTD4 does not revert the protective effect of montelukast on the BBB. Our data suggest that the anticonvulsant role of montelukast is not related to BBB maintenance. **References**: Friedman, A. Blood-brain barrier dysfunction, status epilepticus, seizures,and epilepsy: a puzzle of a chicken and egg? *Epilepsia*, v. 52, n. 8, p. 19. 2011. Heinemann, U., et al. Blood-brain barrier dysfunction, TGFbeta signaling, and astrocyte dysfunction in epilepsy. Glia, 2011. **Sources of research support and Acknowledgements**: CAPES, CNPq, FAPERGS, PRPGP/UFSM, PIBIC/UFSM.

02.013 Trans and n-6 fatty acids increases anxiety-like symptoms induced by DL-amphetamine in rats. Schuster AJ, Kuhn FT, Roversi K, Antoniazzi CTD, Barcelos RCS, Benvegnú DM, Trevizol F, Pase CS, Dias VT, Roversi K, Bürger ME UFSM – Fisiologia e Farmacologia

Introduction: Amphetamine (AMPH) and related drugs are involved in addiction and their abuse can cause neurotoxicity (Iacovelli, 2006). Experimentally, these psychostimulants increase locomotor activity of rodents and lead to self-administration due to their additive property. Despite scientific advances, the AMPH abuse is a serious public health problem that affects the individual, their family and also the society. Chronic abuse of AMPH results in dependence, whose withdrawal symptoms has been related to the development of a hedonia, anxiety, social isolation and depression during its abstinence. Recent studies have focused on the ways in which dietary composition may contribute to the abuse of drugs. Preclinical studies have indicate that the dietary intake of n-3 essential fatty acids (EFA), which are precursor of long chain polyunsaturated FA (LC-PUFA), are able to decrease the anxiety-like behaviors in rodents (Buydens-Branchey, 2006). Since this FA can be incorporated into neuronal membrane phospholipids and to give more fluidity and permeability to membrane (Jump, 2002), we hypothesized that *trans* FA (TFA) may make it more rigid and impair the activity of neuro*trans*mitters in brain. **Methods**: The experimental protocol was approved by the Animal Ethical Committee (UFSM-004/2012), which is affiliated to the Council for Control of Animal Experiments (CONCEA), following international norms of care and animal maintenance. Female rats (32) were supplemented (p.o.3g/kg/day) with fish oil (FO, rich in n-3 FA), soybean oil (SO, rich in n-6 FA) or hydrogenated vegetal fat (HVF, rich in *trans* FA) from the first day of pregnancy until the litter weaning, whose offspring were kept in the same supplementation until postnatal day 40 (PND40). DL-AMPH (4mg/kg/day) was administered once daily, alternating with saline for 8 consecutive days. On PND 51, anxiety-like symptoms were observed in elevated plus maze test (EPM) for 5 min and in open field (5 min), where the locomotor activity also was quantified (Broadhurst, 1960). **Results:** One-way ANOVA followed by Duncan's test showed that rats treated with FO and SO spent more time in the open arms of EPM than HVF group. FO supplemented animals spent lower time in closed arms in relation to other experimental groups. HVF and SO supplemented rats showed higher locomotor activity (crossing) and exploratory (rearing) in comparison to control and FO groups. **Conclusion**: AMPH is an addictive drug which is able to develop anxiety-like symptoms and locomotor agitation, observed in EPM and in open-field task. From this study we concluded that n-6 and *trans* FA are able to increase the anxiety-like symptoms and locomotor activity induced by AMPH administration. Once incorporated into phospholipids, these FA are able to modify the fluidity and permeability of the neuronal membrane, and therefore changing its function. In this time we are evaluating the AMPH additive properties on the 2nd generation of rats supplemented with *trans* and others FA. The authors wish to thank Herbarium® by FO capsules, which were kindly donated. **Financial support**: Fapergs-PRONEM 2011; PROAP/PPG-Farmacologia PRPGP-UFSM. F.T.K and M.E.B. are grateful to CAPES and CNPq, respectively, by the research fellowships.

02.014 Fish oil provides sustained and reproducible antiamnestic effect after transient, global cerebral ischemia: Influence of different treatment regimens. Ferreira EDF[1], Mori MA, Oliveira RMW[2], Milani H[2] [1]UEM – Farmácia e Farmacologia, [2]UEM – Farmacologia e Terapêutica

Introduction: Cerebral ischemia leads to neurodegeneration and cognitive impairment, representing a serious social-economic burden. Fish oil (FO) constitutes a rich dietary source of omega-3 polyunsaturated fatty acids, mainly docosahexaenoic acid (DHA). We found previously that FO administration started prior to ischemia and continued up to 42 days abolish ischemia-induced retrograde amnesia completely.
Objective: This study investigated whether the antiamnestic efficacy of FO would be changed according different treatment regimens in relation to the onset of ischemia and the beginning of memory assessment. Method: Naive rats were trained for 10 days in an eight-arm radial maze task. On day 13 they were subjected to 15-min ischemia (4-VO model). Retention of the previously acquired cognition (i.e., memory) was assessed weekly on days 20, 27, 34, 41, 48 and 55. FO daily administration (DHA 300 mg/kg, p.o.) followed one of three regimens: (regimen 1) from 3 days prior to ischemia up to day 41 postischemia; (regimen 2) from 3 days prior to ischemia up to day 20 postischemia; and (regimen 3) from day 27 up to day 48 postischemia. On day 56 neurodegeneration was examined by Nissl staining. The procedure was approved by the local Ethics Committee on Animal Experimentation (Protocol no. 015/2008). **Results:** Memory performance is reflected by the ability of rats to remember the goal box location learned during the preoperative training, and it is expressed by the parameters latency, number of reference memory errors, and number of working memory errors. In the sham-operated groups, within-group comparisons revealed that memory performance did not change from pre- to postsurgery phases for all the three parameters ($p > 0.05$). After TGCI, however, the latency to find the goal box and the number of errors increased significantly from pre- to postsurgery phases (regimen 1: $p < 0.001$ to 0.05; regimen 2: $p < 0.001$ to 0.01; and regimen 3: $p < 0.01$ to 0.05). This amnestic effect of TGCI is also manifested when between-group comparisons are applied to each memory trial, i.e., on days 20, 27, 34, 41, 48 and 55 (regimen 1: $p < 0.001$- 0.05; regimen 2: $p < 0.0001$; and regimen 3: $p < 0.001$ to 0.01) or to the cumulative data (regimens 1, 2, 3: $p < 0.0001$ to 0.05). All these data indicate that rats subjected to TGCI forgot the task learned previously, an effect that reflects a state of persistent retrograde amnesia. Such ischemia-induced memory impairment was abolished completely by FO treatment according to regimens 1 or 2. This antiamnestic effect was well evident for all the three parameters measured ($p < 0.0001$- 0.05, FO group *vs*. vehicle group). Moreover, this protective/restaurative effect of FO on memory was sustained long after discontinuation of FO administration, as clearly demonstrated after the regimen 2. This cognitive effect of FO occurred in the absence of neurohistological protection. **Conclusion**: Long-term FO treatment afford robust and sustained antiamnestic effect after transient, global cerebral ischemia, an effect that was restricted to regimen 1 and 2, suggesting that FO may acts cognitively by changing some acute, neuropathological process occurring in the periischemic period. Supported by CNPq, Fundação Araucaria and UEM

02.015 Beta1 and beta2 adrenoceptors in the medial amygdaloid nucleus modulate the cardiovascular responses to acute restraint stress in rats. Fortaleza EAT, Scopinho AA, Corrêa FMA FMRP-USP – Farmacologia

Aim: Medial amygdaloid nucleus (MeA) is involved in the cardiovascular and behavior responses on stress situation. Our results previously suggest an inhibitory influence of the beta-adrenoceptors into the MeA on the heart rate (HR) increase evoked by restraint stress (RS) without affect blood pressure (BP) increase. RS causes cardiovascular responses such as increased BP and HR. Therefore, the aim of the present study was to investigate witch of the beta-adrenoceptors into the MeA are involvement on cardiovascular responses to acute RS in rats. **Methods**: We used male Wistar rats weighing (240-280g). Guide cannulae were implanted bilaterally in the MeA for microinjections of drugs or (artificial cerebrospinal fluid aCSF) vehicle. Two days after, animals were anesthetized with tribromoethanol and a polyethylene catheter was implanted into the femoral artery for BP and HR recording. The Institution's animal ethics committee approved the housing conditions and the experimental protocol (no. 057 ⁄ 2009). **Results:** We microinjected the selective beta1-adrenoceptor antagonist CGP20712 and selective beta2-adrenoceptor antagonist ICI118,551 into MeA in the doses of (10 15 and 20nmol/100nL), and submit the animals to acute RS. Acute RS caused BP and HR increases in aCSF treated animals (n=6). Pre-treatment with CGP20712 (20nmoL/100nL) into the MeA significantly reduced restraint-evoked HR increase without significant effect on the BP response, when compared with aCSF-treated animals (DMAP: Treatment: $F_{1,60}$= 3,114; P= 0,0827; and DHR, Treatment: $F_{1,60}$= 4,976, P= 0,0295; two-way ANOVA, n=6). However, the pre-treatment of the MeA with CGP20712 in the doses of the 10 and 15 did not change the restraint-related cardiovascular responses: 10nmol (DMAP: Treatment: $F_{1,66}$= 0,09206, P= 0,7625; and DHR, Treatment: $F_{1,66}$= 3,342, P= 0,0721; two-way ANOVA, n=7); 15nmol (DMAP: Treatment: $F_{1,60}$= 0,7354, P= 0,3945; and DHR, Treatment: $F_{1,60}$= 0,3750, P= 0,5426; two-way ANOVA, n=6). On the other hand, pre-treatment with ICI118,551 in the doses of the 15 and 20nmol/100nL into the MeA significantly enhanced restraint-evoked HR increase without a significant effect on the BP response, when compared with aCSF-treated animals: 15nmoL (DMAP: Treatment: $F_{1,60}$= 1,407, P= 0,2403; and DHR, Treatment: $F_{1,60}$= 20,92, P<0.0001; two-way ANOVA n=6) and 20nmoL (DMAP: Treatment: $F_{1,60}$= 0,1183, P= 0,7321; and DHR, Treatment: $F_{1,60}$= 24,33, P<0,0001; two-way ANOVA n=6). However, the pre-treatment of the MeA with ICI118,551 in the doses of the 10 did not change the restraint-related cardiovascular responses: 10nmol (DMAP: Treatment: $F_{1,60}$= 0,07165, P= 0,7899; and DHR, Treatment: $F_{1,60}$= 1,884, P= 0,1750; two-way ANOVA n=6).**Conclusion**: Our results suggest that beta1-adrenoceptors has a facilitatory influence and beta2-adrenoceptors has inhibitory influence on restraint-evoked tachycardiac responses in rats. **Financial support**: CNPq and CAPES.

02.016 Influence of dietary trans fat on the amphetamine preference in rats. Kuhn FT, Roversi K, Barcelos RCS, Benvegnú DM, Antoniazzi CTD, Trevizol F, Pase CS, Dias VT, Roversi K, Schuster AJ, Bürger ME UFSM – Fisiologia e Farmacologia

Introduction: Western diet is rich in saturated fat and *trans* FA (TFA) and lacking in essential fatty acids (EFA), which compete by desaturation and elongation to form long chain polyunsaturated FA (LC-PUFA), which components in neurons membrane and closely involved in neurotransmission and receptor function (*Mcnamara et al.* 2008). Amphetamine (AMPH) is a psychostimulant drug frequently involved in the abuse due to neuronal release and reuptake inhibition of dopamine (DA) in the brain, thus inducing alertness and motivation. We hypothesized that the continuous consumption of TFA can reduce the fluidity and plasticity of the neuronal membrane phospholipids (*Jump.* 2002) and increasing the preference to psychostimulant drugs.
Aims: This study was designed to determine the influence of supplementation with different fat acids in preference paradigm of DL-AMPH. **Methods**: The experimental protocol was approved by the Animal Ethical Committee (UFSM-004/2012), which is affiliated to the Council for Control of Animal Experiments (CONCEA), following international norms of care and animal maintenance. Female rats (32) were supplemented (p.o. 3g/kg/day) with fish oil (FO, rich in n-3 FA), soybean oil (SO, rich in n-6 FA) or hydrogenated vegetal fat (HVF, rich in *trans* FA) from the first day of pregnancy until the litter weaning, whose offspring were kept in the same supplementation until *postnatal day 40 (PND40).* At the 41 PND, 1 animals of each litter were submitted to conditioned place preference (CPP), whose protocol was developed with one AMPH injection (i.p., 4mg/Kg/day) in the opposite chamber of the basal preference, alternating with saline (injected in the preferred compartment) at intervals of 4 hours (*Thanos et al.,* 2010), for 8 consecutive days. At the end of the protocol (PND49), the influence of the FA supplementation on the AMPH preference was evaluated by one way ANOVA followed by Duncan's test. **Results:** HVF supplementation increased AMPH preference in comparison to control, FO and SO groups, while FO group showed lower preference for AMPH than control and SO groups. **Conclusion**: These preliminary findings point out to the influence of the consumption of *trans* FA on preference to psychostimulant drugs, whose molecular basis may be related to possible change of composition and function of the neuronal membrane phospholipids. Efforts are being invested to confirm and extend these findings. The authors wish to thank Herbarium® by FO capsules, which were kindly donated. **Financial support**: Fapergs-PRONEM 2011; PROAP/PPG-Farmacologia PRPGP-UFSM. F.T.K and M.E.B. are grateful to CAPES and CNPq, respectively, by the research fellowships.

