05 Pain and Nociception Pharmacology

05.001 Transient Receptor Potential Ankyrin 1 (TRPA1) plays a critical role in a metastatic mouse model of cancer pain. de Almeida AS[1], Antoniazzi CTD[1], Nassini R[2], Rigo FK[3], Milioli AM[3], Bellinaso F[1], Camponogara C[4], Silva CR[5], Rossato MF[5], de Logu F[2], Oliveira SM[4], Cunha TM[5], Geppetti P[2], Ferreira J[6], Trevisan G[1] [1]UFSM – Farmacologia, [2]University of Florence – Farmacologia, [3]UNESC – Farmacologia, [4]UFSM – Bioquímica Toxicológica, [5]FMRP-USP – Farmacologia, [6]UFSC – Farmacologia

Introduction: There is a major, unmet need for the treatment of cancer pain, and new targets and medicines are required[1]. The transient receptor potential ankyrin 1 (TRPA1), a cation channel expressed by nociceptors, is activated by oxidizing substances to mediate pain-like responses in models of inflammatory and neuropathic pain[2]. Cancer or chemotherapeutic therapies frequently causes pain, which is difficult to manage[3]. The identification of shared mechanisms and a common pathway for cancer pain and chemotherapeutic-induced neuropathic pain would be important for the clinical development and effectiveness of medicines, needed for improving the quality of patients' life. The transient receptor potential vanilloid 1 (TRPV1), when activated, is also related to the onset of pain, but with a different activation form of TRPA1. Because of this, in this work, it is also tested to know if there is involvement in this metastatic mouse model of cancer pain. As cancer is known to increase oxidative stress, the role of TRPA1 was evaluated in a metastatic mouse model of cancer pain. **Methods:** Fourteen days after injection of B16-F10 murine melanoma cells into the plantar region of the right hind paw, C57BL/6 mice were treated with TRPA1 selective antagonists or antioxidant or TRPA1 oligonucleotide antisense and soon after subjected to behavioral tests, such as: mechanical allodynia, cold allodynia, open field (thigmotaxis behavior), thermal hyperalgesia, eye wiping test. **Results:** Animals exhibited mechanical and thermal allodynia and thigmotaxis behavior. While heat allodynia was partially reduced in TRP vanilloid 1 (TRPV1)-deficient mice, thigmotaxis behavior and mechanical and cold allodynia were absent in TRPA1-deficient mice. Deletion of TRPA1 or TRPV1 did not affect cancer growth. Intrathecal TRPA1 antisense oligonucleotides and two different TRPA1 antagonists (HC-030031 or A967079) transiently attenuated thigmotaxis behavior and mechanical and cold allodynia. A TRPV1 antagonist (capsazepine) attenuated solely heat allodynia. NADPH oxidase activity and hydrogen peroxide levels were increased in hind paw skin *14 days after c*ancer cell inoculation, showing the presence of oxidative stress in this model. The antioxidant, α-lipoic acid, attenuated mechanical and cold allodynia and thigmotaxis behavior, but not heat allodynia. Whereas TRPV1, *via* an oxidative stress-independent pathway, contributes partially to heat hypersensitivity, oxidative stress-dependent activation of TRPA1 plays a key role in mediating thigmotaxis behavior and mechanical and cold allodynia in a cancer pain model. TRPA1 antagonists might be beneficial in the treatment of cancer pain. **References:** [1]Costantini M, et al. Ann Oncol [Internet] 20: 729, 2009. [2]Nassini R, et al. Rev Physiol Biochem Pharmacol Cham: Springer International Publishing, 1, 2014. [3]Plante GE, VanItallie TB. *Metabolism [Internet]* 2010;59: S47–52. Available from: http: //dx.doi.org/10.1016/j.metabol.2010.07.010 **License number of ethics committee**: UFSM, protocol #7658240417 **Financial support**: This work was supported by: Associazione Italiana per la Ricerca sul Cancro (AIRC, IG 19247); Fondazione Cassa di Risparmio di Firenze, Italy (R.N.); National Research Council of Brazil (CNPq; #401437/2014-0).

05.002 Nociceptin/orphanin FQ peptide receptor modulates fibromyalgia-like symptoms in mice. Dagnino APA[1], Silva RBM[2], Chagastelles PC[2], Venturin GT[3], Pereira TCB[2], Bogo MR[2], Greggio S[3], Campos MM[4] [1]PUCRS – Biologia Celular e Molecular, [2]PUCRS – Medicina e Ciências da Saúde, [3]Inscer-PUCRS, [4]PUCRS – Odontologia

Introduction: Fibromyalgia is characterized by widespread pain, being accompanied by functional and affective disorders (Talotta et al., Clin Exp Rheumatol, 105: 6, 2017). This study evaluated the implication of nociceptin/orphanin FQ peptide receptor (NOPr) in a mouse model of fibromyalgia. **Methods:** The local Animal Ethics Committee approved the protocols (15/00487). Fibromyalgia was induced in female CF-1 mice (20-24 g, 4-week-old, total N=151) by reserpine adminstration (0.25 mg/kg; subcutaneous route), once a day, during 3 consecutive days. Control groups received vehicle. Mice were treated with the selective NOPr antagonist UFP101 (1.9 µg/kg), given by intraperitoneal route, during three consecutive days, 30 min after daily reserpine injection. At the 4th day, mice also received UFP101, dosed 30 min before evaluations. The animals were subjected to Von Frey, hot-plate, forced swimming, elevated plus-maze, rotarod and grasping tests. Nociceptin and NOPr expression was determined by RT-qPCR and immunohistochemistry. The [^{18}F]-FDG microPET imaging was used to access the brain activation patterns in reserpine-treated mice. The fiber size distribution of masseter and gastrocnemius muscles was accessed by histological analysis. **Results:** The treatment with UFP101 reduced the mechanical allodynia (37.4 ± 8.5%) and increased the latency time in the hot-plate test (32.2 ± 5%). UFP101 also improved the motor coordination in the rotatod apparatus (13-fold increase in permanence time) and the grasping strenght (21.93 ± 7.7%). UFP101 failed to alter any anxiety or depression parameters. Reserpine-induced fibromyalgia was associated with an increase in nociceptin mRNA expression in the lumbar spinal cord (day 3) and masseter (days 1 and 2), whereas NOPr mRNA expression was increased in the masseter muscle (day 1). Alternatively, NOPr m RNA expression was reduced in the thalamus/hypothalamus (day 3). Immunohistochemistry revealed an incresed expression of NOPr in the dorsal root ganglion (day 4). UFP101 led to a slight decrease in the [^{18}F]-FDG metabolism in cingulate gyrus, superior colliculus, left midbrain, left inferior colliculus and right inferior colliculus of reserpine-treated mice. Additionally, UFP101 prevented reserpine-induced changes in fiber size distribution, according to assessment of masseter and gastrocnemius histological sections. **Conclusion:** The expression of nociceptin and NOPr was altered in the mouse model of fibromyalgia induced by reserpine. Remarkably, UFP101 improved the symptoms of pain, fatigue and adynamia, also recovering the brain activation patterns and the muscle fiber changes in this experimental paradigm. Our data shed new lights on the mechanisms underlying the fibromyalgia pathogenesis, supporting a role for NOPr in this syndrome. **License number of ethics committee**: 15/00487 **Financial support**: FINEP, CAPES, CNPq, PUCRS

05.003 Evaluation of the antinociceptive effect of pregabalin, its associations with antidepressives and pregabalin nanoparticle for chronic pain treatment in Wistar rats. Rodrigues RF[1], Souza GG[2], Carvalho FC[3], Boralli VB[4] [1]Unifal – Análises Clínicas e Toxicológicas, [2]Unifal – Biofísica e Fisiologia, [3]Unifal – Medicamentos e Alimentos, [4]Unifal – Análises Clínicas e Toxicológicas

Introduction: Neuropathic pain is a type of chronic pain that has become a public health problem[1]. The Brazilian Ministry of Health describes the recommended therapeutic option for neuropathic pain, including pregabalin, amitriptyline and duloxetine[2]. In addition, when a controlled drug delivery system is used, its pharmacokinetics and bioavailability may be increased[3]. The main purpose of this study was to evaluate which therapeutic option has a better antinociceptive profile. **Methodology:** Pregabalin nanoparticles (80% m/m) were developed employing chitosan and hydroxymethylpropylcellulose phthalate polymers using the ionotropic gelation method[4]. Male *Wistar* rats (220-250g, n =12/group, 6 sham and 6 operated), had neuropathic pain induced by chronic constriction injury of sciatic nerve[5] and were divided into 8 groups: pregabalin (10mg/kg); amitriptyline (1mg/kg); duloxetine (30mg/kg); pregabalin+amitriptyline (10 mg/kg+1mg/kg); pregabalin+duloxetine (10mg/kg+30mg/kg); pregabalin nanoparticles (10mg/kg); water and empty nanoparticles. On the 14th day after the induction of neuropathic pain, the animals received the substances orally, in a single dose, with a 3-minute interval between administrations. The antinociceptive evaluation was performed by the von Frey hair test immediately before the induction of neuropathic pain, on the 14th day: before the administration of the substances, 1h, 2: 15h, 4h and 8h after the administration of the substances and from 24h to 72h after administrations. The results were compared with the repeated measures ANOVA test. **Results:** Results of antinociceptive test showed that pregabalin in monotherapy presented maximum effect 1h after its administration and the effect lasted until 4h. When compared to the pregabalin group, only pregabalin+amitriptyline and pregabalin+duloxetine groups showed prolonged and higher effect (at 2: 15h ($p<0,05$), 4h ($p<0,05$) and 8h ($p<0,001$) for the first group and at 4h ($p<0,05$) and 8h ($p<0,01$) for the second group administration). At time 2: 15h ($p<0,05$) and 8h ($p<0,05$), the pregabalin+amitriptyline group showed a greater effect than the pregabalin+duloxetine group. The pregabalin nanoparticles group showed similar antinociceptive levels to pregabalin in monoteraphy, however, its effect lasted up to 48 hours after administration ($p<0,05$). The duloxetine, amitriptyline, empty nanoparticles and water groups had no antinociceptive effect. **Conclusions:** Although the pregabalin+amitriptyline group presented a good antinociceptive profile, the animals presented drowsiness as a side effect. The pregabalin nanoparticles group presented a longer effect, besides not presenting drowsiness as a side effect, being more advantageous than the other therapeutic options, and may contribute to a better treatment of chronic pain. **References:** [1]VAN HECKE, O. Pain, v.155, p.654, 2014; [2]BRASIL. Ministério da Saúde. Portaria SAS/MS nº 1083, de 02 de Outubro de 2012; [3]KREUTER, J. Adv. Drug Deliv. Rev., n. 71, p. 2, 2014; [4]CALVO, P. J Appl Polym Sci, v. 63, p. 125, 1997; [5]BENNET, G.J. Pain, v.33, p.87, 1988. **License number of ethics committee**: 57/2016 **Financial support**: FAPEMIG, CAPES

