02. Neuropharmacology

02.001

TRPA1 receptor: a novel approach in Alzheimer's disease. Bicca MA[1], Santos ECS[1], Loch-Neckel G[1], Leal PC[2], Calixto JB[1] [1]UFSC – Farmacologia, [2]UFSC – Química

Alzheimer's disease (AD) is a neurodegenerative and progressive disease for which there is no current cure or efficient treatment. The search for new targets that could be useful models for drug development is indispensable. Transient Receptor Potential Ankyrin 1 (TRPA1) arises as a novelty approach, first considered by our research group to be related to AD due to the fact endogenous molecules that activate this receptor: Reactive Oxygen Species (ROS), Ca^{2+} and products of inflammation are also up-regulated during the initiation and progression of AD. We aimed to investigate the possible role of TRPA1 in experimental models of AD. In order do to that, we used primary neuronal cell culture from cortex (CT) and hippocampus (HP) of rats (E18) cultivated with supplemented neurobasal media at 37°C and 5% CO_2 in an incubator. Cells were treated with TRPA1 antagonist (HC030031) 0.03 µM and Aβ oligomers 1-40 (AβOs) 10 µM, and respective vehicles, for further assessment of ROS and neuronal death. Also, *Swiss* mice (3 months-old) were injected i.c.v. with AβOs (400 pmol) and brains were collected after 6h, 24h, 7 days and 14 days to analyze TRPA1 expression by immunohistochemistry and western blot. Independently, animals were pre-treated i.c.v with HC030031 (10 µg/site), 30 min prior to AβOs injections or 14 days after AβOs injections they were treated orally, once a day, during 14 days with HC030031 (20 mg/kg). After treatments, behavioral analyses were performed using the object recognition (OR) task to assess spatial memory (UFSC Ethics Committee: PP00625/2011). Results showed that cells treated with AβOs presented high levels of ROS formation and consequently neuronal death. Outstandingly, TRPA1 blockage with HC030031 prevented AβOs-induced toxicity alterations in cells, comparable to control levels. Notably, when we evaluated TRPA1 expression in mice CT and HP, we observed low levels of basal expression followed by slightly augmented expression after 6h, higher expression after 24h and still elevated expression after 7 days, returning to expression similar to 6h within 14 days after AβOs-treatment. Immunohistochemical data showed TRPA1 staining surrounding the neuronal cell body and in the microglia, while western blot results confirmed quantifications. To answer robustly if TRPA1 activation is required for AβOs-toxicity, as we have seen *in vitro*, we tested the efficacy of HC030031 *in vivo.* Relevantly, HC030031 pre-treatment prevented AβOs-induced memory deficits, comparable to controls behavior. Besides, another group of AβOs-injected mice showed in the OR after 14 days memory deficits, and then were treated with HC030031 or vehicle for more 14 days and re-analyzed in OR. Of note, HC030031 treatment reversed AβOs-induced memory deficit when compared to vehicle-treated mice, showing a behavior similar to the controls, suggesting the inhibition of TRPA1 as a potential treatment for AD. Summarizing, we are reporting for the first time the significant role of TRPA1 in AD related experimental models. Mostly, the relevance of the receptor activation to ROS formation, cell death and memory impairment induced by AβOs. Our data suggests, from cell biology to animals, TRPA1 as a new and attractive target for the AD therapeutics. Authors thank CNPq, Capes and FAPESC for the financial support.

02.002

Effect of aflatoxin B1 on EEG recordings and Na^+, K^+-ATPase activity after pentylenetetrazol administration in rats Braga ACM[1], Trombetta F[1], Poersch AB[1], Schiefelbein N[2], Lima C[1], Ribeiro LR[1], Furian AF[1] [1]UFSM – Fisiologia e Farmacologia, [2]UFSM – Ciência e Tecnologia dos Alimentos

Introduction: Aflatoxin B1 (AFB1) is a mycotoxin produced by species of *Aspergillus flavus*, *A. Parasiticus* and *A. nomius*, found in cereals, mainly in corn and peanut [1]. This toxin causes cytotoxic effects including hepatic damage, oxidative stress and induces a discoloration of this organ, due to fat accumulation [2]. Some studies report that AFB1 alters specific peripheral and central nervous system neuronal ATPases. In brain, the activity of the enzyme Na^+, K^+-ATPase is essential for maintaining the electrochemical gradient, maintenance of the resting potential and the release and uptake of neurotransmitters and it is known that repeated exposure (chronic) of rats to AFB1 also activated this enzyme [3]. The aim of this study was evaluate the acute effect of AFB1 on seizures and animal behavior, through electroencephalographic (EEG) records and Na^+, K^+-ATPase after pentylenetetrazol (PTZ) administration. **Methods**: 24 male Wistar rats were kept under constant temperature and 12h light/dark cycle. For EEG analysis, rats were anesthetized and two electrodes were placed over the parietal cortex, along with a ground lead positioned over the nasal sinus. The electrodes were connected and fixed to the skull. Antibiotic and analgesic were given to all rats before and after surgery, respectively. Experiments were performed 3 days after surgery and rats were divided into four groups: DMSO and NaCl (control), DMSO and PTZ (30 mg/kg i.p.) (PTZ group), AFB1 (250 µg/kg v.o.) and NaCl (AFB1 group) and AFB1 and PTZ (AFB1-PTZ group). EEG was recorded for 1 hour after AFB1 administration, and then PTZ was administrated and recorded for another 15 minutes. After EEG, animals were killed and cerebral cortex and hippocampus were quickly removed and homogenized for Na^+, K^+-ATPase activity measurement, according to Wyse *et al.* [4]. This project was approved by the Committee on Care and Use of Experimental Animal Resources (process #0048/2013) of the UFSM, Brazil. **Results**: It was observed increased frequency of seizures in EEG records of group AFB1-PTZ when compared to group PTZ and AFB1. In addition, decreased Na^+, K^+-ATPase activity has been observed a after a single administration of AFB1. **Discussion**: EEG recording showed an increase in seizure frequency of group AFB1 + PTZ compared to PTZ group that may be related to other unknown mechanisms of neurotoxicity of AFB1. The reduction of Na^+, K^+-ATPase activity in acute treatment is different in the chronic treatment, where this activity increases [3]. So, acute administration of AFB1 interferes in the PTZ seizure model, but more studies are necessary to give basement to this observation. **Financial Support**: CNPq, FAPERGS, Capes. **References**: [1] Sirajudeen, M. *et al.*, *Environ Toxicol*, v.26, p.153; 2009 [2] Amaya-Farfan, *J Lancet* v.353: p747; 1999 [3] Ikegwuonu, I. F., *Toxicol*, v.28, p.247; 1983 [4] Wyse, A. *et al.*, *Physiol Rev*, v.80, p.1107; 2000

02.003

Chronic *trans* fat consumption facilitate the development of hyperactive behavior. Pase CS, Roversi Kr, Trevizol F, Kuhn FT, Burger ME UFSM

Introduction: Attention-deficit hyperactivity disorder (ADHD) is a serious neuropsychiatric condition (Nigg JT, J Child Psychol Psychiatry 51:58, 2010). Studies have investigated the association between reduced intake of n-3 PUFA and inattention and hyperactivity (Appleton KM, *Nutr Res Rev* 21:13, 2008). In recent decades, the increased consumption of processed foods rich in hydrogenated vegetable fat, has led to a decreased consumption of omega-3 fatty acids and this eating habit provides an increased intake of *trans* fatty acids, which may be related to neuropsychiatric conditions. In this study, we evaluated the potential connection between prolonged *trans* fat consumption and development of hyperactivity-like symptoms in rats. **Methods**: *Experiment 1*: After weaning, rats were assigned to two groups (n=10) and supplemented with 20% soybean oil (SO) or hydrogenated vegetable fat (HVF) incorporated to standard chow. Both experimental groups were submitted to behavioral observations in the forced swimming task (FST) from ten months of dietary consumption. *Experiment 2*: After weaning, rats were assigned to two groups (n=16): 0.1% SO and 0.1% HVF incorporated to tap water. After 8 weeks, half of each group (n=8) received a single daily injection of amphetamine (AMPH 4 mg/kg/ ip) for 8 consecutive days. After the last AMPH administration, the locomotor activity was evaluated in the open field task (OFT). *Experiment 3*: Female Wistar rats were supplemented (3 g/kg; p.o.) with SO or HVF and maintained under the same supplementation during pregnancy and lactation. After weaning, male pup (n=8) was grouped and kept under the same oral treatment until 40 or 90 days of age. At both these times, rats received a single daily injection of AMPH (4 mg/kg/ip) for 8 days and submitted to behavioral observations in OFT. *Experiment 4*: Female Wistar rats were supplemented (3 g/kg; p.o.) with SO or HVF and maintained under the same supplementation until second generation. At day 41, male rats were exposed to the acute restrain stress (AS) procedure and submitted to behavioral assessments. All the experimental protocols were approved by the Animal Ethical Committee (24/2010; 23/2010; 004/2012). **Results**: *Trans* fat intake for 10 months (Experiment 1: 12.88 ± 3.9; 233 ± 6.90; 54.11 ± 8.20), as well as during pregnancy and lactation across two sequential generations of rats, (Experiment 4: 20.1 ± 3.09; 276.33 ± 3.43; 1.58 ± 0.45) induced immobility, climbing and swimming time, respectively, in relation of SO group (Experiment 1: 37.33 ± 8.17; 191.88 ± 8.49; 76.66 ± 10.46 and Experiment 4: 76.9 ± 13.8; 191.08 ± 14.4; 21.5 ± 2.59) on the FST. HVF supplementation was associated with increased locomotion before and after AMPH administration (Experiment 2: 28.42 ± 2.40; 73.87 ± 4.60, respectively) in relation of SO group (13.25 ± 2.76; 51.2 ± 6.48). Similarly, HVF supplementation during pregnancy and lactation were associated with increased locomotion in both young and adult rats (Experiment 3: 70.5 ± 7.68; 63.66 ± 4.00) compared to SO group (48.00 ± 6.42; 41.12 ± 3.27, respectively). Furthermore, *trans* fat intake across two sequential generations increased locomotor and exploratory activities following stressors (Experiment 4: 71.60 ± 6.09; 28.00 ± 4.35, respectively) in comparison of SO group (35.80 ± 5.4; 9.8 ± 0.58). **Discussion**: *Trans* fat consumption from weaning until adulthood and across two generations was found to be associated with increased locomotor activity, impulsiveness and agitation behavior. These data are all the more valuable given the current scarcity of research into the effect of TFA-rich diets on behavioral problems. Our findings open up an exciting perspective for understanding the role of *trans* fat consumption on hyperactivity-like symptoms, which

may indicate a predictability to ADHD development. The authors are grateful to CNPq, Capes and FAPERGS for financial support.

02.004

N-acetilcysteine protects the rat brain against aspartame-induced oxidative stress. Finamor IA[1], Pês TS[1], Ourique GM[1], Londero EP[1], Scheid T[2], Llesuy SF[3], Partata WA[2], Pavanato MA[1] [1]UFSM, [2]UFRGS, [3]UBA

Introduction: Aspartame is metabolized to aspartate, phenylalanine, and methanol (Ranney *et al.* 1976). Its long-term intake at the acceptable daily ingestion dose causes oxidative stress in the rodent brain through the dysregulation of glutathione (GSH) homeostasis (Ruiz *et al.* 2008, Mourad and Noor 2011, Abdel-Salam *et al.* 2012, Iyyaswamy and Rathinasamy 2012, Abhilash *et al.* 2013). N-acetylcysteine provides the cystein required for the production of GSH, being effective in treating disorders associated with oxidative stress (Samuni *et al.* 2013). The aim of this research was to investigate the effects of N-acetylcysteine on the aspartame-induced oxidative stress in the rat brain. **Methods**: The animals received aspartame by gavage for six weeks (40 mg/kg). From the 5th week, N-acetylcysteine (150 mg/kg, via intraperitoneal) was injected for two weeks. Then, they were anaesthetized for blood sample and euthanized for the brain collection. The blood was centrifuged and the serum was separated for glucose measurement. The tissue was homogenized in 1.15% KCl buffer and centrifuged for obtaining the supernatant, which was used to the measurements of oxidative stress biomarkers. All procedures were approved by the Ethics Committee of the UFSM (#020/2012). **Results and Discussion**: Even after N-acetylcysteine administration, glucose levels remained increased in the serum of aspartame-treated rats, indicating that the increase in its levels was not triggered by the aspartame-induced oxidative stress. N-acetylcysteine led to a reduction in the thiobarbituric acid reactive substances and lipid hydroperoxides levels, which were increased in the rat brain after aspartame exposure. Additionally, N-acetylcysteine caused an elevation in the glutathione peroxidase (GPx) and glutathione reductase (GR) activities; non-protein thiols and total reactive antioxidant potential (TRAP) levels, which were decreased in the rat brain after aspartame exposure. N-acetylcysteine treatment prevented all aspartame-induced oxidative injuries perhaps due to its reaction with reactive oxygen species and formaldehyde, diminishing their toxicity (Farbiszewski *et al.* 2000). Furthermore, N-acetylcysteine serves as a precursor of L-cysteine for GSH synthesis (Samuni *et al.* 2013). Hence, we believe that N-acetylcysteine acted as a protective compound in the brain of aspartame-treated rats through the GSH production, and thereby re-establishing GPx and GR activities, and TRAP levels. In conclusion, N-acetylcysteine may be useful for the protection of the rat brain against aspartame-induced lipid peroxidation and dysregulation of GSH metabolism. **Acknowledgements**: The authors are grateful for the financial support of the Comissão de Aperfeiçoamento de Pessoal de Nível Superior and to the Fundo de Incentivo a Pesquisa da UFSM. **References**: Abdel-Salam OME, *Eur Rev Med Pharmacol Sci* 16:2092, 2012. Abhilash M, *Drug Chem Toxicol* 36:135, 2013. Farbiszewski R, *Toxicology* 156:47, 2000. Iyyaswamy A, *J Biosci* 37:679, 2012. Mourad IM, *Int J Pharm Biomed Sci* 2:4, 2011. Ranney RE, J *Toxicol Environ Health* 2:441, 1976. Ruiz NAL, *Arch Neurocien* (Mex) 13:79, 2008. Samuni Y, *Biochim Biophys Acta* 1830:4117, 2013.

02.005

Intrahippocampal infusion of spermidine improves memory persistence: Involvement of protein kinases A and C. Gais MA, Signor C, Girardi BA, Porto GP, Muller M, Rubin M, Mello CF UFSM

Spermidine (SPD) is an endogenous aliphatic amine with polycationic structure that modulates NMDA receptor activity and improves memory. cAMP dependent protein kinase (PKA) and calcium-dependent protein kinase (PKC) play a role in SPD-induced improvement of memory. Recent evidence suggests that systemic administration of spermidine improves the persistence of the long term memory of fear (Signor *et al*, *Eur J Pharmacol*. 730:72, 2014). In the current study we investigated whether the intrahippocampal (ih) injection of spermidine or arcaine, respectively agonist and antagonist of polyamine binding site at NMDA receptor, alters the persistence of the memory of contextual fear conditioning task in rats. We also investigated whether protein synthesis, PKA and PKC play a role in SPD-induced improvement of the fear memory persistence. Adult male Wistar rats, from the animal house of the UFSM, were anaesthetized and received two cannulae placed 1 mm above the CA1 region of the dorsal hippocampus. Five days after, the animals were submitted to the contextual fear conditioning training according to Signor *et al*, (2014) (*Eur J Pharmacol* 730:72, 2014). Twelve hours after training the animals received the ih injections of the drugs. Two or seven days after training, each rat was placed back in the conditioning chamber and the test session was performed. While 12 h post-training injection of spermidine (2 nmol/site, ih) facilitated, arcaine (2 nmol/site, ih) impaired the memory of fear assessed 7 days after training. Arcaine (0.2 nmol/site, ih) prevented the facilitatory effect of spermidine (2 nmol/site, ih) on memory persistence measured 7 days after training. The injection of the protein synthesis inhibitor anisomycin (20 µg/site, ih), the PKA inhibitor H-89 (10 pmol/site, ih), or the PKC inhibitor GF 109203X (1 pmol/site, ih), prevented the memory improvement induced by SPD (2 nmol/site). However, both spermidine and arcaine did not alter fear memory responses when testing was carried out 2 days after training. These results suggest that the improvement of fear memory persistence induced by the delayed (12 hours post-training) intrahippocampal injection of spermidine involves protein synthesis, PKA and PKC pathways, in rats. The experiments were performed with the approval of the Ethics Committee of the UFSM (process number 068/2011). **Financial agencies**: CNPq, FAPERGS, Capes, PRPGP-UFSM.

02.006

Neuromotor impairment and anxiogenic effects in adolescent and adult female rats treated with ethanol following a binge drinking pattern. Fernandes LMP, Santana LNS, Lopes KS, Silva ML, Luz DA, Barros MA, Barros MA, Fontes-Júnior EA, Lima RR, Maia CSF ICS-UFPA

Introduction: Episodic heavy drinking, also known as binge drinking (BD), it is the consumption of high doses of ethanol (EtOH) in a short period of time, followed by an abstinence period. Therefore, the neurotoxic effects of this drinking habit are especially dangerous. In Brazil, binge drinking has increased among the female population, especially the youngest, with consumption frequency of three times a week. Thereby, the aim of this study was to investigate the neuromotor alterations and anxiety pattern caused by EtOH binge drinking in adolescent and adult female rats. **Methods**: Female *Wistar* rats (n=80), 35-days-old, were administered, through gavage, EtOH or distilled water (3g/kg/day) for three consecutive days for one week (E1/C1), four weeks (E4/C4), eight weeks (E8/C8), and for eight weeks followed by 14 days of abstinence (E8Abs/C8Abs); the animals were weighted before every week the administration. After seven and a half hours of the last administration, the animals were submitted to a battery of behavioral assessment which included the *elevated plus maze* (EPM), *pole test* (PT), *beam walking test* (BWT) using quadrangular beams (QB) of 28, 17 and 5cm. Student's t-test considering $p<0,05$ as the statistical significance level was performed. All procedures were approved by the CEPAE- UFPA under license number BIO196-14. **Results**: Change in weight gain was noticed through the third to the seventh week. For the EPM, E1 did not show changes in the number of entries in the closed arm, while E4, E8, and E8Abs presented increased, decreased, and decreased number on entries respectively. On the other hand, there was a decrease in the entries in the opened arm for all groups E1, E4, E8, and E8Abs. In the PT, all groups demonstrate an increase on descent time to the base of platform: E1, E4, E8, and E8Abs. Regarding BWT, E1 and E4 presented increase in escape time and number of slip on QB5, while E8 showed an increase on the same parameters on QB28, 17 and 5. **Discussion**: The animals did not present weight difference in the first two weeks; however, the intoxicated animals presented reduced weight gain during adolescence when compared to the control groups through the third to the seventh week. In the eighth week the intoxicated and control animals presented similar weight, as they both reached adulthood. All groups receiving BD presented anxiety-like behavior even when they experienced abstinence. The spontaneous locomotor activity (SLA) was altered starting on the fourth week, where four binges increased SLA in the adolescent animals, which was subsequently reduced in their adulthood, both under treatment and abstinence. Ethanols also lead the animals to present hypokinetic movements from adolescence to adulthood, which was not normalized after 14 days of abstinence. Nonetheless, the intoxication protocol used impaired the fine motor coordination in adolescence. In addition, it also caused damage to the overall motor coordination after BD chronic in the adult phase, from which the animals were able to recover after 14 days of abstinence. Thus, the BD causes neuromotor impairment and anxiogenic effects in female rats in adolescence to adulthood without full recovery after period of abstinence. **Financial Support**: CNPq and Fapesp.

02.007

Pharmacological Evaluation about the derivate pyrimidinone 4A in the Central Nervous System in a classical model of anxiety. Aquino CQA[1], dos Anjos JV[2], Carvalho MS[3], Gavioli EC[3], Soares Rachetti VP[3] [1]UFRN – Biofísica e Farmacologia, [2]UFPE – Química Fundamental, [3]UFRN – Biofísica e Farmacologia

Introduction: The search for new neuroactive drugs, more specifically, with the anxiolytic action, it has become intense at last years. The synthesis of compounds pyrimidinone has gained prominence due to their high biological relevance[1],[2],[3]. Several pharmacological activities have been described in the literature relating to pyrimidinone heterocyclic derivatives, such as antiviral, antitumor, anti-inflammatory, antihistaminic activities, interferon induces, hypotensive, antipsychotics, among other that are given to the pyrimidinone center[1],[2],[3]. Such importance is gained by these molecules being direct connected to an acid core.[1] Under these perspective, our study proposes to evaluate the actions of the pyrimidinone derivative 4A (series 3,4-dihydro-2,6-diaryl-4-oxo-pyrimidine- 5-carbonitriles), at Central nervous system, such as possible candidates for anxiolytic drugs. **Methodology**: The project has been approved by the Ethics Commission on Animal Use, the Federal University of Rio Grande do Norte, under Protocol No. 022/2013 – CEUA / UFRN. It was used Swiss female mice, weighing 30 to 35g, divided in 5 groups from 12 animals each, distributed as follows: Group I (compound 4A 3 mg), Group II (compound 4A 5 mg), Group III (compound 4A 10 mg), Group IV (Diazepam) and Group V (saline of 0.9%). The animals were immobilized and manually testes drugs, and about control it were administered intraperitoneal 1 hour before its testing behavioral assessment. For the evaluation of anxiety model, the animals were placed individually in High Plus Maze (HPM) and monitored by a camera through the software ANY MAZE, using a period of 5 minutes each. It was analyzed some motors changes by using measurements at how many entries in open and closed arms, time spent in open and closed arms, and total distance traveled in the open and closed arms. After treatment the animals were euthanized. The results were analyzed by ANOVA ate one-way, followed by Turkey test Psst Hoc, assuming values of significance in this study ($p<0.05$). **Results**: The treatment with doses of 3, 5 and 10 mg of Compound 4A did not give any anxiolytic action when compared to Diazepam; the common anxiolytic drug and to the negative control physiological solution 0.9%. In the evaluation parameter %Time in open arms the average values were 36, 844; 21.315; 10,599; 55.031; 42.285, for the doses of 3, 5 and 10 mg, Diazepam and Physiological Solution 0.9%, respectively. For the% Time in closed arms, average values were 51.381; 58.962; 74.472; 33.410; 47.930 for doses of 3, 5 and 10 mg, Diazepam and Solution Physiological 0.9%, respectively. For the %entrance to the open arms, average values were 55.975; 44.225; 36.407; 61.872; 53.210 for doses of 3, 5 and 10 mg Diazepam Solution Physiological and 0.9%, respectively. For the %Entrance in the closed arms average values were 44.025; 55.775; 63.593; 38.128; 46.790 for doses of 3, 5 and 10 mg Diazepam Solution Physiological and 0.9%, respectively. And, for the evaluation of total distance traveled the values from the calculated average was 0.596; 0.994; 0.747; 1,169; 0.740 for doses of 3, 5 and 10 mg Diazepam Solution Physiological and 0.9%, respectively. **Conclusion**: All tested doses of 3, 5 and 10 mg, the acute treatment did not show anxiolytic action, considering these doses one anxiogenic action. The study of the mechanisms of action of the molecule must be noted into account, since that the derivative should take the anxiogenic function control for new experiments in models of induced of the anxiety in experiments *in live.* **Financial Agency**: Capes, CNPq. **Acknowledgement**: PROPESQ/UFRN; Department of Biophysics and Pharmacology/UFRN; Laboratory of Behavioral Pharmacology/UFRN.