02.017 The neostigmine-induced TOFfade depends on activation of inhibitory-M_2 muscarinic receptors on motor nerve terminal which is influenced by level of adenosine in synaptic cleft. Bordignon-Antonio M, Alves-do-Prado W UEM – Farmacologia e Terapêutica

Aim: The degree of neuromuscular transmission blockade is clinically monitored using the train-of-four ratio (TOF_{ratio}). The TOF_{ratio} is the quotient between muscular tension produced by the fourth stimulus (T4) and first stimulus (T1) within a train-of-four stimulus delivered at 2Hz. Reductions in the TOF_{ratio} is TOF_{fade}. It has been shown that the intravenous administration of neostigmina worse the TOF_{fade} and this effect is potentiated by intra-arterial administration of atropine in the cats. Since to investigate the mechanism involved in drug-induced TOF_{fade} might be clinically useful; the roles of muscarinic (stimulatory-M_1, inhibitory-M_2) and adenosine (stimulatory-A_{2A}, inhibitory-A_1) presynaptic receptors in TOF_{fade} caused by neostigmine was investigated in rats phrenic nerve diaphragm muscle preparations. **Material and Methods**: The Ethics Committee for Experimental Studies of the State University of Maringá approved the procedures used in the present study (2600/2011-PRO). Each preparation was immersed in a chamber containing Krebs's buffer at 37ºC and continuously gassed with a mixture of oxygen (95%) and carbon dioxide (5%). Hemidiaphragms were connected to an isometric force transducer (Grass FT 03, West Warwick, RI, USA). Muscle contraction responses were recorded with a PowerLab data acquisition system (Chart Software; AD Instruments, Australia). Data were submitted to ANOVA followed by Bonferroni's post-hoc test. The phrenic nerve was stimulated supramaximally with TOF stimuli delivered at a frequency of 2Hz, which was repeated once every 15s over a period of 15 min. This sequence was repeated three times; the first one in drug-free Krebs buffer condition (control), 2nd in presence of drug-free Krebs buffer (neostigmine control) or antagonists of M_1, M_2, A_1 or $A2_A$ receptors. The 3th sequence of TOF was performed after addition of neostigmine in the bath. The TOF_{ratio} (T4/T1) was taken as a measure of drug-induced neuromuscular transmission failure (TOF_{fade}). The lowest concentration (1.0μM) of neostigmine able to produce 17.0 ± 5.7% (mean±SEM, n=6) TOF_{fade} at first minute after its administration was researched. The neostigmine-induced TOFfade was worsened by blockages of presynaptic muscarinic receptors by atropine (0.2μM; F=6.84, P<0.05; n=4) falicitatory-M_2 presynaptic muscarinic receptors by methoctramine (1.0μM; F=7.32, P<0.05; n=4) inhibitory-A_1 adenosine presynaptic receptors by DPCPX (2.5nM; F=24.24, P<0.01; n=4) or by selective blockage of facilitatory- A_{2A} adenosine receptors on motor nerve terminal by ZM 241385 (10 nM; F=2.6, P<0.05; n=4). Reduction in the level of adenosine in the synaptic caused by adenosine deaminase (ADA, 0.5U/ml) reduced the neostigmina-induce TOF_{fade} (F=15.82, P<0.01, n=4) but the selective blockage of facilitatory-M1 presynaptic receptors by pirenzepine did not cause (F=0.56, P>0.05; n=4) any change in the fade induced by neostigmine. **Conclusion**: The fade induced by neostigmina is dependent on activation of inhibitory-M2 receptors on motor nerve terminal, but the activation of such receptors is influenced by presence of adenosine in synaptic cleft inducing the activation of A_{2A} and A_1 adenosine receptors on motor nerve terminal. **Acknowledgment**: CNPq

02.018 Evaluation of the antioxidant action of drugs used in Parkinson's disease.
Farias CC[1], Bortolasci CC[1], Bonifácio KL[1], Maciel DRK[1], Lavado EL[2], Barbosa DS[1]
[1]UEL – Ciências da Saúde, [2]UEL – Fisioterapia

Introduction: Parkinson's disease (PD) is characterized by degeneration of dopaminergic neurons in the substantia nigra and motor and non-motor symptoms (Mizuno et al., 2008; Kim et al., 2010). Free radicals may play a role in the biochemical events that lead to neuronal death in old age and in neurodegenerative diseases. Furthermore, oxidative stress is apparent in PD. Analyzing the chemical structure of certain anti-parkinsonian drugs, decided to investigate independent of its anticholinergic or dopaminergic agonism effects. Thus, the aim of this work was to evaluate the in vitro antioxidant potential of biperiden and pramipexole. **Methods**: The ethics committee of UEL has approved this study (license number 222/2011).The inhibition of the respiratory burst was performed using an adaptation of the technique described by Huber et al. (2006). The results were expressed as the peak values of the kinetic curves and 1st and 3rd quartiles. Neutrophils buff coat alone as control and with addition of 0.01mg of Biperiden or Pramipexole were used in the reactions. Statistical analysis was performed using the Kruskal-Wallis test complemented by Dunn's test. Differences were considered significant when $p<0.05$. **Results:** The results have demonstrated the ability of Pramipexole and Biperiden to partially inhibit the in vitro respiratory burst. Comparing the results from the addition of Pramipexole and Biperiden to control the Kruskal-Wallis test indicated a statistically significant difference in peak values of the kinetic curves in tests (KW = 40.5, $p<0.0001$). Dunn's test has shown the peak values obtained with amounts of Pramipexole and Biperiden were different from each respective control. The control of Pramipexole have shown a median of 55075.00 (52233.00 – 55413.00) whereas the Pramipexole was 8584.00 (8496.00 – 9362.00) $p<0.002$. The control of Biperiden was 49284.00 (48617.00 – 50774.00) and Biperiden was 13286.00 (12878.00 – 16077.00) $p<0.002$. There was also a significant difference in peak values on the kinetic curves between Pramipexole and Biperiden $p<0.002$. **Discussion**: Pramipexole and Biperiden have demonstrated to have antioxidant effect in vitro and may have a neuroprotective potential because the active molecule of these drugs can cross the blood brain barrier and exert antioxidant effects in the brain. This antioxidant effect may be due to the hydroxyl groups that are present in the chemical structure of the molecule of these drugs. According Schapira (2002) the hydroxylated benzyl ring structure could act by reducing the generation of free radicals and protecting cells from damage caused by oxidative stress favoring neuronal survival in PD patients. **References**: HUBER, K et al. Respiratory burst as a biomarker for stress responses. *Protoplasm*, vol. 229, p. 221, 2006. KIM, J.S. et al. Inhibition of inducible nitric oxide synthase expression and cell death by ()-epigallocatechin-3-gallate, a green tea catechin, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Clin J Neurosci*, v. 17, p. 1165, 2010. MIZUNO, Y. et al. Progress in the pathogenesis and genetics of Parkinson's disease. *Phil. Trans. R. Soc. B*. v. 363, p. 2215, 2008. SCHAPIRA, A.H.V. Dopamine agonist and neuroprotection in Parkinson´s disease. *Eur J Neurol*, v.9, p.7, 2002. **Financial agencies**: We thank CAPES for the fellowship.

02.019 Both alpha1 and alpha2-adrenoceptors mediate the cardiovascular responses to noradrenaline microinjected into the dorsal periaqueductal gray of rats Santana DAR[1], Simões TMG[1], Volpini VL[1], Resstel LBM[2], Corrêa FMA[2], Pelosi GG[1] [1]UEL – Fisiologia e Farmacologia, [2]FMRP – Farmacologia

Introduction: Periaqueductal gray area (PAG) is a mesencephalic region which is involved in the integration of cardiovascular changes associated with defensive responses. In a previous study we showed that noradrenaline (NA) microinjected in the dorsal column of the PAG (dPAG) caused pressor response mediated by vasopressin release. However, the subtype of adrenoceptors that mediates that response is unknown. In the present work, we aimed to evaluate the involvement of alpha adrenoceptors on the pressor response to NA microinjected into the dPAG of unanesthetized rats. **Methods**: Experimental procedures were carried out following protocols approved by the ethical review committee of the State University of Londrina (n.15/2010). A guide cannula was stereotaxically implanted in the dPAG of male Wistar rats (240-260g). A catheter was introduced into the right femoral artery for blood pressure and heart rate recording. First, animals received NA (15nmol/50nL) microinjection into the dPAG. After 24h, dPAG was treated with different doses (4, 8 or 12 nmol/50nL) of the selective alpha1-adrenoceptor antagonist WB4101, or the selective alpha2-adrenoceptor antagonist RX821002 or the combination of both. Ten minutes later NA was microinjected. **Results:** The microinjection of NA into the dPAG of unanaesthetized rats (n=38) caused pressor and bradycardiac response. WB4101: Pretreatment of the dPAG with 4nmol of the selective alpha1-adrenoceptor antagonist potentiated cardiovascular responses to NA (ΔMAP: $F_{(1,320)}$= 83; ΔHR: $F_{(1,320)}$= 150; p<0,0001, n=5; two-way ANOVA). On the other hand, local pretreatment with 8nmol of WB4101 reduced the bradycardiac response, but not the pressor response evoked by NA into the dPAG (ΔMAP: $F_{(1,400)}$= 4,3, p=0,04; ΔHR: $F_{(1,400)}$= 47, p<0,0001, n=6; two-way ANOVA), while the higher dose reduced only the bradycardia (ΔMAP: $F_{(1,480)}$= 2,4, p=0,12; ΔHR: $F_{(1,480)}$= 64, p<0,0001, n=7; two-way ANOVA). RX821002: Pretreatment with different doses of the selective alpha2-adrenoceptor antagonist significantly reduced the pressor response to NA (ΔMAP 4nmol of RX: $F_{(1,320)}$= 45; ΔMAP 8nmol of RX: $F_{(1,320)}$= 54; ΔMAP 12nmol of RX: $F_{(1,320)}$= 169, p<0,0001, n=5/dose; two-way ANOVA). Additionally, pretreatment with 8 or 12 nmol/50nL of RX821002 reduced the bradycardia (ΔHR 4nmol of RX: $F_{(1,320)}$=0,9, p=0,35; ΔHR 8nmol of RX: $F_{(1,320)}$=38, p<0,0001; ΔHR 12nmol of RX: $F_{(1,320)}$=7,7, p=0,006, n=5/dose; two-way ANOVA). WB4101 and RX821002: Pretreatment with the combination of WB4101 and RX821002 (12nmol/50nL) blocked the pressor response caused by NA microinjected into the dPAG (ΔMAP: $F_{(1,160)}$= 64, p<0,0001; ΔHR: $F_{(1,160)}$= 2, p=0,2; n=3). **Discussion**: The results suggest that activation of local alfa1 and alfa2-adrenoceptors mediate cardiovascular responses to NA microinjected into the dPAG of unanesthetized rats. **Financial support**: CNPq

02.020 Time course of histological changes in a chronic brain hypoperfusion stepwise 4-vessel occlusion model in rats: **Comparison between normotensive and spontaneously hypertensive rats**. Romanini CV, Ferreira EDF, Milani H, Oliveira RMMW UEM – Farmacologia e Terapêutica

Introduction: The injury extent and time-course of neuronal death induced by chronic cerebral hypoperfusion (CCH) still remain crucial questions to be answered in order to find the most suitable time-window for therapeutic intervention. The present study was aimed to characterize the time course of histological changes following CCH in rats. We also examined the relationship between CCH and vascular risk factors such as hypertension in spontaneously hypertensive rats (SHR). **Methods**: Adult, male Wistar normotensive rats (NTR) and adult male Wistar-Kyoto SHR were used. The vertebral arteries (VAs) plus the internal carotid arteries (ICAs) were progressively and permanently occluded. Permanent 4-VO (vessel occlusion)/ICA or sham-surgery was performed gradually in two stages according to the following sequence: VA+ ICA→ICA with an interval of 4 days between the occlusions. The NTR subjected to 4-VO/ACI were euthanized at 24 and 48 hours, 7, 15 and 30 days and the SHR were sacrificed at 7, 15, 30 days after surgery. The rats had their brains processed for histological assessment of hippocampus and neocortex. The extent of pyramidal cell loss was quantified bilaterally across the CA1-CA4 subfields of the hippocampus and in the retrosplenial (RS) and parietal association (PtA) neocortices using Nissl´s staining. The thickness of the granular cell layer was measured in the dentate gyrus (DG). Averaged from the various measurements in each individual was transformed into a percentage where the mean of the sham group was considered 100%. Data are present as mean ± SEM and were analyzed by Kruskal–Wallis (K–W) ANOVA followed by Dunn's post hoc test. The procedure was approved by the local Ethics Committee on Animal Experimentation (Protocol no. 059/2011). **Results:** A significant reduction in the number of intact–appearing pyramidal neurons was detected in the hippocampus in NTR, 48 h and 15 days after 4-VO/ICA as compared to sham ($P < 0.0001$). In SHR groups, hippocampal cell loss was seen at 15 days which extended up to 30 days following 4-VO/ICA ($P < 0.001$). In the RS, there was a significant decrease in the number of intact neurons in NTR at 48 h, 7 and 15 days ($P < 0.0001$), and in the SHR, at 15 and 30 ($P<0,001$) days after 4-VO/ICA as compared to sham. Considering PtA, a significant reduction in the number of intact neurons ($P < 0.0001$) was observed at 7 and 15 days after surgery in NTR. In SHR, a significant reduction in the number of intact neurons in PtA ($P < 0.0001$) occurred 7, 15 and 30 days after 4-VO/ ICA when compared to controls. The thickness of the DG granular cell layer was significantly reduced 15 days after 4-VO/ ICA in both NTR ($P < 0.05$) and SHR groups ($P < 0.05$). NTR also presented reduced in the thickness of DG, 7 days after surgery. **Discussion**: The number of intact–appearing pyramidal neurons in NTR was reduced from 48h and kept constant until 15 days after 4-VO/ACI in the hippocampus and neocortex. Otherwise, in SHR the hippocampal and cortical neurodegeneration was kept constant up to 30 days. These results suggest that the presence of a risk factor, such as hypertension, influencing the extent and maintenance of the lesion in the cortex and hippocampus after 4-VO/ ICA. Supported by CNPq.

