03. Psychopharmacology

03.001 5-HT1A receptor activation in the dorsomedial hypothalamus attenuates fear-like defensive behaviors. Biagioni AF, De Oliveira RC, Zangrossi Jr H, Coimbra NC FMRP-USP – Pharmacology

Introduction: Hypothalamic nuclei have been implicated in the organization of defensive responses to aversive stimuli. Moreover, GABAergic dysfunction in dorsomedial hypothalamus evokes panic-like defensive behavior (1). The serotonergic system has been widely suggested to be involved in modulation of panic attacks (2). However, the exact role played by 5-hydroxytryptamine (5-HT) in the dorsomedial hypothalamus (DMH) remains unclear. The aim of the present study was to investigate the involvement of 5-HT_{1A} serotonergic receptor in fear responses elaborated by DMH. **Metodology**: Male Wistar rats (n=6 or 8) were implanted with guide-cannula directed to DMH. After surgery animals were pre-treated with WAY-100635 (0,185nmol), or 8-OHDPAT (1.6nmol), a 5-HT_{1A} receptor antagonist and agonist, respectively. Physiological saline was used as control. After 10 min, bicuculline (40ng/0.2µL), a $GABA_A$ receptor antagonist, or physiological saline was microinjected into-DMH. Immediately after this procedure, each rat was placed in the open-field test where the defensive behaviour was recorded during 10 min. All experimental protocols were approved by local Ethical Committee (protocol number 160/2010). **Results:** Statistical analyses showed that bicuculline injection into the DMH increases the frequency and duration of alertness ($F_{(2,19)}$=11.13; $F_{(2,19)}$=5.89; P<0.05, respectively), escape ($F_{(2,19)}$=12.84; $F_{(2,19)}$=11.37; P<0.001, respectively), defensive backward movements ($F_{(2,19)}$=6.02; $F_{(2,19)}$=4.63; P<0.05, respectively), and the frequency of crossing ($F_{(2,19)}$=17.18; P<0.001). Whereas previous intra-DMH injection of WAY-100635 did not cause any behavioral effect (P>0.05 in all cases), microinjection of 8-OHDPAT significantly impaired the frequency and duration of the escape reaction ($F_{(2,19)}$=12.84; $F_{(2,19)}$=11.37; P<0.01, respectively) evoked by $GABA_A$ receptor blockade in the DMH. **Conclusion**: Despite the fact that microinjection of 5-HT_{1A} receptor antagonist (WAY-100635) showed no effect, 5-HT_{1A} receptors agonist treatment, with 8-OHDPAT, decreased the escape response elaborated by DMH. Thus, serotonin-mediated system might modulate defensive behaviour elaborated by DMH, causing an antiaversive effect, possibly recruiting post-synaptic 5-HT_{1A} receptor. However, additional experiments will be performed to clarify the effect of serotonin on other serotonergic receptor. **References**: [1] Biagioni, A.F., Silva, J.A., Coimbra, N.C. Panic-like defensive behavior but not fear-induced antinociception is differently organized by dorsomedial and posterior hypothalamic nuclei of *Rattus norvegicus* (Rodentia, Muridae). *Braz J Med Biol Res*. 45(4):328-36, 2012. [2] Graeff, F.G., Zangrossi, H. Jr. The dual role of serotonin in defense and the mode of action of antidepressants on generalized anxiety and panic disorders. *Cent Nerv Syst Agents Med Chem*. 10(3):207-17, 2010. **Acknowledgment**: This work was supported by FAPESP 2010/15140-4.

03.002 Transient receptor potential ankirin 1 (TRPA1) mediates antidepressant-like action on forced swimming test in mice. Cavalcante JM[1], Norões MM[1], Soares-Rachetti VP[1], Gavioli EC[1], André E[1] UFRN – Farmacologia Comportamental

Introduction: Transient Receptor potential vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) are involved in many biological processes, including thermal nociception, mechanosensation, and inflammatory hyperalgesia. Some studies have demonstrated the involvement of TRPV1 in psychiatric disorders such as anxiety and depression. However, there are few studies in the literature regarding the expression and function of TRPA1 receptors in the brain tissue. Objective and **Methods**: Thus, the aim of the present study was to investigate a putative role of TRPA1 receptor in the modulation of behaviors related to a psychiatric disorder, the depression, by employing a widely used test to assess the effects of antidepressant drugs, the mouse forced swimming test. The study was approved by UFRN's Ethics Committee on Animal Use (Number: 013/2010). **Results:** The oral administration of TRPA1 receptor agonist, cinnamaldehyde (30 or 50 mg/kg), 90 min before the test, did not affect the mouse behavior in the forced swimming test ($P>0.05$, n=10). Similarly, the treatment with a low dose of antagonist of receptor TRPA1, HC03001 (30 mg/kg, p.o.), 90 min before the test, also did not alter the duration of immobility in the forced swimming test compared to control group. However, at higher doses, the TRPA1 antagonist HC03001 (100 mg/kg and 300 mg/kg, p.o.), 90 min before the test, significantly reduced (117.0±11.00 and 141.5±17.6 sec, respectively, $F_{(2,38)}=7.74$, $p=0.002$, n=13) immobility as compared with control mice (182.1±6.00 sec, n=15). The reduction of immobility induced by the treatment with HC03001 (100 mg/kg, p.o.) was completely reversed by the pretreatment with cinnamaldehyde (50 mg/kg, p.o.) administrated 15 min before of HC03003 ($F_{(3,40)}=10.52$, p=0.0001, n=10). The antidepressant-like effect of HC03003 was not biased by locomotion, since no alterations in the spontaneous ambulation of mice were detected in the open field test. An additional experiment was conducted where a high dose of capsaicin was used to desensitize capsaicin-sensitive sensory neurons. Neonatal animals injected with capsaicin showed less immobility (131.8±13.4 sec, $p<0,018$, n=7) duration as compared with control animals (175.5±8.7 sec). **Conclusion**: In conclusion, our findings suggest that HC03003, a TRPA1 antagonist, orally administered reduced immobility time in the mouse forced swimming test. This effect was reversed by the administration of cinnamaldehyde, a TRPA1 agonist, which was inactive per se, thus suggesting a participation of TRPA1 receptor in mediating the effects of HC03003 in the forced swimming test. Additionally, the antidepressant-like effect of HC03003 was not biased by locomotion, because no alterations in the spontaneous ambulation of mice were detected in the open field test. In addition, the immobility time was also reduced in neonatal capsaicin treated mice, reinforcing the hypothesis that blockage of TRPs channels may be involved in antidepressive-like behavior observed on forced swimming test in mice.. **Financial Support**: Propesq, Reuni, CNPQ, FAPERN.

03.003 Investigation of the involvement of alpha-1-adrenoceptors in the behavioral effects induced by imipramine in the tail suspension and rota-rod tests. Ribeiro CAS, Pupo AS IBB-Unesp – Farmacologia

Introduction: Imipramine is a tricyclic antidepressant drug which is a non-selective inhibitor of norepinephrine and serotonin neuronal reuptake. The adrenoceptor which are the targets for the increased synaptic levels of norepinephrine induced by imipramine are largely unknown. Therefore, this study investigates the involvement of alpha-1 adrenoceptors in the behavioral effects induced by imipramine in the tail suspension (TST) and rota-rod (RRT) tests. **Methods**: All procedures were approved by the local Ethics Committee on Animal Use – CEUA (protocol number418). Male Swiss mice treated with vehicle, imipramine (IMI - 30 mg/kg, IP), prazosin (a selective alpha1-adrenoceptor antagonist, PRA - 0.5 or 1 mg/kg, IP), or the respective association prazosin plus imipramine (IMI+PRA), 30 minutes before being challenged in the TST and RRT. The immobility time was recorded in the TST and the number of falls was recorded in the RRT. Data is presented as mean ± standard error of mean of 8 independent experiments and ANOVA followed by Dunnett test for multiple comparisons was used to check for significant differences. Differences were considered statistically significant when $p \leq 0.05$. **Results:** As expected in the TST, the immobility time of mice treated with IMI (21.0 ± 5.5 seconds/5 min) was lower than that of mice treated with vehicle (98.7 ± 3.3 seconds/5 min, $p<0.05$). However, in mice treated with IMI+PRA the immobility time was not different from that found in mice treated with vehicle (153.0 ± 22.7 seconds/6 min) and 170.3 ± 24.0 seconds/6 min, respectively). Prazosin (1 mg/kg, IP) was not able to affect the performance of mice in the RRT, as the number of falls was not different from that found in mice treated with vehicle (6.8 ± 1.0 falls/3 min and 5.8 ± 1.4 falls/3 min, respectively). These results indicate that the effect of prazosin in the TST is not related to a sedative effect of this drug. **Conclusion**: The results indicate an important participation of alpha1-adrenoceptors in the reduction of the immobility time induced by imipramine in the TST and suggest that alpha-1 adrenoceptors may be one of the targets for the increased synaptic levels of norepinephrine resultant from the inhibition of neuronal reuptake. **Financial Support**: CAPES, FAPESP (08/50423-7 to ASP)

