## 02. Neuropharmacology

**02.001 Schizophrenia-related behavioral changes induced by repeated activation of cannabinoid receptors during brain development in mice.** Gonçalves PFR, Macena MV, Silva FMR, Neves G – UFRJ

**Introduction:** Schizophrenia is a psychiatric disorder characterized by three categories of symptoms: positive (delusions, hallucinations), negative (social isolation, anhedonia) and cognitive (attention and memory deficits). Although schizophrenia's exact etiology is unknown, this disorder has been considered the end stage of abnormal neurodevelopmental processes. Epidemiologic data point to Cannabis spp. use during adolescence as a risk factor to schizophrenia. Whereas these studies provide evidence of a correlation between Cannabis use and psychosis, neither the causal relationship nor the neurobiological processes underlying these observations have been fully elucidated. On the other hand, high nicotine intake among patients with schizophrenia is reported, but there is a lack of knowledge about cholinergic transmission abnormalities that might underlie schizophrenia symptoms. The aim of this study is to investigate behavioral changes related to schizophrenia and on cholinergic neurotransmission induced by repeated activation of cannabinoid receptors ($CB_1$/$CB_2$) during brain development. **Methods:** Male Swiss mice (CECAL/Fiocruz breeding colony) were used. Mice were divided into three experimental groups: 1) control - received vehicle i.p. from post-natal day (PND) 28 to 47; 2) pre-puberty - WIN 55,212-2 (WIN) 2 mg/kg i.p. from PND 28 to 37 and vehicle from PND 38 to 47; and 3) puberty – vehicle from PND 28 to 37 and WIN from PND 38 to 47. Thereafter, animals were left undisturbed until PND 70 (adulthood), when they underwent behavioral testing including locomotor activity, social interaction and social recognition tests, working memory evaluation in the Y maze and prepulse inhibition of the startle response (PPI), in this order. On PND 78, animals were euthanized and theirs frontal cortex, hippocampus and striatum were collected for determination of acetylcholinesterase activity. **Results:** Major behavioral changes were found on the social recognition and PPI tests. Pre-puberty WIN-treated mice showed social memory deficit, since they spent the same amount of time interacting with the familiar and new conspecific. Once again, only pre-puberty treated group showed an improvement in PPI, an unexpected result. Repeated activation of cannabinoid receptors impaired pre-puberty treated mice weight gain during treatment and even after drug discontinuation. These changes were not observed in puberty WIN-treated mice nor in the control group. None of the interventions induced changes on the other performed tasks, as well as no significant change in acetylcholinesterase activity was detected. Conclusion The results of this study indicate pre-puberty period is more vulnerable than puberty period to induction of long-term effects by repeated activation of cannabinoid receptors, since it was the only period wherein was observed long lasting behavioral changes. For now, the behavioral effects observed do not seem related to changes in cholinergic neurotransmission, however, other biochemical evaluation are underway. **Financial support**: CNPq, CAPES. Ethical approval: CEUA/CCS-UFRJ: process n° 075/15.

**02.002 Cognitive decline in the Streptozotocin-induced model of Alzheimer's disease may be related to neuroinflammation and impairment in adult neurogenesis.** Bassani TB[1], Machado MMF[1], Bonato JM[2], Oliveira RMMW[2], Vital MABF[1] [1]UFPR- Farmacologia, [2]UEM – Farmacologia e Terapêutica

**Introduction**: Alzheimer's disease (AD) is a neurodegenerative disorder of unknown etiology characterized by progressive cognitive decline. Neuropathologically, AD presents some features such as amyloid plaques, neurofibrillary tangles, loss of synapses and neurons and dysfunction of neurogenesis. Adult neurogenesis is impaired in AD patients and this may play a role in AD etiopathology. In the adult brain, neurogenesis is essential for both learning and memory and also recovery from neuronal injury. Therefore, abnormalities in neurogenesis may contribute to the development of cognitive disorders such as AD (Qu, 2012). Besides, neuroinflammation, which is known to be increased in the brains of AD patients, can negatively impact neurogenesis (Wang, 2015). The aim of this study was to evaluate the involvement of adult neurogenesis and neuroinflammation in the cognitive decline observed in the rat model of AD induced by intracerebroventricular (ICV) infusion of streptozotocin (STZ). **Methods**: Male wistar rats (n=6-9) received through stereotaxic surgery a bilateral ICV injection of STZ (3 mg/kg dissolved in sterile saline). Sham group received only sterile saline. At days 28-30 after surgery, the animals were tested for cognitive performance in the Object Location Task (OLT), Object Recognition Task (ORT) and Y maze. Right after behavioral tests, the rats were transcardially perfused for immunohistochemical analysis. Immunohistochemistry of the lateral ventricles and hippocampus were performed to assess the expression of Doublecortin (DCX – newborn neurons marker), Iba-1 (ionized calcium binding adaptor molecule - microgliosis marker) and Glial Fibrillary Acidic Protein (GFAP - reactive astrocytes marker). Statistical analysis was performed using two-tailed Student's *t*-test and significance was set at $P<0.05$. **Results**: The STZ group showed a significant deficit in short-term recognition memory in the ORT ($P<0.01$) and also in short-term spatial memory in the Y maze ($P<0.001$) and OLT ($P<0.001$) when compared to the sham group. These cognitive alterations were accompanied by a reduction in hippocampal and subventricular neurogenesis, which is demonstrated by a decrease in DCX-positive neurons in the dentate gyrus of hippocampus ($P<0.01$) and in the lateral ventricles ($P<0.01$) of the STZ group compared to the sham group. Besides, there was a significant increase in the expression of GFAP and Iba-1 in the CA3 region of hippocampus ($P<0.05$) of the STZ group compared to the sham group. **Conclusions**: The STZ model of AD presented impairment on short-term recognition and spatial memories, which was associated with reduction in subventricular and hippocampal neurogenesis and also with increase in neuroinflammation markers in hippocampus. These results suggest that the cognitive decline observed in the STZ model may be related to neuroinflammation and dysfunction in adult neurogenesis. These features make this animal model suitable for the investigation of new drugs that interfere in these mechanisms. **References**: Qu Z-q, PLoS ONE 7(1), e29641, 2012. Wang B, Metab Brain Dis 30, 355, 2015. **Financial support**: CNPq, Capes and Fundação Araucária. Procedures were approved by the Ethical Committee of Animal Experiment of UFPR (protocol 735).

**02.003 α2 $Na^+$,$K^+$-ATPase silencing induces loss of LPS response and ouabain protection in glial cells.** Kinoshita PF[1], Yshii LM[2], Orellana AMM[1], de Sá Lima L[1], Kawamoto EM[1], Scavone C[1 1]ICB-USP – Farmacologia, [2]INSERM

**Introduction**: Ouabain (OUA) is a cardiac glycoside which is as an endogenous hormone produced in hypothalamus and adrenal gland and binds to $Na^+$,$K^+$-ATPase (NKA). NKA is a conserved membrane protein which maintains the cell osmotic balance by the hydrolysis of ATP. The OUA binding can activate signaling pathways in low doses which is not linked to the common effect of the enzyme inhibition. The α subunits of NKA have 4 isoforms which are distributed in a different pattern in the tissues and may have a different physiological role in response to OUA binding. Glial cells which express α1 and α2 isoforms have an important role in the response against injury in the brain and they also control inflammation which is crucial to trigger neurodegenerative diseases. Glial cells play an important role in the response against injury in the brain and they also control inflammation which is crucial to trigger neurodegenerative diseases. OUA can protect against some types of injury in kidney and central nervous system (CNS). The aim of this study is to understand the role of α2 in OUA protection and neuroinflammatory response induced by LPS in mice primary glial cells culture.**Methods**: LDH and MTT were used to test cell viability. EMSA assay and immunofluorescence detected RelA activation and nuclear translocation. While Western blotting and ELISA kits were used to observe the protein expression and cytokines release. **Results**: While LPS treatment (1ug/mL) increased LDH release, OUA did not decrease cell viability. OUA (10uM) blocked LPS – induced activation of the NF-κB in glial cells which was seen in EMSA assay and in immunofluorescence for RelA (NF-κB subunit). To understand the role of the α2 isoform we used a RNAi to silence this isoform and we also used an scramble sequence as a control (scramble). The cells treated with α2 RNAi did not show ERK and NF-κB activation as observed in scramble group to LPS treatment in comparison with the control, OUA and OUA+LPS. In addition, the LPS scramble group has also an increase release of TNF in comparison with RNAi LPS group suggesting that the α2 isoform interacts with the LPS pathway. OUA in the scramble group also increased the release of IL-1β and the lack of α2 also blocked this activation.**Conclusion:** Taken together, the present work suggest that α2-NAK plays an important role in inflammatory response in the brain. **Sponsors**: Fapesp, CNPq and CAPES. CEUA: number 37 page 15 book 3

**02.004 We declare the Nigrostriatal pathway guilty: From sleep disturbances to cognitive deficits.** Targa A[1], Rodrigues LS[1], Noseda ACD[1], Aurich MF[1], Andersen ML[2], Tufik S[2], Lima MMS[1 1]UFPR- Fisiologia, [2]Unifesp – Psicobiologia

**Introduction**: Sleep disturbances and cognitive deficits are among the most disabling non-motor symptoms in Parkinson's disease. The neuronal death in nigrostriatal pathway is the main factor for motor symptoms and recent studies indicate a possible influence in non-motor symptoms as well. However, it is still controversial the level of influence that this pathway has in the sleep disturbances and cognitive deficits. Moreover, memory consolidation and learning processes seem to be largely dependent of sleep. Thus, the objective of this study was to investigate if nigrostriatal pathway has a role in sleep regulation and memory processes in the context of Parkinson's disease and if so, if it influences these functions directly or indirectly, by acting solely on sleep or other functions. **Methods**: Male rats weighting 280-320 g underwent stereotaxic surgery for direct infusion of rotenone in the Substantia nigra pars compacta (SNpc). After seven days, the animals were exposed to 24 hours of REM sleep deprivation (REMSD), followed by dopaminergic D2 receptor agonist (piribedil, 3 µg/µl), antagonist (raclopride, 10 µg/µl) or vehicle (DMSO) infusion directly in the striatum. The animals were submitted to the object recognition test after the REMSD and after the sleep rebound period (REB). The sleep recording occurred during the REB for 24 hours. After all of these procedures, the animals were decapitated and the SNpc, striatum and hippocampus were removed (for neurochemical analysis). **Results**: We observed that Rotenone infusion in the SNpc blocked the sleep rebound and the modulation of striatal D2 receptors did not reverse it. In addition, rotenone administration decreased the time spent in NREM sleep, which was corroborated by positive correlations between dopamine levels in both SNpc and striatum and the time spent in NREM sleep. Regarding the influence in memory, we did not observe any effect of the rotenone infusion in the object recognition test. However, striatal D2 receptors modulation decreased the time spent exploring the novel object, which was unexpectedly prevented by REMSD. Finally, we did not observe statistically significant correlations between the percentage of time spent in NREM or REM sleep and the time spent exploring the novel object. **Conclusions**: These findings suggest that nigrostriatal pathway have a role in both sleep regulation and memory and this may be one of the causes of sleep disturbances and cognitive deficits in individuals with Parkinson's disease. However, our data indicates that the nigrostriatal pathway influence over these functions occurs independently and not by an influence of sleep over memory. **Financial support** and acknowledgments: This paper was supported by Associação Fundo de Incentivo à Pesquisa (AFIP), CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). All of the experiments performed in this study were approved by the ethics committee of Federal University of Paraná (approval ID #655).

**02.005 Quercetin reduces manic-like behavior and brain oxidative stress induced by paradoxical sleep deprivation in mice.** Kanazawa LKS[1], Vecchia DD[1], Wendler EM[1], Hocayen PAS[1], Lívero FAR[1], Stipp MC[1], Barcaro IMR[1], Acco A[1], Andreatini R[1] [1]UFPR-Farmacologia

**Introduction**: Quercetin is a known antioxidant and protein kinase C (PKC) inhibitor. Previous studies have shown that mania involves oxidative stress and an increase in PKC activity. We hypothesized that quercetin affects manic symptoms. In the present study, manic-like behavior (hyperlocomotion) and oxidative stress were induced by 24 h paradoxical sleep deprivation (PSD) in male Swiss mice. **Methods**: Mice were treated with saline (0.9% NaCl; 10 ml/kg, i.p.), lithium carbonate (positive control; 100 mg/kg, i.p.) or quercetin (10 or 40 mg/kg, i.p.) and then underwent the PSD-induced hyperlocomotion model for 24 h. After the sleep deprivation period, the animals were put in the locomotor activity box for evaluation of their locomotor activity. Following the behavioral test, the animals were euthanized and the prefrontal cortex (PFC), hippocampus, and striatum were dissected. The brain samples were used for the evaluation of oxidative stress parameters: reduced glutathione (GSH) and lipid peroxidation (LPO) levels. For all of the experiments, two-way analysis of variance (ANOVA) was used, followed by the Newman-Keuls *post hoc* test if significant main effects or interactions were found in the ANOVA. Pearson's correlation analysis was performed to identify possible relationships between the behavioral and oxidative stress parameters. **Results**: Both 10 and 40 mg/kg quercetin and lithium prevented PSD-induced hyperlocomotion. Quercetin reversed the PSD-induced decrease in glutathione (GSH) levels in the prefrontal cortex (PFC) and striatum. Quercetin also reversed the PSD-induced increase in lipid peroxidation (LPO) in the PFC, hippocampus, and striatum. Pearson's correlation analysis revealed a negative correlation between locomotor activity and GSH in the PFC in sleep-deprived mice and a positive correlation between locomotor activity and LPO in the PFC and striatum in sleep-deprived mice. **Conclusion:** These results suggest that quercetin exerts an antimanic-like effect at doses that do not impair spontaneous locomotor activity, and the antioxidant action of quercetin might contribute to its antimanic-like effects. Acknowledgments: RA and AA are recipients of a research fellowship from CNPq. LKSK, DDV, EMW, PASH, FARL, MCS, and IMRB are recipients of a graduate fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **Financial support**: CAPES, CNPq Research approved by the Animal Research Ethical Committee under the number 733.

**02.006 (+)-Dehydrofukinone inhibits calcium influx in mice cortical synaptosomes.** Garlet QI[1], Pires LC[2], Milanesi LH[1], Mello CF[1], Heinzmann BM[3] [1]UFSM – Farmacologia, [2]UFSM – Farmácia, [3]UFSM – Farmácia e Farmacologia

**Introduction**: (+)-Dehydrofukinone (DHF) is a sesquiterpenoid isolated from *Nectandra grandiflora* (Lauraceae) essential oil that has sedative and anesthetic properties. Early behavioural studies have suggested that DHF inhibits central nervous system activity by GABAergic mechanisms (GARLET et al. Braz J Med Biol Res, 49(1): e4872, 2016). However, no study has addressed whether DHF modulates other cellular events involved in the control of cellular excitability, such as calcium flux. In fact, the calcium ion is a second messenger that plays important role in synaptic vesicles release and modulation of intracellular pathways (ZAMPONI et al. Pharmacol Rev, 67: 821, 2015), and excessive calcium influx has been associated with increased neuronal excitability and seizures (STEINLEIN, O. K. Cell Tissue Res, 357: 395, 2014). Therefore, in the current study we investigated whether DHF alters calcium influx in synaptosomes from cerebral cortex of mice. **Methods**: Animals were sacrificed by decapitation and had their cerebral cortex dissected and homogenized in 320 mM sucrose, 5 mM HEPES and 0.1 mM EDTA at pH 7.4. The homogenate was centrifuged for 10 min at 1130 x g at 4 °C and the supernatant was centrifuged at 16260 x g for 20 min. The resulting pellet was resuspended in aCSF (artificial cerebrospinal fluid) and applied on a Percoll® 10%/16%/23% discontinuous gradient. Synaptosomal fraction placed between the 10 and 16% Percoll bands was incubated with aCSF, $10^{-3}$-$10^{2}$ µM DHF or 400 nM GABA and loaded with a fluorescent probe for 1 hour at 37°C. Synaptosomal calcium influx was measured before and after the addition of aCSF or 10 mM KCl. Fluorescence data were accumulated at excitation wavelength of 485 nm (emission wavelength: 525 nm). Calcium influx was expressed as fraction of maximal free-[$Ca^{+2}$] fluorescence obtained with 1% Triton X-100 by the equation [(F-Fmin)/(Fmax-F)], where Fmin is the mean of baseline points before challenging solutions. The concentration of DHF that reduced calcium influx by 50% ($IC_{50}$) was estimated by non-linear regression. This protocol was approved by the Animal Ethics Committee of Federal University of Santa Maria, Brazil (Process number: 3627041115). **Results**: aCSF application, *per se*, caused a calcium influx of approximately 13.0 ± 2.0% of maximal influx and KCl induced a 2.5-fold increase in calcium influx when compared to aCSF. Preincubation of synaptosomes with 400 nM GABA and with 1-100 µM DHF decreased aCSF- and KCl-induced calcium influx (n= 4, $p<0.001$, $F_{(7, 48)} = 10.54$, Two-way ANOVA). At 1 µM, DHF caused a 4-fold decrease of calcium influx after KCl-evoked depolarization. The $IC_{50}$ values for DHF on aCSF- and KCl-induced calcium influx were 51.0 ± 2.0 nM and 451.9 ± 2.5 nM, respectively. **Conclusion:** DHF decreased aCSF- and KCl-induced calcium influx and its performance was similar to GABA. Therefore, DHF may constitute a pharmacological tool to limit calcium influx, potentially decreasing calcium-induced cytotoxic effects. Financial Support: FAPERGS/PRONEX; CNPq.

**02.007 Hippocampal gene expression profiling reveals anti-epileptogenic targets in a rat model of hyperthermic seizures.** Azevedo H, Khaled N, Santos P, Bertonha F, Moreira-Filho CA FM-USP – Pediatria

**Introduction**: Children who experience febrile seizures (FS) exhibit a higher risk for developing epilepsy at a later age. In immature rats, hyperthermia induces stereotyped seizure behaviors that resemble the clinical condition observed in some infants during high fever. To get insights into the molecular changes that occur in the brain after FS, we carried out here a temporal gene expression profiling of animals submitted to experimental FS. **Methods**: Wistar rat pups at P11 in equal male: female ratio (n = 6-8 per group and time period) were divided into two groups: normothermic controls (CTRL) and animals submitted to hyperthermia-induced seizures (HS). RNA samples were collected from the ventral CA3 hippocampus at 1 (P12), 19 (P30), 49 (P60) and 109 (P120) days after hyperthermic seizures. The temporal endpoints were selected to investigate the acute (P12), latent (P30 and P60) and chronic (P120) stages of the animal model. Gene expression microarray experiments were performed and the differentially expressed (DE) genes were statistically determined at each time point using the significance analysis of microarrays (SAM) algorithm. The DE genes were then enriched to identify overrepresented functions and pathways. **Results and Discussion**: At P12, 21 DE genes were observed between the experimental groups, which were associated with amino acid metabolism, Wnt and Notch pathways, and apoptosis. 48 DE genes were identified at P30, and their enriched functions were amino acid metabolism, serotonergic activity, prostaglandin synthesis and GABA transport. At P60, the 240 DE genes were related to Wnt and MAPK pathways, neuronal differentiation, focal adhesion, bicarbonate transporters and cell migration. At P120, the 526 DE genes were associated with JAK-STAT pathway, Wnt pathway, pluripotency, apoptosis and SLC transporters. Our data collectively suggest that targeting particular genes associated to inflammatory and developmental processes, such as the ones regulated by JAK-STAT, MAPK, Wnt and Notch pathways, could be an interesting strategy for preventing the onset of epilepsy after an initial precipitating insult. Further studies should be conducted to substantiate the above conclusions by using specific inhibitors of these pathways for treating animals submitted to early-life hyperthermic seizures. **Financial Support**: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP (grant 2011/50761-2) and NAP e-Science USP. **Research Ethics Committee Approval**: This study was approved by the ethics committee of FMUSP, under the number 460/13.

**02.008 Effect of ketamine in ultrasonic vocalizations in animal model of Parkinson's disease.** Vecchia DD, Kanazawa LKS, Wendler E, Hocayen PAS, Vital MABF[1], Miyoshi E, Schwarting R, Andreatini R[1 1]UFPR- Farmacologia

**Introduction***:* Parkinson Disease (PD) is characterized by progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) and the most common signals are motor impairments, but most patients also presents other symtoms like depression, olfactive disfunction and voice impairment. Voice deficits in Parkinson disease emerge early in the disease process, but do not improve with standard treatments targeting dopamine. Rats are known to communicate through ultrasonic vocalizations (USVs) in a variety of social situations including mating, and have been used to investigate how PD-related pathology influences vocalizations. Adult rats emit 50-kHz USV in appetitive situations such as social investigation and play or when exposed to drugs of abuse like d-amphetamine, while 22-kHz USV occur in aversive situations. Thus, the present study evaluated the action of ketamine on USVs in animal model of PD. **Methods***:* Male Wistar rats received bilateral intranigral 6-OHDA infusion. USV was performed before surgery (screening), 14 days after surgery and 21 days after beginning treatment. In all these moments, the test was performed in two days (day 1 and day 2) for 5 minutes per day. Rats were individually placed into a clean polycarbonate cage with fresh bedding, where a recording session immediately started. The cage was placed on a desk under a microphone positioned at 35 cm above the center of cage floor. Drug treatments were: vehicle (daily), ketamine (5, 10 and 15mg/kg, ip, once a week) and imipramine (20mg/kg, ip, daily). The rats were divided into 6-OHDA plus vehicle, imipramine or ketamine (3 doses) and SHAM plus the same treatments, totaling 10 groups. This protocol was approved by institutional ethical board (CEUA #786). **Results***:* For statistical analysis, we calculated the resulting mean between two days of analysis and results were expressed as number of calls. It was possible to observe a significant reduction in the number of vocalizations in injured animals with 6-OHDA (n = 24) compared to SHAM animals (n = 25). This can be an indication of vocal impairment, as well as in humans with Parkinson's disease. Previous studies suggest that these changes in speech appear before the classic motor symptoms and are keeped in course of the disease. After treatment with ketamine (all doses), there was no statistical difference in call numbers. The vehicle treated animals also had no increase in the number of calls, indicating that no spontaneous regeneration of the vocal cords. USVs were also classified according to its subtypes (flat, tril, step and mixed). There were no statistical differences in number of each subtype, but it is clear that before and after the surgery and treatment the "flat" type was more abundant. ***Conclusion:*** As a conclusion, it can be suggested that lesion induced by 6-OHDA generates a vocal damage, reducing the number of calls and ketamine does not appear to reverse this condition. **Keywords:** Parkinson's Disease, Ultrassonic Vocalization, Ketamine, Vocal impairment.

