ABSTRACTS

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02. Neuropharmacology

02.001 Pharmacogenetic inactivation of neuronal ensembles of the pre-limbic cortex attenuated the context-induced reinstatement of alcohol seeking. Palombo P[1], Zaniboni CR[1], Moreira J[1], Bianchi PC[2], Leão RM[3], Planets CS[2], Santos PCJL[1], Cruz FC[1] [1]Unifesp – Farmacologia, [2]FCFar-Unesp-Araraquara, [3]UFBA

Environmental contexts previously associated with drug use provoke relapse to drug use in humans and reinstatement of drug seeking in animal models of drug relapse. We examined whether context-induced reinstatement of alcohol seeking is mediated by activation of neuronal ensembles of the pre-limbic cortex. We trained rats to self-administer alcohol in Context A and extinguished their lever-pressing in a distinct Context B. On test day, reexposure to the alcohol-associated Context A reinstated alcohol seeking. To assess a causal role for the prelimbic neuronal ensembles in context-induced reinstatement of alcohol seeking, we used the Daun02 inactivation procedure to selectively inactivate these neurons. We trained c-fos-lacZ transgenic rats to self-administer alcohol in Context A and extinguished their lever-pressing in Context B. On induction day, we exposed rats to either Context A or a novel Context C for 30 min and injected Daun02 or vehicle into prelimbic cortex 60 min later. On test day, 3 d after induction day, the ability of Context A to reinstate alcohol seeking was attenuated when Daun02 was previously injected after exposure to Context A (active lever presses: 16.0 ±4.0 Vehicle drug context vs 4.0±2.0 Daun02 drug context, $p<0.05$). Our data suggest that context-induced reinstatement of alcohol seeking is mediated by activation of context-selected prelimbic cortex neuronal ensembles. **Financial Support**: Fapesp 2013/24986-2 and Capes. Ethics Committee: 2015/01

02.002 Cannabinoid type 1 receptors in the dorsal hippocampus modulate autonomic responses and late behavioral consequences induced by acute restraint stress in rats. Hartmann A[1], Fassini A[1], Scopinho A[1], Correa FM[1], Guimarães FS[1], Lisboa SF[1], Resstel LBM[1] [1]FMRP-USP – Farmacologia

The endocannabinoid (ECB) system, a neuromodulator and stress-buffer system, is widely expressed in the hippocampus (HIP), a limbic structure that has a major role in the modulation of behavioral adaptation to stress. Anandamide (AEA) is an ECB synthesized on demand that acts mainly through cannabinoid type 1 (CB1) receptors, afterward being inactivated by the fatty acid amide hydrolase (FAAH) enzyme. Drugs that enhance ECB signaling usually show anti-stress effects. Our aim was to evaluate if increasing CB1 signaling through pharmacological inhibition of the FAAH enzyme in the dorsal HIP (dHIP) would attenuate the increased autonomic response evoked by exposure to an acute restraint stress (RS) and its later behavioral consequence (anxiety-like behavior). Male Wistar rats (200-230 g) had bilateral cannulae implantation into dHIP by stereotaxic surgery. After 5-7 days, animals underwent surgery for femoral artery cannulation for autonomic evaluations, 24 h before the RS. Tail cutaneous temperature (CT) was recorded with a thermal camera. After the baseline record of mean arterial pressure (MAP) and heart rate (HR), independent groups of rats received 2 microinjections into the dHIP as follow: vehicle (200 nl; 10% DMSO in saline) or AM251 (10, 50, 100 or 300 pmol; CB1 antagonist) followed, 5 min later, by vehicle or URB597 (0.01 nmol; inhibitor of FAAH). After 10 min, the animals were submitted to a 1h-RS period in a restraining tube. For behavioral evaluation, animals underwent the same procedure, except for artery cannulation and autonomic response evaluation. 24 h after the RS, they were submitted to the elevated plus-maze (EPM) for evaluation of anxiety-like behavior. Data, expressed as mean±SEM, were analyzed by one-way ANOVA followed by Duncan post-hoc test. Statistical significance was considered when $p \leq 0.05$. Acute RS increased autonomic response (increased MAP and HR, decreased CT) and induced later anxiogenic-like effect (decreased percentage of time spent and number of entries into the open arms). URB597 administration into dHIP not only prevented the acute RS-induced increase in autonomic activity, but also the occurrence of later anxiety-like behavior. Higher doses of AM251 (100 and 300 pmol) increased autonomic activity during the RS session, indicating tonic modulation of this response by dHIP CB1 receptors. In the EPM, AM251 did not induce effect *per se* in none of the tested doses. Pre-treatment with AM251 attenuated URB597 effects in the autonomic (AM251 50 pmol) and behavioral (AM251 50 and 100 pmol) responses. There was only a stress effect in the number of enclosed arms entries in the EPM, excluding the possibility of enhanced motor activity induced by URB597. The present data showing that FAAH enzyme inhibition in the dHIP attenuates the autonomic activity during an acute stress and its later behavioral consequences, via local CB1 receptors. These data also strengthen previous results indicating that the hippocampal ECB system modulates outcome of stress exposure. Therefore, targeting this system could be therapeutically useful to treat stress-related disorders. Acknowledgements: CNPq/CAPES/FAPESP/FAEPA. University's Ethical Committee Approval (n.127/2011).

02.003 Nitric oxide modulates DNA methylation and DNMT3b expression in the hippocampus. Maciel IS[1], Sales AJ[1], Biojone C[2], Casarotto PC[2], Castrén E[2], Joca SRL[3] [1]FMRP-USP – Farmacologia, [2]University of Helsinki – Neuroscience Center, [3]FCFRP-USP – Física e Química

Introduction: Stress exposure increases glutamate and nitric oxide (NO) levels, and DNA methylation in the hippocampus. However, it is not yet known if there is a causal relationship between these events. Morereover, both nitric oxide synthase (NOS) inhibitors and DNA methylation inhibitiors counteract the behavioral effect of stress. Therefore, our aim was to investigate the effects of NOS inhibitors on stress-induced behavioral changes and DNA methylation in the hippocampus. **Methods:** 1. Rats submitted to learned helplessness-(LH) pretest, treated with inhibitors of NOS 7-nitroindazole (7-NI;60mg/kg,i.p), aminoguanidine (AMG; 30mg/kg,i.p] or vehicle for 7 days and tested 1h after the last injection. Global DNA methylation of hippocampus was measured by immunoassay (ELISA). 2. Primary hippocampal cell culture was challenged with NMDA (30µM,1h), L-arginine (500µM,1h) or dexamethasone (1µM,24h) and pretreated with nNOS inhibitor (NPA, 100nM, 30min before the challenge). DNMT1, 3a and 3b gene expression was assessed by RT-qPCR. The results were analyzed by two-way ANOVA, followed by Bonferroni test. **Results:** Stress exposure increased escape failures in LH, which was attenuated by treatment with AMG (interaction: $F_{1,45}$ = 8.0 4; $p<0.05$) or 7-NI (interaction: $F_{1,42}$ = 8.45; $p<0.05$). Interestingly, the increased DNA methylation in the ventral hippocampus (vHPC) observed in stressed rats was attenuated by treatment with both AMG (interaction: $F_{1,28}$=9.59; $p<0.05$) and 7-NI (interaction: $F_{1,28}$ = 9.15; $p<0.05$). Hippocampal primary cells presented increased DNMT3b mRNA expression in response to challenge with NMDA, L-arginine and dexamethasone, which was attenuated by NPA pretreatment (interaction=$F_{1,32}$=4.851, $p<0.05$; $F_{1,20}$=13.35,$p<0.05$ and $F_{1,20}$=20.52,$p<0.05$; NMDA, L-arginine and dexamethasone respectively). No significant changes were observed in DNMT1 and DNMT3a expression. **Conclusions:** treatment with NOS inhibitors attenuated stress-induced DNA methylation in the vHPC of rat submitted to LH test. NOS inhibition also blocks corticosterone, NMDA and L-arginine-induced DNMT3b mRNA expression in primary hippocampal cell culture. Altogether, our results suggest that glutamate release, leading to NO production during stress may mediate intracellular mechanisms that regulate DNMT3b expression and DNA methylation. Additional experiments are currently under development to investigate further molecular mechanisms involved in the aforementioned effect. **Financial support:** FAPESP (2015/06271-1 and 2015/25067-6), CNPq and ERC (n° 322742). **Local ethical committee** (protocol number: 15.1.285.60.5)

02.004 The endocannabinoid system as a target for novel antipsychotic drugs. Pedrazzi JFC[1], Issy AC[2], Guimarães FS[3], Del Bel EA[2] [1]FMRP-USP – Neurociências, [2]FORP – Fisiologia, [3]FMRP-USP – Farmacologia

The information processing appears to be deficient in schizophrenia which is a highly disabling disease. Prepulse inhibition (PPI), measures the inhibition of a motor response by a weak sensory event is considered particularly useful to understand the biology of information processing in schizophrenia patients. Drugs that facilitate dopaminergic neurotransmission such as amphetamine (AMPH) induce PPI disruption in human and rodents. Clinical effective antipsychotics reverse the AMPH disruptive effect. Cannabidiol (CBD), a non-psychotomimetic constituent of the *Cannabis sativa* plant, has also been reported to have potential as an antipsychotic. Studies demonstrated that CBD is able to attenuate the disruptive effect of AMPH in the PPI. CBD has been reported to act as an agonist of the vanilloid 1 channel in the transient receptor potential family (TRPV1R) and also to inhibit the hydrolysis and cellular uptake of the endogenous cannabinoid anandamide (AEA). TRPV1R are expressed in limbic areas, that are also part of the circuitry regulating sensorimotor gating. Moreover, in a clinical study with schizophrenic pacients, CBD treatment was accompanied by a significant increase in serum AEA levels, which was significantly associated with clinical improvement. Our aim was to investigate the mechanisms enrolled in the CBD effects. To investigate the involvement of TRPV1R in the CBD effects, male *Swiss* mice were systemically treated with either CBD or CBD preceded by the TRPV1R antagonist capsazepine (CPZ) prior to AMPH, and were exposed to PPI test. Since one possible mechanism of CBD action is the facilitation of endocannabinoid mediated neurotransmission through AEA, another group of mice received an AEA hydrolysis inhibitor (URB597) prior to the AMPH. Finally, we investigated the effects of N-arachidonoy-serotonin (AA-5-HT) a dual inhibitor of FAAH and TRPV1R. The PPI test consist of 64 trials irregularly divided into pulse (P, white noise, 105dB), prepulse (PP; pure tone; 7kHz; 80, 85 or 90dB), prepulse + pulse (PP+P) and no-stimuli with white background noise level of 64dB – %PPI=[100-(PP+P/P)*100]. The percentage of PPI was analyzed with repeated measures with the treatment as the independent factor and the prepulse intensity as repeated measure. Duncan´s post hoc test ($p<0.05$) was used to specify differences. Systemic CBD (30 or 60 mg/kg) attenuated the AMPH disruptive effects on PPI test at prepulse intensities of 80 and 85dB). CPZ blocked the ability of CBD to reverse the AMPH. The pretreatment with URB597 dose-dependently (0.3 mg/kg at all prepulse intensities and 1 mg/kg at 90dB) attenuated PPI impairment induced by AMPH. The pretreatment with AA-5-HT (5 mg/kg) failed to block AMPH-induced disruption in PPI. CBD attenuates the AMPH disruptive effects in the PPI test, and this effect may be mediated by TRPV1R as evidenced by the reversal of CBD effect by CPZ. Corroborating the hypothesis that AEA has a role in the CBD antipsychotic-like effects, URB597 has similar effects to CBD in the PPI test. Our results demonstrate that the antipsychotic profile of CBD involves concomitant increase in the availability of AEA and activation of TRPV1R. Comitê de Ética: Processo 2016.1.331.58.6 Support: FAPESP; CNPq; CAPES; NAPNA; USP.