03.004 Evaluation of endogenous and exogenous sexual hormones influences on cocaine-sensitization in female rats. Souza MF[1], Couto-Pereira NS[2], Caletti G[1], Bisognin KM[1], Freese L[1], Olguins D[1], Gomez R[3], Barros HMT[1] [1]UFCSPA – Psicofarmacologia, [2]UFRGS – Bioquímica, [3]UFRGS – Farmacologia

Aim: Chronic intermittent administration of psychostimulants such as cocaine produces behavioral sensitization that is relevant to addictive behaviors. The hormonal differences between males and females may influence cocaine use and its effects, with females experiencing with more intense behavioral effects than males, including higher behavioral sensitization. The aim of this study was verify the influence of female sexual hormones in the behavioral sensitization to cocaine. **Material and Methods:** 120 female Wistar rats (~250g) were used, 96 were submitted to bilateral ovariectomy, and 24 rats to SHAM surgery. The ovariectomized rats were randomly assigned to progesterone 0.5mg/kg (PRO), estrogen 0.05mg/kg (EST), estrogen+progesterone (PRO+EST) or ovariectomized (OVX) groups. The same rats were randomly assigned to control (CTR), acute (ACT) or repeated (RPT) cocaine treatment groups. The hormonal treatment started 24 hours before the first drug administration. Sensitization protocol started ten days after surgery, when CTR and ACT received saline 1 ml/kg i.p. and RPT rats received 15 mg/kg/day cocaine hydrochloride i.p., for 8 consecutive days. After a 10-day pause, ACT and RPT animals received a challenge of cocaine i.p., while CTR received saline. The locomotion was evaluated on 1st, 8th, and 19th days, when rats were individually allocated in a locomotor activity cage with three photocells to monitor horizontal motor activity, during 35 minutes. All experiments were approved by the Ethical Committee for Research of UFCSPA (1034/10). **Results:** In the 1st experimental day, a single dose of cocaine increased locomotion of rats (CTR 132,37±14,42; COC 633,62±55,44), mainly in SHAM and PRO+EST. In this day, 43% of SHAM animals were in the estrus phase. Similarly, 8 consecutive cocaine doses significantly enhanced the locomotion (CTR 104,372±8,95; COC 544,240±66,93), although without significant effect of the hormonal treatment. When compared to 1st day, SHAM and PRO+EST rats treated with cocaine for 8 days presented lower locomotion, while PRO rats presented higher locomotor activity. In the challenge day, the locomotion of RPT animals (420,67±33,54) was significantly higher than ACT animals (282,26±35,02), which, in turn, was higher than CTR group (51,73±35,16). When hormonal condition was analyzed separately, only in SHAM animals did the RPT group have locomotion significantly higher than the ACT group. Additionally, ACT group was significantly higher than CTR only in EST hormonal group. The estrous cycle analysis showed that 48% of SHAM rats were in metestrus in this last day and the hormonal analysis in blood serum indicated higher levels of progesterone (3 times more than OVX). **Conclusion**: Repeated cocaine treatment induced behavioral sensitization in female rats. Moreover, the behavioral response to cocaine in females seems to be differently affected by the female sex hormones. The acute effect of cocaine was potentiated by the concomitant presence of estrogen and progesterone, both endogenously (SHAM) and exogenously (PRO+EST). With eight cocaine treatment days, these two groups presented tolerance to their effects, while progesterone treated rats increased the behavioral effects. Interestingly, sensitization was seen especially in SHAM rats with high levels of endogenous progesterone and estrogen and possibility of cycling. **Financial Support**: UFCSPA, CNPq and CAPES.

03.005 Synergistic interaction between serotonin and opioids in the dorsal periaqueductal gray assessed in the elevated T-maze. Silva PRA[1], Roncon CM[1], Zangrossi Jr H[2], Graeff FG[3], Audi EA[1] [1]UEM – Farmacologia e Terapêutica, [2]FMRP-USP, [3]INeC

Previous results showed that the anti-escape effect of serotonin (5-HT) in the elevated T-maze (ETM) is antagonized by naloxone [1]. To further explore the 5-HT-opoid interaction, this study investigated the association of sub-effective doses 5-HT and morphine administered intra-DPAG in rats submitted to the ETM. The ETM is a model of anxiety that evokes two defensive responses in the same rat, namely inhibitory avoidance and one-way escape, related to generalized anxiety and panic disorder, respectively. Male Wistar rats (UEM Ethics Committee 024/2010) were microinjected (0.5 µL) with 5-HT (2 µg) or saline 5 min before microinjection of morphine (0.3 µg) or saline. The following groups were formed: saline/saline (n=7), 5-HT/saline (n=5), saline/morphine (n=5), 5-HT/morphine (n=6). Rats were submitted to the ETM test, 10 min after the last injection. Locomotion was assessed in an open field, as control for nonspecific motor effects. Repeated-measures analysis of variance (RMANOVA) was used to analyze both avoidance and escape data. Locomotion data were analyzed by one-way ANOVA. When appropriate, the Fisher *post-hoc* test was used. Significance level was set at $p < 0.05$. Sub-effective doses of 5-HT + morphine significantly increased the escape latency (escape 1: sal+sal=8.85±2.23; sal+morf=11.80±2.65 (p=0.38); 5HT+sal=7.80±2.65 (p=0.75); 5HT+morf=18.16±2.41 (p<0.01) and escape 3: sal+sal=7.28±1.98; sal+morf=6.60±2.35 (p=0.84); 5HT+sal=8.00±2.35 (p=0.83); 5HT+morf=16.33±2.14 (p<0.01), indicative of a panicolytic effect while inhibitory avoidance latency unchanged. The distance traveled in open field under the different treatments was not altered when compared to the control groups (p>0.05). These results showed that the association of sub-effective doses of 5-HT and morphine promoted panicolytic effect in ETM and indicate that 5-HT and opioid act synergistically on neurons controlling escape (panic) in the DPAG. **References**: [1] Roncon, C.M., J. *Psychopharmacol* 26, 525, 2012. **Financial Support**: Capes and CNPq

03.006 Naloxone blocks panicolytic-like effect of a 5-HT$_{1A}$-receptor agonist in the dorsal periaqueductal gray: **Evidence from the elevated T-maze**. Roncon CM[1], Biesdorf C[1], Zangrossi Jr H[2], Graeff FG[3], Audi EA[1] [1]UEM – Farmacologia e Terapêutica, [2]FMRP-USP – Farmacologia, [3]INeC

Introduction: Evidence previously obtained in our laboratory indicates that the anti-escape effect of the fluoxetine and 5-HT (serotonin) in the elevated T-maze (ETM) was blocked by intra-DPAG injection of non-selective opioid antagonist naloxone [1]. These results are indicative that serotonergic and opioidergic mechanisms in the DPAG may interact for the regulation of defensive behaviors associated with panic. In this study we investigated whether a previous intra-DPAG injection of naloxone interferes with the behavioral consequences observed in the ETM after intra-DPAG administration of the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agonists, 8-OHDPAT and DOI, respectively. **Methods**: Male Wistar rats (UEM Ethics Committee 024/2010) were microinjected (0.5 µL) with naloxone (0.2 µg) or saline 10 min before microinjection of 8-OHDPAT (1.0 µg), DOI (5.7 µg) or saline. The following groups were formed: saline/saline (n=9), naloxone/saline (n=6), saline/8-OHDPAT (n=6), naloxone/8-OHDPAT (n=7) or saline/saline (n=7), naloxone/saline (n=10), saline/DOI (n=7), naloxone/DOI (n=6). Rats were submitted to the ETM, 10 min after the last injection. Locomotion was assessed in an open field, as control for nonspecific motor effects. Repeated-measures analysis of variance (RMANOVA) was used to analyze both avoidance and escape data. Locomotion data were analyzed by one-way ANOVA. When appropriate, the Fisher *post-hoc* test was used. Significance level was set at $p \leq 0.05$. **Results and Discussion**: 8-OHDPAT significantly decreased inhibitory avoidance latency (avoidance 2: Sal+Sal=71.55±23.22, Sal+8-OHDPAT=17.50±28.44 ($p<0.05$)), indicative of anxiolytic effect and increased escape latency (escape 2: Sal+Sal=5.88±0.96, Sal+8-OHDPAT=10.50±1,17 ($p<0.01$), and escape 3: Sal+Sal=7.11±1.09 , Sal+8-OHDPAT=10.83±1.34 ($p<0.05$)), indicative of panicolytic effect. Naloxone blocked the anxiolytic effect (avoidance 2: Sal+8-OHDPAT=17.50±28.44, Nal+8-OHDPAT=62.57±26.33 ($p<0.05$)) and panicolytic effect (escape 2: Sal+8-OHDPAT=10.50±1.17, Nal+8-OHDPAT=7.00±1.09 ($p<0.05$), and escape 3: Sal+8-OHDPAT=10.83±1.34, Nal+8-OHDPAT=6.14±1.24 ($p<0.01$)) of 8-OHDPAT. DOI significantly increased escape latency (escape 1: Sal+Sal=7.57±1.58, Sal+DOI=13.28±1.58 ($p<0.05$), escape 2: Sal+Sal=5.71±1.69, Sal+DOI=12.14±1.69, ($p<0.05$), and escape 3: Sal+Sal=6.42±2.39, Sal+DOI=14.85±2.39 ($p<0.01$)), indicative of a panicolytic effect, but naloxone not blocked this effect (escape 1: Sal+DOI=13.28±1.58, Nal+DOI=13.16±1.71, escape 2: Sal+DOI=12.14±1.69, Nal+DOI=14.50±1.83, and escape 3: Sal+DOI=14.85±2.39, Nal+DOI=14.50±2.58, ($p>0.05$)) while inhibitory avoidance latency unchanged. 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 suggest that 5-HT$_{1A}$ receptors are involved in 5-HT-opioid interaction in the DPAG. **References**: [1] Roncon, CM, J. Psychopharmacol 26, 525, 2012. **Financial Support**: Capes and CNPq.

