Session 02 − **Neuropharmacology**

02.001

Role of iNOS in the anxiogenic effect induced by withdrawal from chronic ethanol consumption. Padovan D[1], Silva K[1], Tirapelli CR[2], Padovan CM[1] [1]FFCLRP-USP − Psicologia e Educação, [2]EERP-USP − Farmacologia

Introduction*:* The abstinence from chronic consumption of ethanol (CCE) leads to the development of different pathophysiologies. Among them, changes in the functioning of the central nervous system (CNS) appear to involve processes of neurodegeneretion wich are triggered by inflammatory processes due to CCE. Nitric Oxide (NO) has been considered an important factor in the onset of neurodegenerative disorders and CNS, and mainly synthesized by microglial cells induced by the enzyme nitric oxide synthase (iNOS), which are activated by various inflammatory factors. Furthermore, NO is also involved in the pathophysiology of anxiety disorders, wich can be observed in the CCE abstinence. The objective of this study is to investigate whether inhibition of iNOS in the Dorsal Raphe Nucleus (DRN), one of the structures within the neural substrate of anxiety, is able to attenuate the effects anxiogenics induced by withdrawal of CCE. **Methods:** After 48 hours of abstinence from CCE, the animals were injected intra-DRN of 1400W (selective inhibitor of iNOS; 1nmol/0,2ml) or saline (0,2ml) and were submitted to the Elevated Plus-Maze, analyzing the percentages of frequency (%FA) and time spent (%TA) in open arms (two-way ANOVA followed by Duncan´s test, considering the following factors: treatment (TREATY) and drink consumed (DRINK)). The experimental protocol was submitted and approved by the Ethics Committee on Animal Use (number of protocol: 07.1.992.53.2). **Results:** At %FA, statistical analysis showed no significant effects of treatment (TREATY: $F_{1,32}$=1.78; p>0.05) or drink consumed (DRINK: $F_{1,32}$=0,59; p>0,05). However, there was interaction between beverage consumption and treatment (DRINK x TREATY: $F_{1,32}$=11,20; p<0.05). As for the %TA, there was significant effect of treatment (TREATY: $F_{1,32}$=6,87; p<0.05). Significant interaction between beverage consumption and treatment were also observed (DRINK x TREATY: $F_{1,32}$=5,16; p<0.05) **Discussion:** The anxiety induced by withdrawal from chronic consumption of ethanol involves an increase in NO production by action of iNOS, and the administration of a selective inhibitor of the enzyme inducible nitric oxide synthase in the dorsal raphe nucleus attenuates the effects resulting from alcoholic abstinence. **Financial support:** CAPES

02.002

Role of P2X receptors, glia and gap junction in the modulation of glutamatergic transmission in NTS neurons projecting to RVLM. Accorsi-Mendonça D, Bonagamba LGH, Leão RX, Machado BH FMRP-USP − Physiology

Introduction: The peripheral chemoreflex activation produces autonomic and respiratory adjustments. The NTS is the primary site of peripheral chemoreceptor afferents and NTS neurons projecting to RVLM are related to the sympathoexcitatory component of this reflex. In a previous study we demonstrated that ATP modulates the spontaneous and evoked glutamate release on these NTS neurons. Herein, using a pharmacological and electrophysiological approach, we evaluated the subtypes of purinergic receptors involved with this modulation. **Methods:** The experimental protocols were approved by the Institutional Ethical Committee on Animal Experimentation of FMRP-USP (Protocol # 12/02/2003). Adults Wistar rats received microinjection of DiI tracer into RVLM and 24 hours later the labeled NTS neurons projecting to RVLM were identified in transversal brainstem slices. Spontaneous and TS evoked excitatory pos-synaptic currents (sEPSCs and TS-eEPSCs) were recorded using whole cell patch clamp. **Results:** PPADS (100 μM, P2X1/2/3/5/7 receptor antagonist) decreased the frequency of sEPSCs (control: 3.39 ± 0.1 vs PPADS: 1.21 ± 0.1 Hz, n=7), but did not change the amplitude or half-width of events. TNP-ATP (1 μM, P2X1/2/3/4/7 receptor antagonist) also decreased the frequency of currents (control: 4.3 ± 1.1 Hz vs TNP-ATP: 1.6 ± 0.5 Hz, n=8) and did not alter the amplitude or half-width of events. FAC (1mM), an inhibitor of glia, reduced the sEPSCs (control: 2.1 ± 0.33 Hz vs FAC: 1.2 ± 0.43 Hz, n=7) and Carbenoxolone (CNX), a gap junction blocker, also decreased the frequency of sEPSCs (control: 1.7 ± 0.2 Hz vs CBX: 1.1 ± 0.3 Hz, n=4). PPADS, TNP-ATP FAC and CBX decreased the amplitude of TS-eEPSC (23%, 32%, 42% and 37% respectively). **Conclusions:** These data suggest that gap junction and glia are involved in the endogenous ATP release and also that modulation of glutamate release onto NTS neurons projecting to RVLM is due to the activation of P2X1, P2X2, P2X3 or P2X7 subtypes receptor. **Financial Support:** FAPESP and CNPQ.

02.003

Mechanisms involved in the mediation of pressor effects of L-proline injected in the third ventricle of unanesthetized rats. Lopes-Silva S, Scopinho AA, Corrêa FMA USP – Farmacologia

Introduction: The L-proline (L-Pro) is a nonessential amino acid endogenous, neurotransmitter or neuromodulator candidate in the central nervous system. The administration of L-Pro into the brainstem elicits cardiovascular responses in rats. The third ventricle (3V) is a cavity of the brain that had communication with the hypothalamus and brainstem regions involved in the cardiovascular control, in which it was detected the existence of systems of uptake and release of L-Pro. Our goal was to study cardiovascular effects of injection of L-Pro into the supra medullary areas of unanesthetized rats and the peripheral mechanisms involved in these responses. **Methods:** Guide cannulae were implanted in 3V of male Wistar rats to microinjection of L-Pro. Catheters were implanted into the femoral artery or vein for respectively MAP and HR recording and systemic drug treatments. The experimental protocol nº 018/2010 was approved by ethics commission the this institution. **Results:** Injection of the L-Pro (0.6µmol/0.5 µL) into the 3V caused pressor.(ΔMAP= 26.9±0.97 mmHg, t= 25.4, p<0.001) and bradycardic (ΔHR= -48.3±3.3 bpm; t=10.7; p<0.01, paired Student´s t-test, =6) responses. Systemic pretreatment with the vasopressin antagonist dTyr(CH2)5(Me)AVP significantly reduced pressor (L-Pro before: ΔMAP = +28.2 ±3.7 mmHg and after dTyr: ΔMAP +2.0 ± 1.2 mmHg; t= 6.4, p<0.05) and bradycardic responses (L-Pro before: ΔHR= -41.8±4.7 bpm and after dTyr: +3.8±3.7 bpm; t= 7.2, p<0.05, paired Student´s t-test; n=6) to microinjection L-pro into the 3V. Systemic pretreatment with pentolinium significantly potentiated the pressor response (L-pro before: ΔMAP= 21.6 ± 2.2 mmHg and L-Pro after: ΔMAP= 41.3 ± 4.5 mmHg, t= 3.9, p<0.01, n= 8), and blocked the bradycardiac response (L-pro before: ΔHR= -43.1 ± 4.3 bpm and L-Pro after pentolinium: ΔHR= -4.3 ± 3.4 bpm; t=7.1, p<0.01, paired Student's t-test) to the microinjection of L-Pro into 3V. **Conclusion:** The present results indicate that the L-Pro administration in the 3V evoked pressor and bradycardic responses mediated by acute vasopressin release into the systemic circulation. **Financial support:** CNPq, CAPES and FAEPA.

02.004

Central nitric oxide synthase inhibition after 3-amino-1,2,4-triazole into the fourth cerebral ventricle influences parasympathetic response to increase in arterial pressure in spontaneously hypertensive rats. Abreu LC[1], Valenti VE[2], Ferreira C[2] [1]FMABC − Morfologia e Fisiologia, [2]UNIFESP − Cardiologia

Introduction: It was previously demonstrated that exogenous catalase influences neural control of cardiovascular system. Nitric oxide (NO) also modulates autonomic regulation of cardiovascular system; however, we do not know yet if there is any interaction between these substances regarding baroreflex sensitivity (BRS) regulation. Thus, we aimed to evaluate the effects of central catalase and NO inhibition on BRS in conscious spontaneously hypertensive rats (SHR). **Methods:** All procedures were approved by the Ethics Committee in Research of our University (Protocol number 003/2008).We used male Wistar Kyoto rats (WKY) and SHR (16 weeks old), which were implanted with a stainless steel guide cannula into the fourth cerebral ventricle (4^{th}V). The femoral artery and vein were cannulated for mean arterial pressure (MAP) and heart rate (HR) measurement and drug infusion, respectively. After basal MAP and HR recordings, the baroreflex was tested with a pressor dose of phenylephrine (PHE, 8 µg/kg, bolus) and a depressor dose of sodium nitroprusside (SNP, 50 µg/kg, bolus). Baroreflex was evaluated before (control), 5, 15, 30 and 60 minutes after 3-amino-1,2,4-triazole (ATZ, catalase inhibitor, 1 µg/100nL) injection into the fourth cerebral ventricle (4^{th} V), 65 minutes after ATZ injection, L-NAME (NO synthase inhibitor, 10 nmol/µL) was injected into the 4th V and BRS was tested 70, 85, 100 and 130 minutes after ATZ injection into the 4th V (Protocol 1), which represents 5, 15, 30 and 60 minutes after L-NAME injection into the 4th V. The sequence of drugs injection into the 4^{th} V was reversed in Protocol 2. Analyses of variance (ANOVA, one way) for repeated measures followed by the Tukey post test were used for comparisons of the variables. Differences were considered significant when the probability of a Type I error was less than 5% ($p< 0.05$). **Results:** Vehicle treatment did not change BRS responses with respect to WKY and SHR groups. Protocol 1 presented significantly increased bradycardic responses at 130' (-100.7±3.9bpm) compared to 0' (-70±6bpm), 30' (-54.17±6.9bpm) and 60' (-75.17±9bpm) in WKY rats. In relation to SHR group protocol 1 presented significantly increased bradycardic responses at 130' (-79.7±3.9bpm) and 100' (-77.7±3.9bpm) compared to 0' (-44±6bpm). No significant alterations were observed regarding protocol 2 in WKY and SHR groups. **Discussion:** We observed that NO inhibition after catalase inhibition improved parasympathetic responses to increase in arterial pressure. We speculate that in an increased H_2O_2 condition, BH_4 would be consummated due its antioxidant property and, consequently, "uncoupled" NOS would produce superoxide anions and impair baroreflex components. L-NAME could be an alternative to reduce oxidative stress in this situation. In conclusion, central NOS inhibition acutely improves BRS after acute catalase inhibition into the 4^{th} V. **Grants:** FAPESP.

02.005

Physical exercise reduces motor alterations associated to dopamine receptors imbalance in neurointoxicant models of Parkinson's disease. Aguiar-Jr AS[1], Boemer G[1], Rial D[1], Matheus FC[1], Moreira ELG[1], Da Cunha C[2], Prediger RD[1] [1]UFSC − Farmacologia, [2]UFPR − Farmacologia

Introduction: Parkinson's disease (PD) is the most prevalent motor neurodegenerative disease. Dopamine-replacement therapy has dominated the treatment of PD since the early 1960s and although the currently approved antiparkinsonian agents offer effective relief of the motor deficits, they have not been found to alleviate the underlying dopaminergic neuron degeneration, and drug efficacy is gradually lost. Over the last decade, regular physical exercise has been implicated to an increased plasticity in the adult brain and it might also afford protection against dopaminergic neurons death and motor dysfunction elicited in experimental models. **Methods:** Male C57BL/6 mice were assigned to two groups: untrained sedentary and runners, using a moderate-intensity treadmill during 6 weeks. The animals were treated with the neurotoxins 6-OHDA (4 mg), injected into the right midstriatum (anterior 0.4, lateral 1.8, ventral 3.5), or MPTP (65 mg/kg) that was administered intranasally (Prediger et al, Neurotox Res, 17, 114, 2010) or their respective vehicle 48 h after the end of physical program, and 24 h to 4 weeks later they were tested in a battery of behavioral paradigms for the evaluation of motor performance. **Results and Discussion:** In the striatal 6-OHDA model, apomorphine treatment (0.6 mg/kg, s.c.) induced a progressive rotation in sedentary animals, which was not observed in the 6-OHDA trained mice, suggesting a reduced dopamine receptors sensitization in trained mice. Moreover, MPTP-treated groups also showed dopamine receptor sensitization as indicated by a marked increase in climbing behavior induced by a low dose of apomorphine (0.2 mg/kg, s.c.) that was prevented by exercise. Indeed, exercise prevented the catalepsy induced by haloperidol (0.32 mg/kg, i.p.) in MPTP-treated mice. These effects seem not be related to neuroprotective actions of exercise since it did not prevent the MPTP-induced reduction in the levels of dopamine and enzyme tyrosine hydroxylase in the striatum. However, exercised MPTP-treated mice presented decreased striatal DA turnover in comparison to sedentary MPTP-treated mice. Taken together, the present findings suggest that physical exercise can reduce behavioral alterations associated to dopamine receptors imbalance in neurointoxicant models of PD and that this response is independent of the neuroprotective effects frequently associated to exercise benefits. **Financial support**: FAPESC, CAPES and CNPq. This project was approved by the UFSC research ethical committee, protocol CEUA-PP357.

02.006

B_1 and B_2 kinin receptors antagonists modulate the bladder overactivity induced by spinal cord injury in rats. Forner S, Andrade EL, Martini AC, Bento AF, Medeiros R, Koepp J, Calixto JB UFSC − Farmacologia

Objectives: Overactive Bladder (OAB) resulting of spinal cord injury (SCI) is a syndrome characterized by the presence of involuntary contractions of the detrusor muscle during the storage phase and urinary bladder-urethral sphincter dissinergy. Kinins are endogenous peptides present in the urinary bladder (UB), however its role in the OAB after SCI in rats until remains to be elucidated. **Methods and Results**: All procedures were approved by our Institutional Ethics Committee (number 016/CEUA/PRPe/2008. Procedure PP00158). Adult male Wistar rats were anesthetized and the spinal cord injured at 10^{th} thoracic vertebrae by means of an embolectomy catheter. Our findings indicated that SCI induces urinary bladder hypertrophy, increase in IL-1β and IL-6 levels in the 2^{nd} day, but not in the 7, 14 and 28^{nd} days after surgery. Functional studies show that the exposure of naïve and sham UBs to B_2 agonist bradykinin (BK, 0.0001-10 μM), but not to B_1 agonist des-Arg^9-BK (DABK, 0.0001-10 μM) resulted in a concentration-dependent contractile response (CCR). This contractile response was significantly potentiated by the SCI and markedly reduced by the selective B_2 receptor antagonist HOE-140 (30 nM) in 90 ± 2.4%. Interestingly, after SCI, there is a slightly DABK-induced contraction that was inhibited by the B_1 selective antagonist des-Arg-Leu^8-BK (DALBK) (30 μM)(28 ± 12.5%). The real time mRNA level for B_1 and B_2 receptors was assessed in UB, dorsal root ganglion (DRG, L6-S1) and the corresponding segment of the spinal cord from naïve, sham-operated and SCI animals 2, 7 and 14 days after surgery. Following SCI the B_1 mRNA level in UB and DRG were significantly increased on the 2^{nd} day, while in the spinal cord was on the 7^{th} day after surgery. In contrast, there was no significant alteration in B_2 mRNA expression in UB, DRG and spinal cord. The protein expression for B_1 and B_2 receptors assessed by western blot in UB showed an increase of B_1 protein expression in the SCI group on the 7^{th} day and B_2 protein expression on the 14^{th} day after surgery when compared to its respective sham groups. Finally, cystometrics studies on SCI animals revealed that systemic Hoe 140 treatment significantly reduced the amplitude and the number of non-voiding contractions (NVCs) while systemic treatment with DALBK only reduced the number of NVCs. There were no significant changes in basal pressure, threshold pressure, maximum voiding pressure, voided volume and voiding efficiency before administration of vehicle, HOE 140 or DALBK. **Conclusion:** Taken together, these results suggest that upregulation of BK receptors are involved in the mechanism inducing OAB in SCI rats and that a BK antagonist can modulate SCI-induced OAB as indicated by a reduction in NVCs. Thus, selective kinin receptor antagonists could be a relevant therapeutic target for OAB.

02.007

Involvement of muscarinic receptors of subtype M2 in the cardiovascular responses of acetylcholine microinjected into lateral periaqueductal gray area of unanesthetized rats.

Deolindo MV, Corrêa FMA FMRP-USP Farmacologia

Introduction: The periaqueductal gray area (PAG) is a mesencephalic region that is involved in the modulation of cardiovascular changes associated with behavioural responses. Among the neurotransmitters present in the PAG, acetylcholine (Ach) is also known to be involved in central cardiovascular control. In the present study, we attempted the cardiovascular effects of the microinjection of Ach into the lateral portion of the PAG (lPAG) and participation of muscarinic receptors in this response in rats. **Methods:** Male Wistar rats were used. Guide cannulas were implanted in the lPAG for drug injection and a polyethylene catheter was implanted in the femoral artery for arterial pressure (AP) and heart rate (HR) recording using a computerized acquisition system. **Results**: The injection of Ach (45nmol/ 50nl) into the lPAG of unanesthetized rats caused depressor responses accompanied by tachycardia. This responses was blocked dose related with injection of different doses of the 4-DAMP (1, 3, 9nmol/50nl) into lPAG in unanesthetized rats.

Conclusion: The present results indicated that activation of cholinergic receptors within the vlPAG evoked cardiovascular responses and that such responses were blocked with atropine and 4-DAMP.Suggesting the involvement of muscarinic receptors of subtype M2 in the cardiovascular responses of acetylcholine microinjected into lPAG of unanesthetized rats. N approved protocol by CETEA :168/2007

02.008

Intra-bed nucleus of the stria terminalis cannabidiol administration alters cardiovascular changes to acute restraint stress through 5-HT1A receptors. Gomes FV, Crestani CC, Alves FHF, Guimarães FS, Corrêa FMA, Resstel LBM FMRP-USP − Farmacologia

Introduction: Systemic administration of cannabidiol (CBD), a non-psychotomimetic compound from *Cannabis sativa*, is able to attenuate cardiovascular and behavioral responses to restraint stress through the activation 5-HT1A receptors. Despite the brain structures involved in the CBD effects remain poorly understood, previous results from our group suggest that the bed nucleus of the stria terminalis (BNST) could be involved in the antiaversive effects of the CBD. Moreover, it has been proposed that synapses within the BNST influence restraint-evoked cardiovascular changes, in particular by an inhibitory influence on the tachycardiac response associated to restraint stress. Thus, the present work investigated the possible involvement of BNST in CBD effects on cardiovascular changes induced by acute restraint stress and if these effects would involve the local 5-HT1A receptors activation. **Methods:** Male Wistar rats (240-270g, n=5-6) with cannulae implanted bilaterally into the BNST received intra-BNST microinjections, 100nL each side, of vehicle (grape seed oil) or CBD (15, 30 or 60 nmol). A catheter was implanted in the femoral artery to record mean arterial pressure (MAP) and heart rate (HR). Ten minutes after the microinjection of drugs, the animals were submitted to the restraint stress for 60 min. In the second experiment the animals received microinjections of the 5-HT1A receptor antagonist WAY100635 (WAY; 0.37 nmol) 5 min before CBD (30 nmol) treatment. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 88/2009). MAP and HR were analyzed using two-way ANOVA with treatment as the main independent factors, and time as a repeated measurement. **Results:** The microinjection of CBD into the BNST did not affect baseline values of MAP or HR compared with the vehicle group. The exposition to restraint stress increased both MAP [$F(29,390)=18.05$; $P<0.0001$] and HR [$F(29,390)=107.7$; $P<0.0001$]. The microinjection of 30 and 60 nmol of CBD significantly enhanced the restraint-evoked HR increase [$F(3,390)=229.1$; $P<0.0001$], in a dose-dependent manner [$r^2=0.91$, $df=10$, $P<0.05$], without significantly affecting the MAP increase [$F(3,390)=2.45$; $P>0.05$]. WAY by itself did not change the cardiovascular responses to restraint stress, but blocked the effects of CBD (30 nmol). **Conclusion:** These results showed that CBD microinjected into the BNST enhanced the HR increase associated with acute restraint stress without affecting the MAP increase. Although these results are not in agreement with those observed after systemic administration of CBD, they are similar the effects observed after reversible inactivation of the BNST. However, in both situations (systemic and intra-BNST administration) the CBD effects seems to involve 5-HT1A receptor-mediated neurotransmission. **Financial support:** CNPq, FAPESP, CAPES and FAEPA.