**02.009 The effects of ethyl-acetate fraction (EAF) of *Trichilia catigua* (Catuaba) on memory deficit after global cerebral ischemia in rats.** Godinho J, Bacarin CC, Huzita CH, Milani H, Oliveira RMW

**Introduction:** Transient global cerebral ischemia (TGCI) is an immediate and serious outcome observed most frequently after reversible cardiac arrest. Patients who survive long after cardiac arrest may present a broad range of neurologic and cognitive deficits. Trichilia catigua is a popular plant in Brazil and known as “catuaba”. It has been used in folk medicine as a tonic for the treatment of fatigue, stress, impotence and memory deficits. T. catigua has potent in vitro antioxidant activity, and a neuroprotective effect has recently been observed in an in vitro model of ischemia/reperfusion. The present study investigates the effects of an ethyl-acetate fraction (EAF) of T. catigua on the memory deficits caused by TGCI in rats. **Methods:** Male, Wistar rats (3 months-old) were trained for 10 days up to reach asymptotic learning performance in a non-food rewarded, eight-arm radial maze task, and then assigned to one of the following groups: sham-operation (n =13), TGCI + vehicle (n =14), and TGCI + EAF (n=13). TGCI was induced for 15 minutes according the 4-VO model. EAF (400 mg/kg) or vehicle (0.9% NaCl with 1% propylene glycol) was administered by oral route. At the first day of ischemia, two doses of 200 mg/kg each were delivered at 30 min prior to and 1 h after TGCI, respectively. The next doses (400 mg/kg) were given once a day, for 7 days consecutively. Retrograde memory performance was assessed weekly at 18, 25, and 32 days after TGCI and expressed by three parameters: (*i*) the latency to complete the task, (*ii*) the number of reference memory errors and (*iii*) the number of working memory errors. **Results:** TGCI caused the rats to spent more time (latency) to complete the task, and to commit more reference and working memory errors ($F_{2,72} = 22{,}23$ $p < 0{,}001$), indicating they forgot the task that was learned prior to ischemia (i.e., retrograde amnesia). The treatment with *T. catigua* significantly reduced both latency ( $p < 0{,}05$ ) and the number of errors ( $p < 0{,}01$- $0{,}001$ ), indicating a memory-protective effect. **Conclusion:** The ethyl-acetate fraction of *T. catigua* was able to prevent the retrograde amnesia caused by TGCI in rats. Additional examination should determine the presence (or absence) of neurohistological protection by *T. catigua*.

**02.010 Ouabain ameliorates synaptic plasticity and long-Term memory impairments induced by Chronic Unpredictable Stress.** Leite JA, Orellana AMM, Andreotti DZ, dos Santos NB, de Sá Lima L, Kawamoto EM, Munhoz CD, Scavone C ICB-USP – Farmacologia

**Introduction:** Ouabain (OUA), a potent inhibitor of the $Na^+$,$K^+$-ATPase, was identified as an endogenous hormone. It has been demonstrated the involvement of the OUA in the acute stress response, where physical exercise induces an increase in OUA levels in rats, dogs and humans. Repeated life stress precipitates and exacerbates mental illnesses, including depression and anxiety. Chronic stress impairs spatial as well as working memory, suggesting negative effects on prefrontal cortex (PFC) as well as on hippocampal functioning. Memory impairment has been associated with atrophy of neuron and down-regulation of, AMPA, PSD-95, synaptophysin and brain derived neurotrophic factor (BDNF) expression in hippocampus (HPC) of the animals subjected to chronic stress. BDNF, which is induced by LTP (Long-term potentiation) has a critical role in stabilizing synaptic change. **Aims:** The present work investigated the effects of ouabain intermittent administration (each other day during stress protocol) on the impairments induced by Chronic Unpredictable Stress (CUS) on synaptic plasticity and long-Term memory, as well as on depression. **Methods:** Adult male rats were pre-treated intraperitoneally with ouabain (1.8 µg/kg) followed by CUS protocol for 14 days. Serum corticosterone and BDNF levels in hippocampus were measured in rat HPC using ELISA kit. Electrophoretic mobility shift assay (EMSA) and Western Blot were used to evaluate CREB activity, GR levels, AMPA, PSD95 and synaptophysin protein expression. . Moreover the effects of chronic stress on memory and depression were investigated in rats using an object recognition task and sucrose preference. **Results:** Our results showed that after induction of CUS, OUA treatment led reduction in the corticosterone levels. In addition, OUA increased BDNF levels, but does not interfere in the CREB activity and GR level in HPC. Furthermore, analysis of intracellular signaling pathways showed that OUA reversed the CUS-induced low protein levels of synaptic-related proteins PSD95 and AMPA, however does not interfere in synaptophysin levels. Interestingly, we have shown that OUA can prevent the effects of CUS on memory. However no effect on stress-induced in depression behavior was observed, as well as with OUA treatment. **Conclusions:** In conclusion our findings suggest that intermittent chronic treatment with OUA can induce adaptive synaptic protein expression in the hippocampus, besides ameliorate impairments induced by CUS on the memory. Further additional studies are necessary to elucidate a signaling pathway induced by OUA in chronic stress response. Financial Support: FAPESP, CNPq. All procedures were approved by the Biomedical College of Animal Experimentation and the Ethical Committee for Animal Research ICB/USP (fls.19, $n^o$ 52, book 03).

**02.011 Role of brain-derived neurotrophic factor in the basolateral nucleus of amygdala in the modulation of anxiety behaviors.** Matthiesen M[1], Sousa RM[1], Frias AT, Zangrossi Junior H FMRP-USP – Farmacologia

Brain-derived neurotrophic factor (BDNF) is a multifunction growth factor that signals through the TrkB receptor. Perturbed BDNF signaling has been implicated in the pathophysiology of anxiety disorders. One possible mechanism used by this neurotrophin to modulate anxiety behavior is regulation of serotonergic neurotransmission. The basolateral nucleus of amygdala (BLA), a limbic area associated with the neurobiology of anxiety, is densely innervated by ascending serotonergic fibers from the raphe nucleus and contains both BDNF and TrkB receptors. However, the role played by BDNF in mediating anxiety-related defensive responses in the BLA is poorly understood. The present study aimed to investigate the role of BDNF in the BLA in modulating defensive responses evaluated in the elevated T-maze (ETM) and light dark transition test. We also evaluated whether 5-$HT_{2C}$ receptors are recruited for BDNF effects in the BLA. Finally, we investigated whether serotonin depletion would interfere with BDNF behavioral consequences. **Methods:** Male Wistar rats (280–310g) were implanted with bilateral cannulae aimed at the BLA. In experiment 1, seven days after surgery, the animals were intra-BLA injected with BDNF (100, 200 or 400pg/0.2µL) or saline and, thirty minutes later, tested in the ETM. In experiment 2, the animals were previously injected with the 5-$HT_{2C}$ receptors antagonist SB-242084 (0,01nmol/0.2µL) or saline in the BLA and, ten minutes later, injected with BDNF (400pg/0.2µL) or saline in the same structure. Animals were tested in the ETM and ligth dark transition test thirty minutes after the last injection. In experiment 3, independent groups of rats were previously IP treated, during 4 consecutive days, with the serotonin depleting drug PCPA (100 mg/kg) or saline. Twenty-four hours after the last injection, animals of each group were intra-BLA injected with BDNF (400 pg/0.2µL) or saline, and thirty minutes later, they were tested in the elevated T-maze and light-dark transition tests. A repeated measure ANOVA was performed to ETM analysis and an one-way ANOVA was performed to light dark transition test. When appropriate, post hoc analyses were performed by Duncan's test. **Results:** In experiment 1, BDNF 400 pg administrated in the BLA facilitated inhibitory avoidance [treatment: $F(3,26)= 6,14$; $p<0,05$], indicating an anxiogenic effect, without interfering in the escape response. In experiment 2, pre-treatment with SB-242084 in the BLA blocked the anxiogenic effect observed after BDNF administration in the ETM [treatment SB x BDNF: $F(1,20)= 27,18$; $p<0,05$] and in the light dark transition test [treatment SB x BDNF: $F(3,23)= 4,48$, $p<0,05$]. In experiment 3, previous PCPA-induced depletion of serotonin also blocked the anxiogenic effect observed after BDNF microinjection in rats tested in the ETM [treatment PCPA x BDNF: $F(1,20)= 8,30$, $p<0,05$] and showed a tendency in the light dark transition test [treatment PCPA x BDNF: $F(3,21)= 3,98$, $p=0,06$]. **Conclusion:** Our results suggest that BDNF in the BLA increases anxiety by facilitating 5-$HT_{2C}$ receptor-mediated neurotransmission in this limbic area. Financial Support: CAPES Research Approval: 077/2013

**02.012 Effect of riparin IV in cognitive function in mice exposed to chronic stress induced by corticosterone.** Chaves RC[1], Vasconcelos AS[1], Oliveira NF[1], Oliveira ICM[1], Rodrigues GC[1], Lopes IS[1], Valentim JT[1], Fernandes ML[1], Gutierrez SJC[2], Sousa FCF[1] [1]UFC – Fisiologia e Farmacologia, [2]UFPI – Bioquímica e Farmacologia

The Major Depressive Disorder is a world health problem, with a high morbidity and mortality prevalence that affects the functional capability. Mental disorders have a multifactorial etiology and stress presents as one of the causal factors (WANG et al, 2008). In some of those disease, like depression, it's suggested that high cortisol concentration contributes directly to the pathology (TAFET; BERNARDINI, 2003). Since cognitive symptoms are common among patients with major depression, studies suggests difficulties in cognitive functioning such as attention, executive function and learning, processing speed and memory (DARCET et al., 2014). Based on this data, the aim of the study is to evaluate Riparin IV, a synthetic drug riparin I, II and III analogue, in depression model induced by chronic corticosterone injections. Female swiss mice with 25–30g body weight were randomly selected into 4 groups control (Ctrl), stressed (Cort), riparin IV (Cort+RipIV) and fluvoxamine (Cort+Flu). Three groups were administrated subcutaneously (SC) with corticosterone (20 mg/kg) during 21 days, while the control group were administrated only with vehicle. In the last 7 days of treatment, groups were administrated with tested drugs riparin IV (50mg/Kg), fluvoxamine (50mg/Kg) and distilled water, per oral, 30 min after SC injections. After final treatment, animals were exposed to behavioral tests such as forced swimming (FST), open field (OFT), step-down-type inhibitory avoidance and spontaneous alternation behavior (Y-maze). **Results** were analyzed using one-way ANOVA and Student Newman Keul's. All values are expressed as mean ± standard error of the mean (SEM) and $p<0.05$ were considered as statistically significant. Data revels that stress were induced by chronic corticosterone injections and Rip IV and Flu were able to revert the resulting behavioral alterations: Animals displayed a reduction in the immobility time in FST. Mice in OFT didn't show statistical significance between groups what suggests no alterations in locomotor activity. In step-down test, rip IV treated group increased latency of reactions on learning session. Treatment also increased the latency on long memory retention. In Y-maze test, rip IV restored spatial recognition performance of stressed animals to a level comparable to Flu. Antidepressant drug strategies that also target cognitive symptoms could have an impact in patient quality life. Our findings suggest that Riparin IV improves cognitive function after chronic administration and could be and new alternative treatment for depression. **Financial support** and acknowledgments: CNPq and Funcap. Study was approved by Institutional Animal Ethics Committee (Protocol number: 112/2014). DARCET, F. et al. Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. Front Behav Neurosci, v. 8, p. 136, 2014. TAFET, G. E.; BERNARDINI, R. Psychoneuroendocrinological links between chronic stress and depression. Prog Neuropsychopharmacol Biol Psychiatry, v. 27, n. 6, p. 893, 2003. WANG, J. L. at al. A. The relationship between work stress and mental disorders in men and women: findings from a population-based study. J Epidemiol Community Health, v. 62, p. 42, 2008.

**02.013 WNT/β-Catenin as prospective signaling pathway on inflammaging.** Orellana AM, Leite JA, Kinoshita PF, Vasconcelos AR, de Sá Lima L, Andreotti DZ, Munhoz CD, Kawamoto EM, Scavone C ICB-USP – Farmacologia

**Introduction**: In the aging process a low-grade chronic inflammation has been related to an increased susceptibility to many age-related disorders including neurodegenerative disease. The close relationship between aging and low-grade chronic inflammation is called inflammaging (Gabuzda and Yankner, 2013). Inflammaging can be characterized by an increase in cytokines, persistent elevated glucocorticoid levels (GC) and by the activation of the proinflammatory transcription factor NF-κB (Gabuza and Yankner, 2013; Zhang et al. 2013; Orellana et al., 2015). Interesting, both cytokines and NF-κB can be modulated by Glycogen Synthase Kinase 3 beta (GSK-3β) activity, a kinase that can intermediate metabolism and inflammation due to its holes in AKT signaling pathway and WNT/β-CATENIN signaling pathway. The aim of this study was to verify age-related changes in inflammatory status, as well as the status of some signaling pathways as AKT and WNT in hippocampus. **Methods**: Male Wistar rats with 4-, 12- and 24-month-old were used in this study. To verify the biochemical changes ELISA kits, EMSA, Western blotting and qPCR were performed. **Results**: **Results** suggested an age-related increase in neuroinflammation as indicated by NF-κB activation, GCs and TNF-α increased levels. AKT seems to be downregulated leading to an increase in GSK-3β activity and as a consequence a progressive decrease in Wnt activation verified by decreased levels of nuclear β-Catenin translocation, total DVL-2 and in the transcription of Axin 2 gene, in both 12- and 24- month old animals. **Conclusions**: Taken together, results suggest that in hippocampal aging an important increase in inflammatory signaling and a progressive decline in canonical WNT pathway activity happens, with special emphasis to the decrease of DVL-2 levels. Little is known about the DVL-2 regulation but for the first time it was suggested that DVL-2 expression can be changed along aging process. Financial Support: FAPESP 2011/22844-0. Research approved by the Animal Research Ethical Committee, n°194, page 93, book 2. Gabuzda D and Yankner BA. Nature ;497(7448):197. 2013 Zhang G et al., Nature;497(7448):211. 2013 Orellana et al., Aging (Albany NY); 7(12): 1094. 2015

**02.014 Involvement of adrenergic receptors in the dorsal periaqueductal gray matter on behavior of rats exposed to elevated T-Maze.** Estrada VB[1], Matsubara NK[1], Bonancéa AM[2], Soffientini DKM[2], Gomes MV[3], Corrêa FMA[4], Pelosi GG[1] [1]UEL – Ciências Fisiológicas, [2]UENP, [3]UENP- Ciências da Reabilitação, [4]FMRP-USP – Ciências Biológicas

**Introduction**: The dorsal periaqueductal gray matter (dPAG) is involved in the modulation of behavioral response as anxiety and panic[1,2]. Previous studies described the involvement of noradrenaline in the dPAG on modulation of anxiety in rats[3]. Therefore, we aimed identify the role of adrenergic receptors in dPAG on behavioral responses caused by rat exposure to elevated T-maze (ETM), an animal model used to measurement anxiety and panic in the same animal[4]. **Methods**: Wistar male rats (240-260g) were anesthetized (tribromoethanol, 0,25g/Kg i.p.) and subjected to the surgery for implantation of cannula guide directed to the dPAG; seven days later, each animal received only once microinjection of WB4101, RX821002 (selective α1 and α2-adrenoceptors antagonists, respectively), propranolol (non-selective β-adrenoceptors antagonist) at doses of 4, 8, 12nmol/50nl or artificial cerebrospinal into the dPAG and were submitted to ETM followed by open field test to evaluate locomotor activity. For data analysis Two-Way repeated-measures ANOVA was used, with treatment (doses) as the independent and trials (baseline, avoidance 1 and 2, or escape 1 to 3) as the dependent factor; when appropriate, one-way ANOVA followed by the post hoc was used. **Results**: The analysis showed that administration of RX821002 causes differences on trials [$F(2, 56)= 37,62$; $p<0,05$], treatment [$F(3, 28) = 4,87$; $p<0,05$] and interaction between factors [$F(6, 56) = 3,49$; $p<0,05$). Similarly, differences on trials [$F(2, 64)= 31,1$ $p<0,05$], treatment [$F(3, 32)= 3,64$ $p<0,05$] and interaction between factors [$F(6, 64)= 4,36$ $p<0,05$) was observed with propranolol administration. The post-hoc analysis indicates that RX821002 and Propranolol in dose of 12nmol/50nl intra dPAG significantly decreased the inhibitory avoidance 2 ($p<0.05$) compared to the control group, indicating an anxiolytic-like effect without changing on escape response. No effect was observed on exploration activity. **Conclusion:** The data suggest that α2 and β-adrenoceptors play a tonic role on behavioral responses observed during ETM test. **Financial support**: CAPES. CEUA Number: nº 15126.2013.99. References: 1- Pelosi G. G. Behav. Pharmac. v. 20, p.252, 2009. 2- Nashold B. S. J. Neurosurg. v. 30, p.14, 1969. 3- Estrada V. B. Lif. Sci. v. 156, p.94, 2016. 4- Zangrossi H. Brain. Res. Bullet. v. 44, p.1, 1997.

**02.015 Anxiolytic Effects of Riparin III in mice exposed to chronic stress.** Vasconcelos AS[1], Oliveira ICM[2], Oliveira NF[2], Chaves RC[2], Capibaribe VCC[2], Lima FAV[2], Rodriges GC[3], Barbosa Filho JM[4], Araujo MA[2], Silva DMA[2], Lopes IS[2], Valentim JT[2], Fernandes ML[2], Sousa FCF[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Fisiologia e Farmacologia, [3]UFC, [4]UFPB

**Introduction:** Natural products have recently been targeted by many studies due to their great potential as a source of new drugs. Amongst them, Riparin III, a natural alcamide isolated from the green fruit of *Aniba riparia*, stands out as it has displayed diverse pharmacological properties, including antimicrobial activity, spasmolytic effects, and central nervous system activity such as anxiolytic, antidepressant, anticonvulsant and antipsychotic effects, as well as memory restoration, all with a low potential of toxicity. Moreover, even though a vast array of treatments for anxiety disorders is currently available, remedying these diseases remains a challenge in Psychiatry. Therefore, the objective of the present study is to evaluate the anxiolytic effects of Riparin III through behavioral essays and assessment of monoamine levels, performed in a chronic stress model based on corticosterone administration.**Methods:** female *swiss* mice were divided into four groups. The control (Cont) group received saline injections via subcutaneuous (SC) administration during 22 days, and distilled water *per os* from the 14th day of administration onwards. Animals from the other groups received SC injections of 20 mg/kg corticosterone for 22 days, and two of these remaining groups received additional Rip III (Cort + Rip III) or fluvoxamine (Cort + Flu) administration respectively for 8 days. At the end of the administration timeline, the forced swimming (TNF), open field (TCA) and elevated plus maze (LCE) behavioral tests were performed. Next, animals were killed and dissection of the striatum was performed. This region was used for monoamine levels assessment, performed via high-performance liquid chromatography (HPLC). **Results:** the data reveals that it was possible to induce stress with corticosterone, and that Rip III and Flu were able to revert the resulting behavioral alterations: animals displayed a reduction in the immobility time in the TNF (Cont: 52.39± 5.5; Cort: 143.3±8.3; Cort + Rip III: 35.59±4.889; Cort + Flu: 71.72±6.4) and a longer exploration time in the open arms of the LCE (Cont: 38,99±2,1; Cort: 28,35±2; Cort + Rip III: 35,67±3,9; Cort + Flu: 37,63±2), without alterations to locomotor activity, as indicated by the TCA (Cont: 36.67±3.6; Cort: 39,8±4,1; Cort + Rip III: 32,6±3,4; Cort + Flu: 28,18±2,5). HLPC analysis revealed the efficacy of Rip III in restoring noradrenaline (Cont: 1,05±0,04; Cort: 8,25±0,49; Cort +Rip III: 1,45±0,24; Cort+Flu: 3,74±1,33), dopamine (Cont: 2,86±0,57; Cort: 5±0,85; Cort +Rip III: 1,89±0,2; Cort+Flu: 2,06±0,51) and serotonin levels (Cont: 2,37±0,16; Cort: 1,2±0,39; Cort ± Rip III: 4,36±0,81; Cort + Flu: 3,27±0,27).**Conclusion:** this study infers that Rip III presents efficacy in the recovery of alterations induced in this stress model induced by corticosterone, thus this drug bears the potential of being useful in the treatment of anxiety. **Financial support** and acknowledgments: CNPq, CAPES and Funcap. This project started after the approval of the ethics committee (13/2014).