05.005 PPAR gamma activation modulates decrease in gene expression induced by cisplatin in a Chemotherapy Induced Peripheral Neuropathy model *in vitro* Oliveira HR, Duarte DB UnB – Ciências da Saúde

Introduction: The neurotoxicity induced by chemotherapeutic drugs that affect the Peripheral Nervous System (PNS) is named Chemotherapy-induced Peripheral Neuropathy (CIPN). This adverse effect is one the most common that occur within cancer treatment, including platinum derivate (e.g. cisplatin and oxaliplatin), microtubule targeting agents (e.g. paclitaxel and vinka alkaloids), proteasome inhibitors (bortezomib) and antiangiogenic agent (talidomide). CIPN is characterized as dysfunction of the peripheral sensory neurons and manifested as sensory loss, paresthesia, dysesthaesia, numbness, tingling and neuropathic pain. Nowadays there are many proposed mechanisms to explain the establishment of CIPN and changes at the Dorsal Root Ganglia (DRG), such as microglia activation and release of pro-inflammatory cytokines are included. Thus, many strategies are in development to prevent and/or revert CIPN, including neuroprotection. One possible strategy could be the activation of Peroxisome Proliferator-Activated Receptor (PPAR). The activation of this nuclear receptor was already demonstrated to be neuroprotective in many neurodegenerative diseases, such as Alzheimer's disease. In CIPN, the activation of PPAR could be neuroprotective by reducing cytokine expression, once their anti-inflammatory response is well established. **Methods:** To investigate the PPAR activation in an *in vitro* CIPN model, we first characterized the PPAR and TNFα mRNA expression on cisplatin-induced peripheral neuropathy. Thus, DRG cells were isolated from adult naïve Wistar rats weighting 250 – 350 g. DRGs were harvested and the cells dissociated with 1.25 mg/mL collagenase 1A and the reminiscent cells were maintained in HAM F12 culture media with nerve growth factor (NGF -250 µg/mL) for 9 days in 12-well plates. On day 8, cells were treated with cisplatin (3, 10 or 30 µM) for 24 hours with or without rosiglitazone, a PPAR activator (1, 3 or 9 µM). To evaluate gene expression, the mRNA was extracted with trizol and quantified in triplicate by RT-qPCR. The mRNA expression level was normalized to expression of β-actin mRNA. **Results:** 3 µM Cisplatin treatment did not produce mRNA expression change, while 10 µM did not alter TNFα expression, but increase 1.3-fold PPAR mRNA expression ($p < 0.05$). However, the highest cisplatin concentration used (30 µM) increased 2.5-fold TNFα ($p < 0.05$) and reduced 0.72-fold PPAR- mRNA expression ($p < 0.05$) compared to control. Further we investigated whether PPAR activation could have an effect in the cisplatin-induced decrease in PPAR mRNA expression. The co-treatment with 30 µM cisplatin and 9 µM of rosiglitazone prevented the cisplatin-decrease PPAR mRNA expression ($p < 0.05$). We next investigated whether the activation of PPAR could regulate PPAR/ mRNA expression, another isoform of PPAR which also have anti-inflammatory actions. Rosiglitazone also prevented the cisplatin-induced decrease in PPAR/ mRNA expression ($p < 0.05$). CONCLUSIONS: Here we presented that cisplatin treatment modulates TNFα and PPAR mRNA expression. The prevention of cisplatin-induced mRNA decreases in an *in vitro* neurotoxicity model due to rosiglitazone treatment, indicates that PPAR activation could be neuroprotective. **License number of ethics committee**: 55724/2013 **Financial support**: IASP EC 2013 Grant to DBD and FAPDF 1930006682015 to DBD.

05.006 Involvement of the cannabinoid CB_2 receptor in spinal microglia in the control of muscle pain by exercise. Santos RS, Oliveira HU, Souza GG Unifal – Fisioterapia

Muscle pain affects approximately 11 to 24% of the world's population. Studies have shown that physical exercise is a nonpharmacologic therapy that can control pain and that the endocannabinoid system participates in this effect; however, no study has investigated the interaction between cannabinoid CB_2 receptors and spinal microglia in this pain control mechanism. Thus, the present study aimed to investigate whether the cannabinoid CB_2 receptor can inhibit spinal microglia during exercise-induced antinociception. C57BL/6J female mice underwent a muscle pain model by an intramuscular injection of carrageenan into the right gastrocnemius muscle and performed a swimming training protocol. The nociceptive threshold was evaluated by the von Frey filament and hot plate tests. To evaluate the involvement of the cannabinoid CB_2 receptor, endocannabinoids and spinal microglia were injected intrathecally into the AM630, MAFP and minocycline, respectively. In addition, immunofluorescence and western blot assays were used to verify the expression and colocalization of cannabinoid CB_2 receptors and microglia in the dorsal horn spinal cord. Furthermore, the present study used an ELISA assay to evaluate the effect of exercise on pro-inflammatory cytokine (IL-1β and TNF-α) levels in the muscle and a thermography technique to verify changes in the muscle temperature of mice. The muscle pain model induced mechanical allodynia and thermal hyperalgesia, which were reversed after the second and third weeks of swimming. This antinociception was inhibited by AM630 and potentiated by MAFP and minocycline. In addition, exercise increased AEA levels and cannabinoid CB_2 receptor expression and reduced microglia expression in the spinal cord dorsal horn. Muscle TNF-α levels and muscle temperature were also reduced in animals with muscle pain after aerobic training. The present study suggests that aerobic training reduces muscle pain and that this effect occurs by activating the cannabinoid CB_2 receptor, which inhibits the spinal microglia.

Keywords: exercise; antinociception; cannabinoid CB_2 receptor; spinal microglia. **License number of ethics committee**: 628/2015 **Financial support**: CAPES

05.007 Involvement of HSP70/TLR4/IL-6, TNF-α pathway in the mechanical hyperlagesia induced by Delayed-Onset Muscle Soreness. Moraes TR[1], Santos RS[1], Ferreira DW[2], Veras FP[2], Brazil M[2], Lollo PC[3], Amaya-Farfan J[3], Cunha TM[2], Souza GG[1]
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Aim of Investigation: The present study evaluated the involvement of HSP70/TLR4/IL6, TNF-α pathway in the Delayed-onset muscle soreness (DOMS). DOMS is a common myogenic condition characterized by a reduction in the pain threshold to mechanical stimulation, after intensive exercise and is particularly pronounced days after unaccustomed exercise. Several studies have demonstrated a correlation between HSP70 and TLR4 in the genesis of different pathologies. Thus, we hypothesized that HSP70 may activates spinal TLR4, which will lead to production of cytokines, such as IL-6 and TNFα, that contribute to DOMS genesis. **Methods:** To study were used five-month-old male TLR4, MyD88, IL-6, TNFR1, TNFR1/R2-deficient mice (–/–) C57BL/6 background and, theirs wild-type (WT). Initially, we standardized a DOMS model by acute exercise protocol. WT and knockouts mice ran 40 min to a progressive speed, starting 5 m/min until the fifth minute, incrementing to 10 m/min until fifteenth minute, 15 m/min until thirty minute and 17 m/min until forty minutes at 0% grade. Before, 24 and 48 h after exercise the muscle (right gastrocnemius) mechanical nociceptive threshold was measured by Pressure Application Measurement (PAM). Groups composed by nonexercised animals also were used. The anti-HSP70 antibody, TLR4 LPS-RS antagonist and microglial inhibitory minocycline, administered intrathecally before exercise, were used. We also used Western blot assay to evaluate the HSP70 and TLR4 expression, real time quantitative PCR to evaluate the expression and quantification of TLR4 mRNA, and ELISA assay to dosage the IL-6 and TNF-α levels both in the spinal cord and right gastrocnemius muscle. Immunofluorescence assay was also used to co-locate and evaluate HSP70 and spinal microglia expression. **Results:** The results found in this study demonstrated that acute exercise protocol proposed was efficient to produce DOMS. However, this effect was found just in WT animals, in the TLR4, MYD88, TNFR1 and TNFR1/R2 deficient mice the DOMS was not found. Western blot analysis shows that 24 and 48 h after exercise occurs an increase of HSP70 expression in the spinal cord and right gastrocnemius muscle and TLR4 expression in the spinal cord. PCR-RT revealed an increase of nRNA TLR4 expression in the spinal cord just 24h after exercise. However, this effect was not found in the muscle. In addition, immunofluorescence assay revealed an increase of TLR4 and microglia expression in spinal cord. Furthermore, muscle IL-6 and TNF-α levels were increased after 24 and 48 h of exercise in WT mice and in TLR4/- mice these levels not were changed. This increase was also found in spinal cord of WT exercised mice. **Conclusions:** The present study suggest that a single acute exercise progressive session can produce DOMS, even being a moderate exercise. The results demonstrated suggest that the HSP70 released during exercise may activates TLR4 in microglia spinal, leading to production of pro-inflammatory cytokines, such as IL-6 and TNF-α, which contribute for sensitization of muscle pain. **Financial support**: CAPES