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02.008

Spermidine-induced improvement of reconsolidation of memory involves calcium-dependent protein kinase in rats Girardi BA[1], Signor C[2], Muller M[1], Gais MA[1], Schoffer AP[1], Rubin MA[3] [1]UFSM – Farmacologia, [2]UFSM – Bioquímica, [3]UFSM – Farmacologia/Bioquímica

When consolidated memories are reactivated, they become labile and, to persist, must undergo a new stabilization process called reconsolidation. During reactivation, memory is susceptible to pharmacological interventions that may improve or impair it. Intrahippocampal (i.h.) infusion of the protein synthesis inhibitor, anisomycin, shortly after reactivation, prevents reconsolidation of memory. The same treatment with anisomycin, in the absence of memory reactivation, left memory intact. This shows that consolidated fear memories, when reactivated, return to a labile state that requires de novo protein synthesis for reconsolidation. Spermidine (SPD) is an endogenous aliphatic amine with polycationic structure that modulates NMDA receptor activity and improves memory consolidation. Recent evidence suggests that the calcium-dependent protein kinase (PKC) signaling pathway is involved in SPD-induced facilitation of memory consolidation. Other study suggests that systemic administration of polyamine binding site ligands modulate memory reconsolidation. However, no study has addressed whether PKC cascade is involved in the reconsolidation of memory improvement induced by SPD. Thus, in the present study, we investigated whether PKC signaling pathway is activated by SPD in rats. Male Wistar rats were trained in a fear conditioning apparatus using a 0.4 mA footshock as unconditioned stimulus. Twenty four hours later the animals were re-exposed to the apparatus in the absence of shock (reactivation session). Immediately after the reactivation session, PBS, spermidine (2, 20, 200 pmol/site), PKC inhibitor 3-[1-(dimethylamino propyl) indol-3-yl]-4-(indol-3-yl) maleimide hydrochloride, GF 109203X (0.3, 1, 10 e 30 pg/site) or anisomicyn (0.2, 2, 20 pg/site), were injected, (i.h.), and the animals were tested in the same apparatus twenty four hours later. Freezing scores at testing were considered a measure of memory. While spermidine (20 and 200 pg/site) improved, GF 109203X (1, 10 e 30 pg/site) and anisomicyn (20 pg/site) impaired memory reconsolidation. These drugs had no effect on memory when administered in the absence of reactivation. The post-reactivation administration of the PKC inhibitor, GF 109203X (0.3 pg, i.h.) with SPD (200 pmol, i.h.) prevented memory reconsolidation improvement induced by SPD. These results suggest that the improvement of reconsolidation of memory induced by the i.h. administration of SPD involves the PKC in rats. The experiments were performed with the approval of the Ethics Committee of the UFSM (process number 068/2011). Financial agencies: CNPq, FAPERGS, Capes, PRPGP-UFSM.

02.009

Effects of 5-HT3 receptors antagonist ondansetron on anxiety-like behaviour generated by withdrawal from alcohol. Freire BTS[1], Silva CMV[1], Gavioli EC[1], Soares-Rachetti VP[1]
UFRN – Biofísica e Farmacologia

Introduction: The involvement of 5-HT3 receptors in the modulation of anxiety and drug abuse has been previously reported in the literature. Ondansetron is a selective antagonist of 5-HT3 receptors clinically used as antiemetic. Over the past years, this drug has been demonstrating a role in the treatment of other disorders, such as anxiety disorders and drug addiction. Therefore, the aim of this study was to test the hypothesis that 5-HT3 receptors modulate anxiety-related behavioral responses, including those generated by the long-term withdrawal from alcohol, in female rats. **Methods**: Female *Wistar* rats at 90 days old were administered (p.o.) with saline or ondansetron at doses of 10 µg/kg/mL, 100 µg/kg/mL and 1000 µg/kg/mL. In experiment one, animals were submitted to the elevated plus maze test (EPM) sixty minutes after administration of the drug to obtain the dose-response curve. In the experiment two, the animals were submitted to a protocol of chronic alcohol consumption (2% for 3 days, 4% for 3 days and 6% for 15 days), and after 21 days of abstinence, they were exposed to EPM sixty minutes after administration of an ineffective dose of ondansetron. All experiments performed have the approval of the *Ethics Committee on Animal* Use of UFRN, protocol number 056/2012. **Results and discussion**: The dose of ondansetron of 100µg/kg/mL increased the exploration of the open arms of the apparatus [(mean ± SEM of % time on open arms) – control: 25.24 ± 5.49; ond10µg: 32.94 ± 5.45; ond100µg: 45.04 ± 5.19 and ond1000 µg: 23.72 ± 6.20, $F(3,52) = 3.10$; $p= 0.03$, ANOVA], when compared to control group, suggesting an anxiolytic-like effect. Onsansetron in the ineffective dose of 10µg/kg/mL was able to reverse the anxiogenic-like effect promoted by the withdrawal from alcohol, increasing the time spent in the open arms of the apparatus [(mean ± SEM of % time on open arms), water/control: 25.52 ± 8.46; water/ond10µg: 22.25 ± 7.14; alcohol/control: 4.96 ± 1.28; alcohol/ond10µg: 20.58 ± 5.25, $F(3,38) = 2.77$; $p= 0.05$, ANOVA]. Ondansetron in the dose of 1000µg/kg/mL increased the exploration of the enclosed arms of the EPM [(mean ± SEM of entries in the enclosed arms), control: 6.1 ± 0.52; ond1000µg: 8.81 ± 0.60, $F(3,52) = 3.83$; $p = 0.01$, ANOVA]. Our data support the importance of 5-HT3 receptors in the modulation of anxiety, including that one generated by long-term abstinence from alcohol. **Financial Support**: PROPESQ-UFRN

02.010

Effect of cyclooxygenase-2 inhibitors on pentylenetetrazol (PTZ)-induced seizures in mice. Temp FR, Santos AC, Marafiga JR, Jesse AC, Milanesi LH, Hessel AT, Lenz QF, Rambo LM, Mello CF UFSM – Fisiologia e Farmacologia

Introduction: Cyclooxygenase-2 (COX-2) inhibitors reduce the synthesis of prostaglandins (PGs), which play a significant role in inflammation and fever (1). In the past decades research has been focused on the use of coxibs (NSAIDs) and the inflammatory process in the central nervous system (CNS) (2,3,4). However, there are conflicting results in the literature regarding the role of COX-2 in acute seizures. Some studies have shown that COX-2 inhibition decreases seizures, while others have reported that it may facilitate convulsive episodes (3,4,5,6). Current evidence suggests that the effect of COX-2 inhibitors on seizures may vary depending on the convulsant stimulus, the drug dose (3,6), the timing and method of drug administration, and potentially the strain of mouse or rat employed (7). Thus, considering the current discrepancy regarding the pro- or anticonvulsant effect of COX-2 inhibitors, the aim of the current study was to investigate whether the acute administration of the COX-2 inhibitors celecoxib, etoricoxib and nimesulide alter seizures in mice. **Methods**: Adult male Swiss mice were used. Celecoxib, etoricoxib and nimesulide (0.2, 2 or 20 mg/kg, p.o.) or vehicle 0.1% carboxymethylcellulose plus 5% Tween 80, p.o.), was administered 60 minutes before pentylenetetrazol (PTZ, 50 mg/kg, i.p.) injection. After PTZ administration the animals were monitored by 20 minutes for the appearance of seizures. The parameters analyzed were: latency to myoclonic and generalized tonic-clonic seizures, number of seizure episodes, total time spent seizing and Racine scale. **Results**: Nimesulide significantly and dose-dependently decreased the incidence of PTZ-induced myoclonic jerks [$H(3)=11.63$; $p<0.05$], generalized tonic-clonic seizures [$H(3)=9.44$; $p<0.05$] and number of seizure episodes [$F(3,28)=4.2$; $p<0.05$]. However, celecoxib and etoricoxib did not alter the latency to PTZ-induced myoclonic jerks, generalized tonic-clonic seizures and number of seizure episodes. Furthermore, none of the COX inhibitors altered the effect of PTZ on total time spent seizing and Racine scale. **Discussion**: In this study we found that while nimesulide attenuated, celecoxib and etoricoxib did not alter PTZ-induced seizures in mice. This result contrasts with previous studies of the group, who has shown that celecoxib and etoricoxib decrease PTZ-induced seizures in adult rats (3, data not show). Therefore, current data suggest that Swiss mice are resistant to the anticonvulsant effect of coxibs. More studies are needed to elucidate why these mice are sensitive to the anticonvulsant effect of nimesulide. **Source 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 (N°024/2014). **References**: 1. Auriel *et al. Handb Clin Neurol* 119:577 (2014); 2. Hewett *et al. J Pharmacol Exp Ther* 319:1219 (2006); 3. Oliveira *et al. Epilepsy Res* 79:14 (2008); 4. Toscano *et al. Brain Res Bull* 75:598 (2008); 5. Chung *et al. Exp Neurol* 249:95 (2013); 6. Salvadori *et al. Epilepsia* 53:189 (2012); 7. Rojas *et al. Epilepsia* 55:17 (2014).

02.011

Methylprednisolone improves the neuromuscular transmission at 50 Hz only after being transported into the nerve motor. Ambiel CR[1], Dal Belo CA[2], Corrado AP[3], Correia-De-Sá P[4], Alves-Do-Prado W[5] [1]UEM – Ciências Fisiológicas, [2]Unipampa – Farmacologia, [3]FMRP – Farmacologia, [4]UP – Imuno-Fisiologia e Farmacologia, [5]UEM – Farmacologia e Terapêutica

Introduction: Methylprednisolone (MP, 0.3 mM) facilitates neuromuscular transmission acting on motor nerve. Despite a choline transporter has been implicated in MP neuromuscular effect, the contribution of choline to MP facilitatory effect have not been investigated. Since 50 Hz is a physiological pattern of frequency of stimulation that may determines the activation of presynaptic facilitatory (M1 cholinergic) receptors when the neuromuscular preparations are in presence of a drug that increases the acetylcholine release, the effects caused by combined administration of MP, choline and McN-A-343, a selective agonist for M1 receptor, were investigated in the rat neuromuscular preparations. **Methods**: The Ethics Committee for Experimental Animals Studies of the State University of Maringá approved (ECEAS 073/2012) this study. The phrenic nerve-diaphragm preparations of rat were mounted as described elsewhere (Bülbring, *Brit J Pharmacol* 1: 38, 1946). The ratio (R) values was obtained between 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 (R=B/A). R-value was obtained for 5th tetanic stimulus, 60 min after the 1st tetanic stimulus (control). MP (0.3 or 0.6 mM) was administered in Krebs buffer 35 min before 5th tetanic stimulation. The effects of combined administration of MP (0.3 mM) with choline (1.0 µM), McN-A-343 (10 nM), or with the selective blocker of neuronal high-affinity choline transporter (HACT) hemicholinium (HC; 1.0 µM) were tested. These agents were administered 35 min before MP. An inverse sequence to administration of MP with choline or HC was also assayed. McN-A-343 was administered 15 min before of choline. Data were submitted to "t" Student test or ANOVA, followed by Bonferroni test at $P<0.05$ significance level. Results: MP (0.3 and 0.6 mM) caused concentration-dependent facilitatory effect (16.81 ± 1.35%, n=6 and 32.21 ± 4.26%, n=4) in neuromuscular preparations (increase R-values). The facilitatory effect caused by 1.0 µM choline (27.4 ± 1.47%, n=5) was enhanced (from 27.4 ± 1.47% to 44.97 ± 5.4%, n=5) by previous administration of 0.3 mM MP. However, such effect did not occur when the order of administration of drugs was changed. The inhibitory effect (-2.95 ± -2.39%, n=4) caused by HC (1.00 µM) was impaired (from -2.95 ± -2.39% to 22.15 ± 3.7%, n=4) by previous treatment with MP (0.3 mM), but HC (1.0 µM) impaired (~97 % of reduction in R value) the facilitatory effect caused 0.3 mM MP when this agent was administered before. McN-A-343 (3.0 µM) separately facilitated (12.67 ± 1.45%, n=3) the neuromuscular transmission. Nevertheless, such agent produced a reduction (~72 %) in the facilitatory effect caused by 1.0 µM choline. **Discussion**: The facilitatory effect produced by MP in the cholinergic neurotransmission stimulated at 50 Hz frequency depends on transport of MP into cholinergic terminals by HACT system. It is not mediated by activation of facilitatory-M1 receptors on motor nerve terminal, but the activation of such receptors reduces the activity of HACT system.

02.012

Central effects of aqueous extract of yellow and purple passion fruit leaves in mice.

Ayres ASFSJ[1], Lima LA[1], Soares TC[2], Ayres DDJ[1], Rachetti VPS[1], Zucolotto SM[2], Gavioli EC[1] [1]UFRN – Biofísica e Farmacologia, [2]UFRN – Farmácia

Introduction: Exotic species of the genus *Passiflora* has long been used in Brazil for the treatment of insomnia and anxiety. Interestingly, in the genus *Passiflora*, *P. edulis* is the most important economic species used for fresh fruit and for juice*. Despite this economic interest*, *P. edulis varieties are not systematically used with medicinal proposes in Brazil. P. edulis* exhibits significant morphological variability in the color of the fruit rind. Considering these differences, Degener (1933) proposed to name *P. edulis* f. *flavicarpa* (PEF) the population with yellow fruit, while those species with purple fruit were named *P. edulis* f. *edulis* (*PEE*). **Objective**: The aims of this study is to compare the central effects of aqueous extract of PEE and PEF leaves on behavioral tests used for assessing anxiety- and mood-related behaviors in mice. **Methodology**: Male Swiss mice (25-40g) were orally treated with aqueous extract of PEE (100, 300 and 1000 mg/kg), PEF (30, 100, 300 and 1000 mg/kg) or tap water. The extracts were administered 60 minutes before behavioral tests. Animals were assessed in the elevated plus maze (EPM), forced swimming (FST) and open field test. Diazepam (1 mg/kg, ip, 30 min) and nortriptyline (30 mg/kg, ip, 30 min) were used as standard drugs in the EPM and FST, respectively. This project was approved by CEUA/UFRN (Nº 032/2010). **Results**: In the EPM, diazepam increased exploration to open arms compared to control (control=19.3 ± 5.0; diazepam= 51.0 ± 4.5*; $p<0.05$). In a similar manner, the treatment with PEE and PEF induced anxiolytic-like effects in mice. A significant increase in the percentage of open arms entries was detected in mice treated with PEE (control= 30.3 ± 3.3; PEE 100= 36.8 ± 3.3; PEE 300= 41.6 ± 2.0*; PEE 1000= 36.4 ± 2.2; *$p<0.05$) and PEF (control= 32.1 ± 2.6; PEF 30= 29.3 ± 4.1; PEF 100= 43.8 ± 2.0*; PEF 300= 42.2 ± 2.7; PEF 1000= 41.4 ± 3.3; *$p<0.05$) compared to controls. In the FST, nortriptyline significantly reduced the immobility time of mice (control= 176.4 ± 19,4; nortriptyline= 82.3 ± 19.7*; $p<0.05$). Similarly, the acute treatment com PEE (control= 169.3 ± 6.0; PEE 100= 150.8 ± 7.1; PEE 300= 137.0 ± 8.3*; PEE 1000= 175.4 ± 9.4;*$p<0.05$) and PEF (control= 173.8 ± 6.5; PEF 300= 175.4 ± 8.1; PEF 1000= 152.5 ± 6.8*; *$p<0.05$) reduced the immobility time of mice, thus suggesting an antidepressant-like action. Importantly, the aqueous extract of PEF did not affect spontaneous locomotion in the open field test. However, the treatment with PEE significantly reduced distance traveled of mice only at 1000 mg/kg (control= 60.2 ± 8.6; PEE 300= 44.5 ± 8.1; PEE 1000= 34.3 ± 4.8*,*$p<0.05$). **Conclusions**: The aqueous extract of PEE displayed anxiolytic- and antidepressant-like actions at doses up to 300 mg/kg. At higher doses, the PEE extract evoked hypolocomotion in mice. Regarding the PEF extract, anxiolytic activity was observed at 100 mg/kg while the antidepressant-like actions were obtained at 1000 mg/kg. These differences must be explained due to the probable main neuropharmacological active substances available on these species. **Financial agencies**: Capes/ UFRN.

02.013

Effects of topiramate on anxiety related behaviors in ethanol abstinent rats. Ayres DDJ, Soares-Rachetti VP, Gavioli EC, Ayres ASFSJ UFRN – Biofísica e Farmacologia

Alcohol abuse and dependence constitute the third leading cause of morbidity and mortality worldwide. Clinical studies suggest the anticonvulsant topiramate as a promising drug for the treatment of alcohol dependence. However, the behavioral effects of topiramate have been little explored in animal models of alcoholic abstinence. **Objective**: This study aimed to evaluate the effects of topiramate administration on anxiety in naïve and alcohol abstinent rats. **Methods**: Male Wistar rats (±300 g) were acutely (60 min before test) treated with topiramate, and animal behavior was assessed in the elevated plus-maze (EPM) and open-field (OP) tests in naïve and alcohol abstinent rats. Alcohol was mixed in drinking water and it was offered ad libitum to the animals in increasing concentrations (2%, 4%, 6% during 20 days), and, on day 21, alcohol solution was replaced by tap water. Control group received tap water during the same period (i.e., 20 days). The EPM was used to evaluate the levels of anxiety experienced by animals. The number of entries into and time spent in open and enclosed arms were measured for 5 min. In OP, the distance moved in a square arena (60x60x60 cm) was assessed during 15 min. Experimental procedures were performed after approval of CEUA/UFRN (N° 007/2012). **Results**: The treatment with topiramate increased dose-dependently the time spent into the open arms (sal= 93.6 ± 14.3, t10= 115.7 ± 22.1, t40= 169.3 ± 19.3*, *$p<0.05$ vs. saline; ANOVA, Duncan test) in the EPM in naïve rats, suggesting an anxiolytic-like action. Additionally, the administration of topiramate 40 mg/kg reversed the anxiogenic-like behavior evoked by alcohol withdrawal (time into the open arms: H_2O/sal= 96.8 ± 14.6, H_20/t40= 146.1 ± 12.3*, Alc/sal= 50.3 ± 15.0*, Alc/t40= 125.9 ± 21.5$^{\#}$, *$p<0.05$ vs. H_2O/sal; $^{\#}p<0.05$ vs. Alc/sal; two-way ANOVA, Duncan test). In OP, the administration of topiramate did not alter the distance moved by naïve and alcohol abstinent animals. **Conclusion**: The acute administration of topiramate induced anxiolytic-like effects in naïve rats and it also reversed the anxiogenic-related behavior of alcohol withdrawal. Taken together, topiramate seems to be an interesting therapeutic approach to the treatment of anxiety evoked by alcohol withdrawal. **Financial support**: CNPq and UFRN

02.014

Agmatine reverses reserpine-induced orofacial dyskinesia in mice: Role of oxidative stress, nitric oxide and glutamate NMDA receptors. Cunha AS[1], Matheus FC[2], Santos DB[3], Colle D[3], Moretti M[4], Cunha MP[3], Rodrigues AL[3], Farina M[3], Prediger RD[1] [1]UFSC – Farmacologia, [2]UFSC – Farmacologia, [3]UFSC – Bioquímica, [4]UFSC – Farmacologia / Bioquímica

Introduction: Oral involuntary movements are observed in patients with Parkinson's disease and tardive dyskinesia. However, current available treatments present limited efficacy, as well as significant side effects. Thus, the aim of the present study was to investigate the putative effects of agmatine, an endogenous arginine metabolite with antiglutamatergic, antioxidant and neuroprotective properties, in vacuous chewing movements (VCMs) induced by reserpine in mice. **Methods**: All procedures were approved by local Ethical Committee in Animal Research (CEUA PP830/2012). Male Swiss mice (3 months-old) were treated with two reserpine injections (1 mg/kg, s.c.) with an interval of 48 h. Twenty-four hours after the last administration, mice were treated with different doses of agmatine (10, 30 and 100 mg/kg, i.p.); amantadine (1, 10, 40 and 60 mg/kg, i.p.); 7-nitroindazole (7-NI) (0.1, 1, 10 and 100 mg/kg, i.p.) and arcaine (0.005, 0.1 and 1,0 mg/kg, i.p.) (n=6-8 animals per group). It was analyzed the VCMs frequency at 30 min following drugs treatments to define sub-effective and active doses of such compounds required for subsequent experiments. In a set of experiments we analyzed the synergistic effect of treatment with a sub-effective dose of agmatine (10 mg/kg) in combination with a sub-effective dose of amantadine (1 mg/kg) or 7-NI (0.1 mg/kg) on VCMs frequency. Furthermore, we investigated the putative additive effect of agmatine (30 mg/kg) in combination with arcaine (1 mg/kg) on oral involuntary movements in mice. To investigate putative molecular mechanisms associated with the agmatine's antidyskinetic effects, the animals were euthanized for analysis of oxidative stress-related parameters in striatum and prefrontal cortex after the VCM frequency analysis. **Results**: Agmatine (30 mg/kg), amantadine (40 mg/kg) and 7-NI (1 mg/kg) showed a dose-dependent antidyskinetic effects. At all doses analyzed, arcaine reverses VCMs. Agmatine (30 mg/kg) modulated pro-oxidative changes induced by reserpine. It was also observed a synergistic response following the co-administration of sub-active doses of 7-NI (0.1 mg/kg) plus agmatine (10 mg/kg) or the combined administration of 7-NI (0.1 mg/kg) plus amantadine (1 mg/kg) in reducing VCMs frequency in reserpine-treated mice. Agmatine (30 mg/kg) and arcaine (1.0 mg/kg) co-administration maintained the drugs antidyskinetic effect. This result suggest that agmatine acts biding NMDA receptor channel and not in the polyamines site of NMDA receptor. **Discussion**: This is the first study to demonstrate the potential of agmatine to reverse orofacial dyskinesias induced by reserpine in mice. The current findings suggest the involvement of the inhibition NO production, antioxidant properties and blockade of NMDA receptors as possible molecular mechanisms associated with the antidyskinetic effects of agmatine. **Financial support**: CNPq, Capes, FAPESC (PRONEX – Project NENASC), FINEP (IBN-Net #01.06.0842-00), UFSC, CEUA PP830/2012.