02.005 Activity-dependent neuronal klotho production induces astrocytic lactate release through FGFR1 activation and ERK phosphorylation. Mazucanti CH[1], Kawamoto EM[1], Mattson MP[2], Camandola S[2], Scavone C[1] [1]ICB-USP – Farmacologia, [2]NIH-NIA

Mutations of the β-glucuronidase protein α-Klotho have been associated with premature aging, and altered cognitive function. Although highly expressed in specific areas of the brain, Klotho functions in the central nervous system remain unknown. Here, we show that cultured hippocampal neurons respond to insulin and glutamate stimulation by elevating Klotho protein levels. Conversely, AMPA and NMDA antagonism suppress neuronal Klotho expression. We also provide evidence that soluble Klotho enhances astrocytic aerobic glycolysis by hindering pyruvate metabolism through the mitochondria, and stimulating its processing by lactate dehydrogenase. Pharmacological inhibition of FGFR1, Erk phosphorylation, and monocarboxylic acid transporters prevents Klotho-induced lactate release from astrocytes. Taken together these data suggest Klotho is a potential new player in the metabolic coupling between neurons and astrocytes. Neuronal glutamatergic activity and insulin modulation elicit Klotho release, which in turn stimulates astrocytic lactate formation and release. Lactate can then be used by neurons as a metabolic substrate to support their elevated energy requirements.

02.006 The Medial Prefrontal Cortex CRF1 but not CRF2 receptors modulate the tachycardic component of the baroreflex activity. Lagatta DC, Bruffato JPT, Uliana DLM, Borges-Assis AB, Resstel LBM FMRP-USP – Farmacologia

Introduction: The ventral portion of the medial prefrontal cortex (vMPFC) glutamatergic neurotransmission facilitates both tachycardic and bradycardic baroreflex responses through NMDA receptors activation[1]. The corticotropin releasing factor type 1 (CRF1) receptor colocalizes with glutamate vesicles in the vMPFC neurons[2], suggesting that the CRF1 receptors could modulate the glutamate release in this structure. Therefore, we hypothesized that vMPFC CRF1 receptors modulate the cardiac baroreflex responses. **Methods:** Male Wistar rats had stainless steel guide cannulas implanted into the vMPFC using a stereotaxic apparatus. Seventy two hours later, polyethylene catheters were implanted into the femoral artery and vein for cardiovascular recordings and vasoactive compounds infusions, respectively. The experimental protocols were performed twenty four hours later. **Results:** The injection of a CRF1 receptors antagonist (CP376395-4.5 nmol/200nL; n=7) into the vMPFC did not alter the bradycardic reflex (slope before= -1.49 ± 0.18; slope 10 minutes after= -1.97 ± 0.26; t=1.98, P>0.05), whereas the tachycardic component was increased (slope before= -1.74 ± 0.14; slope 10 minutes after= -2.84 ± 0.09; t=7.03, P<0.0001). The administration of an equimolar dose of a CRF2 receptors antagonist (K41498 – 4,5 nmol/200 nL; n=5) did not change both bradycardic (slope before= -1.91 ± 0.42; slope 10 minutes after= -2.09 ± 0.21; t=0.46, P>0.05) or tachycardic (slope before= -2.69 ± 0.36; slope 10 minutes after= -3.04 ± 0.37; t=0.83, P>0.05) baroreflex responses. The microinjection of a non-selective CRF1-CRF2 receptors agonist, urocortine 0.2 nmol/200 nL, into the vMPFC (n=7) reduced the tachycardic response (slope before= -2.46 ± 0.28; slope 10 minutes after= -1.48 ± 0.15; t=3.88, P<0.01), with no alteration in the bradycardic reflex (slope before= -1.79 ± 0.22; slope 10 minutes after= -1.93 ± 0.15; t=0.55, P>0.05). Moreover, the vMPFC pretreatment with an ineffective dose of the CRF1 antagonist (0.45 nmol/200 nL) prevented the effect of urocortine 0.2 nmol/200 nL on the tachycardic response (slope before= -2.97 ± 0.30; slope 10 minutes after= -2.45 ± 0.47; t=1.24, P>0.05; n=6). **Conclusion:** Our results demonstrate that the vMPFC CRF1 but not CRF2 receptors reduce the tachycardic baroreflex response without affecting the bradycardic reflex. **References** 1-“Ferreira-Junior” et al., *The Jour Neurosc Reas*; 91, no. 10 (Obtober, 2013): 1338-48. 2-“Damian Refojo" et al., *Science;* 333, no. 6051 (September 30, 2011): 1903–7.

02.007 The BNST endocannabinoid system modulates learned, but not innate fear in rats. Borges-Assis AB, Uliana DLM, Resstel LBM FMRP-USP – Farmacologia

The endocannabinoid system is widely present in several brain structures involved on fear expression and anxiety-related responses, mostly mediated via CB1 receptors. Nevertheless, the role of the endocannabinoids system role in specific brain structures is not yet completely elucidated. One structure in particular, is the Bed Nucleus of Stria Terminales (BNST), which is a limbic structure responsible for integration of autonomic, neuroendocrine and behavioral information during aversive situations. There is little evidence about the presence and involvement of endocannabinoid system in the BNST on anxiety responses modulation. Therefore, the aim of the present study was to evaluate the role of the endocannabinoid system in the BNST on the modulation of innate and learned aversive responses. Male Wistar rats (240 – 270g) were submitted to stereotaxic surgery for bilateral guide cannula implantation directed to the BNST, for drug administration. Animals received local injections of vehicle, AM251 (CB1-antagonist; 0,1 – 0,3nmol/100nL), URB597 (an inhibitor of FAAH; 0,01 – 0,1nmol/100nL). Five days after the stereotaxic surgery, animals were submitted to the innate response test, the elevated plus maze, for 5 minutes. The percentage of entries and time spent in open and the number of enclosed arms entries were analyzed. After two to three days, animals were submitted to the contextual fear conditioning protocol, performed in three consecutive days. On test day, behavioral (freezing) and autonomic responses (mean arterial pressure, heart rate and tail cutaneous temperature) were recorded for 10 min. Our data suggest that after CB1 receptors blocking or activation in the BNST do not promote changes in innate fear responses. However, during fear learning, CB1 receptor antagonism in the BNST increased freezing behavior and mean arterial pressure. In addition, FAAH inhibition in the BNST, via CB1, reduced freezing behavior and mean arterial pressure in the emotional conditioned response. These results suggest that endogenous cannabinoid system in the BNST can modulate defensive responses in fear learning, but not innate fear responses.

02.008 Extrapyramidal effects induce by metoclopramide in mice. Prieto SG[1], Torres CV[1], França KC[2], Echeverry MB[1] [1]UFABC – Neurociências e Cognição, [2]Universidade Metodista de São Paulo –Ciências Médicas e da Saúde

Introduction Previous studies have shown that the metoclopramide, an antimimetic drug, may induce extrapyramidal effects such as Parkinsonism in humans [1, 2] and rodent [3, 4], similar to haloperidol known as an atypical antipsychotic. Besides, it has been reported that metoclopramide can accelerates dopamine turnover, and stimulates prolactin release into neurons of striatum [5]. On the other hand, some studies show that patients with neurodegenerative diseases increase the metoclopramide binding in striatum, which can be explained by the appearance of tardive dyskinesia due to the metoclopramide treatment [6]. Thereby, the objective of this study was to evaluate the acute motor side effects with different doses and analyze the proto-oncogenes expression in motor structures. **Methods** Twenty swiss adult male mice with 11 weeks old was used. The mice received metoclopramide via (i.p) (four groups) with doses of 5mg/kg, 15 mg/kg, 45mg/kg and saline respectively (n=5/group). The catalepsy test was evaluated 30, 60, 90 and 120 min after injection for each group. The rota-rod and pole tests were performed 60 min after drug administration. Future experiments will be carried out using the same groups distritubion in order to analyze the activation of proto-oncogenes into motor structures such as striatum and pre-frontal cortex. **Results** Behavioral results showed a significant effect in the variable treatment ($F = 92.652$; $p < 0.01$) with significant interaction between time vs treatment ($F = 4.071$; $p<0,01$), indicating that catalepsy effect was maintained for a long time. However, 5 min after injection, the catalepsy time associated with 5mg/kg did not show difference compared to the saline group (Bonferroni's post-hoc $p > 0.05$). The locomotor activity also showed effect ($F = 5.298$; $p < 0.05$), suggesting that the used doses of metoclopramide can lead motor alterations. In fact, the 15mg/kg group presented significant difference when compared with the control group (Bonferroni's post-hoc $p < 0.05$). The pole test does not show any performance difference with the different used doses ($F = 0.563$; $p = 0.647$). **Conclusion** The metoclopramide, an antiemetic drug, induced cataleptic effect using low and high doses after a single administration. **References** 1. Wiholm BE, et. al.; BMJ; 288:545; (1984); 2. Tianyi FL, et. al.; BMC Res Notes; 10:32; (2017); 3. Ahtee L; Br J Pharmacol.; 53:460; (1975); 4. Agovic MS et. al.; Eur J Pharmacol; 587:181; (2008); 5. Hassan MN et. al.; Clin Neuropharmacol, 9:71; (1986); 6. Chen S, et. al.; Synapse; 65:119; (2011). **Financial support** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Federal University of ABC **Animal Research Ethical Committee** Protocol number CEUA 9943080317, under the responsibility of Marcela B. Echeverry and team; Sonia Guerrero Prieto; Victor Ricardo; Karinna Cabral de França

02.009 P2X receptors modulate depressive-related behavior and antidepressant effect. Ribeiro DE[2,1], Stanquini LA[2], Roncalho AL[2], Silva MAP[3], Casarotto PC[4], Biojone C[4], Pereira VS[1], Abildgaard A[1], Müller HK[1], Elfving B[1], Wegener G[1], Joca SRL[3,1,2] [1]Aarhus University – TNU, [2]FMRP-USP, [3]FCRP-USP, [4]University of Helsinki – Neuroscience Center

Introduction: Purinergic P2X7 receptors (P2X7R) are mediators of neuron-glial interactions, with a central role in some stress-related effects, such as neuroroinflammation and neuronal plasticity[1]. P2X7R blockade induce antidepressant-like effect in forced swimming test and tail suspension test [2,3], indicating an involvement of ATP-P2X7 signaling in depression neurobiology and antidepressant effect. However, stress and antidepressant effects on P2X7R expression remain unknown. Additionally, P2X7R antagonist effects and mechanisms in other animal models have not yet been tested. Aims: To investigate: 1. the effects of stress and antidepressants on P2X7R levels on frontal cortex (FC) and hippocampus (HIP) of rats submitted to stress-based animal model, the learned helplessness (LH); 2. P2X7R levels (mRNA and protein) in FC and HIP of flinders sensitive/flinders resistant line (FSL/FRL) rats, an animal model of depression based on selective breeding; 3. the effects of P2X7R antagonists (BBG and A-804598) in LH and FSL/FRL rats; 4. the effects of P2X7R antagonists on brain derived neurotrophic factor (BDNF) levels and downstream signalling proteins on FC and HIP of FSL rats. **Methods:** 1. Male Wistar rats were treated with desipramine, imipramine or vehicle for one (acute) or seven (repeated) days and submitted to stress or no stress in LH paradigm; FC and HIP were dissected and P2X7R levels were evaluated by *western blotting* (WB); 2. Relative mRNA and protein levels of P2X7R were determined by real-time qPCR and WB in FC and HIP of FSL/FRL rats. **3.** Male Wistar rats were treated with vehicle or brilliant blue G (BBG; 25 or 50 mg/kg/day) for one or seven days and exposed to the LH. FSL and FRL rats were treated with vehicle, BBG (25, 50 or 100 mg/kg/day) or A-804598 (3, 10 or 30 mg/kg/day) for one or seven days and were exposed to forced swim test. 4. BDNF, Akt, Erk, mTor and p70 S6 kinase levels were evaluated by WB in FC and HIP of FSL/FRL rats after treatment with A-804598. **Results:** 1. Stress increased and antidepressant decreased P2X7R levels on ventral HIP; 2. FSL rats present increased mRNA levels of P2X7R on FC, dorsal and ventral HIP, while decreased P2X7R protein levels in ventral HIP; **3.** Repeated BBG (50 mg/kg) treatment induced antidepressant-like effect on LH rats, while repeated A-804598 (30 mg/kg) induced antidepressant-like effect on FSL rats. **4.** Stressed FSL rats presented decreased BDNF and p70S6 kinase levels on ventral HIP. A-804598 repeated treatment (30 mg/kg) attenuated stress effects and increased Akt activation on ventral HIP. No significant changes were observed on the BDNF signalling on dorsal HIP or FC. **Conclusion:** Antidepressants effects may involve attenuation of stress-induced P2X7R signalling on ventral HIP and blockade of P2X7R induces antidepressant-like effects in stress-based and genetically-based animal models. P2X7R antidepressant effect is possibly mediated by BDNF-TRKB-AKT-p70S6 kinase signaling in the ventral HIP. Altogether, our data suggest that P2X7R signaling in ventral HIP is involved in depression neurobiology and in antidepressant effect. **References:** [1] Sperlagh, B.; *Trends Pharmacol Sci* **35**(10): 537-547; 2014. [2]Csolle, C.; *PLoS One* **8**(6): e66547; 2013. [3]Pereira, V. S. *Eur Neuropsychopharmacol* **23**(12): 1769. 2013. **Financial Support:** Fapesp, Aarhus University. **AREC number:** 13.1.1506.53.0.