05.008 Peripheral P2X3 receptors are involved in acute muscle pain but not in induction and maintenance of chronic muscle pain. Jorge CO, Azambuja G, Gomes BB, Oliveira-Fusaro MCG [1]Unicamp – Fisiologia

Introduction: Muscle pain is a world health problem with high socio-economic impact. Currently, several mechanisms are known that modulate acute muscle pain. However, the mechanisms underlying transition of acute to chronic pain, as well as, the chronification process are poorly understood. Some hypothesis points out to neuroplasticity in nociceptive pathway. The P2X3 receptors are ionotropic receptors expressed primarily on nociceptive fibers and highly involved in pain process. We have previously demonstrated that peripheral P2X3 receptors are involved in acute muscle pain. Therefore, the present study aimed to investigate whether P2X3 receptors are also involved in induction and maintenance of chronic muscle pain. **Methods:** Initially, we standardized the model of transition of acute to chronic pain (Dina et al., 2008) in gastrocnemius muscle of mice. To this end, carrageenan was injected into gastrocnemius muscle of mice to trigger acute muscle hyperalgesia. After 10 days, PGE_2 was injected into gastrocnemius muscle previously challenged by carrageenan and the nociceptive thresholds quantified for one week to highlight the establishment of chronic muscle pain. Mechanical muscle nociceptive thresholds were quantified by Randall Selitto pressure analgesimeter. To evaluate the involvement of P2X3 receptors in induction and maintenance of chronic muscle pain, A317491, a selective P2X3 receptors antagonist, was injected before carrageenan or PGE_2. Male Swiss mice (6-weeks-old) from CEMIB-UNICAMP were used and all experimental procedures were approved by the Ethics Committee in Animal Research of UNICAMP (4808-1/2018). **Results:** Preliminary results showed that carrageenan (100µg/muscle) induced acute mechanical muscle hyperalgesia for up to 72 hours when compared to 0.9% NaCl ($p<0.05$, Two-way ANOVA, Bonferroni test, n=5). Nociceptive behavioral responses returned to baseline levels 96 hours post carrageenan ($p>0.05$, Two-way ANOVA, Bonferroni test, n=5). Injection of PGE_2 (1µg) into gastrocnemius muscle previously (10 days) challenged by carrageenan induced mechanical muscle hyperalgesia significantly greater than in animals previously challenged by 0.9%NaCl ($p>0.05$, Two-way ANOVA, Bonferroni test, n=5). Mechanical muscle hyperalgesia induced by PGE_2 in animals previously challenged by carrageenan lasted more than one week, while in animals previously challenged by 0.9%NaCl, mechanical muscle hyperalgesia induced by PGE_2 lasted only 24 hours. Pre-treatment with A317491 (60µg/muscle) previously carrageenan prevented the development of acute mechanical muscle hyperalgesia for up to 6 hours ($p<0.05$, two-way ANOVA, Bonferroni test, n=5) and did not prevent the induction of chronic muscle hyperalgesia highlighted by injection of PGE_2 ($p>0.05$, two-way ANOVA, Bonferroni test, n=5). Pre-treatment with A317491 (60µg/muscle) previously PGE_2 did not prevent the maintenance of chronic muscle hyperalgesia ($p>0.05$, two-way ANOVA, Bonferroni test, n=5). **Conclusion:** Our results suggest that P2X3 receptors expressed in muscle tissue are involved in acute muscle pain but not in induction and maintenance of chronic muscle pain. **Financial Support:** São Paulo Research Foundation, FAPESP (Process number: 17/17919-8) **References:** Dina, Neurosc., 152, 521, 2008 **License number of ethics committee**: 4808-1/2018 **Financial support**: FAPESP

05.009 Antinociceptive and anti-inflammatory activities of hydroalcoholic extract and fractions of *Mandevilla moricandiana* (**Apocynaceae**). Dos Santos IS[1], Maciel SASG[1], Pontes RGMS[1], Ferreira LLM[2], Leal IC[2], Raimundo JM[3], Carmo PL[1] [1]UFRJ – Farmacologia e Inflamação, [2]UFRJ – Produtos Naturais e Ensaios Biológicos, [3]UFRJ – Toxicologia e Farmacologia Cardiovascular

Introduction: The objective of this work was to evaluate the antinociceptive and anti-inflammatory activities of the hydroalcoholic extract (HE) and fractions of the leaves of *Mandevilla moricandiana*, not yet described. **Methods:** Protocols approved by ethics Committee on the Use of Animals of the Federal University of Rio de Janeiro-Macaé, protocol MAC038. The experiments were performed on male Swiss mice (18-25g, n= 5-10). DMSO (negative control), HE (2.5, 5 and 10 mg/kg) fractions (10 mg/kg) or positive control was administered intraperitoneally (i.p.), 30 min before the tests. 1) Abdominal writhing test: acetic acid was injected (0.8% v/v, 10 ml/kg, i.p.) and the number of writhes was quantified for 10 min. Indomethacin (4 mg/kg) was the positive control. 2) Formalin test: animals received 20 µl of 2.5% formalin in the right hind paw. The time spent licking the paw timed was during the neurogenic (0-5 min) and inflammatory phases (15-30 min). Morphine (10 mg/kg) was the positive control. 3) Hot plate test: the animals were placed on the hot plate at 54 °C prior to injection and 30, 60, 90 and 120 min after injection of the samples, and the time the animal remained on the plate was measured without licking the paws (time maximum of stay = 30 s). 4) Modified hot plate: 50 µl of saline and carrageenan (500 µg/paw) intraplantar injected were into the right and left hind paws, respectively. Afterwards, the animals placed were on the hot plate at 51 °C, 15, 60, 180 and 360 min after the injection. The time of raising of the paws timed were, and the time difference between the paws was calculated. **Results:** 1) The HE at doses of 5 and 10 mg/kg reduced the number of abdominal writhing from 21.2 ± 1.5 (DMSO) to 8.6 ± 4.4 ($P < 0.05$) and 1.7 ± 0.8 ($P < 0.05$), respectively. The hexane (HF), dichloromethane (DF), ethyl acetate (EAF) and butanolic (BF) fractions were also able to reduce the number of abdominal contortions to 3.3 ± 1.1 ($P < 0.05$); 3.0 ± 1.0 ($P < 0.05$); 3.3 ± 2.1 ($P < 0.05$) and 2.8 ± 1.2 ($P < 0.05$), respectively. 2) Formalin test: HE and HF, DF and EAF reduced the reaction time in the neurogenic phase of the test from 52.3 ± 6.6 (DMSO) to 31.8 ± 8.4 ($P < 0.05$); 20.4 ± 2.0 ($P<0.05$); 28.0 ± 3.7 ($P<0.05$) and 24.4 ± 3.5 s ($P<0.05$); respectively. In the inflammatory phase of the test only the EAF achieved a significant result of 345.7 ± 20.7 (DMSO) to 176.3 ± 38.8 s ($P<0.05$). 3) Hot plate test: HF (10 mg/kg) significantly increased the time the animal remained on the plate 60 min after administration, from 9.3 ± 1.3 (DMSO) to 16.1 ± 1.2 s ($P < 0.05$). 4) Modified hot plate test: EAF (10 mg/kg) reduced the latency variation between the legs at the times of 15, 180 and 360 min, from 4.5 ± 0.8 (DMSO) to 0.0 ± 0.0 s ($P < 0.05$); 6.0 ± 1.4 (DMSO) for 1.0 ± 0.4 s ($P < 0.05$); 10.2 ± 1.7 (DMSO) for 2.5 ± 1.1 s ($P < 0.05$), respectively. **Conclusion:** These results allowed the identification of the antinociceptive and anti-inflammatory activities of *M. moricandiana*. **Financial support**: PIBIC/UFRJ. **License number of ethics committee**: MAC038 **Financial support**: PIBIC/UFRJ.