02.021 Chronic consumption *of* trans fatty acids from the post-weaning period can enhance the movement disorders and locomotor activity in rats adulthood.
Pase CS, Teixeira AM, Dias VT, Bürger ME UFSM – Fisiologia e Farmacologia

Introduction: The increased consumption of industrialized products in the last years, have led to the intake of large amounts of partially hydrogenated vegetable oils rich of trans fatty acids (TFA) (Allison DB, J Am Diet Assoc, 99, 166, 1999). Dietary TFA can be incorporated into membrane phospholipids, decreasing membrane fluidity and altering the biochemical properties and functionality of membrane proteins (Larqué E, J Nutr, 133, 2526, 2003). Therefore, the aim of this study was to evaluate the effects of TFA, in rats supplemented from weaning to adulthood on behavioral parameters. **Methods**: Experiment was conducted with 24 male Wistar rats weighting between 40-60g. The experimental protocol was approved by the Animal Ethical Committee (Universidade Federal de Santa Maria-UFSM-24/2010). Immediately after weaning, the rats were randomly assigned into two experimental groups: soybean oil (SO) or hydrogenated vegetal fat (HVF). Dietary supplementation consisted in the incorporation (20%) of different fatty acids (FA) present in SO (rich in polyunsaturated FA-PUFA) and HVF (rich in *trans*-monounsaturated and saturated FA) on standard chow. After ten and fifteen months of treatment all the animals were submitted to behavioral assessment, with intervals of at least one week between each observation. Data were analyzed by paired samples *t* test and independent *t* test. A value of $p < 0.05$ was considered as statistically significant and results were expressed as mean ± S.E.M. **Results:** HVF dietary increased vacuous chewing frequency (VCM) and facial twitching (FT) in 10 (33.42±3.16; 7.91±1.95) and 15 (44.71±3.44; 14.32±1.01) months of observation, compared with SO10 (24.14±1.89; 2.28±1.39) and SO15 (26.85±5.42; 2.42±1.24) groups and HVF15 group was different of HVF10 group in this parameters. In the open-field test, was decreased of locomotor activity in HVF10 (26.71±1.28) and HVF15 (15.42±1.96) groups as compared to SO10 (33.14±2.15) and SO15 (28.14±4.49) groups, respectively. Similarly, the exploratory activity and central locomotion was decrease in HVF10 (13.28±0.91; 0.85±0.14) group when compared to SO10 (18.71±0.86; 1.85±0.26) and in HVF15 (4.71±0.86; 0.31±0.12) group compared with SO15 (12.42±1.28; 1.63±0.45) group. Still, the locomotor and exploratory activity, and central locomotion was reduced on the HVF15 group compared to HVF10 group. **Discussion**: This study suggests that the predominance of dietary TFA from lifelong may facilitates movement disturbances related to aging. Consequently, further detailed investigations are urgently needed in order to establish nutritional safety to consumption of *trans* fatty acids in animals and humans.
Sources of research support: PROAP – UFSM.

02.022 Pentylenetetrazol-induced seizures alter Na^+,K^+-ATPase activity and phosphorylation state in the mice cerebral cortex. Meier L[1], Marquezan BP[1], Funck VR[1], Oliveira CV de[1], Araújo SM[2], Zarzecki MS[2], Oliveira MS[1] [1]UFSM – Fisiologia e Farmacologia, [2]UNIPAMPA

Introduction: Na^+,K^+-ATPase is a major regulator of brain excitability, and a decrease of its activity enhances brain excitability and trigger seizures. Na^+,K^+-ATPase activity is controlled in multiple ways, and protein phosphorylation is of fundamental importance. The present study aimed to investigate whether the phosphorylation state of two key residues at the Na^+,K^+-ATPase catalytic α subunit, namely Ser943 and Tyr10, is altered by the seizures induced by pentylenetetrazol (PTZ), a classical convulsant agent. In addition, we tested the effect of two protein kinase inhibitors, H-89 (an inhibitor of protein inhibitor kinase A – PKA) and genistein (an inhibitor of non-receptor tyrosine kinases) on PTZ-induced seizures and Na^+,K^+-ATPase activity. **Methods**: Adult male Swiss albino mice (25-30 g) were used. Animals were injected with PTZ (60 mg/kg, i.p.) or its vehicle (0.9 % NaCl, i.p.) and monitored for 15 min for seizure activity. Following, animals were euthanized and their cerebral cortices were used for determination of Na^+,K^+-ATPase activity using a colorimetric method based on ATP hydrolysis in the presence or absence of the specific inhibitor ouabain. Immunodetection of Na^+,K^+-ATPase α subunit and its phosphorylation state at Ser943 or Tyr10 were achieved by western blot. The effect of H-89 or genistein on PTZ-induced seizures and Na^+,K^+-ATPase activity investigated by administrating vehicle (0.9% NaCl, i.p.), H-89 (10 mg/kg, i.p.) or genistein (10 mg/kg, i.p.) 30 min before the injection of PTZ or vehicle. After seizure analysis Na^+,K^+-ATPase activity was measured in the cerebral cortex. Data were analyzed by Student's t test, Kruskal-Wallis test or one or two-way ANOVA, followed by the Bonferroni test, when appropriated. A probability of $p<0.05$ was considered significant. **Results:** PTZ decreased Na^+,K^+-ATPase activity by 37.65 % in the mice cerebral cortex [$t(20)=3.255$; $P<0.05$], but did not alter α subunit immunoreactivity [$t(7)=0.1715$; $P>0.05$]. In addition, PTZ increased phosphorylation of Ser943 by 82.5 % [$t(11)=2.818$; $P<0.05$] and of Tyr10 by 79 % [$t(11)=2.787$; $P<0.05$]. Conversely, H-89 or genistein did not alter PTZ-induced myoclonic seizures [$H(2)=0.7987$; $P>0.05$], generalized seizures [$H(2)=0.3732$; $P>0.05$], seizure duration [$F(2,16)=1.035$; $P>0.05$] or decrease in Na^+,K^+-ATPase activity [$F(2,35)=1.62$; $P>0.05$]. **Discussion**: Here we showed that Na^+,K^+-ATPase activity in the mice cerebral cortex decreases following PTZ-induced seizures, independently of changes in the content of Na^+,K^+-ATPase catalytic α subunit. In addition, phosphorylation of Ser943 and Tyr10 increased after the seizures induced by PTZ. Given the role of Na^+,K^+-ATPase as a major regulator of brain excitability, Ser943 and Tyr10 at Na^+,K^+-ATPase α subunit may represent valuable targets for drug development for seizure disorders. However, other strategies than the use of broad spectrum protein kinase inhibitors should be investigated, since H-89 and genistein had no effect on PTZ-induced seizures. Animal Ethics Committee license number: 051/2010. **Financial support**: FAPERGS, CAPES, CNPq, PBDA/Unipampa

02.023 Time course of cognitive changes and hippocampal neurodegeneration in mice after transient global cerebral ischemia. Soares LM, Schiavon AP, Milani H, Oliveira RMMW UEM – Farmacologia

Introduction: Transient global cerebral ischemia (TGCI) in rodents leads to a reduction of oxygen and glucose to the brain, causing cell death in susceptible brain regions such as the hippocampus. This pattern of neurodegeneration has been associated with behavioral and cognitive deficits. The effects of TGCI in mice are often inconsistent and incomplete, which complicates the interpretation of the experimental data and lacks information about the best time for evaluating neuroprotective effects of drugs. The objective of this study was to characterize the time course of cognitive changes and hippocampal neurodegeneration in mice subjected to TGCI. **Methods**: Male adult albino Swiss mice (30-40g) were used. All the experimental procedures were approved by the Ethical Committee on Animal Research (CEEA 004/2011). The animals were subjected to TCGI by the 2-VO (two-vessel occlusion) method. On the 7^{th}, 14^{th} and 28^{th} days after reperfusion the animals were submitted to a training day in Morris water maze (MWM), for evaluation of spatial learning. The 'latency to find the platform' during 10 consecutive trials was measured. Twenty-four hours later, the 'time spent in the correct quadrant' (where the platform was located on the training session) was analyzed as retention memory (experiment I). A separated group of animals was trained in the MWM and 24h later they were submitted to TGCI in order to evaluate retrograde memory (experiment II). The effects of TGCI on cognition were expressed by the parameters: a) latency to find the platform (learning) and b) time spent in the correct quadrant (memory). The degree of neurodegeneration was measured using Nissl´s and Fluoro-Jade C´s (FJ-C) staining. Data were expressed as mean ± SEM and analyzed by two-way ANOVA or Kruskall Wallis ANOVA (KW), followed by post hoc Duncan's test. **Results:** In experiment I, ischemic animals presented an increase in the latency to find the platform as compared to controls, 7 days after reperfusion (KW=12.87, P=0.0049; ischemic=466.8±36.91, control=337.1±21.95). In experiment II, ANOVA showed a significant decrease in the 'time in correct quadrant` only 14 days after reperfusion as compared to control group ($F_{1,57}$=9.17, P<0.05; ischemic=10.90±0.71, control=15.01±0.87). A significant decrease on the number of intact neurons throughout the hippocampal subfields was detected (KW = 28.13, P <0.0001) at 7 (39.72±12.49), 14 (24.78±6.505) and 28 (32.73±8.577) days after TGCI when compared to sham group (100.0±6.397). Neurodegeneration was detected by an increase of FJ-C positive cells (KW = 9214, P = 0.01) at 7 (100.0±24.29) and 14 (16.78±10.16) days after ischemia. **Discussion**: TGCI caused cognitive deficits at 7 and 14 days after reperfusion. Besides, hippocampal neurodegeneration was detected from the 7^{th} up to 28^{th} day after TGCI in all hippocampal subfields. These results show that the best time for evaluating of cognitive deficits induced by TGCI is between 7 and 14 days after reperfusion. Despite hippocampal neurodegeneration, there was an apparent memory recovery 28 days after TGCI. It is possible that other brain structures but hippocampus are involved in functional recovery after TGCI. Supported by Fundação Araucária, CAPES, CNPq and State University of Maringá.