02.015

Behavioral impairment induced by different doses of reserpine in mice: Relationship with tyrosine hydroxylase levels. Freitas CM[1], Busanello A[1], Leal CQ[2], Peroza LR[3], Schaffer LF[1], Krum BN[2], Fachinetto R[1,3] [1]UFSM – Farmacologia [2]UFSM – Farmácia [3]UFSM – Bioquímica Toxicológica

Introduction: Parkinson's disease (PD) and Tardive dyskinesia (TD) are motor disorders characterized by involuntary abnormal movements. Reserpine, a monoamine-depleting agent, is used to induce motor alterations being considered an animal model for orofacial dyskinesia (OD) by some authors and a Parkinson's disease model by others (Neisewander *et al.*, 1994; Salamone and Baskin, 1996). The aim of the present study was to evaluate the effects of different doses of reserpine on behavioral parameters in mice and if the alterations were accompanied by neuronal changes compatible with OD or with Parkinsonism, by measuring tyrosine hydroxylase (TH) levels. **Methods**: Albino Swiss mice received subcutaneous injections of vehicle or different doses of reserpine 0.1, 0.5 or 1 mg/kg once a day, for 4 consecutive days. The behavioral parameters (vacuous chewing movements – VCM and open-field test) were measured on day 6 (48 h after the 4th injection). After the behavioral tests mice were killed and TH levels were evaluated by western blotting (Trevisan *et al.*, 2013) on the striatum and the region that contains the *substantia nigra*. Experimental protocol was approved by internal ethical commission of UFSM under the number 078/2013. Results: In our study mice treated with 1 mg/kg of reserpine showed an increase on VCM number ($p<0.01$) and the treatment with 0.5 and 1 mg/kg reserpine decreased the locomotor activity when compared with the control group ($p<0.05$ and $p<0.001$ respectively). Pearson's test revealed a negative correlation between VCM values and the locomotor activity ($r = -0.5231$, $p<0,01$). Additionally, these motor alterations were accompanied by a decrease in tyrosine hydroxylase (TH) levels in *substantia nigra*, but any difference was observed in striatum of mice. **Discussion**: Our study showed that treatment with 1 mg/kg reserpine caused motor impairment accompanied by a reduction in TH levels in *substantia nigra*, suggesting that reserpine causes neuronal damage compatible with PD model. **Financial support**: UFSM, Capes, CNPq and FAPERGS. **References**: Neisewander JL, *et al. Psychopharmacology* (Berl). v. 116, 79, 1994. Salamone J, Baskin P. *Pharmacol Biochem Behav*. V. 53, 179, 1996. Trevisan *et al.* Free *Radic Biol Med* V. 72, 200, 2014.

02.016

Is there a central site that modulates peripheral inflammation in the rat? Frade TIC[1], Bakhle YS[2], Francischi JN[1] [1]ICB-UFMG – Farmacologia, [2]Imperial College

Introduction: In a model of oral inflammation in rats (Ladeira *et al.*, 2011), we observed an incomplete anesthesia (light body tremors) in animals treated with a recommended dose of a general anesthetic mixture (ketamine + xylazine; K^+ XYL). To deepen the anesthesia, we increased the dose of the anesthetic and found that this was associated with a reduced inflammatory response. Here we report the effects of K^+ XYL on inflammation following local injection of carrageenan in the rats and the mechanisms involved. **Methods**: Holtzman male rats weighing 160-260 g were injected with 500 μg of carrageenan (CG) or saline either in the subcutaneous tissue of the oral mucosa or the hind paw (ipl). All except one group of animals received a mixture of commercial K^+ XYL solution, given i.p., 5 min (mouth) before or 1 h (paw) following the injection of CG. In the remaining group, K^+ XYL was given intrathecally (i.t.) at two doses: 1200 + 320 and 1600 + 320 μg in 40 μl solution, accordingly to Mestre *et al* (1994), after a brief exposure to isoflurane before CG injection in the mouth. Adequate general anesthesia was assessed by a complete loss of the righting reflex. Oral inflammation was measured as the increased thickness (in mm) of the cheeks obtained with a Mytutoyo caliper at zero, ½, 1, 2, 4 and 24 h. Results are presented as mean ± SEM. This study was approved by the Federal University of Minas Gerais Animal Ethics Committee, Protocol#: 97/2013. **Results**: Injection of K^+ XYL i.p. decreased oral inflammation over 24 h with a maximum effect (about 50 % reduction) at 2 h. A similar reduction was observed 3 h after CG administration in hind paws of rats treated with K^+ XYL. However, when K^+ XYL was given i.t. oral oedema increased depending on ketamine dose, although the depth and duration of anesthesia was not changed. **Conclusions**: 1. Our data suggest that there is an important site for modulation of inflammation in the CNS, which is different from that mediating general anesthesia of the animals; 2. Our data also suggest the participation of NMDA receptors in such modulation. Mestre *et al J Pharmacol. and Toxicol* Meth 32: 197, 1994. **Financial Support**: CNPq

02.017

Riparina III effect of the reversal of anhedonia and abandonment in animals exposed to chronic stress. Vasconcelos AS, Oliveira ICM, Rodrigues GC, Oliveira SC, Vidal LMT, Chaves RC, Castro LA, Lopes IS, Pontes MCD, Sousa FCF UFC – Fisiologia e Farmacologia

Introduction Depression is a serious and prevalent disorder that is triggered by a complex interplay of genetic, developmental, and environmental factors. Its most characteristic symptoms are depressed mood and anhedonia. Currently available antidepressants are effective in many patients, however, all elicit undesirable side effects, some patients remain refractory to pharmacotherapy. In addition to post-treatment relapse, depressive symptoms can even recur in the course of long-term therapy. It is then necessary to search for new effective drugs. Riparin III (Rip III), isolated from *Aniba riparia*, have presented promising Results: In pre-clinic trials, these substances triggered antidepressant and anxiolytic effects in acute and chronic models of depression. Thus, the aim of this study was to investigate the activity of Rip III in mice submitted to the model of depression induced by administration of corticosterone (CORT). **Methods** Swiss female mice were used, weighing between 22-26 g, divided according to the following experimental groups: control (vehicle – saline, 1% Tween80, 1 % DMSO sc, for 14 and 21 days in a row), Stressed (CORT, 20 mg/kg sc for 14 and 21 days), treated with RipIII (50 mg/kg, po for 7 days), treated with fluvoxamine (Flu) (50 mg/kg, po for 7 days). The treatments occurred from the 14th of stress and corticosterone remained concomitant to it. Depression was induced by repeated injections of CORT (20 mg/kg, sc) over the period of 14 days. Behavioral models were evaluated: Forced Swim (FS), Tail Suspension (TS) and Sucrose Consumption (SC). The study was approved by the ethics committee with protocol 13/2014. **Results e Discussion** In FS and TS, the group treated with CORT showed a time of immobility (TI) higher (FS CORT: 143.3 ± 8,37; RipIII: 32 ± 3,2; Flu: 54 ± 4,9/ TS CORT: 140,2 ± 7; RipIII: 87,10 ± 9,03; Flu: 53,69 ± 5,230). In the SC, the treated groups had higher intakes of sucrose (RipIII: 76,72 ± 5,4; Flu: 75,81 ± 5,6) compared with the CORT (CORT: 45,88 ± 1,5). This data is very important considering that anhedonia is one of the most difficult symptoms to treat. The depressive behavior was triggered by the administration of CORT and reversed by treatment with RipIII and Flu. **Financial support**: CNPq, Capes, Funcap. **References**: Millan MJ Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacology & Therapeutics*, v. 110, n. 2, 2006. Sousa F.C. *et al.* Antianxiety and antidepressant effects of riparin III from *Aniba riparia* (Nees) Mez (Lauraceae) in mice. *Pharmacol Biochem Behav.*, v. 78, n. 1, p. 27-33.

02.018

Inosine attenuates inflammatory and nociceptive responses in a murine multiple sclerosis model. Junqueira SC[1,2], Lieberknecht, V[1,2], Albert, TB[2], Peña, MC[1], Coelho, IS[1], Mack, JM[3], Rodrigues, ALS[1], Calixto, JB[4], Santos, ARS[1], Dutra, RC[1,2,3] [1]UFSC – Neurociências, [2]LAIF-UFSC-Araranguá, SC; [3]UFSC – Farmacologia, [4]CIEnP

Multiple Sclerosis (MS) is a chronic, autoimmune and neurodegenerative disease of the central nervous system (CNS) that causes significant disability in young adults. Unfortunately, there is not a definitive therapy for MS and the conventional therapeutic strategies have shown undesirable side effects. Inosine is a dietary supplement available in most health food stores and is taken by some athletes to boost performance. Inosine reduced inflammation in models of pancreatitis, endotoxic shock, allergic lung inflammation, reduced activation of human neutrophils and alleviated inflammation in a hepatitis animal model. Moreover, the antinociceptive activity of inosine has been reported in both acute and chronic pain models. However, it remains unclear whether inosine is involved in neuropathic pain in MS. **Aim**: In the present study, we used behavioral and molecular approaches to investigate the antinociceptive and neuroprotective effects of inosine in the experimental autoimmune encephalomyelitis (EAE) model. We also assessed the underlying mechanisms of inosine in this experimental model. **Method**: Experiments were conducted using female C57BL/6 mice (6-10 weeks old) and were approved by CEUA/UFSC PP00811. EAE was induced by immunization with MOG_{35-55} peptide plus *Mycobacterium tuberculosis* extract H37Ra in incomplete Freund's adjuvant oil (day 0 and 7). The animals received *Pertussis toxin* by intraperitoneally (i.p.) route (day 0 and 2) post-immunization. The animals were weighted and examined daily to observe clinical signs of EAE for 40 days. Inosine (1 and 10 mg/kg) was administrated i.p. twice a day, during 40 days, and vehicle solution was used in EAE-control group. **Results and discussion**: Preventive treatment with inosine (1 and 10 mg/kg, i.p.) significantly reduced the clinical score, weight loss and thermal hyperalgesia induced by EAE ($p<0.05$), although only inosine 1 mg/kg, blocked mechanical hyperalgesia during EAE development ($p<0.05$). Furthermore, inosine treatment was accompanied by a remarkable reduction of A2AR immunocontent ($p<0.001$) and ERK1 phosphorylation ($p<0.001$) in the mouse spinal cord after EAE immunization. However, inosine (1 and 10 mg/kg, i.p.) failed to inhibit the protein levels of A1R and ERK1/2 ($p>0.05$) in the spinal cord. **Conclusion**: Our data constitute convincing preclinical evidence indicating that inosine, an adenosine product, exert a significant role in the establishment of persistent hypersensitivity observed in the EAE model, an action that seems to involve a central autoimmune process, possibly acting by modulating A2AR levels and ERK1 activation. **Sources of research support**: FAPESC, CNPq; Capes; UFSC.

02.019

Administration of 2-araquidonoilglicerol (2AG) in medial pre-frontal cortex induced anxiolytic-like effects in rats. Lima RCO, Almeida-Santos AF, Aguiar DC ICB-UFMG – Farmacologia

Introduction: Anandamide and 2-araquidonoilglicerol (2AG) are the main representatives of the endocannabinoid system. Due to the location of cannabinoid receptors type 1 (CB1r) and type 2 (CB2r) in structures related to defensive behaviours, as medial pre-frontal cortex (mPFC), several works suggest that these endocanabinnoids can modulates defensive responses. Thus, previous studies our group demonstrated that administration of this endocannabinoid into-dorsolateral PAG can induce anxiolytic-like effect in different animal models of anxiety, such as the elevated plus maze (EPM), being this effect mediated through activation of CB1r and CB2r. However, the role of 2AG in behavioral responses mediated by the mPFC is not yet described. Thus the objective of this study was to test the hypothesis that the administration intra-mPFC of the 2AG will exert anxiolytic-like effects in animals submitted to EPM. **Methods**: Male Wistar rats (n= 5-11/group) with bilateral cannula aimed at the mPFC (AP= +3.2 mm bregma, L= +2.5 mm, D= -3.3 mm, angle -23º) received intra-mPFC injections (0.2 µL) of the following treatments: vehicle or 2AG (5 pmol, 50 pmol or 500 pmol). The animals were exposed to EPM 10 minutes after the injection, and the behavior was analyzed by Any-Maze software. **Results**: The administration of 2AG (50 pmol and 500 pmol) significantly increased the number of entries in the open arms of the EPM [vehicle: 18.11 ± 4.49; 2AG (50 pmol): 41.22 ± 4.91; 2AG (500 pmol): 53.46 ± 2.36; $F_{(3,24)}$ = 7.48; Anova followed Bonferroni pos test, $p<0.05$ compared to vehicle group], suggesting an anxiolytic-like effect. Furthermore, no statistical difference was observed in the number of entries into the closed arms of the EPM, suggesting that 2AG did not induce alteration in locomotor activity of animals. **Discussion**: Our results showed that 2AG signaling into-mPFC could also induce anxiolytic-like effects, similar to the effect described by another endocannabinoid, anandamide. The next step of the work will be to investigate which type of cannabinoid receptor is involved in this response. Number of the Animal Ethics Committee: 250/2010. **Financial support**: Capes/FAPEMIG

02.020

Treatment with cilostazol reverts the cognitive impairment caused by chronic cerebral hypoperfusion in rats. Godinho J, Bacarin CC, Ferreira EDF, Zaghi GGD, Oliveira RMW, Milani H UEM – Farmacologia e Terapêutica

Introduction: Chronic and progressive cerebral hypoperfusion (CCH) may be causally related to certain states of aging-related dementias, including the Alzheimer type of dementia. Cilostazol, a selective PDE3 inhibitor, has been used for prevention and/or treatment of brain hypoxia/ischemia-related conditions. Cilostazol exerts distinct effects including antiplatelet aggregation, reduction of oxidative stress, and vasodilation. These effects might be associated to an increased level of intracellular cAMP and endothelial nitric oxide synthase activity. Thus we evaluated if long-term treatment with cilostazol (Cebralat ®) could attenuate both neurodegeneration and memory deficit caused by CCH in middle-aged rats. **Methods**: Middle-aged Wistar rats (12 to 15-month-old) were trained for 15 days up to reach asymptotic learning performance in a non-food rewarded, eight-arm radial maze task, and then assigned to one of the following groups: sham-operation (n = 28), CCH + vehicle (n = 24), CCH + cilostazol long treatment (n = 14) and CCH + cilostazol short treatment (n = 14) . CCH was induced by permanent, stepwise 4-vessel occlusion/internal carotid artery (4-VO/ICA model). Memory performance of the previously acquired cognition was assessed weekly at 7, 14, 21, 28 and 35 days after 4-VO/ICA, and expressed by three parameters: (*i*) the latency to find the goal box, (*ii*) the number of reference memory errors, and (*iii*) the number of working memory errors. Cilostazol (50 mg/kg, p.o.) or vehicle (saline) was administered at once a day, either for 15 days or 45 days, starting soon after the first occlusion stage of 4-VO/ICA. This protocol had the approval of internal Ethical Committee (CEEA No 137/2012). **Results**: Retrograde memory performance was unchanged in the sham-operated groups. CCH elevated all the three parameters in the vehicle-treated group ($p<0,0001$), clearly indicating an state of persistent retrograde amnesia. This sequelae was attenuated by cilostazol, as measured by latency and number of errors ($p<0.0001$), whatever the duration to the treatment. Cilostazol did not alleviate CCH-induced hippocampal and cortical neurodegeneration. **Conclusion**: The results show that Cilostazol reduced the retrograde amnesia caused by CCH in middle-aged rats, an effect that was not accompanied by histological protection. **Research support and acknowledgments**: Capes and UEM (UEM).

02.021

Resveratrol inhibits monoamine oxidase A and B *in vitro*. Barbosa CP[1], Busanello A[2], Freitas CM[3], Reinheimer JB[1], Barbosa NBV[3], Fachinetto R[2,3] [1]UFSM – Farmácia, [2]UFSM – Farmacologia, [3]UFSM – Bioquímica Toxicológica

Introduction: Monoamine oxidase (MAO) inhibitors were the first antidepressant drugs to be prescribed and are still used with relative success, especially in patients resistant to other antidepressants. Nowadays, MAO-B inhibitors have been studied because their promissory potential to treating movement disorders (Youdim & Bakhle 2006). In this study, we evaluated inhibitory potential of resveratrol, a polyphenol compound contained in red grapes and red wine, on *in vitro* MAO-A and MAO-B activities. **Methods**: Resveratrol was tested for its *in vitro* inhibitory potential on rat MAO-A and MAO-B activities in brain mitochondrial preparations by a fluorometric method using kynuramine as a substrate, as previously described (Matsumoto *et al.*, 1985). Briefly, assays were performed in duplicate in a final volume of 500 µL containing 0.45 mg of protein and incubated at 37 ˚C for 30 min. Activities of the A and B isoforms were isolated pharmacologically by incorporating 250 nM pargyline (selective MAO-B inhibitor) or 250 nM clorgyline (selective MAO-A inhibitor) into the reaction mix. The reaction mixture (containing mitochondrial fractions, resveratrol and inhibitors) was pre-incubated at 37 ºC for 20 min, and the reaction was started by the addition of 50 µL of kynuramine (90 µM to MAO-A and 60 µM to MAO-B). Also, resveratrol was tested at a concentration range of 1 to 300 µg/mL, and the IC_{50}value for both MAO isoforms was determined. For kinetic experiments, various concentrations of kynuramine (1-300 µM) were used, and the MAO activity was determined in the absence or presence of different concentrations of resveratrol (0.03, 0.1, 0.3, 1, 3 or 10 µg/mL) for MAO-A and(1, 3, 10, 30, 100 or 300 µg/mL) for MAO-B in order to calculate K_m and V_{max} values. The experimental protocol was approved by internal ethical commission of UFSM under the number 091/2013. **Results**: We observed that resveratrol inhibited both MAO isoforms in a concentration-dependent manner with iC_{50} values of 1.599 (1.2–2.1) µg/mL and 44.45 (31.5–62.6) µg/mL for MAO-A and MAO-B, respectively. Subsequently, kinetic experiments for isoforms activity were carried out using different concentrations of substrate. Resveratrol did not alter the K_m value (29.3 ± 13.92 µM) for MAO-A and (12.47 ± 1.68 µM) for MAO-B and it caused a decrease in the V_{max} value (0.194 ± 0.034 nmol/min/mg protein) for MAO-A and (0.306 ± 0.011 nmol/min/mg protein) for MAO-B compared to K_m and V_{max} values obtained in the absence of resveratrol (13.12 ± 5.35 µM and 0.388 ± 0.043 nmol/min/mg protein, MAO-A; 10.44 ± 1.85 µM and 0.57 ± 0.026 nmol/min/mg protein, MAO-B respectively).In conclusion, the present study, showed that resveratrol has an inhibitory activity for both MAO isoforms of rat brain. **Financial support**: FAPERGS, CNPq, Capes e UFSM. **References**: Matsumoto T, Suzuki O, Furuta T, Asai M, Kurokawa Y, Nimura Y, Katsumata Y, Takahashi I., A sensitive fluorometric assay for serum monoamine oxidase with kynuramine as substrate. *Clin Biochem*. Apr;18(2):126-9, 1985. Youdim, Moussa B.H. & Bakhle, Y.S.., Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness *Brit J Pharmacol* 147: S287–S296, 2006.