02.010 Evaluation of anti-Alzheimer effects of tacrine dimers in animals with peptide Aβ1-42-induced Alzheimer. Gonçalves AE[1], Mariano LNB[1], Silva LM[1], Aquino RAN, Fatima A[2], Andrade SF[1], Souza MM[1] [1]Univali – Ciências Farmacêuticas, [2]UFMG – Química

Introduction: Alzheimer's Disease (AD) is the leading cause of dementia in the world. It is a neurodegenerative disease that causes progressive cognitive impairment. The typical brain lesions in patients with AD are extracellular β-amyloid peptide deposits, as well as the presence of neurofibrillary tangles. Patients also present neuroinflammation and alterations of the oxidative system. Currently, the treatment available for AD includes inhibitors of cholinesterase enzymes and an NMDA receptor antagonist (N-methyl D-Aspartate). These drugs are palliative and only improve patients' cognition, but they do not help to reduce progression of the disease. The aim of this work is to investigate the effect of new tacrine dimers on mice with amyloid peptide ($A\beta_{1-42}$) induced DA, using in vivo behavioral tests and ex vivo oxidative system analysis, and to evaluate possible hepatotoxic effects in animals treated for 15 days. **Methods:** To evaluate the effect of the treatment on the locomotor and exploratory system in animals, the Open Field test was used, and different types of animal memory were analyzed through the Object Recognition (OR), Inhibitory Avoidance (IA) and Morris Water Maze (MWM) tasks. In relation to the effect of treatments on the oxidative and neuroinflammatory system in the cortex and hippocampus of the animals, the levels of reduced glutathione (GSH), superoxide dismutase (SOD) and myeloperoxidase (MPO) and the levels of Lipid hydroperoxide (LOOH) were determined. The effect of treatment on the hepatic system was analyzed by the measurement of transaminases (AST and ALT) in the serum of the animals and histological analysis of the liver, as well as the effect of the dimers on the cytotoxic activity in human hepatoma cells (HepG2). **Results:** All dimers studied (DT1, DT2, DT3 and DT4), which are known to be cholinesterase inhibitors, showed improvement of the cognitive deficit caused by the $A\beta_{1-42}$ peptide in the OR, IA and MWM tasks. DT4 dimer showed decreased locomotion in the Open Field test, as did tacrine itself. Regarding the oxidative system analyzes, all the dimers presented improvement in the levels of GSH in the hippocampus. SOD activity remained decreased with the DT2, DT3 and DT4 dimer treatments. All dimers showed a decrease in MPO activity. Only the DT4 dimer was able to reduce the levels of LOOH. In relation to the hepatic system, DT1 and DT3 dimers raised AST levels as well as tacrine, data confirmed by histological analysis of the liver of the animals. DT2 and DT4 dimers appear to be the ones with the lowest hepatic lesion. No dimer presented cytotoxicity in HepG2 cells. **Conclusion:** The results suggest that the dimers studied show improved cognition of animals with $A\beta_{1-42}$ induced AD, as well as other additional neuroprotection effects. The results also correspond to the DT2 dimer as a promising pharmacological target for the treatment of AD. **Financial support and acknowledgments:** CAPES and UNIVALI. Process number of Ethics Committee on Animal Use - CEUA / UNIVALI: **11/16.**

02.011 Long-term changes in schizophrenia symptoms-related behaviors induced by repeated exposure to a cannabinoid agonist during pre-puberty and puberty in mice. Gonçalves PFR[1], Cardoso AR[2], Castro NG[1], Neves G[1] [1]UFRJ – Farmacologia e Química Medicinal, [2]UFRJ

Introduction: The exact etiology of schizophrenia is unknown, however this disorder is thought to be the end stage of abnormal neurodevelopmental processes beginning years before symptom onset. Current, epidemiologic data point to Cannabis use during adolescence as a risk factor to schizophrenia development and the earlier the age of first use, the higher the vulnerability to the disease. Although these studies provide evidence of an association between Cannabis use and psychosis, it is not clear how prior exposure to Cannabis can lead to schizophrenia symptons at adulthood and if there is a critical period for exposure. Previous data from our group showed mice developed social memory deficit when exposed to a cannabinoid agonist in the pre-pubertal period, but not in the pubertal period. However, it is not clear if this impairment is selective for social recognition or it is a deficit in recognition memory in general. Thus, we aimed at further investigate long-term changes in schizophrenia-related behaviors induced by daily exposure to cannabinoid agonist during pre-pubertal and pubertal development. **Methods:** Male Swiss mice (ICB/UFRJ breeding colony) were assigned to one of the following experimental groups: 1) control - receiving vehicle i.p. from post-natal day (PND) 28 to 47; 2) pre-puberty - WIN 55,212-2 (WIN) 2 mg/kg i.p. at PND 28-37 and vehicle at PND 38-47; and 3) puberty – vehicle at PND 28-37 and WIN at PND 38-47. Thereafter, animals were left undisturbed until PND 70 (adulthood), when we evaluated their performance in novel object recognition, social approach and social recognition tasks. Also, locomotor activity in response to a novel environment and MK-801 induced hyperlocomotion were evaluated. **Results:** Control animals have normal social preference, social and object recognition memory, when compared to published data. Pre-puberty and puberty exposed animals seemed to have social preference and social memory comparable to controls. However, pre-puberty exposed animals seemed to have no preference for the novel object. Besides, none of the interventions affected locomotor response to a novel environment. Data of the hyperlocomotion in response to a MK-801 challenge are under analysis. **Conclusion:** In summary, once more our data indicate pre-pubertal development as a critical period for cannabinoid exposure in male mice. Exposed animals seem to show a non-specific recognition memory deficit. Additional experiments are underway to confirm these observations. **Financial support:** CNPq, CAPES and FAPERJ. **Ethic approval:** CEUA/CCS-UFRJ: process n° 075/15.

02.012 Combined use of alcohol and cigarette smoke increases pro-inflammatory cytokines and decreases BDNF levels in the frontal cortex of rats. Paula LF[1], Quinteros DA[2], Bandiera S[2], Hansen AW[2], Pulcinelli RR[2], Bobermin L[3], Quincozes-Santos A[3], Gomez R[2] [1]UFRGS – Acadêmico, [2]PPGFT-UFRGS – Farmacologia e Terapêutica, [3]UFRGS – Ciência Biológicas: Bioquímica

Introduction: Chronic alcohol use and cigarette smoking are related with inflammatory responses and neurodegeneration. These drugs are frequently used in association and studies exploring the effect of the combined use on pro-inflammatory and neuroproliferative parameters are scarce. Here we studied the effect of the combined use of alcohol and cigarette smoke on pro-inflammatory cytokines (TNF-α and IL-1β) and on the brain-derived neurotrophic factor (BDNF) in the frontal cortex of rats. **Methods:** Adult male Wistar rats (~280 g) were treated with 2 g/kg alcohol, via oral gavage, and exposed to smoke from 6 cigarettes (ALCS group), twice a day, for 28 days. Results from these rats (n=12) were compared with alcohol-air (AL), cigarette smoke-water (CS), or water-air (CT) rats. On day 28, they were euthanized and the frontal cortex was dissected for analyzes of TNF-α, IL-1β, and BDNF. **Results:** Our results showed that combined use of alcohol and cigarette significantly increased both TNF-α and IL-1β ($P < 0.001$) in the frontal cortex of rats. Those pro-inflammatory cytokines were also increased in the AL ($P= 0.027$) but not CS groups. On the other hand, combined use decreased BDNF levels up to 82%, as well AL and CS treatment (38% and 42%, respectively). **Conclusion:** Therefore, combined use of alcohol and cigarette is more deleterious than their isolated use, increasing pro-inflammatory cytokines and decreasing neuroprotector parameter as BNDF in the frontal cortex of rats. Our results suggest higher neurological risks in individuals that combine use of these two drugs of abuse. **Financial Support**: CNPq, CAPES, Propesq-UFRGS. (CEUA-UFRGS #30088). Acknowledgements: Authors are grateful for CNPq, FAPERGS and CAPES fellowships.

02.013 Effect of the combined use of alcohol and cigarette smoke on oxidative stress parameters in different brain areas of rats. Nietiedt NA[1], Quinteros DA[2], Garofalo CB[1], Paula LF[1], Bandiera S[2], Pulcinelli RR[2], Hansen AW[2], Bellaver B[3], Quincozes-Santos A[3], Gomez R[2] [1]UFRGS – Acadêmico, [2]PPGFT-UFRGS – Farmacologia e Terapêutica, [3]UFRGS -: Bioquímica

Introduction: Although the deleterious effect of alcohol use and cigarette smoke habit on health are largely known, few studies explore the neuronal damage related to their combined use. Here we studied the effect of the co-administration of alcohol and cigarette smoke on oxidative stress parameters in different brain areas of rats. **Methods:** Adult male Wistar rats were treated with 2 g/kg alcohol, via oral, and exposed to smoke from 6 cigarettes, twice a day, for 28 days (ALCS group). Results from ALCS rats were compared with alcohol-air (AL), cigarette smoke-water (CS), or water-air (CT) rats (n =12). On day 28, they were euthanized and the hippocampus, frontal cortex, and striatum were dissected for analyzes of glutathione levels and intracellular free radicals using dichlorodihydrofluorescein (DCFH). **Results:** Our results showed that DCFH levels were higher in ALCS than AL, CS, and CT groups in the hippocampus of rats. Curiously, in the striatum, AL and CS decreased free radical production and ALCS treatment counteracted this effect. Glutathione levels were lower in the hippocampus of ALCS, AL, and CS than CT rats. This antioxidant enzyme was also lower in AL than CS, ALCS, or CT groups in the rat striatum. Treatments did not change DCFH or glutathione levels in the rat frontal cortex. **Conclusion:** Thus, our results showed that alcohol, cigarette smoke, or their combined use produces oxidative damage importantly in the hippocampus of rats. **Financial Support**: CNPq, CAPES, Propesq-UFRGS. (CEUA-UFRGS #30088). Acknowledgements: Authors are grateful for CNPq, FAPERGS and CAPES fellowships.

02.014 Investigation of the effects of caffeine on the ansiogenic changes caused by ethanol intoxication in binge pattern in female rats from adolescence to the adulthood. Silva CCS[1], Pinheiro BG[2], Fernandes LMP[2], Melo AS[2], Luz DA[2], Maia CDSF[1] [1]UFPA – Farmácia, [2]UFPA – Neurociências e Biologia Celular

Introduction: Alcohol (ethanol) is one of the most consumed addition drugs in the world in different age groups. Among the patterns of use, the binge pattern, which consists of an occasional and intense consumption of this drug for a short period of time and followed by abstinence, stands out. In Brazil, this pattern of consumption among young woman and teenagers girls has been growing considerably, representing a harmful form of use, causing greater neurotoxic effects for the CNS. Faced with this fact, it was decided to use caffeine in this research to reverse such intoxication damage, because it is one of the most popular psychoactive drugs consumed around the world in the form of caffeinated beverages. Therefore, this research aims to investigate the effects of caffeine on the behavioral changes of the ansiogenic type in rats, intoxicated by ethanol in binge pattern at early teenager till young adult. **Methods**: Adolescent rats (*Rattus novergicus*) were used (n = 50) divided into 5 groups with 10 animals: Control group (received H_2O); Ethanol group (received Ethanol + distilled H_2O); Ethanol + Caffeine group (received ethanol and caffeine); Caffeine group (received caffeine) and Diazepan group(received 1mg/kg; positive control in Elevated Cross Maze (ECM), i.p). EtOH (3g/kg/day) was given by gavage for 3 consecutive days per week. Caffeine (10 mg/kg, v.o) and distilled water (1mL/kg) were given repeatedly and continuously. Diazepan was given 30 minutes before the ECM test. The animals were evaluated after 4 weeks of gavage. The battery of behavioral tests consisted of Open Field Test and ECM. **Results**: In the open field test, the Ethanol group significantly decreased the central distance traveled, as well as the residence time in the central area compared with the control group, while the Ethanol + caffeine group showed increase in the central distance traveled and in the residence time In the central area compared with the control group. In the ECM test, the Ethanol + Caffeine and Diazepan groups increased the rate of the number of open arm entries and the time in open arm compared with control and Ethanol groups. **Conclusion**: The results suggest that chronically administered caffeine can generate anxiolytic-like behaviors, improving the possible anxiogenic effects on animals intoxicated by EtOH in the binge pattern.