02.024 Effect of different atorvastatin treatments on oxidative stress markers in the rat cerebral cortex. Grigoletto J, Oliveira CV, Pereira LM, Funck VR, Oliveira MS
UFSM – Fisiologia e Farmacologia

Introduction: Statins are inhibitors of HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthesis, and have been considered the most effective drugs for the treatment of hypercholesterolemia and atherosclerotic diseases. There is evidence that statins, particularly atorvastatin, are neuroprotective in several conditions. In contrast, abrupt cessation of atorvastatin treatment (i.e. atorvastatin withdrawal) cause deleterious effects. Interestingly, most of the effects of statins seem to be unrelated to changes in cholesterol levels. In order to shed some light on the mechanisms underlying the neuroprotective effects of statins and on the deleterious effects elicited by statin withdrawal, and considering that oxidative stress plays a key role in several diseases, the present study aimed to investigate whether atorvastatin treatment or withdrawal display antioxidant or pro-oxidant properties. **Methods**: Adult male Wistar rats (250-300 g) were used. Animals received either saline solution (0.85 % NaCl) or atorvastatin (10 mg/kg/day) for 7 days by intragastric gavage. Animals were euthanized 30 minutes (atorvastatin treatment) or 24 hours (atorvastatin withdrawal) after the last gavage and had their cerebral cortices used for the oxidative stress markers analyses. Nitrite plus nitrate content (NOx) is an indicative of nitric oxide production and was determined by a colorimetric method based on the Griess reaction. The immunoreactivity for 3-nitrotyrosine (3-NT – marker for nitrosative protein damage), protein carbonyls (marker for oxidative protein damage) and 4-hydroxy-2-nonenal-protein adducts (HNE – marker for protein damage triggered by lipid peroxidation) were determined by slot blot. Data were analyzed by one-way ANOVA, followed by the Newman-Keuls test, when appropriated. A probability of $p<0.05$ was considered significant. **Results:** Statistical analysis revealed a significant decrease in NOx levels in the atorvastatin treatment (27.6 % decrease) and withdrawal groups (34.65 % decrease) [$F(2,31)=5,248$, $p<0.05$]. In contrast, a significant increase in 3-NT levels (33.4 % increase) was found in the atorvastatin withdrawal group [$F(2,21)=5.421$, $p<0.05$]. No changes in protein carbonyls [$F(2,31)=1,970$, $p>0.05$] or levels of HNE-protein adducts [$F(2,26)=0.8280$, $p>0.05$] were found. **Discussion**: Our present results suggest that atorvastatin withdrawal causes nitrosative protein damage, as showed by an increase in 3-NT levels. It is possible that the decrease in NOx levels in atorvastatin withdrawal occurs through increased nitric oxide consumption by superoxide anion, which in turn leads to the formation of peroxynitrite and ultimately to protein nitration. In contrast, decreased NOx levels in the atorvastatin treatment cannot be explained by these mechanisms, and could be related to a direct decrease in nitric oxide production. Further studies are needed to fully understand the mechanisms underlying the currently reported effects of atorvastatin treatment and withdrawal. Animal Ethics Committee license number: 053/2010. **Financial support**: FAPERGS, CAPES, CNPq

02.025 Haloperidol polymeric nanocapsules decrease its adverse motor side effects and oxidative stress markers in rats. Benvegnú DM[1], Barcelos RCS[1], Boufleur N[1], Pase CS[1], Roversi K[2], Segat HJ[2], Dias VT[2], Reckziegel P[1], Flores FC[3], Ourique AF[4], da Silva C B[3], Beck RCR[4], Bürger ME[1] [1]UFSM – Farmacologia, [2]UFSM – Fisiologia e Farmacologia, [3]UFSM – Ciências Farmacêuticas, [4]UFRGS – Nanotecnologia Farmacêutica

Introduction: Haloperidol is the most widely used antipsychotic drug in the treatment of psychiatric disorders (Ponto; **Methods** Find. Exp. Clin. Pharmacol., 32:427, 2010). Despite its satisfactory therapeutic effect, its chronic use is related to severe motor side effects (Andreassen; Prog. Neurobiol., 61:525, 2000). Here, we investigate the incidence of motor side effects of haloperidol-loaded nanocapsules containing fish oil as core (H-NcFO) when compared to free haloperidol (FH) and the relation with oxidative stress development in rats. **Methods**: Haloperidol-loaded nanocapsules formulations (0.25 mg/mL) were prepared and physicochemical characterized. Wistar rats received daily the haloperidol formulations (0.2 mg/kg-ip) administered during 28 days and were submitted to acute and subchronical motor side effects parameters: oral dyskinesia frequency, catalepsy time and locomotor activity. At the end of behavioral observations oxidative stress markers: lipid peroxidation and reduced glutathione levels and catalase activity were evaluated in extrapyramidal brain region. The experimental protocol of this study was approved by Animal Ethical Committee of Universidade Federal de Santa Maria (CIETEA- 22/2010), which is affiliated to CONCEA. **Results:** H-NcFO formulation showed uniform and rounded particles, nanometric size (261±3 nm), negative zeta potential (-13±0.7 mV), low polydispersity indices (0.21±0) and high encapsulation efficiency (95±0.4%). Acutely, lower catalepsy time and oral dyskinesia were observed in H-NcFO-treated group than in FH group; however, both formulations decreased animals' locomotor activity. In experiment performed subchronically, rats injected with H-NcFO showed decreased oral dyskinesia frequency and catalepsy time and no impairment on locomotor activity as compared to FH group. Besides, FH group showed higher oxidative stress, as observed by increased lipid peroxidation and reduced glutathione levels and catalase activity in extrapyramidal brain region. **Discussion**: Our findings showed that nanocapsules may be an efficient form to prevent or minimize haloperidol motor side effects, which are related to OS development, ameliorating psychiatric patients' quality of life. Acknowledgments: This work was supported by grants from CNPq, CAPES and PRPGP (Pró-Reitoria de Pós-Graduação e Pesquisa), PROAP-UFSM.

02.026 Vacuous chewing movements induced by reserpine in rats are related with Na^+,K^+-ATPase activity in striatum – protective effects of gallic acid. Reckziegel P[1], Peroza LR[2], Schaffer LF[1], Ferrari MC[3], Bürger ME[4], Fachinetto F[4]
[1]UFSM – Farmacologia, [2]UFSM – Bioquímica Toxicológica, [3]UFSM – Farmácia, [4]UFSM – Fisiologia e Farmacologia

Aim: Na^+,K^+-ATPase is enzyme with pivotal role in the cellular function, mainly because it is responsible for the maintenance and restore of the membrane potential in excitable cells. At the present moment, few is known about the relation of it in the development of involuntary movements. Then the present study evaluated the activity of this enzyme in striatum, a brain region closely related to the development of movement disorders, in animals with vacuous chewing movements (VCMs) induced by reserpine. In addition, gallic acid (GA), a polyphenyl product, was tested in this context to look for an efficient treatment for involuntary movements. **Methods**: Male Wistar rats weighing 250±20g (about 2-month-old) were treated with reserpine (1mg/Kg, i.p.) or saline (1mL/Kg, i.p.) daily for 3 days, followed by treatment with GA (6.75mg/Kg, p.o.) or water (1 mL/Kg, p.o.) twice a day for 3 days. Thus, the experimental groups of the present study were: control (n=5), reserpine (n=9), GA (n=6) and reserpine+GA (n=10). Twelve hours after the last treatment, VCMs of the animals was counted in glass cages and the animals were killed by decapitation. Brains were dissected and striatum and cortex were used to measurement of Na^+,K^+-ATPase activity. This protocol was submitted and approved by Ethical Intern Commission of UFSM sob number 010/2012. Data were analyzed by two-way ANOVA following Duncan's test (p<0.05), and by Pearson's correlation test. **Results:** It was observed an increase in VCMs in the reserpine-treated animals in relation to the control ones (5.06 times, p<0.05). In addition, reserpine-treated animals showed decrease in Na^+,K^+-ATPase activity in striatum in relation to the control group (24.7%, p<0.05) and any difference was observed in cortex. GA was able to protected the animals that received reserpine of the increase in VCMs as well as of the decrease in Na^+,K^+-ATPase activity in striatum (reserpine+GA group was different of reserpine group in both cases, p<0.05). GA did not change any evaluated parameter *per se*. Negative correlation was found between VCMs and Na^+,K^+-ATPase activity (r=0.48, p<0.05). **Conclusion**: GA decreased VCMs induced by reserpine in rats and its mechanism seems to involve alterations in Na^+,K^+-ATPase activity in striatum. These results suggest that GA may have a promissory use in the treatment of involuntary movements. Further studies to elucidate the mechanisms of reserpine-induced decrease in Na^+,K^+-ATPase activity in striatum and of GA protection in this animal model are needed. Sources of **Financial support**: CAPES, CNPq, FAPERGS, FIPE-UFSM.

02.027 Acute exposure to toxic doses of metamidophos and depression like effects in adult male rats. Araújo SL, Maffezzolli G, Salum N, Zaia RM, Vital MABF, Dalsenter PR UFPR – Farmacologia

Introduction: Brazil is one of the major countries in pesticides buying. The organophosphates are mostly employed against agricultural plagues as insecticides. The organophosphates are frequently related to young and adult neurotoxicity, and are one of the most cited compounds linked to acute and delayed toxicity in humans. Some epidemiological researches try to correlate the amount of suicidal tendencies with the organophosphates spray in agricultural workers. In this work we tried to induce depression like signals in male Wistar rats exposed to metamidophos by gavage, in an acute protocol. This work was licensed by the UFPR Animal Ethics Committees under the number 533. **Methods**: Fifty adult male Wistar rats were exposed to vehicle (control) or four crescent doses of metamidophos(0.125; 0.25; 0.5 and 1.0 mg/kg), in only one time exposure. The animals were recorded and evaluated during 30 minutes post exposure for signals of toxicity (fasciculation, salivation, diarrhea, tremor or convulsion). The animals were evaluated seven days before the exposure and 24 hours, seven days, 14 days and 21 days after the exposure in the open field and in the force swimming test. The voluntary total locomotor activity, the immobility time and the frequency of locomotor activity in the center or periphery of the open field apparatus were analyzed. The immobility, the climb and the swimming behavior were evaluated to analyze the possible depression like signals (immobility behavior).The animals were weighted and decapitated for the organs weight analysis 24 hours after the last behavior test. Results and **Discussion**: The animals exposed to 1.0 mg/kg and 0.5 mg/kg of metamidophos developed fasciculation, diarrhea and salivation (signals of an intoxication by an anticholinesterasic agent). The animals did not show difference in the locomotors performance (open field apparatus); only the habituation was significant for all groups in the test. The locomotors performance was 37% reduced in control group, 40% reduced in the group exposed to 1 mg/kg and 39% reduced in the group exposed to 0,5 mg/kg from day 7 before exposure until day 21 after exposure. The depression like signal was not induced after the exposure (immobility comportment). There were also signs of habituation in the force swimming test. The swimming performance was significantly increased,9% in the control group,42% in group exposed to 1 mg/kg and 32% in the group exposed to 0,5 mg/kg from day 7 before exposure until day 21 after exposure. The acute exposure to toxic doses of metamidophos did not produce an acute depression like signal. The animals exposed were fully recovered from the toxic signs. The pesticide did not cause locomotors impairment 24 hours after the exposure and until 21 days after the exposure in the tested doses. **Financial agencies** and acknowledgments: CAPES/Programa REUNI andCNPq

02.028 N-acetylcysteine prevents spatial memory impairment induced by chronic early postnatal glutaric acid and lipopolysaccharide in rat pups. Rodrigues FS, Gerbatin R, Busanello GL, De Castro M, Fiorin FS, Scherer L, Schopf M, Fichera MR
UFSM – Métodos e Técnicas Desportivas

Introduction: Glutaric aciduria type I (GA-I), an inherited deficiency of glutaryl-coenzyme A dehydrogenase, is characterized by accumulation of glutaric acid (GA) and neurological symptoms. It has been reported that GA-I patients have cognitive impairment after encephalopathic crises, which are precipitated by infectious processes, indicating a potential role of inflammatory cytokines in neurological disorders of this organic aciduria. In this context, drugs with antioxidant and antiinflammatory potential, as N-acetylcysteine (NAC), could be considered a promoting approach to neuroprotection in GA-1 patients. Therefore, we decided evaluated the performance of pup rats injected chronically with GA and/or NAC, in the absence or presence of lipopolysaccharide (LPS), in a spatial memory test and evaluate inflammatory cytokines in hippocampus content. **Methods**: Wistar pup rats, with four days old (5 – 30g) were used. Buffered GA, pH 7.4 (5 µmol g of body weight^{-1}s.c) , was injected s.c. twice per day, from the 5th to the 28th day of life to produce brain [GA] around 0.6 µmol g^{-1}, ~0.72 mM, similar those found in GA-1patients. NAC (150 mg/kg) or vehicle (saline 0.9%), were admistread (i.g.), once per day, for the same period. In order to mimic an infections state, the pup rats were injected with LPS (2 mg/kg; i.p E.coli 055 B5); or vehicle (saline 0.9%), once per day, from 25th to 28th day of life. Spatial memory was evaluated by Barnes maze that consists of four days test. Cytokine levels were measured using a commercially available ELISA Kit from R&D Systems using an antibody selective against rat IL-1β or TNF-α, according to the manufacturer's protocol. **Results:** The Barnes maze consists of four day test. At the second day of test, statistical analysis demonstrated that GA [F(1,57)=14,33; p<0,001] and LPS [F (1,57)=20,25;p<0,001] induced memory deficit characterized by reduction of the latency for escape. Furthermore, NAC prevented memory deficit induced by GA [F(1,57)=23,12; p<0,001] and by LPS[F(1,57)=24,15; p<0,001]. At the third day, statistical analysis showed that GA [F(1,57)=8,51; p<0,01], and LPS [F(1,57)=9,34; p<0,01] induced memory deficit too. However, NAC do not prevented the memory deficit induced by GA [F(1,57)=3,56; p>0,05], but prevented the deficit induced by LPS [F(1,57)=8,61; p<0,05]. Statistical analysis revealed an increase on TNF-α levels in GA [F(1,44)=22,83; p<0,001] and LPS [F(1,44)=14,93; p<0,001]. In addition, NAC prevented the increased TNF-α levels induced by GA [F(1,44)=3,78; p=0,05]. The statistical analyses also showed increase on IL-1β levels caused by GA [F(1,42)=38,86; p<0,001] and LPS [F(1,42)=26,15; p<0,001], and NAC reverted the increase on IL-1β levels induced by GA [F(1,42)=3,95; p=0,05]. This work was approved by ethic committee Federal University Santa Maria (protocol n. 116/2010). **Discussion**: This model of GA-I caused a spatial memory deficit, and the results can be due to an increase in IL-1β and TNF- α cytokines hippocampal in pup rats. On the other hand, chronic treatment with NAC was able to reverse the deficit in spatial memory as well as increased levels of IL-1β and TNF-α. These data suggest that inflammatory cytokines could participate in memory deficit in this model of GA-I. **Financial agencies** and acknowledgments: CAPES-CNPq