**02.016 Maternal physical exercise effects on sociability and anxiety in adult mice.**
Andreotti DZ, Cabral-Costa JV, Scavone C, Kawamoto EM ICB-USP – Farmacologia

**Introduction:** Evidence found in studies show that voluntary exercise benefits could act in some brain functions such as hippocampus neurogenesis and synaptic plasticity and, therefore, improving cognition. It has already seen that physical exercise during pregnancy could ameliorate offspring cerebral function, such as cognition and synaptic plasticity. Although studies have been shown that these effects could extend to the adult age, the mechanisms that could promote these effects are not clearly understood yet. Therefore, this study aims to evaluate the role of exercise during pregnancy in anxiety and cognitive improvement in adult male offspring of these females, through elevated plus maze and social preference tasks. **Methods:** Sixty days-old C57Bl6 females were used. One group was allocated in a cage with a running wheel and the other group in a common cage. After 10 days male mice were placed with each female for mating. During pregnancy and breast-feeding periods females were kept in their respective cage with or without the running wheel. Sixty to ninety days-old offspring were submitted to elevated plus maze and social preference behavioral tasks. **Results:** Preliminary results show that male pups from the dam which was submitted to voluntary running exercise presented an anxious behavior, evidenced by its preference to be more time in closed arms of the apparatus, in comparison to the other pups from sedentary dams. In relation to social preference test, pups from runners preferred a mouse over an object. Besides, pups body masses were measured in different breast-feeding periods. Pups from runners were heavier compared to the pups of sedentary ones in the $17^{th}$ day postnatal. **Conclusion:** In preliminary conclusion, the results showed a potencial benefit of voluntary exercise in offspring from runner dams which seems to extend until adult age. **Financial Support:** University of São Paulo (USP) supported this study. 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).

**02.017 Altered monoamines concentrations in the brain of dystrophin-deficient mice.** Frangiotti MIB[1], Silva JDP[1], Castro-Neto EF[2], Sousa PVV[2], Naffah-Mazzacoratti MG[3], Souccar C[1] [1]Unifesp-EPM- Farmacologia, [2]Unifesp-EPM- Neurologia e Neurocirurgia, [3]Unifesp-EPM-Bioquímica

**Introduction**: Dystrophin is a 427 kDa structural protein found at the inner face of the plasmalemma of striated muscle fibers and postsynaptic regions of neuronal synapses. Mutations in the dystrophin gene and lack of the protein expression leads to a progressive and irreversible muscle degeneration known as Duchenne muscular dystrophy (DMD). One third of DMD patients also present cognitive deficits and neuropsychiatric disorders associated with the lack of dystrophin in the central nervous system. In a previous work, we reported significant changes in the content of amino acid transmitters in the cerebral cortex, hippocampus and cerebellum of dystrophin-deficient (*mdx*) mice, the most studied model of DMD (Frangiotti et al., MSc Thesis, 2015, UNIFESP). This study was aimed to evaluate the influence of dystrophin on the concentrations of monoamines (NE, DA and 5-HT) and their metabolites (MHPG, DOPAC, HVA and 5-HIAA), in homogenates of brain regions that express high concentrations of dystrophin of control and *mdx* mice. **Methods**: The cerebral cortex (CTX), hippocampus (HPC) and cerebellum (CBL) were isolated from 4-months old male control and *mdx* mice. Homogenates of each brain region were used to determine the concentrations of monoamines and their respective metabolites: noradrenaline (NE; MHPG), Dopamine (DA; DOPAC, HVA) and serotonin (5-HT; 5-HIAA), using high performance liquid chromatography with electrochemical detection. **Results** from control and *mdx* groups were compared using the Student´s “t” test, and they were considered different at $p<0.05$. **Results**: Cortical samples of *mdx* mice showed an increased DA content (55%) compared to control values (0.83 ± 0.12 ng/mg wet tissue; means± SEM; n=8), with no alteration of the utilization rate (DOPAC/DA ratio). In the same brain region, the concentrations of NE and 5-HT and respective utilization rates did not differ between control and mdx groups. In mdx hippocampal samples, the concentrations of NE, DA and 5-HT were reduced by 22%, 63% and 55% of control values (0.74 ± 0.02, 0.19 ± 0.05 and 1.04 ± 0.15 ng/mg wet tissue, respectively, n=8). In the same samples, the utilization rate of NE and 5-HT was not affected by dystrophin-deficiency, while that of DA was decreased in mdx mice by 67% of control values (0.80 ± 0.17 ng/mg wet tissue, n=8). In contrast, the cerebellar samples from mdx mice showed increased concentrations of NE (12%) and 5-HT (114%) with no changes of DA, compared to the respective control values. The same samples presented a significant increase in NE (43%) and DA (162%), and a decrease in 5-HT (60%) utilization rate, compared to control values. **Conclusion:** Our data show that dystrophin deficiency affected the concentrations of monoamines in all examined brain regions of mdx mice. The observed changes of NE, DA and 5-HT utilization rate may also reflect an altered release of monoamines associated with dystrophin deficiency. Together, these alterations might contribute to the cognitive deficits and behavioral disorders described in *mdx* mice and patients with DMD. Financial Support: CAPES, CNPq, FAPESP Protocol CEUA Nº 5500081215 (Institutional Ethical Committee)

**02.018 ODQ and Methylene blue as antidyskinetic compounds in 6-OHDA-lesioned rats.**
Bariotto-dos-Santos K[1], Padovan-Neto FE[2], Tumas V[1], Raisman-Vozari R[3], Bortolanza M[4], Del Bel EA[4] [1]FM-USP – Neurociências, [2]University of Medicine and Science North Chicago – Neuroscience, [3]INSERM, [4]FORP-USP – Morfologia, Fisiologia e Patologia Básica

**Introduction:** L-3,4-dihydroxyphenylalanine (L-DOPA), the metabolic precursor of dopamine, is widely used as a pharmacological agent for the symptomatic treatment of Parkinson's disease (PD). However, long-term use results in abnormal involuntary movements (AIMs) known as L-DOPA-induced dyskinesia. Mechanisms by which these effects occur are not clear. There is a strong need to identify new non-dopaminergic mechanisms and intense efforts have been directed toward the development of novel therapeutic agents. Nitric oxide (NO) is a highly diffusible molecule and a small intercellular messenger with many biological functions. The signaling pathway NO/sGC/GMPc could contribute to the pathogenesis of L-DOPA-dyskinesia. **Results** of our group showed that inhibitors of the nitric oxide synthase enzymes (NOS; NG-nitro-L-Arginine [L-NOARG], L-NG-nitro arginine-methyl-ester [L-NAME] and 7-nitroindazole [7-NI]) are able to counteract L-DOPA-induced dyskinesia in rodent models of PD. The enzyme soluble guanylate cyclase (sGC) is an endogenous receptor for NO and emerging as a promising candidate. **Objective:** This study was designed for testing if NO-sensitive inhibitors of sGC (NO/sGC) have a similar effect to that produced by NOS inhibitors on L-DOPA-induced dyskinesia. **Methods:** We examined the effect of systemic (chronic or intermittent; 2, 6 mg/kg i.p.) and intracerebroventricular (acute; 10, 30 mM i.c.v.) administration of the sGC nonseletive inhibitor, methylene blue (MetB), and the acute administration of the selective sGC inhibitor 1H-[1], [2], [4] oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ; 30, 100, 300 nmol i.c.v.) on AIMs induced by chronic treatment with L-DOPA (10 mg/kg, gavage) in 6-hydroxidopamine-lesioned rats. **Results:** We found that acute administration of MetB (30 mM i.c.v.) or ODQ (100 nmol i.c.v.) in L-DOPA primed rats is sufficient to reduce AIMs induced by repeated administration of L-DOPA. Chronic systemic administration of MetB (2 mg/kg, i.p.) was effective in either attenuate the AIMs development (co-administration) or reduce (intermitent administration) AIMs induced by L-DOPA. **Conclusion:** These results give further support to the hypothesis that NO/sGC/cGMP signaling cascades plays a role in motor behavior control mediated, at least in part, by cGMP production, opening new perspectives to modulate the AIMs. Support: FAPESP; CNPq; CAPES; NAPNA; USP. Process number: 094/2011.

**02.019 Effect of naringenin on prevention of oxidative stress in a model of mania induced by lisdexamfetamine.** Rosa LD[1], Nobre CA[1], Gomes MJP[1], Macêdo AJR[1], Turbano MCN[1], Prado SMC[2], Aguiar LMV[2] [1]INTA, [2]UFC

The Bipolar Affective Disorder (BAD) is a chronical condition where the patient presents alternating episodes of mania (or hypomania) and depression. The physiopathology of the disease has not been fully elucidated, but it is believed that oxidative damage of lipids and proteins is one of the possible mechanisms that contribute to the impairment of neuronal and glia cells in BAD. This work aims to evaluate the effects of Naringenin (NAR) on prevention of oxidative stress in a model of mania induced by Lisdexamfetamine in male Wistar rats (150-200g). The Committee on Animal Research and Ethics (CARE) of the Federal University of Ceara approved the experimental protocol with number 01/15. The animals were divided into 5 groups and received saline (SAL) or NAR (10, 25 ou 50 mg/Kg, p.o.) for 14 days and between the 8$^{th}$ and 14$^{th}$ day, the rats received LDX (10 mg/Kg, p.o.). After treatment, the cerebral areas were dissected and the prefrontal cortex (PFC), hippocampus (HC) and basic nuclei (BN) was removed to prepare the homogenate and subsequent determination of reduced glutathione (GSH), concentration of nitrite/nitrate and concentrations of thiobarbituric acid reactive substances (TBARS), express in malondialdehyde (MDA). The result showed an increase in GSH levels in the group NAR10+LDX (1289±191.5; $p < 0.05$) of 43.4% on HC compared with SAL+LDX group. In the PFC of the group SAL+LDX (723.7±28.4; $p<0.05$) there was a decrease of 28% in GSH levels when compared to the animals treaded with SAL. The treatment with NAR in both doses (10 e 25 mg/kg) induced a reduction of the MDA levels and the highest dose showed a better effect. It was evidenced preventive effect on NAR10+LDX (542.3±66.7; $p<0.05$) and NAR25+LDX (240.5±52.8; $p<0.05$) groups, with reduction of MDA levels in 21.1% and 62%, respectively, when compared to the control group of SAL+LDX (637.8±93.84; $p<0.05$). The administration of NAR10+LDX (377.2±42.7; $p<0.05$) or NAR25+LDX (394.8±21.1; $p<0.05$) reduced, respectively, in 40.1% and 37.3% the lipid peroxidation represented by MDA levels in BN when compared to the SAL+LDX (630.2±46.7; $p < 0.05$) group. It was observed an increase of 49%, 48.4% and 52.5%, respectively, in the nitrite concentration in all brain areas investigated in the animal group treated with SAL+LDX (3818±0.1; 4035±0.1; 4035±0.1; $p<0.05$) when compared to the SAL group. In brain areas of animals belonging to groups NAR10+LDX and NAR25+LDX, there was a significant reduction in the concentration of nitrite compared to the control group (SAL+LDX). Thus, the treatment with NAR prevents the increase of oxidative stress and the nitrite concentration of the animals treated with NAR was very close when compared with the rats of saline group. The present study showed that treatment with LDX caused an increase production of free radicals through elevation of nitrate concentrations and an increase of lipid peroxidation by elevated concentrations of MDA in brain areas, leading to lower levels of GSH. Therefore, NAR has a potential antioxidant effect on prevention of oxidative stress expressing a reduction of biomarkers levels mentioned above to values close to the control. Financial suport and acknowledgments: National Consel of Technological and Scientific Development (CNPq). Process number of CARE: 01/15

**02.020 Effects of 5-HT$_{2A}$ antagonist volinaserin on pre-pulse inhibition of startle reflex and working memory deficits induced by MK-801** Macena MV[1], Neves GA[1], Marques AM[1]
[1]UFRJ – Ciências Biomédicas

**Introduction:** Schizophrenia is a relevant psychiatric disorder since its nature is extremely complex and disabling and it usually affects young people in productive age. Nowadays, approximately 1% of world population is diagnosed with schizophrenia. This chronic psychiatric disorder is characterized by three kinds of symptoms: positive (delusions, hallucinations, restlessness), negative (affective blunting, social isolation) and cognitive (attention and memory deficits). Currently, two groups of drugs are used in schizophrenia treatment: the first generation antipsychotics, such as haloperidol; and the second generation antipsychotics, such as clozapine and risperidone. Despite the wide therapeutic arsenal available, many patients do not show any response with drug therapy or develop limiting side effects that leads to noncompliance. Therefore, it is necessary to search for more effective drugs with lower incidence of side effects. The importance of serotonergic receptors in the mechanism of action of second generation antipsychotics is well recognized, especially its potential role in the treatment of negative and cognitive symptoms. Thus, a deeper elucidation of serotonin receptors involvement in schizophrenia symptoms and the investigation of its real role on treatment seems to be a promising approach. **Objective:** In this study, we investigated the effects of the selective 5-HT2A antagonist volinaserin in a pharmacological model of schizophrenia using behavioral tasks related to cognitive symptoms. **Methods:** The study was conducted using the pharmacological model of schizophrenia that involves acute administration of MK-801 (NMDA antagonist) in adult male Swiss mice (CECAL/Fiocruz breeding colony). Volinaserin (or M100907) effects were tested at 0.3, 1 and 3 mg/kg doses i.p., in the following behavioral tasks: prepulse inhibition of the startle response (PPI) and spontaneous alternations in a Y maze (working memory evaluation). **Results:** Volinaserin treatment did not change the startle amplitude of mice, neither when co-administered with MK-801. Regarding PPI, the serotonergic antagonist did not alter significantly this response in animals. As expected, MK-801 induced a decrease in PPI, and the pretreatment with volinaserin failed to inhibit this deficit in PPI. In the Y maze test, volinaserin did not change the number of total arms entries, neither the percentage of spontaneous alternations performed by mice. **Conclusion:** The 5-HT2A receptor blockade had no positive effect on PPI, showing that this pharmacological approach does not seem to be a good strategy for the treatment of this type of deficit in patients. Furthermore, volinaserin did not cause loss of working memory in mice. On the next steps, the effects of 5-HT2A receptors modulation will be tested against working memory impairment induced by MK-801 in the Y maze. **Financial support:** FAPERJ, PIBIC/CNPq. **Ethical approval:** CEUA-UFRJ, no. DFBCICB045.

**02.021 New cholinesterase inhibitors derived from cardanol for Alzheimer's disease.** Boni MS[1], Guimarães MJR[1], Silva FMR[1], Couto GC[1], Castro NG[1], Romeiro LAS[2 1]UFRJ, [2]UCB

Alzheimer's disease is a progressive neurodegenerative disease whose initial symptoms are associated with synaptic dysfunction and death of cholinergic cortical and subcortical neurons. The consequent reduction of acetylcholine levels in the synapses results in lower activation of muscarinic and nicotinic receptors, which might explain the patient's cognitive deficits. Based on this cholinergic hypothesis, the current treatment relies on cholinesterase inhibitors, such as donepezil. However, the available drugs cause significant side effects due to cholinergic stimulation of peripheral muscarinic receptors, mostly M3 receptors. We have studied ten new possible semi-synthetic inhibitors of acetyl and butyrylcholinesterase (AChE and BuChE) derived from cardanol, a major component of the cashew nut shell liquid (CNSL). Using Ellman's spectrophotometric method we have determined the half maximal inhibitory concentration ($IC_{50}$). All the substances inhibited AChE completely, with $IC_{50}$ between 5.7 µM and 19.6 µM. Seven substances were also tested in a BuChE assay, and the $IC_{50}$ were 4.3 µM to 25.7 µM. We investigated the mechanism of AChE inhibition for six compounds, which showed either a linear non-competitive or a mixed type inhibition. In order to evaluate the possible interaction of these six compounds with M3 receptors, we have performed fluorimetric calcium assays in HT29 human colonic epithelial cells. One substance (LDT167) reversibly inhibited carbachol-induced calcium transients at 10 µM. A high-content live-dead fluorescence assay was used to evaluate the cytotoxicity of the compounds (1-100 µM) after 24-hour exposure of HT29 cells. Among the tested compounds only LDT167 showed concentration-dependent cytotoxicity, at 100 µM (ten times above the AChE $IC_{50}$). Thus, the cardanol derivatives from CNSL were efficient non-selective cholinesterase inhibitors. LDT167 seems to have an additional antagonistic effect in M3 receptors, which might be useful to minimize the clinical side effects. Therefore, this substance can be a prototype for developing new, more tolerable drugs for the treatment of Alzheimer's disease.

**02.022 Evaluation of the new anticholinesterasic drug PQM-56 in memory deficit and neurodegeneration induced by Aβ 1-40.** da Silva MCM[1], Bellozi PMQ[1], Junior WOC[1], Campos AC[2], Machado RP[3], Viegas Junior C[3], de Oliveira ACP[1] [1]UFMG – Farmacologia, [2]USP, [3]Unifal

**Introduction:** Alzheimer's Disease (AD) is a neurodegenerative disease characterized by accumulation of amyloid-β (Aβ) and intracellular neurofibrillary tangles. The cholinergic is the main neurotransmitter system affected in AD, which affects learning process, attention and synaptic plasticity. AD treatment is based on the use of reversible inhibitors of cholinesterase. However, there are few treatment options for DA, which demonstrates the urgent need to develop new drugs. Thus, in the present study, we evaluated the effect of PQM-56, a new anticholinesterasic drug, in Aβ 1-40 induced alterations. **Methods:** PQM-56 was administered intraperitoneally in C57Bl/6 mice, aged 10-12 weeks, 1 h before the stereotactic surgery for unilateral intra-hippocampal injection of 400 pmol of Aβ 1-40 or PBS, in a volume of 0.5 µL (coordinates AP = -1.9 mm; LL = -1.5 mm; and DV = -2.3 mm relative to bregma, according to Paxinos atlas). Seven days after surgery, animals were submitted to novel object recognition (NOR) test for evaluation of memory, and underwent intracardiac perfusion to obtain hippocampal slices. Animals were divided into four groups: (1) vehicle + PBS; (2) Aβ + vehicle; (3) Aβ + PQM-56 18,59mg/kg; (4) Aβ + PQM-56 37,18mg/kg. FluoroJade C technique was used for evaluation of neuronal death in groups 1, 2 and 4. Memory was accessed by the recognition index of the new object and neuronal death was calculated by the fluorescence intensity in pixels/µm$^2$. Data were analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test and values were expressed as mean ± SEM. The level of statistical significance was set at $p < 0.05$. **Results:** In the NOR test, the group treated with Aβ + vehicle presented a decrease in recognition index when compared to PBS + vehicle group, which was prevented by the treatment with PQM 56. Aβ induced neuronal death, which was prevented by PQM-56. In CA1 region of hippocampus, there was only a tendency of increase in neuronal death, induced by Aβ, and of recovery, with the treatment with PQM-56. **Conclusion:** Since the current treatments for AD are not effective to prevent the disease progress, therapies to reduce the symptoms and improve patient's life are being investigated. The drug used in this study has an anticholinesterasic action, thus reducing the cognitive deficits caused by the administration of Aβ1-40. It also reduced neuronal death, albeit the mechanism is not clear. These results indicate that the drug may have potential to be used in the treatment of AD and further studies are necessary. **Acknowledgments:** FAPEMIG (Protocol numbers PPM-00372-13 and CEX-PPM-00241-15), and CNPq (Protocol numbers 479254/2013-3, 454088/2014-0) for financial support and fellowships. **Approval:** All procedures were approved by Institutional Ethics Committee protocol nº 39/2012.

**02.023 Auricular electrical stimulation of vagus nerve as an alternative to pharmacological treatment of canine idiopathic epilepsy.** Santos RSS, Carneiro RA EV-UFMG – Clínica e Cirurgia Veterinárias

**Introduction**: Idiopathic epilepsy (IE) is the most common neurological disturb in dogs and is characterized by spontaneous seizures due alterations in neuronal ambient of forebrain. Sympathetic discharges are more predominant than parasympathetic discharges. The aim of treatment is increase quality of life, decrease frequency and gravity of seizures. Phenobarbital (FB) is the first choice drug to treat IE, but in many patients it can cause some side effects that could bring other disturbs. Potassium bromide (PB) is used in association with FB when this drug cannot control seizures as monotherapy or in patients which have hepatic disturbs, as FB causes hepatotoxicity. However, as FB, PB has many side effects and cannot be used in dogs that have renal disturbs. The aim of this paper is to study an alternative method to treat IE in dogs that cannot use FB or PB. **Methods**: A survey was conducted using the databases PubMed and CAPES for the collection of data regarding auricular electrical stimulation of the vagus nerve (A-ESVN) using mainly the keywords: idiopathic epilepsy, seizure, phenobarbital, potassium bromide, vagus nerve stimulation, auricular stimulation, auricular acupuncture. **Results**: Corning, in XIX century, was the first scientist who studied electrical stimulation of vagus nerve (ESVN) using implant of electrodes in left cervical vagus nerve, nevertheless was expensive, hazardous and many side effects. Zabara et al. (1992) used ESVN in dogs and the epilepsy was totally terminated. Peijing et al. (2014) studied IE in humans using transcutaneous electrical nerve stimulation (TENS) on auricular concha and occurred a decrease by 63% seizure frequency. Shu et al. (2004) e Peijing et al. (2015) used electrical acupuncture on auricular concha of rats and their results showed significantly decrease in intensity and duration of seizures. Electrical acupuncture could decrease exacerbated electrical discharges in brain. Shu et al. (2005) showed that electrical discharges in concha had a positive correlation with increase in GABA levels and, therefore, with anti-seizures results. Shu et al. (2004) showed that auricular electrical acupuncture can decrease somatostatin levels on hippocampus and, consequently, confirm the hypothesis of positive correlation between seizures and hippocampal somatostatin concentration. **Conclusion:** A-ESVN is a cheap, simple and effective alternative treatment to IE in patients who cannot use pharmacological treatment and could bring an high quality of life, as A-ESVN almost do not has side effects. This study did not have financial support. This study did not needed approval by the Human or Animal Research Ethical Committee.