02.009

Effect of LASSBio-767 on apoptosis and inhibitory synaptic transmission in neurons. Vieira KST[1], Fraga CAM[2], Barreiro EJ[2], Bolzani V[3], Castro NG[1] [1]ICB-UFRJ − Farmacologia Molecular, [2]FF-UFRJ − LASSBio, [3]NUBBe-UNESP-Araraquara − Química Orgânica

Introduction: The prevalence of Alzheimer's disease (AD) increases gradually with age and the proportion of elderly in the Brazilian population is increasing rapidly, therefore studies about that condition and its treatment are important. LASSBio-767 is a promising candidate drug for the symptomatic treatment of AD, already characterized as an acetylcholinesterase inhibitor with central action (Castro et al., *Eur. J. Pharmacol.,* 580:339, 2008), which also has antimuscarinic effects (Pimentel et al., *Anais do 41º Congresso da SBFTE,* p. 02.002, 2009). Some cholinesterase inhibitors also have neuroprotective properties (Takada-Takatori, Y. et al., *Biol. Pharm. Bull***.**, 32:318-324, 2009; Nordberg et al., *Alzheimer Dis. Assoc. Disord*., 20: 12-18, 2006), which may be beneficial in AD. The objective was to search for a neuroprotective activity of LASSBio-767 and an effect on central inhibitory synapses, which might occur independent of inhibition of acetylcholinesterase. **Methods:** The protective activity was examined in an experimental model of apoptotic cell death induced by staurosporine in rat cortical neurons. Treatment with LASSBio-767 was performed for 48 h with staurosporine. The death was assessed by three tests: lactate dehydrogenase release, resazurin reduction and staining with Hoescht 33342 for the observation of apoptotic nuclei. The effect on the central inhibitory synapses was observed by electrophysiological recordings of miniature inhibitory postsynaptic currents in neurons of rat hippocampus. All procedures with animals were approved by the institutional committee (protocol DFBCICB 029). The membrane currents were recorded by patch-clamp technique in whole-cell mode, with membrane potential set at -80 mV in the presence and absence of LASSBio-767. **Results:** In tests of protection, LASSBio-767 1 microM and 10 microM did not attenuate cell death induced by staurosporine. However, in the concentration of 100 microM there was a significant potentiation of death compared to the group treated only with staurosporine. The release of LDH was 164.1 ± 9.0% larger and the resazurin reduction was 63,3 ± 24,6% smaller in the group treated with LASSBio-767 ($p < 0.01$, ANOVA and Tukey's test, in 3 experiments). In electrophysiological recordings, LASSBio-767 10 microM increased the frequency of synaptic events in 3 neurons, decreased in 2 and in 4 had no significant effect ($p < 0.05$, Mann-Whitney test). **Discussion:** LASSBio-767 showed no protective activity in the models of apoptotic cell death by staurosporine. However, this does not exclude a possible neuroprotective effect in other types of injury, which are worth investigating. Furthermore, electrophysiological results showed a tendency of the drug to act by presynaptic mechanisms, modulating the release of the neurotransmitter GABA. Further experiments are required to clarify whether this effect is direct, by muscarinic receptors, or through another mechanism. **Support:** CNPq, Finep-MCT and Apsen Farmacêutica.

02.010

High and low rearing rats selected in the open field differ in the binding of [^{3}H]RO 15-4513 to the limbic cortex. Alves R, Carvalho JGB, Venditti, MAC UNIFESP − Psicobiologia

Introduction: High (HR) and low (LR) rearing rats differ in the susceptibility to clonic convulsions induced by methyl-6,7-dimethoxy-4-ethyl-b-carboline-3-carboxamide (DMCM), a benzodiazepine inverse agonist and differ in the binding of [^{3}H]Flunitrazepam, a benzodiazepine agonist, to the hippocampus. The aim of this study was to verify if LR and HR subgroups differ in the binding of [^{3}H] RO 15-4513, a benzodiazepine inverse agonist, to the brain regions (frontal cortex, striatum, brainstem, limbic cortex and hippocampus). **Methods:** Naive adults (3 months old), male Wistar rats were submitted to a 3-min session in the open field test. Ambulation and the total number of rearings were scored. Those rats showing ≤9 rearings were selected as the LR (6.5±0.79, n=10; mean±S.E.M) and those showed ≥27 rearings were selected as the HR (31.5±0.91, n=10; mean±S.E.M.). Twenty days after the open field session, the rats were sacrificed by decapitation, the brain regions were dissected, weighed and kept frozen (-20°C) until the preparation of the homogenates. The homogenates (2.5% W/V) were prepared in 0.32 M sucrose. Total [^{3}H] RO 15-4513 binding was carried out at one concentration of the ligand: 5.3 nM. Non-specific binding was determined in the presence of DMCM (10 µM concentration in the assay). The protein concentration was determined according to Lowry et al. (1951) using bovine serum albumin as standard. The data was statistically analysed using the unpaired Student's t test with a significance level set a $p \leq 0.05$, two-tailed. This work was approved by our institution ethics committee (proc. 1023/06). **Results:** The results obtained showed a statistically significant difference in [^{3}H] RO 15-4513 binding between LR and HR subgroups in the limbic cortex (LR: 1225.9±32.4; fmol/mg protein, mean±S.E.M., n=9; HR: 1006±69.8, n=8; $t_{(d.f.=15)}=2.95$, p=0.01, unpaired Student´s t test). There were no statistically significant differences between the subgroups in the binding of all the other brain regions. **Discussion:** Our data suggest that the innate inter-individual differences in exploratory behavior is influenced by inhibitory GABA neurotransmission through the $GABA_A$/BZD site receptor and that this difference may be due to different affinities for specific sites of the receptor complex, as well as differences in their respective distributions. **Reference:** Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Bio Chem*, 1951; 193:265-75. **Support:** FAPESP and AFIP.

02.011

LASSBio-579 prevents hyperlocomotion induced by ketamine A behavior suggestive of atypical antipsychotic activity. Antonio CB[1], Betti AH[1], Neves G[1], Hasse DR[2], Barreiro EJ[3], Fraga CAM[3], Rates SMK[1] [1]UFRGS − Ciências Farmacêuticas, [2]FF-UFRGS − Psicofarmacologia Experimental, [3]FF-UFRJ − LASSBio

Introduction: LASSBio-579 is a *N*-phenylpiperazine derivative, planned through molecular hybridization between clozapine and L-741 (Menegatti *et al., Bioorg Med Chem* 11(22):4807, 2003), that is active in an animal model predictive to schizophrenia positive symptoms (apomorphine-induced climbing) and present a multirecptor profile (binds to D_2-*like*, 5-HT_{1A} and 5-HT_{2A} receptors), characteristic of modern atypical antipsychotics (Neves *et al., Bioorg Med Chem* 18:1925, 2010). Atypical antipsychotics represent an advance in schizophrenia treatment and their main advantages include better tolerability, especially regarding extrapyramidal symptoms, efficacy in a wider range of symptoms (Volavka *et al., Am J Psychiatry* 159:255, 2002) and increase in quality of life (Karow and Naber, *Psychopharmacology* 162:3, 2002). In rodents, N-methyl-D-aspartate (NMDA) receptor antagonists' administration, such as ketamine, produces an increase on locomotor activity. This behavior has been used as an animal model to evaluate atypicality of potential new antipsychotics since ketamine-induced hyperlocomotion can be blocked by atypical but not typical antipsychotics at doses that does not impair the spontaneous locomotor activity (Satow *et al. JPET*, 330:179, 2009). The aim of this work was to evaluate the dose-response relation of LASSBio-579 in the apomorphine-induced climbing behavior; and to investigate a possible atypical profile on the hyperlocomotion model. **Methods:** Male CF1 mice from FEPPS-RS breeding colony were used. All experimental protocols were approved by UFRGS Ethical Committee (protocol 2008220). *Apomorphine-induced climbing* – Animals were treated with saline (1 mL/100g p.o.) or LASSBio-579 (1, 5, 15 or 30 mg/kg p.o.) 30 minutes before the apomorphine administration (4 mg/kg s.c.) or saline (0.5 mL/100g s.c.). The climbing behavior was observed for a period of 30 minutes. *Hyperlocomotion* – Animals received saline (1 mL/100g p.o.), haloperidol (0.01mg/kg p.o.), clozapine (1 mg/kg p.o.) or LASSBio-579 (5 mg/kg p.o.) and 30 minutes later were treated with ketamine (10 mg/kg i.p.) or saline (1.0 mL/100g p.o.). The number of crossings was registered for 20 minutes. **Results and Discussion:** The LASSBio-579 minimal dose able to revert climbing behavior was 5 mg/kg (SAL + SAL 8.167 ± 0.92; SAL + APO 17.538 ± 0.386; L579 1mg/kg + APO 14.455 ± 1.246; L579 5mg/kg + APO 10.625 ± 1.253; L579 15mg/kg + APO 11.5 ± 1.939; L579 30mg/kg + APO 12.125 ± 2.03). At the same dose it blocked significantly (ANOVA one way; $p < 0.001$) ketamine-induced hyperlocomotion (SAL + SAL 262.5 ± 35.8; SAL + KET 641.2 ± 44.6; L579 5 mg/kg + SAL 219.9 ± 32.6; L579 5mg/kg + KET 347.1 ± 44.2). These results demonstrate that LASSBio-579 has a potential atypical antipsychotic profile. **Financial Support:** CAPES, INCT-IM-INOFAR, CNPq.

02.012

Medial prefrontal cortex CB1 receptors are involved with modulation of the baroreflex in rats. Ferreira Junior NC, Alves FHF, Fedoce AG, Corrêa FMA, Resstel LBM FMRP-USP − Farmacologia

Introduction and goals: There are evidences indicating that local neurotransmission present in ventral portion of medial prefrontal cortex (vMPFC) modulates the baroreflex activity. Furthermore, NMDA subtype glutamate receptors are involved in the vMPFC-related modulation of the parasympathetic component of the baroreflex. Cannabinoid CB_1 receptor expression is considerably high in the vMPFC and these receptors can modulate postsynaptic response mediated by glutamatergic NMDA receptors. Thus, the aim of the present study was to verify the involvement of the CB1 receptors within vMPFC on the modulation of the baroreflex activity. **Methods:** Male Wistar rats weighing between 240-280 g had guide cannulae bilaterally implanted into the vMPFC. A catheter was implanted posteriorly into the femoral artery for mean arterial pressure (MAP) and heart rate (HR) recording. Baroreflex activation was induced by infusion of phenylephrine or sodium nitroprusside through a catheter implanted into the femoral vein. The animals received 200 nL of vehicle, each side of vMPFC, or different doses of CB1 receptor antagonist AM251 (10 pmol, 100 pmol or 300 pmol/ 200 nL) into the vMPFC. The Institution´s Animal Ethics Committee approved the housing conditions and experimental procedures (process number: 167/2007). **Results:** All tested doses of AM251 did not affect baseline of either MAP or HR. The dose of 10 pmol of AM251 had no effect on the slope of the linear regression generated 10 min after its administration to both bradycardiac responses (-1.78±0.2 vs -1.93±0.2 bpm/ mmHg; $F=0.36$, $P>0.05$) and to tachycardic responses (-2.7±0.3 vs -2.6±0.6 bpm/ mmHg; $F=0.04$, $P>0.05$). However, 100 pmol of AM251 was able to increase the slope of linear regression of both bradycardiac responses (-1.30±0.1 vs -1.77±0.1 bpm/ mmHg; $F=4.6$, $P<0.05$) and tachycardic response (-2.3±0.4 vs -3.7±0.2 bpm/ mmHg; $F=7.8$; $P<0.05$). Finally, the dose of 300 pmol of AM251 increased only the slope of linear regression of bradycardic responses (-1.7±0.2 vs -2.4±0.3 bpm/ mmHg; $F=4$; $P<0.05$) without change the slope of linear regression of tachycardic responses (-2.7±0.6 vs -2.7±05 bpm/ mmHg; $F=0.04$; $P>0.05$). The effect of AM251 was reversible and the baroreflex activity returned to basal values after 60 min in all doses utilized. **Discussion:** The present findings show that the CB_1 receptors activation within vMPFC has an inhibitory influence on both parasympathetic and sympathetic compound of baroreflex. Moreover, this influence could involve differently these two compounds of baroreflex. **Financial support:** FAPESP, CNPq, CAPES and FAEPA.

02.013

Putative role of Bradykinin (BK) in cognitive deficits in rats. Dong KE[1], Amaral FA[1], Lemos MTR[1], Caetano AL[1], Buck HS[1], Viel TA[2] [1]FCMSCSP − Ciências Fisiológicas, [2]EACH-USP

Introduction: Kinin effects are mediated by the activation of B1 (B1R) and B2 (B2R) receptors. The B2R is preferentially activated by Bk while B1R is activated by des-Arg9BK, a BK metabolite. Whereas B1R is barely detected in most brain regions, B2R is extensively distributed. In closed head trauma it was observed a slight increase of B1R and a decrease of B2R in several brain regions. Also, increased BK concentration in cerebrospinal fluid and increased kinin receptors in brain areas related to cognitive processes where observed after neurodegeneration induced by brain infusion of amyloid-beta peptide. The aim of this work was to evaluate the role of BK on learning, memory consolidation of short and long term memory and memory evocation. **Methods:** Under anesthesia, 2 guide cannulae were bilaterally implanted 1 mm above the hippocampus of male Wistar rats (200-300g; n= 6 to 10 in each group) for the injection (1mL) of artificial cerebrospinal fluid (CSF) or BK (300 pmol). Cognitive and motor activity tests were performed 5 days after the surgery by an inhibitory avoidance apparatus (0,5 mA, 2 sec, maximum 300 sec) and by an activity cage (5 min of observation before the training session), respectively. For effects on learning, BK or CSF were injected 5 min before the acquisition session (AS) and memory evaluated 90 min after that (test session: TS). For short and long term memory evaluation, BK or CSF were injected just after the AS and memory evaluated 90 min or 24 h later, respectively. For memory evocation, BK or CSF were injected 5 min before the TS (90 min later). To determine on which kinin receptor BK is acting, B1 antagonist des-Arg10-Hoe140 or B2 antagonist Hoe140 were injected 5 min before BK injection at equimolar concentrations. Differences were considered statistically significant when $p<0.05$. **Results:** In all the groups injected with CSF a significant increase in latencies to shock was observed, indicating that vehicle injection did not change the cognitive parameters studied. In the same way, significant increases in latencies (in seconds) to shock where observed when BK was injected before the training session, (AS=23.6 [15.1/111.5]; TS=32.5 [25.1/300.0]), in long term memory test (AS=14.6 [11.7/18.8]; TS=36.7 [17.3/243.3]), and memory evocation test (AS=43.2 [15.8/91.7]; TS=228.4 [25.1/298.0]). However, animals injected with BK pos-training did not show significant increases in latencies to shock 90 min (21.3 [11.8/52.2]) after the AS (10.5 [6.6/19.3]). This effect of BK (impairing short term memory) was abolished by previous injection of B1 antagonist (AS=26.8 [15.9/61.6]; TS=298.0 [177.3/298.0]) but not by B2 antagonist (AS=28.4 [20.1/160.5]; TS=56.0 [24.8/194.5]). This was not due to changes in locomotion performance, once no difference was observed among groups. **Discussion:** We suggest that injection of BK in hippocampus did not interfere with learning process, long term memory formation and memory evocation, but caused impairment in short term memory consolidation by the activation of B1R. Considering that increases in expression of B1R and release of BK occur during tissue lesions, this peptide could be associated to cognitive deficits related to neurological lesions. Protocol CEEUA-FCMSCSP nº155. **Financial Support**: FAPESP, CNPq.

02.014

Inhibitory influence of lateral hypothalamus neurotransmission in the cardiac response to fear conditioning to context. Reis DG, Deolindo MV, Guimarães FS, Corrêa FMA, Resstel LBM FMRP-USP

Introduction and goals: The lateral hypothalamus (LH) is a limbic structure involved with both autonomic and behavior modulation during defensive responses. In rats, contextual conditioned fear evokes both freezing immobility and cardiovascular changes, mean arterial pressure (MAP) and heart rate (HR) increases, which are accompanied by LH activation. However, the LH role on autonomic and behavior responses associated to fear conditioning to context has not been fully studied. **Material and Methods**: Male Wistar rats (250g) had guide cannulae bilaterally implanted in the LH for microinjection, 200nL each site, of 1mM of non-selective synapse blocker $CoCl_2$ or vehicle 10 min before the chamber re-exposition (test session). In the conditioning session, the animals were divided into two groups. The conditioned group received 6 foot electrical shock (1.5 mA, 3 s). The Non-conditioned group was placed into the shock chamber but no shock was given. After the conditioning session a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. The Twenty four hours later the animals were submitted to the test session where the freezing immobility and the cardiovascular parameters were registered. The MAP and HR values were continuously recorded in the 5-min-period before (baseline) and during the 10 min test period. MAP and HR changes in the baseline and test periods were analyzed using a repeated measure multivariate analysis of variance, with condition (non-conditioned or conditioned animals) and treatment ($CoCl_2$ or vehicle) as main factors and time as a repeated measurement. When significant interactions between the factors were observed, specific comparisons in each group were made by two-way analysis of variance and Bonferroni's post-hoc test. Freezing was expressed as the percentage of the total test period (10 min). Freezing was analyzed using two-way analysis of variance, with condition (non-conditioned or conditioned animals) and treatment ($CoCl_2$ or vehicle) as main factors. When significant interactions between the factors were observed, specific comparisons in each group were made by analysis of variance followed by the Bonferroni's post-hoc test. The significance level was set at $P<0.05$. **Results:** The administration of $CoCl_2$ in the LH had not effects on behavioral responses in both conditioned (n=6) and non-conditioned (n=6) groups ($F_{1,20}=0.15$, $P>0.05$). However, the non-conditioned group (n=6) which received $CoCl_2$ presented a significantly increase in both (MAP: $F_{1,150}=9.91$, $P<0.05$ and HR: $F_{1,150}=40.42$, $P<0.05$). The conditioned group (n=6) presented no significantly changes in (MAP: $F_{1,150}=2.8$, $P>0.05$) but presented a significantly increase in (HR $F_{1,150}=40.2$, $P<0.0001$).

Conclusion: These results suggest that the LH neurotransmission have an inhibitory role on the cardiovascular response without affect the behavioral response observed during re-exposure to the aversive context. **Approval of the Ethics Committee**: 139/2008. **Financial support**: FAPESP and FAEPA.

02.015

Evaluation of the effect of a *Hypericum polyanthemum* cyclohexane extract in an animal model of Parkinson disease induced by 6-OHDA. Borsoi M[1], Betti AH[2], Batassini C[3], Silvestrin RB[4], Lazzaretti C[1], Pranke M[5], Antonio CB[2], Salles LA[5], Rosa HS[5], von Poser GL[6], Rates SMK[2], Souza TM[3] [1]ICBS-UFRGS, [2]UFRGS − Ciências Farmacêuticas, [3]UFRGS − Bioquímica, [4]ICBS-UFRGS − Neurociências, [5]UFRGS − Farmácia, [6]UFRGS − Produção de Matéria-Prima

Introduction: Parkinson´s Disease (PD) is a neurodegenerative disorder characterized by a loss of dopaminergic neurons of substantia nigra (SN) and consequent depletion of dopamine in the striatum. The available treatments are based on the dopaminergic neurotransmission activation but they do not prevent the disease progression demonstrating the importance of researching new therapies. Previous works from our group demonstrated that *Hypericum polyanthemum* extract inhibits the monoamines reuptake and modulates GTP binding in activated dopamine receptors in the striatum of rats. The aim of this study was to evaluate the effect of the cyclohexane extract obtained from aerial parts of *H. polyanthemum* in a PD model induced by 6-hydroxydopamine (6-OHDA). **Methods:** Wistar rats received two infusions of 6-OHDA (5.5 mL, 3 mg/mL) into the right medial forebrain bundle through stereotaxic surgery. The following treatments with the extract (solvent free) ressuspend in saline and polisorbate 80 (2.5%) were performed: (1) three administrations initiated 24 h after injury, (2) three administrations initiated before the behavioral tests, in which the last administration was performed 1 h before testing. In each scheme, the administration (90 mg/kg p.o. each) was performed in a timeframe of 24 h with intervals of 6 h. Controls received vehicle. The assessment of rotational behavior induced by methylphenidate (MF) (20 mg/kg, i.p.) was performed 10, 45 and 85 days after stereotaxic surgery. The animals were tested for sticky tape 62 days after infusion of 6-OHDA. The assessment of tyrosine hydroxylase (TH) content was performed in animals subjected to treatment 1. All protocols used were approved by UFRGS Research Ethical Committee (project number 2007985). **Results and Discussion:** There was an increase in the number of ipsilateral rotations in animals submitted to treatment 1 (Control: 212.16±36.98 and Treated: 384.36±64.99; mean±ED) [repeated measures ANOVA group effect, $F_{(1,13)}$=6.63, p<0.0185]. In the sticky tape test, the group from treatment 1 took longer to see the tape of the contralateral paw (Control − contralateral paw: med 45, 25%: 29, 75%: 17; ipsilateral paw: med 73, 25%: 30.5, 75%: 47 and Treated − contralateral paw: med 23.5, 25%: 12.25, 75%: 96.5; ipsilateral paw: med 120, 25%: 49.75, 75%: 0) [Wilcoxon test, p<0.005]. In addition, was observed a significant reduction in the content of TH in the substancia nigra pars compacta (SNpc) of the animals submitted to treatment 1 (% of lesion − Control: med 83.05, 25%: 34.38, 75%: 97.06 and Treated: 99.58, 25%: 99.54, 75%: 99.95) [Mann-Whitney test, p=0.0034]. The treatment 2 did not produce any alteration. In conclusion, the administration of the extract at the beginning of the 6-OHDA-induced lesion increased the rotational behavior, impaired performance of animals in the sticky tape test and reduced the content of TH in the SNpc, indicating the toxic effect of the *Hypericum polyanthemum* in the animal PD model.