02.029 Effect of an inhibitor of HMG-CoA reductase, **atorvastatin**, **on the activity of several antioxidant and pro-oxidant enzymes**. Oliveira CV, Pereira LM, Funck VR, Grigoletto J, Oliveira MS UFSM – Fisiologia e Farmacologia

Introduction: Statins comprehend a large group of natural and synthetic drugs which present potent cholesterol-lowering properties, and have been considered the most effective drugs for the treatment of hypercholesterolemia and atherosclerotic diseases. In addition to their cholesterol-lowering and cardiovascular protective properties, several experimental and clinical studies have shown that statins exert a number of pleiotropic and neuroprotective actions, whereas abrupt cessation of statin treatment (i.e. statin withdrawal) cause deleterious effects. In order to elucidate some of the mechanisms underlying the differential effects of statin treatment and withdrawal, we investigated whether atorvastatin treatment or withdrawal display indirect antioxidant or pro-oxidant properties, by measuring the activity of some antioxidant and pro-oxidant enzymes. **Methods**: Adult male Wistar rats (250-300 g) were used. Animals received either saline solution (0.85 % NaCl) or atorvastatin (10 mg/kg/day) for 7 days by intragastric gavage. Animals were euthanized 30 minutes (atorvastatin treatment) or 24 hours (atorvastatin withdrawal) after the last gavage and had their cerebral cortices used for the enzyme assays. The activity of superoxide dismutase (SOD) was determined by a colorimetric method based on the inhibition of auto-oxidation of epinephrine. Glutathione-S-transferase (GST) activity was assayed by measuring the formation of CDNB plus GSH complexes. Catalase (CAT) activity was determined by monitoring hydrogen peroxide consumption. Xanthine oxidase (XOX) activity was determined by measuring uric acid formation in the present or absence of xantine. NADPH oxidase (NADPHOX) activity was measured by monitoring NADPH consumption in the presence or absence of the NADPHOX inhibitor diphenyleneiodonium. Data were analyzed by one-way ANOVA followed by the Newman-Keuls test, when appropriated. A probability of $p<0.05$ was considered significant. **Results:** Statistical analysis revealed a significant increase of SOD (88.61 % increase) [$F(2,18)=4.243$, $p<0.05$] and NADPHOX (39.99 % increase) [$F(2,28)=3.554$, $p<0.05$] activities following atorvastatin withdrawal. In contrast, no significant changes in the activity of CAT [$F(2,17)=0.3044$; $p>0.05$], GST [$F(2,17)=0.6098$; $p>0.05$] or XOX [$F(2,18)=0.2629$, $p>0.05$] were found. **Discussion**: Our present data indicates that atorvastatin withdrawal increases the activity of the antioxidant enzyme SOD in the rat cerebral cortex, which may be an indicative of increased production of superoxide anion. Accordingly, we found an increase in NADPH oxidase activity, a key cellular source of superoxide anion, following atorvastatin withdrawal. Although more studies are needed to demonstrate that these events are causally related, this is an interesting possibility. In addition, increased SOD activity in the absence of parallel increases in CAT or GST activities could explain, at least in part, the pro-oxidant effects of atorvastatin withdrawal. Further experiments are necessary to evaluate the molecular mechanisms underlying our findings as well as its clinical implications. Animal Ethics Committee license number: 053/2010. **Financial support**: FAPERGS, CAPES, CNPq

02.030 Behavioral and neurochemical effects of pentoxifylline in experimental model of Parkinson´s disease. Siqueira RMP, Neves KRT, Tavares KC, Calou IBF, Cavalcante ALC, Cunha GM, Viana GSB UFC – Fisiologia e Farmacologia

Introduction: Pentoxifyilline (PTX) is a methilxanthine derivative that may induce physiologic and pharmacological effects by several mechanisms including inhibition of phosphodiesterases and blockade of adenosine receptors. PTX is also well known to possess anti-inflammatory proprieties. Because these properties, this drug has been used to research in neurodegenerative diseases like Parkinson´s Disease. Objective: Investigate the effects of pentoxifylline (PTX) using a Parkinson's disease (PD) animal model and determinate their effects about neurodegeneration involved in the pathophysiology of this disease. **Methodology**: Male Wistar rats (250-300g) were subjected to unilateral intrastriatal lesion (right side) with 6-hydroxydopamine (6-OHDA). These animals were treated with PTX (25 and 50mg/kg oral) for two weeks, beginning 1 hour after stereotactic surgery treatment. At the end of treatment the rats were evaluated with the apomorphine test, by rotating for 1 h, and locomotor activity (LA) in the open field test, for 5 minutes. After behavioral testing, the animals sham operated group (FO) and 6-OHDA (in the absence or presence of PTX) were decapitated and the brains dissected for the determination of dopamine (DA, ng / g tissue) and of the amino acids glutamate aspartate, GABA and glycine (nmol / g tissue) in the striatum by HPLC. This project was approved by the Ethics Committee for Animal Research (protocol number 23/10). Data were analyzed by ANOVA followed by Student-Newman-Keuls as post hoc test, and values considerate significant with $p < 0.05$. **Results:** The results showed that the group with 6-OHDA lesioned as expected, showed a large number of revolutions (168.9 ± 28.2) compared with the FO (1.3 ± 0.8). The rotations number decreases significantly and dose-dependently in groups treated with PTX (PTX25: 96.1 ± 24.1; PTX50: 39.0 ± 11.5). While the injured group with 6-OHA decreased LA in 24%, this effect was also reversed in the injured group and treated with PTX25, but not the ones treated with PTX50. With respect to the levels of dopamine (DA), while no significant difference was observed in right and left corpora striata group FO, 6-OHDA group showed a significant reduction in striatal DA levels (104.3 ± 47) on the right side when injured compared to his left or to the same side of the group Fo (1970 ± 152), which was partially reversed after treatment with PTX (PTX25: 353.7 ± 180; PTX50: 735.6 ± 109). As to amino acids, the results were similar in left and right sides (injured). Thus glutamate levels in the injured group were increased relative to the group FO and this effect was reversed in both groups treated with injuries and PTX. While glycine levels were reduced in the injured group, these values were close to those of the two groups FO group injured and treated with PTX. While no changes in the values detected in any of aspartate groups, of GABA values shown are reduced on the left side of the injured and treated groups with respect to the injured right side of each group. **Conclusion**: The data indicate a neuroprotective activity of PTX in the model of PD and suggest that this beneficial effect is at least partly related to the dopaminergic system and the levels of glutamate that would be targets of action of PTX. **Financial support**: CNPq

02.031 Neuroinflammatory profile ANS astrocytic morphology in chronic cyclosporine treated rats. Cararo MM[1], Souza DG[2], Andreotti DZ[1], Rodrigues L[3], Lima LS[1], Achaval M[3], Portela LV[2], Souza DO[2], Scavone C[1], Böhmer AE[1] [1]USP – Farmacologia, [2]UFRGS – Bioquímica, [3]UFRGS – Ciências Morfológicas

Introduction: Cyclosporine (CsA) dependent neurotoxicity occurs in up to 60% of transplant patients and the clinical predisposition and mechanisms of CsA-induced neurotoxicity remain controversial and poorly understood. Cerebrovascular complications are most frequent in transplanted patients treated with CsA and we already showed that chronic CsA treatment altered biochemical parameters related to cardiovascular and cerebrovascular risk factors. Ischemia process, excitotoxicity and oxidative stress activated microglial cells and astrocytes which in turn react secreting cytokines and chemokines. This process results in neuronal cell death and increased injury to the ischemic area. Since inflammatory signaling is involved in all stages of ischemic cascade, the present work investigated the release of cytokines and chemokines related to ischemia brain injury and the astrocytes morphology of the hippocampus of rats submitted to a chronic CsA treatment. **Methods**: Adult male Wistar rats were divided in control group (received vehicle - corn oil), and treated group (received 15 mg/kg of CsA), by daily gastric gavage during 8 weeks. Cytokines and chemokines were measured on hippocampus, hypothalamus and cortex and astrocytic cell bodies and processes were identified in a region of interest (ROI) of the CA1 *stratum radiatum*. **Results:** CsA treated rats showed increase expression of RANTES, MCP1, Gro/KC and IL-12 in hippocampus (32, 75, 101, and 65% respectively compared to control: 79.9 ± 1.8, 211.3 ± 25.8, 37.4 ± 7.0 and 94.4 ± 8.6 pg/mL, respectively). And increase expression of Gro/KC and IL-1β in hypothalamus (95 and 730% compared to control: 35.1 ± 5.3 and 19.1 ± 9.3 pg/mL, respectively). Also increase expression of IL-12 in cortex (97% compared to control: 48.8 ± 8.2 pg/mL). Quantitative results showed that the number of astrocytes and glial fibrillary acidic protein (GFAP) density were not affected by CsA treatment. However CsA treatment decreased the number of fusiform astrocytes (2.10 ± 0.19 cells/ROI, $P< 0.0001$) compared to control (4.55 ± 0.26 cells/ROI). At the same time, the astrocytes that predominate at CsA treated rats presented an intermediate shape, between the fusiform and stellate ones (3.67 ± 0.24 cells/ROI, $P< 0.0001$) compared to control (0.61 ± 0.09 cells/ROI). Results are expressed as mean ± SEM; n=5/group. **Discussion**: Considering the key importance of astrocytes in controlling the number and function of neuronal synapses and blood flow in the brain, they are capable to change their shapes due to cerebral insult. The increase expression of pro inflammatory cytokines and chemokines related to ischemia and stroke may give rise to chronic neuroinflammation which has been implicated in several neuropathologies. This research was approved by the Biomedical College of Animal Experimentation (COBEA). All procedures were also approved by the Ethical Committee for Animal Research (CEEA) of the Biomedical Sciences Institute of the University of São Paulo (protocol 20, page 83, book 02). **Financial support**: CNPq /FAPESP (Brazil) / CAPES

02.032 Evaluation of epileptic seizures and neurotrophic factors production induced by intrahippocampal microinjection of pilocarpine in C57BL/6 mice. Lima IVA[1], Campos AC[2], Miranda AS[2], Moraes MFD[3], Teixeira AL[2], de Oliveira ACP[1] [1]ICB-UFMG – Pharmacology, ICB, [2]UFMG – Tropical Medicine and Infection Disease, [3]ICB-UFMG – Physiology and Biophysics

Introduction: Temporal lobe epilepsy (TLE) is the most frequent type of adult human epilepsy. The pilocarpine (PILO) induced animal model of epilepsy is characterized by pathophysiologic and behavioral alterations resembling human TLE. However, this animal model is associated with a high mortality in animals. Although few studies demonstrated that intrahippocampal injection of PILO in rats is a reliable model of TLE, no study has been performed in mice. Therefore, in the present study, we investigated whether PILO microinjection into the hippocampus of C57BL/6 mice induces seizures and alters the production of neurotrophic factors in this brain region. **Methods**: All experiments were performed in accordance with Institutional Ethics Committee (CETEA - UFMG), Protocol 068-2011. C57BL/6 mice (10-12 weeks old) were implanted with a guide cannula in the left and right hippocampus (AP -1.90 mm, LL ±1.5 mm, DV -1.3 mm, bregma as reference) by stereotaxic surgery, and then allowed to recover for 5 days after surgery. "*Status epilepticus*" (SE) were induced by a bilateral intrahippocampal injection of PILO (5, 10 or 20 µg per side) or saline as control. Behavioral activity was recorded for 90 min after the injections. After this period, animals received diazepam (10 mg/kg, i.p.) for interruption of seizures. Scores of seizures were determined based on the scale established by Racine (Racine, R.J., *Eletroencephalogr Clin Neurophysiol*, v.32, p.281, 1972). Mice that received the highest dose of PILO (20 µg per side) were killed by decapitation 24 h after SE and hippocampus was collected for determination of the neurotrophic factors, BDNF and NGF. Statistical analysis was performed using SPSS 16.0.0 software (SPSS Inc., Chicago, IL, USA). **Results:** Mortality was not observed in any group. The highest dose of PILO (20 µg per side) induced SE in 83.3% of the mice, followed by the other two doses (5 and 10 µg per side; 16.6% each). A typical pattern of evolution of limbic seizures during the SE was observed in the animals treated with the highest dose, with the seizures beginning after 19.8 ± 3.8 min and 83.3% of the animals showing the maximal score of seizure. BDNF levels were significantly increased in the hippocampus of the animals: 1.4 ± 0.1 and 3.1 ± 0.6 pg/mg of protein for saline and PILO-treated mice, respectively ($P<0.05$). On the other hand, levels of NFG in this brain region were not altered by PILO injection at this time point. **Discussion**: Here we demonstrated that microinjection of PILO in the hippocampus induced seizures and increased the levels of BDNF. These data indicate that intrahippocampal injection of PILO in mice is a reliable, efficient and low-mortality model that mimics some acute aspects observed after i.p. injection of pilocarpine. Although further investigations are necessary, this might represent an important animal model for the study of the pathophysiology of TLE in different knockout animals that have a C57BL/6 genetic background. **Financial support**: FAPEMIG, PRPq - UFMG