03.007 A 5-HT1A receptor antagonist blocked the panicolytic-like effect of morphine in the dorsal periaqueductal gray of rats tested in the elevated T-maze.
Roncon CM[1], Almeida CB[1], Audi EA[1], Graeff FG[2], Zangrossi Jr H[2] [1]UEM – Farmacologia e Terapêutica, [2]USP

Facilitation of opioid-mediated neurotransmission is reported to alleviate anxiety both in healthy subjects as in patients suffering from anxiety disorders [1]. The serotonergic system in the DPAG is involved panic disorder [2]. This study verified whether the intra-DPAG administration of the 5-HT_{1A} receptor antagonist, $WAY_{100635,}$ antagonizes the effect of morphine in rats submitted to the elevated T-maze (ETM). The ETM is a model of anxiety that evokes two defensive responses in the same rat, namely inhibitory avoidance and one-way escape, related to generalized anxiety and panic disorder, respectively. Male Wistar Rats (UEM Ethics Committee 024/2010) were microinjected (0.5 µL) with naloxone (0.2 µg) or saline 10 min before microinjection of morphine (7.6 µg) or saline. The following groups were formed: saline/saline (n=9), WAY_{100635}/saline (n=6), saline/morphine (n=6) and WAY_{100635}/morphine (n=6). Rats were submitted to the ETM test 10 min after the last injection. Locomotion was assessed in an open field, as control for nonspecific motor effects. Repeated-measures analysis of variance (RMANOVA) was used to analyze both avoidance and escape data. Locomotion data were analyzed by one-way ANOVA. When appropriate, the Fisher *post-hoc* test was used. Significance level was set at $p < 0.05$. RMANOVA showed a significant pre-treatment X treatment interaction [$F_{(1,23)} = 8.28$; $p< 0.01$]. *Post-hoc* comparisons showed that morphine significantly increased escape latencies indicating a panicolytic-like effect. This effect was fully blocked by WAY_{100635}. Inhibitory avoidance in the ETM and locomotion in the open field were not significantly affected by the different treatments employed. These results suggest that the panicolytic effect of morphine is enacted by through 5-HT_{1A} receptor-related mechanisms in the DPAG.
References: [1] Colasanti, A., J Psichopharmacol 25, 1415, 2011. [2] Graeff, F.G., Cent Nerv Syst Agents Med Chem 10, 207, 2010. **Financial Support**: Capes and CNPq

03.008 Effects of cannabidiol on haloperidol-induced catalepsy in mice. Sonego AB[1], Gomes FV[1], Del Bel EA[2], Guimarães FS[1] [1]FMRP-USP – Pharmacology, [2]FORP-USP – Morfology, Physiology and Stomatology

Introduction: Cannabidiol (CBD) is a non-psychotomimetic compound from *Cannabis sativa* plant that has been reported to produce anti-psychotic effects in rodents and humans (1). For example, it reverses L-dopa-induced psychotic symptoms and could improve motor function in Parkinson's patients (2). This latter effect raised the possibility that CBD could have beneficial effects on motor related striatal disorders. To investigate this possibility we verified if CBD would prevent catalepsy induced by haloperidol, a typical antipsychotic drug, which induces motor impairment characterized by Parkinson-like syndrome. The catalepsy test is largely used to investigate impairments of motor function caused by interference on striatal function. **Methods**: Male Swiss mice (25-35 g) received acute pretreatment with CBD (15, 30 or 60 mg/kg, i.p.) or vehicle (Tween 80 2%) 30 min prior to the D_2 receptor antagonist haloperidol (0.6 mg/kg). The mice were tested 1, 2 or 4 h after haloperidol. Catalepsy duration was measured up to 5 min. Data were analyzed by two-way ANOVA for repeated measures followed by Bonferroni's post-test. The protocol (nº056/2012) was carried out in compliance with local ethical committee guidelines for animal research. Results

There was a significant effect of treatment ($F_{5,49}$=14.1; P<0.05), time ($F_{2,49}$=29.4; P<0.05) and treatment x time ($F_{10,49}$=2.4; P<0.05). Haloperidol induced catalepsy throughout the experiment (Bonferroni post-hoc, P<0.05). All three doses of CBD attenuated the haloperidol-induced catalepsy in the second hour (HAL=97,8±17,9; CBD15=59,6±9,1; CBD30=58,6±12,7; CBD60=46,5±4,6; P<0,05), but not 1 and 4 h after haloperidol infection. CBD by itself, did not induce catalepsy (Bonferroni post-hoc, P>0.05). **Discussion**: These findings indicate that CBD can attenuate the catalepsy induced by haloperidol, suggesting that it could be useful in the treatment of striatal disorders. **References**: 1 ZUARDI, A. W. *Rev. Bras. Psiquiatr*., v.30, p. 271, 2008. 2 ZUARDI, A. W. *J. Psychopharm*., v.23, p. 979, 2009. **Financial Agencies and Acknowledgments**: CAPES, CNPq and FAPESP

03.009 Inhibition of inducible nitric oxide synthase (iNOS) present in the dorsolateral periaqueductal gray matter of rats decreases anxiety induced by ethanol abstinence in rats. Contardi EB, Bonassoli VT, Milani H, de Oliveira RMMW
UEM-DFT

Introduction: The dorsolateral periaqueductal gray matter (DLPAG) contains a large amount of nitric oxide (NO) producing neurons which have been implicated in the expression of autonomic, emotional and motor expression of anxiety-like behaviors as well as in the development of ethanol withdrawal syndrome in rats[1]. Ethanol withdrawal activated producing neurons NO in the DLPAG[2]. The goal of this study was to investigate the effect of NO synthase (NOS) nonselective inhibitor, L-nitro-arginine methyl ester (L-NAME), L-arginine, a donor of NO and 1400 W, an inducible NOS (iNOS) inhibitor administered directly into the DLPAG during ethanol withdrawal in rats. **Methods**: Male Wistar rats were subjected to an oral ethanol self-administration procedure, in which they were offered 6 e 8% (vol/vol) nutritionally balanced ethanol solution, as the only source of food, for 15 days followed by ethanol abrupt discontinuation. One week before the treatment end, the animals had a canulla implanted directly into the DLPAG. Twenty-four hours after ethanol discontinuation, rats received microinjections of saline, L-Arginine (L-Arg 100 nmol), L-NAME (50, 100 or 200 nmol) or 1400W (0.3, 1.0 and 3.0 nmol)). Ten minutes later, the animals were placed in a light/dark box for 5 min. The time spent in the light side of the apparatus and the latency for the first entry in the dark compartment were registered. The locomotor activity, expressed by the travelled distance (cm) in an open field over a 10 min, was obtained and evaluated by ANYMAZE software®. All procedures were approved by the local committee on animal ethics (CEAE 007/2010). Data were expressed as mean ± S.E.M. and analyzed by one-way ANOVA followed by the Tukey's test for multiple comparisons. **Results:** L-NAME 200nmol increased the latency for entrying in the dark side ($F_{3,38}$=2.97, P<0.001; saline=20.3±3.44; L-NAME 200nmol=38.8±12.3; saline+L-Arg 100nmol=16.7±2.5; L-NAME 200nmol+L-Arg 100nmol= 14.1±3.1) and the time spent in the light side of the light/dark box as compared with saline group ($F_{3,38}$=2.37, P<0.05; saline=31.3±2.6; saline+L-Arg 100nmol=40.6±6.4; L-NAME 200nmol+L-Arg 100nmol= 33.7±7.2; L-NAME 200nmol=51.2±6.1). A similar effect was detected with 1400W 0,3nmol, which increased the time spent in the light compartment of the light/dark box as compared to saline group ($F_{3,40}$=4.0, P<0.05; saline=32.9±2.7; 1400W 0.3 nmol=57.8±8.5; 1400W 1 nmol=42.6±4.3; 1400W 3 nmol=27.9±7.3). No significant difference was observed on the latency or the travelled distance. **Discussion**: Inhibition of NOS and iNOS in the DLPAG by L-NAME and 1400W, respectively resulted in decrease of anxiety levels during ethanol withdrawal. These findings confirm that NO-producing neurons of DLPAG are involved in the anxiety-like behavior observed during the ethanol withdrawal and suggest that, at least in part, the observed anxiolytic effects may be mediated by iNOS inhibition. This study was supported by Fundação Araucária. **References**: Aguiar, D. C. et al., *J Neurosci Res,* n. 87, p.2418, 2009*.* Bonassoli, V.T. et al., *Alcohol*, v.45, p.641, 2011.