Financial support: CNPq.

02.016

Neuropharmacological profile of parawixin 11, purified from the venom of the social spider *Parawixia bistriata* (Araneae, Araneidae), in Wistar rats. Pereira AC[1], Cunha AOS[1], Fachim H[1], Lopes NP[2], Santos WF[1] [1]FFCLRP-USP − Biology, [2]USP − Physics and Chemistry

Introduction: Our research is focused on the identification and isolation of neuroactive compounds from venomous arthropods, such as spiders. In the present work, we described the anticonvulsant activity of Parawixin11, isolated from the venom of the spider *Parawixia bistriata*, in acute chemically-induced seizures in Wistar rats. In addition, the effects of acute i.c.v. administration of Parawixin11 over motor activity in the open field and rotarod were investigated. Finally, rats treated with Parawixin11 were tested in the Morris water maze, in order to assess deficits in spatial memory. This work was approved by the Ethics Committee for Experimental Animals at the University of São Paulo, Ribeirão Preto Campus (Protocol 08.1.182.53.1). **Methods:** Venom glands were extracted and crushed in a solution of ACN/H_2O (1:1). The extract was filtered in a Millipore filter (cutoff <3000Da), injected in the HPLC and the eluted fractions were tested for anticonvulsant activity. Parawixin11 was then analyzed into a high resolution q-TOF spectrometer. Wistar rats (250g, n=8) were implanted with a guide cannula into the lateral ventricle in order to perform anticonvulsant assays. Experimental animals received i.c.v. injections of Parawixin11 (0.05, 0.1, 0.2µg/µL, 1µL) 10 min before PTZ (85mg/kg- 0.2mL i.p.), strychinine (38µg/µL, 1µL i.c.v.) and pilocarpine (2.4mg/ µL, 1.2µL i.c.v), Parawixin11 (0.1, 0.2, 0.3µg/µL, 1µL) before biccuculine (0.9µg/µL, 1µL i.c.v.), and Parawixin11 (0.003, 0.006, 0.012, 0.025µg/µL, 1µL) before NMDA (17µg/µL, 1µL i.c.v.), whereas control animals received saline (0.9%, i.c.v.) and other group diazepam (3mg/Kg, i.p.). For the open field and Morris water maze tests, rats (n=5) received saline, DZP or Parawixin11 (0.1, 0.2, 0.3µg/µL, 1µL) and for the rotarod test, rats (n= 5) received saline or Parawixin11 (0.2, 0.3 e 0.6µg/µL, 1µL). At the end of the experiments, animals were sacrificed by an anesthetic overdose and perfused through the left ventricle first with saline (0.9%), and then with paraformaldehyde (4%, PBS pH 7.4 at 4°C). After perfusion, the brains were removed, frozen and cut on a cryostat for to check the position of the cannula. Sigmoidal non-linear regression was used to calculate ED_{50} and mean latencies to seizure onset were calculated and tested with One-way ANOVA and Tukey as post test. The spontaneous locomotor activity as well as data from Morris water maze was analyzed by RM-ANOVA. We considered significant values of $p<0.05$. **Results and Discussion:** Our results show that pretreatment with Parawixin11 prevented the onset of seizures induced by all chemo-convulsants in a dose-response manner. However, the most potent effect was observed for NMDA-induced seizures (ED_{50}= 0.01 µg/µL). Also, in lower doses, Parawixin11 significantly increased the latencies to seizures in non-protected animals ($p<0.05$). Parawixin11 was considered well-tolerated as in therapeutic doses. Therefore the RM-ANOVA revealed that it did not cause neurologic alterations in the open Field [$F(4,24)= 2,90$; $p=0,053$] and in the especial memory in Morris water maze [$F(4,20)= 1$, $p=0,431$]. This work has showed that Parawixin11 has an anticonvulsant activity that is probably linked to glutamate receptors, since its ED_{50} in NMDA acid-induced seizures is approximately 10 times lower than in seizures induced by GABA or glycine antagonists. Moreover, Parawixin11 is well tolerated and induces no side-effect in effective doses in rotarod test, open field and Morris water maze. **Financial support:** Fapesp, CNPq and CAPES

02.017

Involvement of serotonergic and dopaminergic neurotransmission in effect of semi-purified constituent from guaraná seeds in the elevated T maze. Roncon CM, Almeida CB, Mello JCP, Audi EA UEL − Farmácia e Farmacologia

Introduction: *Paullinia cupana* (H.B.K. var. sorbilis (Mart.) Ducke), belonging to the family Sapindaceae and popularly known as guaraná, is grown mainly in the central Amazon basin, in Brazil. Guaraná has been popularly used as a stimulant of the central nervous system in cases of intellectual and physical stress. A semi-purified fraction obtained from an extract of guaraná seeds, termed purified extract A (PEA) improved performance and memory speed [1] and produced antidepressant-like effects in rats [2]. The aim of this study was to investigate the involvement of serotonergic and dopaminergic neurotransmissions in the effect of chronic treatment of this semi-purified constituent, PEA, in the elevated T maze (ETM). The ETM is a model of anxiety that´s evokes two defensive responses in the same rat, namely inhibitor avoidance and one-way escape, which has been related to generalized anxiety disorder and panic disorder, respectively [3]. The selective serotonin (5-HT) reuptake inhibitor (SSRI), paroxetine, was used as a positive control. Locomotion was assessed in a circular arena following each drug treatment, as a control for nonspecific motor effects. Ineffective doses of 5-$HT_{2A/2C}$ antagonist receptor, metergoline (2 mg/kg) or of dopaminergic receptor antagonist, sulpiride (20 mg/kg) were associated to effective dose of PEA (8 mg/kg) in the ETM. **Methods:** Male Wistar rats (UEM Ethics Committee (053/2008)), (n=9-15), received repeated oral injections of PEA or paroxetine (3mg/kg) by 24 days. In the last day of treatment, metergoline or sulpiride were acutely administered by intraperitoneal route, 5 min before the PEA or paroxetine. After 60 min of the last injection the animals were exposed to the ETM where inhibitory avoidance (baseline, avoidance 2 and 3) and escape from the open arm (escape 1, 2 and 3) latencies were recorded. Repeated-measures ANOVA was used to analyze the data. When appropriate, one-way ANOVA was applied, followed by Duncan's multiple-range test. **Results and Discussion:** Similar to the SSRI, paroxetine, PEA significantly increased escape latencies (escape 2: VEH+VEH = 9.06±1.64, VEH+PEA = 15.00±2.12 ($p<0.05$), VEH+PAR = 16.66±1.64 ($p<0.01$), indicative of a panicolytic effect, without affect inhibitory avoidance latency. Metergoline reverted the panicolytic effect of PEA, (escape 2: VEH+PEA = 20.23±2.38, MET+PEA = 10.62±3.03 ($p<0.05$), and escape 3: VEH+PEA = 16.30±1.60, MET+ PEA = 10.25±2.04 ($p<0.05$)), as well as the panicolytic effect of paroxetine, (escape 1: VEH+PAR = 16.76±1.83, MET+PAR = 8.50±2.34 ($p<0.05$), escape 2: VEH+PAR = 20.46±2.36, MET+PAR = 9.62±3.03 ($p<0.05$), and escape 3: VEH+PAR = 17.46±1.60, MET+PAR = 12.25±2.04, ($p<0.05$)) in the ETM. Sulpiride reverted the panicolytic effect of PEA, (escape 2: VEH+PEA = 20.23±2.97, SUL+PEA = 11.00±3.78 ($p<0.05$)) but not panicolytic effects of paroxetine (escape 2: VEH+PAR = 20.46±2.97, SUL+PAR = 21.25±3.78). The distance traveled in a circular arena under the different treatments was not altered when compared to the control groups ($p>0.05$). These results showed that chronic treatment with PEA produced a panicolytic effect in the ETM, and that the serotonergic and the dopaminergic neurotransmission systems are involved in this effect. **References**: [1] Otobone, F. *J Braz Arch Biol Technol,* 48, 723, 2005; [2] Otobone, F.J. *Phytotherapy Res* 21, 531, 2007; [3] Graeff, F.G. *Neuro Bio Rev* 23, 237-246. **Acknowledgments:** This study was supported by FINEP

02.018

Kinin receptors blockade ameliorates the neuro-inflammation and the clinical severity in experimental autoimmune encephalomyelitis: the dominant role of kinin B_1 receptor. Dutra RC[1], Leite DFP[1], Manjavachi MN[1], Bento AF[1], Patricio ES[1], Figueiredo CP[1], Pesquero JB[2], Calixto JB[1] [1]UFSC − Farmacologia, [2]UNIFESP − Biofísica

Multiple sclerosis (MS) is a progressive, demyelinating and inflammatory disease of the human central nervous system that still remains without an effective therapy. Kinins are peptides that exert a key role in inflammation and pain processes. Here we investigated the role played by kinin receptors in the modulation of experimental autoimmune encephalomyelitis (EAE), analyzing the preventive and therapeutic effects of the selective blockade of kinin receptors in conjugation with gene deletion for both kinin B_1 and B_2 receptor in the immunological response onset. Experiments were conducted using female C57BL/6, kinin B_1R and B_2R knockout mice (6-10 weeks old) (CEUA/UFSC23080038266/2008-43). EAE was induced by immunization with MOG_{35-55} peptide plus *Mycobacterium tuberculosis* extract H37Ra in incomplete Freund`s adjuvant oil. The animals received pertussis toxin i.p. (day 0 and day 2) post-immunization (p.i.). Mice were observed daily for clinical signs of EAE, locomotor activity, mechanical and thermal hypernociception. After 25 days p.i., cytokines production and proliferative response were evaluated in lymph node and spleen cells. The percentage of $CD4^+CD69^+$ and $CD8^+CD69^+$ T cells was investigated by flow cytometry assay. The inflammatory cell infiltrate, demyelination index, astrocytes, T lymphocyte and transcription factor CREB were evaluated in lumbar spinal cord at day 25 p.i. The real-time PCR and primary astrocytes culture were used to assess the autoimmune inflammation of the CNS. Here, we show, for the first time, that blockade of kinin receptors either by pharmacological treatment or genetic deletion significantly prevented the clinical relevant symptoms and neuroinflammation in EAE model. Our results also showed that the blockade of B_1R modulates the genesis and maintenance of the disease, by interfering with the onset of immune response, mainly by decreasing T lymphocytes activation and proliferation, as well as diminishing T_H1 and T_H17 cytokines, namely IFN-γ and IL-17, respectively produced by MOG-reactive cells. Lymphocytes, mainly T_H17 cells, were found in lower levels in the CNS of both mice treated preventively with B_1 receptor antagonist, in $B_1R^{-/-}$ and $B_2R^{-/-}$ mice. Notably, therapeutic blockade of B_1R initiated after EAE onset consistently impaired the clinical progression of EAE. Moreover, the DALBK and HOE-140 antagonist strongly reduced the IFN-γ induced up-regulate of TNF-α, CXCL1/KC and IL-6 release, as well as COX-2 and NOS2 expression in primary astrocyte cultures. Our results strongly suggest that blockade of kinin receptors, mainly the B_1 subtype, has a preventive and therapeutic effects on the model of EAE, not only by interfering with the genesis of EAE immune response, but also by directly decreasing central inflammatory process. Present results provide new insights indicating that the B_1 selective antagonist might constitute a new and attractive option for the management of neuroinflammatory diseases that still remain without an adequate therapy, particularly multiple sclerosis. **Support**: CNPq; CAPES; PRONEX; FAPESC.

02.019

Dorsal hippocampus glutamate receptors modulate the expression of contextual fear conditioning. Fabri DRS, Reis DG, Hott SC, Corrêa FMA, Resstel LBM FMRP-USP − Farmacologia

Introduction: It has been demonstrated that the dorsal portion of hippocampus (DH) is involved with expression of contextual fear conditioning in rats. There is evidence showing that DH local glutamatergic system is able to modulate aversive responses. Finally, it has been showing that during expression of fear conditioning the levels of glutamate is increased in the DH. The present study investigated the involvement of the local DH glutamatergic receptors on behavioral (freezing) and cardiovascular, increase of mean arterial pressure (MAP) and heart rate (HR), responses of rats re-exposed to an aversive context. **Methods:** Male Wistar rats weighing 210-240 g had guide cannulae bilaterally implanted in the DH. 48 hours before the test session, animals were submitted to conditioning session, when animals received 6 foot electrical shock (1.5 mA, 3 s). After the conditioning session a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. Glutamatergic NMDA antagonist AP7 (5 nmol) or vehicle, 200 nL each site, were administered 10 min before the chamber re-exposition (test session). Time spent in freezing and cardiovascular responses were recorded for 10 min. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 139-2008). **Results and Discussion:** The results showed that conditioned animals which had DH glutamatergic receptors antagonized by AP7 (n=5) presented reduced percentage of time spend in freezing when compared with vehicle-injected animals (n=5) (63.73 ± 8.104 % vs 33.10 ± 9.316 %; t= 8.0, $P<0.05$). Similar reduction was observed in cardiovascular responses (MAP: $F(1, 105)= 105$ $P<0.001$ and HR: $F(1, 90)= 90$, $P<0.001$). The present results suggest that DH glutamatergic neurotransmission modulates the expression of contextual fear conditioning by NMDA receptors activation. **Financial support:** FAPESP, CNPq, Capes and FAEPA.

02.020

Involvement of β-adrenergic receptors in the bed nucleus of the stria terminalis on the expression of contextual fear conditioning. Hott SC, Gomes FV, Reis DG, Fabri DRS, Corrêa FMA, Resstel LBM − Farmacologia

Introduction: The bed nucleus of the stria terminalis (BNST) is a limbic structure associated with autonomic, neuroendocrine and behavioral functions which seems to be critically involved in the expression of anxiety-like responses as contextual fear conditioning. Among the numerous neural inputs to the BNST, noradrenergic synaptic terminals are prominent and some evidence suggests an activation of noradrenergic neurotransmission within the BNST during aversive situations. Thus, the aim of this work was to study a possible involvement of β-adrenergic receptors in the BNST on the modulation of the behavioral and autonomic responses induced by contextual fear conditioning. **Methods:** Male Wistar rats (240-270g) with cannulae implanted bilaterally into the BNST were submitted to a 10 min conditioning session (6 footshocks, 1.5 mA, 3 s). 24 h later, the behavioral and autonomic mean arterial pressure (MAP) and heart rate (HR) responses to the context were measured in a 10 min test session. Bilateral administration, 0.1μL each side, of 7.5 nmol of a non-selective β-adrenergic receptor antagonist propranolol (n=5) or saline (n=6) were administered 10 min before test. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 166-2007). The % of the freezing was expressed as means ± S.E.M. and analyzed by Student´s t-test. MAP and HR values were continuously recorded during the 5 min period before and the 10 min period after exposure to the footshock chamber and were analyzed using two-way ANOVA with treatment as the main independent factors, and time as a repeated measurement. When interactions between the factors were observed, groups were compared using Bonferroni's *post hoc* test. The criterion for statistical significance was considered to be $P<0.05$. **Results:** Propranolol (7.5 nmol) significantly reduced % of the freezing (Saline: 66.2±5.1; Propranolol: 29.5±9.6; $t(10)=3.392$, $P<0.05$). Moreover, this β-adrenergic receptors antagonism attenuated the increased of both MAP [$F(1,135)=11.25$, $P<0.01$] and HR [$F(1,135)=13.52$, $P<0.001$] induced by aversive context re-exposition. **Discussion:** The results showed that β-adrenergic receptor antagonist propranolol injected into the BNST attenuates the expression of contextual fear conditioning. Therefore, our findings suggest that noradrenergic neurotransmission within the BNST, through β-adrenergic receptors activation, is involved in the expression of responses induced by contextual fear conditioning. **Financial support:** FAPESP, CNPq, Capes and FAEPA.

02.021

Glutamate and NMDA modulate A2 adrenergic expression in cell cultures of the medulla oblongata of newborn rats. Silva SM[1], Carrettiero DC[2], Fior-Chadi DR[1] [1]IB − Fisiologia, [2]UFABC − Ciências Naturais e Humanas

Introduction: The neural control of the cardiovascular system is performed mainly through nuclei located in the medulla oblongata. This control is performed by several neurotransmitters, among them glutamate (glu) and the catecholamines norepinephrine and epinephrine. The control of the cardiovascular system through the catecholaminergic system is realized mainly through the α2 adrenoreceptors (α2a). It is well addressed that glutamate is capable of modulating the catecholaminergic system, mainly through NMDA receptors. Previous data from our group showed that glu modulates the α2a in the medulla oblongata. The mechanisms through which this modulation occurs, though, is not known yet. Thus, the objective of this study was to evaluate the modulation of α2a by glu and NMDA in cell cultures of the medulla oblongata of newborn rats. **Methods:** All the procedures and protocols were performed in accordance with the Institutional Guidelines for Animal Experimentation (CEA/IB-USP: 084/2008). Cell culture was prepared from the medulla oblongata of newborn rats (n=30). Cells were enzymatically and mechanically dissociated, and plated in culture dishes pre-treated with Poly-D-Lisine and maintained for seven days in appropriated culture medium. Cells were characterized by immunohistochemical techniques, employing fluorescent antibodies against neurons and astrocytes. Cultures were treated with different concentrations of Glu and NMDA, and protein levels of the α2a were analyzed 24 hours after treatment by Western Blotting. Cell cultures treated with glutamate were also treated with the vital marker Trypan-Blue in order to verify glutamate-induced cell damage or cell death. All data were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni post test. **Results:** Results were expressed as percentage of control ± standard deviation. The percentage of neurons in culture was 15.6 ±2.1. The level of α2a protein in the cells was reduced by Glu treatment, in a dose-dependent manner (control: 100; 0,1uM 88.3 ± 3.8; 1uM: 62.5 ± 8.6; 10uM: 49.9 ± 5.5; 100uM: 72,4 ± 24.2). The level of α2a protein was reduced by NMDA treatment as well (control: 100; Glu 10μM: 68±1; NMDA 10μM: 70±10). Cultures treated with several concentrations of glutamate (0,1μM, 1μM, 10μM, 50μM and 100μM) for 5 minutes did not show significant number of cells marked with the vital marker Trypan-Blue. The experiments suggest that α2a protein expression is modulated by the treatment with Glu and NMDA($p<0.05$). **Discussion:** We showed that glutamate and NMDA is able to modulate α2 adrenoceptor at the protein level in cells from the medulla oblongata of newborn rats. We suggest that this modulation might be important to the neural mechanisms of blood pressure control. This study was supported by grants from FAPESP and CNPq.

02.022

L-arginine into the CA1 hippocampal subfield did not change retention of inhibitory avoidance task in rats. Yoneyama B, Contardi EB, Milani H[1], Oliveira RMMW UEL − Farmácia e Farmacologia

Introduction: Nitric oxide (NO) has been shown to have a number of important physiological roles within the central nervous system (CNS) under normal conditions, including pain perception, synaptic plasticity, long-term potentiation (LTP) and learning. Expressive densities of the enzyme that synthesizes NO in the CNS, the neuronal NOS (nNOS), are described in several brain regions including the hippocampus, which has been implicated in several types of learning and memory formation, as inhibitory avoidance learning. Since the CA1 region of hippocampus presents high level of nNOS, interference with NO neurotransmission could modulate hippocampal function, hence affecting memory processes. **Objective:** The aim of this study was to evaluate the effect of NO precursor, L-Arginine, in a passive inhibitory avoidance task using the step-down test. **Methods:** Male Wistar rats (280-310 g) were divided into 3 groups and bilaterally implanted with guide cannulae aimed at the CA1 region of hippocampus. Animals received pre-retrieval injections of vehicle (saline) or different doses of L-Arginine (200 nmol and 400 nmol) and were tested in the step-down apparatus. The apparatus consisted of an acrylic box (12 cm×30 cm×15 cm), whose floor consisted of parallel 1.0mm diameter stainless steel bars spaced 1.0 cm apart. A 10 cm wide, 3.0 cm high, 6.0 cm long platform occupied the right side of the grid floor. In the training session (day 5 after surgery), immediately after stepping down their paws on the grid the animals received a 0.8mA 2 s scrambled foot shock. Then, the animals were put again in the platform and if it stepped down their paws again on the grid, it received another shock. If it not did that in 3 min it was withdrawn from the cage. Forty-eight hours later, in the text session, no foot shock was given and the step-down latency (seconds) was used as a measure of memory retrieval (to a ceiling of 180 seconds). Data (mean±SEM) were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons. The experimental procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, (CEEA n^{o} 003/2008). **Results:** Our results indicated that step-down through latency of memory retrieval was not changed in L-Arginine treated groups, as compared to that of control group (saline=117.9±28.5; L=-Arginine 200 nmol=94.14±30.86; L-Arginine 400 nmol=94.20±30.11 $F_{2,19}$=0.22, *P*=0.80). **Discussion:** Herein, intra-hippocampal of L- Arginine (200 and 400 nmol) not affected the step-down latency. This result was somehow unexpected since L-Arginine is a NO precursor and NO has been consistently involved in short and long-term memory processes. However, the present results are in accordance with others that have shown that intra-hippocampal injection of L-Arginine produces no any significant effect on the step-though latency test but is able to revert the effects of the NOS inhibitor L-NAME when administered on pre-training session (Harooni et al., *Behav. Brain Res*., 201:166, 2009). Future experiments using NO scavengers and association between L-Arginine and NOS inhibitors will be conducted in order to clarify the role of NO on hipocampal memory processes. This study was supported by Fundação Araucária and UEL (UEM).