**02.024 Cheek injection of the selective TRPV4 agonist GSK1016790A elicited scratching behavior in mice.** Cruz JVR[1], Matias OD[2], Dias FC[2], Alves VS[2], Miranda ALP[2], Figueiredo CP[2], Costa R[2] [1]ICB-UFRJ, [2]UFRJ – Farmácia

**Introduction:** Chronic itch is a debilitating condition usually associated with skin diseases such as psoriasis, dermatitis and dry skin. However, the cellular and molecular mechanisms underlying itch sensation are not completely understood. Due to the low efficacy of available drugs for chronic itch, it has become necessary to found new molecular targets to develop more efficient drugs. Among the molecules that were recently associated with itch, Transient Receptor Potential (TRP) channels have stood out. TRP channels work as cellular sensors that are involved in sensorial pathway. TRPV1 and TRPA1 have been implicated in both nociceptive and pruriceptive transmission. Also, TRPV4 channel was shown to be involved in the pruriceptive response elicited by histamine, compound 48/80, endothelin-1, chloroquine and serotonin. Additionally, the intradermal (i.d.) injection of the selective TRPV4 agonist (GSK1016790A) into the nape of the mouse neck elicited scratching behavior (Akiyama T. J. Invest. Dermatol., 136: 154, 2016; Chen Y. J. Biol. Chem., 291: 10252, 2016). However, the injection into the nape of the neck does not discriminate between pain and itch in the mouse. Thus, it was recently proposed a new mouse model to differentiate pain and itch, the "cheek model of itch" (Shimada S.G. Pain, 139: 681, 2008). In this study we investigated the ability of GSK1016790A to elicit pain- and itch-like behaviors after its injection in the mouse cheek ("cheek model of itch"). **Methods:** Female Swiss mice (25 – 30g, 8 weeks old, n = 05 per group) were injected with GSK1016790A (0.3, 3 or 10 nmol/site), compound 48/80 (C48/80; 10 µg/site), capsaicin (10 µg/site) or vehicle (1% DMSO and 1% ethanol) into the right mouse cheek. Wiping and scratching behaviors were quantified during 30 minutes after stimuli as indicatives of pain- and itch-like responses, respectively. **Results:** I.d. injection of C48/80, a well-known pruritogenic agent, predominantly elicited scratching behavior in mice. Conversely, i.d. injection of capsaicin, a potent algogen, mainly elicited wiping behavior in mice. GSK1016790A given at 0.3 or 3 nmol/site did not cause any significant response in mice. However, when injected at 10 nmol/site GSK1016790A significantly elicited scratching behavior in mice, with negligible wiping behavior. **Conclusions:** Our present findings indicate that the direct activation of TRPV4 by selective agonists predominantly causes itch-like behavior in mice. Thus, TRPV4 could be an interesting target to develop new therapeutic strategies against chronic untreatable itch. Animal Ethics Committee (CEUA/UFRJ): 054/14 Financial Support: FAPERJ, CNPq e CAPPES. **Key-words:** Itch, TRPV4 and GSK1016790A.

**02.025 Etoricoxib blunts pentylenetetrazole-induced seizures and proinflammatory cytokine levels increase in mice.** Londero AL[1], Temp FR[1], Marafiga JR[1], Duarte T[1], Jesse AC[1], Milanesi LH[1], Hessel AT[1], Mello CF[2 1]UFSM, [2]UFSM – Fisiologia e Farmacologia

**Introduction**: Central nervous system inflammation may be either the cause or the consequence of convulsive seizures (VEZZANI, A. et al. Exp Neurol, 244, 11, 2013). Cytokines produced during the inflammatory process may cause secondary brain damage, increasing the risk for recurrent seizures (VEZZANI, A.; GRANATA, T. Epilepsia, 46: (11), 1724, 2005). Nonsteroidal anti-inflammatory drugs (NSAIDs), such as etoricoxib, are usually indicated for the treatment of exacerbated inflammation (KUMMER, C. L.; COELHO, T. C. Rev Bras Anestesiol, 52: (4), 498, 2002). NSAIDs block the activity of cyclooxygenase (COX) enzymes, which have a fundamental role in thromboxane and prostanoid synthesis (BOMBARDIER, C. et al. N Engl J Med, 343: (21), 1520, 2000). Although prostaglandins are increased in seizures by pathways involving cytokines signaling (OTTO, J. C.; SMITH, W. L. J Lipid Mediat Cell Signal, 12: (2-3), 139, 1995), it is unknown whether COX-2 inhibitors modulate the cytokine levels post-seizure and seizure behavior. Therefore, the objective of this study was to investigate if the subchronic administration of the COX-2 inhibitor etoricoxib alters cytokine levels in hippocampus and cerebral cortex of mice subjected to seizures induced by pentylenetetrazole (PTZ). **Methods**: Adult male Swiss mice received vehicle (0.1% carboxymethylcellulose plus 5% Tween 80, p.o.) or etoricoxib (0.2, 2 or 20 mg/kg, p.o.), daily for 14 successive days. On the 15$^{th}$ day mice were challenged with PTZ (50 mg/kg, i.p.). After PTZ administration animals were monitored for 20 minutes for the appearance of myoclonic jerks and generalized tonic-clonic seizures. The number of seizure episodes, total time spent seizing and Racine scale score were recorded. After behavioral analysis animals were euthanized and cerebral cortex and hippocampus were dissected and homogenized according to manufacturer's protocol for posterior interleukins analysis (IL-1β, TNF-α, INF-□, IL-6 and IL-10) by ELISA. **Results**: Subchronic administration of etoricoxib significantly increased the latency to PTZ-induced generalized tonic-clonic seizures [$H_{(3)}$=10.28; $p<0.05$]. However, etoricoxib did not alter the latency to PTZ-induced myoclonic jerks, number of seizure episodes, total time spent seizing and seizure severity. Furthermore, the increase in INF-γ [$F_{(1,11)}$=21.71; $p<0.01$] and TNF-α [$F_{(1,11)}$=29.86, $p<0.01$] levels in cerebral cortex and hippocampus induced by PTZ was reverted by etoricoxib. Moreover, etoricoxib administration reverted the increase in IL-1β [$F_{(1,11)}$=9.95, $p<0.01$] levels in cerebral cortex induced by PTZ. However, PTZ administration increased IL-6 [$F_{(1,11)}$=319.44, $p<0.01$] and IL-10 [$F_{(1,11)}$=135.46, $p<0.01$] levels in cerebral cortex and hippocampus, and etoricoxib did not prevented or reverted such alterations. **Conclusion:** These results suggest that etoricoxib has a protective effect on PTZ-induced seizures through reducing the increase of pro-convulsant cytokines in both hippocampus and cerebral cortex. Hence, inhibition of COX-2 is a possible pharmacological strategy to manage seizures. **Financial support** and acknowledgements: CAPES, CNPq, FAPERGS, PRPGP/UFSM, PIBIC/UFSM. The protocols followed the official Government Ethics Guidelines and were approved by the University Ethics Committee (N°024/2014).

**02.026 Effect of naringenin on reversion of oxidative stress in a model of mania induced by lisdexanfetamin.** Macêdo AJR[1], Nobre CA[2], Rosa LD[1], Gomes MJP[1], Campêlo JAC[1], Araújo AB[2], Aguiar LMV[2] [1]INTA, [2]UFC

The Bipolar Affective Disorder (BAD) is between the 10 more incapacitating medical conditions in the world. It is divided in two subgroups; the type I is the classical form and the patient has alternated episodes of depression and mania. The type II is characterized by not having episodes of mania, but the patient has hypomania with depression. The Naringenin (NAR) is an important flavonoid that has antioxidant, anti-inflammatory, antidepressant, and neuroprotection activity. Studies indicate that administration of Lisdexamfetamine (LDX) in rats can be a safe model for mania, that mimic some aspects of the disease in humans such as hyperactivity, increase of oxidative damage and neuroinflammation. The aim of this work is to evaluate the effects of NAR on reversion of oxidative stress in a model of mania induced by LDX in rats. Male Wistar rats were obtained from the Central Animal Laboratory of the Federal University of Ceará (UFC), weighing between 150-200 g. The Committee on Animal Research and Ethics (CARE) of the Federal University of Ceará approved the experiment with protocol number 01/15. The animals were divided in 5 groups, each one contained 18 rats. Each group received a daily oral dose of LDX (10mg/kg) or saline solution during 14 days. In the 8th day of treatment the animals in the saline and LDX groups additionally received oral administration of NAR (10, 25 or 50mg/kg) once a day or a saline solution with 1 hour of interval between the treatments. The cerebral areas were dissected, removing the prefrontal cortex (PFC), hippocampus (HC) and basic nuclei (BN). The level of lipid peroxidation was measured determining the concentrations of thiobarbituric acid reactive substances (TBARS). In order to determinate the concentration of nitrite/nitrate, dilutions of sodium nitrite were made in series and then the Griess reagent was utilized. It was possible to observe that the administration of LDX reduced, significantly, the levels of glutathione (GSH) when compared with Saline group. LDX+NAR25 (1536±158.6) recuperate the concentrations of GSH, increasing their levels in 154% on HC, when compared with the LDX group (604.3±82.4) ($p<0.05$). On PFC was observed that the administration of LDX (545.8±86.4) reduced the levels of GSH in 46.6% compared with animals treated with saline (1003±99.4) for 14 days ($p <0.05$). However, LDX+NAR25 (1.249±128.8) could increase the GSH levels in 128.8%, when compared to LDX group. The same was observed on BN where both doses of NAR tasted promoted recovery of GSH levels, wherein the dose of LDX+ NAR25 obtained an increase of 180.2%. On HC, the administration of LDX+NAR10 (464±26.4) and LDX+NAR25 (488.8±28.2) reduced in almost 50% the lipid peroxidation when compared with LDX group ($p < 0,05$). Thus it was possible to prove the effect of NAR against the neurochemistry alterations in a model of mania induced by LDX. In this experiment the substance was able to revert the oxidative stress and lipid peroxidation demonstrating a possible neuroprotective effect with potential use as an adjunct in the treatment of mania. **Financial support and acknowledgments**: Concelho Nacional de Desenvolvimento Científico e Tecnológico - National Consel of Technological and Scientific Development (CNPq). Process number of CARE: 01/15

**02.027 Effect of naringenin on prevention and revertion of neuroinflammation through the tumor necrosis factor α dosage in a model of mania induced by lisdexanfetamin.**
Gomes MJP[1], Nobre CA[2], Turbano MCN[1], Rosa LD[1], Macêdo AJR[1], Val DR[2], Aguiar LMV[2]
[1]INTA, [2]UFC

Bipolar Affective Disorder (BAD) is a chronical disease characterized by alternated episodes of depression and mania. Although not completely understood the physiopathological process of the disease, it is known that oxidative damage of lipids and proteins are involved in the death of neurons and glia cells. Moreover, certain cytokines and pro-inflammatory mediators also have the potential of causing toxicity and apoptosis in these cells, showing an association between the neuroinflammation and BAD. The aim of this work is to analyze the effect of Naringenin (NAR) on prevention and reversion of neuroinflammation through the TNF-α dosage in basal ganglia (BG), hippocampus (HC) and prefrontal cortex (PFC) of animals submitted to the mania model induced by Lisdexamfetamine (LDX). The Committee on Animal Research and Ethics (CARE) of the Federal University of Ceara approved the experimental protocol with number 01/15. Male Wistar rats (150-200g) were used and received LDX (10 mg/Kg, p.o.), NAR (10, 25, 50 mg/Kg, p.o.) or saline (SAL). For the reversion protocol, the rats received LDX during 14 days and between the 8th and 14th day, received NAR or SAL. For the prevention protocol, the rats received SAL or NAR during 14 days and between the 8th and 14th day, received LDX. The animals that survived to the process were subjected to decapitation and their cerebral areas were dissected, removing the PFC, HC and BG. These areas were homogenized and centrifuged for obtainment of the supernatant. Following, 96-well polystyrene plates were incubated with antibodies against TNF-α of rats, with later wash and incubation in solution with 1% of bovine serum albumin. Subsequent to blocking and wash of the plates, the standard curves in various dilutions or the samples were added and incubated during 2h. The plates were washed and specific antibodies, tested. After incubation, the plates were washed and the streptavidin-HRP, added. The enzymatic reaction was interrupted with H2SO4 and the absorbance was determined in 450 nm. The analysis of the results demonstrated a decline in the levels of TNF-α in all the areas analyzed with 55.7%; 20.7% and 27.3% in the HC, PFC and BG, respectively, in the animals pre-treated with NAR50+LDX (25.3±2; 55.5±3.8; 39±6.6; $p<0.05$) when compared to the control with SAL+LDX (57.2±5.3; 70±5.2; 53.7±4,2; $p<0.05$). However, in the reversion results, the rats treated with NAR50+LDX caused a reduction of the concentrations of TNF-α only in the HC and BG with values 55.7% and 27.3% (20.8±25.5; 41.75±3.1; $p<0.05$), respectively, when compared to the control with LDX. Thus, the study showed that the treatment with NAR was capable of preventing and revert the increase of the levels of TNF-α. This effect may be related to the reduction of oxidative damage and consequently the reduction of the induction of the inflammatory process, or still be related to its action in certain transcription factors, as NFκB, that induces the genic transcription of pro-inflammatory molecules. Therefore, the drug demonstrated a neuroprotective effect with potential use as adjuvant in the treatment of BAD. Financial support and Acknowledgments: National Council of Technological and Scientific Development – CNPq Process number of CARE: 01/15

**02.028 NOS enzymes play a role in oxidative stress of hippocampal cells injured by glutamic acid or conditioned medium of microglia activated by Interferon gamma.**
Montenegro NA, Titze-de-Almeida SS, Titze-de-Almeida R UnB

**Introduction:** Glutamate is a neurotransmitter that plays an important role in neurological disorders. Glutamate induces glutathione (GSH) depletion and increases reactive oxygen species (ROS) production in HT22 cells, all of which lead to cell death. Besides glutamatergic transmission, inflammatory cytokines like IFN-gamma also contributes to the loss of neuron cells. Excessive production of inflammatory mediators, like nitric oxide, produced by NOS enzymes (NOS), may lead to cell injury. No previous work, however, has evaluated whether NOS enzymes affect the viability of HT22 cells exposed to conditioned medium (MC) from BV-2 microglial cells. In the present study we evaluate the role of NOS enzymes in the injury of HT22 cells caused by glutamic acid or CM from BV-2 cells activated by IFN-gamma. **Methods:** First, total RNA from HT22 cells injured by glutamic acid (2mM) or CM was exctracted with a commercial kit (RNeasy® PlusMiniKit, Qiagen, Germany). Then, we performed the RT-qPCR in a QuantStudio 12K Flex (Applied Biosystem, USA). The forward and the reverse primers for nNOS were 5'-GTGGAGGTGCTGGAGGAGTT-3' and 5'-CGGGTATGGTAGGACACGAT-3' respectively. The endogenous primer used was poly(A) polymerase alpha (PAPOLA). Conditioned medium was obtained by activation of BV-2 cells using IFN-gamma® (37.5 ng/mL, Invitrogen). After 24 hours, this medium was filtrated and frozen in -80ºC freezer. The cell viability of HT22 cells injured by CM was determined using MTT assay. For that, we used 15 µL of the MTT-labeling reagent (0.5 mg/mL, Invitrogen) to each well. The plate was maintained at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air for an additional 3h-period. The insoluble formazan was dissolved with dimethylsulfoxide and the MTT reduction was measured at 595 nm. To determine the reactive oxygen species (ROS) the cells were submitted to a treatment of CM or L-NAME (NG-nitro-L-arginine methyl ester) –an inespecific blocker of NOS enzymes- for 4 hours or 8 hours. After that, DCFH-DA reagent was added to each well and incubated for 30 minutes at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air. The medium was removed and the cells were washed with PBS. The ROS levels was measured with excitation and emission wave lengths set at 485 and 538, respectively. **Results:** The nNOS expression was induced in HT22 cells treated with MC from BV-2 cells activated by IFN-gamma. Hippocampal cells also presents a increase in oxidative stress after injury by CM (21.2%, $P<0.05$) that was reduced 11.8% after L-NAME blocking at 4h. **Conclusion:** The present study reveal a role for nNOS enzymes in the interaction of microglial cells (BV-2) and hippocampal cells (HT22). The results suggest that NOS enzymes play a role in hippocampal cell death and oxidative stress provoked by both injuries, glutamic acid and conditionate medium from BV-2 cells activated by IFN-gamma. **References:** Fukui, M et al. European journal of pharmacology, 1;617(1-3):1-11, 2009. Henn, A. et al. Altex 26(2):83-94, 2009. Moncada & Bolaños. J. Neurochem 97:1676–1689, 2006. Titze-de-Almeida, SS et al. Neurochem Res 39:2452-64 (2014). **Financial support:** CNPq.

**02.029 MIR-7 And MIR-34A are modulated in the rat striatum after injury by rotenone.** Horst CH, Montenegro NA, Rocha AP, Domingues ACM, Sousa LL, Schlemmer F, Titze-de-Almeida SS, Titze-de-Almeida R UnB

**Introduction:** Parkinson's disease is a progressive and neurodegenerative disorder characterized by the loss of dopaminergic neurons. Degeneration particularly occurs in dopaminergic cells that present Lewy bodies, which are alpha-sinuclein and ubiquitin aggregates. In this regard, only the rotenone model results in the development of this hallmark of Parkinson. Previous works shown that microRNAs are important in physiology and development of the nervous system. Deregulation in miRNA networks may produce pathological alterations found in brain diseases, including Parkinson. As one miRNA controls many target genes, an abnormal expression assumes increased relevance. The present study aimed to evaluate microRNAs expression in the rat striatum after injury by rotenone. **Methods:** In this work we used male albino Wistar rats, young adults, with 200-250 grams. The animal experiments were approved by the Committee for Ethics in Animal Use of the University of Brasília. The rats were randomly divided into control group and the rotenone group. Rats were injected intraperitoneally with rotenone (2.5 mg/Kg I.P.), for 10 days, dissolved in sunflower oil; control group received only the vehicle. Behavior and motor signals due to rotenone injury were analyzed weekly by Open-field Test. After 36 days from the first injection, rats were euthanized. The rat brain was removed to fixation in 4% paraformaldehyde or dissection. Dopaminergic neurons present in striatum and substantia nigra was quantified by imunocitochemistry, using tyrosine hydroxylase antibodies. Levels of microRNAs (miR7, miR34a, miR26-a, miR132, miR-382 and Let7a) in the rat striatum were measured by the Real Time qPCR assay. **Results:** The behavioral test showed significant motor alterations at the 7 and 14 day after the last injection of rotenone ($P < 0.05$). The motor alterations were followed by dopaminergic cells lost. The expression of microRNAs in rotenone injury triggered a 2.2 fold increase in a microRNA that downregulates the potassium channel Eag1 (miR34a). On the other hand, miR-7, that have recently been shown to target α-synuclein presented a 0.5 fold decrease. **Conclusion:** Our data suggest that microRNAs miR34a and miR-7 could be involved in the dopaminergic cell lost. Further studies related to microRNAs inhibitors or mimics to miR34a and miR-7, respectively must be done in rotenone rodent model, to evaluate its ability to protect nigrostriatal neurons and revert motor signs found in parkinsonism. Financial Support: CNPq. Research approval by the Committee for Ethics in Animal Use of the University of Brasília, under license number 22267/2015. **References:** Agid Y. Lancet, 337:1321, 1991. Baek D et al. Nature, 455:64, 2008. Bassani TB et al. Brain Research, 1593:95, 2014. Bové J et al. NeuroRx, 3:484, 2005. Dauer W et al. Neuron, 39:889, 2003. Kawasaki A. et al. Neuroscience, 160:61, 2009. Kosik KS. Nat Rev Neurosci, 7:911, 2006. Selbach M. et al. Nature, 455:58, 2008. Uversky VN, Cell Tissue Res, 318:225, 2004.