03.010 Evaluation of arginase pathway and oxidative status in platelets from patients with major depressive disorder. Oliveira MB[1], Mury WV[1], Pinto NO[1], Costa CA[1], Resende AC[1], Brunini TMC[1], Mendes Ribeiro AC[2] [1]UERJ – Farmacologia e Psicobiologia, [2]UERJ / UNIRIO – Farmacologia e Psicobiologia

Introduction: Major depressive disorder (MDD) is characterized by endothelial and platelet dysfunction and is a nontraditional cardiovascular risk factor. (1,2). We have previously demonstrated that patients with MDD have an impairment of NO production associated with hyperaggregability (1). L-arginine is converted into nitric oxide (NO) by a family of enzymes referred as NO synthase (NOS) (1,2). This amino acid is also used by arginase as a substrate in the urea cycle and is thought to be diverted from NO production (2). Moreover, the generation of reactive oxygen species (ROS) by NADPH oxidases can reduce the half life of NO (3). The purpose of this study was to investigate if the oxidative stress and arginase pathway in platelets from patients with MDD may affect NO bioavailability. **Methods**: Nine patients with moderate MDD without medication (31±2.3 years) and eleven healthy controls (C) (31±2.1 years) participated in this study. Expression of arginase II in platelets was accessed by Western Blotting and activity was analyzed through the conversion of [C^{14}]-L-arginine into [C^{14}]-urea. Catalase (CAT) activity was measured in terms of the rate of decrease in hydrogen peroxide at 240 nm. NOX2, the prototype of NADPH oxidase, was analysed by Western Blotting, where immunoreactivity toward the $p47^{phox}$ subunit was found. The Pedro Ernesto Hospital Ethical Committee approved this study (1436–CEP/HUPE), and informed consent was obtained from each participant. Values are means ± S.E.M. compared by the Student's t-test. **Results:** The expression of arginase II in platelets was not affected by MMD, however, this enzyme activity was significantly increased in this disorder (MDD:30.8±5 vs. C:14±3 pmol urea/mg protein/2h). NADPH $p47^{phox}$ (arbitrary units) was overexpressed in MDD (0,2902 ± 0,04112) compared to controls (0,04038 ± 0,01595) Catalase activity (C:0,22 ± 0,03 vs. MDD:0,27 ± 0,04 U/mg of protein) in platelets and in serum (MDD:0,12±0,03 vs. C:0,14±0,02 U/mg of protein) was similar between patients with MDD and controls. **Discussion**: Thus the present study revealed an activation of arginase which probably shifted L-arginine towards the urea cycle in MDD, reducing intraplatelet NO synthesis. Moreover, overexpression of NADPH $p47^{phox}$ can generate ROS in platelets, increasing NO degradation. These data suggest that in platelets arginase II and NADPH may be involved in the activation of platelets detected in patients with MDD. Keywords: Major depressive disorder, Nitric oxide, Arginase, Oxidative Status, Platelets. **Financial Support**: FAPERJ and CNPq. **References:** 1. Pinto VL, et al. Low plasma levels of l-arginine, impaired intraplatelet nitric oxide and platelet hyperaggregability: Implications for cardiovascular disease in depressive patients. *J Affect Disord,* 2012. 2. Pinto, V. L., T. M. Brunini, et al. Depression and cardiovascular disease: role of nitric oxide. *Cardiovasc Hematol Agents Med Chem*6(2): 142-9, 2008. 3. Bedard, K, Krause, K-H, The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev.* 87: 245-313, 2007.

03.011 Role of the endocannabinoid system in defensive responses mediated by the dorsolateral periaqueductal gray of rats. Viana TG, Aguiar DC, Moreira FA
UFMG – Pharmacology

Introduction: The glutamate plays a role in modulating defensive responses, possibly facilitating the reactions of anxiety and panic. Several brain regions are involved in these responses, including the dorsolateral periaqueductal gray (dlPAG). Studies indicate that endocannabinoid system could modulate glutamate levels and reverse the panicogenic-like effect caused by the excitatory amino acid. Thus, this study investigated the involvement of cannabinoids receptors in an animal model of panic attacks: NMDA injection into the dlPAG. **Methods**: Male Wistar rats (n=4-6/group) underwent stereotaxic surgery for implantation of the cannula guide into dlPAG and after a recovery period were subjected to behavioral tests. In the first protocol, animals received injections of vehicle or ACEA (a CB1 agonist; 0.005;0.05; 0.5 pmol/0.2μL) followed by NMDA 1nmol/0.2μL. In the second protocol the animals received injections of vehicle or URB 597 (endocannabinoid-hydrolysis inhibitor; 0.3;1; 3 nmol/0.2μL) followed by NMDA 1nmol/0.2μL. All previously described groups were placed in an observation box and recorded by 2 minutes.The third protocol consists in evaluate if the pretreatment with AM 251 (a CB1 antagonist) could reverse URB effects.The rats received AM 251(1nmol) 5 minutes before URB 597(3nmol). After 10 minutes, the animals received NMDA and were placed in an observation box. The parameters evaluated were crossings and jumps. c-Fos expression was analyzed for the groups Vehicle-Vehicle, Vehicle-NMDA, URB (3nmol) - NMDA and Vehicle-URB (3nmol) The results were analized by Kruskal-Wallis followed by Mann–Whitney and expressed by median ± I.R. **Results:** The NMDA injection produced flight reactions, characterized by increase of crossings (29.00 / 36.50 / 46.00; p= 0.0089, Kruskal-Wallis) and jumps (18.25 / 29.00 / 34.25; p= 0.0043, Kruskal-Wallis).The pretreatment with ACEA reduced the number of crossings and jumps in all dosis used. The pretreatment with URB 597 0.3 nmol, 1 nmol, 3 nmol significantly reduced the number of crossings (p=0.0411, p=0.0281 and p= 0.0043 respectively; Mann–Whitney).The jumps were reduced only by URB597 0.3 nmol and 3 nmol (p=0.0079 e p=0.0041 respectively; Mann–Whitney). The pretreatment with AM 251 reverse the effect of URB 597(3 nmol) (p=0.0195 and p=0.0179 for crossings and jumps, respectively; Mann-Whitney). NMDA significantly increased cFos positive cells in the contralateral dlPAG (19.50/31.00/44.00 p=0.0248; Kruskall-Wallis), ipsilateral dlPAG (59.00/94.00/130.0, p=0.0030; Kruskall-Wallis) and ipsilateral vlPAG (28.00/34.00/73.00; p=0.0076). No differences were found in dmPAG and contralateral vlPAG. The pretreatment with URB 597 (3 nmol) attenuated Fos expression in contralateral dlPAG, ipsilateral dlPAG and ipsilateral vlPAG (p=0.0212, p=0.0159, p=0.0159, respectively; Mann Whitney). **Discussion**: These results suggest that the endocannabinoid system plays an important role in defensive responses induced by NMDA injections. Substances which increase the endocannabinoids levels reduce flight responses and this effect is mediated by CB1 receptors. In conclusion, it appears that cannabinoids receptors can modulate defensive responses mediated by dlAPG possibly inhibiting glutamatergic neurotransmission. Number of the Animal Ethics Committee: 059/11. **Financial Support**: CAPES/FAPEMIG

03.012 Ethanol withdrawal after chronic consumption induces anxiety-like responses without altering locomotion or motor coordination in mice. Maciel SX, Guimarães RAM, André E, Gavioli EC, Soares-Rachetti VP [1]UFRN – Biofísica e Farmacologia