05.010 Angiotensin II Type 2 receptor antagonism as treatment of acute gouty attack, **including those precipitated by the use of antihypertensive drugs**. Vieira TN[1], Silva CR[1], Ferreira J[2] [1]UFU – Biotecnologia, [2]UFSC – Farmacologia

Introduction: Individuals with gout arthritis frequently experience a range of comorbidities where hypertension has been one of the most common, affecting 60-80% of gouty patients. Clinical studies previously demonstrated that antihypertensive drugs as angiotensin I-converting enzyme inhibitors (ACEi) and AT1 receptor blockers (BRAT1) increase the risk of acute gouty attack. ACEi can modulate the renin-angiotensin system resulting in Angiotensin II type 2 Receptor (AT2R) activation, which could be the mechanism targeting the inflammatory responses induced by MSU. Recently, the expression of AT2R in the synovial tissue of individuals with rheumatoid arthritis and osteoarthritis was confirmed, so that AT2R antagonism presents as a promising therapeutic strategy for patients with arthritis such as gout. Thus, the objective of this study is to evaluate the analgesic potential of the AT2R antagonist in acute gout models, including those precipitated by the use of antihypertensive drugs. **Methods:** Adult male Wild type C57BL/6 mice (20-25 g) were used and our Institutional Ethics Committee approved all procedures (process number 080/16). Firstly, animals were treated with the AT2R antagonist, PD123319 (10 mg/kg v.o), administered 0.5 hours before IA MSU (30 µg/articulation) injection. In addition, animals were pre-treated with antihypertensive drugs BRAT1 valsartan (20 mg/kg) or ACEi enalapril (3 mg/kg), given v.o., 0.5 hours before IA MSU Low dose, without effects alone (1 µg/articulation), or MSU Low dose plus PD123319 (30 nmol/articulation). Also, animals were IA co-administered with PD123319 (30 nmol/articulation) plus angiotensin II (0,05 µg/articulation). All groups were analyzed for mechanical allodynia, thermal hyperalgesia, overt pain-like behaviors and ankle edema development several times after IA injections. **Results:** At the tested dose, oral PD123319 treatment was active in prevent mechanical allodynia (44% of prevention) and overt pain-like behaviors (56% of prevention) from 1 up to 6 h after IA MSU injection. MSU low dose alone did not induced pain neither edema. Animals treated with antihypertensive drugs plus MSU low dose developed mechanical allodynia and overt pain-like behaviors from 2-6 hours after the injections. IA co-administration of PD123319 prevented mechanical allodynia (28% of prevention) and overt pain-like behaviors (67% of prevention) precipitated by antihypertensive drugs plus low MSU dose treatments. IA Angiotensin II induced mechanical allodynia and overt pain-like behaviors from 0.5 up to 6 h after injections, and pain was prevented by PD123319 IA co-injection 4 up to 6 h after administrations. **License number of ethics committee**: CEUA 080/16 **Financial support**: Research support: This study was supported by Conselho Nacional de Desenvolvimento Científico (CNPq) and FAPEMIG (Fundação de Apoio a Pesquisa do Estado de Minas Gerais)

05.011 Evaluation of electroacupunture and physical exercises in monoarthritis model in rats. Rebelo IN[1], Martins GA[2], Ferraz AG[1], Witz MI[1], Souza AH[1] [1]Ulbra – Farmacologia, [2]Ulbra – Genética e Toxicologia Aplicada

Introduction: Osteoarthritis (AO) is defined as a disorder involving movable joints characterized by cellular stress and degradation of the extracellular matrix initiated by lesions that activate improper repairs, including pro-inflammatory pathways of innate immunity. One of the main aspects of the disease is chronic pain that can lead to psychic decay and poor quality of life. The financial costs of treatment and management of AO are a major problem, requiring the discovery of new therapeutic strategies and different combinations of treatments, including complementary and alternative treatments such as physical exercise, physical therapy, acupuncture and electroacupuncture, among others. Acupuncture and transcutaneous electric nerve stimulation (TENS) are recommended as non-pharmacological interventions. The therapeutic effects of electroacupuncture (EA) are well documented, such as pain control by endogenous opioids and their μ and δ receptors and attenuations of substance P levels and proinflammatory cytokines, but the mechanisms of action are not well elucidated. Physical exercise (EX) has positive effects in the treatment of AO, since they demonstrate important ways of modulating pain. **Methods:** In this study, male Wistar rats were randomly divided into 5 groups, including control, saline, EX, EA and association (EX + EA). The monoarthritis in the animals of the last three groups was induced by injection into the intra-articular space of the left ankle of Complete Freund's Adjuvant (CFA) containing 1 mg/mL of heat killed mycobacterium. Disease incidence and severity was evaluated through measure of the paw edema. The duration of the treatment after CFA injection were two times of 5 sessions of EA or EX + EA (2 sessions EA and 3 sessions EX) or 3 sessions of EX (forced swims) per week, 7 days after induction of monoarthritis. The animals were evaluated through nociceptive tests: paw-flick (heat hyperalgesia), cold simulation (cold hyperalgesia) and motor activity evaluation, in the day 0 (basal), 7 (after CFA injection and before sessions), 13 and 20 (after sessions). **Results:** After 10 sessions, the EX group showed good results in controlling the evolution of the inflammation induced by CFA when compared to EX + EA group, reducing paw edema. The EX + EA group relieved the inflammatory pain ascertained by mechanical allodynia. The EA group presented improvement of the heat hyperalgesia after 10 sessions when compared to the other groups. It also showed improvement of the cold hyperalgesia when compared to the EX + EA group after 10 sessions. There wasn't significant improvement in the therapeutic protocols in the results after 5 sessions, except in the motor activity evaluation, where the EX + EA group presented greater motor activity than the group receiving the EA treatment. **Conclusion:** The present study demonstrates, for the first time, the relevance of this treatments (EX, EA and EX + EA) for pathways underlying the OA, serving as a screening for the detection of new treatments. Additional studies are under development to further characterize the common mechanisms involved in this response. **Acknowledgments:** CAPES and CNPq for **Financial support**. The experimental protocols have been approved by the Ethics Committee of Animals from ULBRA (nº2017/252). **License number of ethics committee**: nº2017/252
Financial support: CAPES and CNPq

05.012 Antinociceptive and anti-inflammatory effects of Bixin, **a carotenoid extracted from the seeds of** *Bixa orellana* **Linné**. Gasparin AT[1], Golin SD[2], Jesus CHA[1], Sotomaior BB[1], Cunha JM[1] [1]UFPR – Farmacologia, [2]UFPR – Pharmaceutical Sciences

Introduction: Bixin is a FDA-approved food colorant and additive, used as cosmetic and textile colorant [(1)]. Annatto (common name of the *Bixa orellana* species*)* has also been used in South America to treat infectious and inflammatory diseases of the skin, prostate, gastrointestinal tract, and others [(2)]. Since carotenoids are compounds with antinociceptive and anti-inflammatory effects, the aim of this work was to evaluate the potential antinociceptive and anti-inflammatory effects of bixin in murine models of acute pain and inflammation. **Methods:** Male Wistar rats (180–220 g) or Swiss albino mice (18–35 g), n=6-8 were used in the present study, previously approved by the UFPR's Committee on the Ethical Use of Animals (CEUA/BIO-UFPR; #1087). After a 12 hour (h) fasting period, all animals were orally treated with bixin (15 or 30 mg/Kg), or vehicle (corn oil), 1h before all behavior tests. For assessment of potential antinociceptive effect, rats received an intraplantar injection (i.pl) of formalin (2.5%, 50 μL/paw). The number of flinches was recorded for 60 minutes after injection, divided into neurogenic phase (0-10 min) and inflammatory phase (15-60 min). This potential was also evaluated in the acetic acid-induced writhing model in mice. For this, mice were treated with equivalent doses of bixin (27mg/Kg and 53mg/Kg) or vehicle, and 1h after received an intraperitoneal injection of acetic acid (0.6%; 10 mL/Kg). The number of writhing was counted for 30 minutes. For assessment of a possible central analgesic effect, rats were placed on the hot plate apparatus (50 ± 1°C) and the latency until a typical distress reaction was recorded. To evaluate spontaneous locomotor activity, the number of squares crossed in the open field apparatus was counted for 5 minutes. For assessment of the potential anti-inflammatory effect, rats received i.pl injection of carrageenan (Cg; 200 μg/paw; 0.1mL), 1 h after bixin or dexamethasone (Dexa; 1 mg/Kg; positive control) treatment. The variation in paw thickness (indirect measurement of paw edema) was recorded using a caliper rule at 1, 2, 3 and 4h after Cg injection. **Results:** When compared to vehicle-treated group, the treatment with bixin (at the both tested doses) significantly reduced: 1) number of formalin-induced flinches in both phases of the test; 2) the number of acid acetic-induced writing. Only the higher dose of Bixin (30 mg/Kg) induced: 1) an increase in the latency on the hot plate apparatus; 2) a significant decrease of Cg-induced paw edema 1 and 2 h after Cg injection, while Dexa inhibited the paw edema at all time points. There was no significant difference between the groups in the number of crossings in the open field test. **Conclusion:** Our data, for the first time in the literature, show that isolated bixin presents the antinociceptive and anti-inflammatory effects in pre-clinical models of pain and inflammation. This effect seems not to be related to locomotor deficit. Further studies are in progress to characterize the mechanisms of action involved. **References:** [1]Jondiko, I. J. O. et al. *Phytochemistry*, *28*(11), 57, 1989; [2]Shahid-ul-Islam, et al.*Journal of Advanced Research*, *7*(3), 499, 2016. **License number of ethics committee**: CEUA/BIO-UFPR #1087

05.013 Involvement of Transient Receptor Potential channels (TRPs) in the pharmacological effects of trans-anethole. Santos LG, Oliveira JRJM, André E UFPR – Farmacologia