02.022

Reserpine effects on dopaminergic neurons morphology. Reckziegel P[1], Aschner M[2], Fachinetto R[3] [1]UFSM – Farmacologia, [2]Albert Einstein College – Molecular Pharmacology, [3]UFSM – Fisiologia e Farmacologia

Introduction: Several disorders with involuntary movements show dopaminergic neurodegeneration. As an example, patients with Parkinson's disease show a decrease in the number of dopaminergic neurons in *substantia nigra*. Reserpine is used as an animal model of involuntary movements; however its relation to dopaminergic neurodegeneration is not clear yet. *Caenohabditis elegans* is a useful tool on neurodegeneration studies due its transparency, simple genome and a simple and well described nervous system. Fluorescent proteins (as green fluorescent protein, GFP) can be coupled to specific dopaminergic genes and, as result, the morphology of dopaminergic neurons can be observed in alive worms. Thus, the present study evaluated some dopaminergic neurodegeneration parameters on reserpine-exposed worms. **Methods**: Worms expressing GFP coupled to dopamine transporter gene (*dat-1*) (*dat-1*:GFP(*vtIs1*) *V* strain) were exposed to vehicle, 30 or 60 µM reserpine on NGM/OP50 agar plates for 8 days. Some worms were removed from reserpine exposure on day 4. The fluorescence intensity of the cephalic CEP neurons of the worms was captured by confocal microscope and quantified by ImageJ software. Neuronal defects on CEP dopaminergic neurons were evaluated with an epifluorescence microscope. Worms were considered with intact CEPs neurons when they did not show any shrunken soma, loss of dendrites or loss of somas in any one of the 4 CEP neurons. In addition, the expression of *GFP* and *dat-1* genes was evaluated using real-time PCR. Data were analyzed by ANOVA followed by Tukey's test. Results were considered significant when $p<0.05$. **Results**: As result, 60 µM reserpine (and no 30 µM) decreased the fluorescence intensity of CEP neurons in relation to control ($p<0.05$). In addition, both concentrations of reserpine decreased the number of worms with intact CEP neurons, and it increased the number of shrunken soma per worm in relation to control on day 4 and 8 ($p<0.01$). Reserpine (30 and 60 µM) increased the expression of *GFP* and *dat-1* genes after 4 and 8 days of exposure ($p<0.05$). These alterations on the morphology of dopaminergic neurons and gene expressions were recovered after 4 days without reserpine exposure (day 8, $p<0.05$). **Discussion**: Some observations suggest that reserpine induces degeneration on CEP dopaminergic neurons: 1) the decrease in fluorescence intensity, 2) the decrease in the number of intact neurons per worm and 3) the increase in the number of shrunken soma per worm. In addition, gene expression results show that these observations were not false-positive results, because reserpine did not decrease the expression of *GFP* and *dat-1* genes. By the contrary, reserpine increased the expression of these genes. However, after the end of reserpine exposure, the dopaminergic neurons showed normal morphology and expression of *GFP* and *dat-1* genes, suggesting that the observed effects were not dopaminergic neurodegeneration. These curious effects of reserpine on dopaminergic neurons morphology in *C. elegans* should be better studied. **Financial support**: FAPERGS, Capes/PDSE

02.023

Pharmacological evaluation of a newly synthesized piperazine derivative-LQFM104 with antidepressant activity. Rodrigues ORL[1], Galdino PM[2], Menegatti R[3], Costa EA[1] [1]ICB-UFG – Ciências Fisiológicas, [2]UFSC – Farmacologia, [3]UFG – Farmácia

Introduction: Mood disorders, including depression, are becoming increasingly common and prevalent in the developed world. Furthermore, treatment of depression therapeutics, still has many side effects and high refractory rate, and the mechanism of action mainly influencing the serotonergic and adrenergic systems, is considered insufficient. In an attempt to improve these problems, the Laboratório de Química Farmacêutica Medicinal designed and synthesized a new piperazine derivative (LQFM104). This study aimed the pharmacological evaluation in the central nervous system and antidepressant-like effect of LQFM104. **Methods**: The animals used were adult male *Swiss* mice weighing 35 – 40g (n= 8). The central nervous system and antidepressant-like effect of LQFM104 were evaluated by the open-field test, chimney test, pentobarbital-induced sleep test, forced swimming test and tail suspension test. All experimental protocols were approved by the Research Ethics Committee of the UFG (Protocol Nº. 182/10). **Results and Discussion**: Treatment with LQFM104 at doses of 25, 50 or 100 µmol/kg (p.o.) in the open-field test did not alter the number of grooming behavior, the number of fecal boluses, the total number of crossings, immobility time, number of rears, the percentage of crossings in the central area and the time spent in the center. None of the doses tested with LQFM104 was able to change the time spent in the chimney test. In pentobarbital-induced sleep test, the treatment with LQFM104 25, 50 or 100 µmol/kg (p.o.) did not affect sleep latency, while the sleep duration has increased by 65%, 64.4% and 78.6% respectively compared to the control group treated orally with vehicle 10 ml/kg (28.8 ± 2.9 minutes). In the forced swimming test just LQFM104 50 µmol/kg (p.o.) was able to reduce the immobility time in 19.8% compared to the control group (263.2 ± 6.7 seconds) and increased the latency to immobility in 43%, compared with the control (70.6 ± 6.5 seconds). Similarly in the tail suspension test, also had only LQFM104 50 µmol/kg (p.o.) reducing immobility time in 32.2% compared to the control (216.1 ± 13.2 seconds). The results with LQFM104 in the open-field test indicated no changes in spontaneous locomotor activity, in emotionality and not a anxiogenic activity. The chimney test did not reveal impairment in motor coordination. The pentobarbital-induced sleep test increased sleep duration without reducing the latency, thus suggesting a sedative action. The forced swimming test and the tail suspension test confirmed for LQFM104 50 µmol/kg (p.o.) an antidepressant activity in mice.

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02.024

Influence of trans fat supplementation crossover two generations of rats on an amphetamine-induced mania-animal model. Roversi KR[1], Trevizol F[2], Dias VT[1], Roversi K[2], Burger ME[2] [1]CCS-UFSM – Farmácia, [2]UFSM – Farmacologia

Introduction: Dietary fatty acids may change the composition of the neuronal membranes, especially during pregnancy and lactation, affecting their fluidity, plasticity and function (Jump, 2002). Changes in feeding habits of Western countries have provided an excessive consumption of processed foods, which are rich in *trans* fatty acids (TFA) in detriment of omega-3(n-3) (Baggio and Bragagnolo, 2006), which may increase the vulnerability to develop neuropsychiatric conditions, such as bipolar disorder (Hamazaki *et al*., 2009). Here we studied the influence of different fats, which were supplemented during gestation and development of two sequential generation of rats on behavioral and molecular status considering an mania animal model amphetamine (AMPH)-induced. **Methods**: Two sequential generations of female rats were orally supplemented (3g/kg/day) with soybean oil (SO-C, rich in n-6 FA, control group), fish oil (FO, rich in n-3 FA) and hydrogenated vegetable fat (HVF, rich in *trans* FA) from pregnancy and lactation. In adulthood (90 days old) half of each group of male offspring was exposed to an AMPH-induced model animal model (AMPH once a day, 4 mg/kg/mL-ip) for fourteen days. Male rats were submitted to behavioral evaluations (Novel Object Recognition Task –NORT) (de Lima, 2005) after 1 and 24h the last AMPH injection, which was follow by euthanasia. Hippocampus was removed for dopamine transporter (DAT) levels and BDNF- mRNA expression (Bustin *et al*, 2009). This experimental protocol was approved by the Comissão de Ética no Uso de Animais (CEUA-UFSM, number 23/2010, at 14 June, 2010), which is affiliated to the Council for Control of Animal Experiments (CONCEA). **Results**: While AMPH increased short-term memory (NORT-1h) in FO, it was able to reduce this parameter in HVF in comparison to SO-C-supplemented group. In addition, both FO and SO-C showed better long-term memory (NORT 24h) when compared to HVF group. FO supplementation increased the DAT levels in both vehicle and AMPH-injected animals, while this drug was able to decrease this molecular marker in HVF group. Additionally, AMPH-treatment increased the BDNF mRNA expression in FO and reduced in HVF group, with no changes in SO-C group. **Discussion: and conclusion**: The consumption of processed foods, which are rich in TFA, crossover two generations of rats facilitated the mania-like symptoms development, compromising short- and long-term memory. So behavioral outcomes may be related to TFA incorporation in hippocampus (0.2%; data not shown), also affecting dopamine transporter (DAT) and neurothrophic factor expression such as BDNF mRNA. In this context, the prolonged consumption of *trans* fats over two generations may be favorable to the development of neuropsychiatric conditions, which has been increased in Western societies in the last decades. **Acknowledgment**: We are grateful to CNPq, Capes, FAPERGS and PRPGP (PROAP) for the fellowships and financial support and we are grateful to Herbarium® for their donation of fish oil capsules. Baggio SR, Bragagnolo N. *Food Chem* (2006) 95:611. Bustin *et al. Clin Chem* (2009) 55:1-12. Jump DB. *Curr Opin Lipidol* (2002) 13:155–64. Hamazaki K *et al. J Psychiatr Res* (2009) 44:688

02.025

Screening effects of reserpine in *Caenorhabditis elegans*. Ferrari MC[1], Reckziegel P[2], Aschner M[3], Fachinetto R[2,4] [1]UFSM – Curso de Farmácia, Centro de Ciências da Saúde, [2]UFSM – Farmacologia, [3]Albert Einstein – Molecular Pharmacology, [4]UFSM – Fisiologia e Farmacologia

Introduction: *Caenorhabditis elegans* is a nematode that has been used for screening tests of drugs, helping to reduce the number of rodents in science. Reserpine is an alkaloid that decreases monoamine levels through its effect on vesicular monoamine transporter. However, the effect of reserpine has not been explored in *C. elegans*. Thus, the present study performed screening tests for checking survival, development, basal slowing response and dopamine levels on reserpine-exposed *C. elegans*. **Methods**: Wild-type N2 and cat-2 [CB1112, *cat-2(e1112) II*] synchronized L1 worms were exposed to vehicle, 30 or 60 µM reserpine on NGM plates with *Escherichia coli* OP50 during different times. Survival, development and locomotor rate on food were checked daily for 4 days, and dopamine was measured on adult worms. cat-2 worms, *knockout* for tyrosine hydroxylase, were used for behavior and dopamine quantification. Data were analyzed by one and two-way ANOVA followed by Tukey's multiple comparison test ($p<0.05$). **Results and discussion**: Reserpine (60 µM) showed a toxic effect on *C. elegans* by decreasing its survival after 2 days of exposure (11.4%, $p<0.05$) and during all exposure time (day 3: 15%, $p<0.001$; day 4: 23%, $p<0.001$) in relation to control group. In addition, considering the necessary time for having more adult worms on plates, 60 µM reserpine group needed 4 days, and control and 30 µM reserpine groups, 3 days, showing a reserpine-induced delay on development. The locomotor rate on food of reserpine-exposed worms (30 and 60 µM) was higher in relation to control during all exposure times (day 1 to 4, $p<0.001$), and lower than cat-2 group ($p<0.05$). Worms usually decrease their locomotor rate when they are in presence of food; thus, the results of the present study suggest alterations on dopaminergic system in reserpine-exposed worms. In accordance, dopamine content measurement showed that cat-2 and 60 µM reserpine groups had less dopamine contents in relation to control group (40.7 and 79.5% respectively, $p<0.05$). **Conclusion**: The screening tests performed in the present study suggest that exposure to 60 µM reserpine on plates for 4 days is toxic for *C. elegans*, and also causes dopamine depletion. More screening studies evaluating the effects of reserpine on other monoamines are necessary. **Acknowledgements**: Capes/PDSE, FAPERGS.

02.026

Na^+, K^+-ATPase activator delays pentylenetetrazol-induced myoclonic seizures in a mice model of temporal lobe epilepsy. de Souza TL[1], Funck VR[1], de Oliveira CV[1], Grauncke ACB[1], Grigoletto J[1], Larrick JW[2], Ribeiro LR[1], Furian AF[3], Oliveira MS[1] [1]UFSM – Physiology and Pharmacology, [2]Panorama Research, [3]UFSM – Food Science and Technology

Introduction: Epilepsy is considered a neurological disorder and the most common human's type of this disease is the Temporal Lobe Epilepsy (TLE). It has been characterized by an initial injury named *status epilepticus* (SE) [1], which is followed by the occurrence of spontaneous and recurrent seizures [4]. It is known that Na^+,K^+-ATPase activity is decreased in epileptic patients [2]. So, the objective of this study was to evaluate the effect of an activator of this enzyme on convulsive behavior of an experimental model of TLE. **Methods**: To develop this study 30-45 day-old C57BL/6 mice were used to induce a model of TLE through a single injection of Pilocarpine (320 mg/kg in 0.9% NaCl; intraperitoneal). At 55 day after the induction of SE the animals were anesthetized and subjected to a surgical procedure to place the electrodes over the parietal cortex and into the hippocampus [3] and, from 60^{th} day, each animal had its electroencephalographic (EEG) register recorded. After a baseline recorder (15min), animals received polyclonal antibody DRRSAb (1µg, intrahippocampal) [5] or control IgG (1µg, intrahippocampal), and 30 minutes later, were injected with pentylenetetrazol (PTZ – 30 mg/kg, intraperitoneal) and behavioral parameters as myoclonic seizures, and latency and duration of generalized seizures, were observed. The EEG was offline analyzed to wave amplitude and spontaneous spike frequency. Data were analyzed by two-way repeated measures ANOVA or unpaired t test when appropriated and a probability of $P<0.05$ was considered significant. **Results**: Administration of DRRSAb did not change wave amplitude or spontaneous spike frequency in hippocampal [respectively, $F(1,8)=0.4052$ and 4.789] or cortical recordings [$F(1,8)=1.540$ and 2.031] compared to control group. Moreover, was observed that DRRSAb is capable to delay the onset of PTZ-induced myoclonic seizures [$t=4.39$; $P<0.05$], without change latency to generalized seizures [$t=1.118$], its duration [$t=0.922$] or wave amplitude in parietal cortex [$t=0.464$] and hippocampus [$t=0.789$] during generalized seizure compared to IgG-treated group. **Discussion**: The hippocampal administration of DRRSAb in a mice model of TLE induced by Pilocarpine delays the onset of convulsive behavior induced by pentylenetetrazol. Therefore, this study point to a novel treatment of seizure susceptibility in TLE, using Na^+,K^+-ATPase as a drug target. **References**: 1. Acharya *et al*., *Prog Neurobiol* 84, 363-404, 2008. 2. Moseley *et al*., J *Neurosci* 27, 616-626, 2007. 3. Paxinos and Franklin, The Mouse Brain in stereotaxic coordinates, 2007. 4. Scholl *et al*., *Neuroscience* 252, 45-59, 2013. 5. Xu, *Biochem Biophys Res Commun*. 338, 1669-1677, 2005. Animal Ethics Committee license number: 037/2012 and 098/2012. Work supported by CNPq and Capes.

02.027

Increased nitration and alterations of Na^+,K^+-ATPase activity in a mice model of temporal lobe epilepsy. Grauncke ACB[1], Funck VR[1,2], de Oliveira CV[1,2], de Souza TL[1], Grigoletto J[1], Ribeiro LR[1,2], Furian AF[1,2,3], Oliveira MS[1,2] [1]UFSM – Physiology and Pharmacology, [2]UFSM – Pharmacology, [3]UFSM – Department of Food Science and Technology

Introduction: Temporal lobe epilepsy (TLE) is the most common type of human epilepsy and knowledge about the mechanisms underlying seizure activity is fundamental to discovery of new drug targets. It has been suggested that Na^+,K^+-ATPase plays a role in several neurological disorders, including seizure activity and epilepsy. Therefore, the present study aimed to verify oxidative modifications of Na^+,K^+-ATPase and its activity in hippocampus of an experimental model of TLE. **Methods**: Hippocampi of control and epileptic male C57BL/6 mice (60 days after SE) were dissected and Na^+,K^+-ATPase activity was measured by a colorimetric method based on the differential sensitivity of α isoforms to the specific inhibitor ouabain, using different ATP concentrations (1). The Michaelis-Menten constant (K_m) for ATP was calculated using the nonlinear fit function of Graphpad Prism 5.0 (1). Oxidative modifications in the Na^+,K^+-ATPase α subunit were determined by measuring levels of HNE, protein carbonyls and 3-NT by slot blot following immunoprecipitation (3). Data were analyzed by unpaired t test and a probability of $P<0.05$ was considered significant, statistical values are shown only when significant. **Results**: We found that total [t=2.873, $P<0.05$] and α1 [t=3.240, $P<0.05$] Na^+,K^+-ATPase activity is decreased in the epileptic hippocampus two months after SE, but α2/α3 isoforms weren't altered [t=2.373]. Functional analysis of the ATP dependence of Na^+,K^+-ATPase activity revealed a significant increase in the K_m for ATP the α2/α3 isoforms 60 days after SE [t=2.722, $P<0.05$] but to α1 and total Na^+,K^+-ATPase activity were not altered [t=0,7234 and t=1.898, respectively]. In Na^+,K^+-ATPase α subunit immunoprecipitated we found an increase in the nitration of Na^+,K^+-ATPase α subunit [t=3.016, $P<0.05$] but, on the other hand, HNE or protein carbonyls content didn't change in pilocarpine-treated animals [t=0.04241 and t=0.2937, respectively]. **Discussion**: Since irreversible posttranslational modifications such as nitration are usually associated with permanent loss of function (2), nitration of Na^+,K^+-ATPase α subunit could represent a new mechanism for cellular dysfunction in TLE. Another interesting point to consider is the increase in the Michaelis-Menten constant (K_m) for the ATP, main substrate to Na^+,K^+-ATPase, which was selective for Na^+,K^+-ATPase α 2/3 isoforms, and suggests that their affinity for ATP is reduced in the hippocampus of epileptic mice. **References**: 1. Clapcote *et al.*, *Proc Natl Acad Sci USA* v106, p140850, 2009. 2. Dalle-Donne *et al.*, *Clin Chem*. v52, p601, 2006. 3. Joshi *et al.*, *Neurochem Int* v48, p318, 2006. **Financial support**: FAPERGS, Capes, CNPq Animal Ethics Committee license number: 037/2012 and 098/2012

02.028

In vitro **modulation of neuromuscular transmisson induced by riluzole**. Rangel DL[1], Lucho AP[1], Perin AP[1], Porciúncula L[2], Rocha-E-Silva TAA[3], Rodrigues-Simioni L[4], Dal Belo CA[1], Vinade L[1] [1]Unipampa, [2]UFRGS, [3]FCMSCSP, [4]Unicamp

Introduction: Riluzole is currently the main drug for the treatment of Amyotrophic Lateral Sclerosis (ALS). One of the main symptoms of ALS is the general neuromuscular weak associated to the degeneration of central and peripheral motor neurons. Despite Riluzole is thought to stabilize the ASL clinical evolution, the investigation of its direct effect at neuromuscular junctions (NMJ) has been neglected. The aim of this work was to investigate the effects of Riluzole at mouse and avian skeletal NMJ. **Materials and Methods**: Chicks of 1 to 10 days and mice of 25-30g were used. The animals were maintained in 12-h light/dark cycle, with food and water *ad libitum*. The experiments were carried out according to the Ethics Committee for the Use of Animals-CEUA/Unipampa, no. 01/2012. Chick *biventer cervivis* (CBCP) and mouse phrenic-nerve diaphragm (PND) preparations were mounted according to (Dal Belo *et al.*, *Toxicon* 46, 736p, 2005). The muscles were stimulated indirectly by electrical supramaximal pulses of 3-6V (0.1Hz, 0.2ms). The muscles were maintained in Krebs solution, aerated with 5% CO_2/95%O_2 at 37ºC. The preparations were allowed to stabilize during 15min before the addition of treatments, which were recorded for 120 min. The results were expressed as the mean percentage ± S.E.M and statistics were done using ANOVA/MANOVA. **Results**: In both preparations, Krebs solution alone did not change the muscle twitch tension. Riluzole (5,10 and 20μM) induced dose and time responses in both preparations. Thus, for CBCP the concentration of 5μM induced a decrease in the twitch tension (11 ± 0.5%, $p<0.05$, n=6). On the contrary, 10 μM riluzole increased significantly the amplitude of twitches (11 ± 0.2%, $p<0.05$, n=6). At 20μM riluzole there was no change in the muscle tension. The exogenous application of ACh (110 μM) and KCl (20 μM), before and after treatment with 10μM riluzole showed a significative ($p<0.05$) increase in the amplitude of ACh and KCl. At CBCP 10 μM riluzole was able to partially reverse the neuromuscular blockade induced by 10 μM d-Tc (n=3, $p<0.05$). The incubation of CBCP with 0.5 μM neostigmine, 15min previous to 10 μM riluzole, significantly increased the twitch tension (30 ± 1%, n=3, $p<0.05$) when compared with neostigmine (5 ± 0.3%) or riluzole (11 ± 0.2%) alone. At PND preparations, riluzole only induced dose and time-dependent decrease of twitch tension. Riluzole 5, 10 and 20 μM induced 10 ± 1%, 20 ± 1% and 30 ± 0.5% decrease, respectively ($p<0.05$, n=6). **Discussion**: The results indicate that riluzole has different mechanisms of action at peripheral nervous system. The increase in the amplitude of muscle twitch tension associated with the anticurare activity and the summation of anticholinesterase activity of neostigmine suggests a positive interaction with the muscle ACh receptors (Reali *et al.*, *Toxicon* 41, 657p., 2003). The increase in the contracture of KCl also suggests a direct action of the riluzole at the skeletal muscle. **Acknowledgements**: To Capes for financial support to A.P.Lucho.

02.029

Cognitive and emotional benefits of simvastatin and pravastatin in an animal model of Parkinson's disease. Schamne MG[1], dos Santos AFC[2], Latyki C[2], Ferro MM[3], Moretti M[1], Prediger RD[1], Miyoshi E[2] [1]LEXDON-UFSC – Farmacologia, [2]UEPG – Ciências Farmacêuticas, [3]UEPG – Biologia Geral

Introduction: Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*. Besides dopamine, other neurotransmitters system may also be impaired, such as serotonin and noradrenaline, leading to the onset of emotional and cognitive symptoms, such as depression, and learning and memory disabilities. On the other hand, recent studies have demonstrated the neuroprotective properties of statins against different insults in the CNS (central nervous system). Therefore, in the present study we investigated the effects of repeated treatment with simvastatin and pravastatin on the cognitive and emotional deficits observed in an experimental model of PD. **Methods**: This study was approved by the Ethics Committee on the use of animals (Protocol: CEUA-UEPG 3593/2013). Three months old male Wistar rats were divided in control SHAM-operated groups and 6-hydroxydopamina (6-OHDA)-lesioned groups (infused bilaterally with 4 µg of 6-OHDA into the medial forebrain bundle). Animals were treated with vehicle, simvastatin or pravastatin (both 10 mg/kg, p.o.), during 10 days (from 3 days before and until 7 days after 6-OHDA lesion). At 7 and 14 days after 6-OHDA lesion, the short-term memory and depressive-like behavior were addressed, respectively, in the social recognition and forced swimming tests. After behavioral studies, the animals were euthanized for the quantification of striatal tyrosine hydroxylase (TH) levels by Western blotting analysis. **Results**: In the social recognition test, the 6-OHDA + vehicle group showed a higher RID (ratio of investigation duration) in comparison to SHAM + vehicle group ($p \leq 0.001$) indicating social recognition memory impairments. Both simvastatin and pravastatin reduced RID when compared with 6-OHDA + vehicle group ($p \leq 0.05$). In the forced swimming test, the animals of the 6-OHDA + vehicle group showed a reduced swimming time when compared to SHAM + vehicle group [$F(3.49)=4.75$; $p \leq 0.001$] indicative of a depressive-like behavior. This reduction in the swimming time was reversed by treatment with simvastatin or pravastatin. Importantly, at this time no significant alterations on the locomotor activity of the animals were observed. Surprisingly, no significant differences were observed between the groups regarding the striatal TH levels. **Discussion**: The present study provides new evidence of the occurrence of short-term memory impairments and depressive-like behavior in 6-OHDA-lesioned rats that seem not related to dopaminergic neurodegeneration in the nigrostriatal pathway. More importantly, our findings demonstrate the benefits of the repeated treatment with simvastatin and pravastatin against the cognitive and emotional alterations induced by 6-OHDA. **Acknowledgments**: Capes, UEPG, UFSC

02.030

Neuromuscular activity of Jack Bean Urease (JBU) on the nervous system of the cockroach *Nauphoeta cinerea*. Freitas TC[1], Perin AP[2], de Almeida CGM[2], Heberle MA[2], Breda RV[3], Salamoni SD[3], Dal Belo CA[2], da Costa JC[3], Carlini CR[1] [1]UFRGS – Biofísica, [2]LANETOX/Unipampa, [3]InsCer-PUCRS

Introduction: *Canavalia ensiformis* urease (Jack Bean Urease, JBU) displays entomotoxic effects whose mechanism of action is still not fully understood. The aim of this work was to investigate the effects of JBU on the peripheral nervous system of *Nauphoeta cinerea.* **Material and Methods**: Male cockroaches were reared with water and food *ad libitum* (22-25ºC). The biological activity of JBU was accessed using the *in vivo* cockroach coxal-abductor methatoracic muscle preparation (CCMMP) according to (Martinelli *et al*., *BBA Gen*, 1840, 935, 2014) and the *ex-vivo* electrophysiological recordings of cockroach leg sensorial action potentials (LSAP). All protocols were in agreement with the CONCEA. For the LSAP, after anesthesia by chilling, the third methatoracic leg was removed and fixed in a Sylgard-filled petri dish using Ag/AgCl needles connected to a Probe (Axon Instruments, Sunnyvale, CA, USA). The signals were amplified (2000x) using a differential amplifier Axoclamp 2B (Axon Instruments, Sunnyvale, CA, USA) and digitalized (300Hz) using a A/D converter model Digidata 1200A (Axon Instruments, Sunnyvale, CA, USA). JBU was dissolved in PBS buffer and diluted in insect physiological solution consisting of in mM: 150 NaCl; 10 KCl; 4 CaCl2; 2 MgCl2; 4 NaHCO3; 5 HEPES; adjusted to pH 7.2 using HCl (in mM), for the assays. Test solutions were prepared daily and injected into the third abdominal segment of the insect (20 µL) using a Hamilton syringe. Statistics were done using ANOVA/MANOVA or Student "t" test. **Results**: The effect of JBU (0.75, 1.5 and 3µg/animal) at insect neuromuscular junction was time-dependent. The lowest dose induced a transient increase in the twitch-tension, followed by an irreversible neuromuscular blockade, after 120 min recordings (40 ± 2%, n=6, $p<0.05$). At the highest dose assayed, there was a blockage of (65 ± 2% n=6, $p<0.05$) the twitches, at 120min. The insect physiological saline alone did not change the normal twitches baseline. JBU (0.75µg/g) also induced a significative decrease in the frequency of the spontaneous (LSAP) (1425 ± 52, n=5) compared to the control saline (3685 ± 200, n=6, $p<0.05$) in 120min. At this concentration, JBU decreased the mean duration and decay time of (LSAP) (22 ± 2ms and 13,8 ± 2ms at control saline and 1,92 ± 0.02ms and 3,72 ± 2ms JBU-treated, respectively) (n=5, $p<0.05$). **Discussion**: This work shows that the insecticidal activity of JBU may rely, at least in part, on a direct effect on the insect neuromuscular junction. Additionally, the significative decrease in the decay time and duration of spontaneous LSAP suggests a direct influence of the JBU at the insect nerve potassium channels. **Acknowledgements**: The authors thank Edital Toxinologia 063/2010 of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Capes for financial support.