Introduction: Previous evidences suggest that withdrawal after chronic abuse of ethanol can generate anxiety-related symptoms in humans [Saitz, Alcohol Health Res World. 22(1):5, 1998]. Preclinical data have been showing that ethanol withdrawal after chronic consumption generates in rodents a wealth of behaviors related to human symptoms, such as agitation, anxiety- and depression-related responses [Kliethermes, *Neurosci Biobehav Rev*. 28(8):837, 2005; Ribeiro-Carvalho et al., *Behav Brain Res*. 221(1):282, 2011]. The purpose of the present study was to evaluate if the withdrawal of ethanol after being chronically consumed by mice would alter behaviors in the EPM, locomotor activity in the open field test and motor coordination in the rota-rod test. **Methods**: Male Swiss mice (60 days-old) were submitted to chronic consumption of ethanol (increasing concentrations: 2% (3 days), 4% (3 days) and 6% (15 days) during 21 days as the only source of liquid diet, while control group received water. Both ethanol and control groups received a free solid diet during all the experiment. On the 21st day after chronic exposure, ethanol was substituted by water (withdrawal). Three days after withdrawal, animals were submitted to elevated *plus-maze during 5 minutes and percentage of time and entries in the open arms and the entries in the enclosed arms of the maze were analyzed*. In the two days following the EPM testing (5 days after withdrawal), mice were submitted to open field test to assay general locomotor activity. In this test it was analyzed distance travelled by the animals during 15 minutes by using ANY-MAZE software (Stoeling, USA). Thirteen days after withdrawal, mice were submitted to the rota-rod training during three days and, sixteen days after withdrawal, the test was performed by subjecting animals to crescent rotation speeds and the latency to fall from the rotation bar was recorded. This study was approved by Local Ethics Committee (Protocol N° 019/2010). **Results and Discussion**: Data are represented by mean±standard error of the mean (SEM). Results obtained in the EPM showed that ethanol withdrawal group explored less the open arms of the apparatus, suggesting an anxiogenic-like effect [% of time spent in the open arms of EPM: Control (20.66±2.78), n=8; Ethanol withdrawal (11.21±1.81), n=12, ANOVA followed by Student *t* test, $p<0.05$]. This effect was not influenced by alterations in the locomotor activity or in the motor coordination since there was no difference between groups in the distance travelled in the open field test and in the performance of the mice in the rota-rod test. In conclusion, by using this animal model of ethanol withdrawal it would be possible to study future therapeutic options for ethanol addiction, especially in respect to anxiety, that has been related to relapse [Trevisan et al., Alcohol Health Res World 22(1):61, 1998]. **Financial Support**: PROPESQ-UFRN, FAPERN.

03.013 The ethanol withdrawal after chronic consumption generates anxiogenic-like responses in both female and male rats. Ali MS[1], Santos RO[1], Souza Pinto IA[1], Santana IH[1], André E[1], Padovan CM[2], Gavioli EC[1], Soares-Rachetti VP[1] [1]UFRN – Biofísica e Farmacologia – Farmacologia Comportamental, [2]FFCLRP-USP – Psicobiologia

Introduction: Clinical data have been showing that acute withdrawal after chronic abuse of ethanol can generate anxiety-related symptoms [Adinoff et al., Med Toxicol Adverse Drug Exp;3(3):172, 1988]. Preclinical studies have been investigating the brain areas and neurotransmitters related to anxiety caused by ethanol withdrawal by using animal models of chronic ethanol consumption followed by testing in animal models of anxiety, such as elevated plus maze test (EPM) [Kliethermes, *Neurosci Biobehav Rev*;28(8):837, 2005; Ribeiro-Carvalho et al., *Behav Brain Res*;221(1):282, 2011]. Despite a lower ethanol consumption has been described among women, its impact may be greater than among men, as assessed by health problems associated with alcohol [Bongers et al., *Subst Use Misuse* 32 (11): 1491, 1997]. However, most part of preclinical studies is conducted in male gender and the results are extrapolated to both gender disregarding physiological differences related to the estrous cycle and hormonal changes experienced by females. This study aimed to observe whether the withdrawal of ethanol after chronic consumption generates anxiety-related behaviors in the test of EPM in female and male rats. **Methods**: Female (n=10-15) and male (n=6-8) *Wistar* rats (60 days old) were submitted to increasing concentrations of ethanol (2% during the 3 first days, followed by 4% during 3 days and 6% during 15 days, WITHDRAWAL group) or to water (CONTROL group) as the only source of liquids and received a solid diet. Females were mapped in their estrous cycle phases by daily vaginal smears at least 7 days before testing. On the 21st day, ethanol was substituted by water and after 72 hours of withdrawal, animals were individually submitted to the EPM test to evaluate percentage of entries, time spent in the open arms and the number of entries in the enclosed arms during 5 minutes. This study was approved by Local Ethics Committee (Protocol N° 019/2010). **Results and Discussion**: Data showed that ethanol withdrawal after chronic consumption decreased the exploration in the open arms of the EPM independently of the gender of the rats. As previously described [Simpson et al., *Prog Neuropsychophar. Biol* Psy.;37(2):227, 2012] female rats spent more time in the open arms when compared with male rats [female rats (mean±SEM):43.34±4.65, male rats: 12.8±3.82, ANOVA, $p<0.05$]. Female rats submitted to ethanol withdrawal spent less time in the open arms of the EPM, when compared with female control group [CONTROL (mean±SEM):43.34±4.65, WITHDRAWAL: 28.36±5.73, ANOVA, $p<0.05$] and the same result was observed for male rats. Data here obtained suggest that despite differences observed between genders in behaviors of control rats in the EPM, this model of chronic consumption of ethanol followed by withdrawal promotes an anxiogenic-like effect in both male and female rats. This model would allow the elucidation of the role of brain areas or neurotransmitters systems related to anxiety during ethanol withdrawal. **Financial Support**: PROPESQ-UFRN, CAPES, FAPERN.

03.014 Effect of alcohol and tobacco association on behaviors in the open field test in rats. Santos CF[1], Quinteros DA[1], Caletti G[2], Wieczoreck MG[3], Schneider R[4], Gomez R[1,2,3] [1]UFRGS – Farmacologia, [2]UFCSPA – Farmacologia, [3]UFRGS – Fisiologia, [4]UFRGS – Neurociências

Introduction: Alcohol and tobacco are licit drugs of abuse and frequently used in association. However, few studies evaluate their concomitant use in animals. Our study aimed to evaluate the effect of alcohol and tobacco association on behaviors of rats in the open field test. **Methods**: Male, adults, Wistar rats were divided into four groups (n = 8/group): control (CTR), alcohol (ALC), tobacco (TAB) and association (ALCTAB). They were treated for 28 days, twice a day, with 2g/kg ethanol solution (20% w/v) (ALCTAB and ALC groups) or a 5% glucose solution (CTR and TAB), orally administered immediately before they were placed in chambers with circulating ambient air or air saturated with the smoke of 6 cigarettes burned in the course of two hours, during the morning and during the afternoon. Therefore, the rats received 4 mg/kg/day of alcohol and were exposed to smoke from 12 cigarettes/day. On the 28th day, after the 3rd cigarette was burned, and about 60 min of alcohol or glucose administration, in the morning, the animals were observed for 5 min in the open field test. The behaviors were recorded by a video camera and subsequently evaluated for central and peripheral crossings, rearing, grooming and fecal boli. Results were analyzed by an ANOVA-one way, followed by the Bonferroni test to identify differences between groups, with P values < 0.005. (CEUA-UFRGS # 19566). **Results:** Our results showed that the association between alcohol and tobacco (ALCTAB group) increased peripheral crossing ($P < 0.001$) when compared to CTR, ALC or TAB groups ($P < 0.001$). ALCTAB and ALC groups increased the central crossings ($P < 0.001$), whereas the association ALCTAB increased rearing and reduced the number of fecal boli. **Discussion**: Therefore, our results show that the association ALCTAB increases motility and exploratory behaviors, observed by the rearing increases, without losing the anxiolytic properties of alcohol, observed by the central crossings and reduction of fecal boli. Such effects may account for the elevate association of these two drugs of abuse, because user experiment pleasure effects without experience the depressant effects of alcohol. **Financial Support**: UFRGS, CNPq

03.015 Facilitation of 2-araquidonoilglicerol (2AG) signaling in the dorsolateral periaqueductal gray in rats induced anxiolytic-like effects. Almeida-Santos AF, Gobira PH, Moreira FA, Aguiar DC UFMG – Pharmacology