02.023

The selective A-type K^+ current blocker Tx3-1 rescues memory of mice submitted to a model of Alzheimer´s disease. Gomes GM[1], Dalmolin GD[2], Ferreira J[1], Gomez MV[2], Rubin MA[1] [1]UFSM − Química, [2]UFMG − Farmacologia

Introduction: Potassium channels play a key role in many neuronal functions, including regulation of neuronal excitability and synaptic plasticity, contributing, in this way, to mnemonic process. In particular, A-type potassium currents (IA) seems to be involved in hippocampal synaptic plasticity, as inhibition of this current facilitates the induction of long term potentiation (LTP). Besides, IA seems to be enhanced in an experimental model of Alzheimer disease. Therefore, blocking selectively potassium IA current would improve memory storage. The peptidic toxin Tx3-1, extracted from the venom of the spider *Phoneutria nigrieventer*, is able to block selectively A-type K+ currents. The present study aimed to evaluate the effect of Tx3-1 in memory of naive mice and mice submitted to a model of Alzheimer´s disease. **Methods:** To Alzheimer disease induction, male Swiss mice were injected by intracerebroventricular (icv) route with Aß peptide 25-35 (3 nmol/3 µl) and its inverted sequence (Aß 35-25: 3 nmol/3 µl) was injected as control group. The behavioral test was carried on 6 days after injection of Aß peptides. Cognitive behavior was evaluated through object recognition test, which consist of 3 sessions: habituation, training and retention. On the first day, animals were habituated to experimental apparatus for 10 min in absence of objects. On the second day, training session was carried out, in which two identical objects (A) were placed in the box and the amount of time that animal explored both objects was recorded. Immediately after training session, Tx3-1 (10, 30, 100, and 300 pmol/site), 4-aminopyridine, a non-selective blocker of voltage activated potassium channels (4-AP: 30, 100 and 300 pmol/site) or PBS (5 µl/site) were administered by icv route. In retention session, one object (A) was changed for a new object (B) and the time that animal spent exploring the new object was recorded. The discrimination index used to assess memory was calculated as the difference in time exploring the novel and familiar object, expressed as the ratio of the total time spent exploring both objects. All protocols employed have been approved by the Ethics Committee of UFSM (process number: 23081.008569/2006-60). **Results and Discussion:** Administration of Tx3-1 (10-300 pmol/site, icv) improved long term memory in object recognition test with Emax of 44.9% of discrimination index by administration of 300 pmol/site and ED_{50} of 40.3 (10.3 – 158.4) pmol/site. Animals previously administered with Aß peptide 25-35, showed impaired performance in retention session when compared with control group (1.8±1.9% and 25.1±3.5% of discrimination index for mice injected with Aß 25-35 and Aß 35-25, respectively). Interestingly, Tx3-1 exhibited a higher potency to improve memory of mice submitted to a model of Alzheimer disease when compared to naive mice (ED_{50} of 2.0 (0.8 – 5.4) pmol/site. Aiming to compare the effect of Tx3-1 with other potassium channel blocker, we administered 4-AP (30-300 pmol/site) in mice. This non-specific potassium channel blocker did not improved long term memory retention and, at least in doses tested, caused toxic side effects such as shaking, cycling and tonic-clonic seizures in the higher dose tested (300 pmol/site). These results shown that Tx3-1 improved long-term memory retention and rescue memory deficit in animal model of Alzheimer disease, suggesting the involvement of IA currents in the cognitive deficit of Alzheimer disease. **Fellowship:** Instituto do Milenio MCT/CNPq, Capes, Pronex, Fapemig

02.024

Ketamine/fentanyl administration in infant rats induces anxiolysis until adult life. Medeiros LF[1], Souza A[1], Rozisky JR[1], Santos VS[1], Netto CA[2], Battastini AMO[2], Torres ILS[1] [1]UFRGS − Farmacologia, [2]UFRGS − Bioquímica

Objective: Evidence from the literature indicates that early exposure to anesthetic agents can be detrimental to the development of the CNS of mammals, resulting in behavioral changes until adulthood. Several therapeutic agents are used in pediatry, we highlight anesthetics and analgesics. Ketamine has a unique anesthetic state; it promotes dissociation between the limbic system and thalamus. While fentanyl, an opioid widely used as an adjunct to general anesthesia. The objective was evaluating locomotor activity and anxiety of the animals submitted to the administration of general anesthetic, with or without a surgery procedure at P14. **Methods:** We used litters of rats with 14 days-old (P14) divided into 3 groups: control (C), ketamine S+/fentanyl (KF), ketamine S+/fentanyl+surgery (KF+SUR). We used 0.09 mg/kg fentanyl and 20 mg/kg ketamine S+. The surgery procedure model was used described by Levine, modified by Rice et al. (Ann Neurol 9:131, 1981), without production of hypoxia-ischemia. In P14 pups were evaluated six hours after intervention. The behavioral assessment for 5 min in the Open Field (OF) at P14, P30 and P60 (n=9-16); and in the Plus Maze (EPM), at P30 and P60 (n=12-18). Behaviors analyzed in OF: latency to leave the first quadrant (s), number of locomotion and rearing. Behaviors, in EPM: number of entries in open arms (EBA) protected head-dipping (PHD), non-protected head-dipping (NPHD) and time (s) to spend in the open arms (TBA). Data were analyzed by one-way ANOVA followed by SNK. The results expressed as mean±SEM and considered significantly different with P<0.05. This study was approved by Ethical Committee of HCPA (n°08149). **Results:** In OF, at P30 there was an increase in the number of crossings of the KF (86.19±8.01) and KF+SUR groups (93.33±8.76) compared to C (66.00±3.35,ANOVA/SNK, *P*<0.05). EPM: at P30, the KF group showed an increase in the number of EBA (2.94±0.24) and NPHD (5.22±0.68), associated with an increase of TBA (34.27±4.75) when compared to C (EBA:1.53±0.24; NPHD:1.82±0.32; TBA:11.41±2.11, ANOVA/SNK, *P*<0.05). The KF+SUR group showed an increase in the number of NPHD (6.94±1.11) in relation to C group (1.82±0.32), with an increase in the EBA (4.38±0.57) and TBA (50.55±6.88) compared to other groups (ANOVA/SNK, *P*<0.05). At P60, the KF group showed an increase in the EBA (3.27 ± 0.71), PHD (6.2 ± 0.92), NPHD (4.67±0.97) and TBA (35.93±8.72) in relation to other groups (ANOVA/SNK, *P*<0.05). This study was approved for Ethical Committee of HCPA (n°08149). **Conclusions:** Behavioral changes analyzed together suggest adaptations in neurotransmitter systems involved in anxiety, the anxiolytic effect induced by association fentanyl/ketamine S+ it was observed until P60. It is known that structures such as hippocampus and periaqueductal gray (PAG) participate in the regulation of anxiety state, and that ketamine or DAMGO (μ agonist) when applied directly to the PAG have anxiolytic effects. Ketamine also interacts with the monoamine reuptake transporters, increasing its synaptic concentration. Future studies are needed to clarify the relationship of the state of anxiety about the imbalance in the neurotransmitter systems. **Financial Support:** CAPES; FIPE/HCPA, PROPESQ/UFRGS; FAPERGS.

02.025

Kinin B2 receptor can play a neuroprotective role in Alzheimer's disease. Caetano AL[1], Amaral FA[1], Dong KE[1], Baraldi T[2], Viel TA[2], Buck HS[1] [1]FCMSCSP − Ciências Fisiológicas, [2]EACH-USP

Introduction: Alzheimer's disease (AD) is characterized by neurodegeneration associated with senile plaques, neurofibril tangles and memory impairment. We described memory impairment in rats and C57Bl/6 mice (WT) after chronic infusion of amyloid-beta peptide (AB) in lateral ventricle. The memory disruption was potentiated in mice lacking B2 receptor (koB2) and abolished in mice lacking B1 receptor (koB1) suggesting that B2 receptor (B2R) could be neuroprotector and B1 receptor (B1R) could be neurodegenerative. The aim of this study was to evaluate the density of binding sites for B1R and B2R in WT, koB1 and koB2 mice (12 weeks old) after chronic infusion of AB, in 10 months old transgenic mice expressing amyloid precursor protein (B6.Cg-Tg(PDGFB-APPSwInd)20Lms/2J) (TG10) and in aged matched C57Bl/6 mice (WT10). **Methods:** Males WT, koB1 and koB2 were chronically infused with AB (0,46 nmol – AB group) or vehicle, for 5 weeks, by a mini-osmotic pump connected to a stainless steel cannula implanted in the animal's lateral ventricle. Animals were killed at the end of infusion period and their brains were removed and frozen at -80°C until use. Serial frozen sections (20µm) were obtained and incubated with 200 pM [^{125}I]HPP Hoe-140 (B2 antagonist) or [^{125}I]HPP-Des-Arg^{10}-Hoe-140 (B1 antagonist). Non-specific binding was determined in the presence of 2nM of cold ligand. Differences were considered statistically significant when $p<0.05$. **Results:** ABWT animals showed significant increase in B2R specific binding sites (fmol/mg of tissue) in lateral portion of accumbens shell (LAcbSh, 0.29±0.04), CA1 (0.17±0.03), CA2 (0.19±0.03) and CA3 (0.24±0.04) areas of hippocampus in comparison with the WT control group (LAcbSh= 0.13±0.06; CA1=0.08±0.02; CA2=0.07±0.02 and CA3=0.11±0.02). When compared to the koB1 control group, ABkoB1 mice showed significant increase of B2R binding sites in anterior part of the anterior commissure (0.54±0.04 vs. 1.3±0.09), caudate putamen (0.03±0.01 vs. 0.49±0.03), Cortex (0.11±0,02 vs. 0.67±0.04), core (0.37±0.12 vs. 0.77±0.06) and shell (0.2379±0.06 vs. 0.82±0.07) portion of accumbens nucleus, LAcbSH (0.14±0.06 vs. 0.67±0.04), CA1 (0.08±0.01 vs.0.28±0.08), CA2 (0.08±0.02 vs. 0.26±0.05) and CA3 (0.10±0.02 vs. 0.23±0.06). In comparison to wt10, transgenic mice showed significant increase of B2R densities in the caudate putamen, cortex, CA1 and CA3 areas at similar values observed in koB1 infused with AB. All strains, except koB1 mice, showed very weak specific biding sites to B1R in several brain areas and no statistical differences were observed between groups. **Discussion:** Several studies suggest the participation of kallikrein-kinin system in the pathogenesis of Alzheimer's disease. Data obtained in transgenic mice reinforces this participation. It has been suggested by our group a protective role for B2R. The present data showing the absence of memory impairment in koB1 mice infused with AB accompanied by an increase in B2R density in brain structures related to memory process let us suggest that B2R could play an important role in neuroprotection. In addition, the unchanged B1R density observed in koB2 infused with AB indicate that the severe memory impairment observed could be due to the absence of B2R and not by an increase in B1R. Protocol CEEUA nº 155. **Financial support**: FAPESP, CNPq

02.026

Medial prefrontal cortex muscarinic receptors modulate the expression of contextual fear conditioning. Fedoce AG, Ferreira Junior NC, Reis DG, Corrêa FMA, Resstel LBM FMRP-USP − Farmacologia

Introduction: It has been shown that the ventral portion of medial prefrontal cortex (vMPFC) is involved with expression of contextual fear conditioning in rats. There is evidence showing that vMPFC local cholinergic system is able to modulate aversive responses. Finally, it has been show that during expression of fear conditioning the levels of acetylcholine is increased in the vMPFC. The present study investigated the involvement of the local vMPFC muscarinic receptors on behavioral (freezing) and cardiovascular, increase of mean arterial pressure (MAP) and heart rate (HR), responses of rats re-exposed to an aversive context. **Methods:** Male Wistar rats weighing 230-270 g had guide cannulae bilaterally implanted in the vMPFC. Twenty four hours before the test session, animals were submitted to conditioning session, when animals received 6 foot electrical shock (1.5 mA, 3 s). After the conditioning session a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. Muscarinic antagonist atropine (6 nmol) or vehicle, 200 nL each site, were administered 10 min before the chamber re-exposition (test session). Time spent in freezing and cardiovascular responses were recorded for 10 min. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 139-2008). **Results and Discussion:** The results showed that conditioned animals which had vMPFC muscarinic receptors antagonized (n=5) presented reduced percentage of time spend in freezing when compared with vehicle-injected animals (n=4) (21 ± 10 % vs 74 ± 9 %; t= 5.0, P<0.001). Similar reduction was observed in cardiovascular responses (MAP: F= 6,5, p<0.001 and HR: F= 11,52, P<0.001).The present results suggest that vMPFC cholinergic neurotransmission modulates the expression of contextual fear conditioning by muscarinic receptors activation. In conclusion, the vMPFC local cholinergic system is crucial for the expression of the conditioned fear response. **Financial support:** FAPESP, CAPES, CNPq and FAEPA.

02.027

Morphological changes in rat skeletal muscle during atrophy caused by amyotrophic lateral sclerosis. Figueiredo LB, Barnabe GF, Mello LE, Godinho RO UNIFESP − Farmacologia, [2]UNIFESP − Fisiologia

Introduction: Skeletal muscle atrophy is a devastating condition seen in many catabolic diseases such as cancer, diabetes, AIDS, denervation, and sarcopenia as well as in neuromuscular disorders. Recent studies in mice and rats with amyotrophic lateral sclerosis (ALS) have shown a dramatic atrophy of skeletal muscles that resembles those of human patients, although the degree of atrophy during the evolution of disease had not been assessed. In the present study, we evaluated the pattern of skeletal muscle morphological and changes of acetylcholinesterase (AChE) activity during the progression of disease in the transgenic rat model of ALS (G93A ALS rats). **Methods:** Adult (160-180 days old) male G93A ALS rats were divided in mild symptomatic (MS) and end-stage (END) groups and compared to wild type Sprague Dawley rats (W_T) (n = 3-5). Soleus (Sol) and extensor digitorum longus (EDL) muscles from both hindlimbs were obtained from rats euthanized with chloral hydrate (9.9mg/kg, i.p). For morphometric histological analysis, 8 µm muscle sections were stained with hematoxylin and eosin and 600-800 fibers/muscle were photographed using a capture-and-image-analysis system microscope. The Basso, Beattie, and Bresnahan (BBB) open field locomotor scale, developed to measure hindlimb motor function of spinal cord-injured rats was adapted for ALS models. AChE activity was measured by colorimetric method. **Results and Discussion:** During ALS disease progression, there was a high correlation (r^2 = 0.89) between decline in hindlimb locomotor function, obtained using BBB analysis, and reduction of body mass. Body (W_T = 561.8 ± 17.3 g), EDL (W_T = 244.5 ± 5.1 mg) and Sol (W_T = 252.5 ± 8.4 mg) masses were also reduced by up to 45%, 55% and 41%, respectively. No change was observed in the relative muscle mass (rmm = [muscle mass x 1000]/body mass) of Sol, whereas the EDLrmm was decreased by 16%, indicating that loss of body mass is mainly related to skeletal muscle atrophy. The mean diameters of EDL and Sol fibers, from MS and End groups, were respectively 24%-26% and 4%-39% smaller than control ones (EDL W_T = 56.59 ± 0.43 µm; Sol W_T = 61.56 ± 0.47 µm). Finally, at the end stage of disease, acetylcholinesterase activity of EDL muscles was reduced by 54%, in comparison to control values (24.3 ± 0.9 arbitrary units/ mg of tissue; n = 3-4). These results show that G93A ALS rats animal model of ALS develop symptoms that resembles those of human disease, but differently affect fast (EDL) and slow (Sol) muscles. Further studies will be necessary elucidated the mechanisms involved in those differences. UNIFESP Ethics Committee in Animal Research (approval # 0239/05). **Financial support:** Fapesp and CNPq.

02.028

“Anxious” and “non-anxious” subgroups of rats selected in the elevated plus maze do not differ in the density of [^{3}H]-flunitrazepam binding in the hippocampus and limbic cortex.

Carvalho JGB, Venditti MAC UNIFESP − Psicobiologia

Introduction: The elevated plus maze test is widely used to the evaluation of “anxiety” in laboratory animals (Psychopharmacol.,112:13, 1993). A lower open arms exploration in this apparatus is associated to a higher level of anxiety. Previous study reported that “anxious” compared to the “non-anxious” rats presented a significantly lower number of benzodiazepine sites in the brain cortex (Rago, Arch. Pharmacol. 343: 301, 1991). The present work sought to verify if subgroups of rats with low (LA) and high activity (HA), selected according to their “anxiety” level in the elevated plus maze, differ in the binding of the benzodiazepine full agonist [^{3}H]-Flunitrazepam, in the hippocampus and limbic cortex. **Methods:** The elevated plus maze was the same as the one described by Pellow and File (Pharmacol. *Biochem. Behav*., 24: 525,1986) with minor modifications in the recording parameters. Adult, male, Wistar naive rats (N=86) were submitted to a single 5-min session in the elevated plus maze test. Total time spent (TS) and the total number of sectors crossed (SCR) in the open arms were scored (*Behav. Brain Res*., 39: 63, 1990). Those rats showing TS ≤ 6.0 sec. (Mean – 1SD) and SCR ≤ 0.9 (Mean – 1SD), were selected as low activity (LA, N=11). Those rats showing TS ≥ 101.6 sec. and SCR ≥ 32.7 (Mean + 1 SD), were selected as the high activity group (HA, N= 11). Fifteen days after the elevated plus maze test, the rats were sacrificed by decapitation, the hippocampus and limbic cortex were dissected. The homogenates were submitted to a sequence of differential centrifugation to obtain a rich synaptosomes fraction. The binding assay with [^{3}H]-flunitrazepam was carried out at 4nM (*Chronobiol. International*, 3: 91,1986). This work was approved by our institution ethics committee (proc. 1023/ 6). **Results:** The statistical analysis by the unpaired Student’s t test indicated that there were no significant differences between LA and HA subgroups of rats in the [^{3}H]-flunitrazepam binding in the hippocampus (LA:359.0±19.3 pmol/mg protein ± SEM; N=8 and HA: 368.1±16.8; N =10; p=0.73) and in the limbic cortex (LA: 251.2±16.8 pmol/mg protein ± SEM; N=10 and HA: 238.1±17.4; N=10; p= 0.59). **Discussion:** Our results suggest a lack of relation between the “anxiety” level and the benzodiazepine sites in the hippocampus and in the limbic cortex. The absence of difference in the [^{3}H]-flunitrazepam binding may be due to a lack of selectivity of flunitrazepam to the different alfa isoforms (mainly $alfa_2$/$alfa_3$) specifically related to the anxiety processes. **Financial support:** AFIP.

02.029

Binding of [^{3}H]-flunitrazepam and [^{3}H]-MK-801 in brain regions of rats with different sensitivity to the convulsant effect of a benzodiazepine inverse agonist. Conto MB[1], Carvalho JGB, Venditti MAC UNIFESP − Psicobiologia

Introduction: The $GABA_A$/benzodiazepine site complex, as well as the NMDA glutamatergic receptor are neurochemical entities which seem to be related to the pathophysiology and maybe even to the pathogenesis of disorders such as epilepsy (Corda, JPET, 262,792,1992; Rocha, Epilepsy Behav.,24,65, 1996). In previous studies we demonstrated that rats with lower threshold to the convulsions induced by DMCM, a benzodiazepine inverse agonist, presented a higher level of anxiety in animal models of anxiety (Contó, *Pharmacol. Biochem. Behav*., 82, 417, 2005), as well as a lower sensitivity to the hypnotic effect of diazepam (data not published), suggesting the existence of a neurochemical common mechanism predisposing the coexistence of anxiety and insomnia in epileptic patients. In the present study we sought to investigate if rats with high and low threshold to the clonic convulsion elicited by DMCM, differ in the binding of [^{3}H]-flunitrazepam, a full benzodiazepine agonist, and in the binding of [^{3}H]-MK-801, a NMDA receptor channel blocker, in different brain regions. **Methods:** Naïve adult, male, Wistar rats, aged 3 months, were administered with 2 intraperitoneal injections of a CD_{50} of DMCM (one-week interval between them). The rats which presented clonic convulsions in both the administrations were termed SC group (susceptible to convulsions) and those which did not present any sign of motor disturbances in both the expositions were termed NSC group (non-susceptible to convulsions). Twenty-five days after the second drug administration, the selected subjects were sacrificed. The brains to be used in the autoradiography with [^{3}H]-flunitrazepam at 3 nM (n=10/group) were dissected and kept frozen. The brains to be used in the binding experiment with [^{3}H]-MK 801 at 4 nM (n=10/group) were dissected in different regions. This work was approved by our institution Ethics Committee on Animal Research (Proc. 1058/06). **Results:** The analysis of the data by unpaired Student's t test showed statistical differences between the subgroups in the specific binding of [^{3}H]-flunitrazepam at 3 nM in the subregion CA2 of the ventral hippocampus (SC: 13,87 ± 0,53 pmol/g of tissue, mean ± SEM; NSC: 15,3 ± 0,62; $p<0,05$) and in the specific binding of [^{3}H]-MK 801 at 4 nM in the hippocampus (SC subgroup: 136,6 ± 10,03 fmol/mg of protein, mean ± SEM; NSC subgroup: 164,6 ± 8,3; $p<0,05$); in the frontal cortex (SC subgroup: 75,69 ± 8,81; NSC subgroup: 122,6 ± 8,63, $p<0,005$); and in the striatum (SC subgroup: 87,93 ± 13,16; NSC subgroup: 127,9 ± 10,29, $p<0,05$). It was not found a statistical difference between the subgroups in the amygdala + limbic cortex (SC subgroup: 215,6 ± 6,28; NSC subgroup: 219,1 ± 8,2, $p>0,05$). **Discussion:** The results suggest that the more excitable phenotype of SC group is probably related to a lower GABAergic inhibition mediated by $GABA_A$/benzodiazepine complex and to a higher glutamatergic excitability mediated by NMDA receptors. Further research need to be carried out, aiming a better comprehension of the neurochemical aspects related to the neurobiology of comorbidities such as epilepsy/anxiety and epilepsy/sleep disorders. **Financial support**: AFIP and Capes.