02.033 Repeated caffeine administration by oral or intraperitoneal routes prevents working memory deficits in the intranasal MPTP rat model of Parkinson's disease. Wopereis S[1], Rial D[1], Moreira ELG[2], Bertoglio LJ[1], Prediger RD[1]
[1]UFSC – Farmacologia, [2]UFSC – Neurociências

Introduction: Recently our group demonstrated that rodents treated with a single intranasal infusion of MPTP suffered working memory deficits observed in the early pre-motor phase of Parkinson's disease (PD). On the other hand, caffeine consumption is negatively correlated with the development of PD, possibly because its neuroprotective effects can counteract the dopaminergic neurodegeneration, avoiding the onset of disease. As the drinking coffee is the main source of caffeine in humans, the aim of the present work is to establish a comparison, on the scope of neuroprotection, between the oral and the intraperitoneal routes of caffeine administration. **Methods**: Male Wistar rats were submitted to two schedules of caffeine administration; the first group were pretreated by intraperitoneal (i.p.) route with vehicle (saline) or caffeine (10 mg/kg) once daily during 5 consecutive days, two hours after the last injection of caffeine, the animals were infused intranasally (i.n.) with a single bilateral dose of MPTP (1 mg/nostril). The second group was allowed to drink a solution of 0,3 mg/ml of caffeine dissolved in drinking water or vehicle (tap water) during all the experimental period and received MPTP infusion as described above, 21 days after the beginning of caffeine treatment. Animals were evaluated in cognitive (social recognition), motor (open field) and emotional (elevated plus maze) tests and biochemical assays (western blot for tyrosine hydroxylase) from 7 to 31 days after i.n. MPTP infusion. Ethics committee protocol: CEUA-UFSC: PP00615/2011. **Results:** MPTP-treated rats displayed similar investigation times in the 1st and the 2nd juvenile exposure. More importantly, independently of the treatment regimen, caffeine was able to prevent the working memory impairment (investigation times (i.p. groups): cont:46.9±7.9; caf:46.5±4.0; MPTP:61.4±6.2; caf+MPTP:42.7±6.7; (oral groups): cont:62.9±13.2; caf:40.9±13.2; MPTP:84.3±14.2; caf+MPTP:51.1±14.2). At this time, locomotor parameters were unaltered by the treatments (distance (m) (i.p. groups): cont:14.7±1.5; caf:18.4±2.1; MPTP:16.8±2.1; caf+MPTP:14±0.8; (oral groups): cont:11.2±0.9; caf:12.8±1.4; MPTP:14.5±1.0; caf+MPTP:12.8±1.5). On the other hand, none of the treatments were able to promote changes in anxiety-like parameters, showing that the caffeine dosage used prevented the toxic effects of MPTP without induce anxiogenic-like effects (Open Arms Time (%) (i.p. groups): cont:7.8±2.9; caf:7.5±2.9; MPTP:5.2±3.1; caf+MPTP:7.9±2.8; (oral groups): cont:11.5±2.3; caf:8.1±1.1; MPTP:7.7±2.2; caf+MPTP:8.8±2.3). The MPTP-induced working memory impairments were associated with a marked reduction of TH-positive neurons in the substantia nigra (reduction of TH in relation to control: cont: 0%; caf: 0%; MPTP: 49%; caf+MPTP: 12%). Of high importance, both schedules of caffeine treatment prevented this dopaminergic dysfunction. **Discussion**: These results indicate that caffeine may represent a promising therapeutic tool in PD, thus preventing non-motor early symptoms of PD together with its neuroprotective potential. **Financial support**: CAPES, CNPq, FAPESC, Brazil.

02.034 Relationship between thresholds to convulsions induced by a benzodiazepine inverse agonist and glutamatergic receptors in membranes of brain regions. Conto MB, Carvalho JGB, Venditti MAC Unifesp – Psicobiologia,

Introduction: The generalized epilepsies, both idiopathic and symptomatic, seem to have a strong genetic predisposition (Berkovic, Trends Neurosci, 29, 391, 2006), and an unbalance between the GABAergic and the glutamatergic neurotransmissions are believed to be involved in the etiology of epilepsies. A previous study has observed a higher concentration of an endogenous benzodiazepine inverse agonist in the plasma of epileptic patients compared to controls (Ferrarese, Epilepsy Res, 29, 129, 1998), and it has been suggested that endogenous benzodiazepine inverse agonists play a role in the pathophysiology of epilepsies (Polc, Epilepsia, 37, 1007,1996). To study the possible participation of glutamatergic receptors in the susceptibility to convulsions induced by the benzodiazepine inverse agonist DMCM, we sought to determine if rats with different thresholds to clonic convulsion induced by DMCM present possible differences concerning the binding of [^{3}H]-L-glutamate in membranes of discrete brain regions. **Methods**: Naïve adult male Wistar rats, aged 3 months, were administered with 2 intraperitoneal injections of a CD_{50} of DMCM (one-week interval between them). The rats which presented clonic convulsions in both the administrations were termed LTR (low threshold rats) and those which did not present any sign of motor disturbances in both the administrations were termed HTR (high threshold rats). Twenty-five days after the second drug administration, the selected subjects were sacrificed, and the brain structures dissected and stored at -20^{o}C until the preparation of the homogenates. The [^{3}H]-L-glutamate binding assay of membrane fraction was conducted at the concentration of 50 nM. This work was approved by our institution's Ethics Committee on Animal Research (Proc. 1058/06). **Results:** The analysis of data by unpaired Student's t test demonstrated statistical differences between the groups (N = 8/group) in the specific binding of [^{3}H]-L-glutamate at 50 nM in the in the hippocampus (LTR group: 1353.0 ± 97.80 fmol /mg protein, mean ± SEM; HTR group: 1862.0 ± 161.50; $p<0.05$); in the frontal cortex (LTR group: 1305.0 ± 107.40 fmol /mg protein; HTR group: 1741.0 ± 152.60; $p<0.05$); and in the amygdala plus limbic cortex (LTR group: 1755.0 ± 88.70 fmol /mg protein; HTR group: 2375.0 ± 180.90; $p<0.01$). It was not found a statistical difference between the groups in the striatum (LTR group: 1322.0 ± 114.90 fmol /mg protein; HTR group: 1299.0 ± 146.20; $p>0.05$). **Discussion**: The role of glutamatergic receptors in the epilepsy has been a matter of controversy. The lower [^{3}H]-L-glutamate binding from the LTR in our study may represent a compensatory mechanism from a higher basal glutamate release and/or a lower reuptake from synaptic cleft. Thus, a higher glutamatergic excitability inherent to brain networks involving hippocampus, frontal cortex and amygdala plus limbic cortex maybe responsible for a lower threshold to DMCM-induced convulsion and possibly underlies the susceptibility to generalized convulsions. **Financial support**: AFIP and Capes.

02.035 Effects of cannabidiol on hippocampal neurodegeneration and neurogenesis after transient, global cerebral ischemia in mice. Schiavon AP[1], Soares LM[1], Milani H[1], Guimarães FS[2], Oliveira RMMW[1] [1]UEM – Farmacologia e Terapêutica, [2]FMRP-USP – Farmacologia

Introduction: Transient global cerebral ischemia (TGCI) is reported to cause delayed cell loss of pyramidal neurons in the hippocampus in mice. Concomitantly, TGCI has been shown to trigger neurogenesis in the dentate gyrus (DG). It has been suggested that increased neurogenesis may be a compensatory adaptive response to brain injury which could counteract the effects of neurodegeneration and cell death. Cannabidiol (CBD), a non-psychoactive component of marijuana, seems to be a neuroprotective since it reduced brain damage after middle cerebral artery occlusion model of ischemic injury in mice. The aim of this study was to investigate whether treatment with CBD, could prevent hippocampal neurodegeneration as well as stimulate neurogenesis in DG after TGCI in mice. **Methods**: TCGI was induced by using the 2-vessel occlusion (2-VO) technique in which the common carotid arteries were occluded for 17 min. Sham operated animals underwent the same surgical procedure, but without occlusion of the arteries. CBD (10 or 30 mg/Kg) was administrated intraperitoneally 30 min before, 3, 24 and 48 h after TGCI. Five days after the last administration, the animals were sacrificed and had their brains removed. Fluoro-Jade C (FJC)-staining, doublecortin (DCX) and MAP2 immunohistochemistry were performed in order to evaluate neurodegeneration, neurogenesis and dendritic sprouting, respectively. Pyramidal intact-appearing cells were quantified using Nissl´s staining. 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 042/2012), Paraná, Brazil. **Results:** Data are presented as mean ± SEM and were analyzed by one way analysis of variance (ANOVA) followed by the Bonferroni or Tukey´s tests. A significant decrease in the number of FJC-positive neurons was detected in the hippocampal subfields of CBD-treated animals as compared to ischemics ($F_{3,18}$=8.19, P=0.001; ischemic=344.6±116.5; CDB 10 mg/Kg=46.1±31.7; CBD 30 mg/Kg=14.6±14.6). Immunohistochemistry for DCX did not detect significant differences between groups ($F_{3,20}$=1.99, P=0.152; sham=259.1±25.7; ischemic=281.3±60.3; CDB 10 mg/Kg=386.7±37.1; CBD 30 mg/Kg=255.0±21.6). A significant decrease on the number of intact-appearing pyramidal cells in ischemic group ($F_{3,28}$=5.0, P=0.007; sham=606.5±102.3; ischemic=254.8±77.2; CDB 10 mg/Kg=717.8±112.4; CBD 30 mg/Kg=606.2±84.1) was detected as compared to sham group. Finally, ANOVA did not show significant differences in number of MAP2-positive dendrites in the CA1 subfield of all groups ($F_{3,16}$=0.90, P=0.466; sham=13.7±0.2; ischemic=12.2±1.5; CDB 10 mg/Kg=13.9±0.6; CBD 30 mg/Kg=14.3±0.3). **Discussion**: The present findings showed that CBD at 10 and 30 mg/Kg prevented cell loss and reduced hippocampal neurodegeneration in ischemic mice. Additional studies are needed to evaluate which properties of CBD are related to its protective effects after TGCI. Keywords: transient global cerebral ischemia, neurodegeneration, neurogenesis, cannabidiol, mice **Financial support**: CAPES, UEM and FMRP/USP

02.036 Evaluation of neurotransmitters involved in the anxiolytic and panicolytic effect of the aqueous extract guaraná in the T-maze. Rangel M[1], Mello JP[2], Audi EA[1] [1]UEM – Pharmacology and Therapeutic, [2]UEM – Pharmacy

Introduction: The anxiety disorder comprises distinct pathological conditions such as generalized anxiety disorder, panic disorder, social phobias and posttraumatic stress disorder [1].The treatment of these disorders is defined by the use of antidepressants. However, they have limitations, requiring new therapeutic strategies. *Paullinia cupana* (H.B & K. var. sorbilis [Mart.] Ducke), belonging to the family Sapindaceae and popularly known as guaraná [2], and has been used as CNS stimulant. The ethanolic semi-purified fraction obtained from guaraná seed extract, (EPA; State University of Maringá, patent pending PI0006638-9) produced antidepressant-like effects in rats in the forced swimming test [3]. The objective of this study was to evaluate the anxiolytics and panicolytic effects of the aqueous fraction of guarana (FAQ) in rats treated for 21 days and submitted to the elevated T-maze (ETM) test, and to understand the mechanisms involved in the effects produced by FAQ. **Methods**: Male Wistar rats (n=8-13) were treated orally for 21 days with FAQ (8mg/Kg), paroxetine (PAR-3mg/Kg) or saline (NaCl 0.9%). On the test day the animals received single intraperitoneal administration of receptor antagonist serotoninergic (metergoline-MET;3mg/Kg), dopaminergic (SUL-sulpiride; 20mg/Kg) or glutamatergic (ketamine-QUET; 0.125mg/Kg) and were exposed to ETM. Repeated-measures analysis of variance (RMANOVA) was used to analyze both avoidance and escape data. Locomotion data were analyzed by one-way ANOVA. When appropriate, the Duncan post-hoc test was used, significance level was set at $p<0.05$. The experimental protocol was approved by the ethics committee (No.107/2011). Results and **Discussion** Post-hoc comparisons showed significant effect on the avoidance both FAQ and PAR decrease in the inhibitory avoidance latencies of the ETM at baseline, avoid 1 and 2 ($p<0.05$) indicating anxiolytic effect for the FAQ and PAR. The anxiolytic effect produced by FAQ was blocked by MET, SUL and QUET, where as the anxiolytic effect produced by PAR was blocked only by MET. Post-hoc comparisons showed that FAQ increased escape 1, 2 and 3 ($p<0.05$) and that PAR significantly increased escape 1, 2 and 3 ($p=0.001$) compared to the control group, indicating panicolytic the effect. The panicolytic effect produced by FAQ was blocked by both MET and SUL, whereas the panicolytic effect produced by PAR was blocked only by MET. These results show that chronic treatment with FAQ produces an anxiolytic effect during the ETM test, and that the serotonergic, dopaminergic and the glutamatergic neurotransmission systems are involved in this effect, and also produces a panicolytic effect during the ETM test, with dopaminergic and serotonergic neurotransmission systems are involved in this effect. **Conclusions:** In conclusion the results show that FAQ produced a similar profile to the PAR, with involvement of serotonergic, dopaminergic and glutamatergic in its pharmacological effect, and it could be an alternative therapy in the treatment of conditions such as anxiety and panic. **Reference:** 1-Kessler, R.C. Arch Gen Psychiatry, 62, 593-602, 2005. 2-Henman, A. J. *Ethnopharmacol*, 6, 311-338, 1982. 3-Otobone. *Phytother* Res *21*: 531, 2007.