Introduction: Anandamide and 2-araquidonoilglicerol (2AG) are the main representatives of the endocannabinoid system. Anxiolytic-like effects were described for anandamide in different animal models of anxiety, such as the elevated plus maze (EPM). These effects are mediated through activation of cannabinoid receptors type 1 (CB1r), which are highly expressed in several brain regions related to defensive behavior, such as the periaqueductal gray (PAG). However, the role of 2AG in behavioral responses mediated by the dlPAG is not yet described. Thus the objective of this study was to test the hypothesis that the administration intra-dlPAG of the 2AG or increase endogenous levels of 2AG, by inhibiting monoacylgycerol lipase (MAGL), will exert anxiolytic-like effects in animals submitted to the EPM. The mechanism involved in this behavioral response was also investigated. **Methods**: Male Wistar rats (n= 5-12/group) with cannula aimed at the dlPAG (AP-lambda 0 mm, L-1,9 mm, P-4,3 mm, angle-16^0) received inta-dlPAG injections (0.2 µL) of the following treatments: Experiment 1: vehicle (veh) or 2AG (5pmol, 50pmol, 500pmol). Experiment 2: veh or URB602 (MAGL inhibitor, 30pmol, 100pmol or 300 pmol). Experiment 3: veh or AM251 (antagonist CB1r, 100pmol) followed 10 minutes later by injection of veh or 2AG Experiment 4: veh or AM630 (antagonist CB2r, 1000pmol) followed 10 minutes later by injection of veh or 2AG (50pmol). The animals were exposed to EPM ten minutes after the last injection for 5 minutes. **Results:** The administration of 2AG (50pmol) significantly increased the number of entries in the open arms of the EPM (veh: 11.52 ± 4.72; 2AG (50pmol): 37.65 ± 3.71, $F_{(3,33)} = 4.2$, $p = 0.01$; Duncan, $p < 0.05$ compared to vehicle group), suggesting an anxiolytic-like effect. Likewise, the intra-dlPAG injection of URB602 (100pmol) induced a significant increase in time spent in the open arms of the EPM (veh: 9.01 ± 3.20; URB602 (100pmol): 30.71 ± 6.86, $F_{(3,26)} = 1.2$, $p = 0.1$; Duncan, $p < 0.05$ compared to vehicle group). Both pretreatment with CB1 (AM251) and CB2 (AM630) attenuated the anxiolytic-like effect induced by 2AG. However, the effects of MAGL inhibitor were blocked only by CB2 antagonist. **Discussion**: Our results showed that the augmentation of 2AG signaling could also induce anxiolytic-like effects. But these effects were mainly related trough CB2 activation, since the effects of MAGL inhibitor were blocked by CB2 antagonist but not by CB1 antagonist. Number of the Animal Ethics Committee: 250/2010. Financial Agencies: CAPES/FAPEMIG APQ-01883-10.

03.016 Subchronic administration of *Trichilia catigua* ethyl-acetate fraction promotes antidepressant-like effects and increases hippocampal cell proliferation in mice. Bonassoli VT, Chassot JM, Longhini R, Milani H, Mello JCP, Oliveira RMMW [1]UEM – Farmácia e Farmacologia, [2]UEM – Farmacologia e Terapêutica,

Introduction: *Trichilia catigua* preparations have been popularly used in Brazil as a tonic for the treatment of fatigue, stress, impotence, and memory deficits. We recently demonstrated an antidepressant-like effect of acute administration of the ethyl-acetate fraction of *T. catigua* (EAF) in mice. The aim of the present study was to evaluate whether subchronic EAF administration maintains its antidepressant-like effects and whether these effects are related to hippocampal neurogenesis. **Material and Methods:** EAF (200 and 400 mg/kg), saline or imipramine was orally administered to mice for 14 days. The animals were tested in the forced swim test (FST) and tail suspension test (TST) during 6 min sessions. After behavioral testing, the animals received bromodeoxyuridine (BrdU; 200 mg/kg, i.p.) and were euthanized 24 h, 7 days, or 15 days later. The brains were assayed for BrdU to detect cell proliferation/survival. All procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 042/2007). Data were expressed as mean ± S.E.M. and analyzed by one-way ANOVA followed by the Newman Keuls *post hoc* test. This study was supported by CAPES and Fundação Araucária. **Results:** Subchronic administration of 400 mg/kg EAF decreased immobility time in the FST compared with the saline group ($F_{3,42}$ = 12.1, p< 0.0001, Saline=270,5±15.49; Imipramine=118,4±13,39; EAF 200=250,5±19,27; EAF 400mg/kg=204,3±19,17). No difference was detected among the experimental groups in the latency to the first immobility episode in this test ($F_{3,42}$ = 1.99, p = 0.13). In addition, 400 mg/kg EAF decreased the immobility time in the TST compared with the saline group ($F_{2,29}$ = 20.10, p<0.0001, Saline=185,3±9,50; Imipramine=98,0±8,29; EAF 400mg/kg=154,0±10,30). No significant effect on latency was observed in any of the experimental groups ($F_{2,26}$ = 0.54, p = 0.59). The antidepressant-like effect was accompanied by an increase in cell proliferation in the dentate gyrus (DG) of the hippocampus 24 h after the treatments were discontinued ($F_{2,14}$ = 18.76, p = 0.0002, Saline=21,65±1,44; EAF400mg/kg=27,74±1,20; Imipramine= 31,30±2,47). No significant effect was observed 7 or 15d groups. **Conclusion**s: These results show that EAF administration produced antidepressant-like effects in mice and did not present tolerance after repeated administration. In addition to its pharmacological effect, EAF induces hippocampal cell proliferation. Therefore, EAF could potentially be used as a coadjuvant treatment of mood disorders.

03.017 Evidence for simultaneous anxiolytic-like and aversive effects of pulegone, **a dual behavioral activator and depressant**. Silveira NS, Prado LCS, Cunha JM, Bispo-da-Silva LB UFU – Pharmacology

Introduction: Pulegone possesses psychostimulant effects that are sensitive to dopamine receptor antagonists. Considering the important role of dopamine in the regulation of both movement and reward, we hypothesized that pulegone has both psychostimulant and reward properties. We also analyzed pulegone for anxiolytic-like effects. **Methods:** The experiments were conducted using male Swiss mice (CEUA/UFU license number: 170/10 and 202/11) treated with Pulegone (100 – 800 mg/kg, i.p.) or vehicle (olive oil). General mouse activity (locomotion and immobilization) was determined in the open field. The anxiolytic-like activity, motor coordination and strength force were evaluated using the elevated plus maze (EPM), rotarod test and grasping test, respectively. Pulegone motivational properties were evaluated by pairing the drug effects on the mice with the less preferred compartment (previously determined) of a conditioned place preference (CPP) apparatus. **Results:** Pulegone increased mouse locomotor activity at the dose of 200 and 400 mg/kg (146±12 vs. 210±19 and 244±21, vehicle, pulegone-200 and -400 mg/kg, respectively; $P<0.05$) and immobilization time only at the dose of 800 mg/kg (5.00±2.3 vs. 163.9, vehicle and pulegone-800, respectively; $P<0.05$). Haloperidol greatly decreased the psychostimulation induced by pulegone (184±9 vs. 205±29, vehicle and pulegone-400+haloperidol, respectively; $P>0.05$). Pulegone caused motor incoordination (243.4±48.4 vs. 30.3±12.8 and 7.1±7.1, in s; vehicle and pulegone-400 and -800 respectively; $P<0.05$) and weakness (92.8±2.9 vs. 41.5±20.9 and 24.6±16.1, in g; vehicle and pulegone-400 and -800 respectively; $P<0.05$). Moreover, pulegone (200 and 400 mg/kg) increased the time spent in the open arms (in %) of the EPM (16.6±3.6 vs. 24.9±2.2 and 28.4±3.0, vehicle and pulegone-200 and -400, respectively; $P<0.05$) and flumazenil pre-treatment did not alter this effect (29.4±4.9 vs. 27.9±3.1, pulegone-400 vs. pulegone-400+flumazenil; $P>0.05$). None of the doses tested induced CPP (vehicle: 262±23 vs. 305±25; $P>0.05$. Pulegone-100 mg/kg: 270±32 vs. 208±28; $P<0.05$. Pulegone-200 mg/kg: 281±25 vs. 265±44; $P>0.05$). **Discussion:** Pulegone either produced no CPP or induced conditioned place aversion and pulegone has a dual effect on mouse behavior, acting either as a stimulant or as a depressant. Although the stimulant effect involving dopamine D2 receptor activation, pulegone has negative reinforcing properties and appears to possess anxiolytic-like actions unrelated to the benzodiazepine site of the GABAA receptor. **Financial Support:** PROPP/UFU and CAPES.

03.018. **The influence of alcohol withdrawal on anxiety and S100B serum concentrations in rats**. Schneider R[1,2], Quinteros DA[1], Ferreira C[1], Silva J[1], Brolese G[2], Gonçalves CA[2], Gomez R[1,3] [1]UFRGS – Farmacologia, [2]UFRGS – Neurociências, [3]UFRGS – Fisiologia