02.030

The paraventricular nucleus of the hypothalamus mediates pressor response to acute restraint stress in rats. Busnardo C[1], Tavares RF[1], Resstel LBM[1], Elias LLK[2], Corrêa FMA[1]
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Introduction: The paraventricular nucleus of the hypothalamus (PVN) has been implicated in several aspects of neuroendocrine and cardiovascular control. Acute restraint is an unavoidable stress situation that evokes corticosterone release and autonomic changes, which are characterized by elevated mean arterial pressure (MAP), intense heart rate (HR) increases and superficial tail temperature decreases. The present work studied the possible involvement of local PVN neurotransmission in the mediation of restraint stress-induced cardiovascular changes. **Methods:** Male Wistar rats were used (250-270g). Guide cannulas were implanted into the PVN. A catheter was introduced into the right femoral artery for blood pressure and heart rate recording**.** We submitted rats to acute restraint and studied the effect of PVN neurotransmission chemical inhibition with $CoCl_2$ on increases in blood pressure and heart rate induced by stress. Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (Protocol number 042/2009). **Results:** The cobalt ($CoCl_2$, n=6) or artificial cerebrospinal fluid (aCSF, n=6) administration in the PVN did not change the basal levels of MAP ($CoCl_2$:107 ± 3 mmHg vs 106 ± 3 mmHg, t=0.1, P>0.05 and aCSF: 101 ± 2 mmHg vs 100 ± 2 mmHg, t=0.2, P>0.05), or HR ($CoCl_2$: 347 ± 4 bpm vs 348 ± 5 bpm, t=0.1, P>0.05 and aCSF: 354 ± 9 bpm vs 350 ± 8 bpm, t=0.3, P>0.05). Moreover, $CoCl_2$ treatment of the PVN significantly reduced the restraint-evoked increase in MAP response ($F_{1,460}$= 997.9, P<0.0001) without a significant effect on the HR ($F_{1,460}$=0.5, P>0.05) response, when compared with aCSF-treated animals. **Conclusion:** These results show that local PVN neurotransmission is involved in the neural pathway which is involved with pressor response observed during acute restraint stress. **Financial Support:** FAPESP (2009/05308-8) and FAEPA.

02.031

Pre-synaptic nicotinic cholinergic receptor increases neurotransmitter release in cultured cells from the medulla oblongata. Matsumoto JPP, Martins EAC, Fior-Chadi DR IB-USP − Physiology

Introduction: Nicotine acts on nicotinic cholinergic receptor (nAChR), which is found in abundance in brain regions involved in cardiovascular control, including medulla oblongata. Neuronal nAChR are assembled from five transmembrane protein subtypes that are arranged around a central water-filled pore forming a selective ionic channel permeable to sodium and calcium. Pre-synaptic nAChR is able to facilitate neurotransmitter release. Superecliptic synapto-pHluorin (SpH) is a fusion between the vesicular associated membrane protein (VAMP2) and the mutant pH-sensitive green fluorescent protein (GFP) used for neurotransmitter exocytosis studies. We used SpH to determine whether pre-synaptic nAChR activation can facilitate neurotransmitter release in neurons from medulla oblongata. **Methods:** All the procedures were performed in accordance with the Institutional Guidelines for Animal Experimentation (CEA/IB-USP protocol number 065/2008). Cell cultures were made using the medulla oblongata of one-day old Wistar rat (n=10). Seven day-old culture (n=4) was transfected with SpH using lipofectamine. Twenty four hours after transfection cells were treated with 100μM of nicotine, followed by 10μM α-bungarotoxin (α-BTX), an irreversible nAChR antagonist followed again by 100μM nicotine and 90 mM KCl to evaluate cell responsiveness as control. SpH fluorescence from neuronal terminals was measured using a custom-built confocal-imaging system in line-scan mode, through a 63x 1.3 numerical aperture Zeiss using 515-560nm emission and 510nm dichroic filters. Images were analyzed in Image J using Time Series plugins. All data were evaluated by Student`s t-test. **Results:** Results are expressed as mean ± standard error. We found that SpH fluorescence was increased after treatment with nicotine (158.1±4.2 arbitrary units) compared to basal situation (140.4±3.8). Furthermore, α-BTX treatment (135.4±3.9) was able to abolish the SpH fluorescence increase evoked by nicotine (136.7±3.1). After KCl treatment (168.1±4.1) it was also observed an increase in SpH fluorescence, which validated cell responsiveness. Neurotransmitter release in the cell culture was increased in response to nicotine treatment ($p<0.05$). **Discussion:** Pre-synaptic nAChR, which is α-BTX sensitive or insensitive, modulates neurotransmitter release in several areas of central nervous system (CNS), such as, cerebellum and striatum. Medulla oblongata has a dense population of α7 and α4β2 nAChRs which might be involved in nicotine-induced neurotransmitter release. α-BTX abolished the increase in neurotransmitter release induced by nicotine treatment, which suggests that α-BTX-sensitive nAChR is involved in this modulation. In conclusion, we demonstrated that nicotine increase neurotransmitter release in cultured cells from medulla oblongata and this modulation is mediated via α-BTX-sensitive nAChR. Nevertheless, the mechanism involved in this modulation needs further investigation. This study was supported by grants from FAPESP, CAPES and CNPq.

02.032

Facilitation of endocannabinoid-mediated neurotransmission in the dorsal hippocampus induces anxiolytic effects in rats submitted to the Vogel conflict test. Nejo P, Lisboa SF, Resstel LBM, Guimarães FS FMRP-USP

Introduction: Glutamate-mediated neurotransmission in the hippocampal formation has been related to anxiety. The endocannabinoid (eCB) system modulates glutamate release and systemic administration of CB1 receptor agonists induces anxiolytic-like effects. The aim of the present study, therefore, was to investigate if facilitation of eCB-mediated neurotransmission in the dorsal hippocampus (DH) would induce anxiolytic effects in rats submitted to the Vogel conflict test (VCT), a widely used animal model of anxiety, and if the effects were mediated by the activation of CB1 receptors. **Methods:** Male Wistar rats (250 g, n=5-8) with cannulae aimed at the DH received a firtst bilateral microinjections of AM251 (CB1 antagonist; 100 pmol) or vehicle (500nL), followed 5 min later by a microinjection of AM404 (an inhibitor of anandamide metabolism and uptake, 50 pmol) or URB597 (an inhibitor of the fatty acid amide hydrolase enzyme, which metabolizes anandamide, 0.01 nmol) and 10 min later were submitted to the VCT session. In this test 24 h water-deprived animals were first pre-exposed to the apparatus and allowed to drink for 3 min. After another 24 h of water deprivation, they were placed into the apparatus for the 3-min test session where an electrical shock (0.5 mA, 2s) in the spout of the drinking bottle was delivered at every twenty licks. The Institution´s Animal Ethics Committee approved housing conditioned and experimental procedures (protocol nº 143/2007). **Results:** AM404 increased the number of punished licks (vehicle: 91±26 vs AM404: 381±48, $P<0.05$), an effect that was mediated by CB1 receptors because the pretreatment with the antagonist AM251 attenuated this effect ($P>0.05$). A similar effect was observed with URB597 (URB: 224±66, $F(2.17)=9.96$, $P<0.05$), but we did not yet tested the participation ofCB1 receptors. The effects of both AM404 and URB597 were specific to anxiety because in the controls experiments, the water consumption and tail-flick tests, these drugs did not altered water consumption and pain, respectively. **Discussion:** These results suggest that the eCB system present in the DH could modulate defensive responses, probably by activating CB1 receptors and decrease glutamate release. **Financial support:** FAPESP, CNPq. **Animal Experimentation Ethics Committee** FMRP-USP (protocolo nº 143/2007).

02.033

Possible role of the angiotensin (1-7) in the hippocampus in a model of epilepsy. Pereira MGAG[1], Souza LL[1], Becari C[2], Camacho F[1], Oliveira JAC[3], Salgado MCO[2], Garcia-Cairasco N[3], Costa-Neto CM[1] [1]FMRP-USP − Biochemistry and Immunology, [2]FMRP-USP − Pharmacology, [3]FMRP-USP − Physiology

Introduction: Data of our lab showed that losartan (AT1 receptor antagonist) treatment led to significant seizures severity decrease in Wistar Audiogenic Rat (WAR) animals with audiogenic kindling (AK), a model of temporal lobe epilepsy. Thus, our purpose was to investigate the possible role of renin-angiotensin system (RAS) in the hippocampus in this model. **Methods:** We examined the expression of two receptors of the RAS (AT_1 and Mas receptor) in the hippocampus of naïve WARs and WARs with AK by RT-PCR. The metabolism toward angiotensin I (AngI) in the hippocampus was investigated by HLPC. Pull-down assay was made with endopeptidase 24.15 antibody to determine the participation of this enzyme in Ang I metabolism. All experiments were conducted in accordance with the local Animal Care and Use Committee (protocol FMRP-USP 200/2005). **Results and Discussion**: Hippocampal AT_1 and Mas mRNA levels were upregulated (8- and 4-fold, respectively) in AK WARs when compared to naïve WARs. The HPLC and pull-down assay, respectively, showed that the Ang (1-7) is the main derived peptide of Ang I and that endopeptidase 24.15 is the major Ang (1-7) forming enzyme in this process. Furthermore, Ang I metabolism is higher in AK WARs then naïve WAR. Interestingly, Ang (1-7) is the main ligand of Mas receptor and also, with less affinity, of AT1 receptor, both upregulated in AK WARs hippocampi. In this study we showed that Ang (1-7) may have a role as neuroactive peptide in the seizures in a model of epilepsy. **Support**: FAPESP, FAPESP-CInapce, CNPq, CAPES and FAEPA.

02.034

Enriched environment stimulus improves spatial and aversive-related memory performance in an animal model of severe Alzheimer's disease. Schowe NM[1], Oliveira EM[1], Souza LHJ[1], Sousa AMA[2], Amaral FA[2], Lopes ASA[2], Caetano AL[2], Rocha MN[3], Buck HS[2], Viel TA[1] [1]EACH-USP, [2]FCMSCSP − Ciências Fisiológicas, [3]FCMSCSP − Medicina Molecular

Introduction: Alzheimer's disease (AD) is mainly characterized by memory impairment and progressive neurodegeneration that increase along the course of the disease. It has been shown that rodents submitted to an enrichment of their living environment have their cognitive performance improved. The aim of this work was to evaluate if cognitive stimulation could improve the memory performance of transgenic mice with a severe form of AD. **Methods:** 3 months-old male Tg(PDGFB-APPSwInd – The Jackson Laboratories) mice were exposed to enriched environment (cages containing toys, spin wheel and wooden blocks) during two months (TG-EE, n=9). At the same time C57Bl/6 mice (WT, n=10) and another group of TG (n=8) were kept in standard cages with water and food *ad libitum*. Spatial memory was evaluated using the Barnes maze (5 blocks of two trials). Aversive-related memory was evaluated using the inhibitory avoidance test (0,5 mA, 2 sec, max latency of 300 sec) and locomotion was measured in an automatic activity cage. **Results:** In the Barnes maze test, TG and WT mice had a significant difference in time spent to find the escape box along the five blocks [$F(1,89)=12.52$, $p < 0.0001$], indicating that both groups learned the task. However, no significant difference was observed between them along the five blocks. When comparing TG with TG-EE, a significant difference was observed in time [$F(4,84)=3.85$, $p < 0.01$] and between groups [$F(1,84)=79.88$, $p < 0.0001$]. In the last day, the performance of the three groups was similar (WT: 92.8 ± 17.4 sec; TG: 107.3 ± 21.8 sec; TG-EE: 48.4 ± 13.1 sec).). In the inhibitory avoidance test the three groups showed improvement of memory in test session (TS), when compared to the acquisition session (AS), as follows: WT: AS=12.2 [9.0/20.3] sec), TS=300.0 [298.0/300.0] sec, $p < 0.01$; TG-EE: AS= 11.6 [6.8/30.4] sec, TS= 300.0 [205.3/300.0] sec, $p < 0.01$; TG: AS= 30.6 [11.5/39.1 sec, TS= 300.0 [180.7/300.0] sec, $p < 0.05$. TG-EE mice locomotion was 35% and 58% greater when compared respectively to WT or TG performance (613.5 ± 12.5 unities and 511.5 ± 37.1 unities, respectively). **Discussion:** Exposure of humans or animals to enriched environment increases neuroplasticity as well as cognitive functions. Tg(PDGFB-APPSwInd) mice (TG) present a more severe form of AD, since at 5 months-old animals show a decrease of more than 30% in the number of synapses and neurons, and a decrease of more than 40% in synaptic transmission. The exposure of TG mice to enriched environment improved greatly the spatial memory. The greater locomotion performance is highly related to this since the greater the animal explores the maze, the faster it finds the escape box. Improvement in aversive stimulus-related memory was not so striking, once this task involves a highly emotional reinforcement. We suggest that cognitive stimulation can be an efficient strategy to prevent cognitive deficits even when neuronal losses are already significant. As these animals present a greater neurodegeneration as they get older, these memory behaviors will be re-analyzed when the animals complete 7 months-old, in order to verify the maintenance of these responses. **Financial support**: FAPESP. CEEA/FCMSCSP protocol number: 175

02.035

Acute but not chronic administration of pioglitazone promoted behavioral and neurochemical protective effects in the MPTP model of Parkinson's disease. Barbiero JK, Santiago RM, Lima MMS, Ariza D, Morais LH, Andreatini R, Vital MABF UFPR − Farmacologia

Objectives: Recently, studies focusing on agonists of peroxisome proliferator-activated receptor gamma (PPAR-g) showed neuroprotective effects, mainly involving anti-inflammatory and anti-oxidative properties unleashed by PPAR-g's activation. PPAR-g is one isoform of the PPARs family, which is found to be highly expressed in various brain regions including the striatum, SNpc, cortex and hippocampus. The present study investigated the neurochemical, motor and cognitive effects of pioglitazone, a PPAR-g agonist, in a rat model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). **Methods and Results**: The animals (80 male Wistar rats) were randomly distributed in the following groups: sham+saline (n=10), sham+pioglitazone 30mg/kg (n=10), MPTP+saline (n=10), and MPTP+pioglitazone 30mg/kg (n=10). In this experiment, we administered MPTP (1mmol MPTP − 2,1mL de saline, 0,35mL/min) in the pars compacta substantia nigra. We demonstrate that 30 mg/kg of pioglitazone was capable of restoring striatal dopamine (DA) concentrations and motor behaviors. The experiment was conducted to test the effects of two protocols (acute and chronic) of pioglitazone 30 mg/kg administration in the open-field test, two-way active avoidance task and in the DA and metabolites levels. The acute protocol consisted of a single oral administration 1 hour after MPTP, whereas the chronic protocol was performed with daily administrations starting 1 hour after MPTP and ending 22 days after that. Results showed that neither protocol were able to reverse the cognitive impairment promoted by MPTP. We also demonstrated that acute treatment generated some level of neuroprotection, as confirmed by the absence of DA reduction in the group treated with pioglitazone in comparison to the sham group. By contrast, chronic treatment leaded to a reduction of striatal DA, close to MPTP administration alone. These findings suggest that acute administration of pioglitazone (30 mg/kg) was more efficient in generating beneficial effects on motor behaviors and in striatal DA levels. Nevertheless, we cannot demonstrate that pioglitazone administration improved performance on a dopamine-related cognitive task after MPTP. **Conclusions:** We demonstrate that 30 mg/kg of pioglitazone was capable of restoring striatal dopamine (DA) concentrations and motor behaviors. **Acknowledgments:** REUNI for financial support.

02.036

The noradrenergic neurotransmission in the MeA modulates the cardiovascular responses to acute restraint stress in rats. Fortaleza EAT, Scopinho AA, Corrêa FMA FMRP-USP

Introduction and Goals: The aim of present study was investigate the involvement of the medial amygdaloid nucleus (MeA) and noradrenergic neurotransmission therein mediate cardiovascular responses to acute restraint stress in rats. Bilateral microinjection of the non-specific synaptic blocker CoCl(2) (1mM/100 nL) into the MeA enhanced the heart rate (HR) increase without affecting the blood pressure (BP) increase during exposure by acute restraint, indicating that synapses within the MeA influence restraint-evoked HR changes. **Methods:** We used Wistar rats weighing (240-280g). It was done implanting bilateral guide cannula into the MeA to microinjections of drugs or (artificial cerebrospinal fluid aCSF) vehicle. Two days after, animals were anesthetized with tribromoethanol and a polyethylene catheter was implanted into the femoral artery for blood pressure recording. **Results:** We pretreatment the MeA with the selective alpha(2)-adrenoceptor antagonist RX821002 and with of alpha(1)-adrenoceptor antagonist WB4101 or the beta-adrenoceptor antagonist propranolol in the doses of 15 nmol/100nL, submitting the animals to acute restraint. Acute restraint caused BP and HR increases in aCSF treated animals (n=12). The RX821002 treatment significantly enhanced restraint-evoked tachycardiac response (DHR: treatment: $F_{1.96}$ = 10.38, P<0.0001, n=6) without significant effect on the pressor response (DMAP: treatment $F_{1.96}$=0,1281, P=0,7212, n=6), when compared with aCSF-treated. Pretreatment in the MeA with WB4101 caused no significant differences in the restraint-related MAP or HR increases when compared with aCSF-treated animals (DMAP: treatment $F_{1.108}$=0,07092, P=0,7905; and DHR: treatment: $F_{1.108}$ = 1,938, P=0,1667, n=8), neither the pretreatment of the MeA with Propranolol (DMAP: treatment $F_{1.102}$=0,1824, P=0,6702; and DHR: treatment: $F_{1.102}$ = 3,833, P=0,0530, n=7) did not affect restraint-related cardiovascular responses, reinforcing that alpha(2)-adrenoceptors mediate the MeA-related inhibitory influence on HR responses. **Conclusion:** Thus, our results suggest an inhibitory influence of the MeA on the HR increase evoked by restraint stress, and that this is mediated by local alpha(2)-adrenoceptors. **Financial Support:** CNPq, CAPES and FAEPA.