02.031

Determination of amino-peptidergic striatum after the of use 6-styryl-2-pyrone the model convulsion by bicuculline. Nonato DTT[1], Nascimento FLF[1], Maia MLCL[1], Aragão GF[1], Filho JMB[2], Vasconcelos SMM[3], Chaves EMC[1] – [1]ISCB-UECE, [2]UFPB – Tecnologia Farmacêutica, [3]UFC – Farmacologia

The *Lauraceae* family has the *Aniba* gene, that has among its constituents neolignans, pyrones and flavonoids, consisting of 52 species found in the Amazon and Central Guyana. The *Aniba panusirensi* presents a styril pyrone isolated from natural fruit. Studies on genus Aniba have demonstrated anxiolytic, anticonvulsant and depressant actions of bicuculine in the central nervous system (CNS) model. The aim of this study is to identify amino acid – peptidergic – concentrations in the striatum (ST) in mice after seizures induced by bicuculline and pretreatment with the molecule of 6-styryl-2-pyrone (STY). The study was approved by the Ethical Committee of UFC (10/2008). Male Swiss mice (25- 30grams) were used in each experiment, in groups (6-8). The STY was dissolved in Tween 80 by 4% and administered intraperitoneally (i.p.), STY (1, 10 and 20 mg/kg), and the control group. After 30 minutes the bicuculline was administered (12 mg/kg i.p.). The ST was removed so as to determine the concentration of amino acids, aspartate (ASP), glutamate (GLU), glycine (GLY), taurine (TAU) and amino-butyric gamma acid (GABA). All the values were expressed as the mean ± and the standard error of the mean. The ASP increased at the highest dose (STY20: 0.64 ± 0.10) compared to the controls (0.17 ± 0.08), [$F(3,26)=5.890$; $p<0.0039$]. There was an increase in the concentration of GLU (STY20: 0.65 ± 12.15), compared to the controls (0.10 ± 0.01), [$F(3,27)=12.8$; $p<0.001$]. The concentration of GLI increased considerably (STY20: 0.83 ± 12.25), compared to the controls (0.0 ± 0.0), [$F(3,27)=8.669$; $p<0.0004$]. TAU increased in the dose of STY20: 1.22 ± 12.26, compared to the controls (0.01 ± 0.00), [$F(3,26)=8.15$; $p<0.0001$]. The GABA concentration increased in a dose of STY20: 1.20 ± 0.29, compared to the control (0.00 ± 0.00), [$F(3,27)=10.64$; $p<0.0001$]. There wasn't any difference in the concentrations of amino acids in other doses. Pretreatment with STY was able to increase the concentrations of the excitatory amino acids (ASP and GLU) and inhibitory (GLI, TAU and GABA) in the striatum at a dose of 20 mg/kg after the administration of bicuculline. These results suggest that STY has a similar action as the benzodiazepine drugs that activate GABAergic receptors belonging to a family of ion channels, o STY, which promotes increasing opening of the chloride channel, leading to hyperpolarization of neurons and consequently to an inhibitory action. Therefore the peptidergic amino acids involved act via GABAergic and glycinergic. **Acknowledgment**s: This study was supported by grants from the National Council of Scientific and Technological Development (CNPq) and from the Amendment Coordination of High Degree Personal (Capes), Brazil. The authors are grateful to Dr. José Maria Barbosa Filho, Laboratory of Pharmaceutics Technology, UFPB, Brazil, for the experimental drug (ripl) used in this research.

02.032

Nociceptive threshold by combined use of alcohol and tobacco smoke in rats. Bandiera S[1], Nunes EA[1], Santos CF[2], Pulcinelli R[2], Schneider R[3], Torres ILS[1,2], Gomez R[1,2,3] [1]UFRGS – Fisiologia, [2]UFRGS –Farmacologia, [3]UFRGS – Neurociências

Introduction: Although studies point that nicotine administration may reduce pain perception in smokers and alcohol use decreases acute pain threshold in alcoholics, the interaction between alcohol and cigarette smoke on pain perception after acute or chronic combined use remains unknown. **Objective**: Our objective here was to investigate changes on pain threshold in the tail-flick test after acute or chronic combined use of alcohol and tobacco smoke in rats. **Methods**: Forty male Wistar rats (~280 g) were assigned to 4 groups and treated with alcohol (ALC: 2 g/kg by gavage) and/or immediately exposure to the smoke from 6 cigarettes (TBC or ALTB) by inhalation in hermetic cameras along 2 h. Before treatments, rats were tested in the tail-flick to obtain the pain baseline values (TF0). After acute treatment, rats were retested in the tail flick (TF1) and again 24 h from baseline (TF24). Next, they were treated daily, twice a day (at 9 AM and 2 PM) for 28 days (4 g/kg/day alcohol and 12 cigarette smoke/day). In the 28th day, after the morning treatment, rats were tested for chronic effect of alcohol and smoke in the tail flick test (TFC). A two-way ANOVA was performed to detect statistical significance, followed by the Bonferroni test, considering $p<0.05$. (CEUA-UFRGS # 25022) **Results and Discussion**: Our results showed that acute treatment increased the latency to tail flick only in the TBC group (TF1: CTR: 6.4 ± 0.5; ALC: 6.3 ± 0.4; TBC*: 7.6 ± 1.2; ALTB: 7.1 ± 0.8 sec; *P = 0.039), while the ALC group decreased the latency after 24 h from the last administration (TF24: CTR: 6.0 ± 0.6; ALC*: 5.1 ± 0.5; TAB: 5.5 ± 0.5; ALTB: 5.5 ± 1.0 sec; P = 0.031). However, there was no differences in the tail-flick after chronic treatment (TFC). According to our results, combined use of alcohol and tobacco abolished the analgesic effect from the tobacco smoke after acute treatment and decreased the algesic effect from alcohol withdrawal in rats. After chronic treatment, absence of nociceptive effect suggests a desensitization in neurotransmitter systems, such as GABA and glutamate, involved in the pain perception. **Financial support**: CNPq, Capes, Propesq-UFRGS

02.033

From blood to brain: Can graphene oxide nanoparticle cross the blood-brain barrier?

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The major challenge for drug development to treat diseases of the central nervous system is the inability to cross the blood–brain barrier (BBB). Nanotechnology, however, may offer an innovative solution to this problem. In recent years, graphene family has attracted significant interest in the area of biomedicine, as their extremely large surface area can interact with various biomolecules, therefore providing useful as biosensor, drug and gene delivery [1]. Although graphene oxide (GO) is water soluble, GO functionalized with polyethylene glycol (PEG) is more stable in physiological solutions [2]. However, the *in vivo* capacity of GO, whether dispersed in water (GO-H_2O) or PEGylated (GO-PEG), to cross the BBB has not yet been reported. In the present study, we used MALDI imaging mass spectrometry, a powerful analytical tool capable of providing high resolution spatial information of small particles or molecules, to investigate a possible presence of GO-H_2O and GO-PEG into the brain. The brain sections of six-week-old male Wistar rats (*Rattus norvegicus*) were examined 15min, 1h, 3h and 7 days after the systemic administering of 7 mg/kg of GO-H_2O and GO-PEG (CEUA/protocol: 2884-1). In addition, the activation of astrocytes was analyzed through the induction of glial fibrillary acidic protein (GFAP), and neuron viability was examined by neuron-specific nuclear protein (NeuN) expression in the hippocampus, tested by western blot. Both types of GO-based nanoparticles were found in specific regions of the rat brains, including the cortex, hippocampus and thalamus. Although the spatial distribution of GO-PEG and GO-H_2O was similar, clearance of GO-PEG was much faster than GO-H_2O. There was no difference between the expression of GFAP at 15min and 1h in GO-H_2O and GO-PEG-treated rats, however a significant decrease was observed at 3h ($p<0.05$) and 7 days ($p<0.01$) after treatment with GO-H_2O and 7 days after treatment with GO-PEG ($p<0.01$). Contrastingly, expression of NeuN increased significantly at 15min after GO-H_2O ($p<0.05$) treatment and 1h after GO-PEG ($p<0.01$) treatment, and was similar to baseline values at 3h and 7 days. Histologically, astrocytes and neurons show no visible alteration compared to control. Typically, when neurons are injured, GFAP rise rapidly, predicting gliosis occurrence. A decrease in GFAP, although less common, has been reported in response to nicotine treatment, toluene and hormonal serum levels [3]. The significance of the GO-induced decrease of GFAP and its consequence for the nervous system requires further studies aiming at identifying the mechanisms involved. These findings demonstrate that MALDI-MSI has a high spatial resolution that is powerful enough to detect the localization of nanoparticles, confirming the previous hypothesis that GO can cross the BBB, and can therefore be applied to the field of brain drug delivery. **References**: [1] Zhang,Y., *et al. Nanoscale*, 4(13), 3833-42, 2012. [2] Liu, Z., *et al. J. Am. Chem. Soc.*, 130(33), 10876-7, 2008. [3] Malkiewicz, K., *et al. Environ Toxicol Pharmacol.* 21(1), 51-5. 2006. **Acknowledgements**: Fapesp and CNPq for the Financial support.

02.034

Effects of Atorvastatin (Citalor®) on the behavioral sequelae after chronic cerebral hypoperfusion in middle-aged rats. Zaghi GGD, Godinho J, Ferreira EDF, Oliveira RMW, Milani H UEM

Introduction: Chronic cerebral hypoperfusion (CCH) may be involved in the pathophysiology of aging-related dementias, including Alzheimer's disease. CCH risk factors include hypertension, diabetes, dyslipidemia, obesity and cardiopathies, which prevention represents the primary recommendation to reduce the prevalence of those neurodegenerative diseases. Once CCH occurs, however, an important question is whether pharmacological treatment can attenuate the progression of neuropathological and functional sequelae. Statins constitute a class of drugs that, besides its inhibitory properties on HMG CoA-reductase, has been show to increase NO bioavailability that regulates cerebral perfusion and improves endothelial function. Other possible mechanisms include antioxidant properties, and anti-inflammatory effects. Statins may also be protective in acute ischaemic stroke. **Methods**: Naïve rats (12-month old) were trained for 15 days in the aversive radial maze, then subjected to permanent occlusion of the vertebral artery (VA) followed by the occlusion of the internal carotid artery (ICA), according to the sequence VA-ICA-ICA, with a 4-day interstage interval (-). Atorvastatin (10 mg/kg, p.o.) was given since the first occlusion stage up to the end of behavioral testing. Memory retention of previously acquired cognition begun to be tested 15 days after CCH, and continued for 5 weeks, at a rate of 1 session/week. Memory performance was expressed by the parameters 'latency', 'number of reference memory errors', and 'number of working memory errors'. Ethical approval was confirmed by the Protocol 038/2013-CEUA. Kruskal-Wallis ANOVA was used for between-group comparisons. **Results**: In the sham-operated group, memory performance did not change from pre- to post-surgery trails. The groups subjected to CCH and treated with vehicle showed a significant increase in all the three parameters, whatever the testing day ($p<0.01$ to 0.05), indicating that CCH caused persistent retrograde amnesia. This amnesic effect of CCH was completely abolished by atorvastatin ($p<0.005$ – 0.05, vs. vehicle). Supportted by Capes and UEM.

02.035

Hoodia gordonii: Involvement with monoamine system. Citó MCO[1], Santos LKX[1], Fernandes ML, Melo FHC, Silva MIG, Sousa FCF [1]UFC – Fisiologia e Farmacologia

Hoodia gordonii, used as an appetite suppressant, is it consumed in many countries, but there are few studies on this species. Considering this, the present study aimed to investigate the possible central action (forced swimming test) of *Hoodia gordonii* (*HD*) in mice. The project was approved by the Animal Ethics Committees of the Federal University of Ceara through the protocol 05/12. For this, male *Swiss* mice were used in each experiment. *Hoodia gordonii* (extract) was imported from China by Pharma Nostra Comercial LTDA, dilution of 20:1. The extract was administered orally, in the doses of 25 and 50 mg/kg for 1 day. The animals were submitted to the forced swimming test to investigate the possible effects induced by this substance. The results are presented as mean ± SEM. Data were analyzed by ANOVA followed by Newman Keuls as *post hoc* test. Values were considered statistically significant at $^{a}p<0.05$, compared to the control, and $^{b}P<0.05$ compared to the *Hoodia gordonii*. It was observed that *HD*25 mg/kg (38.00 ± 3.53*[8]) and 50 mg/kg (42.13 ± 4.24*[8]), decreased the immobility time, as compared to the control (70.17 ± 7.24 [8]), suggesting an antidepressant-like effect. Additionally, it was also assessed if this antidepressant-like effect of *HD* in mice was linked with the monoaminergic systems. The results showed that, the antidepressant-like effect of *HD*50 mg/kg was prevented by pre-administration of noradrenergic (yoimbine and prazosin)[control: 87.75 ± 12.83 (8); *HD*: 46.00 ± 11.48^{a} (8); PZS: 111.7 ± 6.85 (8); *HD* + PZS: 83.89 ± 9.31^{b}(8); YOHI: 109.8 ± 4.32(8); *HD* + YOHI: 70.89 ± 6.00^{b}(8)], serotonergic (PCPA, NAN, ondansetron and ritanserin)[control: 98.14 ± 16.16 (7); *HD*: 42.13 ± 4.24^{a}(8); PCPA: 136.30 ± 6.48(8); PCPA + *HD*: 118.10 ± 13.78^{b}(7); NAN: 51,75 ± 13,03; NAN + Hoodia: 100,30 ± 19,69; ondansetron: 103.10 ± 15.43 (7); ondansetron + *HD*: 81.83 ± 11.13^{b}(7); ritanserin:102.00 ± 13.53(7); ritanserin + *HD* 103.00 ± 12.08^{b} (7); fluoxetine: 45.64 ± 3.01^{a}(8); fluoxetine + PCPA: 107.50 ± 7.54(8)] and dopaminergic antagonists (sulpiride and SCH 23390) [Control: 91.63 ± 15.44 (8); *HD*:42.13 ± 4.24^{a} (8); sulpiride: 75.15 ± 10.31 (7); sulpiride + *HD*: 83.14 ± 5.79^{b} (8); SCH: 75.90 ± 11.51 (8); SCH + *HD*: 87.43 ± 6.18^{b} (8)]. In conclusion, *Hoodia gordonii* presented antidepressant-like effects in the forced swimming test, and this effect seems to be dependent on the interaction with the monoaminergic system. **References**: Van Herdeen, *J Ethnoparmacol*, 119, 434, 2008. Melo, *Fund Clin Pharmacol*, 27, 104, 2013. **Financial support**: Capes, CNPq and FUNCAP.

02.036

Antipsychotic and antidepressant-like effects of Riparin I in the corticosterone-induced depression model in mice. Oliveira ICM[1], Vasconcelos AV, Vidal LMT, Castro LA, Lopes IS, Rodrigues GC, Lima AEL, Sousa PB, Pontes MCD, Sousa FCF UFC – Physiology and Pharmacology

Introduction: Psychotic Major Depression is a difficult-to-treat illness that is associated with high functional impairment and that has significantly higher mortality than nonpsychotic major depression. Traditional treatment has been combination of an antipsychotic and a tricyclic antidepressant but response rates with this regimen are only between 30% and 40% [1]. In this context, natural products can represent a potential source of new therapeutic agents. Riparin I (Rip I), one alcamide isolated from *Aniba riparia*, has presented promising Results: In acute stress behavioral models, it has unchained predictive effects of antidepressant and anxiolytic activities [2]. Facing that, the goal of the trial was to investigate the activity of Rip I in mice exposed to corticosterone-induced depression model, suggested by Iijima *et al*., 2010 as supposed model of psychotic depression [3]. **Methods**: The project was approved by the Animal Ethics Committees of the Federal University of Ceara through the protocol in 58/2013. Swiss female mice were used, weighing 22-25 g, divided according to the following experimental groups: Control group (Vehicle1: saline containing 0.1% dimethyl sulfoxide and 0.1% Tween-80, sc, for 14 or 21 days and Vehicle 2: distilled water emulsified with 2% Tween-80, orally, for 8 days); Stressed group (corticosterone (cort), 20 mg/kg, sc, during 14days or 21 days + Vehicle 2, orally, for 8 days) Rip I group (50 mg/kg, po for 8 days) and Fluvoxamine group (Flu) (50 mg/kg, po for 8 days). Positive psychotic symptoms (prepulse inhibition and locomotor activity) and negative (social interaction) and depressive symptoms (hopelessness and anhedonia) were evaluated. Treatments were made from the 14th of stress by corticosterone and remained concomitant to it for 8 days. Behavioral tests occurred in 14th, 21st and 22nd days of treatment, 1 hour after administration of the last substance. Statistical analysis of the data was performed by one-way ANOVA, followed by Newman-Keuls test. Data are expressed as mean ± SEM and, in parentheses, number of animals per group. **Results**: Both administrations of Rip I and Flu significantly reversed the impairment in pre-pulse inhibition caused by corticosterone in prepulse intensities of 70 and 75 dB ($P < 0.001$) and reduced the immobility time in the forced swimming test after 21 days [Control: 41.18s ± 7.498 (11); Cort: 153.5s ± 7.940 (12); Cort + RipI: 39.73s ± 4.317 (11), $p<0.001$; Cort + Flu: 70.59s ± 6.716 (17), $p<0.001$]. **Discussion**: The results indicate the potential antidepressant and antipsychotic effects of Rip I in the corticosterone-induced depression model in mice and these effects are similar to the Flu that has shown high response rate in clinical. This experimental evidences opens perspectives for further studies that may lead to future therapeutic use of Rip I in the treatment of Psychotic Major Depression. **Financial support**: CNPq, Capes, Funcap. **References**: [1] Stahl, Stephen M. CNS Spectr. 10(4):319. 2005. [2] Oliveira, Iris C. M. Dissertation. Federal University of Ceará. 2012. [3] Iijima, Michihiko. *Brain Res* 1359: 75. 2010.