Introduction: Trans-anethole is a substance naturally found in anise, star anise and fennel essential oils. Several studies have shown that anethole may present antifungal, antibacterial, anti-inflammatory and antinociceptive properties[1] but the responsible mechanisms for these effects are still unclear. Many plant-isolated compounds that have sweet tasty or spicy have been discovered to be targets for Transient Receptor Potential channels (TRPs)[2]. Considering these similarities, we hypothesized that TRPA1 could be involved in the pharmacologic actions of anethole. **Methods:** The nociceptive response, ear edema as well as sensitization/desensitization process after topical application of anethole or capsaicin in mice was analyzed. The nociceptive behavior was evaluated during 20 minutes after intraplantar injection (100, 250 e 500 nmol/20 µl/paw) of anethol. Additional experiments involved intraplantar treatment with TRPA1 antagonist HC-030031 (300 µg/20 µl/paw). The ear edema was measured 60 minutes after the topical application of anethole or capsaicin. We also investigated whether repeated ear exposure to anethole or capsaicin could induce the sensitization/desensitization process to its edematogenic effect. Thus, mouse ears were topically pretreated on days 1 and 3 with anethole (500 mmol/20µl/ear) or capsaicin (200 µg/20 µl/ear) in separate groups. After 3 days, the animals in each group were challenged with anethole (500 mmol/20 µl/ear) or capsaicin (200 µg/20 µl/ear) and the development of edema was assessed. All protocols have been approved by the Ethics Committee of the Federal University of Paraná (Protocol n°: 1125). **Results:** The intraplantar injection of anethole promoted nociception (22,5±5,3 s and 47, 8±11,9 s) only in the higher doses (250 and 500 nmol/20 µL, respectively) as compared to vehicle (0,8±0,2 s). The HC030031 administration was able to reduce this response by 96,2±2%. Topical application of anethole (500 mmol/20 µL/ear) or capsaicin (200 ug/20 µL/ear) in the mouse ear induced edematogenic response (0,118±0,01 mm, 0,068±0,008 mm, respectively), compared to vehicle (0,007±0,005 mm). In ears pre-treated with anethole on days 1, the edematogenic response of anethole or capsaicin on day 3 was significantly inhibited (69,2±7,7%, 50±3,3%, of reduction, respectively). In addition, pretreatment with capsaicin on days 1 also reduced the edema evoked by capsaicin or anethole on day 3 (60±9,7%, 94,9±3,4% of reduction, respectively). **Conclusion:** Anethole promote nociception response, at least, by TRPA1 activation. The edematogenic and anti-edematogenic response induced by anethole appear occur by activation and desensitization, respectively, of TRP-expressing sensory neurons. 1-RITTER et al. **Hind.Pub.Corp**, 2014. 2-JULIUS, D. **Annu.Rev.Cell.Dev.Biol**., 29: 355, 2013. **License number of ethics committee**: 1125 **Financial support**: CNPq

05.014 Comparative analysis of different antioxidants in oxaliplatin-induced peripheral neuropathy in mice. Agnes JP, Santos VW, Gonçalves RM, Delgobo M, Macedo Júnior SJ, Ferreira J, Zanotto-Filho A UFSC – Farmacologia

Introduction: While cytotoxic chemotherapeutics are among the most used pharmacological strategies, their low selectivity for cancer cells results in various adverse effects, such as chemotherapy-induced peripheral neuropathy (CIPN). CIPN affects a significant percentage of patients treated with drugs such as oxaliplatin (OXA), bortezomib and paclitaxel. Clinical manifestations include pain and numbness in hand and foot as well as increase in cold sensitivity, which culminate in interruption, delay or modification of the therapeutic protocol. Some antioxidants were reported as having anti-nociceptive effects in CIPN models. On the other hand, the redox balance is important for tumor biology. In this context, studies have reported that antioxidants may protect tumor cells from chemotherapeutics, thus making the use of antioxidants in anticancer therapy controversial. The aim of this study was comparatively evaluating the impact of three differing antioxidants, namely N-acetylcysteine (NAC), lipoic acid (LA) and tocopherol, on mechanical, heat and cold-induced nociceptive behavior and tumor growth in a mice models of CIPN induced by OXA and Paclitaxel. **Methods:** To CIPN induction, 3-month-old Swiss mice were treated with 5 mg/kg of OXA or Paclitaxel intraperitoneally each other day, for a total 14 days. The antioxidants NAC, LA and tocopherol were administered at the dose of 50 mg/kg/day (gavage) either from the first day of CIPN induction or after CIPN establishment in order to compare their preventive versus therapeutic potential. Von Frey test, adapted cold plate test and Hargreaves were used to assess mechanical, cold and heat nociceptive thresholds. The impact of antioxidants on OXA antitumor efficacy (tumor growth and volume/weight) was evaluated by treatment of Swiss mice implanted with Ehrlich cells. **Results:** Treatment with antioxidants reduced both mechanical and cold allodynia induced by OXA and Paclitaxel; heat sensitivity was not altered by OXA/Paclitaxel. Long-term antioxidant administration prevented CINP whereas single injection was not capable of attenuating nociceptive behaviors. On the hand, repeated administration of antioxidants post-CINP induction attenuated cold and mechanical allodynia. With regard to tumor growth, the antioxidants did not alter OXA effects upon tumor growth kinetics and size. Finally, evaluation the oxidative parameters in kidney, heart and liver confirmed that antioxidants attenuated OXA-induced oxidative at the used doses. **Conclusion:** Treatment with NAC, AL and tocopherol were efficient in attenuating mechanical and cold allodynia induced to OXA and Paclitaxel without impairing their antitumor activity. While direct effect of antioxidants upon nociceptive pathways does not seem to be involved in their anti-CINP effect in our model, further studies will address their protective effect upon oxidative damage in peripheral nociceptive neurons in order to delineate potential mechanisms. **Acknowledgment:** Thanks to LAMEB-UFSC for technical assistance and CAPES for **Financial support**. **License number of ethics committee**: 3722260417 **Financial support**: CAPES

05.015 α-Phellandrene/ β-cyclodextrin presents antinociceptive activity in a model of subacute inflammatory pain in rats. França ARS[1], Pinheiro-Neto FR[1], Nogueira MRS[2], Acha BT[2], Feitosa EL[2], Sousa DP[3], Lima SG[4], Almeida FRC[1] [1]UFPI – Biochemical Pharmacology, [2]UFPI – Bioquímica e Farmacologia, [3]UFPB – Pharmaceutical Sciences, [4]UFPI – Organic Geochemistry

Introduction: Inflammatory pain occurs as a result of the activation of the immune system after lesions or tissue infections due to the increased release of inflammatory mediators. Studies have already demonstrated the anti-inflammatory and antinociceptive activity of free α-phellandrene (α-PHEL). Thus, we sought to develop an inclusion complex with β-CD, in order to improve its effects on the Complete Freund Adjuvant (CFA)-induced inflammatory pain model. **Methods:** The inclusion complex was prepared with α-PHEL and β-CD in the ratio 1: 9 g under magnetic stirring, dried using a spray dryer and analyzed in an infrared spectrometer. CFA (50 µl i.pl.) was administered to the right hind paw of female Wistar rats (170-240g, n=6-8). Acute evaluation was performed at oral doses of α-PHEL/β-CD (3.125, 6.25 and 12.5 mg/kg), dexamethasone (0.5 mg/kg) or vehicle at different time intervals (1, 2, 4, 6, 8, 12 and 24 hours) and for subacute investigation, animals were treated and evaluated for allodynia once daily for 10 days using the digital von Frey method. In order to evaluate the participation of opioid receptors in the antihypernociceptive effect of α-PHEL (100 mg/kg p.o.), naloxone (3 mg/kg i.p.) and morphine (5 mg/kg i.p.) were used. The results were analyzed using ANOVA one-way followed by Bonferroni test, $p < 0.05$. All protocols were approved by Ethics Committee on the Use of Animals (ECUA/UFPI No. 274/16). **Results:** The complexation efficiency of α-PHEL in β-CD was 70.7±1.5%. The results of the Fourier transform infrared spectroscopy allow us to verify complex formation. In the evaluation of the acute pain treatment, no significant increase was observed. On the other hand, during subacute treatment, all doses were effective in reducing mechanical allodynia. A dose of 12,5 mg/kg had a maximum effect on day 3 (44,7±1.5 g), vehicle (25,1±0,9), while doses of 6,25 and 3.12 mg/kg showed maximum inhibition of allodynia on the 5 th day (41,6±1,1 and 42,5±2,2 g, respectively), vehicle (24,3±1,2). The interruption of treatment from day 6 to day 8 did not show the development of tolerance, led to a decrease in the mechanical nociceptive threshold, and the α-PHEL/β-CD presented antiallodynic effect again on day 9, when the treatment was reestablished and lasted until the 10 th day. The following results showed the participation of the opioid system in the anti-hypernociceptive effect of α-PHEL on the mechanical hypersensitivity induced by CFA: Vehicle (17,2±0,5), morphine (44,4±1,2), naloxone plus morphine (21,2 ± 1,7), naloxone (19,6±1,3), α-PHEL (40,8±2,4), naloxone plus α-PHEL (21,3±1,2). **Conclusion:** In conclusion, our findings suggest that α-phellandrene has potential antinociceptive effect against subacute inflammatory pain and the opioid system participates in this effect. **Financial support:** UFPI/CAPES/CNPq. **License number of ethics committee**: (ECUA/UFPI No. 274/16) **Financial support**: UFPI/CAPES/CNPq

05.016 Ultrasonic vocalization analysis for the study of the affective component of acute orofacial pain and emotional contagion of pain in rats. Barroso AR, Araya EI, Chichorro JG UFPR – Farmacologia