02.037

Inhibition of spinal c-Jun-N-terminal kinase (JNK) after spinal cord injury improves locomotor performance. Martini AC[1], Forner S[1], Koepp J[2], Rae GA[1] [1]UFSC − Pharmacology, [2]UFSC − Chemical and Food Engineering

Introduction: Traumatic spinal cord injury (SCI) is a complex devastating neurological disorder that compromises major motor, autonomic and reflex functions, leading to permanent homeostatic disabilities. SCI affects 2.5 million people worldwide (~ 250,000 in Brazil) and remains an important cause of mortality (Thuret et al., *Nat. Rev. Neurosci*., 7: 628, 2006). Mitogen-activated protein kinases (MAPKs) exert control over various cellular functions, including mitosis, metabolism and apoptosis, and hence may be implicated in spinal cord inflammation and neuronal death after SCI (Neary., IUBMB Life, 57: 711, 2005). In particular, c-Jun-N-terminal kinase (JNK) is a stress-activated MAPK implicated in many aspects of cellular regulation including proliferation, gene expression and programmed cell death (Johnson et al., BBA, 1773:1341, 2007). The present study assesses changes in spinal levels of MAPKs and inflammatory cytokines after SCI, and the potential role of spinal JNK activity in promoting the motor impairment inflicted by the lesion. **Methods:** A moderate degree of paraplegia was induced in anesthetized male Wistar rats (270-300 g) by inserting a Fogarty 2F embolectomy catheter (3 mm diameter, 5 mm length) at the T10 level and inflating it for 1 min (Vanický et al., J. Neurotrauma, 18: 1399, 2001). At 2, 6, 24 and 72 h after SCI or sham surgery, spinal cord segments (T8 to T12; ~ 2 cm long) were collected for Western blot analysis of expression of total and phosphorylated forms of ERK1/2, JNK and p38 MAPK proteins, myeloperoxidase (MPO) activity assays and ELISA analysis of IL-1b and TNF-a levels. Rats were treated intrathecally twice with SP600125 (a JNK inhibitor; 150 nM) or vehicle at 1 and 4 h after SCI. Recovery from motor disturbance was graded every other day, for 4 min, during 14 days, using a neurological deficit scoring system (BBB scale) (Basso et al., J. Neurotrauma, 12: 1, 1995). All protocols were approved by UFSC's Committee on the Ethical Use of Animals (23080.008708/2010-41). **Results and Discussion:** Relative to sham-group values, expression levels of activated p38 MAPK was increased by 200 ± 41% at 6 h after SCI, those of activated JNK levels rose by 260 ± 41% and 299 ± 52% at 6 and 24 h, respectively. Activated ERK1/2 expression increased by 31 ± 4, 51 ± 7 and 27 ± 12% at 2, 6 and 24 h after SCI, respectively. Spinal cord MPO levels at 6, 24 and 72 h after SCI increased by 417 ± 95, 437 ± 82 and 233 ± 40%, respectively. SCI also increased the spinal levels of IL-1b (411 ± 81, 466 ± 48, 286 ± 38%, at 2, 6 and 24 h, respectively) and TNF-a (286 ± 95%, at 2 h only). More importantly, SCI rats treated with SP00125 displayed higher BBB scores than the vehicle-treated SCI controls as from 8th day after SCI onwards (BBB scores at 2, 8 and 14 days after SCI: SP600125 group 0.64 ± 0.42, 6.36 ± 1.21, 9.36 ± 1.24; vehicle group 0.0 ± 0.0, 2.83 ± 0.95, 5.92 ± 0.89). Experiments examining recovery at later time points are ongoing. These results show a close association between activation of spinal MAPKs and initiation of inflammatory responses after SCI. Moreover, they suggest that inhibition of JNK activation in the spinal cord might hold therapeutic potential for functional recovery from SCI. **Financial Support:** CNPq, FAPESC, PRONEX, CAPES.

02.038

Medial prefrontal cortex NMDA-Nitric oxide pathway modulates anxiety-behavior in rats submitted to the Vogel conflict test. Resstel LBM, Lisboa SF, Guimarães FS FMRP-USP

Introduction: It was demonstrated using the Vogel conflict test (VCT) that the ventral portion of medial prefrontal cortex (vMPFC) of the rat is involved in anxiety behavior. Local vMPFC interaction between glutamatergic and nitregic system is involved in modulation of fear conditioning, a model of anxiety. The present study was realized to better understand the role of the MPFC-glutamatergic and nitrergic system on the VCT behavior response. **Methods:** Male Wistar rats (250 g) rats were water deprived for 48 h before the test. After the first 24 h of deprivation they were allowed to drink freely for 3 min in the test cage in order to find the drinking bottle spout. Twenty-four hours later bilateral microinjections of NMDA-antagonist LY235959 (4 nmol/ 200 nL, n=5), the specific nNOS inhibitor N-Propyl-L-arginine (N-Propyl − 0.08 nmol/ 200 nL, n=5), the NO scavenger Carboxi-PTIO (C-PTIO, 2 nmol/ 200 nL, n=6) or vehicle (200 nL, n=7) were bilaterally microinjected in the vMPFC. After 10 min, the animals were placed into the test box. The test period lasted for 3 min and the animals received a 0.5 mA shock in the bottle spout every 20 licks. The number of punished licks was registered. The Institution´s housing conditions and the experimental procedures were previously approved by the local Animal Ethics Committee (process number: 067-2009). **Results:** Compared to vehicle group (6±1, $F(3,31)$: 4.59, $P<0.01$) the LY235959 increased the number of punished licks (17±4, $P<0.05$). Similar to LY235959, both N-Propyl (19±3, $P<0.05$) and C-PTIO (16±4, $P<0.05$) also increase the number of punished licks. No changes were observed when LY235959, N-Propyl and C-PTIO (n=6, $P>0.05$) were microinjected into vMPFC surrounding structures such as the cingulate cortex area 1, the corpus callosum and the tenia tecta. The drugs also did not change the number of unpunished licks ($P>0.05$). **Discussion:** The results show that NO in the vMPFC modulates anxiety-behavior in the VCT by controls punished behavior. Moreover, this NO modulation could be associated with local glutamatergic activation through NMDA receptors. **Financial support:** FAPESP, CNPq and FAEPA

02.039

Aged and young rats respond differently to permanent, 3-stage 4-vessel occlusion: An analysis of learning, neurodegeneration and β-APP expression. Ferreira EDF[1], Romanini CV[2], Albertin M[1], Mori MA[3], Oliveira RMW[1], Milani H[1] [1]UEM − Farmácia e Farmacologia, [2]UEL − Farmácia e Farmacologia, [3]UEM − Ciências Biológicas

Introduction: Previous works from our laboratory introduced the permanent, 3-stage, 4-vessel occlusion model of chronic cerebral hypopperfusion (CCH), in which bilateral occlusion of the vertebral arteries (VA) is followed by stepwise ligation of the internal carotid arteries (ICA), according to the sequence VA®ICA®ICA, with a 7-day interstage interval (ISI, ®). Under these conditions, 4-VO/ICA did not cause neither hipocampal damage nor spatial learning/memory deficit when measured 30 days after 4-VO/ICA in young rats. When imposed to aged rats, however, 4-VO/ICA was able to provoke spatial learning deficit, despite the absence of hippocampal damage. We hypothesized that a 7-day ISI could be longer enough to allow the brain to compensate for cerebral blood flow reduction, thus preventing neuronal and behavioral outcomes. Therefore, this study was designed to evaluate whether permanent, 3-stage 4-VO/ICA, with ISI shorter than 7 days, could become effective to cause both learning and memory impairments, as well as hippocampal and/or cortical neurodegeneration. In addition, we investigated for the first time whether chronic 4-VO/ICA could elicit β-APP overexpression. **Method:** In a first experiment, young rats were subjected to chronic 4-VO/ICA with an ISI of 5, 4, 3 or 2 days. Ninety days later they were tested for learning and memory ability in the aversive radial maze. In a second experiment, aged rats were subjected to 4-VO/ICA with an ISI of 4 days, and tested for learning/memory performance 90 days later. Cognitive performance was expressed by the following parameters: (i) latency to find the goal box, (ii) number of reference memory errors, and (iii) number of working memory errors. At the end of behavioral testing, the brains were examined histologically for damage to the hippocampus and cerebral cortex, and immunohistochemically for the expression of β-APP in the hippocampus, cortex and thalamus. This study was approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, Paraná (CEEA 045/2009). **Results and Discussion:** Chronic 4-VO/ICA with an ISI of 5, 4, 3 and 2 days, did not cause learning/memory impairments in young rats ($p>0.05$), despite extensive hippocampal damage in the all sectors of the hippocampus ($p < 0.001$). In contrast, aged rats were cognitively affected by chronic 4-VO/ICA ($p < 0.05$). Robust neurodegeneration was observed in both hippocampus ($p < 0.0001$) and cerebral cortex ($p < 0.05$) in aged rats. β-APP immunorreactivity was only marginally expressed in the 4-VO/ICA group when compared with positive control (ischemia + hyperglycaemia) or negative control (sham-operation). The data suggest that 4-VO/ICA with a 4-day ISI and chronicity of 90 days may represent a suitable model of neurodegenerative diseases associated to chronic cerebral hypoperfusion in aged rats. Importantly, previous data from our laboratory demonstrated that permanent 4-VO/ICA did not cause histomorphological damage to the retina, thus avoiding misinterpretation concerning the behavioral data. Supported by CNPq, Fundação Araucária and UEM.

02.040

Alpha2-adrenoceptors in the lateral septal area modulates cardiovascular responses evoked by restraint stress in rats. Scopinho AA, Reis DG, Resstel LBM, Corrêa FMA
FMRP-USP

Introduction: The lateral septal area (LSA) is involved in cardiovascular responses connected with emotional behavior (Reis et al., Learn. Memory, v.17, p. 134, 2010; Scopinho et al., Brain Reserc., v.1122, p. 126, 2006). Restraint stress (RS) evokes expression of c-*fos* in LSA and an increase in blood pressure that was blocked by bilateral microinjections muscimol (Kubo et al., *Neurosc. Letter*s, v.318, p.25, 2002). These findings suggest that the LSA is involved in the stress-induced pressor response. Studies using microdialysis have shown that the levels of noradrenaline (NA) are increased during RS in several limbic regions involved in behavioral and autonomic responses, including LSA (Morilak et al., *Progress in Neuro-Psychop. Biol. Psyc*., v.29, p.1214, 2005). Then, the aim of our study was to investigate the involvement of the noradrenergic system of LSA on the cardiovascular responses caused by the RS. **Methods:** Wistar rats were used (240-280g). Guide-cannulae were implanted bilaterally in the LSA for microinjection of alpha2-receptor antagonist RX 821002 (10nmol/100nL) or vehicle artificial cerebrospinal fluid (aCSF). A catheter was implanted in the femoral artery to record arterial pressure and heart rate. Ten minutes after the microinjection of drugs, the animals were submitted to the RS for an hour. The Institution's Animal Ethics Committee approved the housing conditions and experimental protocols, nº 150/2007. **Results:** The microinjection of RX821002 into the LSA did not affect baseline values of MAP (before RX 821002=103±4 and after=103±1 mmHg, t=0.7, p>0.05, n=7) or HR (before RX 821002=337±7 and after=336±8 bpm, t=1.1, p>0.05, n=7) compared with the vehicle group (n=5). Acute restraint caused significant increases in both MAP ($F_{35,360}$=5.2; P < 0.001) and HR ($F_{35,360}$=7.56; P < 0.001). RX821002 treatment significantly reduced the MAP increase ($F_{1,360}$ = 32.66, P < 0.0001) and tachycardiac response ($F_{1,350}$ = 84.96, P<0.0001) evoked by RS. **Discussion:** These results suggest that noradrenergic alpha2-receptors in the LSA has facilitatory role in controlling cardiovascular responses to RS. **Financial Support:** CAPES, FAPESP and FAEPA.

02.041

Involvement of glutamate AMPA receptors in the hypothalamic mechanisms triggered by paracetamol on the supression of LPS-induced fever. Campos EMB[1], Moraes TP[1], Kanashiro A[2], Malvar DC[3], Souza GEP[2] Iyomasa MM[1], Rosa ML[1] [1]FAMECA-FIPA − Neurociências, [2]FCF-USP − Física e Química, [3]UFRRJ − Ciências Fisiológicas

Introduction: It has been shown that the hypothalamic glutamatergic neurotransmission is involved in the febrile response. The concentration of glutamate in this area and the core temperatures were simultaneously increased following systemic administration of LPS[(1)] in rabbits. The aim of this study was to investigate the involvement of glutamate AMPA receptors on the hypothalamic mechanisms activated by pretreatment with the antipyretic paracetamol in rats after i.p. injection of LPS. **Methods:** Male Wistar rats (200g; n=3-6) received vehicle (ethanol 10% in saline plus 20µl tween 80) or paracetamol (200mg kg^{-1}) i.p. 30 min before (0.5 ml) of LPS (50µg kg^{-1}) or saline (control group). The rectal temperature (^{o}C) was measured every 30 minutes for up 3h by telethermometry. The fever induced by LPS (3 h: 1.4 ± 0.06 ^{o}C) was reduced by treatment with paracetamol (3 h: 0.3 ± 0.08 ^{o}C). Saline injection did not alter the basal temperature of the animals (3 h: 0.1 ± 0.08 ^{o}C). After 3 hours the animals were deeply anaesthetized (50ml/kg of urethane 25%), perfused with paraformaldehyde 4% and their brains removed. 40-µm sections were used for immunohistochemistry. The immunopositive cells (IC) were counted bilaterally, in 3 sections/rat of the lateral area of the hypothalamus. Data were compared by one-way ANOVA followed by Duncan test (p<0.05). **Results:** Pre-treatment with paracetamol induced a significant increase in GluR1-IC in hypothalamus of animals that received LPS when compared to saline (76%) or LPS alone (138%; p=0.02). A non-significant reduction (26%) was observed in LPS group when compared to saline. Although the number of GluR2-IC in hypothalamus was higher than GluR1, no change was induced by either LPS or pretreatment with paracetamol. **Discussion:** The results suggest that the glutamatergic mechanism in hypothalamus through GluR1 AMPA receptors is involved on the antipyretic effect of the paracetamol during LPS induced fever. Support: FAPESP and FPA. Committee on Animal Research and Ethics − USP/Ribeirão Preto, Protocol Number: 136/2007 Huang et al., *Eur J Pharmacol*. 593:105, 2008.

02.042

Comparison of cognitive stimulation during life-time and during the elderly: effects on spatial memory and on neuroplasticity of mice. Baraldi T[1], Amaral FA[2], Caetano AL[2], Albuquerque MS[1], Buck HS[1], Viel TA[1] [1]EACH-USP, [2]FCMSCSP − Ciências Fisiológicas

Introduction: Aging process involves many alterations in physiological functions (Fichman et al., 2005). The degree of decline in memory retrieval depends on the quantity and quality of stimuli received during life-time (Riley e col., 2005; Segovia e col., 2009). This study evaluated the spatial memory and the synaptic density of brain areas related to learning and memory of mice submitted to cognitive stimulation during life-time or during the elderly. **Methods:** Male C57Bl/6 mice (two months old, n = 10) were exposed to enriched environment (cages containing toys, spin wheel and wooden blocks) during 15 months (EE-15). An aged-matched C57Bl/6 group was left in regular cages with no objects during the same time (control, n = 9: C-15). Spatial memory was evaluated using the Barnes maze (5 blocks of one trial) at 2, 5, 11 and 17 months of age. Besides, more 20 mice were left in cages until the age of 15 months old, when the group was divided: 10 mice were placed in enriched environment for two months (EE-2) and 10 mice were left in regular cages (C-2). These groups were submitted to Barnes maze when they were 15 and 17 months old. At the end, brains were extracted and 20 mm slices were obtained on a cryostat (−18ºC). Synapses densities from three animals per group were evaluated by imunohistochemistry using primary synaptophysin antibody (1:2000) incubated during six hours, followed by incubation with the biotinylated secondary antibody. The immunolabelings were detected by avidin-biotin system conjugated to horse radish peroxidase. The density of immunolabelings was quantified using a MCID image analysis system. **Results:** At 2 and 5 months old, no difference in memory was observed between C-15 and EE-15. However, at 11 months old, mice from both groups presented a significant difference in time [$F(4, 99) = 17{,}16$, $P < 0{,}0001$], showing that learning has occurred in spite of the cognitive stimulation. When animals were resubmitted to Barnes maze at 17 months old, it was observed a significant difference between the groups [$F(1{,}94) = 6{,}61$, $P = 0{,}0119$] and between time [$F(4, 94) = 3{,}32$, $P = 0{,}0142$]. At 15 months-old, C-2 and EE-2 showed no difference in spatial memory. However, after two months stimulation of EE-2 in the enriched environment, a significant difference between these groups was observed [$F(1, 99) = 43{,}86$, $P < 0{,}0001$]. Imunohistochemistry analysis for synaptophysin in cortex, hippocampus, striatum and nucleus accumbens, showed a significant increase of 15.4% in the number of synapses in the cortex of EE-15, when compared to C-15 (0.222 ± 0.008 ROD, $P < 0.05$). No difference was observed concerning the other groups and other brain areas. **Discussion:** Cognitive stimulation along life-time was effective to improve spatial memory of adult mice (11 months old), when compared to animals with no stimulation. The increase in synaptic terminals in cortex of EE-15 animals supports this observation, as the memories are stored in cortical areas. Apparently, animals that were stimulated only in the aged phase of the aging process showed greater effect on spatial memory recovery. These results let us suggest that cognitive stimulation applied to elderly is as important as that one started earlier in the life-time, which supports the efforts made by social groups to preserve the cognitive function of aged people.

References: Fichman HC, Caramelli P, Sameshima K, Nitrini R. Declínio da capacidade cognitiva durante o envelhecimento. *Rev Bras Psiquiatr*. 27(12):79-82 2005. Riley KR, Snowdon DA, Desrosiers MF, Markesbery WR. Early life linguistic ability, late life cognitive function, and neuropathology: findings from the Nun Study. *Neurobiol. Aging* 26: 341-347, 2005. Segovia G, Arco AD, Mota F. Environmental enrichment, prefrontal cortex, stress and aging of the brain. *J. Neural Transm*. 116:1007–1016, 2009. **Financial support**: PIBIC/CNPq; FAPESP. CEUA/FCMSCSP protocol number: 173

02.043

Characterization of glycinamide as a co-agonist of NMDA receptors. Montenegro VM[1], Setti-Perdigão P, Guimarães MZP, Castro NG ICB-UFRJ − Farmacologia Molecular

Introduction: The NMDA receptor (NMDAr) is a major component in synaptic integration, being responsible for the modulation many neural functions. The activation of the NMDAr requires the binding of an agonist, glutamate being the main endogenous and NMDA the main exogenous one, and of a co-agonist, glycine and d-serine being the two main endogenous ones. These receptors are functionally expressed as hetero-tetramers and are mainly composed by subunits of two separated subfamilies of genes – GluN1 (NR1) e GluN2 (NR2). It has been shown that glycinamide (Gly-NH_2) acts as an agonist of the glycine (Gly) site and it can lead to increased levels of cGMP in the central nervous system in *in vivo* assays. In these studies glycinamide was shown to have greater efficacy than glycine (Rao et al., *Neuropharmacol*., 29(11), 1075, 1990). However, in binding experiments using [^{3}H]TCP, a pore ligand of the NMDAr, glycinamide has shown an efficacy of 73% of the glycine response and a EC_{50} of 39.3 microM against 0.72 microM from glycine (Monahan et al., J. Neurochem., 53(2), 370, 1989). Our goal in this study is to evaluate glycinamide's potency and efficacy using a functional and molecular approach. **Methods:** We used the patch-clamp technique in the whole cell configuration to measure the transmembrane currents elicited by short applications of NMDA at 50 microM alone and in the presence of Gly at 10 microM or Gly-NH_2 at 10 microM or 100 microM. We also tested Gly-NH_2 at 10 or 100 microM alone. Experiments were conducted in primary cultures of rat hippocampal neurons and the membrane potential was held at −70 mV. All drugs were applied using a parallel tubes system that allows quick exchange between test solutions. All procedures with animals were approved by the institutional comittee (Protocol: DFBCICB 029). **Results:** Both NMDA and Gly-NH_2 showed no measurable response when applied alone in this model. However, the application NMDA 50 microM and Gly-NH_2 100 microM concomitantly elicited a peak current of 309 ± 102 pA (mean ± SD, n = 3) which was 31 ± 8 % (mean ± SD, n = 3) of the control response (NMDA 50 microM + Gly 10 microM) measured in the same cell. The response to NMDA plus Gly-NH_2 10 microM was evaluated in just one cell and its peak current was similar to that elicited by NMDA + Gly-NH_2 100 microM in the same cell. **Discussion:** In our model, glycinamide acted as a co-agonist of the NMDAr. The currents elicited by applications of NMDA 50 microM + Gly-NH_2 at 10 and 100 microM were similar, suggesting that these concentrations are saturating for this binding site. That being the case, we could say that Gly-NH_2 is less efficacious than Gly when activating the receptors expressed in these hippocampal neurons. **Financial Support:** CNPq, CAPES and FAPERJ **Ethics Committee Protocol:** DFBCICB 029

02.044

The expression of mRNA encoding flip and flop isoforms of GLUR1 is increased in hippocampus of isolated young adult rats. Pereira MTR[1], Tonso VM[2], Limonte FH[2], Oliveira FS[3], Iyomasa MM[1], Rosa ML[1] [1]FAMECA-FIPA − Neurociências, [2]FAMECA-FIPA − Bioquímica, [3]USP – Farmacologia

Introduction: It has been shown that the glutamatergic neurotransmission in hippocampus is part of the brain mechanisms involved on depression. Several studies in postmorten human brains with depression and animal models have reported that AMPA receptors may underlie the glutamatergic mechanisms in this disorder. The social isolation is a chronic affective stress largely used as experimental model of psychiatric disorders like depression. The aim of this study was to investigate the changes in the expression of GluR1 in hippocampus induced by social isolation of young adult rats. **Methods:** Two groups of male Wistar rats (140g, n=6/each) were used. They were housed in a temperature-controlled room (23ºC), on a 12:12-h light:dark cycle, with free access to food and water. The rats were allocated randomly to one of two conditions: 1) grouped, housed 4 per cage and handled 3 times a week; 2) isolated, housed individually and handled once a week. After 10 weeks the animals were deeply anaesthetized, perfused with paraformaldehyde 4% and their brains removed. GluR1 subunit expression was evaluated by immunohistochemistry (40-um sections). The immunopositive cells (IC) were counted by 2 examiners independently, bilaterally, in 3 sections/rat. The average was compared by Student *t*-test ($p<0.05$). Gene expression of flip and flop isoforms of GluR1 were evaluated by quantitative real-time PCR. All experiments were done in triplicate and submitted to Mann-Whitney test ($p<0.05$). **Results:** A significant increase in GluR1-IC was induced by social isolation in CA3 (90%, $p=0.004$) and CA1 (270%, $p=0.001$) of the hippocampus, while no change was found in the hillus of dentate gyrus ($p>0.05$). In agreement with these findings, additional results from real-time PCR have shown that 10 weeks of social isolation resulted in significant upregulation of the genes encoding both isoforms of GluR1, flip (250%, p=0,03) and flop (280%, p=0,03), in the whole hippocampus. **Discussion:** All findings together, the increased expression of GluR1 subunit and the upregulation of the genes encoding flip and flop isoforms in hippocampus induced by isolation, suggest that both isoforms of GluR1 AMPA receptor may underlie the hippocampal glutamatergic mechanism involved on the brain alterations in psychiatric disorders like depression.**Committee on Animal Research and Ethics**: CEUA-School of Medicine of Catanduva, 01/08. **Financial Support**: FAPESP and Padre Albino Foundation.