02.037

Catecholaminergic inputs to the retrotrapezoid nucleus. Oliveira LM[1], Moreira TS[2], Takakura AC[1] [1]USP – Farmacologia, [2]USP – Fisiologia

The rat retrotrapezoid nucleus (RTN) contains chemoreceptor neurons with cell bodies located in the region defined as the marginal layer (ML) (Stornetta at al., 2006; Takakura *et al.*, 2008). Evidences suggest that the RTN is the site of the major neuroanatomic defect in Congenital Central Hypoventilation Syndrome (CCHS). Previous study has shown that there are numerous and often excitatory catecholaminergic terminals in the ML, demonstrating catecholaminergic innervations within the RTN (Rosin *et al.*, 2011). Application and action of catecholamines (noradrenaline) in other neural regions is controversial for respiratory control (Viemari & Hilaire, 2002). Depending on catecholaminergic effect in RTN, it could be a target for CCHS treatment. The aim of this study was to identify the source of those catecholaminergic innervations to RTN. The retrograde tracer FluorGold (2%) was injected into the RTN in adult male Wistar rats (250-300g, n = 5). The animals were sacrificed 7 days later, perfused and the brains were removed for immunohistochemistry process to tyrosine hydroxylase (TH), a limiting enzyme in the synthesis of catecholamines. Procedures were in accordance with Animal care and use committee (Approval number 14, p. 15, b. 3). The results showed that there are projections from several catecholaminergic nucleus to RTN. We found that 8.6 ± 2.0% of TH^+-A1/C1 neurons, 13.0 ± 1.4% of TH^+-A2/C2 neurons and 9.7 ± 2.0% of TH^+-A5 neurons project to RTN. There was no projection from TH^+-A6 neurons to RTN. We concluded that A1/C1, A2/C2 and A5 regions in the brainstem are some of the catecholaminergic sources to RTN. More catecholaminergic nucleus need to be investigated, and functional activity of catecholaminergic inputs need to be assessed. **Financial support**: Fapesp, CNPq, Capes/PROEX

02.038

Trans fat supplementation crossover two generations modifies lipid profile in brain areas and predisposes to amphetamine preference. Kuhn FT, Bürger ME, Roversi Kr, Schuster AJ, Metz VG, Rosa HZ UFSM – Fisiologia e Farmacologia

Introduction: Fatty acids (FA) are constituents of the CNS and polyunsaturated FA (PUFA) are easily incorporated into neural membrane phospholipids, providing more fluidity and permeability to it (Jump, 2002). Studies have shown that dietary n-3 essential FA (EFA), which are precursors of long chain polyunsaturated FA (LC-PUFA), are able to decrease anxiety-like symptoms in rodents (Buydens-Branchey, 2006), while *trans* FA (TFA) may increase the rigidity of these neural membranes, impairing the brain neurotransmission. Amphetamine (AMPH) is a psychostimulant drug involved in addiction, and their use has been related to neurotoxicity (Iacovelli, 2006). Experimentally, this drug increases locomotor activity in rodents, showing solid additive properties. Chronic use of AMPH causes dependence, which is characterized by development of anhedonia, anxiety, social isolation and depression following its withdrawal. We hypothesized that *trans* fat consumption crossing two generations allows the TFA incorporation in brain, thus facilitating the AMPH-preference in rats. **Methods**: Female adult rats (n=24) were orally supplemented once a day (3g/kg) with fish oil (FO, rich in n-3 FA), hydrogenated vegetable fat (HVF, rich in TFA) or soybean oil (SO, rich in n-6 FA). Supplementations were initiated at pregnancy and maintained during lactation and growing (until 40 days old). While male from 1st generation were exposed to experimental protocol (n=8), female rats were maintained in the same supplementation until adulthood, when they were mated (same protocol described for 1st generation). Male pups (40 days old) from 2nd generation were also submitted to experimental design. The experimental protocol was approved by the Animal Ethical Committee (UFSM-UFSM-24/2010). Rats from 1st and 2nd generations were conditioned with AMPH (DL-amphetamine 4 mg/mL/kg; once a day) in conditioned place preference (CPP) paradigm (Thanos *et al.*, 2010) for 8 consecutive days. The incorporation of the FA was quantified by gas chromatography (Hartman & Lago, 1973). **Results/Discussion**: SO and HVF supplemented rats showed increased AMPH-CPP in relation to their respective groups of 1st generation, while FO group of 2nd generation showed lower AMPH-preference. We suggest that these behavioral changes may be closely related to different hippocampal PUFA incorporation, since that comparisons between the two generations showed an increased LA incorporation in SO and HVF groups (16 and 45.9%, respectively), and an increased TFA incorporation (185%) in HVF group, in comparison to the 1st generation. In addition, a decreased DHA incorporation (8%) was observed in SO group. FO supplementation was related to reduce incorporation of total n-6 PUFA (7.3%), together with a lower n-6/n-3 PUFA ratio (12.2%). **Conclusion**: The higher LA incorporation in both SO and HVF, as well as the increased TFA incorporation in HVF supplemented rats from 2nd generation were sufficient to modify the fluidity of neural membranes thus affecting the neurotransmission, which was increased by AMPH-treatment. In this sense, these animals showed higher AMPH-preference. On the other hand, FO group was related to lower n-6/n-3 PUFA ratio, which was related to minor AMPH-preference. We hypothesized that the chronic consumption of processed foods through the generations may facilitate drug addiction due to different FA composition in the membrane phospholipids. **Acknowledgements**: CNPq, Capes (by research fellowships), Herbarium® (by donation of fish oil capsules

02.039

Infusion of neuropeptide Y into dorsal hippocampus disrupt the memory reconsolidation for contextual fear conditioning. Linartevichi VF, Lima TCM UFSC – Farmacologia

Introduction: The encoding of emotional memories may occur inappropriately resulting in pathological conditions such as the Post-Traumatic Stress Disorder (PTSD). The potential relevance of manipulations of memory reconsolidation for the treatment of mental disorders such as PTSD is acknowledged. Also, the role of Neuropeptide Y (NPY) in experimental anxiety has been demonstrated, even though its role in mnemonic processes still remains unclear. **Methods**: We investigated the effects of NPY infused into the dorsal hippocampus of adult male Wistar rats (300-350 g, 14-16 weeks-old) tested in an aversive memory reconsolidation paradigm. All procedures were approved by the Institutional Ethical Committee for the Care and Use of Laboratory Animals of the UFSC (PP798). One week after bilateral guide cannula implantation, contextual fear conditioning was performed and freezing was the parameter evaluated. Each animal was placed in a Context A for 3 min, during five consecutive days, as following: familiarization, conditioning session (3 electrical footshocks of 1.0 mA), reactivation (without unconditioned stimulus presentation) followed by intra-hippocampal infusion of NPY (0.6 uL / side / min), Test A and Test B (Context B). To access anxiety-like levels an independent group of rats was infused with NPY 10 min prior the evaluation in the Elevated Plus-Maze (EPM) test for 5 min. For statistical analysis only rats that presented successful bilaterally surgery were considered. **Results**: One-way ANOVA revealed no significant difference ($F_{4,30}$=0.17 P=0.94 n=7) between groups during the reactivation session. During Test A, one-way ANOVA showed significant differences ($F_{4,30}$=3.54 P=0.017) between groups [(mean ± SEM) Vehicle=79.7 ± 7.8 n=7; NPY 1 pmol=74.2 ± 9.1 n=7; NPY 3 pmol=32.6 ± 6.5 n=7; LP-NPY (Y1 selective agonist) 1 pmol=84.7 ± 8.2 n=7; ANI (Anisomycin – positive control) 80 ug=40.1 ± 7.5 n=7]. Newman-Keuls post hoc test showed that NPY-3pmol and ANI groups expressed statistical (p<0.05) less freezing compared to other groups. Statistics showed no difference on freezing behavior ($F_{4,30}$=0.7 P=0.59) when memory reactivation was omitted. Moreover, statistics showed no difference on freezing behavior ($F_{4,30}$=0.05 P=0.99) when exposed to Context B. On EPM, one-way ANOVA showed no statistical difference between groups for open-arm time exploration ($F_{4,30}$=2.09 P=0.10 n=7) (anxiety index) and enclosed-arms entries ($F_{4,30}$=0.17 P=0.94 n=7)(locomotor activity index). **Discussion**: Altogether, our results suggest that the infusion of NPY into dorsal hippocampus may impair aversive memory reconsolidation, without interfere with anxiety levels nor locomotor activity. **References**: Decressac M, Exp Neurol, 238:265, 2012; Lach G, Neurobiol Learn Mem, 103:26, 2013; Parsons RG, Nat Neurosci, 16:146, 2013; Sah R, *Biol Psychiatry*, 66:705, 2009. **Financial support**: Capes, CNPq, UFSC.

02.040

Potential anxiolytic-like effect of riparin I in corticosterone-induced depression model in mice. Vidal LMT, Oliveira ICM, Vasconcelos AS, Castro LA, Rodrigues GC, Alencar RN, Lima AEL, Sousa FCF UFC – Fisiologia e Farmacologia

Introduction: Data supporting treatment selection for anxiety disorders comorbid with depression are limited and specific to the type of anxiety disorder. Antidepressants are now first-line treatments for anxiety disorders, with or without comorbid depression. However, high doses are required for efficacy when an anxiety disorder is present and the medication should be titrated slowly to efficacy [1]. In this context, natural products can represent a potential source of new therapeutic agents. Riparin I (rip I), an alcamide isolated from *Aniba riparia*, has presented promising Results: in acute stress behavioral models, it has caused predictive effects of antidepressant and anxiolytic-like activities [2]. Facing that, the goal of the trial was to investigate we investigated the potential anxiolytic and antidepressant-like effects of rip I in mice exposed to corticosterone-induced depression model. The project was approved by the Animal Ethics Committee of the Federal University of Ceará through the – protocol 58/2013. **Methods**: Swiss female mice, weighing 22-25 g, were used and divided according to the distributed in following experimental groups: Control group (Vehicle1: saline containing 0.1% dimethyl sulfoxide and 0.1% Tween-80, sc, for 14 or 21 days and Vehicle 2: distilled water emulsified with 2% Tween-80, orally, for 8 days); Stressed group (CORT, 20 mg/kg, sc, during 14days or 21 days + Vehicle 2, orally, for 8 days); rip I group (50 mg/kg, po for 8 days) and fluvoxamine group (flu) (50 mg/kg, po for 8 days). The mice were exposed to the behavioral: Open Field, Plus Maze, Forced Swimming and Tail Suspension tests. Statistical analysis of the data was performed by one-way ANOVA, followed by Newman-Keuls test. Data are expressed as mean ± SEM and number of animals per group. **Results**: Both administrations of rip I and flu significantly reduced the immobility time in the Tail Suspension test after 21 days [Control: 56.33s ± 5.060 (15), $p<0.001$; Cort: 143.3s ± 6.813 (14); Cort + Ripl: 59.19s ± 5.723 (16), $p<0.001$; Cort + Flu: 53.69s ± 5.230 (16), $p<0.001$]. Chronic administration of corticosterone for 22 days decreased the parameters of anxiety of stressed animals compared to control animals. On the other hand, the administration of rip I or flu for 8 days, prevented the decrease of these parameters in the groups of animals subjected to these treatments [Time of permanence in the open arms: Control: 55.33 ± 5.471 s (15), $p<0.01$; Cort: 34.58 ± 3.630 s (12); Cort + Rip I: 60.50 ± 6.193 s (14), $p<0.01$; Cort + flu + : 59.93 ± 4.685 s (14), $p<0.01$]. **Discussion**: The administration of rip I and the flu triggered anxiolytic and antidepressant-like effects at the same dose. In clinical, serotonin and norepinephrine reuptake inhibitor are preferred due to their greater tolerability and safety profiles, but flu is not among the most prescribed. This experimental evidence opens perspectives for further studies that may lead to future therapeutic use of rip I in the treatment of anxiety disorders comorbid with depression.

02.041

Intranigral 6-hydroxydopamine and intraperitoneal rotenone exposures cause hypolocomotion and anxiolytic-like effect in male Wistar rats. Vieira JCF, Bassani TB, Zanoveli JM, Vital MABF UFPR – Farmacologia

Introduction: Anxiety in Parkinson's disease (PD) may represent a psychological reaction to the development of other symptoms during the progress of the disease, but there is increasing evidence that anxiety disorders may be related to neurochemical changes in PD. Over the past 30 years an increasing number of studies have been devoted to the investigation of anxiety in both PD patients and animal models of PD. However is necessary more data involving validation of methods that can feature anxiety in animal models of PD (Prediger *et al.*, 2012). **Methods**: Male Wistar rats from our breeding colony weighing 280–320 g at the beginning of the experiments were used. Our protocol complies with the recommendations of University Federal of Paraná and was approved by the University Ethics Committee (Protocol No.697). 40 rats were distributed randomly into 4 groups: sham, 6-hydroxydopamine (6-OHDA), rotenone and its vehicle (sun flower oil). 6-OHDA (6 mg/ml) dissolved in artificial cerebral spinal fluid (aCSF) was injected bilaterally into the SNpc. The sham group received only (aCSF). The rotenone group received daily intraperitoneal injections of rotenone (2,5 mg/kg) dissolved in sun flower oil for 10 days. The Open-Field Test was conducted at 1 and 21 days after the end of rotenone exposure and the intranigral injections of 6-OHDA. The same animals were tested in the Elevated T Maze (ETM) test 21 days after exposure to both neurotoxins. **Results and Discussion**: In our experiments, 1 day after exposure to the neurotoxins, locomotion frequency was reduced in the rotenone and 6-OHDA groups compared with the control and sham groups ($P<0.05$), on the other hand 21 days after the neurotoxins, locomotion frequency was reestablished, suggesting a recovery of motor function. Furthermore in ETM we observed that both rotenone and 6-OHDA-treated animals spent more time in the open arms compared with sham and control groups ($P<0.05$). These results suggest an anxiolytic effect of both neurotoxins. Considering rotenone, there are no previous studies showing a similar effect. With regard to 6-OHDA, some studies are controversial by using different methodologies. Some authors claim that anxiety does not seem to be a common feature of this PD model (Carvalho *et al.*, 2013) and in a study of Branchi *et al.* (2008) the 6-OHDA injury produced a 36% loss of striatal dopamine levels and a reduction in anxiety-like behavior in rats. This feature is not entirely understood, but it seems to be related to the serotonin content in brain areas such as periaqueductal gray matter (Graeff and Zangrossi, 2010). **Financial Agencies and acknowledgments**: This work was supported by grants from CNPq and Capes that had no further role in the study design. **References**: Branchi, I. *Neurosci Res*, v.86, p.2050, 2008. Carvalho, MM *Mol Neurodegener*, v.8, p. 11, 2013. Graeff, FG, Zangrossi, H. Jr. *Cent Nerv Syst Agents Med Chem*, v. 10, 2010. Prediger, RDS Neuropharmacology, v. 62, p. 115, 2012.

02.042

The effects of a single acute dose of carvacrol on monoamine levels in mice striatum.

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Carvacrol (CVC) is a monoterpenic phenol present in the essential oil of many aromatic plants (AZIZI *et al.* 2012). Previous studies have reported that CVC has anxiolytic (MELO *et al*, 2010) and antidepressant (MELO *et al*, 2011) effects in mice, when administered orally, with involvement of GABAergic transmission and the doparminergic system, respectively. The present work was undertaken to investigate the effects of single acute dose of CVC by measuring the levels of monoamines and their metabolites in mice striatum using high performance liquid chromatography (HPLC). Animals were treated in accordance with the current law and the National Institutes of Health Guide for Care and Use of Laboratory Animals. The protocols of the experiments were approved in the Ethical Committee on Animal Research number 04/2012 at Federal University of Ceará, Brazil. Carvacrol was administered orally in mice at single doses of 25 and 50 mg/kg. For determination of monoamine levels, control and CVC groups (25 and 50 mg/kg) were sacrificed 1h after treatment, and the striatum was dissected on ice. Concentrations of noradrenaline (NE), dopamine (DA), serotonin (5-HT), and their metabolites 4-hydroxy-3-methoxy-phenylacetic acid (DOPAC), homovanilic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were detected electrochemically using HPLC.The results are presented as mean ± S.E.M. Data was analyzed by ANOVA followed by Student-Newman-Keuls's post hoc test. Results were considered significant at $P<0.05$. A significant increase was observed in DA levels in both doses compared to control: DA [Control: 1.089 ± 0.4135(8);CVC-25: 5.598 ± 1.384(8);CVC-50: 5.954 ± 10.8468(8)]. There was also an increase in 5-HT and DOPAC levels, but this increase was not significant compared to control; 5-HT [Control: 2.148 ± 0.5730(8);CVC-25: 4.810 ± 1.017(8);CVC-50: 4.976 ± 1.455(8)]; DOPAC [Control: 3.755 ± 0.92(8);CVC-25: 5.470 ± 0.6968(8);CVC-50: 4.897 ± 1.088(8)]. There were no significant changes in NE, HVA and 5-HIAA levels. These results are consistent with the hypothesis that the antidepressant activity of carvacrol in the study described above for (MELO, 2011) may be dependent on an increase in dopamine levels in the CNS. **Financial support**: FUNCAP, Capes, CNpQ. **References**: Azizi Z, Ebrahimi S, Saadatfar E, Kamalinejad M, Majlessi N. Cognitive-enhancing activity of thymol and carvacrol in two rat models of dementia. *Behav Pharmacol* 2012; 23(3):241-9. Melo FHC, Venâncio ET, de Sousa DP, de França Fonteles MM, de Vasconcelos SM, Viana GS *et al.* Anxiolytic-like effect of Carvacrol (5-isopropyl-2-methylphenol) in mice: involvement with GABAergic transmission. *Fundam Clin Pharmacol* 2010; 4:437-43. Melo FHC, Moura BA, de Sousa DP, de Vasconcelos SM, Macedo DS, Fonteles MM *et al.* Antidepressant-like effect of carvacrol (5-Isopropyl-2-methylphenol) in mice: involvement of dopaminergic system. *Fundam Clin Pharmacol*. 2011; 25(3):362-7.

02.043

Acute postnatal administration of methylmalonate provokes memory deficit in mice: involvement of inflammatory and apoptotic markers. Funghetto MP[1], Gabbi P[2], Ribeiro LR[2], Cardoso A S[1], Haupental F[1], Fighera M R[3] [1]UFSM – Bioquímica do Exercício, [2]UFSM – Farmacologia, [3]UFSM – Neuropsiquiatria

Introduction: The methylmalonic acidemia is an inborn error of metabolism (IEM) characterized by methylmalonic acid (MMA) accumulation in body fluids and tissues, causing neurological dysfunction, mitochondrial failure, oxidative stress and neuroinflammation (2, 3, 5). Although neurological evidence demonstrate that infection and/or inflammation mediators facilitate metabolic crises in patients, the involvement of neuroinflammatory processes in the neuropathology of this organic acidemia is not yet established. **Methods**: In this experimental study, a single intracerebroventricular (i.c.v.) dose of MMA (2.5 μmol/g) was administered at postnatal day 0 (P0) to induce an acute, transient rise of MMA levels in the central nervous system (CNS). In the following days (21^{st} – 33^{th}) animal behavior was assessed in the radial maze test (6). The animals were euthanized and cerebral cortex was removed and homogenized for the following analyzes: measurement of necrosis factor-alpha (TNF-α) levels (5), acetylcholinesterase (Ache) activity (1) and caspase levels (4). Data were analyzed by analysis of variance (ANOVA) or Mann-Whitney test when appropriated. **Results**: Behavioral tests showed that animals injected with MMA had a reduction in the working memory test [$F(1,32)= 42.00$, $p<0.05$], but no in the reference test [$F(1,32)= 0.30$; $p>0.05$]. Furthermore, MMA increased levels of TNF-α ($t =13.71$, $p<0.001$,), Ache activity [$t = 5.9$, $p<0.0001$] and activation of caspases 1, 3 and 8 ($t = 25.54$; $t=38.6$; $t=57.9$; $p<0.0001$) in cerebral cortex.

Conclusions: The overall results indicate that MMA increased pro-inflammatory and apoptotic markers in the cerebral cortex and these coincided with the memory deficit found in young mice. **References**: 1. Ellman *et al*., *Bioch Pharmac*, v7, p88, 1961. 2. Fenton, The Metabolic Bases of Inherited Disease, MacGraw – Hill, New York, 1995. 3. Kolker *et al*., J *Inherit Metab Dis*, v23, p355, 2000. 4. Peres and Curi, "Como Cultivar Células." v1, p3, 2005. 5. Ribeiro *et al*., *Immunobiology*, v218, p1175, 2013. 6. Schmitt, *et al*., *J Neurosc*, v23, p3953, 2003. Animal Ethics Committee license number: 112/2010. Work supported by CNPq and Capes.

02.044

Repeated intranasal administration of the fungicide Ziram induces motor deficits in adult mice: support for the olfactory vector hypothesis of Parkinson's disease. Mack JM[1], Schmitz AE[2], Scarpa P[1], Souza LF[2], Dafre AL[2], Prediger RDS[1] [1]UFSC – Farmacologia, [2]UFSC – Bioquímica

Introduction: Parkinson's disease (PD) is characterized by motor symptoms that are related with the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta and consequent striatal dopamine depletion. The etiology of PD is still unknown, but probably it involves genetic and environmental factors, including agricultural chemicals, toxins and dietary nutrients (Prediger *et al.*, Neurotox Res, v. 21, p. 90, 2012). The presence of smell loss and the pathological involvement of the olfactory pathways in the early stages of PD are in accord with the tenants of the olfactory vector hypothesis (Braak *et al.*,Cell Tissue Res, v. 318, p. 121, 2004). Ziram (zinc dimethyldithiocarbamate) is a fungicide widely used in crop protection with activity of inhibiting the ubiquitin proteasome system (UPS) and it has been associated with an increased risk of developing PD (Chou *et al.*, The *J Biol Chem*, v. 283, p. 34696, 2008). In the present study we investigated the effects of repeated intranasal (i.n.) administration of NaDMDC (sodium dimethyldithiocarbamate), a more soluble salt of dimethyldithiocarbamate, on motor performance and antioxidant enzymes activities on brain areas in mice. **Methods**: All procedures were approved by local Ethical Committee in Animal Research (CEUA PP830/2012). Male Swiss mice (3 months-old) were lightly anaesthetized with isoflurane (0.96%) and the NaDMDC (1 mg/nostril) or vehicle (NaCl 0.9%) were administered by i.n. route during 4 consecutive days. The animals were submitted to a battery of behavioral paradigms after the last day of NaDMDC administration that included the neurological severity score (NSS) (3^{rd} and 6^{th} days), open-field (4^{th} day), grip strength (5^{th} day) and rotarod (7^{th} day) tasks. In the 8^{th} day after treatment, animals were euthanized and the hippocampus and striatum were collected for evaluation of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) activities. **Results and discussion**: The i.n. NaDMDC administration caused significant motor deficits in mice. In the NSS test, the animals administered with NaDMDC demonstrated a worse general neurological state ($p<0,05$). In the rotarod, grip strength ($p=$ and open-field tests, the i.n. NaDMDC administration significantly reduced the coordination, strength and locomotion, respectively ($p<0,05$). The activities of the GPx, GR and GST in the hippocampus and striatum were not altered by the administration of NaDMDC. These results are in accordance with literature showing that this class of fungicides does not cause their neurotoxicity via oxidative stress. Therefore, the motor deficits observed may be associated with other mechanisms including the inhibition of UPS and α-synuclein accumulation in motor areas. In conclusion, our data demonstrated that i.n. administration of NaDMDC causes motor deficits in male Swiss mice without modifying the activity of antioxidants enzymes on glutathione system. These results suggest that the NaDMDC may represent an important tool for PD study in animal models. Finally, the current findings reinforce the olfactory vector hypothesis that postulates that some forms of PD may occur due environmental agents that enter the brain via olfactory mucosa. **Financial support**: CNPq, Capes, FAPESC (PRONEX – Project NENASC), FINEP (IBN-Net #01.06.0842-00), UFSC.