02.015 Central administration of cyclic Glycine-Proline (cGP) induced antidepressant-like effect in the mouse forced swimming test. Cavalcante GTS, Souza FMA, Santos-Neto JG, Nicácio DCSP, Mendes RA, Dias-Batista JB, Maciel DM, Duzzioni M UFAL – Farmacologia

Introduction: The cyclic glycine-proline (cGP) neuropeptide is derived from the natural cleavage (N-terminal position) of insulin-like growth fator-1 (IGF-1). Peripheral administration of cGP has been shown to improve memory and reduce anxiety-like behavior. However, the role of cGP after intracerebroventricular (ICV) administration in mediating depression-like behavior remains to be elucidated. Thus, this study aimed to investigate the effects of central administration of cGP on the depression-related behaviors in the mouse forced swimming test. **Methods:** Adult Swiss mice were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg), and mounted in a stereotaxic apparatus. A stainless steel 8-mm guide was implanted in the lateral ventricle (coordinates, posterior - 0.2 mm, lateral ± 1.0 mm, and ventral -2.0 mm). After surgery, the animals were allowed to recover for at least 7 days. The animals were ICV injected with PBS (1µL) or cGP (0.1, 1 or 10 pg, 1µL), and after 5 min were submitted to forced swimming test (FST). **Results:** Central administration of cGP significantly reduced the time of immobility in the FST (cGP 0.1pg 43.11±17.75; cGP 1pg 10.38±5.37; cGP 1µg 25.89±8.40, $P < 0.005$) in relation to the control group (PBS, 102±18.81). **Conclusion:** Our results demonstrated for the first time an antidepressant-like effect of the cGP neuropeptide. **Financial Support**: CNPq. This work was approved by the Animal Ethics Committee of the Federal University of Alagoas (22/2016).

02.016 PTEN deletion effects on neuronal morphology. Mello NP, Cabral-Costa JV, Mazucanti C, Scavone C, Kawamoto E ICB-USP

Introduction: Literature data show that the absence of the PTEN protein results in neuronal morphological alterations, which leads to dysfunctions in the neuronal development and, consequently, compromise its regular network. Understanding the pathways involved in this situation can contribute to improve the outcomes from some Central Nervous System (CNS) diseases and to generate new strategies that could revert or at least retard the disease process. PTEN (Phosphatase and Tensin homolog on chromosome ten) is a tumor suppressor protein that is related to growth, proliferation and cell survival. Due to its importance to the cell development, animals with total deletion of this protein cannot survive. In this study we aimed as starting point to evaluate PTEN expression and the cell morphology changes during neuronal development *in vitro*. **Methods:** PTEN conditional knockout mouse is obtained by the Cre-LoxP system. The Cre recombinase enzyme is guided by enolase promoter and its expression is homogenous mainly in the cortex and hippocampus. To get the homozygous (HO) and wild-type (WT) genotypes, animals $PTEN^{loxp/+}$; Cre^{+} were mated. E16.5 embryos cortex from PTEN conditional knockout mice were used to get primary neuronal culture. Cells were fixed in 7, 14, 21 and 28 days and, after that, it was performed immunofluorescence. **Results:** Preliminary data suggest a reduction in the PTEN expression and an increase of soma size over the time in the HO animals, while in the WT animals the expression appears to be relatively constant. Besides, data also suggest that PTEN deletion seems not to be occurring in all neurons. **Conclusion:** The decrease in the PTEN expression over the time is expected since the HO animals can survive during the first days of life, but not much longer than that. However, as the project is developed in embryonic culture, is it extremely important to define the necessary time in vitro to occur the deletion that leads to the morphological neuronal alterations characteristics of this model. Besides, not all the neurons from HO animals appears to present an increased soma size, which could indicate that the deletion is not occurring in the same way in all the neurons. **Ethics Committee on Animal Use:** Protocol nº 75/2016. **Financial Support:** FAPESP

02.017 (−)-β-caryophyllene suppresses motor paralysis and neuroinflammation in a murine model of multiple sclerosis through CB2 cannabinoid receptor Alberti TB[1], Gonçalves ECD[1], Barbosa WLR[2], Vieira JLF[3], Raposo NRB[3], Dutra RC[1] [1]UFSC – Ciências da Saúde, [2]UFPA – Ciências Farmacêuticas, [3]UFJF – Ciências da Saúde

Introduction: (-)-Beta-caryophyllene (BCP), a dietary non-psychoactive CB2 receptor ligand sesquiterpene, has showed analgesic, anti-inflammatory and neuroprotective effects (Sharma, C. et al. Curr pharm des; v. 22, n. 21, p. 3237, 2016). Nevertheless, the role of CB2 receptor agonists in general and BCP in particular, in the prevention and treatment of demyelinating diseases, has not previously been studied. In the present study, we sought to investigate the therapeutic potential of BCP on experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS); and attempted to elucidate some of the mechanisms through which BCP modulates the pro-inflammatory scenery of the central nervous system (CNS) and demyelination during MS experimental. **Methods:** C57BL/J6 mice were immunized with $MOG_{35–55}$ and given BCP (25 or 50 mg/kg) daily p.o. from day 0 until the end of the experiment (30 post-immunization). *In vivo* and *ex vivo* immunological responses were evaluated by ELISA, immunohistochemistry, immunofluorescence and flow cytometry. **Results:** Our findings demonstrate that BCP preventively or therapeutically blocked the development and progression of clinical and pathological parameters of EAE. Moreover, data hereby presented indicates that the effect underlying BCP immunomodulatory activity on EAE occurs through activation of CB2 receptor. BCP inhibited immune cell activation, migration of encephalitogenic CD4+ T lymphocytes to the CNS, as well as expression of pro-inflammatory cytokines, also modulating Th1/Treg immune balance. Furthermore, it led to a corresponding decrease in axonal demyelination, neuroinflammation and clinical score. **Conclusion:** Withal, our study strongly supports BCP, a non-psychoactive phytocannabinoid, as a novel molecule to target the cannabinoid receptor CB2 in the development of effective therapeutic agents for MS. BCP represents significant implications for clinical research of MS, as well as other autoimmune and neuroinflammatory diseases. **Acknowledgments:** This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio a Pesquisa do Estado de Santa Catarina (FAPESC), Programa de Pós-Graduação em Neurociências (PGN/UFSC), and Faculdade de Ciências Farmacêuticas (UFPA), all from Brazil. All procedures used in the present study followed the “Principles of laboratory animal care” and were approved by the Animal Ethics Committee of the Universidade Federal de Santa Catarina (CEUA-UFSC, protocol number PP00956).

02.018 Time-dependent dual effect of pilocarpine in non-convulsive mice in the forced swimming test. Souza FMA[1], Santos-Neto JG[1], Nicácio DCS[1], Cavalcante GTS[1], Mendes RA[1], Dias-Batista JB[1], Duarte FS[2], Lima TCM[3], Duzzioni M[1] [1]UFAL – Farmacologia, [2]UFPE – Farmacologia e Fisiologia, [3]UFSC – Farmacologia

Introduction: Studies suggest the existence of genetic and neurobiological similarities between depression and anxiety disorders. Recently, our research group showed that a single systemic injection of pilocarpine (PILO) triggered long term anxiogenic-like behavior in non-convulsive rats and mice. This work aimed to evaluate the short and long-term effects induced by a single injection of PILO in non-convulsive mice in the forced swimming test. **Methods:** Male and female adults mice received methyl-scopolamine (1 mg/kg s.c.) followed 30 min later by a single systemic injection of saline (NaCl 0.9% i.p.) or PILO (75 or 150 mg/kg i.p.). 24 h or 1 month after the last treatment, animals were submitted to the forced swim test (FST, 6 min). Animals were also submitted to rotarod test to rule out any nonspecific motor effect. **Results:** Our results showed that PILO (75 mg/kg i.p.) significantly decreased the immobility time 24 h after the treatment in both male (n=25; 41.50±14.55; $P < 0.05$) and female (n=34; 59.08±12.34; $P < 0.05$) mice submitted to the FST, indicating an antidepressant-like effect. In contrast, the same dose of PILO significantly increased the immobility time 1 month after the treatment in only female mice (n=26, 165.6 ± 14.40; $P < 0.05$) submitted to the FST, indicating a depressive-like effect. 24 h and 1 month after PILO treatment, no significant difference was detected in the rotarod test. Conclusions: Our results showed a time-dependent dual effect of PILO in non-convulsive mice when evaluated in the FST, indicating that behavioral and neurobiological changes related to depressive-like states happen over time, especially in female mice. **Financial Support**: CNPq. This work was approved by the Animal Ethics Committee of the Federal University of Alagoas (CEUA: 22/2015).

02.019 Effect of repeated taurine administration on voluntary alcohol consumption and on behaviors in rats. Pulcinelli RR[1], Nietiedt NA[2], Paula LF[2], Garofalo CB[2], Bandiera S[1], Hansen AW[1], Almeida RF[3], Fontella FU[3], Gomez R[1]
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Introduction: Taurine is a sulfonated β-amino acid that is highly abundant in the CNS and plays a role in various physiologic processes, such as osmoregulation, neuroprotection and neuromodulation. Acute taurine administration reduces ethanol consumption in rats. The aim of this study was to evaluate the effects of repeated taurine administrations on chronic alcohol voluntary consumption in rats and its effect of the combined use after 60 min from the first taurine administration. **Methods:** Male adult Wistar rats (280-300 g) were free to choose from a bottle containing saccharin solution 0.08% or another one containing alcohol 20% w/v with saccharin 0.08% for 4 weeks. Likewise, control group was allowed to choose between 2 bottles containing saccharin 0.08%. The daily liquid consumption of both groups was monitored. On day 22 they were divided into 4 groups (n=12/group) to receive 100 mg/kg taurine, intraperitoneally, once a day, for 7 days, or saline. One hour after the first taurine administration, rats were placed in the open field test and their behaviors were recorded to evaluate the acute effect of taurine. **Results:** Total volume of liquid consumed per week was 1.5 higher in the alcohol than control group, with no effect of taurine treatment. After 7 days of taurine intraperitoneal administration, we did not find a significant change in alcohol voluntary consumption nor in the preference for alcohol in rats. Taurine decreased total ambulation in both alcohol and control rats ($P = 0.024$) after 60 min from the first administration. **Conclusion:** Repeated taurine administration did not affect the voluntary alcohol consumption in rats. Prolonged treatment or higher doses of taurine could be determinant for the appearance of its anti-addictive effect. **Financial Support**: CNPq, CAPES, Propesq-UFRGS. (CEUA-UFRGS #32850). Acknowledgements: Authors are grateful for CNPq, FAPERGS and CAPES fellowships.