2.045

Imipramine facilitates adaptation to chronic stress in animals with lesions of serotonergic neurons of the median raphe nucleus. Silva K, Padovan D, Padovan CM FFCLRP-USP − Psicologia e Educação

Introduction: Repeated exposure to aversive events leads to the development of tolerance to stress, which appears to involve the serotoninergic pathway Median Raphe Nucleus (MnRN) – Dorsal Hippocampus (DH). Mal functioning of this pathway leads to learned helplessness in animals and depression in humans. Lesions of the MnRN serotoninergic neurons impair the development of tolerance when animals are submitted to chronic stress (Deakin & Graeff, 1991). The aim of this study was to investigate the effects of chronic treatment with Imipramine (IMI), a tricyclic antidepressant, in rats with lesions in the MnRN and that were exposed to chronic restraint stress. **Methods:** Male Wistar rats with (LESION) or without (vehicle, VEH) lesions of MnRN serotoninergic neurons by local administration of the neurotoxin 5,7-DHT were submitted to chronic restraint (CHRON_RESTR; 2 hours/day/seven consecutive days). Immediately before and 12 hours after each period of restraint, rats received an i.p. injection of Imipramine (IMI, 15mg/kg) or saline (SAL, 1mL/kg). On the eighth day the animals were tested in the Elevated Plus-Maze (EPM). In acute restrained group (AC_RESTR), rats with or without lesion received the i.p. chronic treatment as described before, but were submitted to acute restraint on the last of treatment being tested in the EPM 24 hours later. Percentage of entries (% EOA) and time spent (% TOA) in the open arms of the EPM were recorded and analyzed by a three-way ANOVA followed by Duncan´s test, considering the following factors: LESION, i.p. treatment (TREAT) and stress (acute x chronic) and are represented by Mean±SEM (Standard Error of the Mean). All procedures were previously approved by local ethical committee on animal research (CEUA Protocol 06.1.1131.53.0). **Results:** When submitted to AC_RESTR, IMI (34.1±5.1), increased %TOA when compared to control (17.8±7.6; F1,43=5.7; p<0.05) in intact rats. No differences were observed between groups with MnRN lesions (LESION/IMI: 32.5±2.8; LESION/SAL: 27.0±2.7). In lesioned rats, chronic restraint decreased %EOA (LESION/SAL: 18.8±3.2) and %TOA (LESION/SAL: 14.1±0.7) when compared to VEH/SAL group (%EOA: 33.1±1.8; F1,46=10.4; p<0.05; %TOA: 22.1±5.1; $F_{1,46}$=4.6; p<0.05). IMI did increase %EOA (F1,46=26.7, p<0.05; LESION/IMI: 40.6±2.,0) and %TOA (F1,46=10.4, p<0.05; LESION/IMI: 35.4±4.6) when compared to the other groups (% EOA: LESION/SAL=18.83±3.22; VEH/SAL=33.09±1.83; VEH/IMI=42.06±3.50; %TOA: LESION/SAL=22.1±5.13; VEH/SAL=14.1±3.7; VEH/IMI=33.5±4.6). **Discussion:** Our results showed that lesion of the MnRN serotoninergic neurons did not change the behavior of rats in the EPM after a single episode of restraint, but did decrease open arm exploration after chronic restraint, as previously described in the literature (Kennett e cols. 1985, 1987). This effect on chronically stressed rats with lesions in the MnRN was attenuated after chronic treatment with IMI. These results suggest that integrity of MnRN is not essential for the antidepressant effects of IMI on the development of tolerance to repeated stress. **References:** Deakin, J.FW.; Graeff, F.G. 5-HT Anf mechanisms of defense. *J Psychopharmacology*, v. 5, p. 305-315, 1991; Kennet GA, Dickinson SL, Curzon G. Enhancement of some 5-HT dependent behavioral responsesfollowing repeated immobilization in rats. *Brain Research*. v. 330, p. 253-263, 1985. Kennet GA, Dourish CT, Curzon G. Antidepressant-like action of 5-HT1A agonists and conventional antidepressants in an animal model of depression. *Eur J Pharmacol*. v. 134, p. 265-274, 1987. **Financial support**: FAPESP and CAPES

02.046

Allopregnanolone antidepressive and stress activity evaluation after nucleus *accumbens* administration in rats. Ferri MK[1], Dalpra WL[1], Azeredo LA[1], Couto-Pereira N[2], Nin MS[1], Gomez R[1], Barros HMT[1] [1]UFCSPA − Farmacologia, [2]UFCSPA − Ciências Fisiológicas

Introduction: Under certain conditions the levels of allopregnanolone (ALLO), a GABAergic positive modulator, may reach concentrations sufficient to positively modulate the $GABA_A$ receptor[1]. Animal models of depression, such as the social isolation and the forced swimming test (FST), confirm the ALLO involvement in the etiology of depression[2,3]. In the FST there is a reduction in immobility time after intracerebral or intraperitoneal ALLO administration in rats[4,5,6], with changes in mRNA $GABA_A$ receptor subunits[3]. Animal studies suggest a circuitry of brain areas for ALLO effect, such as striatum, hippocampus, frontal cortex[7], hippocampus[8,3] and the *nucleus accumbens*[9]. Treatment with direct and indirect GABA agonists decreases the grooming frequency, while selective $GABA_A$ receptor antagonists increase grooming. Sometimes, anxyolitics do not alter classical measures of grooming activity, but alter more specific grooming sequence measurements[10,11,12]. The aim of the present study was to verify the antidepressive and stress effect of bilateral intra-nucleo-accumbens (intraNA) administration of ALLO, in the FST and in the grooming test. **Methods:** Wistar male rats were treated with control solution (SolC): 2-hydroxypropil-b-cyclodextrin 20% w/v in artificial cefalo-rachidian liquid fluid; ALLO 1.25 mg/rat; ALLO 2.5 mg/rat or ALLO 5.0 mg/rat. The microinjection was carried out in bilateral cannulae (1.6, 1.8 and 6.6, anterior, lateral and ventral from bregma, respectively), 0.5 ml/side and 0.25 ml/min. Each rat received ALLO or SolC, 24, 5 and 1 h before the test section, and behaviors were videotaped during 300s for posterior analysis. At the end of the experiments, the animals were decapitated and brains were removed and freezed for posterior histological analysis. The results are showed at mean ± SD. One Way ANOVA, followed by SNK test was used when $p<0.05$. All experiment followed the guidelines of the International Council for Laboratory Animal Science (ICLAS) and were approved by the Ethical Committee for Research of FFFCMPA (nº 834/09). **Results:** Bilateral intraNA ALLO administration reduced immobile behavior ($F_{(3, 38)} = 3.36$; $p = 0.029$), comparing the control group to the 5.0 mg/rat (SolC: 202.0±48.0; ALLO5.0: 129.1±63.6; $p=0.019$) and increased climbing behavior ($F_{(3, 38)} = 4.26$; $p=0.011$) between the same groups ($p=0.007$). Overall, grooming behavior was decreased after ALLO treatment analyzing the % of Correct Transitions ($F_{(3, 29)}= 3.34$; $p=0.037$), were the control (SolC: 55.7±4.5) is higher than ALLO1.25 (ALLO1.25: 37.9±8.8; $p<0.005$) and than ALLO5.0 group (ALLO5.0: 42.4±10.4; $p<0.005$). The classical grooming analysis did not show any difference ($F_{(3, 29)}= 1.74$; $p=0.180$). There was no significant correlation between FST and grooming behaviors effects. **Discussion:** Intra-NAc ALLO 5.0 mg/rat administration presents antidepressive-like effect, confirming the potential participation of neurosteroids in depression etiopathology. The assessment of grooming microstructured test assured a better sensitivity to detect lower stress-like behaviors modulated by GABA agents. Further studies should try to detect why the decrease in depression and stress by the neurosteroid are not correlated. **Financial Support:** UFCSPA, CAPES, CNPq, AAPeFATO. **References:** 1. Paul SM, Purdy RH *FASEB J*, v6, p2311, 1992. 2. Matsumoto K. *et al*. *Neuropharm,* v38, p955, 1999. 3. Nin MS *et al. J. Psychopharmac*, v22, n5, p477, 2008. 4. Khisti et al. *Pharmac Biochem Behav*, v67, n1, p137, 2000. 5. Molina-Hernández, *et al*. *Prog Neuropsychopharm Biol Psych*, v28, n8, p1337, 2004. 6. Rodriguez-Landa, *et al*. *J Psychopharmac,* v21, n1, p76, 2007. 7. Uzunov, D.P. *et al*. *Prot Nat Acad Sci USA*, v93, p12599, 1996. 8. Frye CA, Walf AA *Horm Behav*, v41, p306, 2002. 9. Molina-Hernández. *Pharmacol Biochem Behav,* v80, p401, 2005. 10. Barros HM *Pharmacol Toxicol*, v.74, p339, 1994. 11. Kalueff AV, Tuohimaa P. *Brain Res Protoc,* v.13, p.151, 2004. 12. Kalueff, AV, Tuohimaa P. *J Neurosc Meth*, v.143, p.169, 2005

02.047

The expression of mRNAs encoding the flip isoforms of GLuR1 and GLuR2 are decreased in hippocampus of rats reared in isolation from weaning. Trindade LB[1], Sestito RS[1], Kerbauy LN[1], de Souza RG[1], Limonte FH[1], Iyomasa MM[2], Rosa ML[2] [1]FAMECA − Bioquímica, [2]FAMECA-FIPA − Neurociências

Introduction: There is growing evidence implicating the glutamate (Glu) system in the pathophysiology of schizophrenia. Flip and flop isoforms of GluR2 AMPA receptor have been shown to be decreased in the hippocampal formation in schizophrenics. Rats reared in isolation from weaning have been used as an experimental model of affective disorders like schizophrenia. In this model it was reported a decrease in the expression of GluR1 and GluR2 subunits in rat hippocampus. This study aimed at evaluating the changes in mRNA expression encoding GluR1 and GluR2, flip and flop isoforms, in rat hippocampus induced by isolation rearing. **Methods:** Two groups of Wistar rats (n=5-8/each) were used. In both groups the pups remained with their mothers (6 pups per mother) until weaning (21 days − 40g) when they were allocated randomly to one of two conditions: 1) grouped, housed 3 per cage and handled 3 times a week; 2) isolated, housed individually and handled once a week for cleaning purpose. After 10 weeks all animals were deeply anaesthetized, perfused and their brains removed. 12-um sections of the hippocampus were used for radioactive in situ hybridization (^{35}S). The signal was quantified bilaterally in 3 sections/rat and the average for grouped and isolated compared by Student *t*-test ($p<0.05$). **Results:** Isolation rearing induced a significant decrease only on flip isoform of both GluR1 and GluR2 in hippocampus while no significant changes were observed on flop isoform. For GluR1 flip the reduction was 9% in dentate gyrus, 11% in CA3 and 12% in CA1 ($p=0.01$). For GluR2 flip the reduction was observed only in dentate gyrus (12%, $p=0.04$). **Discussion:** These results confirm that GluR1 and GluR2 mRNAs are reduced in hippocampus of rats reared in isolation from weaning and indicate that these reductions occur specifically in the flip isoform of both subunits. These findings suggest that isolation rearing induces an alteration in the hippocampal flip:flop ratio, which probably changes the kinetic properties of the receptor. These alterations are similar to those reported for human brains with schizophrenia. Committee on Animal Research and Ethics: CEUA-USP/Ribeirao Preto: 05.1.769.53.0. **Financial Support:** FAPESP (05/01501-7; 06/53343-9; 06/53345-1; 06/53342-2; 06/53344-5) and FPA.

02.048

Neuroprotective effect of propofol in model of hippocampal ischemia in rats. Binda NS[1], Pessoa FLC[2], Pinheiro ACN[1], Silva JF[3], Lavor MSL[4], Gomez RS[6], Gomez MV[3] [1]UFMG − Farmacologia, [2]UFMG − Medicina, [3]UFMG − Farmacologia Bioquímica e Molecular, [4]UFMG− Clínica e Cirurgia Veterinária, [5]UFMG − Cirurgia

Studies in laboratory animals have shown that anesthetic agents reduce infarct size and improve neurologic outcome after transient focal and incomplete hemispheric ischemia (Gelb and cols, 2002). Propofol (2,6-diisopropylphenol) is an intravenous sedative–hypnotic agent commonly used in anesthesia and intensive care that has been tested as a neuroprotective agent in models of cerebral ischemia. Both positive (Velly and cols, 2003) and negative (Qi and cols, 2002; Feiner and cols, 2005) results have emerged from *in vitro* studies. This current study evaluated the neuroprotective effects of propofol in rat organotypic hippocampal slices exposed to oxygen deprivation and low glucose (ODLG). **Methods:** Experimental Animal Ethics Committee: 4201092005-6. The neuroprotective effects of propofol were evaluated in rat organotypic hippocampal slices exposed to ODLG, an *in vitro* model of cerebral ischemia. To investigate its possible mechanism of action were used the following pharmacological tools: tetrodotoxin (TTX), EGTA and ω conotoxin MVIIC. After ODLG insult, cell viabilility in hippocampal slices was assessed by fluorescence microscopy using the ethidium homodimer. **Results:** In all concentrations tested (1-300 µM), propofol was able to reduce neuronal death in the CA1 region of the hippocampus. The maximum effect of propofol in neuronal death reduction (47,02±1,47%) was obtained with the concentration of 100 µM. Imaging of CA1 region of rat hippocampal slices subject to ischemic insult treated with TTX, propofol and propofol with TTX showed a decrease in cell death that amounted to 35,60±2,84%, 25,58±2.11% and 43,30±3.62%, respectively. Cell protection effect was also observed when the slices subject to ischemic insult treated with EGTA (38,70±0,37%), propofol (33,67±3,95%) and both drugs (44,02±3,04%). In addition, rat hippocampal slices subject to ischemic insult treated with ω conotoxin MVIIC , propofol and both drugs showed a decrease in cell death that amounted to 32,84±1,19%, 31,54±1,66% and 44,05±0,32%, respectively. **Discussion:** These results indicate that propofol neuroprotection seems to be dependent on the influx of Na^+ through the Na^+ ion channels sensible to voltage and extracellular Ca^{2+} influx. ω conotoxin MVIIC and propofol presented additional effects regarding neuroprotection indicating that neuroprotetion by propofol could be independent of blocking voltage-dependent -N and -P/Q type Ca^{2+} channels. Therefore, this study suggests that propofol presents neuroprotector effects in hippocampus slices submitted to deprivation of glucose and oxigen. The mechanism of this neuroprotection seems to involve the reduction of the neuronal Na^+ and Ca^{2+} influx independent of -N, -P/Q type Ca^{2+} channels. **References**: Gelb AW and cols, *Anesthesiology* 96:1183–90 (2002); Velly LJand cols, *Anesthesiology* 99:368–75 (2003); Qi S and cols, Anesth. Analg. 94:655–60 (2002); Feiner JS and cols, *Anesth. Analg*. 100:215–25 (2005). **Supported by**: FAPEMIG, CNPq, Instituto Milênio.

02.049

Glial cells are important in protecting neurons after ischemia induced by glucose deprivation. Lopes DCF, Matsubara CS, Franco LAM, Sá Lima L, Scavone C, Munhoz CD
ICB-USP − Farmacologia

Introduction: Cerebral ischemia is a major neurological insult that disrupts brain function and causes neuron death. The role of glial cells in the development of neurodegenerative damage is a complex phenomenon that comprises positive and negative responses for neuronal survival. If in one hand, microglial and astrocytic activation during neuronal damage are sought to have detrimental effects and potentiate neuronal damage, on the other hand, a more recent view suggests that glial cells are involved in a variety of physiological functions, such as regulation of neuronal metabolism, neuronal activity, plasticity, and synaptic transmission, exerting even a protective role in some neurodegenerative conditions. In this study we verified whether the presence of glial cells in the cerebral cortical primary cultures was important for the neuronal protection after ischemia induced by glucose deprivation (GD). **Methods:** Primary cortical cultures were obtained from newborn rats (P1-P4) as described previously (Ahlemeyer, *J Neurosci* **Methods**, 149:110, 2005). Mixed cultures were maintained in DMEM High Glucose media supplemented with 10% Fetal Bovine Serum, 10% Horse serum and 0,1% penicillin/streptomycin. Enriched neuronal cultures were maintained in DMEM High Glucose media supplemented with 2% B27 and 0,1% penicillin/streptomycin. Experiments were performed on days 10 to 12 after culturing. For GD, cultures were washed once with balanced salt solution without glucose to remove trace amounts of glucose, and the cells were maintained in aCSF (artificial cerebrum-spinal fluid) media lacking glucose (Medvedeva, *J Neurosci*, 29(4):1105, 2009). Control cultures were treated the same way except they were maintained in aCSF media with 10 mM glucose. After 24 h of GD, cells were reperfused with 20 mmol/L glucose. After cultures were reperfused for 24 and 48 h, cells were submitted to LDH assay to measure cellular viability. For immunofluorescence assays, cells were cultured in cover slips, fixed with cold methanol, and stained using a neuron specific marker (MAP2), astrocytic marker (GFAP), and a microglial marker (Iba-1) as described previously (Piccioli, *J Neurosci Res*, 66:1064, 2001). **Results:** The mixed cultures were composed by 20% to 30% neurons, 20 to 30% microglia, and 50% to 60% astrocytes. The phenotype of our enriched neuronal culture was 60% to 70% neurons, 10% to 20% microglia, and 20% to 30% astrocytes. In mixed cultures, 24 hours of GD followed by 24 hours reperfusion did not induce cellular death (control 7,6 ±0,2%; control GD 15,5 ±1,3%; GD24/RP24 13,5 ±1,1%) while 48h reperfusion induced significant damage to the cells (control 19,3 ±0,4%; control GD 22,8 ±1,1%; GD24/RP48 27,5 ±0,2%). In enriched neuronal cultures, 24 hours of GD followed by 24 hours reperfusion already damaged the cells (control 4,8 ±0,4%; control GD 13,1 ±2,2%; GD24/RP24 18,0 ±0,5%), which was potentiated when reperfusion was increased to 48 hours (control 10,3 ±0,2%; control GD 21,1 ±0,9%; GD24/RP48 35,7 ±0,8%). **Conclusions:** Our results show that the culture conditions used here are a good model for providing two different phenotypes of cells population in culture and suggest that glial cells are important in the maintenance of neuronal survival after an ischemic insult. **Apoio Financeiro:** FAPESP, CNPq e CAPES

02.050

Interaction between 5-HT_{1A} receptor and a nitric oxide donor, sin-1, ON locomotor activity of rats. Gualda LBS[1], Martins GG[2], Guimarães FS[3], Oliveira RMMW[1] [1]UEL − Farmácia e Farmacologia, [2]UEM − Farmácia e Farmacologia, [3]FMRP-USP

Introduction: The dorsal raphe nucleus (DRN) has been considered an important component of the brain circuit that mediates anxiety- and depression-related behaviors (Abrams et al., Ann. NY Acad. Sci.1018:46-57, 2004). It contains a large proportion of nitric oxide (NO)-producing neurons (Onstott et al., *Brain Res*. 610:317-324, 1993). Recently, it was demonstrated that direct injection of L-Arginine, a NO precursor into the DRN, resulted in an anxiolytic-like effect. This effect, however, was limited and the dose-response curve presented an inverted U shape. Otherwise, the NO synthase (NOS) inhibitor L-NAME into the DRN decreased the general motor activity of the animals, measured in the elevated plus maze model of anxiety. Recently, it has been demonstrated that animals expressing high level of 5-HT_{1A} receptor presented anxiety-like behavior concomitant with a decreased exploratory activity when exposed to a novel environment (Bordukalo-Niksic et al., *Behav Brain Res*., 2010 doi:10.1016/j.bbr.2010.05.002). This became more interesting and complex the question about the influence of NO on modulation of 5-HT neurotransmission. **Objective:** The aim of this study was to compare the locomotor effects produced by direct administration of the NO donor, SIN-1, and the 5-HT_{1A} agonist 8-OH-DPAT and a 5-HT_{1A} receptor antagonist WAY-100635, into the DRN of rats submitted to the open field model. **Methods:** Male Wistar rats (280-310g) with stainless steel cannulae aimed at the DRN received intra-DRN microinjections of WAY or 8-OH-DPAT and 10 min later receive SIN-1 150 nmol and immediately were submitted to the open field model for 10 min. The behavior was videotaped and the distance travelled (cm) was calculated for each animal with the aim of Ethovision software. Data (mean±SEM) were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons. The experimental procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, (CEEA n^{o} 003/2008). **Results and Discussion:** SIN-1, a NO donor, at 150 nmol significantly increased the distance traveled by rats in the open field ($F_{2,31}$ = 11.28, p = 0.002). Overall, the effects of the pretreatment with 8-OH-DPAT and WAY-100635 before SIN-1 administration showed significant difference. The 8-OH-DPAT ($F_{3,30}$ = 4.12, p = 0.015) blocked the effects of SIN-1 on distance travelled. However, no significant effect was detected with WAY-100635 pretreatment (p > 0.05, Newman Keuls' *post hoc* test). None of the drugs administered alone produced significant effects on locomotor activity (p > 0.05, Newman-Keuls *post hoc* test). Further studies using NO scavengers will be conducted in order to elucidate the mechanism involved in the locomotor effects induced by NO and to address the question if they can be produced independently of anxiolytic-like effects.