Introduction: Alcohol withdrawal leads to neuronal injury, neurochemical and behavioral changes[1]. Increased serum levels of S100B has been correlated with affective disorders that co-occur with alcoholism[2,3]. However, changes in serum levels of this astroglial protein in according to the time of withdrawal and its relationships with anxiety are not well understood. The aims of this study were to evaluate changes in anxious behavior and serum levels of S100B after 20 hours or 5 days of withdrawal in rats chronically treated with alcohol. **Methods**: Adult (~ 300 g), male Wistar rats (n=26) were divided in ETOH group, treated with ethanol (2 g / kg) dissolved in 3% glucose (w / v), and CTR group treated only with 3% glucose solution for 30 days, orally, twice a day. Anxious behavior was assessed after 20 hours and after 5 days of withdrawal in the open-field test (OF) for 5 min followed by the elevated plus-maze test (EPM), as well for 5 min. Behaviors were recorded in a video camera for later analysis by a blinded and experienced evaluator. In the OF we analyzed the central and the peripheral crossings, rearing and grooming. In the EPM, we evaluated the frequency of open and closed arms entries, as well as the time spent in each arm and in the central area. Serum levels of S100B were measured by ELISA after euthanasia by decapitation. Data were analyzed by the Student's t-test for independent samples and paired t-test. We applied the Mann-Whitney and Wilcoxon tests for variables which did not present a normal distribution. This study was approved by the animals ethics committee of Universidade Federal of Rio Grande do Sul (CEUA-UFRGS: # 23069). **Results:** Under our experimental conditions, ETOH treated rats only showed a decreasing on rearing (P = 0.004) comparing to CTR group in the OF test after 20 h of withdrawal. However, after 5 days, rats from the ETOH group showed a lower number of rearing (P = 0.002), grooming (P = 0.001) and total crossings (central plus peripheral crossings) (P = 0.003) than CTR rats. Moreover, in the EPM test, 5 days ETOH withdrawal rats presented lower frequency of entrance (P = 0.018) and time spent in the open arms (P = 0.015). Serum levels of S100B in the ETOH group were significantly higher at 20 hours of withdrawal compared with 5 days of withdrawal ($p < 0.01$). **Discussion:** These results suggest that anxiety increases in 5 days of withdrawal but not in 20 hour. However, S100B serum levels increase only at the beginning of withdrawal (20 hours), decreasing significantly over time. Regarding the neurotrophic function of S100B, its increase in the serum may be due to an attempt of the central nervous system to revert the cellular damage induced by alcohol withdrawal more studies are needed to elucidate the role of this peptide on anxiety and neurochemical pathways correlated with drug-addiction and relapse. **References**: [1]McKeon et al..*J Neurol Neurosurg Psychiatry*,79, 854. 2008. [2]Andreazza et al. *J Psychiatr Res*, 41, 523. 2007. [3]Arolt et al., Eur Neuropsychopharmacol,13, 235. 2003. **Financial Support:** CNPq, UFRGS

03.019 Influence of withdrawal syndrome to use of alcohol and cocaine in neurotransmission peripheral adrenergic. Bomfim GHS, Verde LF, Jurkiewicz NH, Jurkiewicz A Unifesp – Farmacologia

Introduction: The withdrawal syndrome is characterized as a set of organic modifications that occur due to the abrupt removal and or reducing the consumption of a drug. Some drugs trigger excessive release of catecholamines, which may possibly lead to changes in peripheral systems. The aim of our study was to investigate the possible effects of withdrawal ofter use of ethanol (EtOH) and cocaine (COCA) in autonomic neurotransmission, *in vitro* during periods of 1h, 24h, 48h and 120h. **Methods**: The rat vas deferens (VD) that has rich adrenergic innervation was used. Isometric contractions induced "in vitro" by adrenergic drugs and nerve-induced contraction by pulses of 5.0 Hz 60 V for 1 ms were performed. Additionally, the activity of α-2 adrenoceptor was tested by guanfacine on a tonus of 0.2 Hz for 50v 3ms. Through these protocols, we measured the parameters of apparent affinity (pD_2), maximum effect (E_{max}) and pIC_{50} of drugs, as well as values of the phasic and tonic response. We used adult *Wistar* rats with male body weight of 250 ± 50 g. EtOH by intragastric way (gavage) was administered in animals (T) at doses of 7g, 8g, 9g 10g/kg/day in the 4th day of treatment. The control group received a vehicle (H_2O). The animals treated with COCA received by intraperitoneal way 10mg, 15mg, 20mg, 25mg and 30mg/kg/day in the 5th day of treatment and control group vs were treated with vehicle (saline 0.9%). The animals were sacrificed by decapitation; the VD was surgically removed and mounted in isolated organ bath for recording isometric contractions. Initially, were performed cumulative concentration-effect curves to adrenergic agonists: dopamine, phenylephrine and norepinephrine$^{\#}$ in the presence of cocaine (6μM), corticosterone (10μM) and propranolol (0.1mM) 30' before curves. Electrical field stimulation and inhibition curve with guanfacine were also evaluated. Significance: * $p < 0.05$ minimum 8 experiments, non-parametric *Test T Student*. This study was approved by the ethics and research committee of UNIFESP under number 1168/11. **Results:** Animals with EtOH withdrawal in periods of 24h had an increased pD_2 values to norepinephrine$^{\#}$, dopamine and E_{max} for phenylephrine, compared with control: (T) 7.30 ± 0.07* and (C) 7.11 ± 0.05; (T) 1.78 ± 0.11* and (C) 1.48 ± 0.06; (T) 1.92 ± 0.08* and (C) 1.60 ± 0.06, respectively. However, the tonic response of nerve-evoked contraction was diminished: (T) 0.63 ± 0.07* and (C) 0.90 ± 0.04. The pIC_{50} values to guanfacine were also decreased in this period: (T) 7.59 ± 0.08* e (C) 7.81 ± 0.03. Animals after cocaine abstinence presented an increased E_{max} to norepinephrine$^{\#}$, dopamine, phenylephrine and tonic component of nerve-evoked contraction after 48h: (T) 2.22 ± 0.09* and (C) 1.37 ± 0.06; (T) 2.36 ± 0.09* and (C) 1.88 ± 0.08; (T) 1.91 ± 0.06* and (C) 1.40 ± 0.05; (T) 0.98 ± 0.04* and (C) 0.80 ± 0.03, respectively. The pIC_{50} values to guanfacine were decreased in this period compared with control: (T) 7.40 ± 0.07 * and (C) 7.81 ± 0.04. **Discussion**: Our data suggested that the period of abstinence to EtOH and COCA influence the adrenergic neurotransmission in VD, especially in periods of 24h and 48h. The origin of the exacerbation is possibly explained by a failure in the release of monoamines and autoceptors α-2 activity, which is reflected in the hypereactivity postsynaptic. Support: FAPESP and CAPES.

03.020 Evaluation of the anxiolytic activity of essential oil of *Citrus limon (L.)* Burm. F. orally in mice. Cardoso RM[1], Viana MDM[1], Silva NKGT[1], Falcão MAP[1], Silva WL[2], Sant´Ana AEG[2], Alexandre-Moreira MS[1], Campesatto EA[1] [1]UFAL – Fisiologia e Farmacologia, [2]UFAL – Química e Biotecnologia

Introduction: Several herbal medicines are recognized as active in the central nervous system (CNS), and they have at least a hypothetical potential to affect chronical conditions such as anxiety, depression, headaches or epilepsy, that do not respond well to pharmacological conventional treatments[1,2]. Preparations from peel, flowers and leaves of many species of genus *Citrus* (Rutaceae) are popularly used in order to minimize central nervous system disorders. The aim of the present study was to evaluate the anxiolytic-like effect obtained orally in two different concentrations (100, 300mg/kg) in two experimental models: Open-Field and Plus-Maze. **Methods**: The experimental procedures were performed with essential oil (EO) of peels of *Citrus limon* in the way it is marketed (Ferquima Ind. e Com. Ltda distributor, São Paulo). By GC-MS analysis was possible to guarantee the quality and purity of the EO, as well as identify some components, whose main compounds are β-pinene and (-)-D-limonene. Swiss male mice (25-35g, n=6) were divided in three groups. The first was treated intraperitoneally with diazepam 1.5 mg/kg; the second, with saline 0,9% orally in dose of 10mL/kg; and the third group with the oil in the same dose, in the following concentrations: 100 and 300mg/kg. The animals of diazepam group were evaluated in the Elevated Plus-Maze Test and Open-Field Test 60 minutes after treatment, whereas the experimental and control group were evaluated 30 minutes after oral administration. All experiments were approved by the Ethics Committee for Animal Research of UFAL (protocol number nº 024094/2011-14). **Results and Discussion**: In the Elevated Plus-Maze test, both doses provided a significant reduction ($p < 0.0001$) in time spent in closed arms (EO 100 = 80.2 ± 14.9s; EO 300 = 57.5 ± 5.5) compared to control (162.7 ± 14.8s) and consequently a greater permanence in open arms (EO 100 = 182.5 ± 19.5; EO 300= 200.0 ± 13.8; SAL = 95.0 ± 12.7) presenting very close results to the average of the diazepam group (DZP = 220.0 ± 2.0). Others parameters (number of entries in open or closed arms and time spent in open arms) were also affected by the administration of oil dose-dependent effect. In the Open-Field test, two doses were able to reduce ($p < 0.0001$) not only the ambulation, observed by the number of segments crossed (EO 100 = 14.8 ± 5.2; EO 300 = 11.8 ± 4.3; DZP = 27.1 ± 3.2, vs control = 62.7 ± 2.3), but also the number of rearings, grooming and faeces; as well as significant increase ($p < 0.0001$) in immobility time (EO 100 = 234.7 ± 19.2; EO 300 = 251.6 ± 10.8; SAL = 32.9 ± 3.9; DZP = 188.8 ± 16.7) showing, then, a sedative effect such as the standard drug used, diazepam. These results were able to demonstrate that both doses in this study presented psychopharmacological effects suggestive of anxiolytic action, once they were active in experimental animal models typical for this purpose, reducing satisfactorily their ambulation, confirming, therefore, dose-dependent action. **References**: [1]Phillipson, J.D. *Phytochem.*, 56, 237, 2001. 2 Carlini, E.A. *Pharmacol. Biochem. Behav*., 3, 501, 2003. **Financial Support**: CNPq and FAPEAL.