02.020 A single systemic injection of pilocarpine induces short- and long-term anxiogenic-like effects in non-convulsive mice. Nicácio DCSP[1], Souza FMA[1], Santos-Neto JG[1], Cavalcante GTS[1], Mendes RA[1], Dias-Batista JB[1], Maciel DM[1], Duarte FS[2], Lima TCM[3], Duzzioni M[1] [1]UFAL – Farmacologia, [2]UFPE – Farmacologia e Fisiologia, [3]UFSC – Farmacologia

Introduction: Recently, our research group showed that a single systemic injection of pilocarpine (PILO) triggered short- and long-term anxiogenic-like behavior in non-convulsive rats. This work aimed to evaluate the short and long-term effects induced by a single injection of PILO in non-convulsive mice submitted to different anxiety-tests. **Methods:** Male and female adults mice received methyl-scopolamine (1 mg/kg s.c.) followed 30 min later by a single systemic injection of saline (NaCl 0.9% i.p.) or PILO (75 or 150 mg/kg i.p.). After 24 h or 30 days, animals were submitted to elevated plus maze (EPM) and light/dark box (LDB) tests to evaluate anxiety-related behaviors. Animals were also submitted to open field and rotarod tests to rule out any nonspecific motor effect. **Results:** A significant decrease in the entries into the open arms (male; n=22, 25.99±3.85; $P < 0.05$), and in the time spent on the open arms (female; n = 23, 19.79 ± 2.25; $P < 0.05$), as well as an increase in the number of protected stretch attend postures (male; n= 22, 15.88 ± 2.04; $P < 0.005$), was found 24 h later the administration of PILO (75 mg/kg) in the EPM, indicating an anxiogenic-like effect. PILO (75 mg/kg) was also found to induce anxiety-like behavior in the LDB test, in which time spent in the light compartment of the LDB apparatus was significantly decreased at 1 month (female, n = 24, 100,9 ± 7,74; P < 0,005) post-PILO treatment. 24 h and 30 days after PILO treatment, no significant difference was detected in the open field and rotarod tests. **Conclusions**: Our results showed that a single systemic injection of PILO triggered short- and long-term anxiogenic-like behavior in non-convulsive mice, extending our proposed model to evaluate the anxiety trait. **Financial Support**: CNPq. This work was approved by the Animal Ethics Committee of the Federal University of Alagoas (CEUA: 17/2014).

02.021 Intrahippocampal injection of OUA triggers dendritic branching in neurons and memory improvement in adult rats. Orellana AM[1], Leite JA[1], Kinoshita PF[1], Andreotti DZ[1], Sá LL[1], Kawamoto EM[1], Scavone C[1] [1]ICB-USP – Farmacologia
Introduction: Ouabain (OUA) is a well-known endogenous cardiotonic steroid that binds to Na,K-ATPase (NKA) and can trigger through intracellular Ca^{2+} levels oscillation the activation of signaling pathways such as NFκB (Aizman *et al.*, 2001). Particularly in neurons, the NFκB signaling has been described to have an important role in molecular switch from short to long-term memory (Kaltschmidt and Kaltschmidt, 2015) also regulating PKA/CREB signaling cascade (Kaltschmidt *et al.*, 2006). Furthermore, CREB activation can lead to an increase in BDNF that can modulate AKT and Wnt/β-Catenin signaling pathways (Yang *et al.*, 2015). The aim of this study was to verify whether intrahippocampal injection of OUA (10 nM) was able to modulate the principal signaling pathways involved in morphological plasticity and memory formation in hippocampus of male adult rats. **Methods:** Wistar adult male rats were submitted to estereotaxic surgery followed by intrahippocampal injection of OUA 10 nM or Saline 0,9%. All the procedures were performed according to Ethics Commitee of Animal Use (CEUA). Results were obtained from Western Blot assay, PCR, ELISA, immunofluorescence, Golgi-Cox stainig and Moris Water Maze behaviour test. **Results:**For the first time we suggest that intrahippocampal injection of OUA 10 nM can activate WNT/β-Catenin signaling pathway as well as CREB/BDNF, AKT and NFκB leading to important changes in cellular microenvironment that results in increased dendritic branching in CA1 and DG neurons, with spatial reference memory improvement. **Conclusion:** The molecular mechanism trigged by low doses of OUA seems to be a promising therapeutic strategy to improve morphological plasticity and cognition in adult hippocampus. **Financial Support**: Fapesp 2011/22844-0 CEUA: Number 144/ Fls13, book3. References: Aizman, O. PNAS, 98, 13420, 2001. Kaltschmidt, B. Front Mol Neurosci, 8, 69, 2015. Yang, J. W. Neuropeptides, 54, 35, 2015.

02.022 Evaluation of pain sensitivity in mice submitted to sporadic Alzheimer's-like dementia. Souza MM[1], D'Aquino MS[2], Solanha RL[2], Gonçalves AE[2] [1]Univali – Bioquímica e Farmacologia, [2]NIQFAR-Univali

Introduction: Recently, several studies in the clinical area have demonstrated that patients with Alzheimer's disease (AD) are more sensitive to pain when compared to individuals without pathology. Little is found in the literature the study of pain in animals submitted to Alzheimer's models. **Objective.** In this context, the main objective of this study was to evaluate the nociceptive response of rats submitted to Sporadic Alzheimer's disease, the streptozotocin model (STRZ). **Methods:** In this study all experiments were conducted using female Swiss mice (25–35 g), with access to food and water ad libitum. The experiments were performed after approval of the protocol by the Institutional Ethics Committee (CEUA 23/2014 /UNIVALI) and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious. The animals were submitted to Streptozotocin-induced Alzheimer's model (2 µl of 2.5 mg / ml solution) and 15 days after memory tests (inhibitory avoidance and recognition of objects). Sham (false induceds), Naive (untreated) and morphine (5 mg / kg, i.p.) groups were used to compare the nociceptive response. Detected total memory impairment animals were divided into groups and submitted to pain models induced by: capsaicin (20 µL , 1.6 µg/paw), acetic acid (0.6%), glutamate (30 nmol/paw), formalin (2.0%) (20 µL/paw) or heat (hot-plate test). **Results:** The results indicate that animals with streptozotocin-induced AD induced a statistical increase in nociception induced by: capsaicin (Vehicle / 9322.15 s, Naive 62.70 ± 2.4 s), acetic acid (Vheicle / 62-4.2 ; Naive 38.7 ± 2.4) and Glutamate (Vehicle / 152 ± 3.4 s; Naive 102.0 ± 4.1 s). Increased sensitivity to pain was also observed in the first phase of formalin-induced pain (Vheicle / 62-4,2, Naive 84.2.2 ± 2.4) and hot plate test (Vehicle / 7.4 ± 3.2 Naive 13.7 ± 1.6).The results show that animals with AD had a high nociceptive response when compared to the Naive group when nociception was induced by acetic acid (99.05%), capsaicin (53.57%), and heat (45.03%). Formalin (38%). Glutamate 42.36% when compared to the group without AD induction. **Conclusions:** The results allow us to suggest that animals with STZ-induced AD showed a higher nociception when compared to the group of normal animals (Naive) in several pain models, partially validating what is observed in the clinical setting that AD patients exhibit greater pain sensitivity. **Financial support:** CNPq/ UNIVALI

02.023 New piperazine derivate (LQFM183) improves cognitive dysfunction and oxidative stress in aluminium chloride-induced model in mice. Neri HFN[1], Costa EA[1], Santos FCA[2], Menegatti R[3], Ghedini PC[1] [1]UFG – Farmacologia, [2]UFG – Histologia, [3]UFG – Faculdade de Farmácia

Introduction: The oxidative stress is associated with several neurodegenerative disorders, causing cell death and leading to development of neurodegenerative diseases, as Alzheimer's and Parkinson's disease. In the present investigation, protective effect of LQFM183, a new candidate molecule of drugs prototype with antioxidant properties, was evaluated in a model of neurotoxicity and cognitive impairment induced by aluminum in mice. **Methods:** Swiss male mice (35-45 g) were randomized into four groups (n=10): I and II received Tween 80, 2,0% (10 mL/kg); III and IV were treated with LQFM183 (200 µmol/kg). One hour after, the animals received a second treatment: I and III (distilled water 10 mL/kg); II and IV ($AlCl_3$ 750 µmol/kg). The treatments were given once daily through oral gavage for 40 days. After this period, the short-term memory (STM) and long-term memory (LTM) were evaluated using passive avoidance test and the motor activity by open field and chimney tests. Posteriorly, the animals were euthanized and the hippocampus was dissected for determination of catalase (CAT) and acetylcholinesterase (AChE) activities and levels of malondialdehyde. The results were expressed as the mean ± SEM and the statistical analysis was performed using one way ANOVA followed by Newman-Keuls or Dunnett post-hoc tests. Significant difference was considered when $p<0.05$. All procedures were approved by the Institutional Ethics in Research Committee at the Federal University of Goiás, Goiás, Brazil (Protocol CEUA/UFG 053/2016). **Results:** Increase of oxidative stress marker and cognitive dysfunction were present in all $AlCl_3$ treated groups. Pretreatment with LQFM183 protects against $AlCl_3$ damage, observed by increase of the latency time in STM (7.22±0.91s) and LTM (16.71±1.87s) of group IV when compared with II group (STM= 4.63±1.04s; LTM= 5.28±1.07s, $p<0.001$). CAT activity was increased in groups III and IV (0.33±0.02; 0.17±0.01 mmol/mg proteins, respectively) when compared with group II (0.09±0.01 mmol/mg proteins, $p<0.01$). The AChE activity was decreased in both groups III and IV (23.51±0.95; 21.71±1.63 nmol/min/mg proteins, respectively) when compared with group I (38.41±1.52 nmol/min/mg proteins, $p<0.001$) and group II (33.24±1.5503 nmol/min/mg proteins, $p<0.001$). LQFM183 decreased the MDA levels in groups III and IV (30.07±0.3; 36.58±0.28 pmol/ mg proteins, respectively) when compared with group II (47.03±0.44 pmol/ mg proteins, $p<0.05$). The open field and chimney tests showed that the locomotor activity was not affected by LQFM183 or $AlCl_3$ treatments. **Conclusion:** The data suggest that LQFM183 protected against neurotoxicity induced by $AlCl_3$ showing that this compound may be a potential therapeutic approach in the treatment of oxidative stress diseases associated with neurotoxicity. **Financial support:** CAPES.

02.024 Evaluation of the influence of maternal voluntary physical exercise on synaptic plasticity and cognition in animals with PTEN deletion. Andreotti DZ, Cabral-Costa JV, Scavone C, Kawamoto EM ICB-USP

PTEN (*Phosphatase and Tensin Homolog*) was study initially as tumor suppressor gene and acts in order to antagonize pathways related to Akt (protein kinase B). By your broad expression in neurons, PTEN mutations could change the morphology of these cells, phenotype related to neuropsychiatric disorders, such as Autism spectrum disorder. It is possible that Non-pharmacological interventions, like voluntary physical exercise, ameliorate the phenotype related to these disorders, acting at synaptic phenomena and cognitive improvement. Besides, physical exercise during pregnancy seems to ameliorate cerebral function in offspring, being that effects could extend to adulthood.. Based on these facts, the present study aimed to evaluate the role of maternal voluntary physical exercise over cognition and synaptic plasticity in mice with PTEN depletion. Sixty days-old female mice were used, being one group allocated individually in a cage with a running-wheel and another group was in a simple cage. After 10 days, a male mouse was allocated with the females for mating. When the male offspring was between 60 and 90 days-old, they were submitted to behavioral tasks (open field, elevated plus maze, social recognition, social novelty, Morris water maze and inhibitory avoidance) and euthanasia followed by and their cortex and hippocampus were collected for biochemical assays, as western botting and Elisa kits. Preliminary results showed that maternal exercise could have generated anxious behavior in offspring, whether or not PTEN deletion occurs in animals. On sociability tasks, maternal physical exercise seems to improve the social interaction only in wild type mice. The Morris water maze task, in turn, showed a learning deficit to find the hidden platform localization in animals with the deletion of PTEN, and maternal exercise seems to ameliorate this deficit. Biochemical markers related to synaptic plasticity, as NR1 subtype of NMDA receptor (N-methyl-D-aspartate) and the neurotrophin BDNF (brain-derived neurotrophic factor), showed an increase in their concentration in mutant mice whose progenitor were submitted to voluntary physical exercise. Based on these data, we suggest that maternal exercise could have the potential to counteract phenotypes that may be caused PTEN deletion. **Financial suport:** This study was supported by FAPESP (Process number 2011/21308-8) and University of São Paulo (USP). This study was approved by the institute's Animal Research Ethical Committee and followed the required guidelines for animal manipulation (Protocol number CEUA/ICB-USP 114/14).