**02.030 Investigation of the effects of Riparin IV in the oxidative stress markers.** Valentim JT, Silva DMA, Oliveira NF, Vasconcelos AS, Chaves RC, Lopes IS, Oliveira ICM, Capibaribe VCC, Sousa FCF UFC – Fisiologia e Farmacologia

**Introduction**: The free radicals are involved in several pathological conditions including psychiatric disorders. Oxidative stress generates chain reactions causing damage to polyunsaturated membrane lipids, proteins and DNA. Thus, the neurons suffer injury or even death. Increased oxidative stress occurs in major depression, evidenced by decreased antioxidant defenses in conjunction with the increase in lipid peroxidation. Thus, it plays an important role in the pathophysiology of depression. Some parameters can predict the oxidative stress, as the levels of reduced glutathione enzyme (GSH), which is one of the most important agents of the antioxidant defense cell system, the nitrite levels, which causes brain damage and malonyldialdehyde levels (MDA) which is a cytotoxic product formed by the lipid peroxidation test and is identified by the TBARS called. In this context, natural products potential may represent a source of new therapeutic agents. The riparin IV, isolated of alcamida *Aniba riparia*, has shown promising results. In behavioral models of acute stress, it triggered predictive effects of antidepressant and anxiolytic activities. This study aimed to study the potential antioxidant effect of riparin IV through the GSH levels, the nitrite content and lipid peroxidation in three areas very related to depression: the prefrontal cortex (PFC), hippocampus (HP) and striatum (CE) in mice. **Methods**: Were used mice Swiss males, weighing between 20-35 g, from the Central Animal Laboratory of the Federal University of Ceara (UFC). The animals were divided into two groups. One group was treated orally with doses of riparin IV 50mg/kg and the control group, received only saline. In both groups, 60 minutes after administration, the animals were sacrificed for dissection of the cerebral areas and the homogenate preparation for subsequent determination of GSH, nitrite and TBARS. **Results**: Compared to the control group, levels of GSH significantly increased in the hippocampus (HP) of the animals treated with riparin IV. [RIPARIN IV (HP = 974.3 ± 124.5); CONTROL: (HP = 615.9 ± 24.70)]. There was also these animals, decrease in nitrite in the hippocampus and prefrontal cortex [RIPARIN IV: (HP = 2.307 ± 0.02013); (PFC = 2.420 ± 0.2308); CONTROL: (HP = 11.38 ± 1.927); (PFC = 5.279 ± 1.258)]. The levels of TBARS decreased in all areas of the animals treated with IV riparin [RIPARIN IR: (HP = 41.19 ± 4.849); (PFC = 39.96 ± 4.016); (CE= 60.04 ± 9.533); CONTROL: (HP = 76.32 ± 7.992); (PFC = 72.55 ± 7.230); (CE = 179.4 ± 29.12)]. $P < 0.05$. **Conclusion:** The results of this study allowed us to verify the reduction of nitrite production and lipid peroxidation and increasing the levels of glutathione in the areas, revealing that riparin IV exerts antioxidant effects, which may explain, at least in part, to its neuroprotective actions are important for its antidepressant action mechanism in mice. Acknowledgements and **Financial support**: CAPES, CNPq, FUNCAP. This study was approved for committee of animal ethic in the Federal University of Ceara with number 68/2014.

**02.031 Antioxidant effect of citronelyl acetate in mice: involvement of reduced glutathione.** Silva DMA, Santos LKX, Carmo MOC, Fernandes ML, Melo FHC, Lopes IS, Valentim JT, Sousa FCF UFC – Fisiologia e Farmacologia

**Introduction**: Stress is a state generated by the perception of stimuli that cause emotional arousal and disturb homeostasis. This form, to protect yourself from oxidizing agents the cell has a defense system that can act before the agent causes injury, consisting of reduced glutathione (GSH), superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and vitamin E. This study aims to investigate the antioxidant involvement citronelyl acetate by determining the activity of GSH (reduced glutathione). **Methods**: They used male Swiss mice, weighing 20-35 g, from the Central Biotery of the Federal University of Ceara. The animals were divided into two groups. The group that underwent the stress induction by the forced swimming test and the group that did not receive the stress induction. In the first group, animals received citronelyl acetate 50 and 100 mg/kg or vehicle and after 60 minutes was subjected to forced swimming and 60 minutes later were killed by cervical dislocation dissection of brain areas (striatum, hippocampus and cortex pre- front) and preparation of the homogenate and reading of GSH in the ELISA device. In the second group of animals received citronelyl acetate 50 and 100 mg/kg or vehicle and after 120 minutes were sacrificed and their brains were dissected to remove all brain areas. **Results**: In the striatum, pretreatment of animals with citronelyl acetate did not alter the GSH levels in the group that was not subjected to forced swimming test, however there was a significant increase in GSH levels at doses of 50 and 100 mg/kg of citronelyl acetate [CIT-50: 1,321 ± 99.37 (8); CIT-100 1.089 ± 131.9 (8); control: 407.00 ± 31.24 (8) $p < 0.01$] in animals subjected to forced swimming. In the prefrontal cortex, pretreatment of animals with citronelyl acetate at a dose of 50 mg/kg did not alter the levels of GSH and citronelyl acetate 100 mg/kg increased the GSH levels in the animals were not submitted the forced swimming. Likewise, there was no increase in GSH levels at a dose of 50 mg/kg [CIT-50: 644.6 ± 49.63 (8) $p < 0.05$], but there was an increase in GSH levels with citronelyl acetate 100 mg/kg [CIT-100: 1.114 ± 133.9 (8); control: 490.4 ± 30.33 (8) $p < 0.01$] in animals subjected to the forced swimming. In hippocampus, pretreatment of animals with citronelyl acetate at a dose of 50 mg/kg decreased the levels of GSH and citronelyl acetate 100 mg/kg increased the GSH levels in animals that did not perform the forced swimming. However, there was an increase in GSH levels at doses of 50 and 100 mg/kg citronelyl acetate [CIT-50:1,098 ± 41.98 (8); CIT-100: 1.344 ± 156.3 (8); control: 365.80 ± 48.26 (8) $p < 0.001$] in animals subjected to forced swimming. **Conclusion:** This suggests that citronelyl acetate possibly has its antidepressant effect via an antioxidant action mediated by increased activity of the reduced glutathione (GSH) in the brain. Acknowledgements and **Financial support**: CAPES, CNPq, FUNCAP. This study was approved for committee of animal ethic in the Federal University of Ceara with number 07/2012.

**02.032 The antiretroviral drug efavirenz induces depressive-like behavior in rodents and affects monoamines levels in striatum.** Oliveira JVS, Cavalcante GIT, Filho AJMC, Souza DAA, Carvalho MAJ, Gaspar DM, Fonteles MMF UFC – Farmacologia e Fisiologia

**Introduction**: The antiretroviral drug Efavirenz (EFV) is one of the most frequently employed drugs in the combined regimens used to treat HIV infection, due to its great antiretroviral efficacy and favorable pharmacokinetics profile. However, there are major concerns about the EFV safety. Patients treated with EFV frequently experience major neuropsychiatric adverse effects, which often lead to replacement of therapy or its discontinuation. Despite this, the mechanisms involved in the central action of EFV are intrinsically nuclear. **Methods**: Male adult Wistar rats (200-250g) received EFV (25 or 50 mg / kg) or vehicle (distillated water) by gavage for 14 days. On the 14th day, the animals were submitted to the behavioral analysis by forced swimming test. After this, the animals were sacrificed and the striatum was dissected. The monoamines levels, serotonin (5-HT), dopamine (DA) and norepinephrine (NE), and their non-conjugated metabolites 3,4-hydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), and 5-hydroxy-indoleacetic acid (5-HIAA) were assayed by reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection. Based on these data, 5-HT turnover rate (5-HIAA/5-HT) and DA turnover rate (DOPAC+HVA/DA) was calculated. The results were analyzed by one-way ANOVA followed by Student-Newman-Keuls as post hoc test. The significance level was set at $p<0.05$. **Results**: In the forced swimming test, we detected a significant increase in the total immobility time in the EFV-treated groups, EFV 25 mg/kg and EFV 50 mg/kg, compared to control group ($P < 0.5$). The group treated with the highest dose of EFV (50 mg/kg) also showed a significant increase in immobility time when compared to the group treated with the lowest one (25 mg/kg) ($P < 0.05$). Regarding the monoamines levels, a significant decrease in 5-HT and DA levels was found in the striatum of EFV- treated groups at both doses tested compared to controls ($P < 0.05$). In the EFV 50 mg/kg group, a significant decrease in NE levels was also noted compared to controls ($P < 0.05$). Furthermore, a significant increase in 5-HT turnover rate was found in EFV-treated groups at both doses tested. However, just in EFV 50 mg/kg group, an increase in DA turnover rate and HVA levels was evidenced ($P < 0.05$). **Conclusion:** The present study show that the EFV treatment induce important behavioral changes in rodents, notably depressive-like behavior. Furthermore, our results demonstrate that EFV can affect the monoaminergic neurotransmission in striatum, reducing the monoamines levels and increasing their turnover rate in this brain area. Together, these findings can evidence a possible mechanism involved in the neuropsychiatric effects of EFV, and open perspectives to the improvement of antiretroviral therapy. **Financial support**: The authors thank the Brazilian Institutions CAPES, FUNCAP and CNPq for the financial support of this study. This study was performed with the approval of the ethical committee of Federal University of Ceará, with Protocol Number 09/2011.

**02.033 Lutein prevents ethanol-induced memory deficit in rats.** Tonding FF[1], Geiss JMT[2], Sagae S[3], Bonfler ML[4], Fariña LO[5], Paz EDR[4], Freitas ML[6], Souto NS[7], Furian AF[7], Oliveira MS[6], Guerra GP[2] [1]UNIOESTE, [2]UTFPR – Tecnologia de Alimentos, [3]UNIOESTE – Biofísica e Fisiologia, [4]UNIOESTE – Fisiologia, [5]UNIOESTE – Ciências Médicas e Farmacêuticas, [6]UFSM – Farmacologia, [7]UFSM – Tecnologia e Ciência dos Alimentos

**Introduction**: Excessive consumption of alcohol affects the central nervous system, resulting in memory and learning deficits. Accordingly, lutein, a carotenoid, known for its antioxidant properties, is believed to be able to prevent neurodegenerative diseases and cognitive deficits. **Methods**: We evaluated the effect of lutein on ethanol-induced memory deficits in the object recognition task in adult rats, as well as the possible involvement of oxidative stress. **Results**: The results showed that lutein administration (100 mg/kg) improved the memory of rats in the recognition task objects [$F_{(3,34)}$ = 7.13; $p < 0.05$], while doses of 15 or 50 mg / kg showed no effect; the sub-chronic administration of ethanol (3 g/kg) caused memory deficit in rats recognition task objects [$F_{(3,37)}$ = 3.06; $p < 0.05$]; and lutein (50 mg/kg) prevents the memory deficit induced by ethanol [$F_{(3,39)}$ = 7.64; $p < 0.05$]. **Conclusion:** The administration of lutein, ethanol, and a co-administration of lutein and ethanol did not alter the parameters of oxidative stress (superoxide dismutase, thiobarbituric acid reactive substances, and non-protein thiol), evaluated in the cortex and hippocampus. This suggests that the prevention of memory deficits induced by ethanol does not involve oxidative stress in the cortex and hippocampus. Thus, based on the results obtained, lutein may be considered an alternative in the treatment of memory deficits induced by ethanol, however, more studies are needed to evaluate the mechanism involved in this effect. **Financial support**: CNPq 306469/2005-0. Research approved by the Human or Animal Research Ethical Committee: 01/2016.

**02.034 Analysis of nitrergic system in astrocytes after stimulation of ATP receptors: involvement of A1 adenosine receptor.** Marra KL[1], Vaz S[2], Sebastião AM[2], Fior-Chadi DR[1]
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Adenosine plays a fundamental role in neural stimulation and synaptic transmission in central nervous system (CNS), as an endogenous modulator. A1 and A2a receptors are highly distributed in CNS and have great affinity for adenosine. Nitric Oxide (NO) is a free radical synthesized by nitric oxide synthase (NOS) and it is known to modulate several CNS functions and neurotransmitter systems, especially the actions mediated by the purinergic system. ATP, adenosine and NO are known to modulate astrocyte function through Ca2+ intracellular release. Despite synaptic activity and modulation by neurotransmitters, the control of NO synthesis in astrocytes is not fully established in different systems. Thus, the purpose of this study was to evaluate the possible involvement of the A1 adenosine receptor in the astrocyte Ca2+ intracellular release induced by ATP and NO donor. To accomplish this, astrocyte cultures from rat brain were stimulated with ATP and NO donors, and changes in A1R was evaluated after treatment. Astrocyte cultures were made using cortex of one-day old Wistar rats. Cultures were replated after 14 DIC and then charged with Fura-2 acetoxymethyl ester. The plaques were analyzed on inverted microscope with optical epifluorescence (Axiovert 135TV, Zeiss) and intracellular Ca2+ release was evaluated after ATP application. The culture was treated with NO donors/inhibitors and ATP stimulations were done to observe any alterations on Ca2+ release amplitude. The concentration of NO donor used (SNAP 25µM) was capable to increase baseline about of 12% (0.44 ± 0.10 n=149) when compared to control (0.39 ± 0.058 n=149). Furthermore, the NO inhibitor (L-NAME 10µM) was capable to decrease Ca2+ amplitude around 36% (0.40 ± 0.022 n=159) when compared to control (0.63 ± 0.032, n=159). At this point there is only a trend suggesting a time dependent involvement of A1R on astrocyte Ca2+ release-induced by NO donor. Astrocyte cultures were treated by SNAP for 6h (0.703 ± 0.166 n= 6), 12h (0.5806 ± 0.4246 n=3) and 24h (0.9180 ± 0.4264 n=4) compared to control (0.4218 ± 0.1662 n= 3). Thus, NO is involved in Ca2+ release in astrocytes. A1R are possibly involved in this response, although more experiments are needed to finish this conclusion. Acknowledgements: This study was supported by grants from FAPESP. This research was approved by Animal Research Ethical Committee (224/2015).

**02.035 Changes in α-Na,K-ATPase isoform expression and NMDAR-NOS signaling in hippocampus of klotho mutant mice, a genetic model of aging.** Cararo MM, Mazucanti CH, Sala T, Andreotti D, de Sá Lima L, Scavone C, Kawamoto EM ICB-USP – Farmacologia

**Introduction**: Klotho protein has anti-aging function, and klotho hypomorphic mice (*kl*$^{-/-}$) show premature aging features, being considered a model of accelerated aging that can provide important information about Klotho function in CNS (central nervous system) during the aging process. Previous studies from our laboratory reported an age-related decline of $Na^+,K^+$-ATPase activity associated to cGMP–PKG (cGMP-dependent protein kinase) pathway in CNS. N-methyl-D-aspartate receptor (NMDAR) is the main regulator of neuronal nitric oxide synthase (nNOS) and cGMP in brain, and its disfunction has been associated with cognitive decline and neurodegeneration during aging process. The aim of the present study is to investigate whether alterations in NMDAR signaling could influence $Na^+,K^+$-ATPase activity in hippocampi of klotho hypomorphic mice. **Methods**: Male *kl*$^{+/+}$ and *kl*$^{-/-}$ 8 weeks-old mice had their hippocampi dissected and processed for enzymatic and molecular assays. α subunit expression and N-methyl-D-aspartate receptor (NMDAR)-associated parameters were acessed by western blotting analysis and enzymatic activity of NOS and $Na^+,K^+$-ATPase were determined. Student t-test was used for statistical analysis with significance considered for $p \leq 0.05$. **Results**: **Results** showed an increase in $\alpha_2$ and a decrease in $\alpha_3$ $Na^+,K^+$-ATPase isoforms expression in *kl*$^{-/-}$ mice, but no significant difference in $Na^+,K^+$-ATPase activity was detected. In addition, GluN2A subunit of NMDAR, GluN1 NMDAR subunit phosphorylation and NOS activity were increased in *kl*$^{-/-}$ mice, otherwise no differences in NOS expression were observed. **Conclusion:** These results can indicate an increased NMDAR deleterious function, which agrees with the increased oxidative status seen in *kl*$^{-/-}$ mice, and it might be negatively impacting $Na^+,K^+$-ATPase function, during the aging process. Financial Support: FAPESP (#2014/14199-6), CAPES and CNPq. Research approval by Local Animal Research Ethical Comittee (CEUA ICB/USP#79, p.21, book #3).

**02.036 DNA methylation inhibitors modulate neuritogenesis in SH-SY5Y neuroblastoma cells** Cantelmo RA[1], Santos NAG[2], Santos AC[2], Joca SRL[1] [1]FCFRP-USP – Ciências Farmacêuticas, [2]FCFRP-USP – Toxicologia

**Introduction:** Environment can significantly affect the risk and progression of many neurodegenerative diseases through epigenetic mechanisms [1, 2]. DNA methylation is one of the major epigenetic mechanisms controlling gene transcription [1]. It is a process catalyzed by DNA methyl transferases (DNMT) in which a methyl group is added to a cytosine before a guanine in CpG islands, a modification frequently associated with gene silencing [1-3]. Studies suggest that DNA methylation is an important biological mechanism that regulates the expression of genes involved in controlling neural plasticity in the brain [3]. However, little is known about how drugs that target DNA methylation affect neuroplastic processes [1], including neuritogenesis, in healthy and diseased brain. The present study addressed this issue in SH-SY5Y cells, a widely used neuronal cell model. **Objective:** The aim of this study is to evaluate the effect of two DNMT inhibitors (5-aza-cD, 5-aza-2´-deoxycytidine and RG108, n-phthaloyl-l-tryptophan) and a methyl donor (SAM, S-adenosil-metionine) on the neuritogenesis of SH-SY5Y cells previously stimulated (or not) with retinoic acid, which induces the expression of neurotrophin receptors (trkB and trkC) in these cells. **Materials and Methods:** Growth medium: F12 Ham plus 15% fetal bovine serum (FBS) and 1% antibiotics (PSN). Neurite outgrowth assays: $1x10^5$ cells/well in 24-well plates for 24h; medium was replaced by F12K plus 1% FBS with (or without) 10 µM RA and incubation for 5 days. Additions: RG108 (2000 µM, 100 µM and 4 µM), 5-aza-cD (0.5 µM and 0.005 µM) or SAM (10 nM) and incubation for 72h. Quantitation: inverted-phase-contrast microscopy and Image J open source software. Statistics: one-way ANOVA with Tukey multiple comparisons test or t-test for pairs, $p < 0.05$, GraphPad Prism software. **Results:** RG108 100 µM and 4 µM significantly increased the neuritogenesis of RA-differentiated cells ($F_{3,30}$=47.61, p<0.0001) and RG108 4 µM in non-RA-differentiated cells ($F_{3,30}$=20.55, p<0.0001) whereas RG108 2000 µM decreased the neuritogenesis. 5-aza-cD 0.5 µM significantly increased the neuritogenesis of RA-differentiated cells ($F_{2,23}$=3.936, p=0.0339) and non-RA-differentiated cells in 0.005 µM and 0,5 µM concentrations ($F_{2,20}$ = 10.72, p = 0.0007). SAM 10 nM increased the neuritogenesis of RA-differentiated cells ($t_{14}$ = 5.518, p<0.0001) and non-RA-differentiated cells ($t_{14}$ =5.341, p=0,0001). **Conclusion:** DNA methylation inhibitors are able to increase neuritogenesis in SH-SY5Y cells regardless of the stimulation by retinoic acid, which suggests their ability to modulate plastic changes in differentiated and undifferentiated cells. Pharmacological modulation of DNA methylation might represent an important target to modulate neuroplasticity and the underlying mechanisms should be further investigated. **Acknowledgements: Financial support** from FAPESP, CAPES and CNPq. **References** [1] Wang, Y.*, et al.*, CNS Neuroscience & Therapeutics 19 (2013) 183. [2] Coppedè, F., Frontiers in Genetics, Epigenomics and Epigenetics, 5 (2014) 1. [3] Kaidery, N. A.*, et al.*, Neurotherapeutics, 10 (2013) 698.