02.045

Tactile stimulation and neonatal isolation modify oxidative status during cocaine abstinence in young rats. Antoniazzi CTD, Roversi KR, Dias VT, Bürger ME UFSM – Fisiologia e Farmacologia

Introduction: Neonatal isolation (NI) is an animal model of early life stress, which has been related to facilitation for acquisition, maintenance and relapse to psychostimulant drugs in adulthood (Imanaka, 2008). Tactile stimulation (TS) is closely related to maternal liking and care acting as "enrichment" for the developing brain. Thus, TS has emerged as a tool able to recover deficits in the neonatal period and prevent effects of NI (Lovic, 2006). This study aimed to evaluate a possible influence of distinct forms of neonatal handling such as TS and NI on oxidative status during cocaine abstinence in young rats. **Methods**: Animal Ethical Committee (UFSM-106/2010), affiliated to the Council for Control of Animal Experiments (CONCEA), approved the experimental protocol. After delivery (PND1) of female Wistar rats, male pups were assigned in one of four groups (n=14): unhandled (UH, not touched), tactile stimulation (TS), in which pups were individually held and stroked with the index finger on the dorsal surface for 10min (Boufleur, 2012), neonatal isolation 10min (NI_{10}), and 60min (NI_{60}), in which pups were put in an individual plastic box for 10 or 60 min, respectively. Neonatal procedures were applied daily from PND1 to PND21 and UH group remained in their nest without any touch by human hand. Pups were weaned (PND22) and left undisturbed until PND40, when half of each group (n=7) received cocaine (20 mg/kg, i.p) or vehicle (0.9% NaCl, i.p) for 10 days. After 96h from the last cocaine or vehicle administration, all animals were anesthetized with pentobarbital (80 mg/kg body weight i.p) and euthanized for tissues removal. Brain areas (cortex, striatum and hippocampus) were removed, dissected, and used for protein carbonyl (PC) levels and catalase (CAT) activity determination. Data were analyzed by two-way ANOVA followed by Duncan's test ($p<0.05$). Results were expressed as mean ± S.E.M. **Results**: In vehicle-treated animals, NI_{10} *per se* showed increased PC levels and reduced CAT activity in cortex, while in TS group were observed decreased CAT activity in cortex. NI_{60} exposure *per se* was associated with increased PC levels in both cortex and hippocampus, and increased CAT activity in striatum and hippocampus. In cocaine-treated animal, TS decreased PC levels in cortex, while NI_{60} increased this oxidative marker in striatum and hippocampus. CAT activity was increased by TS in all evaluated brain areas, while UH, NI_{10} and NI_{60} showed similar CAT activity to each other. **Discussion**: These findings suggest that while NI_{60} is able to stimulate the HPA axis and release monoamines, which are vulnerable to auto-oxidation and generation of pro-oxidant metabolites, TS is negatively related to these events, offering protection against oxidative damage in brain tissues. In this scenario, TS was able to stimulate the antioxidant defense system and attenuate the cocaine-induced oxidative damage, confirming the protective role of TS through lower oxidative damage to proteins, even in cocaine-treated rats. **Financial support**: FAPERGS/ PROAP/ PRPGP–UFSM. **Acknowledgements**: The authors are grateful to Capes and CNPq by their fellowships. **References**: Boufleur, N. *Brain Res* 1474:50. 2012. Imanaka, A. *Behav Brain Res* 186:91. 2008. Lovic, V. *Pharm Biochem Behav* 84: 497. 2006.

02.046

Chronic ethanol intoxication exacerbates the losses generated by cerebral ischemia.

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Stroke is the second largest cause of death in the world, with 87% of deaths due to ischemic processes. Chronic ethanol consumption contributes directly to cerebral ischemia by worsening the neurological deficits. Although clinical cases combining both pathologies are relatively frequent, there are no data available from animal models. Thus, this study aims to analyze the damage caused by ischemia in the motor area of chronic alcoholic individuals. Wistar female rats (10) were chronically exposed to ethanol (6.5 g/kg/day) from puberty to adulthood (ethics committee – BIO007-09). By the end of the intoxication treatment, focal ischemia was induced in the motor cortex with endothelin-1 (ET-1), and seven days later the animals were submitted to the open field, inclined plane and rota rod tests. After that, five animals underwent perfusion and their brains were collected for histopathological analysis of neuronal death through (anti-NewN), astrocytic (anti-GFAP) and microglial/macrophage (Anti-ED-1) activation. The brains of five non-perfused animals were immediately dissected and the cortex area was collected for further nitrite levels and lipid peroxidation evaluation. Chronic ethanol administration promoted significant losses on motor function, mainly on balance and motor strength. In addition, a reduction in cell density, number of NewN + cells in the motor cortex, and elevation of ED1 + and GFAP + cells were also noted. The chronic intoxication with ethanol also lead to increased levels of lipid peroxidation. The focal ischemic process stimulated ambulation reduction, as well as reduced tolerance for elevated angles, and reduced time to fall during the first cycle of the rota rod test, followed by recovery in the second cycle. Ischemia also induced significant loss of NewN + cells, intense increase of ED1 + and GFAP + cells, and increased levels of nitrite and lipid peroxidation. The interaction between stroke and ethanol intoxication provoked a more expressive decrease in ambulation and potentiated deficit in the first rota rod cycle, which persisted in the second. A partial recovery was noted in the third cycle. The alcoholism also potentiated lipid peroxidation generated by stroke. Our results revealed that chronic alcohol intoxication exacerbates motor deficit and tissue damage in animals subjected to focal ischemia. This process seems to be associated with microglial/astroglial activation and oxidative stress (lipid peroxidation and induction of nitric oxide synthesis). **Support**: Fundação Amazônia Paraense de Amparo à Pesquisa – Fapesp

02.047

Control of breathing automaticity by activation of the retrotrapezoid nucleus/parafacial region in adult anesthetized rats. Lucena EV[1], Takakura AC[2], Moreira TS[1] [1]USP – Physiology and Biophysics, [2]USP – Pharmacology

Introduction: It is well established that the parafacial region (pFRG) of the medulla oblongata regulates breathing. This heterogeneous region also includes the retrotrapezoid nucleus (RTN), a group of glutamatergic, noncatecholaminergic Phox2b-expressing neurons that contribute to central respiratory chemoreception. The RTN neurons recruit inspiratory and expiratory muscles activities. However, in the present study we seek to understand whether stimulation of RTN/pFRG neurons activates the three major components of breathing (inspiration, post-inspiration and active expiration). **Methods**: Mean arterial pressure (MAP), diaphragm EMG (dEMG), abdominal EMG (abdEMG) and central vagus nerve activity (cVN) were recorded in urethane-anaesthetized, vagotomized, sino-aortic denervated and artificially ventilated male Wistar rats (250-300 g, n = 5). **Results**: Unilateral injection of glutamatergic NMDA receptor agonist (5 pmol/50 nl) into the RTN/pFRG produces an increase in MAP (13 ± 6 mmHg), dEMG amplitude (18 ± 6%) and frequency (25 ± 5%) and post-inspiratory activity (57 ± 9%). NMDA in the RTN/pFRG was able to generate active expiration. **Discussion**: These results suggest that RTN/pFRG neurons may be important to regulate breathing automaticity by controlling inspiration, post-inspiration and active expiration. All experiments was in accordance with the guidelines approved by the Animal Experimentation Ethics Committee of the Institute of Biomedical Science at the University of São Paulo (ICB/USP) (n ° 008, in fl. 02, book 03). **Financial support**: Fapesp, CNPq, Capes/PROEX

02.048

Antidepressant like activity of citronellyl acetate in mice: involvement of noradrenergic and serotonergic system. Santos LKX, Citó MCO, Fernandes ML, Melo FHC, Aguiar JA, Sousa PB, Lopes IS, Santos APX, Sousa FCF UFC – Pharmacology and Physiology

Introduction: The citronellyl acetate is a monoterpene present in various essential oils. Studies confirm that many essential oils and plant extracts possess antibacterial, antifungal, anti-inflammatory and antidepressant activities. This form the study purpose was to demonstrate the antidepressant effects in male mice on animal models of depression of citronellyl acetate. **Methods**: In each test were used mile Swiss mice (25-30 g). The animals were provided by the biotery of the Federal University of Ceara. This study was approved for committee of animal ethic in the UFC with number 07/2012. The test used was the forced swimming test (FST). In this test each mice was observed to be immobility time. The drugs used were citronellyl acetate (50 and 100 mg/kg, p.o), imipramine (10 mg/kg, i.p.), Prazosin (1mg/kg, i.p.), Yohimbine (1mg/kg, i.p.), p-chlorophenylalanine methyl ester (PCPA) 100 mg/kg, i.p. **Results and discussion**: In this study groups treated with citronellyl acetate (50 and 100 mg/kg) and imipramine, induced a significant decrease in the immobility time in mice, when compared to control[control:102.30 ± 08.65;CIT-50:57.00 ± 05.13;CIT-100:43.17 ± 05.91;IMP-10:31.38 ± 02.64].Pretreatment of mice with the inhibitor of 5-HT synthesis PCPA (once a day on 4 consecutive days) affect the antidepressant-like effect of citronellyl acetate (50 and 100 mg/kg, p.o.), when compared to control group [control:100.80 ± 11.88;CIT50:41.88 ± 06.88; CIT-100:33.00 ± 04.75;PCPA:138.40 ± 09.72; CIT-50 + PCPA:105.70 ± 19.61;CIT-100 + PCPA:100.90 ± 10.55].Pretreatment of mice with the α1-adrenoceptor antagonist prazosin and the α2-adrenoceptor antagonist yohimbine able to reverse the antidepressant-like effect of citronellyl acetate (50 and 100 mg/kg) in the forced swimming test when compared to control group [control: 120.70 ± 09.61; CIT-50: 53.50 ± 7.02; CIT-100: 36.50 ± 04.44; PRZ-1: 119.70 ± 08.03; YOIM-1: 109.80 ± 04.32; CIT-50 + PRZ-1: 97.29 ± 13.30; CIT-100 + PRZ-1: 71.50 ± 08.57; CIT-50 + YOIM-1: 101.30 ± 13.52; CIT-100 + YOIM-1: 130.30 ± 09.30]. These results demonstrate that citronellyl acetate possible issue antidepressant like effect dependent of noradrenergic system with interactions of α1 and α2 adrenergic receptors and issue um possible involvement with serotonergic system. Acknowledgements and **Financial support**: Capes REUNI, CNPq, FUNCAP.

02.049

7-fluoro-1,3-diphenylisoquinoline reverses motor and non-motor symptoms induced by MPTP in mice: Role of neuroinflammation. Sampaio TB[1], Sari MHM[2], Pesarico AP[2], Mantovani A[2], Prediger RD[1], Nogueira CW[2] [1]UFSC – Pharmacology, [2]UFSM – Chemistry

Introduction: Parkinson's disease (PD) is characterized by slow and progressive loss of dopaminergic neurons in the nigrostriatal pathway, which leads to the development of irreversible and incapacitating motor symptoms. Moreover, parkinsonian patients also display non-motor symptoms, such as cognitive and emotional disorders. Several biochemical and molecular mechanisms seem to be involved in the PD pathogenesis, including oxidative stress, neuroinflammation and mitochondrial dysfunction. *in vitro* and *in vivo* studies have revealed that 7-fluoro-1,3-diphenylisoquinoline (FDPI) is a selective MAO-B inhibitor with antioxidant and antidepressant properties. In this study, the effects of FDPI on motor and non-motor symptoms in MPTP-induced mouse model of PD were investigated, as well as its putative anti-inflammatory mechanism. **Methods**: Adult male C57Bl/6 mice were divided into four groups: I) Control; II) FDPI; III) MPTP and IV) MPTP + FDPI. In the first day, mice received four injections of MPTP (20 mg/kg, i.p.) or saline (vehicle) with an interval of 2 h. On the next day, the animals were treated with FDPI (10 mg/kg/day; 10 ml/kg, i.g.) or vehicle (canola oil) and were treated once a day during eight consecutive days. The behavioral tests were carried out at 7th and 8th days after MPTP administration to evaluate the motor and non-motor symptoms. After that, the animals were killed and their nigrostriatal tissues were collected for the evaluation of dopaminergic and inflammatory markers. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources and with the approval of the Animal Use Committee (047/2014), of UFSM, Brazil. **Results and Discussion**: MPTP-treated mice showed impairment in motor skills, as assessed on rotarod and challenging beam tests, which were reversed by FDPI treatment. The spontaneous locomotor activity evaluated on the activity chamber was not altered by any treatment. Interestingly, FDPI treatment was effective to reverse the short-term memory deficits and depressive-like behaviors induced by MPTP in mice addressed, respectively, in the object recognition and forced swimming tests. The anti-inflammatory effect of FDPI was indicated by the reversion of MPTP-induced increase of nigrostriatal cyclooxygenase-2 levels and myeloperoxidase activity. However, FDPI was not effective to reverse the increase of inducible nitric oxide synthase levels and the decrease of tyrosine hydroxylase levels in MPTP-treated mice. Altogether, our findings demonstrate for the first time the potential of FDPI to reverse both motor and non-motor symptoms induced by MPTP in mice. These beneficial effects of FDPI seem to be mediated by anti-inflammatory mechanisms, most likely to the modulation of cyclooxygenase-2 levels and myeloperoxidase activity. **Financial support**: Capes, CNPq.

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Antidepressant-like effects of piroxicam in the rat forced swim test are associated with serotonin. Santiago RM[1], Bassani TB[1], Zaminelli T[1], Boschen S[1], Lima MMS[2], Da Cunha C[1], Andreatini R[1], Vital MABF[1] [1]UFPR – Farmacologia, [2]UFPR – Fisiologia

Introduction: The pathophysiology of depression is far from being fully understood. However, the inflammatory hypothesis has received increasing attention, suggesting that inflammation mediated by immune cell activation is a key factor in depression (Maes *et al.*, 2009). This hypothesis is supported by the fact that patients with depression have elevated levels of proinflammatory cytokines in plasma and cerebrospinal fluid (Smith, 1991) Some authors have contended that depression is characterized by activation of the inflammatory response through an increase in the production of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor α (TNF-α), and prostaglandin E2 (PGE2) (Dowlati *et al.*, 2010). The aim of the present study was to investigate the possible antidepressant-like effect of piroxicam, a nonsteroidal anti-inflammatory drug (NSAID), with a focus on serotonergic neurotransmission. **Methods**: Male Wistar rats were randomly distributed into seven groups: 0.9% saline control group, 3 mg/kg pizotifen (nonselective serotonin receptor antagonist), 10 mg/kg sertraline (selective serotonin reuptake inhibitor), 10 mg/kg piroxicam (NSAID), 10 mg/kg sertraline + 10 mg/kg piroxicam, 10 mg/kg sertraline + 3 mg/kg pizotifen, and 10 mg/kg piroxicam + 3 mg/kg pizotifen. All of the drugs were dissolved in 0.9% saline. Three administrations of the drugs (piroxicam and sertraline) were performed, 1, 5, and 24 h before testing the animals in the open field followed by the forced swim test (FST). Piroxicam and sertraline were administered orally by gavage, and pizotifen was administered intraperitoneally 30 min before gavage. Immediately after the FST, the animals were dissected for neurochemical analysis. Our protocol complies with the recommendations of UFPR and was approved by the University Ethics Committee (Protocol #470) **Results and discussion**: Acute treatment with piroxicam promoted an antidepressant-like effect in the FST, which was strongly associated with an increase in serotonin levels in the hippocampus. This effect was potentiated in the piroxicam + sertraline group but counteracted by administration of the nonselective serotonin receptor antagonist pizotifen. These results suggest that the antidepressant-like effect of piroxicam in the FST is at least partially mediated by the serotonin system. The potentiation of the antidepressant-like effect of sertraline by piroxicam might have potential therapeutic value. **Financial support and acknowledgments**: This work was supported by grants from CNPq, and Capes that had no further role in the study design, in the collection, analysis and interpretation of data, in writing of the report, and in decision to submit the paper for publication. RA, CC and MABFV are recipient of CNPq fellowships. **References**: Dowlati, Y. *Biol Psychiatry*, v.67, p. 446, 2010. Maes, M. Metab. *Brain Dis*, v. 24, p.27, 2009. Smith, R.S. *Med Hypotheses*, v.36, p.178, 1991.

02.051

Neuroanatomical evidences of the control of expiratory activity by the retrotrapezoid nucleus. Silva JN[1], Moreira TS[2], Takakura AC[1] [1]USP – Farmacologia, [2]USP – Fisiologia

Introduction and Objectives: CO_2-sensitive, glutamatergic neurons located in the retrotrapezoid nucleus (RTN) of the rat are candidates to central respiratory chemoreceptors. RTN is involved in the control of inspiratory activity. Besides, evidences suggest the involvement of chemosensitive-RTN neurons also in the control of expiratory activity of premotor neurons in the caudal ventrolateral medullary respiratory grouping (cVRG). Thus, the goal of this study was to clarify by anatomical approach, the existence of a projection from RTN to expiratory neurons of the cVRG and neurotransmitters involved in this projection. **Methods and Results**: The experiments were performed on male Wistar rats weighing 250 – 400 g (n = 11). Procedures were approved by the University of São Paulo's Animal Care and Use Committee (CEUA 029/2012). The anterograde tracer biotinylated dextran amine (BDA, 10%) was injected into the RTN (n = 4) and the retrograde tracer FluorGold (FG, 2%) was injected into the cVRG (n = 4). Seven to ten days after the injections the animals were perfused with paraformaldehyde and had the brains removed and cut to immunohistochemical procedures. Animals with FG injection were exposed to a hypercapnia breathing mixture (7% CO_2, 21% O_2, balanced with N_2) in a small flow-through environmental chamber for 3 hours to stimulate the central chemoreflex. The anterograde tracer results showed the presence of varicosities with BDA immunoreactivity in the cVRG region. Most of the varicosities were immunoreactive for VGLUT2 (60 ± 9%) whereas only 28 ± 5% sampled varicosities were GAD67-ir. The retrograde tracer results showed the presence of FG cell bodies in the RTN region. The retrograde tracer results showed that the RTN neurons that were immunoreactive for FG (i.e project to cVRG) contained Fos (32 ± 8%) or Phox2 (31 ± 9%), but none of them were immunoreactive for tryptophan hydroxylase. **Conclusion**: The present results suggest a direct excitatory input from RTN neurons to the premotor neurons within the cVRG neurons during central chemoreceptor activation. **Financial Support**: Fapesp, CNPq and Capes

02.052

Noradrenergic degeneration contributes to the rapid onset of L-DOPA induced dyskinesia in rats. Lopes SC[1], Oliveira PA[1], Lopes MW[2], Leal RB[2], Takahashi RN[1], Prediger RD[1] [1]UFSC –Farmacologia, [2]UFSC – Bioquímica

Introduction: Parkinson's disease (PD) is mainly characterized by a slow and progressive degeneration of dopaminergic (DAergic) neurons in the substantia nigra. However, recent evidence suggests that the loss of noradrenergic (NAergic) neurons in locus coeruleus may be antecedent to progressive degeneration of DA neurons. Therapy with L-DOPA, a dopamine precursor, remains as the gold standard treatment for PD. Nevertheless, the chronic administration of L-DOPA does not prevent the progression of the disease and its long-term use is associated with undesirable dyskinesia.The aim of this study was to investigate the role of the Naergic degeneration in the development of L-DOPA-induced dyskinesias (LIDs) and effectiveness of dopamine replacement therapy with L-DOPA in motor dysfunction of rats submitted to a single lesion (DAergic) compared to animals double lesion (DAergic and NAergic). **Methods**: All procedures were approved by local Ethical Committee in Animal Research (CEUA PP830/2012). Male Wistar rats were divided in two groups, where both were lesioned with unilateral microinjection of the 6-hydroxidopamine (6-OHDA, 24 µg) in the medial forebrain bundle. One group received only 6-OHDA (double Daergic and Naergic lesion), while the other, in order to protect NAergic fibers was administered with desipramine (20 mg/kg, i.p.) 30 min before the 6-OHDA injection (single Daergic lesion). Three weeks after lesions, animals were treated with L-DOPA (12 mg/kg) plus benserazide (3.25 mg/kg) by oral route once a day for 7 consecutive days. The LIDs scores were measured on alternate days. The anti-parkinsonian effect of L-DOPA was assessed by the cylinder test, before and after L-DOPA treatment. **Results**: The L-DOPA treatment for seven days of 6-OHDA hemiparkinsonian rats induced the appearance of dyskinesias. The double lesioned animals showed high scores of LIDsin comparison with that with only DAergic lesion, either in axial, forelimb and orolingual dyskinesias or locomotor LIDs. No significant differences were observed regarding the efficacy of L-DOPA treatment to alleviate PD-related motor symptoms in both groups. Single and double lesioned animals presented a significant improvement in the use of the contralateral paw injury after treatment with the L-DOPA. **Discussion**: These results indicate that damage of NAergi neurons contributes to the rapid onset of LIDs in 6-OHDA lesioned rats. However, the combined (Naergic + Daergic) lesion does not influence on the efficacy of L-DOPA treatment in motor symptoms. TAltogether our findings corroborate a new approach to the management of L-DOPA-therapy in PD treatment, also considering NArgic system as a new interpellation in LIDs development. **Financial agencies**: CNPq, Capes, FAPESC, FINEP, UFSC.