02.025 Sulfated polysaccharides from red seaweed *Gracilaria cornea* protects against neurodegenerative damage in 6-OHDA model in rats. Frota AF[1], Soares VVM[1], Sousa RM[1], Nascimento HG[1], Silva Júnior PN[1], Aguiar LMV[2], Benevides NMB[1]
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Sulfated polysaccharides (SP) isolated from marine organisms has demonstrated pharmacological potential several *in vitro* and *in vivo* models. SP agaran type isolated from the red seaweed *Gracilaria cornea* has shown a possible action in central nervous system in animal models of nociception and anti-inflammatory action. However, analyses of the effects of these polysaccharides in Parkinson's disease are still required. This study aimed to evaluate the neuroprotective effects of agaran from *G. cornea* in model Parkinson's disease induced by 6-OHDA in rats. This study is identified under number 45/13 by Ethics Committee of Animal Research of the UFC. The alga was collected on the Flecheiras beach, Trairí-Ce/Brazil. SP were obtained by enzymatic digestion. Male Wistar rats (250-300 g) were randomically divided in five groups (n = 7 animals per group). Rats were anesthetized and submitted to unilateral intrastriatal injection of 6-OHDA (21 µg) or Saline (0.9%) (Sham group). After 24 hours, animals were treated with SP (0.3; 3.0 or 30 mg/kg) or saline (0.9%) by gavage, for 14 days and maintained under *ad libitum* feeding conditions. At 14st day, all animals were submitted to behavioral tests (Open-field test, Cylinder test and Rotation test induced by apomorphine [3 mg/kg, i.p.]). Then, animals were euthanized and striatum were removed and used for neurochemical analysis. In Open-field test, SP (0.3 mg/kg) increased the locomotor activity (40.2±5.8 number of crossing lines), in relation to 6-OHDA group (34.2±3.4 number of crossing lines). In the cylinder test the groups treated animals with SP 0.3 mg/kg, the appeared to perform ($p<0.05$) better than that control group (3.2±0.9 and 0.3±0.1% use of left paw, respectively). Rotational test SP (0.3; 3.0 and 30 mg/kg) decreased ($p<0.001$ and $p<0,01$) number of rotations in 94, 73 and 45%, respectively, in comparison with 6-OHDA group. Analysis of nitrite/nitrate revealed a return to basal conditions in parkinsonian's rats treated with SP 0.3 mg/kg in comparison Sham group. SP (0.3; 3.0 and 30 mg/kg) reduced levels of nitrite/nitrate (0.9±0.1; 1.0±0.08 and 1.1±0.1, respectively) in comparison with 6-OHDA group (1.3±0.1). The levels of lipid peroxidation were reduced after treatment with SP in Parkinsonian rats. Sulfated polysaccharides of *Gracilaria cornea* presented effects against locomotive and neurochemical disorders induced by 6-OHDA in model of Parkinson's disease. Suggesting a possible neuroprotective effect. Support by CAPES and CNPq.

02.026 Taurine counteracts the neurotoxic effects of chronic hyperglycemia in diabetic rats. Bandiera S[1], Caletti G[1], Pulcinelli RR[1], Hansen AW[1], Steffens L[2], Herrmann AP[3], Moura DJ[2], Barros TMH[2], Gomez R[1] [1]UFRGS – Farmacologia, [2]UFCSPA – Farmacologia, [3]UFFS – Patologia

Diabetes is a chronic metabolic disease associated with oxidative stress and neuroinflammation. Taurine, a sulfur-containing amino acid, has neuroprotective properties that might prevent neural injury induced by chronic hyperglycemia. Taurine shows antidepressant-like effect and decreases up to 18% glycemia in diabetic rats. We evaluated here the effect of chronic taurine treatment on oxidative stress parameters and cytokines levels in the frontal cortex and hippocampus of diabetic and non-diabetic rats. Diabetes was induced by streptozotocin administration (60 mg/kg, ip) in half of 24 rats. After diabetes confirmation, rats were randomly allocated to receive taurine (100 mg/kg, i.p.) or saline, once a day for 28 days (n = 6/group). On day 29, rats were euthanized and the frontal cortex and hippocampus were dissected and stored for analyses. Our results showed that taurine counteracted the increased levels of reactive oxygen species (ROS), measured by dichlorofluorescein (DCF fluorescence), in the frontal cortex and hippocampus of diabetic rats. Taurine also counteracted the increased levels of IL-6, IL-12, TNF-a, and IFN-g in the frontal cortex and hippocampus of diabetic rats. Supporting our hypothesis, taurine treatment reduced ROS and inflammatory cytokine levels, evidencing its beneficial effects against toxicity and neuroinflammation associated with diabetes. Ethics Committee: CEUA-UFRGS 26303 **Financial Support**: CNPq, CAPES, Propesq-UFRGS

02.027 A_{2A} receptor facilitates neuromuscular transmission activating PKA, thereby increasing the high-affinity choline transporter rate. Castellão-Santana LM[1], Abiko PY[1], Ambiel CR[2], Correia-de-Sa P[3], Alves-Do-Prado W[1] [1]UEM – Farmacologia e Terapêutica, [2]UEM – Ciências Fisiológicas, [3]Universidade do Porto – Ciências Biomédicas

Introduction: Acetylcholine (ACh) released from motor nerve terminal (MNT) also can act pre-synaptically to regulate its own release through M_1, M_2, A_1 and A_{2A} receptors. Presynaptic facilitatory M_1 receptors are typically coupled to protein kinase C (PKC) activation, whereas the presynaptic facilitatory adenosine A_{2A} receptors are positively coupled to protein kinase A (PKA) activation. Choline is taken up from the cholinergic terminal through the (HChT). As it has been shown that there is a close correlation between the release of ACh from MNT and the activity of HChT to HC-3, in the present study were investigated the influences of M_1, M_2, and A_{2A} receptors on the HChT in neuromuscular transmission. **Methods:** Phrenic nerve–diaphragm muscle preparations from rats (CEUA/UEM- n^o 7227300915) were isolated. Preparations were indirectly stimulated at 0.2 Hz and six tetanic stimuli (50 Hz) were applied at 20 min intervals. The initial tetanic tension at the beginning (A) of the tetanic stimulus and tension at the end (B) of the tetanic stimulus (after 10.0 s; B) was recorded and the ratio (R) B/A calculated. The value of lowest concentration of HC-3 (4.0 µM) able to produce effect in R values were researched and it's separately effect, or in presence of others drugs, was analyzed at T= 45 min. The others drugs (ZM241385, CGS21680, methoctramine, McN-A-343c, phorbol (PMA) and forskolin (FSK) were administered 20 min after the administration of HC-3. Experiments were also performed with inverted order administration of HC-3 and FSK. In this case, the PKA activator was administrated 20 min before HC-3. **Results:** The inhibitory effect in the R-value (-4.46± 1.04%, n=5) caused by HC-3 (4.0 µM) was worsened by ZM241385 (from -4.46±1.04% to -12±0.97%, n=4), CGS21680 (from -4.46±1.04% to -9.67± 0.46% n=4) or by McN-A-343c (from -4.46± 1.04% to -49.0± 1.41%, n=4). FSK separately increased R-Value (11.0±0.35%, n=5), but it was not able to change the inhibitory effect caused by HC-3 when it was administered after HC-3 (from -4.46±1.04% to -4.2±1.31%, n=4). In contrast, the inhibitory effect caused HC-3 was reduced when FSK was the agent previously administrated (from -4.46±1.04% to -2.0±0.35%, n=4). PMA, an activator of PKC (from -4.46±1.04% to 8.0±4.9%, n=4), and methoctramine (from -4.46±1.04% to -2.0±0%, n=4) antagonized the inhibitory effect produced by HC-3. Data indicate that whereas the facilitatory effect caused by the presynaptic A_{2A} receptors activation seems to be mediated by PKA increasing the HChT rate, as the facilitatory effect caused by FSK and CGS disappeared when the neuromuscular preparations were treated with HC-3. It is also shown that appears to be no coupling between HChT and PKC activation, or HChT and M_1 receptors, as the facilitatory effect caused by PMA was only reduced by HC-3, but the facilitatory effect determined by McNA-343c was changed to a dramatic reduction in R-value. **Conclusion:** Whereas the facilitatory effect caused by the A_{2A} receptors seems to be mediated by PKA increasing the HChT rate, the effects caused by M_1, M_2 receptors blockage, and PKC activations do not seem to depend on a coupling with HChT. **Financial Support**: FADEC/UEM.

02.028 Presynaptic adenosine (A2A) receptor reduces the neostigmine-induced fade by diminishing both desensitization of neuronal nicotinic receptor and inhibitory effect caused muscarinic (M2) receptor activations on motor nerve terminal , Andreo PHM[1], Ambiel CR[1], Castellão-Santana LM[2], Correia-de-Sá P[3], Alves-do-Prado W[2] [1]UEM – Ciências Fisiológicas, [2]UEM – Farmacologia e Terapêutica, [3]Universidade do Porto

Introduction: The neostigmine-induced fade (NEO-FADE) may be induced by acetylcholine accumulation at synaptic cleft to be able to trigger inhibitory-M_2 receptors ($^{-}CM_2R$) on motor nerve terminal (MNT). Since neostigmine is not a pure AChE inhibitor, as itself also desensitizes facilitatory (**+**) neural nicotinic receptors (nCNR) on MNT, and taken into account that presynaptic $^{-}CM_2R$ and adenosine (A) A_{2A} receptors ($^{+}A_{2A}R$) play keys roles in the neostigmine-induced TOF_{fade}, the roles of presynaptic nCNR (blocked by hexamethonium, HEXA) and $^{+}A_{2A}R$ (blocked by ZM 241385, ZM) in the NEO-FADE were investigated in the phrenic nerve diaphragm muscle preparations of rats. As the effects caused by $^{+}A_{2A}R$ and $^{-}CM_2R$ involve activation or inhibition of adenylate cyclase (AC), respectively; the influence of forskolin (FSK) in the NEO-FADE was also researched. **Methods:** Phrenic nerve–diaphragm muscle preparations from rats were isolated (CEUA/UEM- n^{o} 7781201016). Preparations were indirectly stimulated at 0.2 Hz and six tetanic stimuli (50 Hz) were applied at 20 min intervals. The initial tetanic tension at the beginning (A) and that obtained at the end (B) of the tetanic stimulus (50 Hz during 10s) was taken as ratio (R) B/A. The lowest concentration of neostigmine (0,1 µM), fasciculin (FAS; 0,1 µM) and the oxotremorine (OXO; 5.0 µM) able to change R values were researched. HEXA (270µM), methoctramine (MTC, 0.1µM), pirenzepine (PZP, 10nM), ZM (10nM) and FSK (3.0µM) were administered 20 min after neostigmine. The effect caused by neostigmine separately, or in presence of others drugs, was analyzed at T= 65 min. Taken into account that FAS is a pure AChE agent and that the shape of tetanic contraction caused by FAS is mainly generate by ACh accumulation in the synaptic cleft, FAS was used to compare what would be the shape of tetanic fade generated by pure and a non pure (neostigmine) anticholinesterase agent. Data were submitted to ANOVA, followed by Bonferroni test ($P<0.05$). **Results:** Neostigmine (0.1 µM), FAS (0.1 µM) and OXO (5.0 µM) produced -86.0± 1.30% (n=6), -26.40± 1.10% (n=5) and -50.80± 1.30% (n=5) reduction in R-values, respectively. HEXA (from -86.0± 1.30%, n=6 to -14.20± 0.75%, n=5), MTC (from -86.0± 1.30%, n=6 to -69.30± 1.5%, n=5) and ZM (from -86.0± 1.30%, n=6 to -81.7± 1.1, n= 5) reduced NEO-FADE. NEO-FADE was not only prevented, as it was turned in facilitation, by previous treatment of preparation with FSK (from -86.0± 1.30%, n=6 to +23.2± 0.75%, n=5) or with simultaneous administration of HEXA and MTC (from -86.0± 1.30%, n=6 to +7.97± 0.73%, n= 5). In contrast, PZP worsened NEO-FADE (from -86.0± 1.30%, n=6 to -93.7± 0.78, n= 5). The fade induced by OXO was reduced by MTC (-50.8± 1.30%, n=5 to -37.2 ± 2.5, n= 5). **Conclusion:** NEO-FADE seems to be caused by desensitization of $^{+}$nCNR and activation of $^{-}CM_2R$ on MNT by NEO and ACh accumulation, respectively. These effects would be mediated by PKA (via $^{+}A_{2A}R$ activation reducing $^{+}$nCNR desensitization) and AC activation (reducing $^{-}CM_2R$ effect) on MNT. **Financial Support**: FADEC/UEM