**02.037 The inhibitory effect caused by choline in neuromuscular transmission is mediated at 50 HZ by activation of A1 and A2A receptors on motor nerve terminal.**
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**Introduction**: Alfa$_7$ neuronal (n) nicotinic (N) receptors (Rs) ($\alpha_{7\text{-}n}$NRs) are present in Schwann cells (SC) and skeletal muscle, but $\alpha_{7\text{-}n}$NRs found in SC are functionally more actives, as the $\alpha_{7\text{-}n}$NRs in skeletal muscle are only activated in pathological conditions. Although choline (CHO) and acetylcholine are molecules able to activate $\alpha_{7\text{-}n}$NRs, it has been shown that CHO expresses higher affinity for such receptors. Since the acetylcholine release from motor nerve terminal (MNT) may be modulate by presynaptic Rs for adenosine (A), such as the inhibitory-$A_1$R and the facilitatory-$A_{2A}$R, we hypothesized that CHO might play an important role in control of acetylcholine release from motor nerve terminal, as it has already been reported that activations of $\alpha_{7\text{-}n}$NRs in SC might be able to increase the level of ATP and/or adenosine in synaptic cleft. **Methods**: The Ethics Committee for Experimental Animals Studies of the State University of Maringá approved (ECEAS 7227300915) this study. The phrenic nerve-diaphragm preparations of rat were mounted as described elsewhere (Bülbring, Brit. J. Pharmacol. 1: 38, 1946). The muscular tension (A) registered at the beginning of high-frequency (50 Hz) elicited by electric indirect stimulation was the parameter analyzed. The tetanic (T) stimulation was applied each 20 min intervals, during 10s. A-value control was obtained after the muscular tetanic contraction to be stable. CHO (1.0 µM) was administered in Krebs buffer 45 min before the tetanic ($T_{4th}$) stimulation analyzed. The effects of combined administration of $A_1$R (DPCPX, 2.5 nM,) or $A_{2A}$R (ZM241385, 10.0 nM) antagonists with choline (1.0 µM) were tested. These agents were administered 20 min before CHO. The lowest concentration of choline able to cause significant effect in A-value was researched and used in all experiments. Similar experimental design was also followed when the experiments were performed with PNU282987 (1.0 µM), a selective $\alpha_{7\text{-}n}$NRs agonist. Data were submitted to ANOVA, followed by Bonferroni test at $p<0.05$ significance level. **Results**: CHO (1.0 µM) caused inhibitory (reduction in A-values) effect (-23.4± 3.0%, n= 4) in neuromuscular transmission. The inhibitory effect caused by 1.0 µM CHO (-23.4± 3.0%, n= 4) was reduced by previous administration of 2.5 nM DPCPX (from -23.4± 3.0%, n= 4 to -15.0± 4.24%, n=6) or 10.0 nM ZM241385 (from -23.4± 3.0%, n= 4 to -14.5± 0.7%, n=4). The selective activation of $\alpha_{7\text{-}n}$NRs by 1.0 µM PNU282987 also reduced (-15.3±0.6%, n=4) A-value. The previous treatment of preparation with 2.5 nM DPCPX or 10.0 nM ZM241385 reduced ($P< 0.05$) the effect caused by PNU282987 (from -15.3±0.6%, n=4 to -13.0±0.7%, n=4 case of DPCPX; from -15.3±0.6%, n=4 to -11.9±1.55%, n=4, case of ZM 241385). **Conclusion:** Data indicate that the inhibitory effect caused by CHO in the cholinergic neurotransmission stimulated at 50 Hz frequency seems depend on activation of $\alpha_{7\text{-}n}$NRs by CHO in SC, thereby inducing activation of $A_1$R and $A_{2A}$R on MNT by adenosine. **Financial Support**: FADEC

**02.038 Pioglitazone reduces the activation of the NF-κB in the 6-OHDA model of Parkinson's disease.** Machado MMF[1], Moura ELR[1], Bassani TB[1], Cóppola V[2], Zanata S[2], Vital MABF[1] [1]UFPR- Farmacologia, [2]UFPR- Patologia

**Introduction:** The neuroinflammation is one of the main factors related to the progression of neuronal death in Parkinson's disease (Hirsch, 2012). The NF-κB is a nuclear transcription factor involved in the inflammation process and in its resting state remains in the cytoplasm bound to an inhibitory protein (IκB). To occur the activation of the NF-κB is necessary the phosphorylation of the IκB releasing the dimer p60/p65 which is translocated to the nucleus where perform the transcription of genes involved in the inflammatory process (Gilmore, 2006). Pioglitazone (pio) is an insulin sensitizing drug used in the treatment of type 2 diabetes, characterized pharmacologically as agonist of peroxisome proliferator-activated receptor gamma (PPARγ). When the PPARγ is activated it is able to inhibit the NF-κB activation promoting a reduction in the inflammatory response (Sauer, 2015). The aim of the study was to assess the neuroprotective effect of the pioglitazone in the 6-OHDA animal model of Parkinson's disease. **Methods:** Male Wistar rats were divided into 4 groups: sham+vehicle, sham+pio, 6-OHDA+vehicle e 6-OHDA+pio (n = 8-10/group). Bilateral infusions of 6-hidroxydopamine (6-OHDA) (6 µg dissolved in aCSF; 1µl per site injection) in the SN was performed through stereotaxic surgery. The rats were treated for 5 days with pioglitazone 30 mg/kg (pio) or vehicle (veh) orally. These animals were submitted to the open field test (OFT) which was held on 1, 7, 14, 21 days after the surgery. After that they were euthanized with removal of substantia nigra (SN) and striatum (ST) for later analysis total IκB and NF-κB p65 by western blot. Statistical significance was performed using two-way ANOVA with repeated measures followed by Tukey's test and *t*-test to western blot analysis. **Results:** In the OFT the 6-OHDA group presented hypolocomotion in the $1^{st}$ and $7^{th}$ days after toxin infusion when compared to the sham group ($P < 0.05$). However, the animals of the 6-OHDA+pio group present hypolocomotion only in the $1^{st}$ day ($P < 0.05$). Moreover, in the 7, 14 and 21 days the rats of the 6-OHDA+pio group showed a significant increase in locomotion in comparison with the 6-OHDA+veh ($P < 0.05$). In the ST the 6-OHDA+veh group showed a significant increase of the IκB ($P < 0.05$) compared with the sham+veh group, while the 6-OHDA+pio group did not present difference compared with the sham+veh group. No difference was observed between the groups in relation to NF-κB p65 levels ($P > 0.05$). In the SN both the 6-OHDA+veh and 6-OHDA+pio groups showed a significant increase of the IκB ($P < 0.05$) compared to the sham group. The 6-OHDA+veh exhibited an increase of the NF-κB p65 levels compared to the sham group ($P < 0.05$), while no difference was observed between the 6-OHDA+pio and sham+veh groups ($P > 0.05$). **Conclusions:** The study shows that pioglitazone in 6-OHDA model of PD was able to restore the animals' hypolocomotion. This effect may be related to a anti-inflammatory action of the pioglitazone by reducing the NF-κB activation and thus preventing the transcription of genes linked to inflammatory response. This study was approved by Institutional Ethics Committee (no. 881). **References:** Hirsch, E., Parkinsonism Relat Disord, 18S1, 210, 2012. Gilmore, T., Oncogene, 25, 6680, 2006. Sauer, S., Trends Pharmacol Sci, 36, 688, 2015. **Financial support** and acknowlegments: CNPq, Capes and Fundação Araucária.

**02.039 Effects of neuronal PTEN haploinsufficiency on memory and synaptic markers.**
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**Introduction:** The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) was initially described as a tumor suppressor. However, due to its crucial role on cellular metabolism, proliferation, migration and survival, it has also been associated with many important processes in the central nervous system, including neurogenesis and synaptic plasticity. Considering that a complete knockout of PTEN culminates in embryological lethality, the use of alternative tools, such as conditional knockouts (*e.g.*, through the Cre-loxP system), is of great value for studies that aim to assess site-specific functions of PTEN. **AIMS:** This study aimed to characterize the effects of neuronal PTEN haploinsufficiency on cognition and synaptic markers, thus giving support to the assessment of PTEN effects on synaptic plasticity. **Methods:** Male mice of 4 months of age with a neuronal heterozygous deletion of PTEN (HT) from the *PTEN$^{loxP/+}$;NSE-Cre* lineage were submitted to behavioral assays (*e.g.* Morris water maze and passive avoidance) and, after euthanasia, had the hippocampus and cortex dissected and processed for molecular assays (*e.g.* western blotting). Their wild-type (WT) littermates were used as controls. Student t-test or two-way ANOVA were used for statistical analysis, with significance considered for $p \leq 0.05$. **Results:** There was no statistical difference between WT and HT animals regarding their performance on spatial memory – neither in the learning curve nor in the 4 h probe from the Morris water maze. However, HT animals did not present the expected latency increase – observed in WT mice – in the 24 h probe from the passive avoidance test. In the hippocampus, we could not detect an increase on AKT phosphorylation in both S473 and T308 sites of HT animals. On the other hand, we did found the expected increase on AKT activation in the cortex. The results of expression and/or activation of the synaptic markers studied (PSD-95, synaptophysin, and NMDA and AMPA receptors) were still insufficient to obtain a conclusive statistical analysis. **ConclusionS:** The PTEN neuronal heterozygous deletion impaired contextual fear memory, but not spatial memory. Considering the data present on the literature, this effect might be correlated with a disbalanced synaptic plasticity. Although we could not observe a significant effect on the synaptic markers studied, further molecular analyses – as well as an expansion of the sample size for the western blotting assay – will enable a better understanding of the mechanisms involved in the role of PTEN on cognition and synaptic plasticity. **Financial Support:** This study was supported by FAPESP (#2011/21308-8 and #2014/18689-8) and approved by the local Animal Research Ethical Committee (CEUA ICB/USP #167, p. 113, book #2).

**02.040 Biochemical and behavioral effects of the pre-treatment with the inverse agonist of CB1 in the inflammatory signaling triggered by LPS in mice.** de Souza BLS, Andreotti DZ, Scavone C, Kawamoto EM ICB-USP – Farmacologia

**Introduction:** The endogenous cannabinoid system seems to play a modulatory function in many neurobiological processes, including neuroprotection, neuronal plasticity and neuroinflammation. Most of the literature of this area has a focus in the anti-inflammatory and neuroprotective actions of this system, using agonists of the receptors $CB_1$ and $CB_2$. However the effects of the pre-treatment with cannabinoids compounds in the inflammatory response are not so present in the literature. In this context, we used a new approach to promote the neuroprotection, activating signaling pathways that modulate adaptive responses. In this study we pre-treated the mice with an inverse agonist of the receptor $CB_1$, in order to evaluate this neuroprotection through adaptive responses. **Aims:** This study aimed to characterize the behavioural and biochemical effects of the pre-treatment with the inverse agonist of the receptor $CB_1$ in the inflammatory signaling triggered by LPS. **Methods:** Male mice (C57BL/6J) of 3 months of age were pre-treated during 4 days with daily injections of 3mg/Kg of AM251 ($CB_1$ inverse agonist) or with vehicle, intraperitoneally (i.p.). Twenty-four hours after the last AM251/Vehicle injection, the animals were injected i.p. with LPS (500 µg/kg) or saline. Twenty-four hour after the LPS/Saline injection, animals were submitted to behavioural tests: Morris Water maze and inhibitory avoidance task. In another batch, animals were euthanized 4 hours after LPS injection, to accomplish the biochemical tests (e.g. Western Blotting and ELISA) with the dissected structures: hippocampus and prefrontal cortex. Data were analyzed using One Way ANOVA with Student-Newman-Keuls post-test. Training and mean speed data of the Morris Water maze were analyzed with Two-Way ANOVA followed by Bonferroni Post-test. Inhibitory avoidance data was analyzed by Mann-Whitney test. To all statistic analysis significance was considered for $p \leq 0.05$. **Results:** We found that the pre-treatment with the $CB_1$ inverse agonist seems to be potentiating the inflammatory response. AM251 pre-treated animals exposed to LPS showed a higher memory deficit in the behavioural tests, and biochemically higher concentrations of TNF- α, and higher expression of p-IκB/IκB, indicating the potentiation of the inflammatory response triggered by LPS. The inverse agonist of $CB_1$ by itself does not present any pro-inflammatory effects nor behavioural effects. Animals treated only with LPS present a memory deficit in the behavioural tests, as expected by literature, but less than the group pre-treated with AM251 and LPS. **ConclusionS:** Our hypothesis was that the pre-treatment with the inverse agonist of $CB_1$ was going to act as a moderate stressor, leading to a neuroadaptation (mainly by glutamatergic pathways). However exactly the opposite happened; the pre-treatment potentiated the pro-inflammatory signaling pathway. We are currently trying to explain by which mechanisms this phenomenon is acting. One of the hypothesis is that the endogenous cannabinoid system can modulate glucocorticoids, and with this, our pre-treatment may be working not as a moderate stressor but as a deleterious stressor, such as unpredictable chronic stress. Further assays will be done in order to better understand the mechanism. **Financial Support:** This study was supported by CAPES and approved by the local Animal Research Ethical Committee (CEUA ICB/USP #030, p. 04, book #3).

**02.041 The interesterified fat consumption during early life periods can impair responses related to morphine administration in adult rats.** Milanesi LH, Roversi K, Antoniazzi C, Davila LF, Kronbauer M, Segat H, Trevizol F, Burger ME UFSM – Farmacologia e Fisiologia

**Introduction:** The interesterified fat (IF) is an alternative process used to replace *trans* fatty acids (FA) in processed foods. Interesterification rearranges the FA in the glycerol molecule resulting in physical and chemical characteristics improvement important for food industry [1]. However, nearly no data about the brain health effects of IF intake are available. The dietary FA are largely incorporated into neuronal membranes during cerebral development and neural functions may be affected [2]. Drug addiction is one of the biggest public health problems in our society, and specially the chronic use of morphine (MOR) leads to physical and psychological dependence, causing aversive symptoms known as abstinence syndrome during its withdrawal [3]. Considering this, the aim of this study was to investigate the influence of IF supplementation during pregnancy, lactation and postweaning on behavior parameters of MOR addiction in rats. **Methods:** Female Wistar rats were assigned into 2 experimental groups that received soybean oil (SO, control group) or interesterified fat (IF) by gavage (3g/kg, p.o, once a day) during pregnancy and lactation. Pups were maintained in the same maternal supplementation until adolescence. On post-natal day (PND) 39, animals were subjected to morphine conditioned place preference (CPP), when received morphine (4,0 mg/kg, i.p) or saline once a day during 4 days. To evaluate anxiety-like symptoms, animals were observed in the elevated plus-maze (EPM). Thermal nociception was assessed on the hot plate test. **Results:** IF supplemented rats showed no preference for MOR when compared to SO group. During MOR withdrawal, SO group showed higher anxiety-like symptoms than IF, as observed by decreased the time spent in the open arms of the EPM. In fact, IF supplemented group showed a minor anxiety index than SO group. In addition, Animals of both saline and morphine groups that were supplemented with IF showed lower pain threshold than SO group, as observed by minor latency time in hot plate test. **Conclusions**: Our findings show experimental and clinical relevance , since the chronic consumption of IF may impair responses to opioid drugs such as MOR, ,requiring higher doses of the drug, with greater risk tolerance and respiratory depression.. **Financial support:** CNPq, CAPES. All experimental protocols were approved by the institutional animal care and use ethics committee (nº 036497/2014). 1. Farfan, M. Food Chemistry 139:571, 2013. 2. Bourre, J.M. J Nutr 119:1880, 1892. 3. O'Brien, C.P. Science 278:66, 1997.

**02.042 Investigation of Thymol on behavioral models of depression in mice: involvement of serotonergic and noradrenergic system.** Capibaribe VCC, Fernandes ML, Melo FHC, Santos LKX, Cito MCO, Lopes IS, Silva DMA, Vasconcelos AS, Chaves RC, Oliveira NF, Valentim JT, Oliveira ICM, Sousa FCF UFC – Farmacologia

Thymol (2-isopropyl-5-methylphenol), isomer of carvacrol, a monoterpene extracted from essential oil of various aromatic plants, is the main component of the essential oil of Alecrim-pimenta (Lippia sidoides), constituting approximately 48% of its composition. Essential oils are substances very studied for their pharmacological properties as anti-inflammatory actions, microbiological, anticonvulsant, anxiolytic and antidepressant activities. Based on this, this study aimed to evaluate the behavioral actions of thymol in animal models of depression in mice. The animals were provided by the biotery of the Federal University of Ceará. The test used was the forced swimming test (FST). In this test the immobility time (sec) was observed each mouse. The drugs used were thymol (25 and 50 mg/kg, p.o), imipramine (10 mg/kg, i.p.), p-chlorophenylalanine methyl ester (PCPA) (100mg/kg, i.p.), Prazosin (1mg/ kg, i.p.), Yohimbine (1mg/ kg, i.p.). **Results** were analyzed using one-way ANOVA and Student Newman Keul's. All values are expressed as mean ± standard error of the mean (SEM) and $p<0.05$ were considered as statistically significant. In this study groups treated with thymol (25 and 50mg/kg) and imipramine, a significant decrease in the immobility time in mice, when compared to control was observed (Control:105,4 ± 14,21; THYMOL 25: 51,22 ± 10,60; THYMOL 50: 35,00 ± 7,730; IMP 10: 35,00 ± 7,730. Pretreatment of mice with the inhibitor of 5-HT synthesis PCPA (once a day on 4 consecutive days) affected the antidepressant-like effect of thymol (50 mg/kg, p.o.), when compared to control (Control: 169,4 ± 11,03; PCPA100: 121,0 ± 5,837; THYMOL 50: 37,40 ± 5,357; THYMOL 50 + PCPA 100: 198,4 ± 11,42, FLU 35: 42,82 ± 3,811; FLU 35 + PCPA 100: 121,0 ± 4,89). Pretreatment of mice with the α1-adrenoceptor antagonist prazosin and the α2-adrenoceptor antagonist yohimbine were able to reverse the antidepressant-like effect of THYMOL (50 mg/kg) in the forced swimming test when compared to control group (control:108,9 ± 9,182; THYMOL-50: 15,17 ± 5,089; PRZ-1: 111,7 ± 6,856; THYMOL-50 + PRZ-1: 141,4 ± 9,279; (control: 136,9 ± 15,46; THYMOL 50: 36,00 ± 6,586; YOIM: 117,9 ± 8,998; THYMOL 50 + YOIM: 78,67 ± 16,03). The results confirm an antidepressant effect of thymol, and suggest that this effect may be related to serotonergic and noradrenergic systems. **Financial support**: CAPES, CNPq, FUNCAP. Study was approved by Institutional Animal Ethics Committee in the Federal University of Ceará (Protocol number: 30/2011). MATOS**,** F.J.A. ET AL. J. Essent. Oil Res., v.11, p.666, 2000. MELO FHC et al. Fund. and Clin. Pharm. Jun; 25(3):36, 2011. SOUSA, F.C.F.: SOUSA, D.P. Chapter 7, p123 Nova Biomedical, 2012

**02.043 LQFM181 ameliorates aluminum chloride-induced cognitive dysfunction via alleviation of hippocampal oxidative stress.** Neri HFS, Brito AF, Costa EA, Santos FCA, Ghedini PC, Menegatti R UFG – Ciências Biológicas

**Introduction**: LQFM181 is a new candidate molecule of drugs prototype that has antioxidant properties. The oxidative stress is associated with several neurodegenerative disorders, where the excess of reactive oxygen species (ROS) can cause cell death and the establishment of characteristic diseases such Alzheimer's(AD) and Parkinson's(PD) disease. Aluminium has been reported to cause oxidative stress-associated damage in the brain. In the present investigation, protective effect of LQFM181 against aluminium chloride (AlCl3)-induced neurotoxicity and cognitive impairment was studied in male adult Swiss albino mice. **Methods**: The animals were randomized into four groups (n=10), according treatment used: I and II (Tween 80, 2.0%, 10 mL/kg); III and IV (LQFM181 200 µmol/kg). One hour after, the animals received a second treatment: distilled water 10ml/kg (I and III groups) or $AlCl_3$ 750 µmol/kg (II and IV groups). The treatments were administered once daily through oral gavage for 40 days. After the treatment period, the long-term memory (LTM) was evaluated using passive avoidance test and motor activity by open field and chimney tests. Posteriorly, the animals were euthanized and the hippocampus was dissected for determination of catalase activity (CAT). The results were expressed as the mean ± SEM and the statistical analysis was performed using 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 Goias, Goias, Brazil (Protocol CEUA/UFG 205/2009).
**Results**: Cognitive dysfunction and increase of oxidative stress markers were found in $AlCl_3$-treated groups. Animals treated with LQFM181(III and IV groups) showed similar latency time to escape platform (LT) (77.2 ± 14.4s and 14.7 ± 2.3s, $p <0,001$, respectively) with Group I (10.4 ± 1.6 s, $p <0,001$), but they were significantly higher when compared with the Group II (5.3 ± 1.1s). There were no differences among groups in the open field and chimney tests. CAT activity was significant increased in the Groups I, III and IV (0.17 ± 0.01,0.16 ± 0.02, and 0.21 ± 0.02, $p <0,01$ respectively mmol/mg proteins) when compared to Group II (0.09 ± 0.01, $p <0,05$ mmol/mg proteins). **Conclusion:** The data suggest that LQFM181 offers neuroprotection in $AlC1_3$-induced neurotoxicity and it may be a potential therapeutic approach in the treatment of oxidative stress diseases associated with neurotoxicity. **Financial support**: CAPES.

**02.044 Prelimbic cortex mediates context-induced relapse to alcohol.** Palombo P[1], Leão RM[2], Bianchi PC[1], Carneiro-de-Oliveira PE[1], Planeta CS[1], Cruz FC[3] [1]FCFar-Unesp-Araraquara – Farmacologia, [2]UFBA – Biorregulação, [3]Unifesp – Farmacologia

Evidence indicates that drug relapse in humans is often provoked by exposure to the self-administered drug-associated context. An animal model called "*ABA renewal procedure"* has been used to study context-induced relapse to drug seeking. In this procedure, rats are first trained to self-administer drug in one context. Next, drug-reinforced lever responding is extinguished in a different (non-drug) context. Subsequently, context-induced reinstatement of drug seeking is assessed by re-exposing rats to the drug-associated context. In this study, we examined whether context-induced reinstatement of alcohol seeking is mediated by activation of prelimbic cortex neurons. On test day, re-exposure to the alcohol-associated context reinstated alcohol seeking (active lever presses: 6.8 ± 2.30, extinction context and 33.1 ± 5.10 drug context; $p<0.05$, n=16 per group) and increased expression of the neural activity marker Fos (Fos $mm^2$: 42.4± 6.8, extinction context and 110 ± 13.8 drug context; $p<0.05$). The percentage of neural activation in prelimbic cortex was 3.4% in extinction context and 7.7% in drug associated context. Reversible inactivation of neural activity in prelimbic cortex using the GABA agonists Muscimol (M) and baclofen (B) decreased context-induced reinstatement (active lever presses: 3.7 ± 1,9, extinction context-saline; 4 ± 0.3, extinction context- B+M; 11.67 ± 3.33, drug context-saline; 1.33 ± 1.33 drug context -B+M). Our results demonstrate an important role of prelimbic cortex in context-induced reinstatement of alcohol seeking. Keywords: self-administration, dependence, ethanol. **Financial support**: FAPESP 2103 / 24986-2 and CAPES. Ethics Committee: USP 01/2015

**02.045 Roles of TLR4 on biochemical and behavioral effects of intermittent fasting.** Paixão AG[1], Vasconcelos AR[1], Mattson MP[2], Scavone C[1], Kawamoto EM[1] [1]ICB-USP – Farmacologia, [2]NIA

**Introduction**: Intermittent fasting (IF) protocol, in the absence of desnutrition, is able to induce a moderate stress to the body, stimulating stress proteins, modulating transcription factors such as nuclear transcription factor kappa B (NF-κB) and cAMP response element binding (CREB) and making the organism more resistant to a more severe stimuli. Recent evidences have suggested that Toll-like receptor (TLR) 4 plays an important role in the development of metabolic disorders such as obesity and insulin resistance and also in mediating oxidative stress in situations of ischemia/reperfusion and hemorrhagic shock. However, little is known about the involvement of TLR4 on the beneficial effects induced by the IF protocol as well as the molecular mechanisms underlying this processes. Aim: The aim of the present work was to investigate the effects of IF on memory and on the signaling mechanisms associated with the transcription factors NF-κB and CREB in TLR4 knockout (KO) mice. **Methods**: Adult male mice were divided into 4 groups: C57Bl6 Tlr4+/+ (wild type) control, C57BI6 Tlr4+/+ subjected to IF, C57BI6 Tlr4-/- (KO of TLR4) control and C57BI6 Tlr4-/- subjected to IF. Mice were subjected to IF or ad libitum control diets for 30 days. After that, mice were subjected to Morris water maze (MWM) and inhibitory avoidance (IA) tests. Hippocampus was used for electrophoretic mobility shift assay (EMSA), Western blot to measure 4-hydroxynonenal (4-HNE) levels and multiplex assay to measure cytokines levels. **Results**: In the absence of TLR4, IF leads to an increase in oxidative stress levels (4-HNE), which may contribute to the observed reduction in the activity of the transcription factors NF-κB and CREB. Consequently, there is a reduction of the expression of target genes modulated by NF-κB and CREB, namely Bdnf1, IL-12, IL-15 and RANTES. These results may contribute to the memory impairment of TLR4 KO mice submitted to IF observed in the MWM and IA tests. **Conclusion:** The present study suggests for the first time that TLR4 participates in the modulatory effects of IF. This data allow a better understanding of the physiological processes that aim at developing new strategies for pharmacological interventions to promote longevity and healthy aging, as well as for the treatment of neurodegenerative disorders. **Financial support**: FAPESP, CNPq, and CAPES. All procedures were approved by the Biomedical College of Animal Experimentation and the Ethical Committee for Animal Research ICB/USP (fls. 89, no 60, book 02).