02.037 Effects of atorvastatin treatment and withdrawal on Na^+, K^+-ATPase activity. Funck VR, Grigoletto J, Oliveira CV, Pereira LM, Oliveira MS UFSM – Fisiologia e Farmacologia

Introduction: Statins are selective inhibitors of 3-hydroxyl-3-methyl-glutaryl coenzyme A reductase, the rate-limiting enzyme of the mevalonate pathway for cholesterol biosynthesis. Increasing evidence indicates that statins, particularly atorvastatin, are neuroprotective in several conditions, including stroke, cerebral ischemia, traumatic brain injury, excitotoxic amino acid exposure and seizure activity. In contrast, abrupt cessation of atorvastatin treatment (i.e. atorvastatin withdrawal) causes detrimental effects. In both cases, the underlying mechanisms are still unclear. One possible target for statin-mediated neuroprotection is Na^+,K^+-ATPase, an eletrogenic pump which plays a key role in the regulation of brain excitability. Regarding this point, and given the emerging role of Na^+,K^+-ATPase as an target for the treatment of several neurological disorders, the present study aimed to investigate whether atorvastatin treatment and withdrawal alters Na^+,K^+-ATPase activity in the rat cerebral cortex. **Methods**: Adult male Wistar rats (250-300 g) were used. Animals received either saline solution (0.85 % NaCl) or atorvastatin (10 mg/kg/day) for 7 days by intragastric gavage. Animals were sacrificed 30 minutes (atorvastatin treatment) or 24 hours (atorvastatin withdrawal) after the last gavage and had their cerebral cortices used for the determination of Na^+,K^+-ATPase activity and phosphorylation state. Na^+,K^+-ATPase activity was measured by a colorimetric method based on the differential sensitivity of alpha isoforms to the specific inhibitor ouabain. Immunodetection of Na^+,K^+-ATPase α subunit and its phosphorylation state at Ser943 were evaluated by western blot. Data were analyzed by one-way ANOVA. *Post hoc* analyses were carried out by Bonferroni test, when appropriated. A probability of $p<0.05$ was considered significant. **Results:** We found that atorvastatin withdrawal decreased Na^+,K^+-ATPase isoform α2 and α3 activity, without changing total or isoform α1 activity [$F(2,33)=9.866$; $p<0.05$]. Interestingly, atorvastatin withdrawal also decreased the immunoreactivity of Na^+,K^+-ATPase α subunit by 35 % [$F(2,24)=3.641$; $p<0.05$] and increased Na^+,K^+-ATPase α subunit phosphorylation at Ser943 by 44 % [$F(2,10)=14.28$; $p<0.05$]. **Discussion**: Our present results indicate that atorvastatin withdrawal decreases Na^+,K^+-ATPase in the rat cerebral cortex, and this effect could be related to a decrease in the immunoreactivity of α subunit. Interestingly, atorvastatin withdrawal increased Na^+,K^+-ATPase α subunit phosphorylation at Ser943, a residue which is crucial for the regulation of enzyme catalytic efficiency, since its phosphorylation decreases substrate affinity and alters membrane targeting of Na^+,K^+-ATPase. Additional studies are necessary to evaluate the molecular mechanisms underlying our findings as well as its clinical implications. Animal Ethics Committee license number: 053/2010. **Financial support**: FAPERGS, CAPES, CNPq

02.038 Behavioral and neurochemical alterations produced by the standardized extract of *Myracodroun urundeuva* (Aroeira-do-Sertão) in an experimental model of Parkinson disease. Calou IBF[1], Lopes MJP[2], Siqueira RMP[1], Pinto NB[1], Rodrigues DL[2], Tavares AF[2], Uchoa MMA[2], Gonçalves DO[1], Tavares KR[1], Viana GSB[1] [1]UFC – Fisiologia e Farmacologia, [2]Estácio – FMJ

Introduction: *Myrocodroun urundeuva* Allemão (Anacardiacea family), is popularly used in Brazil as anti-inflammatory, mainly in genitourinary diseases. The anti-inflammatory, analgesic and antiulcer activity has been proved as well as a neuroprotective activity in an *in vitro* model of Parkinson disease (PD) (1-3). PD is characterized by locomotor commitments and selective degeneration of dopaminergic neurons in the substantia nigra (4). **Methods**: The standardized extract of *Myracrodruom urundeuva* (SEMU) (5,10 and 20 mg/kg,p.o), was tested in a experimental model of PD induced by a stereotaxic unilateral injection of 6-hidoxidopamine (6-OHDA). The toxin was injected, at two points, in the right striatum (12 µg/µl, each point, supplemented with acid ascorbic). Male Wistar rats (200 g) were divided in four groups (n=5), as follows: Sham operated (SO), lesioned group and 6-OHDA treated with SEMU (5 and 20 mg/kg) for 15 days. At the end of the treatments, the open field, for the evaluation of locomotor activity, and the rotational test induced by apomorphine (1mg/kg,s.c),for assessment of the brain 6-OHDA lesion, were performed and, hereafter, the animals were sacrificed and had their striata withdrawn for analysis of monoamines (DA and DOPAC, results in ng/mg tissue-right striatum) levels on HPLC. The results were expressed as means ± SEM. One-Way ANOVA and the Student Newman Keuls as the *post hoc* test were used and results considered significant at $p<0,05$. The study followed all standards rules for the use of laboratory animals and was approved by the committee on ethics in animal research (104/2011). **Results:** In the open field test none of the analyzed parameters, which included number of crossings, rearings and groomings, showed significant differences among the lesioned group before and after SEMU treatments or the sham operated group. In the rotational test, SEMU at the doses of 5,10 and 20 mg/kg reduced the number of contralateral rotation/h in a significant way (138,8±20,4; 104,4±26,73 and 33,8±6,46, respectively) as compared to the lesioned 6-OHDA group (188,4±15,13). The SO group did not show any rotation. The levels of dopamine increased significantly at lesioned group treated with the dose of 20mg/kg (1915,5±146,2) but not with the smaller doses as compared to 6-OHDA-lesioned group (763,2±94,49). Striatal DA concentrations in the SO group were 4186±572,3ng/mg tissue. The DOPAC levels did not show difference statistic between the groups. **Discussion**: At the used doses, the SEMU improved the observed parameters as related to the lesioned and untreated group indicating the potential of this medicinal species as an alternative treatment Parkinson disease. The mechanism of action by witch SEMU increased the DA concentrations in parkinsonian rats is not clear but could be, in partly, due to the anti-inflammatory and antioxidant activities of the drug as already observed by us. **References**: 1.Viana GSB. *Phytomedicine* 10: p. 189, 2003. 2.Sousa sm. *Phytother Res*. 21: p.220, 2007. 3.Nobre-Junior HV. *Neuroch Res* 34;p.1066, 2009. 4.Hirsch EC.J Neural Transm Suppl 65:p.89, 2003. **Financial support**: CNPq

02.039 Influence of neonatal handling on amphetamine-conditioned place preference of young rats. Antoniazzi CTD, Boufleur N, Dolci GS, Kuhn FT, Benvegnú DM, Pase CS, Roversi K, Roversi K, Dias VT, Bürger ME UFSM

Introduction: Adolescence is characterized by enhanced social cue salience, which can predispose to use and abuse of drugs. While behavioral changes are related to early life stress and includes an altered response to drugs, neonatal isolation (NI) has been used as an animal model of early life stress (Imanaka, A. Behav Brain Res. 186:91. 2008) in rats. Thus, conditioned place preference (CPP) has been widely used to assess rewarding effects of different substances of abuse (Tzschentke, T.M. Addict Biol. 12:227. 2007) such as amphetamine (AMPH). In rats, tactile stimulation (TS) is closely related to maternal liking and care in rats. Such TS acts as "enrichment" for the developing brain, which is favorable to face challenging experiences later in life (Lovic, V. *Pharm Biochem Behav*. 84:497. 2006). **Methods**: The experimental protocol was approved by Animal Ethical Committee (UFSM-106/2010), which is affiliated to the Council for Control of Animal Experiments (CONCEA). After delivery (PND1) of seven Wistar female rats, male pups from different litters were distributed into three experimental groups (n=7): unhandled (UH, not touched), TS and NI. Postnatal handling was applied from PND1 to 21 and consisted of: TS, in which pups were individually held and stroked with the index finger on the dorsal surface for 10min. NI pups were put in an individual plastic box lined with soft paper and warmed with an incandescent lamp for 10min. On PND22, pups were weaned and left undisturbed until PND40, when animals were submitted to CPP, which consisted in habituation for 15min at each chamber of the apparatus. At PND41 a pretest was done to determine initial preference with free access for 15min. From PND42 to 49, conditioning phase was conducted and all rats received AMPH (dl-AMPH 4mg/Kg i.p.). They were placed in opposite chamber of its preference for 25min. AMPH and saline (0.9%) were administered on alternating periods with 4h of interval. At PND50, rats were tested (similar to pretest) for CPP to AMPH and at PND53 they were tested again. Data were analyzed by one-way ANOVA followed by Duncan's test ($p<0.05$). **Results:** Results were expressed as mean±S.E.M. and values are presented as percentage of time spent in each chamber of apparatus. Results of CPP showed that in the test day UH (45.5±5.9), TS (42.5±4.9) and NI (38±3.8) showed no difference in time spent at the chamber opposite to that associated with AMPH, while the time spent at the chamber associated with drug was increased for NI (66.6±0.8) in relation to UH (50.3±5) and TS (52.6±2.5). At withdrawal test day (PND53), no difference of time spent at chamber opposite to those related to drug was observed in UH (41±4.2), TS (48.1±4.3) and NI (42.6±1.6). However, the time spent at the chamber associated with AMPH was lower in TS (46.2±1.8) when compared to UH (58.9±4.2) and NI (57.3±1.3). **Conclusion**: Our study showed that TS exerted beneficial effects on behavior parameters related to psychostimulant drugs on the CPP, while NI may facilitate the drug preference of young rats. Biochemical studies involving action mechanism should be performed. **Financial support**: FAPERGS/PRONEN-2010; PROAP/ PRPGP–UFSM. **Acknowledgements**: The authors are grateful to CAPES and CNPq by their fellowships.

02.040 Effect of creatine on spatial and non-spatial retention memory in rats. De Castro M, Souza MA, Gerbatin R, Busanello GL, Fiorin FS, Royes LF UFSM – Bioquímica do Exercício

Creatine (Cr) (*N*-[aminoiminomethyl]-*N*-methyl glycine) is a guanidine compound endogenously synthesized from glycine, arginine and S-adenosylmethionine in kidneys, liver, pancreas and the brain. Cr present neuroprotective effects in some models of neurodegenerative disease such as Alzheimer and Parkinson and improve memory in some cognitive tests. Recently we have demonstrated that Cr improve spatial memory in Barnes Maze test. The objective of this work was evaluated the effect of intrahippocampal Cr administration in an Object exploration task (OET) and on anxiolitic-like behavior. Adult male Wistar rats (270–300 g n= 289) were used in the present study. Rats were surgery implanted with two 27-guage guide cannulas, which were placed 1mm above the CA1 region of the dorsal hippocampus. One week after surgery, animals were trained to solve the OET paradigm (n=11-12) and elevated plus maze (n=7-8).