02.029 Effects of enrichment environment in female rats and its effects on choice between cocaine and saccharine in a CPP model. Heidrich N[1], Almeida FB[2], Fernandes PR[2], Fonseca AR[1], Costa L[1], Silva FFS[1], Freese L[1], Barros HMT[2,1]
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Psychostimulants, such as cocaine, have significant abuse potential and studies shows that women are more vulnerable to addiction development, probably due to the influence of sex hormones. On the other side, in rats, enriched environment (EE) has a protective role and prevents the development of addiction. Conditioned place preference (CPP) evaluates food and drugs' reinforcement effects. In this model, the animal is able to choose to remain a greater amount of time in a drug-paired chamber. Its well know that cocaine induces CPP-conditioning in animal models. Choice is a model that has been used in self-administration, in which animals are able to choose to drink a sweet solution (e.g. saccharine) or to self-administer a cocaine injection. The goal of this study was to verify whether the protective effects of the environmental enrichment affects choice between a sweet solution or cocaine in female rats in an adapted CPP protocol. 24 female rats (PND 21) were allocated between two housing conditions: control group (CTR - n=12) and enriched environment (EE - n=12). From PND 35, estrous cycle was assessed daily untill the end of the experiment. **Results:** only CTR group individuals had conditioned to cocaine (p=0,038), showing EE's protective effect. Phases of estrous cycle were distributed regularly. No correlation was found between estrous cycle and vulnerability to cocaine-seeking. This study showed that, although females are more vulnerable to psychostimulants, it was possible to determine a protective effect of enriched environment. More studies are needed to better understand the effects of choice on females, as well as the link between estrous cycle and the greater vulnerability to cocaine-seeking in CPP models.

02.030 Anxiolytic-like effect of [BMZPdCl]2, an organometallic bromazepam-palladium(II) derivatives, in the mouse elevated plus maze. Santos-Neto JG[1], Souza FMA[2], Nicácio DCS[2], Silva AHQ[3], Maciel DM, Cavalcante GTS[4], Souza GF[2], Santos-Vieira MP[2], Silva OBS[2], Meneghetti MR[3], Duzzioni M[2] [1]UFAL, [2]UFAL – Farmacologia, [3]UFAL – Química, [4]UFAL – Farmacologia

Introduction: Clinical results with current pharmacological treatment of anxiety disorders are far from ideal. Therefore, new research is still necessary to search for alternative anxiolytic-selective compounds which are more effective, safer and with less possibility of adverse reactions. A promising area for the development of new drugs is the organometallic chemistry. This work aimed to evaluate the effects of intraperitoneal (i.p.) administration of the metal complex derived from bromazepam $[BMZPdCl]_2$ on fear and anxiety-related behaviors in mice. **Methods:** Female Swiss mice (30-35g) received saline (NaCl 0.9%, i.p.; control group) or $[BMZPdCl]_2$ (0.05; 0.5 or 5 mg/kg) and 30 minutes after, animals were submitted to anxiety [elevated plus maze (EPM, 5 min)] or memory [step-down (SD, 6 min)] tests. Animals were also submitted to open field test (OFT, 5 min) or rotarod test (RR, 3 sessions) to rule out any nonspecific motor effect. **Results:** Our results showed that $[BMZPdCl]_2$ at the dose of 0.05 mg/kg increased the percentage of time [$F(3, 33)=8,64$; $P < 0.005$)] and number of entries [$F(3,33)=3,44$; $P < 0.05$)] in open arms of EPM, indicating an anxiolytic-like effect. No significant difference was detected in the memory test (SD) or locomotors tests (OFT and RR), indicating absence of amnesic and sedative effect. Conclusions: Our results showed an anxiolytic-like effect of $[BMZPdCl]_2$ on mice when evaluated in EPM, without impairment in memory or locomotion. **Financial Support**: CNPq. This work was approved by the Animal Ethics Committee of the Federal University of Alagoas (CEUA: 22/2017).

02.031 Na^+,K^+-ATPase and NMDA receptor-related alterations in cerebellum of Klotho hypomorfic mice – a genetic model of aging. Cararo-Lopes MM, Mello PS, Mazucanti CH, Sá Lima L, Andreotti DZ, Scavone C, Kawamoto EM ICB-USP – Farmacologia

Introduction: Klotho protein has anti-aging function, and *klotho* hypomorphic mice (*kl*$^{-/-}$) present premature aging features, including cognitive and motor impairments. Studies from our laboratory reported an age-related decrease of Na^+,K^+-ATPase activity linked to cGMP–PKG (cGMP-dependent protein kinase) in cerebellum, which are signaling pathways closely related to N-Methyl-D-aspartate receptor (NMDAR) function. Many other studies support the idea of Na^+,K^+-ATPase and NMDAR-related alterations leading to learning, memory, affective and motor disturbances during the aging process. In addition, increasing evidence further cerebellum involvement in such disturbances. Although, whether Klotho reduction could influence these pathways on cerebellum remains unknown. **Aim:** Therefore the present study aims to assess NMDAR and Na^+,K^+-ATPase subunit composition and function in cerebellum of *kl*$^{+/+}$ and *kl*$^{-/-}$ 8 weeks-old mice. **Results:** Current results revealed an increase in α_2,Na^+,K^+-ATPase subunit levels and decreased Na^+ pump activity. Moreover, we found diminished levels of GluN1 NMDAR subunit phosphorylation and a change in the GluN2A/GluN2B ratio. These results were followed by a reduction in NOS activity, but no significant changes in cGMP levels. Changes in apoptotic proteins (Bax/BCL-2) were also observed, which could be related to NMDAR hypofunctioning. According to literature data, pro-apoptotic proteins have a key role in the proper functioning of NMDAR-dependent long-term depression (LTD), an imperative mechanism to motor coordination refinement. **Conclusion:** Taking together, our data suggest that the drastic reduction in Klotho protein levels, led to significant molecular changes in cerebellum, therefore, corroborating previous data on Na^+,K^+-ATPase and NMDA relevance to age-related alterations and proper cerebellar function. Also, these results provide an insight of a possible explanation for motor coordination deficits found on these mice. Nevertheless, further investigation is required to precise whether these alterations are affecting LTD and motor coordination in Klotho hypomorphic mice. **Financial Support**: FAPESP, CNPq. All procedures were approved by the Biomedical College of Animal Experimentation and the Ethical Committee for Animal Research ICB/USP (Number 79 –October 16, 2014).

02.032 The role of GCs signaling via GR in BLA on stress-induced late anxiety-like behavior in rats. Bueno-de-Camargo LM, Novaes LS, Munhoz CD ICB-USP – Farmacologia

Introduction: It has been noted, year after year, an increase in the incidence of psychiatric disorders, with a greater emphasis on anxiety-related ones. These, in turn, are closely linked to stress, a factor that is increasingly present in our daily life, especially on large cities. Because of the high rates of violence, labor competitiveness and low quality of life, Brazil has figured as one of the countries where these diseases had the highest annual incidence increase, which justifies the importance of this study. Data from the literature describe the amygdala, more precisely, the basolateral amygdala complex (BLA), as the encephalic central structure to the emotional behavior expression. It is known that, in response to stressful events, the hypothalamic-pituitary-adrenal axis (HPA) promotes the release of glucocorticoids (GCs), mainly corticosterone (CORT, main GC in murine), which returns to its basal levels by a negative feedback system. However, on anxiety disorders, including post-traumatic stress disorder (PTSD), this feedback is impaired, resulting in a HPA axis hyperactivation. Some studies suggest, as a possible neurobiological mechanism for the development of anxiety-like behavior, the GCs' action through their receptors, mineralocorticoids (MR) and glucocorticoids (GR), expressed at high levels on BLA neurons. In addition, is has been shown that acute immobilization stress (2h) promotes, 10 days later, anxiety-like behavior and dendritic hypertrophy in BLA neurons of rats. Corroboratively, a previous study from our group showed that the administration of metyrapone (a GCs synthesis inhibitor) previously to acute immobilization stress prevented anxiety-like behavior in rats. Therefore, this study sought to verify the role of GCs/GR signaling in BLA on late anxiety-like behavior in rats triggered by 2 hours of acute immobilization stress. **Methodology:** For this, cannulas were implanted bilaterally intra-BLA of adult male rats, and 5 days later, RU486 (mifepristone) - an antagonist of GR or saline – was injected immediately before 2 hours of immobilization. After 10 days of the stress, the anxiety-like behavior was verified by the elevated plus maze (EPM) test. For the statistical analysis, only the animals with cannulas correctly implanted intra-BLA were considered. **Results and Conclusion**: Our data indicate that the GR antagonism intra-BLA prevents the occurrence of the stress-induced anxiety-like behavior in animals 10 days later, suggesting that this stress-induced disorder is dependent on GR activation in rat BLA. *Acknowledgments to FAPESP for the* Financial Support

02.033 Study of new multi-target acetylcholinesterase inhibitors of greater tolerability for treatment of Alzheimer's disease. Guimarães MJR[1], Viegas CJ[2], Castro NG[1] [1]ICB-UFRJ, [2]Unifal

Alzheimer's disease (AD) is a progressive neurodegenerative disease that initially affects cognitive abilities due to the dysfunction and death of entorhinal and hippocampal neurons. A cholinergic hypothesis proposes that neurodegeneration in the basal forebrain causes the levels of acetylcholine to fall in the cortex and hippocampus, possibly leading to their dysfunction.. This contributes to the cognitive symptoms evident in those with the disease. In agreement with this hypothesis, AD is currently treated through the use of anticholinesterase substances, such as galantamine, donepezil and rivastigmine. However, the high cost and a high presence of side effects, mainly due to activation of peripheral muscarinic receptors, drive the demand for new drugs. We have planned and synthesized novel AD multi-action drug candidates with anticholinesterase and anti-inflammatory properties, designed by hybridization and molecular modification from the framework of donepezil with an acylhydrazone spacer unit, which in addition to the anticholinesterase activity unit could also have other beneficial activities in AD, interacting with other targets. One of the goals is to add an inhibition of M_3 receptors, which mediate adverse effects such as nausea and diarrhea related to increased gastrointestinal secretion and bowel motility. All samples were first analyzed for their inhibitory effect on cholinesterases by the Ellman method with purified acetylcholinesterase of E. electricus (AChE) and equine serum butyrylcholinesterase (BuChE). Calcium fluorimetry assays were performed with the ratiometric indicator fura-2 to determine a possible antagonistic action of the M3 muscarinic receptor on human intestinal epithelial cells (HT-29 strain). Cytotoxicity assays were performed using a Live / Dead methodology for calcein AM and propidium iodide fluorimetry. Twelve of 13 substances that were screened at 30 μM presented an inhibitory action on AChE greater than 50% and were selected to obtain concentration-response curves. The IC_{50} were between 3.3 and 22.3 μM for AChE and between 12.8 and 18.9 μM for BuChE. Thus, most of the analyzed substances demonstrated selectivity between cholinesterases. We obtained the concentration-response curve of carbachol in standardization tests of the calcium assay and began the evaluation of the antagonistic effect, preincubating the cells with the phenylpiperidine derivatives before the addition of carbachol. None of the compounds (at 30 μM) seemed to inhibit carbachol-induced calcium mobilization in HT-29 cells. None of samples evaluated was cytotoxic (at 30 μM). The novel donepezil analogues were active as AChE inhibitors, but additional structural modifications in our current series of compounds may be required to incorporate a significant antimuscarinic activity. Support: FAPEMIG, CNPq and a CAPES fellowship.