03.021 Chronic alprazolam treatment induces anxiolytic and panicolytic-like effects in rats. de Bortoli VC[1], Zangrossi Jr H[2] [1]CEUNES-UFES – Ciências da Saúde, [2]FMRP-USP – Farmacologia

Introduction: Electrical or chemical stimulation of the dorsal periaqueductal gray matter (DPAG) induces defensive reactions, such as escape behavior, that have been related to panic attacks [1]. The microinjection of the 5-HT_{1A} receptor agonist 8-OH-DPAT or the preferential 5-HT_{2A} agonist DOI into this area inhibits the escape reaction evoked by local electrical stimulation. Chronic treatment with antipanic drugs such as imipramine and fluoxetine facilitates the anti-escape effect caused by the intra-DPAG injection of these 5-HT agonists [2]. Furthermore, it has been shown that chronic treatment with the benzodiazepine agent alprazolam also facilitated the inhibitory effect of 8-OH-DPAT and DOI on escape induced by electrical stimulation [3]. In the present study we investigated whether chronic treatment with alprazolam may interfere on escape expression generated by an ethologically based model of anxiety, the elevated T-maze (ETM). This test also allows the measurement of inhibitory avoidance, which has been related to generalized anxiety disorder. **Methods**: Male Wistar rats (230-250 g) were treated daily with alprazolam (1, 2 or 4 mg/Kg, i.p.) or vehicle solution for 13 days. In the next day, thirty minutes after the last injection of alprazolam or vehicle, the animals were tested in the ETM. Commission ethical protocol nº 077/2008 – CETEA-FMRP/USP. **Results:** The results showed that chronic treatment with alprazolam (1, 2 and 4 mg/Kg) impaired inhibitory avoidance, suggesting a anxiolytic effect [(mean ± SEM, seconds): vehicle = 129.25 ± 50.28; alprazolam 1mg = 10.13 ± 1.13; alprazolam 2mg = 5.00 ± 1.29; alprazolam 4mg = 4.13 ±1.30]. Besides the animals treated chronically with alprazolam (1 and 4 mg/Kg) had longer ($p < 0.05$) escape latencies than those systemically injected with vehicle solution, suggesting a panicolytic-like effect [vehicle = 5.0 ± 0.91; alprazolam 1mg = 16.5 ± 1.17; alprazolam 4mg = 17.25 ± 2.53]. **Discussion**: Alprazolam, a clinically effective drug in treating panic disorder, also induced anxiolytic and panicolytic-like effects in the elevated T-maze. **References**: [1] Graeff FG and Zangrossi HJr. *Textbook of biological psychiatry: animal models of anxietydisorders*, p. 879, 2002. [2] Guimarães FS et al. *Handbook of the behavioral neurobiology of serotonin*, p. 667, 2010. [3] de Bortoli VC et al. *Psychopharmacology* 198, p. 341, 2008. **Financial Support**: FAPESP, PRPPG-UFES.

03.022 High- and low-rearing rats differ in the brain excitability controlled by the allosteric benzodiazepine site in the GABAA receptor. Alves R, Carvalho JGB, Venditti MAC Unifesp – Psicobiologia

Rearing is an exploratory behavior induced by novelty, such as exposure to an open field. Stimulation of certain brain regions, including the hippocampus, induces both rearing and clonic convulsions. Brain excitability is controlled by gamma-aminobutyric acid (GABA) inhibitory neurotransmission through its ionotropic GABA /allosteric benzodiazepine site. Drugs that decrease GABAA receptor fast inhibitory neurotransmission induce clonic convulsions and rearing when injected into the hippocampus. Therefore, individual differences in rearing behavior may be related to the susceptibility to clonic convulsions, which could involve differences in brain excitability controlled by GABAA/allosteric benzodiazepine site receptors. **Material and Methods:** Adult, male Wistar rats were divided into high- (HR) and low-rearing (LR) groups based on the number of rearings in the open field test. Groups of HR and LR rats were challenged with convulsant drugs that antagonize GABA neurotransmission via different mechanisms of action (3-mercaptopropionic acid, a glutamate decarboxilase inhibitor; bicuculline, a GABAA receptor antagonist; pentylenetetrazol and picrotoxin, both GABAA receptor chloride channel blockers and DMCM, a benzodiazepine inverse agonist). The convulsant doses that induced 50% of clonic convulsions were determined for each drug. The LR rats had a higher susceptibility (a lower convulsant dose 50%) to clonic convulsions induced by DMCM than the HR rats, but there were no differences between the groups in the susceptibility to tonic convulsions induced by the same drug. This work was approved by our institution ethics committee on animal research (proc. # 0938/03). **Results:** There were no significant differences in the convulsant dose 50% for clonic convulsions between the groups for all other drugs injected. In another experiment, additional HR and LR rats were injected with a sedative-hypnotic dose of diazepam, which caused a significantly higher hypnotic effect (sleeping-time) in the LR rats than in the HR rats. The LR group was also shown to have a significantly lower density of [3H]-Flunitrazepam bound to the GABAA receptor in hippocampal membranes. **Conclusion**: The data obtained in this study suggest that the differences in rearing behavior induced by novelty are related to differences in the hippocampal allosteric benzodiazepine site in the GABA receptor. Further work is needed to determine possible differences in the GABAA receptor assembly between the groups of HR and LR rats. **Financial Support:** FAPESP and AFIP

03.023 Effect of vitamin E on oxidative stress and behaviors related to anxiety and depression in streptozotocin-induced diabetic rats. Morais H, Pasquini CS, Ferreira DM, Silva LM, Beltrame OC, Cunha JM, Zanoveli JM UFPR – Farmacologia

Diabetes mellitus is a chronic disease with patients showing a high incidence of psychiatric disorders such as depression and anxiety. Studies have suggested that these associations may be a direct consequence of the biochemical changes induced by hyperglycemia such as an increase of oxidative stress.

We evaluated the effect of vitamin E (vit E) treatment, an antioxidant compound, on the oxidative stress and behavioral responses in animals submitted to experimental models of depression and anxiety. Male *Wistar* rats (180-250 g; n=6-10) treated with citrate buffer (10mM, pH 4.5, normoglycemic group-N) or streptozotocin (50 mg/kg, i.p, diabetic group-DBT) were submitted to a chronic treatment during 28 days with vit E (300 mg/kg, v.o.) or vehicle (VEH). The increase in the immobility time (IT) and decrease in immobility latency time (ILT) in the forced swimming test (FST) were scored as depressive-like behaviors. The decrease of crossings number in the center (NCC) of the open field (OF) was scored as anxiogenic-like effect. Additionally, the general locomotor activity was also observed in the OF. Immediately after the tests, both N and DBT rats were euthanized and the hippocampus (HIP) and pre-frontal cortex (PFC) were dissected for further lipid peroxidation (LPO) levels quantification. As a positive control to the antidepressant and anxiolytic-like effects, N and DBT animals were treated sub-chronically (3 days) with the imipramine (IMI, 15 mg/kg/ml; ip). In the FST the animals were submitted to two sessions with pre-test (15 min) occurring 24 h before the test session (5 min). The procedures were approved by the Ethics Animal Experiment Committee of Federal University of Paraná (#576). When compared to N rats (mean±SEM, seconds; IT: 206,3±16; ILT: 52,5±14,9), DBT animals showed: 1) a significant increase in IT (263,5±10,2) and decrease in ILT (17±4,4) when evaluated in the FST; 2) a reduced exploration in the NCC of the OF (42%) without altering the general locomotor activity; 3) a significant reduce in weight gain (52%) and 4) an increase in the LPO levels (HIP: 44%; PFC: 43%). Moreover, compared to DBT rats treated with VEH (IT: 265,9±9,2; ILT: 5,5±1,5), the vit E treatment significantly ameliorated the depressive-like behaviors in DBT rats (IT: 213,5±16,4; ILT: 30,8±7,7), as well as the anxiogenic-like effects (increase of 96% in NCC). This treatment also induced a significant weight gain in DBT animals (52%) as well caused a significant reduction in LPO levels (HIP: 16%; PFC: 21%). Interestingly, vit E was not able to alter these behavioral and biochemical parameters in N rats. Differently, IMI treatment prevented the depressive-like effect in both N and DBT animals (N rats - decrease of 32% in IT, increase of 103% in ILT; DBT animals: decrease of 24% in IT, increase of 376% in ILT), but it was not able to modify significantly the anxiogenic-like effect in DBT animals, maybe due to short-treatment regimen. All these data were analyzed by one-way analysis of variance followed by Newman Keuls test. This study indicated that vit E exert neuroprotector effects in the brain areas extremely related to depression and anxiety disorders as HIP and CPF. Additionally, vit E supplementation can be an alternative, as an adjunct, in the treatment of diabetic patients suffering of disorders like depression and anxiety. Support: CAPES, CNPq, Brazil.