**02.046 Involvement of $H_2S$ pathway in behavioral changes in pilocarpine-induced seizure model.** Rios ERV[1], Silva AH[2], Carvalho AMR[1], Vasconcelos LF[1], Carvalho MAJ[1], Souza DAA[1], Oliveira JVS[1], Fonteles MMF[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Farmácia

**Introduction**: Hydrogen sulfide (H2S) is a small molecule permeable by membranes and endogenously produced by several enzymes, especially cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). Regarded as a neuromodulator involved in various functions and affecting various neurotransmitter systems related to seizure models such as glutamate, GABA and catecholamines. So, the aim of this work was to clarify the role of endogenous pathways H2S production in behavioral changes pilocarpine-induced seizures in mice. **Methods**: Male Swiss mice (25-32g), kept in a room with controlled temperature and luminosity, were used in all experiments, and the tests were conducted with 6-8 animals per group. It were observed the effects of pretreatment with H2S donors (NaHS - 32, 102.4 or 307.2 µMol/kg, ip. - or Lawesson's Reagent – 9, 27 or 81 µmol/kg, ip.), inhibitor CSE (DL-propargylglycine - 14.7, 46.9, 150 mg/kg, ip.) or inhibitor CBS (aminooxyacetic acid - 3, 9 or 27 mg/kg, ip.) in behavioral changes in animals subjected to seizure model induced by pilocarpine 400mg/kg ip., the parameters being observed: latency of first convulsion and latency of death. **Results** and conclusion: Only the donor H2S, NaHS, the higher but not in lower doses, was able to decrease the latencies of first convulsion and of death (102.4 mMol/kg - 403.7 seconds / 474 seconds; 307.2 mMol/kg - 357.9 seconds / 428.3 seconds; control - 518.5 seconds / 592 seconds), while with the Lawesson's reagent there was no change in these parameters when compared with the control. Blockade of the CSE or CBS was able to increase time to onset of convulsions (highest dose - 952.8s / 824.6s; Control - 695.2s / 610.8s) and survival (DL-propargylglycine - all doses 50-90% survival; Control - 0% survival) and (aminooxyacetic acid - 9 mg/kg - 40% and 27 mg/kg - 50% survival). With our results we can conclude that the increase of H2S in mice worsened chemical-induced seizures and the blocking of its endogenous synthesis had protective role against the effects of pilocarpine. Therefore, the H2S production pathway seems to be involved in proconvulsant effect of the pilocarpine. **Financial support**: CNPq, CAPES and UFC. Approval protocol ethics committee on animal research - UFC: 95/2014

**02.047 Memantine prevents brain damage induced by *Zika virus* infection.** Costa VV[1], Del Sarto JL[2], Rocha RF[2], Marques RE[2], Esper L[2], Ribeiro LS[3], Ribeiro F[2], Ribeiro F[2], Vieira THF[2], Souza DG[3], Ribeiro F[2], Teixeira MM[2] [1]UFMG – Bioquímica e Imunologia, [2]UFMG – Bioquímica e Imunologia, [3]UFMG – Microbiologia

**Introduction:** ZIKA virus (ZIKV) infection has emerged as an important disease with public health concern. There is no approved treatment or vaccine. Memantine, which is a potent neuroprotective drug, is largely used in clinical practice, acts by blocking N-methyl-D-aspartate receptors (NMDARs) and decreasing excessive $Ca^{2+}$ entrance, which can promote neuronal cell death through excitotoxicity. Here we propose to evaluate the possible neuroprotective effects of the treatment with Memantine (MEM) after *Zika virus* (ZIKV) infection. **Material and methods:** We evaluated the ability of ZIKV to replicate and cause damage in primary murine neurons by plaque assay and immunostaining analysis, respectively. In parallel, mice deficient in type I interferon receptors (IFNα/β$R^{-/-}$) were intravenously infected with ZIKV and treated with MEM (30 mg/kg B.I.D). Body weight loss; hematocrit index and platelet levels were analyzed in moribund mice. Leukocyte infiltration in blood and tissues were assessed by differential counts and biochemical assays, respectively. Histopathological analyses and viral load were also performed. **Results:** ZIKV actively replicates in primary neurons and glia cells of mice and virus replication was directly associated with massive neuron death. Interestingly, treatment with MEM was able to prevent neuronal death without interfere with the ability of ZIKV to replicate in those cells. *In vivo* experiments demonstrated that MEM treatment reduced disease parameters such as total of leucocytes in blood and neutrophil infiltration into brain. Most importantly, MEM treatment massively reduced neurodegeneration and microglial activation in the brain of infected mice. **DISCUSSION:** ZIKV infection and its neurological commitments are rising as a major public health concern. There is no available treatment or vaccine for ZIKA. Our results strongly demonstrate a neuroprotective role of MEM treatment after ZIKV infection *in vitro* and *in vivo*. **Conclusion:** MEM treatment was able to prevent neuronal damage induced by ZIKV infection. Association of MEM with antiviral drugs could be an ideal treatment against ZIKV infection. **Financial Support:** FAPEMIG, CAPES, CNPq. INCT em Dengue. **CEUA/UFMG** approval number: 169/2016.

**02.048 Kinin B2 receptor as a target for the treatment of Alzheimer's disease** Nunes MA[1], Dong-Cresti KE[1], Baraldi-Tornisielo T[1], Schöwe NM[2], Cheloni JA[1], D'Amaro G[1], Caetano AL[1], Farah D[3], Irigoyen MCC[3], de Angelis K[4], Gobeil F[5], Viel TA[6], Buck HS[1] [1]FCMSCSP – Ciências Fisiológicas, [2]USP – Ciências Farmacêuticas, [3]InCor-HC-USP – Hipertensão Experimental, [4]Uninove, [5]Université de Sherbrooke – Pharmacology, [6]EACH-USP

**Introduction**: Alzheimer's disease is characterized by cognitive decline, amyloid beta peptide aggregates and neurofibrillary tangles. We have described an increase in bradykinin (BK) in the cerebrospinal fluid and in densities of B1 and B2 receptors in brain areas related to memory, after chronic infusion of AB peptide in rats, accompanied by memory disruption and neuronal loss. Memory impairment was observed in C57BL/6 mice (WTAB) occurred earlier in mice lacking B2R (KOB2RAB) was absent in mice lacking B1R (KOB1RAB). Increase in B2R density was observed in both WTAB and KOB1RAB. Increase in AB deposits in KOB2RAB was observed. Memory preservation observed in KOB1RAB, could be due a neuroprotective role for B2R. Thus, the aim of this study was to evaluate the participation of kinin B2 receptor in AD, using transgenic mice hyperexpressing human APP treated with the synthetic and biostable kinin B2 agonist FGB2A. **Methods**: Male, 12 months-old transgenic mice (TG) for Alzheimer's disease (APPSwInd) and their littermates (WT) were infused subcutaneously with FGB2A or vehicle, during 8 weeks, using a mini-osmotic pump (0.11 µL/h) implanted in the animal's back. Before and after the treatment, the animals were submitted to the Barnes maze for the evaluation of spatial memory, Von Frey test for the evaluation of mechanical nociception or sensibility and, only at the end of infusion period, inhibitory avoidance for the evaluation of aversive-related memory. In a third group, comprised by WT mice, the B2 agonist FGB2A was infused for 14 days in order to evaluate possible alterations in the cerebral blood flow (CBF). The CBF was evaluated through the infusion of fluorescent microspheres via the femoral artery and quantification of microspheres deposition in the target organs. The fluorescent microspheres were quantified in a fluorescent spectrophotometer (λ = 465 nm).**Results**: In the behavioral evaluation, TG animal treated with the B2 agonist showed improved spatial memory when comparing the last Barnes maze test (85.00 ± 15.28 N=5) before the treatment with the first test (29.40 ± 6.038 N=5) after the treatment, indicating that the B2 agonist infusion improved the spatial memory performance. No differences were observed in the others groups. Also, no difference in the aversive-related memory after the treatment with the B2 agonist were observed. Transgenic mice treated with FGB2A showed significant less sensibility to pain (2.04 ± 0.05 arbitrary units; AU, n=5, P<0.05) than untreated ones (1.64±0.13 AU, n=4). In the littermates, no difference in the sensibility was observed. Animals treated with NG291 showed a significant increase of 1.5 times in the flow (2.68 ± 0.79 mL/min, n=7, P < 0.05) when compared to untreated animals (1.76 ± 0.51 mL/min, n=6). **Conclusions**: The treatment with the B2 receptor agonist FGB2A improved spatial memory, decreased the sensibility to pain of transgenic animals and increased the cerebral blood flow of wild type animals. Financial Support: FAP Santa Casa; FAPESP nº 2013/13656-1. CEUA nº 2014/001

**02.049 A novel potential target to Alzheimer's disease: Transient Receptor Potential Ankyrin 1 (TRPA1).** Bicca MA[1,2], Santos ECS[1], Viola KL[2], Loch-Neckel G[1], Klein WL[2], Calixto JB[1] [1]UFSC – Farmacologia, [2]Northwestern University – Neurobiology

**Background**: TRPA1 is a member of the transient receptor potential (TRP) superfamily well known to be expressed in the spinal horn and other tissues, and recognized to mediate a diversity of pain and inflammatory states. Alzheimer's disease (AD) is a degenerative disease characterized by accumulation of both tau and Aβ peptides, and for having the disease course affected by progressive oxidative stress and brain inflammation. Products of inflammation, notably reactive oxygen species (ROS) and $Ca^{2+}$ are augmented during AD initiation and progression. Intriguingly, these are endogenous molecules known to be able to activate TRPA1. However, the possible role of TRPA1 in AD pathogenesis is still unknown and we aimed to investigate it. **Methods:** We used different approaches (primary cell culture treated with AβOs, AβOs-injected Swiss mice, 5XFAD TG AD mouse model and AD human brains; including respective controls to each) and also two distinct preparations $AβOs_{40}$ and $AβOs_{42}$. Besides behavioral assessments, a variety of molecular and biochemical techniques, namely the evaluation of ROS formation, mitochondrial membrane potential determination, immunocytochemistry, immunofluorescence in brain slices, western blotting, co-immunoprecipitation, electron microscopy, and others. **Results:** Here we report TRPA1 is largely expressed in neurons and microglia in the brain. TRPA1 is relevant to AβOs binding and AβOs-induced oxidative stress/death in neuronal cells. We demonstrated the correlation between the up-regulation and spreading of both AβOs and TRPA1, in all the approaches used. Herein, we are also reporting TRPA1 augmented expression in the microglia and its possible role in the inflammation process. Data indicate that AβO-induced TRPA1 activation facilitates microglial phenotype switch. Of note, TRPA1 selective antagonist (HC030031) oral treatment improved memory deficits in the different mouse models of approach. Besides, reduced Aβ burden in plaques and oligomers with consequent improvement on AβOs-induced synaptic loss. Preliminary data suggest TRPA1 inhibition contributes to a pro-resolution microglial M2 phenotype. **Conclusion:** We propose TRPA1 is essential to AD pathogenesis given its importance to AβOs-induced toxicity and further memory impairment. TRPA1 could serve as a novel potential target to Alzheimer's disease. **Financial Support:** FAPESC, CNPq and CAPES. Ethical Committee from both universities approved the employed procedures (UFSC: PP0625) (Northwestern University: 2014-3406). **Key words:** TRPA1 channel, AβOs-toxicity; neuroinflammation; Alzheimer's disease;

**02.050 Topic Dexamethasone impairs protein synthesis and neuronal regeneration in the olfactory epithelium.** Crisafulli U[1], Xavier AM[2], Cambiaghi TD[3], Santos FB[2], Castilho BA[3], Porcionatto M[2], Malnic B[1], Glezer I[2] [1]USP – Bioquímica, [2]Unifesp-EPM – Bioquímica, [3]Unifesp-EPM – Biologia Celular e Molecular

**Introduction:** Chronic inflammatory process in the nasal mucosa is correlated with loss of smell perception. Over-activation of immune cells is generally associated with loss of olfactory epithelium (OE) function, and topical steroidal anti-inflammatory drugs have been largely used for treating such condition. It is not definitively recognized whether these drugs impact the regenerative process in the OE. **Methods:** We evaluated the effects of these drugs in OE lesion models through intranasal infusion of gram-negative bacteria lipopolysaccharide (LPS) or intraperitoneal methimazole in C57/Bl6 mice. After OE injury completion, topical application of the corticosteroid dexamethasone (DEX) was performed in acute or consecutive days (1, 2 and 3 days post-lesion) and OE regeneration, protein synthesis signaling and neuronal progenitor activity were analyzed by immunofluorescence (Olfactory Marker Protein–OMP, Bromodeoxyuridine-BrdU labeling and puromycin incorporation), immunoblotting, and *in vitro* experiments using OE-derived neurospheres (NE). **Results:** DEX short-term treatment reduced the overall restoration of OMP and BrdU-positive cells at the site of drug infusion. The corticosteroid also affected protein synthesis, which is crucial for cell proliferation, as shown by reduced puromycin incorporation and decreased mTOR activity, as determined by lower levels of the phosphorylated p70-S6 kinase protein. *In vitro* assays corroborated an effect on proliferation as inferred by a dose-dependent reduction of NE numbers. **Conclusions:** Our results suggest that DEX can interfere with the regenerative cellular mechanisms of the olfactory neuroepithelium, including signals for proliferation and protein synthesis. **Financial support:** FAPESP (2007/53732-8; 2013/07937-8) and CNPq (484869/2012-4) **Approval of Animal Care Committees** (CEUA–UNIFESP 1427-11 and COBEA-USP).

**02.051 Test of ONO-8713, a PGE2 EP1 selective receptor antagonist, on potential benefits in Alzheimer mouse models subjected to stroke.** Mendes FR[1], Doré S[2] [1]UFABC – Ciências Naturais e Humanas, [2]University of Florida – Anesthesiology, Neurology, Psychiatry, Psychology, Pharmaceutics, Neuroscience

**Introduction:** Stroke and Alzheimer disease (AD) are major neurodegenerative diseases that may worsen the prognosis of each other, but co-factors shared by these two diseases remain unclear. Neuroinflammation has been recognized as an important player in etiopathology of these neurovascular diseases. The most recognized pathway in mediating neuroinflammation is the $PGE_2$ pathway. The $PGE_2$ proinflammatory and neurotoxic effects have been associated with the GPCR $PGE_2$ EP1 receptor. Here, we examined the efficacy of a selective EP1 antagonist on the beta-amyloid (Aβ) load along with lesion volume and changes in neurobehavioral outcomes in the setting of the permanent middle cerebral artery occlusion (pMCAO) ischemic model. **Methods:** We used transgenic APP/PS1 (4.5 months), 3xTg-AD (3 months) and their respective age-matched wildtype (WT) controls. Mice were maintained at the University of Florida animal housing facility on a 12-h reversed dark/light cycle and the behavioral tests carried out during the dark phase. Each group of mice was divided in pMCAO and sham treated with ONO-8713 (EP1 selective antagonist, 10µg/kg ip) or vehicle. The pMCAO procedure was performed under anesthesia and the pharmacological treatment was made immediately after surgery and daily during 14d. Before the surgery (basal) and at days 1, 3 and 7, the animals were evaluated in open field (OF) and cylinder test (CT) and at days 13 and 14 in passive avoidance task (PA). The animals were sacrificed at day 14 and the brains collected for quantification of infarct volume by crezyl violet staining and for Aβ measured by ELISA. **Results:** The pMCAO and sham surgery induced reduction on OF activity in both cohorts of mice, and there were only limited differences between the experimental groups. Preliminary analyses suggest that there is no significant biological difference found for the CT. In the PA, APP/PS1 mice submitted to the pMCAO surgery appear to present shorter latency retention compared to sham group under vehicle treatment which would suggest worse memory (APP/PS1+sham+Veh = 300±0s vs APP/PS1+pMCAO+Veh = 222±19s*; *=$p<0.05$ Kruskall-Wallis/Mann-Whitney). On the other hand, the APP/PS1+pMCAO had latency relatively similar to controls suggesting the treatment protected the mice against memory deficits. In regard to Aβ quantification the Aβ40, and the Aβ42 load revealed mild concentration of Aβ in cortex of APP/PS1 mice, but there was no difference among the experimental groups. This pMCAO model produces relatively small cortical lesion volumes and we are completing detailed statistical analyses. **Conclusion:** The pMCAO ischemic lesion was discrete and localized such that it produced limited to no major motor and cognitive deficits either in transgenic or WT mice, except by worsening the memory in APP/PS1. The ONO-8713 pre-treatment appears to attenuate the memory impairment in these mice, and no sticking significant behavioral changes were observed. This ongoing investigation suggests the potential of the selective $PGE_2$ EP1 antagonist ONO-8713 as therapeutic agent in neuroinflammation in AD mouse models after ischemic stroke. Animal ethical approval: IACUC study #201305020 **Financial support**: FAPESP grant#2014/18702-4 (FM) and NIH (SD)

**02.052 Evaluation of the anxiolytic-like behavior and density of kinin B1 and B2 brain receptors in knockout Mice for kinin receptors.** Barald-Tornisielo T[1], Dong-Krest KE[1], Schöwe NM[2], Lopes ASA[1], Sousa AMA[1], Caetano AL[1], Nunes MA[1], Viel TA[3], Buck HS[1]
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**Introduction**: The brain aging is associated to the increase in neuroinflamation, characterized by the increase of activated microglia, inflammatory cytokines and decrease in anti-inflammatory molecules. Kinin B1 and B2 receptors are classically associated to the peripheral and central inflammatory responses. These receptors could have an antiinflammatory function when expressed in some glial cells Recently our research team showed changes in memory processing of B1R or B2R knockout mice (KOB1, KOB2) submitted to the chronic infusion of 1-40 amyloid-β (Aβ) peptide. Besides, in an observation along the aging process, KOB2 mice showed memory impairment at 6 months-old whereas C57Bl/6 mice showed the same impairment at 12 months-old. However, KOB1 mice showed memory impairment only at 18 months-old, suggesting that the absence of B1 receptor preserves the memory and also the presence of B2 receptor improves the memory. The aim of this work was to evaluate the behavioral profile of knockout mice for kinin receptors related to anxiety and to analyze the possible histological alterations in the brain. **Methods**: For the behavioral analysis, C57Bl/6, KOB1 and KOB2 male mice with six and 18 months-old were used. The anxiety behavior was observed in the elevated plus maze apparatus where the number of entries and the time of permanence in the open and closed arms were registered. The compulsive behavior was analyzed with the marble test, where the number of hidden marbles were counted. For the histological alterations in the brain, samples from C57Bl/6, KOB1 and KOB2 with 6, 12 and 18 months old, from a previous work, were used. Autoradiographic analysis for B1 receptors were done using 150 pM de [$^{125}$I]HPP(des-Arg$^{10}$-HOE140 (B1 antagonist) and 250 pM de [$^{125}$I]HPP-HOE140 (B2 antagonist). Non-specific binding were determined with 2µM of the unlabeled peptide. **Results**: Animals with six months of age were considered as a reference and comparisons where performed inside each strain. It was observed a decrease in the behavioral anxiety of 18 months-old KOB1 animals evidenced by the increase in the ratio of time of permanence and entries in the open arm of the elevated plus maze. For the three strains no changes in the compulsivity-like behavior were observed. Autoradiography for B1 receptors showed no labeling in all groups. For the B2 receptors, an increase in the density was observed in KOB1 mice in the cingulate gyrus, external capsule, anterior commissure and corpus callosum in the 12 and 18 months old-animals, when compared to 6 months old-mice. No decrease in the synapses density was observed in the 18 months-old KOB1 mice, different from the other strains. **Conclusions**: It is suggested the participation of the B2 receptor in the modulation of the anxiety-like behavior in the aged mice. Also, the genetic deletion of the B1 receptor leaded to the increase of B2 receptor density in the bundles and tracts associated to the anxiety behavior. **Financial Support**: FAP Santa Casa; FAPESP nº 2013/13656-1. CEUA Santa Casa nº 001/2014.