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02.051

Glutamatergic neurotransmission within the hypothalamic paraventricular nucleus is involved in the cardiovascular response evoked by noradrenaline microinjected into the dorsal periaqueductal gray area of rats. Pelosi GG[1], Busnardo C[2], Tavares RF[2], Corrêa FMA[2] [1]UEL − Farmacologia, [2]FMRP-USP − Farmacologia

Introduction: The dorsal periaqueductal gray area (dPAG) is an important brain region involved in cardiovascular modulation. Previously, we showed that noradrenaline (NA) microinjection into that mesencephalic structure caused pressor response mediated by vasopressin release which involves a relay in the hypothalamic paraventricular nucleus (PVN). However, the local PVN neurotransmission involved in its mediation is unknown. Glutamate is the main excitatory neurotransmitter in the central nervous system. In this direction, in the present study we evaluated the involvement of PVN glutamatergic receptors in the cardiovascular response to NA microinjection into the dPAG of unanesthetized rats. **Methods:** Experimental procedures were carried out following protocols approved by the ethical review committee of the School of Medicine of Ribeirão Preto, University of São Paulo (007/2007). Male Wistar rats were used (240-260g). Guide cannulas were stereotaxically implanted in the dPAG and PVN of all animals. A catheter was introduced into the right femoral artery for blood pressure and heart rate recording. **Results:** The microinjection of the selective NMDA receptor antagonist LY235959 (2 nmol/100nL) into the PVN did not affect the cardiovascular response (before, □MAP: +38 ± 5 mmHg and □HR: -36 ± 4 bpm; and after, □MAP: +39 ± 6 mmHg and □HR: -36 ± 2 bpm, n=6) evoked by NA injection (15nmol/50nL) into the dPAG. Otherwise, PVN pretreatment with the selective AMPA receptor antagonist NBQX (2 nmol/100nL) significantly reduced the pressor and cardiac response caused by NA microinjection into the dPAG (before, □MAP: +36 ± 3 mmHg and □HR: -50 ± 7 bpm; and after, □MAP: +13 ± 4 mmHg and □HR: -24 ± 7 bpm, n=8; One-Way ANOVA followed by Bonferroni post hoc test; PAM: F=52 and FC: F=5.2;* $p<0.05$). Twenty four hours later, the cardiovascular response to NA into the dPAG was restored (□MAP: +39 ± 4 mmHg; □HR: -37 ± 6 bpm, n=8). **Discussion:** The results suggest the involvement of local PVN glutamatergic synapses in the pressor pathway activated by NA microinjection into the dPAG of unanesthetized rats. In addition, our data point to the participation of non-NMDA glutamate receptors in that mediation. **Financial Support:** FAPESP (proc.: 05/57227-0 and 02/14147-9).

02.052

Relationship of long-term memory evocation and cholinergic markers in hippocampus, along the aging process of rats. Oliveira EM[1], Souza LHJ[1], Schowe NM[1], Albuquerque MS[1], Baraldi T[1], Chambergo FS[1], Pina dos Santos VP[2], Araújo MS[2], Buck HS[3], Viel TA[1]
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Introduction: Memory impairment is a frequent complaint of the elderly. Memory loss is linked to either normal aging or progressive dementias and can be related to deficits in cholinergic function. The aim of this work was to evaluate long term memory evocation of rats with different ages and to evaluate the density of some markers of the cholinergic system in the hippocampus of those animals. **Methods:** 3, 6, 12, 18 and 22 months old (m-o) male Wistar rats were submitted to inhibitory avoidance equipment (0.5 mA, 2 sec, max of 180 sec) 24 hours (test session – TS) after the acquisition session (AS), to evaluate long term memory (LTM). After that, brains were extracted and freezed at -80ºC. Half part of them was cut in a cryostat (20 mm, -18ºC) and thaw-mounted on gelatin coated slides. Autoradiography (ARG) for α7 nicotinic acetylcholine receptor (nAChR) was conducted using [^{125}I]-α-bungarotoxin (5 nM, 90 min, 25°C). Non-specific binding was assessed using 2 uM of the unlabelled toxin. The other half part was used for western blotting analysis. 100 ug of protein extracted from hippocampus of rats with different ages were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were incubated overnight at 4º C with primary choline acetyltransferase (ChAT) antibody (1:1500), incubated with a biotinylated secondary antibody and processed by avidin-biotin horseradish peroxidase system. The density of α7 nAChR or immunobloting was analyzed using a MCID image analysis system. **Results:** 3, 6 and 12 m-o rats (but not 18 and 22 m-o animals) showed a significant increase in LTM (8.3, 2.1 and 1.8 fold, respectively, $P < 0.05$). ARG for α7 nAChR in hippocampus showed a significant increase in density of this receptor ($P < 0.01$) along aging, with a distribution similar to an inverted U-shaped curve, mainly in the pyramidal cells, ventral caudal hippocampal area and polymorphic layer of the dentate gyrus. The lowest values were observed for 3 (1.70±0.15 fmols/mg, 32.45±0.78 fmols/mg and 5.28±0.39 fmols/mg, respectively) and 22 m-o rats (1.15±0.90 fmols/mg, 21.61±0.23 fmols/mg and 6.12±0.28 fmols/mg, respectively). Western blotting for ChAT showed a significant increase of 1.93 fold ($P < 0.05$) in abundance of this protein in hippocampus of 12 m-o rats, when compared to 3 m-o animals (0.78 ±0.03 x 10^7 ROD). No difference was observed in samples from other age groups. **Discussion:** Cholinergic system can modulate long term potentiation and, therefore, formation of LTM. We observed an increase in LTM along aging until the age of 12 m-o and a decrease in memory in 18 and 22 m-o rats. In the same way, an increase in α7 nAChR and in ChAT expression was verified in animals until 12 m-o, suggesting an influence of the cholinergic system in the formation of LTM along aging. In the same way, a loss in the maintenance of the functionality of this system in older rats can be related to the memory impairment observed. **Financial support**: FAPESP. CEUA/FCMSCSP protocol #: 175

02.053

Effects of isolation rearing on the expression of B-amyloid precursor proteins, isoforms 695 and 751/770, in rat hippocampus. Kerbauy LN[1], de Souza RG[2], Trindade LB[1], Sestito RS[1], Limonte FH[2], Iyomasa MM[2], Rosa ML[2] [1]FAMECA-FIPA − Bioquímica, [2]FAMECA−FIPA Neurociências

Introduction: The isoforms 695 and 751/770 of the amyloid precursor protein (APP) have been shown to be involved in physiological and pathological processes in the brain. However, the roles of these APPs are unknown. Rats reared in isolation from weaning have been used as experimental model of affective disorders like schizophrenia. This study aimed at evaluating the changes induced by isolation rearing on the expression of both isoforms of APP 695 and 751/770 in rat hippocampus. **Methods:** Two groups of Wistar rats (n=12/each) were used. In both groups the pups remained with their mothers (6 pups per mother) until weaning (21 days – 40g) when they were allocated randomly to one of two conditions: 1) grouped, housed 3/cage and handled 3 times/week; 2) isolated, housed individually and handled once/week for cleaning purpose. After 10 weeks all animals were deeply anaesthetized, perfused and their brains removed. The expression of APP695 and APP751/770 were evaluated by immunohistochemistry (40-um sections, n=6/group). The immunopositive cells (IC) were counted by 2 examiners independently, bilaterally, in 3 sections/rat. The average was compared by Student *t*-test ($p<0.05$). The expression of mRNAs were evaluated by radioactive in situ hybridization (12-um sections, n=6/group). The signal was quantified bilaterally in 3 sections/rat and the average for grouped and isolated compared by Student *t*-test ($p<0.05$). **Results:** Isolation rearing induced a significant decrease in APP695-IC (43%) only in the hillus of dentate gyrus ($p=0.001$) while no difference was seen in any other hippocampal area. APP751/770-IC were noted only in CA2 area of the hippocampus, where a significant reduction (38%) was induced by isolation rearing ($p>0.001$). However, these findings were not seem on the expression of mRNAs by in situ hybridization in any area of the hippocampus. **Discussion:** Isolation rearing induces changes in the expression of APP695 and APP751/770 in hippocampus of rats, suggesting that both isoforms of APP in this area may be involved on the brain alterations that occur in schizophrenia. Committee on Animal Research and Ethics: CEUA-USP/Ribeirão Preto: **05.1.769.53.0** Financial Support: FAPESP (05/01501-7; 06/53343-9; 06/53345-1; 06/53342-2; 06/53344-5) and FPA.

02.054

Influence of glucocorticoids in the increase of CRF_2 mRNA levels in the lateral septal nucleus of rats submitted to chronic unpredictable stress. Malta MB[1], Sita LV[2], Silva JM[2], Bittencourt JC[2], Scavone C[1], Munhoz CD[1] [1]ICB-USP − Farmacologia, [2]ICB-USP − Anatomia

While acute stress initiates neuronal responses that prepare an organism to adapt to challenges, chronic stress may lead to maladaptive responses that could result in psychiatric syndromes such as anxiety. Corticotropin-releasing factor (CRF), acting through its receptors CRF_1 and CRF_2, is a neuropeptide responsible for initiating the endocrine, autonomic and behavioral responses to stress. The aim of this work was to investigate whether chronic unpredictable stress (CUS) could modulate the CRF_2 mRNA levels in the intermediated part of the lateral septal nucleus (LSi) of male Wistar rats. Adult male Wistar rats (300-350g) were randomly assigned to either the control or CUS group. CUS paradigm was applied as described in Munhoz et al. (*J. Neurosci.;* 26(14):3813, 2006). Twenty four hours after the last stress session, the animals were anesthetized with chloral hydrate (35%, 1mL, i.p.) and perfused via ascending aorta with saline followed by 4% formaldehyde). All experiments were conducted in accordance with the ethical principles in animal research adopted by the Institute of Biomedical Sciences – Ethical Committee for Animal Experimentation (102/06/CEEA ICB-USP). In situ hybridization showed that CUS increased CRF_2 mRNA in the LSi when compared to control ($p<0.05$), an effect mediated by corticosterone, since metyrapone, a compound that blocks corticosterone synthesis by inhibiting the enzyme 11-beta-hydroxylase, was able to prevent this increase. Therefore, the present data indicates that CUS induces changes in CRF_2 mRNA levels in the LSi of the rat brain, suggesting that these receptors might be involved in modulatory responses associated to CUS in central nervous system, an effect mediated at least partially by the glucocorticoids hormone.

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02.055

Overactive bladder induced by spinal cord injury: implication of TRPA1 receptor. Andrade EL[1], Forner S[1], Bento AF[1], Leite DFP[1], Dias MA[2], Leal PC[3], Koepp J[4], Calixto JB[1] [1]UFSC − Farmacologia, [2]ISCAL − Neurocirurgia, [3]UFSC − Química, [4]UFSC − Engenharia Química

Introduction: Spinal cord injury (SCI) causes serious alterations in lower urinary tract function, as the development of overactive bladder (OB). The relevance of TRPA1 (ankyrin-repeat transient receptor potential 1) was investigated in OB following SCI. **Methods and results**: Spinal cords of anesthetized male Wistar rats (270-300g) were injured at T10 level by inserting a Fogarty 2F embolectomy catheter (ethical committee process 000156). SCI resulted in locomotor disturbance and urinary bladder (UB) damage as urothelial cell loss, edema formation, vessels congestion, fibrin deposition and neutrophil infiltration. The neutrophil migration to UB was confirmed by means of the increase in mieloperoxidase activity (70-fold; day 2 after SCI). Immunohistochemistry analysis revealed the TRPA1 expression up-regulation in UB on days 7, 14 and 28 after SCI (4.3; 2.9 and 3.2-fold increase respectively) as well in the dorsal root ganglion (DRG) neurons (L6-S1) (7d: 48; 14d: 52-fold increase), but not in corresponding of spinal cord segment. This increase was associated with the enhancement in mRNA levels in BU and DRG neurons (7d: 9- and 2-fold increase, respectively). TRPV1 (transient receptor potential vanilloid 1) expression was also up-regulated in UB (28d: 8.5-fold increase), DRG neurons (7d: 21.5-fold increase) and corresponding segment of spinal cord (2d: 1.7; 7d: 2.5-fold increase) after SCI. Moreover, the functional studies showed that the TRPA1 agonist cinnamaldeyde (300 μM) or TRPV1 agonist capsaicin (0.1 μM)-induced contraction were significantly higher in SCI UB in comparison to sham UB (14d: 43 ± 14% and 28d: 61 ± 18% or 14d: 98 ± 46%; 28d: 155 ± 34% respectively). The pre-incubation of the UB preparations on day 14 after surgery with HC-030031 (TRPA1 antagonist; 10-100 μM) or with SB-366791 (TRPV1 antagonist; 10-300 μM), significantly reduced the cinnamaldehyde-induced contraction in sham and SCI groups. The mean IC_{50} values were: HC-030031 [sham: 46 (24 − 9); SCI: 31 (9 − 53) μM] and SB-366791 [sham: 46 (11 − 81); SCI: 60 (1 − 119) μM]. Comparatively to the sham group, the SCI group showed increase in the amplitude of the BU spontaneous activity, which was significantly reduced (53 ± 10%) by the intrathecal treatment with the TRPA1 antisense oligodeoxynucleotide (AS-ODN: 0.5 nmol/μl). Moreover, TRPA1 AS-ODN reduced by 53 ± 7% and 52 ± 7% the cinnamaldehyde-induced contraction in SCI and sham UB, respectively. During the BU filling, SCI animals showed more non-voiding contractions (NVCs) (13 ± 2) than sham-operated animals (1 ± 0.3). The intravenous treatment with HC-030031 decreased the amplitude and number of NVCs by 42 ± 15% and 67 ± 9%, respectively, while the intrathecal treatment with TRPA1 AS-ODN decreased the number of NVCs by 52.1 ± 15.6%. **Discussion:** These results suggest that the TRPA1 and TRPV1 up-regulation is involved in the mechanism inducing OB in SCI rats, and that TRPA1 blockade could modulate SCI-induced OB. Thus, TRPA1 might constitute a potential target to develop a new therapy for OB. **Financial Support:** CAPES, CNPq, FINEP, FAPESC, PRONEX.

02.056

Neonatal morphine exposure alters E-NTPDase activity and gene expression pattern in spinal cord and cerebral cortex of rats. Rozisky JR[1], Silva, RS[2], Adachi LNS[1], Bogo MR[2], Bonan CD[2], Torres ILS[1] [1]UFRGS − Farmacologia, [2]PUCRS − Biologia Celular e Molecular

Introduction: Studies have shown that exposure to opioids in early life can have implications for nervous system development. It has been proposed that adenosine is involved in opioid antinociception, and ATP is involved in central and peripheral mechanisms of nociception. Extracellular nucleotides can be hydrolyzed by E-NTPDases and ecto-5'nucleotidase, which present the functions of removing ATP and generating adenosine. In this study, we evaluated ATP, ADP, and AMP hydrolysis in synaptosomes from spinal cord and cerebral cortex after repeated morphine exposure in early life. Additionally, we evaluated E-NTPDases and ecto-5'nucleotidase gene expressions. **Methods:** were utilized male *Wistar* rats, which were divided into two groups: control (C) and morphine (M), which received saline (0.9% NaCl) or morphine (5 µg s.c. in the mid-scapular area) at postnatal day 8 (P8), once a day for 7 days. At P16 the animals were killed and the chosen structures were removed for enzyme assays and analysis of gene expression. The synaptosomes were isolated as described by Nagy et al. (Nagy, J Neurochem. 43:1114, 1984). The reaction medium used to assay ATP, ADP and AMP hydrolysis was performed as described by Battastini et al. (Battastini, Neurochem Res 16:1303, 1991). The enzyme assays were performed on spinal cord and cerebral cortex (6 animals per group). Analysis of E-NTPDase expression (E-NTPDase 1, E-NTPDase 2 and E-NTPDase 3) and of ecto-5'nucleotidase was carried out with a semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay, and it was analyzed in spinal cord and cerebral cortex (3 animals per group). Data were expressed as mean ± SEM for the enzyme activities and percentage for E-NTPDases expression. Enzyme activities were expressed as nmolPi.min^{-1}.mg^{-1} of protein. Statistical analysis were made by Student's *t* test, considering $P<0.05$ as significant. This study was approved by the Ethics Committee at the Institution of UFRGS (2006661 and 2007715). **Results:** we observed a decrease in ADP hydrolysis in spinal cord (ADP: C=82.55±1.6, M=51.85±10.3, Student's test, $P<0.05$; ATP: C=155.6±10, M=142.3±14; AMP: C=8.6±1.9, M=6.5±1.1; Student's test, $P>0.05$) and an increase in ATP hydrolysis in the cerebral cortex (ATP: C=161.6±27.5, M=213±30.9, Student's test, $P<0.05$; ADP: C=95.9±19.5, M=104.3±17.6; AMP: C=12.4±1.9, M=14.5±2.8, Student's test, $P>0.05$). Expression levels of E-NTPDase 1 decreased in cerebral cortex (28%, optical density for C=230.08, and for M=165.3; Student's *t* test, $P<0.05$) in the M group when compared to C group, and increased in spinal cord (23%, optical density for C=120.61, and for M=148.26; Student's *t* test, $P<0.05$) in the M group when compared to C group. There were no differences in other E-NTPDases (2 and 3) and ecto-5'nucleotidase expression between the groups in both structures. **Discussion:** Our findings highlight the importance of the purinergic system in young rats submitted to repeated morphine exposure by showing that in the neonatal period such exposure is capable of affecting the control system for nucleotide levels, which can promote changes in modulation or transmission of painful stimuli. **Financial Support:** CNPq, FAPERGS, Propesq-UFRGS.

02.057

Fish oil prevents orofacial dyskinesia, memory loss and lipid peroxidation induced by haloperidol in rats. Bürger ME[1], Barcelos RCS[2], Benvegnú DM[1], Müller LG[2], Reckziegel P[2], Pase C[2], Emanuelli T[3], Boufleur N[2] [1]UFSM − Farmacologia, [2]UFSM − Fisiologia e Farmacologia, [3]UFSM − Tecnologia dos Alimentos

Introduction: Haloperidol is a typical neuroleptic widely used for treatment of mental disorders, but it produces movement disorders and cognitive impairment. The brain is susceptible to oxidative damage because by its high levels of membrane lipids and low antioxidant defenses. Omega-3 (w-3) is an essential fatty acid (EFA) that participates of neuronal membranes, and modifies the membrane fluidity and the cell permeability, affecting the brain physiological functions especially the dopamine system. **Methods and results:** During this experiment, the record on animal ethics committee was not required in UFSM, whose demand occurred in late 2009. Our aim was investigate the possible benefit of fish oil (FO-23% of w-3 EFAs) supplementation (p.o., *ad libitum* in the place of drinking water) on the orofacial dyskinesia and memory dysfunction haloperidol-induced. Data were analyzed by two-way ANOVA followed by Duncan's test ($p<0.05$). Haloperidol (12mg/kg/week-4 weeks) induced vacuous chewing movements (VCM= 56.3±12) and the intake of FO prevented this effect (34,0±19,0). Haloperidol showed impairment in water maze task (22.1±6.7s to find the platform), and this effect was attenuated by FO (13.3±10.5 seconds). Haloperidol increased lipid peroxidation (by TBARS) in striatum and hippocampus (255.3±105.6 hmol and 345.42±37.05 hmol MDA/g tissue, respectively), and the FO reduced it (107.7±58.4 and 214.76±53.16 hmol MDA/g tissue, respectively). **Discussion:** We suggest that the motor and cognitive impairments haloperidol-induced may be closely related to lipid peroxidation in striatum and hippocampus, respectively, and that FO supplementation may decrease the side effects of neuroleptic treatment (Barcelos, *Neurotox Res* DOI: 10.1007/s12640-009-9095-0). **Financial Support:** PROAP – UFSM. Protocol nº 23081.007166/2010-80