02.053

Bed nucleus of the stria terminalis noradrenergic system modulates contextual fear conditionig: involvement of CRF1 receptors and NMDA-NO pathway. Hott SC, Gomes FV, Uliana DLM, Resstel LBM FMRP-USP – Pharmacology

Introduction: Some evidence showed that during aversive situations noradrenaline releases is increased in bed nucleus of the stria terminalis (BNST) [1-5]. Previous studies showed that BNST noradrenergic neurotransmission is involved with conditioned emotional response (CER) by activation of both α1 and β1-adrenergic receptors [6]. In addition, BNST β1-adrenergic receptors activation increases the local release of glutamate in a manner dependent of CRF1 receptors [7]. Thus, we investigated the participation of the CRFergic, glutamatergic and nitrergic systems in the modulation of CER by the BNST noradrenergic neurotransmission induced by contextual fear conditioning (CFC) in rats. **Methods**: Male Wistar rats (240-270g) with cannulae implanted bilaterally into the BNST were submitted to a 10min conditioning session (3 footshocks, 0.85 mA, 2s). 24h later, the behavioral and autonomic responses (mean arterial pressure – MAP; heart rate – HR and cutaneous temperature of the tail – CT) evoked by aversive context were measured during test session. The Institution's Animal Ethics Committee approved the housing conditions and experimental procedures (process number: 119/2010). It were administered 0.1μL of saline, reboxetine (2nmol; n=6-9), a potent and selective inhibitor of noradrenaline uptake or CRF agonist urocortin (0.01nmol; n=5-7). Moreover, it were administrated vehicle, α1 and β1-adrenergic receptors antagonist WB4101 (1.7nmol; n=5) and CGP20712 (4.5nmol; n=6) respectively, CRF1 antagonist CP376395 (2.7nmol; n=6-7), NMDA receptor antagonist AP7 (1nmol; n=6) and neuronal NO synthase inhibitor NPLA (0.4nmol; n=5-6) 5 min before the reboxetine or urocortin. Results: Compared to control animals, reboxetine significantly increased freezing behavioral and autonomic responses after re-exposure to the aversive context. These effects were blocked for WB4101 and CGP20712 (freezing: $F_{5,29}$=4.7, P<0.01; PAM: $F_{5,435}$=30.8, P<0.001; FC: $F_{5,435}$=56.8, P<0.001; TC: $F_{5,435}$=29.9, P<0.001), CP376395 (freezing: $F_{3,18}$=13.0, P<0.001; MAP: $F_{3,270}$=46.4, P<0.001; HR: $F_{3,270}$=69.5, P<0.001; CT: $F_{3,270}$=18.4, P<0.001), AP7 (freezing: $F_{3,20}$=5.4, P<0.01; PAM: $F_{3,300}$=81.9, P<0.001; FC: $F_{3,300}$=63.4, P<0.001; TC: $F_{3,300}$=57.7, P<0.001) and NPLA (freezing: $F_{3,17}$=19.2, P<0.001; PAM: $F_{3,255}$=51.5, P<0.001; FC: $F_{3,255}$=71.2, P<0.001; TC: $F_{3,255}$=81.5, P<0.001). Similar to reboxetina, urocortin significantly increased the CER after re-exposure to the aversive context. These effects were blocked for CP376395 (freezing: $F_{4,30}$=8.2, P<0.001; MAP: $F_{4,450}$=34.2, P<0.001; HR: $F_{4,450}$=8.8, P<0.001 and CT: $F_{4,450}$=20.5 P<0.001) and also for AP7 (freezing: $F_{3,22}$=18.3, P<0.001; PAM: $F_{3,330}$=53.4, P<0.001; FC: $F_{3,330}$=23.2, P<0.001; TC: $F_{3,330}$=46.1, P<0.001) and NPLA (freezing: $F_{3,18}$=11.3, P<0.001; PAM: $F_{3,255}$=24.2, P<0.001; FC: $F_{3,255}$=88.8, P<0.001; TC: $F_{3,255}$=23.9, P<0.001), but not WB4101 and CGP20712 (P>0.05). **Discussion**: These results show that the stress increases the release of noradrenaline which act on α1 and β1-adrenoceptor and promotes direct depolarization of CRF neurons in the BNST. The increase in extracellular CRF levels lead to a facilitation of NMDA-NO pathway on BNST, suggesting the existence of a BNST norepinephrine-CRF-glutamate pathway modulating the expression of CFC in rats. **Financial support**: Fapesp, Capes 1. Cecchi, M., H. Khoshbouei, and D.A. Morilak, Modulatory effects of norepinephrine, acting on alpha 1 receptors in the central nucleus of the amygdala, on behavioral and neuroendocrine responses to acute immobilization stress. *Neuropharmacology*, 2002. **43**(7): p. 1139-47. 2. Fendt, M., S. Siegl, and B. Steiniger-Brach, Noradrenaline transmission within the ventral bed nucleus of the stria terminalis is critical for fear behavior induced by trimethylthiazoline, a component of fox odor. *J Neurosci*, 2005. **25**(25): p. 5998-6004. 3.

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02.054

Protective effect of riparin III on oxidative stress in a corticosterone-induced stress model in mice. Capibaribe VCC, Vasconcelos AS, Oliveira ICM, Vidal LMT[1], Pontes MCD, Castro LA, Lopes IS, Sousa PB, Lima AEL, Sousa FCF UFC – Fisiologia e Farmacologia

Introduction The efficacy of the most used antidepressants is limited by monoamine hypothesis. Other biological factors results in the development of depression: factors involved in neurodevelopment, epigenetic modulation, neuroendocrinology, immunology, and exposure to toxins and reactive oxygen species have also been reported to play roles in the pathogenesis of depression. Really, this limitation is highlighted by consistent reports of low remission rates associated with the current antidepressants. Therefore, new therapeutic agents are garnering increasing interest for the treatment of depression especially, agents that may intervene in the damaging process of oxidative/nitrosative stress and inflammatory event. **Methods** Riparin III (Rip III), isolated from *Aniba riparia*, have presented promising Results: In pre-clinic trials, triggered antidepressant and anxiolytic effects in acute and chronic models of depression, in addition to trigger an anti-inflammatory effect in dextran -induced paw edema test. The goal of this work was to examine the effects of Rip III in corticosterone (CORT)-induced animal model of depression on oxidative stress parameters. Facing that, the goal of this work was to examine the effect of Rip III incorticosterone (CORT)-induced animal model of depression on oxidative stress parameters. In the experiment, were used Swiss female mice, weighting 22-25g, divided as the following groups: control (vehicle – saline, 1% of tween 80, 1% of DMSO, s.c., for 14 and 21 consecutive days), stressed group (CORT, 20 mg/kg, s.c, for 14 or 21 days), group treated with Rip III (50 mg/kg, o, for 7 days) and group treated with fluvoxamine (Flu 50 mg/kg, o, for 7 days). The administration of Rip III and Flu began from the 14th day of corticosterone-induced stress and remained simultaneously with the administration of corticosterone until the 21st day. The study was approved by the ethics committee with protocol 13/2014. **Results and discussion** The administration of riparin, reduced levels of malondialdehyde (*MDA*), in the prefrontal cortex (PC) (CONT: 15,82 ± 3,131; CORT: 74,96 ± 10,37; CORT + Rip III: 4,418 ± 1,370; Flu: 13,87 ± 2,173), in the hippocampus (HP) (CONT: 11,86 ± 1,697; CORT: 24,11 ± 2,304; CORT + Rip III: 9,104 ± 1,469; Flu: 18,20 ± 1,547) and in the the corpus striatum (CS) (CONT: 10,27 ± 1,330 CORT: 17,93 ± 2,708; CORT + Rip III:8,853 ± 1,084; Flu: 5,999 ± 1,080) and reduced the nitrite levels in PC (CONT: 3.724 ± 1,94; CORT: 4,932 ± 0,95; CORT + RipIII: 4.082 ± 1,25; Flu: 4,220 ± 0,870), in HP (CONT: 2.223 ± 0,279; CORT: 2,918 ± 0,565, CORT + RipIII: 2,915 ± 0,656; Flu: 2.915 ± 0,656) and in CS (CONTROL: 3.575 ± 1,129; CORT: 6,267 ± 1,682; Cort + RipIII: 2,398 ± 0,643; Flu: 1,874 ± 0,581). Our study further supports the antidepressant-like activity of Rip III. Pae C.U. *et al. CNS Drugs* 25:109. 2011. Sousa F.C. *et al. Pharmacol Biochem Behav.* 78(1):27. 2004. **Financial support**: CNPq, Capes, Funcap

02.055

Investigation of the effect of meropenem in astrocyte culture. Lima CNC[1], Venancio ET[1], Filho AJMC[1], Feitosa ML[1], Lopes KS[2], Sousa A[1], Lima KA[1], Macedo DS[1], Martins AMC[2], Fonteles MMF[1,2] [1]UFC – Farmacologia e Fisiologia, [2]UFC – Farmácia

Introduction: Astrocytes are also the target of inflammatory molecules which, through the activation of specific receptors, may aggravate astrogliosis and amplify the proepileptogenic inflammatory signaling (Aronica and Crino, 2011; Farina *et al.*, 2007; Sofroniew and Vinters, 2010).).In this context, we aim to investigate the effect of meropenem on cell viability of astrocytes in culture. **Methods**: The astrocyte were maintained in a medium containing fetal bovine serum (10%) and antibiotics (100.000U/mL penicillin, 10mg/ml streptomycin) and incubated at 37 ˚C in an atmosphere of 95% humidity and 5% CO2. The cells were placed in a 96-well plate (1 x 10^5 cells / ml) and treated with five different concentrations of meropenem (MERO) (1, 2, 4, 8, 16 mM/ml) for 24h. After the substrate was removed from the cultures and were added 10µL of 3-(4,5-dimetilazil-2-il)-2,5 difenil tetrazólico (MTT) dissolved in phosphate buffered saline (PBS) (500 µg/mL), and then incubated for 4 hours. This method is based on the metabolic activity of viable cells which are capable of converting the MTT salt in formazam crystals, a colored and water-insoluble product. Finally, was added sodium dodecil sulfate (SDS) (10%) in HCl (0.01 N) to solubilize the formazan crystals presents in the medium of culture. Plates were incubated for 17h, and then the cell viability was quantified in a micro plate reader at 570 nm (MOSMANN, 1983). For the statistical analyze ANOVA was performed followed by Bonferroni post hoc test. Probability (P) values less than 0.05 were considered significant. This study was realized under the consent of the Committee of Ethics in Animal Research,Federal University of Ceará(Protocol 08/11). **Results and discussion**: Our findings showed an increase in astrocytes viability's in culture, in a dose dependent manner, only with the two largest concentrations under study: 8 mM/ml (136,3 ± 9,605 vs 100,0 ± 5,608 in the control group, $p<0.05$) and 16 mM/ml (168,0 ± 7,771 vs 100.0 ± 5,608 in the control group, $p<0.0001$) : Recent evidences has proposed the idea that dysregulation of the immune-inflammatory astrocyte function by reactive astrogliosis is a factor that may predispose or contribute directly to the generation of seizures or neuronal damage related to seizures in epilepsy of various etiologies (ARONICA *et al.*, 2012). Thus, it is suggested that reactive astrogliosis may is a likely mechanism for the pro-convulsant effect of MERO, however further studies are needed to confirm this association.

02.056

Evaluation of sedative, anxiolytic, and anticonvulsant activity of the essential oil of *Piper tuberculatum* in mice. Rodrigues CKS[1], Sales VS[1], Figueirêdo FRSDN[1], Nascimento EP[1], Delmondes GA[1], Cruz LP[1], Tintino SR[1], Silva RER[1], Amaro EN[1], Rodrigues LB[1], Monteiro AB[1], Cesário FRAS[1], Lemos ICS[1], Menezes IRA[1], Barbosa R[1], Felipe CFB[2], Kerntopf MR[1] [1]URCA – Biological Chemistry [2]UFPB – Molecular Biology

Introduction: The *Piper tuberculatum* (monkey pepper) is used in popular medicine for sedation and analgesia of stomach disturbances. It was aimed to investigate the sedative, anxiolytic, and anticonvulsant activity of the essential oil of *Piper tuberculatum* (EOPT). **Methodology**: Female Swiss mice were subjected to behavioral tests such as; **elevated** plus **maze** (EPM); open field test (OF) and convulsions induced by Pentilenotetrazol. They were divided into 5 groups of 8 mice, treated with saline solution, diazepam(Dzp) 1 mg/kg i.p. and EOPT (25, 50, 100, 200 and 400 mg/kg, i.p.) for anxiolytic activity. In EPM, the observed parameters were: time spent in the open arms (TSOA), and the number of entries in the open arms (NEOA). In OF: number of crossings (NC) among the quadrants, the grooming number (GN) and the rearing number (RN) . For the induced convulsions test by PTZ, it was administered diazepam 2 mg/kg and OEPT in (100, 200 and 400 mg/kg, i.p.). Thirty minutes after it was administered PTZ (80 mg/kg,i.p.), and analyzed the latency of the first convulsion and the death. Approved work for CEUA of URCA, n° 211/2013.1. **Results**: In open field test (OF), the results observed for the number of crossings (CN) among the quadrants, the grooming number (GN) and the rearing number (RN) did not show significance. In elevated plus maze (EPM), the number of TPBA: The EOPT 200 (72,0 ± 6,4) presented moderate anxiolytic activity when compared with group controle (28,5 ± 2,5) e DZP 1 (179,2 ± 15,6), and NEBA confirmed this probable anxiolytic effect: control (2,6 ± 0,3), Dzp 1 (12,4 ± 1,1), EOPT 25 (4,6 ± 0,4), EOPT 50 (4,6 ± 0,2), EOPT 100 4,2 ± 0,4), EOPT 200 (5,1 ± 0,2) and EOPT 400 (5,1 ± 0,5). In the test of convulsions the latency of first convulsion was: control (139,1 ± 10,6), Dzp 2 (1800 ± 0), EOPT 100 (170,6 ± 17,5), EOPT 200 (269,9 ± 58,0) and EOPT 400 (197,7 ± 38,1),); for latency of death an increase was observed in the largest concentrations of OEPT: control (466,1 ± 97,9), Dzp 2 (1800 ± 0), EOPT 100 (840,3 ± 132,9), EOPT 200 (1369 ± 215,5) and EOPT 400(1505 ± 179,5).**Discussion**: Observed the sedative effect and anxiolytic in elevated doses (200 and 400 mg/kg) suggesting a possible anxiolytic effect of the type benzodiazepines. The results of the anticonvulsant activity indicate that EOPT does not alter the latency for convulsion; however it promotes neuroprotetor effect by increasing death latency similar to diazepam. **References**: Chaves MCO.; Júnior AGF; Santo BVO. Amides from *Piper tuberculatum* fruits. *Fitoterapia* 74: 181-183, 2006. Araújo-Júnior, JX; Chaves, MCO; Cunha EVL; Gray AI Cepharanone B from *Piper tuberculatum.* Biochem Syst Ecol, v. 27: 325-327, 1999. **Financial Agency**: FUNCAP.

02.057

Gene expression of myoinositol co-transporters in nervous tissue during the development of experimental diabetes. Uchoa PN[1], Fonteles MC[1], Nascimento NRF[1], Santos CF[1], Bindá AH[2], Farias VX[1], Prata MMG[2], Britto LRG[3], Lessa LMA[1] [1]ISCB-UECE, [2]UFC – Fisiologia e Farmacologia, [3]ICB-USP

Disturbances in the transport and metabolism of inositol may be involved in the genesis of pathological conditions, such as diabetic neuropathy. Sodium/myoinositol cotransporters (SMIT-1 and SMIT-2) and hydrogen/myoinositol transporter (HMIT) regulate intracelular concentrations of myoinositol. Myoinositol is an essential compound for cell survival and is a precursor of structural molecules of the plasma membrane, helps to regulate the osmolarity of the cell and contributes on fetal development. The objective of this study was to investigate and compare the gene expression of myoinositol co-transporters in the central nervous system (cerebral cortex, cerebellum, hippocampus) and peripheral nervous system (sciatic nerve and dorsal root ganglia -DRG) during the development of experimental diabetes. For this purpose, adult male Wistar rats (200-250g), kept in light/dark cycle and free access to water and food, were subjected to induction of diabetes by streptozotocin (STZ, 60 mg/kg;i.p). The experimental protocol was approved by the ethics committee with the number 11518153-9/68. Euglycemic and 4, 8 and 12 weeks long diabetic rats were sacrificed and sciatic nerve, DRG, hippocampus, cerebellum, and cerebral cortex were collected for analysis of SMIT-1, SMIT-2 and HMIT mRNA expression by RT-PCR. Statistical analysis was performed using ANOVA for comparison between groups. Results, in the null hypothesis had a probability of occurrence less than 5% ($p<0.05$), were considered significant. An initial screening was performed to check the expression of SMIT-1, SMIT-2 and HMIT mRNA in different tissues of the nervous system, where it was observed that three co-transporters studied were expressed in all evaluated tissues, excluding SMIT-2 in sciatic nerve. It was observed significant decrease in expression of SMIT1 in DRG, cerebral cortex, cerebellum and hippocampus (55%, 58%, 52%, 69%, respectively) compared to euglycemic group, 4 weeks after the course of disease. For the sciatic nerve, it was observed a reduction of the SMIT-1 expression (68%) in 4 weeks group . In contrast, in sciatic nerve and DRG, there was a progressive increase of HMIT gene expression (117%) after 4 weeks and (70%) after 12 weeks of the disease and (70%) of SMIT2 in GRD with during the course of the disease, suggesting a possible compensatory mechanism for the decreased expression of SMIT1, as an attempt to maintain the transmembrane transport of myoinositol. There is no significant difference in gene expression of SMIT2 in the cerebral cortex and cerebellum of diabetic rats compared to euglycemic. In the hippocampus, the mRNA expression of HMIT was significantly lower throughout the course of the disease, reaching a reduction of 74% after 8 weeks of diabetes. In the hippocampus of 4 weeks diabetic animals, there was down regulation of SMIT2 (57%), while 12 weeks after disease induction was observed up regulation by 154%, suggesting a possible mechanism for compensatory reversal. Our present data show that there are differences in myoinositol transporters expression during the development of experimental diabetes in peripheral and central nervous system and this system is very weel regulate to keep myoinositol transport.

02.058

Anxiolytic-like effects of lipossomal formulation containing nimodipine: possible involvement of serotoninergic transmission. Viana HKMMV[1], Almeida VPA[1], Rodrigues JBR[1], Moreno LCGAI[2], Santos-Magalhães NS[2], Freitas RM[1], Rolim HML[1] [1]UFPI, [2]UFPE

Introduction: Liposomes containing nimodipine (NMD-Lipo) have shown promising results in the treatment of anxiety. Thus, it is a perceived improvement in the pharmacokinetics of nimodipine, both in plasma and in brain tissue. The present study aims to investigate the mechanism of anxiolytic action of NMD- Lipo and the involvement of the serotonergic system through the use of serotonin antagonists. **Methods**: NMD-Lipo was prepared by hydrating the lipid film as described by Moreno *et al* (2014). The experimental protocols and procedures were approved by the Ethics Committee on Animal Experimentation of the UFPI (CEEA/UFPI No.014/11). The mice were treated acutely intraperitoneally with 0.9% saline (negative control), buspirone 5 mg/kg (positive control), free nimodipine (10 mg/kg, po) and NMD-Lipo(10 mg/kg). To investigate the possible involvement of NMD with liposomes containing 5HTA receptor antagonist WAY-100635 (10 mg/kg ip) was used. Behavioral evaluations in the light and dark test and elevated plus maze test (EPM) were performed after administration of the last drug. **Results**: In the light and dark box test (LDBT), the animals treated with buspirone showed a significant increase in the time spent in the light box (TSLB) when compared to the control group. The mice treated with free NMD showed no change in time spent in the light field. For their part, rodents treated with NMD-Lipo were shown to spend a significantly longer time in the light box than the control group. The mice pretreated with WAY100635 and subsequently given buspirone and NMD-Lipo showed reduction in the TSLB, compared to animals that did not receive the antagonist. In the EPM, the animals treated with buspirone and the mice that received free NMD showed a significant increase in the time spent in the open arms (TSOA) when compared to the control group. The rodents treated with NMD-Lipo exhibited a significant increase in the number of of open arms entries (NOAE) when compared to the control group. The mice pretreated with WAY100635 and subsequently given buspirone and NMD-Lipo showed a reduction in the NEOA and TSOA, compared to animals that did not receive the antagonist. **Discussion**: The LDBT evaluates the anxiety rodent behavioral level. It is based on the spontaneous exploratory behavior of rodents forward to a new and bright field. The EPM test use as a measure of anxiety is based on the natural aversion of rodents for open spaces. Thus, the increased TSLB in the LDBT and the increase of the NOAE and TSOA in the EPM of the animals treated with NMD-Lipo is evidence anxiolytic-like activity. Which suggests that the encapsulation of the drug into liposomes promoted its controlled release, increasing its bioavailability and improving its effect. Furthermore, the reduction in TSLB in the LDBT and the reduction in the NOAE and TSOA in the EPM test of the mice treated with the combination of WAY100635 and NMD-Lipo, compared with the animals treated only with NMD-Lipo, suggests that NMD-Lipo act on serotoninergic receptors. **References**: Moreno LCGA. I. *et al. Pharmacol Biochem Behav*, v. 116, p.64, 2014. **Acknowledgements**: Capes

02.059

Involvement of dopaminergic system in antidepressant like activity of monoterpene citronellyl acetate. Silva FCC, Santos LKX, Citó MCO, Fernandes ML, Melo FHC, Sousa FCF UFC – Fisiologia e Farmacologia

Introduction: The citronellyl acetate is a monoterpene present in various essential oils. Studies demonstrate antibacterial, antifungal, anti-inflammatory and antinociceptive activity this monoterpene, however the effects in central nervous system no weren't described. This form the study purpose was to demonstrate the antidepressant effects in male mice on animal models of depression of citronellyl acetate and evaluate your involvement with dopaminergic system. **Methods**: In this study were used mile Swiss mice (25-30 g). The animals were provided by the biotery of the Federal University of Ceara. This study was approved in committee of animal ethic in the UFC with protocol number 07/2012. The test used was the forced swimming test (FST). In this test each mice was observed to be immobility time for five minutes. The drugs used were citronellyl acetate (50 and 100 mg/kg, p.o), imipramine (10 mg/kg, i.p.), SCH 233390 (15µ/kg, i.p.), Sulpiride (50 mg/kg, i.p.) and Bupropion (30 mg/kg p.o.). **Results and discussion**: The groups treated with citronellyl acetate (50 and 100 mg/kg) and imipramine, induced a significant decrease in the immobility time in mice, when compared to control [control: 102.30 ± 08.65; CIT-50: 57.00 ± 05.13; CIT-100: 43.17 ± 05.91; IMP-10: 31.38 ± 02.64]. These demonstrate antidepressant like activity that substance. For corroborate with dates, we make other experiment for evaluate involvement of dopaminergic system. The animals were pretreated with the antagonist of receptor D1 dopaminergic (SCH 23390) and antagonist of receptor D2 dopaminergic (Sulpiride), after were administrated citronellyl acetate (50 and 100 mg/kg, p.o.). The results issue that pretreatment of mice with the antagonist D1 receptor (SCH) and the antagonist D2 receptor able to reverse the antidepressant-like effect of citronellyl acetate (100 mg/kg) [CIT-100: 31.63 ± 4.86 (8)]; CIT-100 + SCH-15: 68.75 ± 8.28 (8) $p<0.05$; CIT-100 + sulpirida-50: 120.4 ± 11.55 (8) $p<0.001$] in the forced swimming test when compared to control group. Although, the citronellyl acetate (50 mg/kg) had the effect antidepressant like able for SCH 23390 (D1 receptor antagonist) [CIT-50: 51.13 ± 5.37 (8); CIT-50 + SCH-15: 156.70 ± 12.57 (8) $p<0.001$; CIT-50 + SCH-15: 156.70 ± 12.57 (8) $p<0.001$; CIT-50 + sulpirida-50: 57.50 ± 7.19 (8)] but not able for sulpiride (D2 receptor antagonist). These results demonstrate that citronellyl acetate issue antidepressant like activity dependent of dopaminergic system. Acknowledgements and **Financial support**: Capes REUNI, CNPq, FUNCAP.