Introduction: Considering the unique features of craniofacial pain, it has been necessary to propose new methods to increase the translational aspect in the basic research on pain. The ultrasonic vocalizations (USVs) have been related with emotional states of rats and may indicate the affective component of pain. Studies of emission of USVs in rats submitted to the formalin test injected in the paw demonstrated an increase of USVs emission, which was suppressed by morphine treatment. Besides, there is growing evidence that USVs can elicit emotional contagion, a form of empathy, which can modify the perception of pain. Nonetheless, the influence of changes in the social context in the emission of USVs in pain models has not yet been investigated. Hence, the aim of the present study was to evaluate if the emission of USVs represents a parameter for the study of the affective component of pain, as well as if the social interaction modulates USVs emission. **Methods:** All experiments were performed with male Wistar rats (200-220g) and the protocols approved by the local Ethical Committee (CEUA/BIO #1151). Formalin (2. 5%/50 µL) was injected into the right upper lip and the USVs was monitored by a microphone (UltraSoundGate Condenser Microphone, CM16; Avisoft Bioacoustics, Berlin, Germany) along with the register of the facial grooming for 30 min. The same protocol was repeated in an independent group of rats to evaluate the effect of systemic morphine (2.5mg/kg) on USV and facial grooming behavior induced by formalin. The modulation of the USVs was evaluated by assessing the formalin-induced-nociceptive behavior of one rat in the presence of a cagemates, non-cagemates and a cagemates separated with a visual barrier. **Results:** Formalin injected into the right upper lip induced an increase of aversive USs (i.e 22 kHz) emission in comparison to control rats. The increase was significant at 9-18 min interval, which corresponds to the final of the interphase and the beginning of late phase of formalin test. Morphine treatment resulted in a complete inhibition of USVs emissions as well as, facial grooming behavior. Rats that received formalin and were evaluated in the presence of cagemates and non-cagemates showed an increase of aversive USVs emissions in the interphase of the formalin test. However, when rats were separated with a visual barrier the USVs were not detected. **Conclusion:** Although the peaks of the grooming behavior and USVs emission evoked by formalin are not coincident, both are reduced by morphine, suggesting they represent different orofacial pain measures. In addition, USVs emission induced by formalin is susceptible to social modulation, indicating that the analysis of this parameter may contribute to explore the affective dimension of orofacial pain. **License number of ethics committee**: local Ethical Committee (CEUA/BIO #1151)

05.017 Involvement of Endothelin-1 in Hyperalgesia and sickness behavior induced by Lipopolysaccharide Lomba LA, Leite-Avalca MCG, Cruz JV, Maba IK, Correia D, Zampronio AR UFPR – Farmacologia

Introduction: Infectious diseases present a common set of signs and symptoms such as fever, hyperalgesia, loss of appetite, and somnolence that is called sickness syndrome. This sickness behavior and associated symptoms result in saving energy and help to fight infection. The central mechanisms involved in sickness behavior are still unclear, but it is known that endothelin-1 (ET-1) is an important central mediator of the febrile response induced by lipopolysaccharide (LPS), and that this response is not dependent on prostaglandin (PG) production. ET-1 is also involved in several types of pain. The aim of this study was to evaluate the participation of central ET-1 in sickness behavior. **Methods:** Male Wistar rats (200-250 g) were used and when necessary a guide cannula was implanted in the brain for intracerebroventricular (i.c.v.) administration of drugs. Intraperitoneal (i.p.) injection of LPS (50 µg/kg) or ET-1 (0.1, 0.3 or 1.0 pg/2 µl, i.c.v.) was used to induce sickness syndrome. Animals were treated with the ET_A (BQ123, 3 pmol/2 µl) and ET_B (BQ788, 3 pmol/2 µl) receptor antagonists 30 min before the stimulus. Mechanical (eletronic analgesimeter) and thermal (hot plate apparatus at 48ºC) hyperalgesia, sucrose preference index and the motor performance in the open field were evaluated. **Results:** Peripheral administration of LPS induced mechanical and thermal hyperalgesia in the rat hind paw that lasted for at least 6 h. The i.c.v. treatment with BQ123 abolished while the ET_B receptor blockade with BQ 788 decreased in 60% the LPS-induced mechanical hyperalgesia. In LPS-induced thermal hyperalgesia, the blockade of the ET_A receptor reduced the response by 50.62% in the third hour after the administration of LPS. BQ 788 treatment reduced the hyperalgesic response in 70%. Intracerebroventricular injection of ET-1 also induced thermal hyperalgesia in the paw which lasted around 3 h.Treatment with BQ123 and BQ788 reduced the response in the first two hours in about 50% . Mechanical hyperalgesia was also induced by central injection of ET-1 which persisted for approximately two hours and was reversed by the administration of BQ123 but not by the administration of BQ788. In the sucrose preference index, the LPS treated animals had a lower preference for the sucrose solution when compared to vehicle-treated animals. Only the ET_B receptor antagonist BQ788 inhibited the LPS-induced decrease in sucrose preference. LPS also decreased the motor performance of the rats and treatment with BQ788 was also able to reverse this decrease. **Conclusion:** These data suggest that central ET_A and ET_B receptor are involved in the mechanical and thermal hyperalgesia induced by LPS. However, while both receptors are involved in thermal hyperalgesia induced by central ET-1, only ET_B receptors are involved in ET-1-induced hyperalgesia. Additionally, only the ET_B receptor is involved in the other sickness behaviors evaluated. **Financial support**: CNPq and CAPES. Animal Research Ethical Committee (protocol # 1090) **License number of ethics committee**: 1090 **Financial support**: CNPQ and CAPES

05.018 Spinal kynurenine monooxygenase-expressing astrocytes mediate the maintenance of neuropathic pain. Maganin AGM, Souza GR, Silva RL, Lopes AH, Alves-Filho JC, Cunha FQ, Cunha TM FMRP-USP – Farmacologia

Introduction: Neuropathic pain is a chronic pain caused by injury or disease in the nervous system. Previous study from our group has identified that after peripheral nerve injury there is a up regulation of kynurenine metabolic pathway, leading to an increase in kynurenine in the plasma, which seems to be involved in the maintenance of neuropathic pain. However, the mechanisms by which peripheral kynurenine (Kyn) mediates neuropathic pain is unknown. Kynurenine-3-monooxygenase (KMO) is the rate-limiting downstream enzyme in the kynurenine pathway that oxidatively metabolizes Kyn into 3-Hk and could be involved in maintenance of neuropathic pain. **Objectives:** The aim of the present study was to test the hypothesis that peripheral Kyn reaches the spinal cord and maintain neuropathic pain through its metabolism by KMO that forms downstream nociceptive metabolites. **Methods:** Spared Nerve Injury (SNI) model of neuropathic pain was induced in C57BL/6 mice and the following test and methods were used: von frey filaments nociceptive test, spinal cord were harvested for Real-time PCR and western blotting. KMO activity was pharmacologically (Ro-18048) and genetically (ShRNA) inhibited. Astrocytes primary cultures were also used. This study was approved by Local Ethical Commission in Animal Research: Protocol n°045/2013. **Results:** SNI induced mechanical allodynia in a time dependent manner, which peaked from 7 up to 21 days. SNI-induced mechanical allodynia was associated with an increase in the expression (protein and mRNA) of KMO in the spinal cord, mainly at day 10 and 14 after peripheral nerve injury. KMO expression was restrict to spinal cord astrocytes, but was not detected in microglia and neurons. Functionally, pharmacological inhibitor and ShRNA against KMO injected intrathecally after SNI reduced mechanical allodynia. As a control, ShRNA against KMO reduced KMO expression. Kyn injected systemically (i.v) also promoted mechanical allodynia, which was also reduced when KMO was pharmacological inhibited in the spinal cord. *In vitro*, primary cultured astrocytes stimulated with TNF increased the expression of activation cell makers including GFAP and also of KMO. **Conclusions:** In summary, **t**hese results indicated that after peripheral nerve injury spinal astrocytes-expressing KMO plays a critical role in the development of neuropathic pain. In conclusion, these data reveal a previously unappreciated role for the kynurenine metabolic pathway as a critical link between peripheral nerve injury, spinal cord glia cells (astrocytes) and the maintenance of neuropathic pain. **License number of ethics committee**: 045/2013 **Financial support**: CAPES, CNPq, FAPESP

05.020 Delayed muscle blood flow perfusion after cuff occlusion in rats subjected to an experimental model of eccentric exercise. Souza-Silva E, Ascenso R, Tonussi CR, Da Silva-Santos JE UFSC – Farmacologia