Statistical analysis showed that there was no difference in time of exploration between the objects 1 and 2 [$F(1,14)=0.17$; $P>0.05$] as well as the recognition index for object exploration [$F(1,14)=0.05$; $P>0.05$]. On the other hand, we showed that intrahippocampal administration of Cr (2.5 nmol/hippocampus) (post-training; session 2) improved the spatial version of OET [F (1, 14) =5.83; $P<0.05$]. In Elevated Plus Maze test, intrahippocampal Cr administration did not alter percent of time [$F(1,14)=0.04$; $P>0.05$] and percent of entries in open arm [$F(1,14)=0.517$; $P>0.05$]. In addition, statistical analysis revealed that intrahippocampal Cr administration did not alter percent of time [$F(1,14)=0.248$; $P>0.05$], entries on enclosed arm [$F(1,14)=0.485$; $P>0.05$;] and time spend on middle [$F(1,14)=1.740$; $P>0.05$;] indicating that this treatment had no effect on anxiety-like behavior. In the present study we demonstrate that Cr did not alter anxiety measures of the animals indicating that Cr affects spatial memory in OET task and does not alter the motivational aspects of learning. Supported Ethics Committee: Committee Ethics Universidade Federal de Santa Maia (115/2010). Work Supported by CNEPq and CAPES.

02.041 Influence of different fatty acids supplementation on the vulnerability of the 1st generation of rats to develop an animal model of mania. Trevizol F[1], Roversi K[2], Dias VT[2], Roversi K[2], Barcelos RCS[1], Benvegnú DM[1], Kuhn FT[1], Bürger ME[1]
[1]UFSM – Farmacologia, [2]UFSM – Fisiologia e Farmacologia

Introduction: Fatty acids (FA) are constituents of the neuronal membrane phospholipids and are fundamental to the development and functioning of the brain (Haag M., *Can J Psychiatr*, v.48 p.195, 2003; Yehuda S, Humana Press, p. 99, 2005). Since the peak of neuronal growth occurs during the last week of gestation and lactation period, a rapid accumulation of long chain fatty acid requires an abundant supply of essential fatty acids (EFA) during this period in order to contribute to normal fetal development and ensuring the development of neurological functions. In the last decades, changes in dietary habits in Western countries have been observed, mainly due to increased consumption of *trans* fatty acids (TFA) and omega-6 (n-6 FA) at the expense of omega-3 (n-3) (Pfeuffer M, *Internat Dairy J*, v.16 p.1383, 2006; Baggio SR, Food Chem, v.95 p.611, 2006). Such changes may result in an increase of oxidative damage affecting the neuronal plasticity; increase the vulnerability to neuropsychiatric diseases such as bipolar disorder (Hamazaki K, *J Psychiatr Res*, v.44(11) p.688, 2009). **Methods**: The experimental protocol was approved by the Animal Ethical Committee (UFSM) (23/2010), which is affiliated to the Council for Control of Animal Experiments (CONCEA). Wistar female rats were orally supplemented (3g/kg/day) with fish oil (FO, rich in n-3 FA), soybean oil (SO, rich in n-6 FA) and hydrogenated vegetable fat (HVF, rich in *trans* FA) from the pre-conception until adulthood of the litter (90 days), which received daily injections of amphetamine (AMPH -4 mg/kg/mL-ip) for the last week of oral supplementation. Locomotor activity in the open field was observed 2h after the last AMPH dose, which was followed by euthanasia (after thiopental anesthesia-50 mg/kg). Brain was removed to dissection of striatum and hippocampus for protein carbonyl (PC) (Levine RL, Meth Enzymol, v. 86 p.464, 1990) levels determination. Results and **Discussion**: TFA and SO groups showed higher locomotor activity than the FO. AMPH increased PC levels in hippocampus and striatum of SO and HVF-supplemented rats, while this was not observed in the FO group. FO supplementation was able to prevent the development of AMPH-induced hyperactivity as well as the striatal and hippocampal protein oxidative damages. Our results suggest that highest consumption of processed foods rich in TFA, saturated and n6-FA is able to interfere on the development of neuropsychiatric disorders as mania in the next generation, especially if this high consumption occurs during the gestational period and during development. Acknowledgment: Herbarium® Laboratório Botânico Ltda by FO capsules kindly donated. **Financial support**: FAPERGS; CNPq; CAPES.

02.042 Caffeine effects on antioxidant status and behavioral parameters altered by a pentylenetetrazol challenge. Busanello GL, Souza MA, Rodrigues FS, Gerbatin R, de Castro M, Fiorin FS, Scherer L, Royes LF UFSM – Métodos e Técnicas Desportivas

Introduction: Seizures are the main clinical manifestation of epilepsy, that is a neurological condition with incidence in 1% of word population. About 20 to 30% of patients do not respond to the treatment with classical antiepileptic drugs available. In this context, studies aiming find out therapeutic adjuvants, eg. caffeine supplementation, are required in the sense of attenuate seizures. Caffeine is derived from metylxantine, a compound that has shown neuroprotector effect in some situations, eg. Parkinson and Alzheimer disease, sleep disturbances and aging. However, the effects of caffeine in PTZ-induced seizures (GABAergic antagonist) still controversial. The objective of this research was evaluate the effects of chronic administration of caffeine (3mg/kg) on PTZ-induced seizures and oxidative damage in rats. **Methods:** Male Wistar rats (250 – 300 g) were used. PTZ i.p (60mg/kg) was administrated and animals were observed for twenty minutes for appearance of seizures characterized by myoclonic jerks and generalized tonic-clonic seizures. After death by decapitation, cortex was removed and Na^+,K^+-ATPase activity, DCFH-DA oxidation and SH content was measured. **RESULTS:** Caffeine administration decreases PTZ-induced time spent on generalized tonic-clonic seizures [U=7,5; p< 0,05], but does not change latencies to myoclonic jerks and generalized tonic-clonic seizures. Furthermore, PTZ induced Na^+,K^+-ATPase activity inhibition [F(1,27)= 8,87 *p*< 0,05] and caffeine treatment prevents this effect [F(1,27)= 8,87 *p*< 0,05]. PTZ also increases DCFH-DA oxidation [F(1,27)= 12,4,87 *p*< 0,05], a marker of reactive species generation, but caffeine was not able to attenuate this effect. However, caffeine increases SH levels per se [F(1,27)= 7,00 *p*< 0,05], while PTZ reduces basal levels of SH [F(1,27)= 6,22 *p*< 0,05]. **Discussion:** The present results show that low doses of caffeine chronically administrated attenuated PTZ-induced seizures and protected from Na^+, K^+-ATPase activity inhibition. Furthermore, caffeine treatment increased the levels of non-proteic sulfhydryl groups. However, caffeine have no effect on the production of mitochondrial reactive species. Together, these results indicate possible protective effect of caffeine PTZ-induced seizures, which can be associated to levels of non-proteic sulfhydryl groups and maintenance of Na^+, K^+-ATPase activity. Ethic Committee: This work was approved by ethic committee Federal University Santa Maria (115/2010). Sources of Research Support: CAPES-CNPq

02.043 Extinction of fear memory: **on the participation of different neuromodulatory systems in the hippocampus**, **basolateral amygdala and ventromedial prefrontal cortex**. Fiorenza NG[1,2], Rosa J[2], Izquierdo I[1], Myskiw JC[4,1]
[1]INNT-PUCRS, [2]UFRGS – Medicine and Health Sciences, PUCRS, [4]IGG – PUCRS

Introduction: Extinction memory consists of the inhibition of retrieval of a previously acquired memory. The CA1 region of dorsal hippocampus (CA1), basolateral amygdala (BLA) and ventromedial prefontral cortex (vmPFC) participate in the extinction of contextual fear conditioning (CFC). Here, we studied the effect of drugs acting on receptors involved modulation of extinction in this task. **Methods:** The present study was approved by The Ethics Committee on Animal Use (CEUA 11/00262), PUCRS, Brazil. Naïve male Wistar rats (300-330 g) underwent stereotaxic surgery for the implantation of guide cannulae bilaterally aimed to the CA1, the BLA and the vmCPF. After having recovered from surgery, these animals were trained in a task of CFC (2 shocks 0,5 mA for 2 sec). Twenty four hours thereafter, they were re-exposed CFC box's in the absence of reinforcement, which consisted on the extinction session. The drugs were given bilaterally into the mentioned areas immediately after the first extinction session of task. The doses used are known to influence memory consolidation of the original task. Their effects were evaluated on a second extinction session 24 h later, and assumed to result from influences on the consolidation of extinction. The CFC extinction data were analyzed by student t test for $p \leq 0.05$. **Results:** When given bilaterally into the CA1, AP5 (5 µg/side), a NMDA-antagonist, ($t=2.3$, $p<0.05$, $n=10$), ranitine (17.5 µg/side), a H2-histaminergic antagonist ($t=3.2$, $p<0.05$, $n=11$) and SCH 23390 (1.5 µg/side), D1 antagonist receptor, ($t=4.2$, $p<0.05$, $n=11$) impaired the extinction of CFC. Extinction improved by D-serine (50 µg/side), a NMDA receptor stimulant, ($t=2.2$, $p<0.05$, $n=11$), by SKF38393 (12.5 µg/side), D1 agonist receptor ($t=2.1$, $p<0.05$, $n=11$) and by SKF91488 (12.5 µg/side), a histamine methyl-transferase inhibitor, ($t=2.6$, $p<0.05$, $n=11$). When given bilaterally into the BLA, AP5 ($t=2.2$, $p<0.05$, $n=12$) and ranitidine ($t=2.9$, $p<0.05$, $n=12$) impaired, and D-serine ($t=1.9$, $p=0.05$, $n=13$), timolol (1 µg/side), a β-adrenergic antagonist, ($t=2.5$, $p<0.05$, $n=11$) and SKF91488 ($t=2.6$, $p<0.05$, $n=12$) enhanced extinction. When given bilaterally into the vmPFC, AP5 ($t=1.9$, $p=0.05$, $n=10$), ranitidine ($t=2.4$, $p<0.05$, $n=11$) and norepinephrine (1 µg/side), a β-adrenergic agonist, ($t=2.5$, $p<0.05$, $n=11$) impaired extinction of the CFC task. D-serine ($t=2.4$, $p<0.05$, $n=10$), SKF91488 ($t=2.3$, $p<0.05$, $n=10$) and timolol ($t=2.0$, $p=0.05$, $n=10$) enhanced extinction. **Discussion:** The present study demonstrates that extinction of CFC is modulated by various systems, which bears upon the behavioral and pharmacological treatment of fear-motivated brain disorders. **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional Científico e Tecnológico (CNPq).

02.044 Beta-amyloid peptide modulates NOS and Na,K-ATPase activities in rat hippocampus. Vasconcelos AR[1], Lima LS[1], Böhmer AE[1], Andreotti DZ[1], Yshii LM[1], Russo LC[2], Ferro ES[2], Munhoz CD[1], Scavone C[3], Kawamoto EM[4] [1]ICB-USP – Pharmacology, [2]ICB-USP – Cell and Developmental Biology, [3]ICB-USP, [4]ICB-USP – Pharmacology –Neurosciences / NIA

Introduction: The neuritic plaque in the brain of Alzheimer's disease patients consists of an amyloid composed primarily of Beta-amyloid (Ab), a peptide that plays a key role in the pathogenesis of the disease. Ab has been shown to cause synaptic dysfunction and can render neurons vulnerable to excitotoxicity and oxidative stress. Nitric oxide synthase (NOS) is an enzyme which has been linked to both survival and apoptosis. Sodium, potassium pump (Na,K-ATPase) plays an important role to maintain cell ionic equilibrium. Disruption of NOS and Na,K-ATPase activities could lead to oxidative stress process which could be detrimental to the cells. Our aim was to evaluate the signaling pathways of Ab in relation to N-Methyl-D-aspartate (NMDA)-NOS-cyclic GMP pathway and Na,K-ATPase activity in rat hippocampal slices. **Methods**: 4-month-old male Wistar rats hippocampi were dissected and immediately sliced. The samples were pre-treated with L-NAME (NOS inhibitor, 100 μM) or MK-801 (NMDA receptor antagonist, 1 μM) for 15 minutes to study the participation of NOS and NMDA receptor in the effects mediated by Ab. Then, samples were incubated with Aβ (200 nM and 2 μM) for 1 hour. NOS and Na,K-ATPase activities were performed to measure Ab effects in this preparation and MTT reduction assay was used to evaluate tissue viability. This research was approved by the Biomedical College of Animal Experimentation (COBEA). All procedures were also approved by the Ethical Committee for Animal Research (CEEA) of the Biomedical Sciences Institute of the University of São Paulo (protocol 89, page 60, book 02). Results and **Discussion**: Ab induced dose dependent activation of NOS activity in rat hippocampal slices which was completely blocked by MK-801 and L-NAME pre-treatment. The Ab treatment also decreased Na,K-ATPase activity which was reverted by L-NAME but not MK-801 pre-treatment. The decrease in enzyme activity induced by Ab was isoform specific since only α_1-Na,K-ATPase was affected. MK-801 and L-NAME pre-treatment in the presence or absence of Ab did not cause cell death based on MTT reduction assay. Taken together, these findings suggest that the activation of NMDA-NOS signaling cascade linked to $\alpha_{2/3}$-Na,K-ATPase activities may mediate an adaptive, neuroprotective response to Ab in rat hippocampus. **Financial support**: CNPq /FAPESP (Brazil)