02.034 Study of neuroprotective effect of arylnitrones in *In vitro* models of stroke. Boni MS, Castro NG ICB-UFRJ – Farmacologia e Química Medicinal

Introduction: and Stroke is a neurologic disease that represents the third most frequent cause of deaths in the whole world. Focal ischemia leads to excessive release of excitatory neurotransmitters, such as glutamate, triggering the excitotoxicity process, which is the main responsible for neuronal injury in the ischemic core. Alteplase (a thrombolytic agent) is the only pharmacologic therapy available for the acute treatment of ischemic stroke. However, blood reperfusion leads to a massive production of reactive oxygen (ROS) and nitrogen species (RNS), promoting neuronal death in the penumbra zone and lesion propagation. Arylnitrones related to the antioxidant alpha-phenyl-tert-butyl-nitrone (PBN) synthetized by Laboratório de Química Bioorgânica from UFRJ showed a protective effect in a peripheral model of ischemia followed by reperfusion (Sothea Kim, Bioorg Med Chem, v. 15, p. 3572, 2007). **Aim:** Considering the redox imbalance in neural ischemia-reperfusion, our aim was to study the possible antioxidant and neuroprotective effects of the novel arylnitrones in *in vitro* models of stroke. **Methods:** Using a rat cortex primary neuronal cell cultures, we analyzed the effect of arylnitrones in excitotoxicity induced by a brief exposure to 500 µM of glutamate + 10 µM of glycine through colorimetric quantification of lactate dehydrogenase (LDH) released. A high-content live-dead fluorescence assay was used to evaluate the cytotoxicity of the compounds (50-1000 µM) after 24-hour exposure of HT29 human colon epithelial cells. **Results:** Glutamate in the presence of glycine induced an increase of 3.4 times in LDH release (26.9% of total content) compared with the control group (cells not exposed to glutamate) (8.7% of total). Initially we performed a screnning of six arylnitrones in the concentration of 500 µM, and the compounds LQB 123 and DS 127 showed respectively 58% e 55% of protection compared with the glutamate group (mean of three independent experiments in triplicates). The cLog P of the compounds determined *in silico* (ChemDraw program) ranged between 0.241 and 4.303. The substances LQB 123 and DS 127 showed the highest cLog Ps of 3.420 and 4.303, respectively, which is promising regarding possible brain penetration. None of the tested compounds was cytotoxic to HT29 cells in the concentrations tested. **Conclusion:** Thus, the arylnitrones were not toxic in a large range of concentration and two compounds showed an interesting protective effect in the excitotoxicity assay. On the next steps, it would be interesting to investigate whether neuroprotection is associated with an antioxidant effect of these compounds using more complex models of stroke and appropriate biochemical endpoints. **CECEUA-CCS protocolo**: DFBICB029 **Financial Support**: CNPq-MS-MCTI, CNPq and CAPES fellowships.

02.035 The Role of 5-HT2A receptors in the dorsal raphe nucleus in panic-like behaviours and antinociception evoked by chemical stimulation of the inferior Colliculus . Soares-Junior RS, Falconi-Sobrinho LL, Garcia TA, Coimbra NC FMRP-USP – Neurociências

Introduction: There is evidence that N-methyl-d-aspartic acid (NMDA) microinjections in the inferior colliculus (IC) of rats elicit defensive behavioural reactions. The defensive behaviour elaborated by dorsal midbrain neurons is followed by an antinociceptive response. However, there are no studies showing the role of IC neurons on fear-related hypoalgesic mechanisms. Moreover, it has been suggested that these fear-related defensive responses triggered by midbrain tectum neurons can be modulate by the dorsal raphe nucleus (DRN) 5HT system. The purpose of this study was to investigate the role played by DRN 5-HT2A receptors in the modulation of panic-like behaviour and fear-induced antinociception elicited by IC chemical stimulation. **Methods**: Male Wistar, weighing 250–300g (n=6 per group) were used. Guide-cannulae were stereotaxically implanted in the DRN and IC. Before the experiment the rats were submitted to baseline measurements of tail-flick. In experiment I, the animals received microinjection of vehicle (0.9%NaCl/0.2µL) or 6, 9 and 12nmol NMDA into the IC. In experiment II, it was performed the pretreatment of DRN with microinjections of vehicle or the 5HT2A receptor selective antagonist R-96544 in a dose of 10nM. Ten minutes later, NMDA (12nmol) was injected in the IC. In both experiments, the defensive responses displayed by rats in the circular arena were quantitatively analyzed for 10min and then the tail-flick withdrawal latencies were measured at 10min intervals for 70min. At the end of the experiment, each animal was perfused for confirmation of injection sites. One way-ANOVA followed by Newman-Keuls' post hoc tests were used to analyse the ethogram and two-way RM-ANOVA followed by Bonferroni's multiple comparison tests were used to analyze nociceptive threshold ($p<0.05$ was considered statistically significant). **Results**: Exp.I: Treatment with NMDA at 6, 9 and 12nmol in the IC evoked a dose-related response on frequency [$F(3,20)=10.95$, $p<0.001$] and duration [$F(3,20)=11.97$, $p<0.001$] of freezing, frequency [$F(3,20)=16.44$, $p<0.001$] and duration [$F(3,20)=12.64$, $p<0.001$] of running and on frequency [$F(3,20)=3.95$, $p<0.05$] of jumping. In addition, intra-IC injections of NMDA caused an antinociceptive effect [$F(3,20)=8.66$, $p<0.001$], time [$F(8,160)=82.36$, $p<0.001$] and treatment versus time interaction [$F(24,160)=9.44$, $p<0.001$]. The dose of 12nmol of NMDA was the most effective in causing panic-like reactions and much higher hypoalgesia. Exp.II The pretreatment of the DRN with R-96544 at 10nM decreased the frequency [$F(3,20)=18.27$, $p<0.001$] and duration [$F(3,20)=12.93$, $p<0.001$] of freezing, frequency [$F(3,20)=10.54$, $p<0.001$] and duration [$F(3,20)=9.41$, $p<0.001$] of running and on frequency [$F(3,20)=6.58$, $p<0.001$] of jumps. This pretreatment also significantly decreased fear induced antinociception [$F(3,20)=82.39$, $p<0.001$], time [$F(8,160)=48.7$, $p<0.001$] and pretreatment versus time interaction [$F(24,160)=31.93$, $p<0.001$]. **Conclusion**: The blockade of DRN 5-HT2A receptors decreased both panic-like defensive behaviour and fear-induced antinociception evoked by chemical stimulation of the IC neurons with NMDA. **Financial Support**: CAPES. **Research Approval**: CEUA-FMRP-USP; proc. 017/2016.

02.036 N-type Ca^{2+} channels are affected by full-length mutant huntingtin expression in a mouse model of Huntington's disease. Santos RPM[1], Silva FR[2], Miranda AS, Olmo IG, Zamponi GW, Dobransky T, Cruz JS, Vieira LB[1], Ribeiro MR
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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion in the amino-terminal region of the huntingtin (htt) protein. In addition to facilitating neurodegeneration, mutant htt is implicated in HD-related alterations of neurotransmission. Previous data showed that htt can modulate N-type voltage-gated Ca^{2+} channels ($Ca_v2.2$), which are essential for presynaptic neurotransmitter release. Thus, to elucidate the mechanism underlying mutant htt-mediated alterations in neurotransmission, we investigated how $Ca_v2.2$ is affected by full-length mutant htt expression in a mouse model of HD (BACHD). For this we performed measurement of continuous glutamate release, neuronal primary culture, electrophysiology, biotinylation, coimmunoprecipitation and immunoblotting experiments. Our data indicate that young BACHD mice exhibit increased striatal glutamate release, which is reduced to wild type levels following $Ca_v2.2$ block. $Ca_v2.2$ Ca^{2+} current-density and plasma membrane expression are increased in BACHD mice, which could account for increased glutamate release. Moreover, mutant htt affects the interaction between $Ca_v2.2$ and 2 major channel regulators, namely syntaxin 1A and $G_{\beta\gamma}$ protein. Notably, 12-month old BACHD mice exhibit decreased Cav2.2 cell surface expression and glutamate release, suggesting that Cav2.2 alterations vary according to disease stage. Número da licença do Comitê de Ética: CEUA/UFMG 139/2013 Apoio finaceiro: Fapemig; CNPq; Capes; PrPq/UFMG

02.037 Evaluation of cytokines in patients with temporal lobe epilepsy. Silva MCM, Martins FMA, Gonçalves AP, Teixeira AL, Vieira ELM, Vieira ELM, Oliveira ACP, Oliveira ACP – UFMG

Introduction: Epilepsy is a neuronal disorder with a high prevalence. Researches have been demonstrated the possibility that inflammatory process can induce a neuronal damage and contribute to the etiopathogenesis and recurrence of seizures in epilepsy. The proinflammatory mediators have proconvulsant effect in various seizure animal models, because they can affect the physiological functions of the astrocytes and microglial cells, and alter the neuronal excitability. In this context, we investigated if patients with temporal lobe epilepsy have alterations in plasma cytokines levels when they are compared with control subjects. **Methods:** Experiments were performed with plasma samples from patients with Temporal Lobe Epilepsy (TLE) and subjects without epilepsy, aged between 18 and 65 years old, who agreed to participate by signing the Informed Consent. In addition to the peripheral blood, we collected the medical history and sociodemographic data, as well as we measured the weight and height of the patients. Peripheral blood was collected in vacuum tubes containing sodium heparin and was centrifuged (300rpm, 4ºC, 10 min). Evaluation of cytokines was performed using Human Inflammatory CBA method. This project was approved by the Research Ethics Committee - CEP (Nº 147543/2013). **Results:** The cytokines IL-10, IL-6, IL-1 and IL-8 were elevated in the patients with TLE, when they were compared with healthy subjects ($p<0,001$). On the other hand, no difference between the patients with ELT and controls was observed in the cytokines IL-12 and TNF levels. **Conclusion:** In the present study, we demonstrated that both pro- and anti-inflammatory mediators can be altered in patients with epilepsy in the interictal period. The alteration in the levels of these mediators could contribute for the pathogenesis of epilepsy and for the refractoriness to the treatment. However, further studies are necessary to better understand the role of these cytokines in the pathophysiology of TLE. **Acknowledgments**: FAPEMIG, CNPq and CAPES

02.038 Role of TLR2 in biochemical effects induced by intermittent fasting. Paixão AG, Vasconcelos AR, Scavone C, Kawamoto EM ICB-USP – Farmacologia

Introduction: Some diet protocols such as intermittent fasting (IF) seem to induce a moderate nutritional stress to the organism stimulating body's defense mechanisms by enhancing oxidative stress resistance, regulating inflammatory response and thus making cells or the organism more resistant to severe stress. Toll-like receptor (TLR) 2 classically excite immune responses related to pathogen recognition, although physiological and pathological roles not associated with inflammation has been revealed. Interestingly, little is known about the molecular mechanisms underlying the involvement of Toll-like receptor (TLR) 2 on the beneficial effects induced by IF. **Aim:** The present work investigated the effects of IF on the signaling mechanisms associated with the transcription factors NF-κB and CREB in TLR2 KO mice. **Methods:** Adult male mice were divided into 4 groups: *Tlr2*$^{+/+}$ (wild type) control, *Tlr2*$^{+/+}$ subjected to IF, *Tlr2*$^{-/-}$ (knockout of TLR2) control and *Tlr2*$^{-/-}$ subjected to IF. Mice were subjected to IF or *ad libitum* control diets for 30 days. Hippocampus was used for electrophoretic mobility shift assay (EMSA), reverse transcription polymerase chain reaction (RT-PCR) and multiplex assay. All procedures were approved by the Ethical Committee for Animal Research (CEEA) of the Biomedical Sciences Institute of the University of São Paulo (Nº108/2011). **Results:** In the absence of TLR2, IF leads to a decrease in activity of the transcription factors NF-κB, CREB and NRF2. Consequently, there is a reduction in the expression of BDNF and HO1. Also, IF was able to reverse the increased levels of the IFN-γ, IL-1β and RANTES as well as the levels of corticosterone in the absence of TLR2. **Conclusion:** The present study suggests that TLR2 participates in the modulatory effects of IF and allows a better understanding of the physiological processes that aim at developing new strategies for therapeutical interventions to promote longevity and healthy aging. **Key-words:** Intermittent fasting, Tlr2, inflammatory response. **Financial support:** FAPESP (Process 2011/12255-8; 2016/07376-4) and CAPES.