Introduction: Delayed onset muscle soreness (DOMS) occurs within 1-2 days after eccentric exercise, but the mechanisms mediating the muscle pain is unclear. This study hypothesized that eccentric exercise impairs the blood flow (BF) response following cuff occlusion, which may result in accumulated algesic substances being a part of the sensitization in DOMS. **Methods:** Female Wistar rats (90 days) were anesthetized (ketamine/xylasine 100/20 mg/kg;) and subjected to an experimental model of DOMS by inducing eccentric contraction (ECC) to the tibialis anterior (TA) muscle. For this, electrodes were placed directly in the skin above the TA muscle. The leg was exposed to 500 sets of electrical stimulation (ES) (50 Hz, 1 ms pulse), which was accompanied by stretching of the TA muscle. Each set of ES lasted 1 s followed by 3 s of resting. The current intensity of the ES was set as three times the threshold for twitch contractions. The ECC was achieved in the right leg, while the left leg was used for control. The function of the TA muscle in ECC leg was assessed in a electronic apparatus by counting the paw elevation time (PET; s) by 60 s of forced walk, and measuring the distribution of body weight (DW; g) between the hind legs in day 0 (pre-exercise), day 1 and day 2 (post-exercise). In addition, in day 2 the animals were anesthetized (ketamine/xylasine, 100/20 mg/kg), an incision was made in the both legs, and a laser probe connected to a laser Doppler blood flow monitor was placed directly on the TA muscle. A cuff was wrapped separately in legs before the knee and inflated to 300 mmHg/5 min to BF occlusion in the TA muscle. The BF before 9 s and 75 s after the cuff occlusion release was recorded by laser Doppler on the tissue of TA muscle. Data (mean ± SD; n=8-10) for PET, DW and BF were analyzed by repeated measures (RM) one-way or two-way ANOVA and *t test* when appropriate. The Newman Keuls (NK) test was used when interaction between factors (days and legs) was observed. **Results:** The ECC no promoted difference in PET (RM ANOVA: P= 0.15) among day 0 (11.77 ± 2.7), day 1 (15.77 ± 5.5) and day 2 (14.61 ± 5). Although not difference was observed in WD among day 0 (85 ± 11.4), day 1 (77.5 ± 7.7) and day 2 (71.56 ± 10.16) to ECC leg and between legs in day 0 (86 ± 10.4 and 85 ± 11.4) or day 1 (86.4 ± 5.6 and 77.5 ± 7.7), the ECC reduced the WD in day 2 on the exercised leg (71.56 ± 10.16) compared with control leg (90,80 ± 14, 89) (RM ANOVA: P= 0.03, NK: P=0.03). Not found difference to the baseline BF between legs (45.74 ± 22.6 and 43.39 ± 16.24) on day 2 (P= 0.8). After cuff occlusion release the BF increased during 75 s (5402 ± 2674 and 5907 ± 3723), measured in the ECC and control legs respectively and no difference was observed between legs (P = 0.7). Although, no difference was found between ECC (64.65 ± 28.32) and control (74.75 ± 39.06) legs in day 2 (P=0.5) after cuff occlusion release in the peak of BF, the time to peak BF after cuff occlusion was later (P=0.03) in ECC leg (6 ± 3.29) compared with control leg (3.75 ± 3.05). **Conclusion:** These results showed that ECC promoted the reduction in weight distribution only in the exercised leg and delayed the hyperemic response after cuff occlusion release in TA muscle 48 h after eccentric exercise. These results reinforce our hypothesis that BF impairment may contribute for accumulation of algesic substances and increasing muscle sensitivity after eccentric exercises. **License number of ethics committee**: CEUA PP00566. **Financial support**: Capes, with a PNPD fellowship to Souza-Silva E, and a Ph.D. fellowship to Ascenso R.

05.021 Zinc deficient diet increases nociceptive pain; **however, it reduces inflammatory pain**. Lima CKF[1], Silva RV[1], Silva VDCS[1], Oliveira JT[2], Lima LMTR[3], Miranda ALP[1] [1]LEFEx-UFRJ – Farmácia, [2]UFRJ – Biofísica, [3]UFRJ – Farmácia

Background and Aims: Nutritional influences on epigenetics could play a determinant role in the onset and progression of neuropathic pain (NP). Zinc (Zn) is a micronutrient essential for the physiology of nervous system, memory and learning processes, pain and also as a mediator of synaptic plasticity (Frederickson et al, 2005; Sensi et al, 2009; Jo et al, 2008). Therefore, our work **Aims:** to investigate the effects of zinc homeostasis unbalance on the development of pain. **Methods:** Zn homeostasis unbalance was induced by submitting 3 weeks old mice to different diets: AIN-93 (with 30mg/Kg of Zn) – control group; and AIN-93 (with 20mg/Kg of Zn) - Zn(-) group (n=6-8 animals /group). Once a week, for 4 weeks, mechanical allodynia was measured using Von Frey hairs. At the 3rd week, plantar assays for cold allodynia, heat allodynia, formalin-induced nociception and carrageenan-induced mechanical allodynia were performed. At the end, samples of plasma and DRG were removed for cytokines quantification and Western blot analysis. *p<0.05 (Student's *t* test, Anova two-way Bonferroni post-test). Animal ethics committee: CEUA/UFRJ 011/15. **Results:** Two weeks after diet intervention onset, mechanical allodynia was detected in the Zn(-) group, being gradually enhanced until 3rd week. Zn(-) group also presented cold and heat allodynia. Nociceptive sensitivity is significantly increased when compared to the control group. In formalin test it was detected an increased sensitivity in Zn(-) group at phase 1 (neurogenic phase), however, surprisingly a reduced response at phase 2 (inflammatory pain). The same hypersensitivity reduction was observed after carrageenan intraplantar injection. Plasma TNF was reduced in Zn(-) group. Analysis of ATF-3 and GFAP expression at the DRG showed an increase in ATF-3 and reduction of GFAP expression, corroborating the hypothesis of enhanced neuronal activation, but reduced immune activation. Also, DRG SOD-1, a hallmarker of oxidative stress, expression was increased in animals submitted to Zn(-) diet. **Conclusion:** Zn restriction diet alters basal sensitivity to pain in mice. Reduction of Zn intake seems to interfere in pain circuits, reducing inflammatory pain, however enhancing nociceptive pain. Accordingly, Zn unbalance could be predisposing factor for NP development. **License number of ethics committee**: CEUA UFRJ 011/16
Financial support: FAPERJ CNPQ

05.022 Research of therapeutic action of PHα1β toxin, **a calcium channel blocker**, **in model braquial plexus avulsion in rats**. Pires CS[1], Angelo SG[1], Dallegrave E[2], Souza AH[1] [1]Ulbra – Farmacologia, [2]UFCSPA – Farmacologia e Toxicologia

Introduction: Neuropathic pain due to brachial plexus injury is caused by avulsion of the medullar root and produces chronic, intermittent and often intractable pain. This is the most serious injury affecting the upper extremity, which can cause pains of difficult control, including paralysis of the arm, causing motor and sensory deficits, leading to social and economic losses. Neuropathic pain is still a health problem, because opioids and other analgesics, which are commonly used in the clinic to reduce pain, have limited efficacy in this type of pathology. The toxin derived from the venom of the spider *Phoneutria nigriventer* Phα1β presents antinociceptive actions in several models of pain in rodents, both acute and chronic. In this study, we verified the antinociceptive potential of intrathecal Phα1β in models of neuropathy induced by the brachial plexus avulsion surgery in Wistar rats, comparing it with the drugs currently used as treatment of the pathology (morphine and ωconotoxin MVIIA). The surgery was performed to induce brachial plexus injury, where the upper trunk of the plexus was dissected to the spinal cord, inducing neuropathy after 17 days. The following treatments were administered: PBS (10 µl / site), morphine (1000 pmol / site), Phα1β (200 pmol / site) and ω-conotoxin MVIIA (100 pmol / site) and effects 1, 3 and 5 hours after administration. To evaluate nociception, the hot plate test was performed with the objective of evaluating the acute pain caused by a stimulus at a temperature of 52 ° C ± 0.1. The animals were placed one by one in a transparent cylinder on the heated plate surface and the time between placing the animals and the paw withdrawal was recorded as a latency response in seconds. In mechanical allodynia Von Frey filaments were used where tactile sensitivity was assessed by the application of a stimulus represented by a light and constant pressure required to determine the withdrawal of the right hind paw. Cold allodynia was induced with a micropipette of 250 µl of acetone. , the central part of the surface of the hind paw (ipsilateral to the lesion). The response was evaluated with the help of a scale of 0 to 3 after the single application of acetone. The Phα1β toxin produced long lasting antinociception in the mechanical allodynia. This effect was verified for up to five hours (26.40 ± 4.02) and acetone induced cold allodynia for up to two hours (0.44 ± 0.18). Greater effectiveness of Phα1β was found at its third hour following intrathecal administration. In addition, the analgesic actions of both toxins, Phα1β (0.9597 ± 0.2345) and ω-conotoxin MVIIA (0.9723 ± 0.2133), as well as morphine (0.8395 ± 0.1371) were related to the inhibition of calcium evoked pro-nociceptive glutamate release in the spinal cord. Thus, the present study demonstrated that Phα1β toxin demonstrated therapeutic effect in neuropathy model, suggesting that this toxin may have potential to be used as a drug for the control of neuropathic pain. **License number of ethics committee**: O projeto foi aprovado pela Comissão de Ética no Uso de Animais na Universidade Luterana do Brasil (CEUA, protocolo: 2017/250). **Financial support**: CAPES, FAPEMIG.

05.024 Essential metals deprivation diet induces mechanical allodynia in mice. Matias DO[1], Martins AF[2], Santos BLR[2], Miranda ALP[2], Lima LMT[1] [1]UFRJ – Bioquímica, [2]UFRJ – Ciências Farmacêuticas

Introduction: Essential metals, as divalent ions copper, zinc, magnesium and iron, play pivotal roles in homeostasis maintenance. Enzymatic catalysis and oxy-reduction reactions that occur in energy metabolism are some examples of their relevant physiological actions. The unbalance of divalent ions has been related with changes in inflammatory, immune and behavior response, some of key process to neuropathic pain development (TAMBA *et al.,*2013). Neuropathic pain (NP) is caused by a lesion or disease of the somatosensory system that leads to cognitive and emotional changes and to symptoms such as allodynia and hyperalgesia (WOOLF, 2009). NP can be originated by different diseases, drugs as chemotherapeutic agents and genetic, environmental and nutritional factors (POSSO *et al*, 2016). **Goals:** The present study aimed to investigate the impact of the reduction of essential metals bioaccessibility in the onset and development of NP symptoms. **Methods:** To reduce the bioavailability of divalent ions in the diet, a chelating agent (inositol hexaphosphate 1% - IP6) was added to the standard rodent feed AIN93. Three weeks old male Swiss mice were divided in two experimental groups: control - AIN93 and intervention - AIN93 + IP6 (n=6 animals/ group). The animals received the respective diets immediately after weaning (21 days) and for 8 weeks with water *ad libitum*. The nociceptive response, mechanical allodynia (von Frey test), cold allodynia (acetone test) and heat hyperalgesia (Hargreaves test), was evaluated weekly. Object recognition and open field tests were performed at week 8 to evaluate eventual implications of intervention on learning and locomotion behavior. Animal weight and glycemia were measured weekly and at the 8th week the glucose tolerance test (TTG) was performed. The metabolic pattern of animals was determined throughmetabolic cage and liver, kidney and abdominal fat were collected for macro analysis. **Results:** The group that received the diet with IP6 intervention showed a significant reduction of mechanical threshold compared to control group at weeks 3, 5 and 8 (* $p < 0.05$ at 3rd and 5th, ** $p < 0.01$ at 7th week). There was no statistical difference in the development of thermal sensitivity to cold and heat stimulus. IP6 intervention reduced the RotaRod latency time (** $p < 0.01$), showing a decreased in locomotor activity. However, this effect was not confirmed by open field test. No differences were observed in cognition or memory performance. Body weight, glycemia, glucose tolerance, food intake and excretion were also not affected by the intervention. However, the intervention group showed an increase of abdominal fat when compared to the control group (* $p < 0.05$), suggesting that essential metals deprivation can affect lipid metabolism. We also observed a significant decrease of TNF levels in spinal cord. **Conclusion:** The present study showed for the first time that the diet with multiple divalent ions restriction alters the normal development of mechanical sensitivity to pain without interfere on the development of thermal hyperalgesia, both present in NP. We also showed that intervention diet does not modify metabolic parameters, suggesting that allodynic response is happening as a result of lower bioavailability of essential metals. More analyzes are underway to better understand the action of the IP6 and its effects on neuropathic pain and metabolic parameters. **License number of ethics committee**: 016/18 **Financial support**: PIBIC, CNPq, CAPES, FAPERJ