**02.053 Programming of dopaminergic neurons by neonatal estradiol exposure reduces dopamine transporter expression and amphetamine-induced conditioned place preference in adult female rats.** Selva M[1], Sanguinetti N[1], Silva RA[1], Martínez J[1], Cruz G[1], Andrés ME[2], Renard GM[1], Sotomayor-Zárate R[1] [1]Universidad de Valparaíso – Neurobiology and Brain Plasticity, [2]Pontificia Universidad Católica de Chile – Cellular and Molecular Biology, Faculty of Biological Sciences

**Introduction**: The programming concept is defined as the physiological redirection of an organ or tissue due to an early insult during sensitive developmental periods. In this context, our laboratory has shown that neonatal exposure to estradiol valerate (EV) increases the amount of dopamine (DA) in brain circuits associated with reward and locomotion in adult rats. Unexpectedly, amphetamine-induced DA release (systemic and intra-nucleus accumbens) is significantly lower in EV-treated females than control female rats. The objectives of this study were to evaluate the expression of the DA transporter (DAT) in dopaminergic neurons of the Substantia Nigra (SN) and Ventral Tegmental Area (VTA), and the expression of conditioned place preference (CPP) to amphetamine in adult male and female rats exposed to EV at postnatal day (PND) 1. **Methods**: Sprague-Dawley rats of both sexes were used. At PND1, they received a single dose of EV (0.1 mg/50 µL s.c. in sesame oil) or an equivalent volume of sesame oil (Control rats). At PND60, both, EV-treated and control rats were randomly assigned for DAT mRNA expression by Q-RT-PCR in SN and VTA or to a seven days CPP protocol. CPP protocol consisted of a pretest day, five days of conditioning with a daily dose of amphetamine (1 mg/Kg i.p.), and a test day. Time spent in the compartment associated with amphetamine is an index of the reinforcing value of the drug. **Results**: **Results** show a significant reduction of DAT mRNA expression in SN and VTA of EV-treated female rats. Consistently with neurochemical results, EV-treated female rats did not express CPP to amphetamine while control females, control males, and EV-treated males showed a significant preference for the amphetamine-associated compartment at the test day. **Conclusion:** These results suggest that neonatal administration of EV does not produce CPP behavior in adult female rats due to reduced expression of the DAT (amphetamine molecular target) in midbrain dopaminergic neurons. **Financial Support and Acknowledgments:** FONDECYT Grant N° 116-0398 to RS-Z **Research Approval:** All experimental procedures were approved by Ethics Committee of Universidad de Valparaíso (N° 001-2016) and the Science Council (FONDECYT) Chile.

**02.054 P2X2 Receptors potentiate the amyloid beta peptide toxicity inducing a synaptic failure and mitochondrial dynamic dyshomeostasis** Fuentealba J[1,2], Barra K[1], Celis T[1], Godoy P[1], Panes J[1], Silva-Grecchi T[1], Fuentes-Villalobos F[3], Castro A[3], Guzman L[1]
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**Introduction:** Alzheimer's Disease (AD) is a neurodegenerative disorder and there are different hypotheses to explain its pathogenic mechanism. The amyloidogenic theory has been studied in depth and it is accepted that the amyloid-β peptide (Aβ) that is generated from the proteolytic processing of the amyloid precursor protein (APP); which also generates the amyloid intracellular domain (AICD), is one of most important agents responsible for AD. The Aβ peptide has many toxic effects such as altering the function and expression of different proteins, and dyshomeostasis of intracellular $Ca^{+2}$ which modifies mitochondrial activity and finally produces neuronal death. Additionally, we and others have demonstrated changes in purinergic P2X receptors (P2XR) and Fe65 (a multidomain adaptor protein) levels which could have an impact on APP processing and Aβ physiopathology. Therefore, our main objective was to evaluate the changes in P2X2R and Fe65 levels in neurons and cell lines after chronic treatment with the Aβ peptide. **Methods**: Using molecular biology, we demonstrated changes in different key proteins related with the toxicity of chronic (24h) soluble $Aβ_{1-40}$ oligomer (SOAβ) treatment. Additionally, immunocytochemistry and confocal techniques were used to study the changes in localization and co-localization of proteins of interest in hipocamppal neuron cultures and cell lines (PC12 and HEK). Also, mitochondrial morphology and distribution were evaluated *in vivo* using site-directed mitochondrial pericam (2mt-per) and super-resolution microscopy. The functional consequences of these alterations were quantified using patch clamp techniques and measuring the spontaneous synaptic activity. **Results**: Our data show that SOAβ treatment (0.5 μM) increases P2X2R expression about 43±16% over the control (in PC12 cells) without significant changes in Fe65 levels. Additionally, we observed a specific interaction between P2X2a isoforms and Fe65 that induce a significant reduction (25% over the control) in PGC-1α. These data are tightly correlated with the changes observed in mitochondrial network morphology and key proteins associated with mitochondrial dynamics (Mfn1 and DRP); specifically with P2X2a instead of the P2X2b isoform. **Conclusion**: Our results suggest that an enhanced P2X2R-FE65 interaction could play a key role for this receptor in the expression levels of the coactivator PGC-1α, and that P2X2R could participate in the potentiation of Aβ toxicity by markedly altering mitochondrial dynamics and biogenesis. Care of animals and the experimental protocols of this study were approved by the Institutional Animal Use Committee of the University of Concepción and conducted according to the ethical protocols established in the Guide for the Care and Use of Laboratory Animals promulgated by the U.S. National Institutes of Health by the National Institutes of Health (NIH) and the National Committee of Science and Technology (CONICYT). This Work Has Been Funded By grants FONDECYT 1161078, 1130747

**02.055 The REM-enhancing ventral pontine reticular area is inhibited by tuberomammillary histaminergic neurons.** Garzon M[1], Diez-Garcia A[1], Gonzalez-Escobar S[1], Nuñez A[1] [1]Universidad Autónoma de Madrid – Anatomía, Histología y Neurociencia

Tuberomammillary nucleus (TMN) within the posterior hypothalamus is the only source for Histamine neurons (HA) in the central nervous system. Histaminergic system is involved in sleep-wakefulness cycle (SWC) regulation through promotion of cortical activation and wakefulness generation. The aim of the present study was to examine in rats (1) the electrophysiological effect of TMN electrical stimulation on neuronal activity of REM sleep-on centers such as the oral pontine reticular nucleus (PnO), and (2) the anatomical presence of immunohistochemically-identified HA axons and HA receptors (H1 and H3) in this area. Procedures were approved by the Ethics Board of the Universidad Autónoma de Madrid and Madrid Regional Government in accordance with the European Communities Council guidelines (2012/63/UE) on the ethical use of animals. Electrical stimulation of the TMN with single pulses elicited orthodromic responses in electrophysiologically characterized PnO neurons. TMN stimulation with pulse trains produced a decrease in PnO neuron activity. This inhibitory effect in PnO was blocked by the systemic (i.p.) administration of the H1 receptor antagonist pirilamine. Immunohistochemical studies showed small varicose HA-immunolabeled axons through the oral pontine tegmentum, including the PnO. H1 and H3 receptors were also identified in PnO. Thus, our results indicate that TMN HA neurons have inhibitory actions on PnO. This suggests that TMN HA projections to PnO could be part of a neuronal network modulating SWC by suppression of REM sleep in PnO while enhancing wakefulness in some other areas, such as the Locus Coeruleus. Synergic TMN actions in REM-suppressing and wake-enhancing areas are likely mechanisms in hypothalamic sleep-wake modulation through the pontine tegmentum. Supported by MINECO BFU2013- 43741-P. Ethical Committee approval ref: PROEX 004/15.

**02.056 Study of depression model in rats treated with corticosterone in the postnatal period.** Viana GKB[1], Araujo EP[1], Mesquita DS[2], Barriga JRM[2], Jucá MM[3], Vasconcelos SMM[3], Honório Júnior JER[2] [1]Unichristus – Enfermagem, [2]Unichristus – Biomedicina, [3]UFC – Farmacologia

**Introduction**: Glucocorticoids are essential for the maintenance of a wide variety of physiological and behavioral processes in animals. However, lasting exposure to glucocorticoids, such as that occurs during periods of chronic stress leads to anxiety and/or depression, the dysfunction and death of hippocampal and striatal neurons. Currently, the study of animal behavior on models of depression, present acute symptoms and do not show all the symptoms of major depression. The aim of this work is to develop an animal model that allow to study depression-like behavior in rats using corticosterone administration. **Methods**: Wistar rats were used (*Rattus norvegicus*) from the Central Animal Laboratory of the University Center Christus for procreation. The rat pups were divided into 2 groups control (CRT), corticosterone (CORT) 20 mg / kg, (ip), each group having an average of 10 puppies of both sexes, all infants were weighed and measured, and isolated from mother after 21 days. The rat pups received two stimuli, the first was the corticosterone administration i.p. between the 15th and the 21st day of born. The second stimulus with stress factors from the 30th day to the 40th day of born. From day 50 to day 60 the animals were assessed using behavioral tests (Open Field, Elevated Plus Maze and Forced Swim). **Results:** The locomotor activity tests showed a decrease in their mobility within the arena in treated animals [CRT 25.6 ± 3.0 (9) and CORT 12.3 ± 1.6 (11)]. The treated animals in the maze tests showed anxiolytic activity when compared to the control [CRT 6.3 ± 2.9 (8) and CORT 18.0 ± 2.8 (8)]. In the forced swimming test, rats treated with corticosterone had depressive activity compared to controls [CRT 88.5 ± 13 (6) and CORT 134.2 ± 11.7 (12)] **Conclusion:** Thus, through this studied model, we can conclude that the animals showed depression and anxiety-like behaviors. However, it's necessary more research to indicate this model as a new model for chronic assessment of depression-like behavior. **Financial support**: CAPES – CNPq. Number of CEUA Approved: 73/2013

**02.057 Discovering the role of vasopressin system in the lateral septum of amphetamine-conditioned male and female rats.** Mendez AM, Bahamondes C, Tapia S, Tobar F, Cruz G, Sotomayor-Zárate R, Renard GM Universidad de Valparaíso – Centro de Neurobiología y Plasticidad Cerebral – Instituto de Fisiología – Facultad de Ciencias

**Introduction:** Research in the neurobiology of addiction has been focused on the effects of drugs of abuse on the reward system. In the last years the lateral septum (LS) has regained importance in this field as a modulatory nucleus of addictive behaviors. LS is a relay station that integrate different brain areas regulating several behaviors. In this sense, our group is interested in how vasopressin (AVP) action on the LS is involved in addictive behavior. The role of vasopressin (AVP) in addictive behavior has not been widely studied. The aims of the present work were to study the effects of amphetamine (AMPH) administration on AVP levels in the LS in conditioned female and male rats and also study the effects of intra-LS AVP microinjection in modulating AMPH-induced conditioned place preference (CPP). **Methods:** Female and male Sprague Dawley rats (55-60 days old) were used according protocols approved by the Bioethical and Biosecurity Committee of Universidad de Valparaíso. We examined the rewarding effects of AMPH with the CPP test. The CPP protocol consisted in a pretest day, four days of conditioning with a daily dose of AMPH (1.5 mg/Kg i.p.), and a test day. Time spend in the compartment associated with AMPH was an index of the reinforcing value of the drug. After the CPP protocol we measured LS AVP levels by Elisa. To examine the role of AVP in AMPH-induced CPP, the animals received AVP intra-LS microinjection (0.1 ng/0.5 uL), 3 min before the administration of AMPH (1.5 mg/kg, i.p.) during a 4-days conditioning phase. **Results:** Our results showed a significant decrease in LS AVP levels in AMPH-conditioned males while no changes were observed in females. However, intra-LS injection of AVP did not alter the expression of AMPH place conditioning. **Conclusion:** Therefore, AMPH treatments could produce an increase in LS AVP release in male rats, which produce a reduction in the AVP tissue levels. **Financial support and Acknowledgments:** FONDECYT Grant N° 111-40065 to GMR. Certificate N° 027-2014 of the Bioethical Committee of Universidad de Valparaíso.

**02.058 Orbitofrontal cortex mediates context-induced relapse to alcohol.** Leão RM[1], Bianchi PC[2], Palombo P[2], Carneiro-de-Oliveira PE[2], Planeta CS[2], Cruz FC[3] [1]ICS-UFBA – Biorregulação, [2]FCFar-Unesp-Araraquara – Farmacologia, [3]Unifesp – Farmacologia

**Introduction**: In humans, exposure to environmental context previously associated with alcohol use often provokes relapse after prolonged withdrawal periods. We examined whether context-induced reinstatement of alcohol seeking is mediated by activation of the orbitofrontal cortex (OFC). **Methods:** 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 (active lever presses: 16.61 ± 2.59, context B and 34.78 ± 6.32, context A; n=14 per group). Context-induced reinstatement of alcohol-seeking was accompanied by increased Fos expression (fos/mm$^2$ - extinction context: 56.60 ± 8.8 n=6 and training context: 112.9 ± 23.6; n=6). To assess a causal role for the OFC in context-induced alcohol seeking, Baclofen+Muscimol ($GABA_{A\ and\ B}$ agonists) were injected into the OFC prior to exposure to alcohol self-administration context or the nondrug (extinction) context. **Results:** Muscimol+baclofen injections into OFC decreased context-induced reinstatement of alcohol seeking (active lever presses: 7.5 ± 3.2, context B-saline; 10 ± 5.3, context B-B+M; 41.1 ± 9.2, context A-saline; 16.3 ± 4.7 context A-B+M). **Conclusion: Results** suggest that OFC is critical for context-induced reinstatement of alcohol seeking. **Financial support:** FAPESP 2014/02296-7 e 2013/24986-2; **Research approval by the Human or Animal Research Ethical Committee:** USP 01/2015.

**02.059 Role of amygdala neuronal ensembles in context-induced reinstatement of alcohol self-administration in rats.** Cruz FC[1], Tavares LC[2], Bianchi PC[3], Palombo P[3], Carneiro-de-Oliveira PE[3], Planeta CS[3], Leão RM[4] [1]Unifesp – Farmacologia, [2]IFSC-USP, [3]FCFar-Unesp-Araraquara – Farmacologia, [4]ICS-UFBA – Biorregulação,

**Introduction:** High rates of relapse to drug use during abstinence is a defining feature of drug addiction. In abstinent drug users, drug relapse is often precipitated by acute exposure to drug-associated environmental context. Previous studies indicate that specific patterns of sparsely distributed neurons, called neuronal ensembles, mediate and likely encode learned associations between drug-associated contexts and drug-taking. In the present study, we investigated whether context-induced reinstatement of alcohol seeking is mediated by activation of neuronal ensembles in the amygdala. **Methods:** Rats were trained to self-administer alcohol (10%) 1 h/day for 16 days; alcohol deliver was paired with a discrete tone-light cue. Subsequently, lever responding was extinguished over 10 days in the presence of the discrete cue in a non-drug context with different sensory features than the drug self-administration context. Rats were then re-exposed to the cocaine-associated context (or the non-drug extinction context as the control condition) and lever-pressing was assessed under the same extinction conditions for 60 min as a measure of alcohol seeking. Neuronal ensembles in the amygdala that were activated during context-induced reinstatement were identified using Fos immunohistochemistry. We also determined the proportion of amygdala neurons expressing Fos during the reinstatement test by double-labeling Fos and the neuron-specific protein marker (NeuN). **Results:** Re-exposure to the alcohol-associated context, but not the non-drug context, increased lever pressing (lever pressing: 22±3, Context A and 7±3, Context B; $p<0.05$). However, Fos-immunoreactivity in the basolateral amygdala was increased in Context A as well as in Context B, (Fos positive nuclei; Basolateral: 124,2±14.99 Context A and 138.88±20.2, Context B). In the central amygdala Fos-immunoreactivity was only increased in context B: (Fos positive nuclei; 81.9±9.11, Context A and 122.01±14, Context B; $p<0.05$). The percentage of neurons activation in Context A and B in central and basolateral amygdala were: 0.4%, and 1.0%; 1.6% and 1.8%, respectively. **Conclusion:** These data suggest that activation of amygdala neuronal ensembles might mediate the extinction and context-induced reinstatement of alcohol self-administration. **Financial support:** FAPESP 2013/24986-2; **Research approval by the Human or Animal Research Ethical Committee:** USP 01/2015.

**02.060 Corticotrophin Releasing Factor (CRF) and Protein Kinase A (PKA) role in hippocampus: anxiety-like behaviors evaluation of mice exposed to elevated plus maze.**
Miguel TT, Bertagna NB, Queiroz RM, Fernandes GJD UFU – Farmacologia

**Introduction:** anxiety disorders involve behavioral reactions of coping several aversive stimuli. Different neurotransmitters and modulators as well as proteins associated to functions, are able to act in different brain limbic areas such as hippocampus. Kinases such as PKA, PKC and PKG are activated by different neurotransmitters and/or modulators. Among them, CRF produces anxiety-like behaviors and one of possible act mechanism is cAMP/PKA pathways activation. **Objective:** evaluation of CRF and PKA role into the hippocampus subnuclei dorsal (DH) and ventral (VH), in mice exposed to the elevated plus maze (EPM). **Methods:** male Swiss mice (Mus musculus) (30-40g, N= 38, aged 5-8 weeks) underwent stereotaxic surgery for cannulae implantation bilaterally in the hippocampus (VH or DH). Four to seven days after, animals received microinjection of: A) vehicle (saline) or CP (CRF1 receptor antagonist, dose: 3.0nmol, into the VH and DH), or B) vehicle or H-89 (inhibitor of PKA, dose: 2 5nmol, only into the VH). After ten minutes, each animal were exposed to EPM for analysis of behavioral responses. Tests were recorded and measures related to anxiety were assessed. Spatiotemporal: percentage of time and entries into the open arms and locomotion index assessed through closed arms entries, and complementary measures: head dipping (protected and unprotected), stretched attend posture (protected and unprotected) and LCE open arm end entries. Data were expressed as mean ± SEM and analyzed by Student's t test (significance $p<0.05$, n=6-8/group). **Results:** intra-VH CP treatment provoked anxiolytic effect through percentage increase of time spent on the EPM open arms [Saline: 20.73 ± 6.85; CP3.0: 38.0 ± 3.05; $t(9)= -2.21$; $p<0.05$] and a strong tendency to increase the percentage of entries in the same arms [Saline: 34.53 ± 5.86; CP3.0: 46.69 ± 3.33; $t(9) = -2.08$; $p=0.06$]. No effects on complementary measures were observed. In DH, there were no significant effects of CP neither percentages of time spent [Saline: 27.07 ± 6.24; CP3.0: 36.11 ± 6.29; $t (9)= -0.86$; $p>0.05$] nor entries in the open arms [Saline: 30.96 ± 5.85; CP3.0: 34.87 ± 3.30; $t(9)= -0.51$; $p>0.05$]. However, unprotected head dipping behavior increased after injection of CP [Saline: 8.40 ± 2.91; CP3.0: 23.67 ± 3.10; $t(9)= -3.03$; $p<0.05$]. Treatment with H-89, accomplished first in the VH because only in this site CP produced effect on spatiotemporal measures, showed an increase in the percentage of time spent [Saline: 27.03 ± 4.49; H-89 2.5: 46.27 ± 4.03; $t(14)= -3.18$; $p<0.05$] and entries in the open arms [Saline: 41.0 ± 6.60; H-89 2.5: 58.85 ± 4.29; $t(14)= -2.26$; $p<0.05$]. Moreover head dipping was less observed in H-89 group [Saline: 15.88 ± 3.08; H-89 2.5: 8.25 ± 1.77; $t(14)= 2.14$; $p<0.05$]. **Conclusions:** results showed CRF seems to participate in the mediation of anxiety-related behavior in VH, but not DH and cAMP/PKA pathway in VH showed also important. Further studies should be performed with the PKA inhibitor and CRF agonist to associate PKA mediation in the CRF action, since this protein may be activated by other neurotransmitters. **Financial Support:** CNPq and UFU. **Animal Research Ethical Committee:** Experiments were approved by CEUA/UFU protocol 100/14.

**02.061 Role of Accumbens core in context-induced reinstatement of alcohol-seeking in rats.** Tavares LC[1], Bianchi PC[2], Leão RM[3], Palombo P[2], Carneiro-de-Oliveira PE[2], Planeta CS[2], Cruz FC[4] [1]USP-São Carlos, [2]FCFar-Unesp-Araraquara – Farmacologia, [3]UFBA – Ciências da Saúde, [4]Unifesp – Farmacologia

In human addicts, relapse is often precipitated by re-exposure to contexts that were previously associated with drug use.The ABA renewal procedure has been used as a preclinical animal model to study relapse to drug seeking induced by context. Here, we assessed the involvement of accumbens core in context-induced reinstatement of alcohol-seeking in rats. Male and Female Long Evans rats were first given home-cage access to 20% ethanol. Next, they were trained to self-administer 10% ethanol in one context (context A).Subsequently, we extinguished their lever-pressing in a distinct Context B.Rats were then tested for relapse to alcohol seeking under extinction conditions in contexts A or B.Neuron activation in accumbenscore during context-induced reinstatement were identified using Fos immunohistochemistry. We also determined the proportion of accumbens core neurons expressing Fos during the reinstatement test by double-labeling Fos and the neuron-specific protein marker (NeuN).Finally, we determined a causal role of accumbens core in context-induced reinstatement of alcohol seeking by using a mixture of muscimol+baclofen (GABAa+GABAb agonists) to inactivate accumbens core neurons prior to context-induced reinstatement testing.Reexposure to the ethanol-associated context reinstated alcohol seeking (active lever presses: 16.61 ± 2.59, context B and 34.78 ± 6.32, context A; n=14 per group) and increased expression of the neural activity marker Fos in accumbens core (fos/mm$^2$ - extinction context: 56.60 ± 8.8 n=6 and training context: 112.9 ± 23.6; n=6). Reversible inactivation of accumbens coreattenuated context-induced reinstatement (active lever presses: 7.5 ± 3.2, context B-saline; 10 ± 5.3, context B-B+M; 41.1 ± 9.2, context A-saline; 16.3 ± 4.7 context A-B+M). Our results suggest that accumbens core contributes to context-induced reinstatementof alcohol-seeking. **Financial support:** FAPESP 2013/24986-2; **Research approval by the Human or Animal Research Ethical Committee:** USP 01/2015.