05.025 Dorsal Root Ganglion Produce CXCL1 in response to TLR2 and TLR4 activation Via AP-1. Zanata GC, Silva RL, Lopes AHP, Manganin AGM, Fonseca MDM, Cunha TM FMRP-USP – Farmacologia

Introduction: It is well known that peripheral noxious stimulus is transmitted to central nervous system through of nociceptors. The somas of these nociceptors are localized into the dorsal root ganglia (DRGs), a central structure to the modulation of these stimuli. DRGs are composed of multiple cells types, including neurons, glial satellite cells (SGCs), and macrophages. Additionally, Toll–like receptors (TLRs) are involved in the hyperalgesic process in several models of chronic pain. However, how TLRs and SGCs are interconnected to mediate mechanical sensitization is still unknown. Here, we investigate the relation of SGCs in the chemokine production into DRG in response to TLR2 and TLR4 agonists, and the signaling pathway involved. **Methods:** This study was approved by the Ethics Committee on the use of Animals from Faculty of Medicine of Ribeirao Preto (42/2015). Male C56Bl/6 wild type mice (WT), TLR2$^{-/-}$ or TLR4$^{-/-}$ weighing between 20 and 25 grams were used in the experiments. A total of five animals were used per group in all the experiments. TLR4 and TLR2 agonists, lipopolysaccharide (LPS; 0.05-5 µg) and peptidoglycan (PGN; 0.1-10 µg) were injected intrathecally (i.t.) in WT, TLR2$^{-/-}$ or TLR4$^{-/-}$ male mice, respectively. The hyperalgesia was measured by using Electronic von Frey. DRGs were removed for analyzes of the time course expression level of CXCL1 and GFAP by PCR-RT. Lastly, SR11302 (AP-1 inhibitor) was injected via intrathecal in WT, followed by LPS injection to the measurement of the hyperalgesia, and then DRGs were removed to access the CXCL1 and GFAP expression. Statistical analyses were made by using one-way ANOVA followed by Tukey pos-hoc test. **Results:** LPS or PGN when administrated intrathecally induce hyperalgesia starting from 3 h and remaining for more than 48 h after the injection (~52% and ~45%, respectively; $p<0.001$). Additionally, these stimuli induce the expression of CXCL1 at 4h (increase of LPS =13.5 times and PGN = 92.2 times; $p<0.01$), but returning to the basal levels after 24 h. Moreover, GFAP expression levels were temporally correlated with expression levels in DRGs (at 4h LPS induced an increase of 181 times and PGN 29 times; $p<0.05$). Given that GFAP is present in SCGs which have increased expression when are activated, these data suggest that SGCs are the source of CXCL1 into DRG. Conversely, the augmented expression of CXCL1 and GFAP were impaired when LPS and PGN were injected in TLR4$^{-/-}$ and TLR2$^{-/-}$, respectively. Finally, the pretreatment with the AP-1 inhibitor decreases pain (~25%; $p<0.05$) and CXCL1 expression (~52%; $p<0.05$) induced by LPS, but do not reduce GFAP expression. **Conclusion:** Satellite cells express functional TLRs which when activated mediate CXCL1 production and pain via AP-1. **License number of ethics committee**: 089/2016 **Financial support**: CNPQ; FAPESP

05.026 Involvement of Succinate Receptor GPR91 in the pathophysiology of paclitaxel-induced peripheral neuropathic pain. Gomes FIF, Kusuda R, Silva NR, Guimarães RM, Lopes AHP, Silva NIQ, Alves JCF, Cunha FQ, Cunha TM FMRP-USP – Farmacologia

Introduction: Paclitaxel is a chemotherapeutic agent employed in the treatment of solid tumours, but the ensuing peripheral neuropathy is a dose-limiting side-effect and a major cause of treatment discontinuation. The pathogenesis of paclitaxel-induced neuropathic pain is not fully elucidated, can cause metabolic changes and the levels of succinate can rise in such conditions. Succinate binds and transduces signals through G-protein receptor 91 (GPR91) activation. In the immune system, GPR91 is expressed on macrophages and, albeit its role in inflammatory conditions has been described, e.g. rheumatoid arthritis, its involvement in neuropathic processes is unknown. Here, we aimed to study the GPR91 involvement in the pathophysiology of paclitaxel-induced peripheral neuropathic pain. **Methods:** GPR91$^{-/-}$ and wild type C57BL/6 mice (20-20g) received four doses of paclitaxel (4 mg/kg; i.p.) on days 0, 2, 4, and 6. Negative control animals received saline solution (i.p.). Paw withdrawal thresholds by Von Frey filaments and time spent (s) on nociceptive responses - paw licking, elevation, and shaking during 1 minute to cold stimuli (acetone instillation on the right paw) - were assessed 4 and 24 hours after the injections and on alternate days from day 8 till day 30. In another series of experiments, C57BL/6 wild type mice received paclitaxel as mentioned, were euthanized on days 7, 14, and 21 after the last paclitaxel injection, and dorsal root ganglia were collected for quantification of mRNA relative expression levels of GPR91 and Kreb´s cycle enzymes (qRT-PCR). **Results:** Paw withdrawal threshold values of GPR91$^{-/-}$ mice increased compared with paclitaxel-treated animals ($p<0.001$) and the time spent on nociceptive behaviour did not differ from the negative control, suggesting these animals were partially protected from paclitaxel-induced peripheral neuropathic pain. qRT-PCR revealed no significant changes in the relative levels of GPR91 mRNA, but the expression of succinate CoA synthetase rose whilst the succinate dehydrogenase expression fell on days 14 and 21 after the last paclitaxel injection ($p<0.05$), respectively. **Conclusion:** These findings suggest that succinate might accumulate during paclitaxel-induced neuropathic pain, playing a role in its physiopathology, which is supported when its receptor was absent, a condition in which neuropathic pain was less prominent in knockout mice. **Keywords:** succinate; GPR91; paclitaxel; neuropathic pain. **License number of ethics committee**: CEUA FMRP-USP - 225/2017 **Financial support**: Fundação de Amparo à Pesquisa do Estado de São Paulo (grant number 2017.23815-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Center for Research in Inflammatory Diseases (CRID).

05.027 Hyperalgesia induced by central prostaglandin E2 is not dependent on EPAC signaling in ovariectomized female rats. Maba IK, Cruz JV, Zampronio AR
UFPR – Farmacologia

Introduction: Sexual dimorphism in pain is well known, although differences related to gender in hyperalgesia observed during sickness syndrome are poorly understood. Sickness syndrome is a group of signals and symptoms that include fever and generalized hyperalgesia besides other behavioral, autonomic and endocrine changes, which are an adaptive response that enables the body's host to fight infections (Saper et al, Nat. Neurosc., 2012, 15, p.1088-95). Considering the lack of studies involving gender differences and the high prevalence of chronic and acute pain conditions reported in humans, this study **Aims:** to investigate the hyperalgesia during sickness syndrome in female rats and the involvement of sexual female hormones in this response. **Methods:** Sham-operated and ovariectomized (OVX) female Wistar rats, weighing between 180-120g were used 21 days after the surgery. For prostaglandin E_2 (PGE_2) intracerebroventricular (i.c.v.) injection, the rats were implanted with a guide cannula in the lateral ventricle one week earlier. Mechanical and thermal hyperalgesia was evaluated in the same animals using an electronic analgesimeter and a hot plate apparatus (48□C) in the right hindpaw. Hyperalgesia was induced by an intraperitoneal (i.p.) injection of lipopolysaccharide (LPS, 50 µg/Kg) or by i.c.v. PGE_2 (125 ng/2 □l) and the mechanical threshold or the time spent in the hot plate was evaluated 2, 4 and 6 h or 0.5, 1, 1.5 and 2 h after the injection of LPS and PGE_2, respectively. In another set of experiments, animals were treated with the exchange protein activated by AMPc (EPAC) inhibitor ESI-09 (350ng/2µl) or vehicle (DMSO) 15 min before the injection of PGE_2. All the procedures were approved by CEUA/BIO UFPR protocol #1075 #1155). Results and **Conclusions:** No differences were found in the basal mechanical and thermal threshold of sham-operated and OVX animals. Similarly, no difference between the thermal or mechanical hyperalgesia produced by the i.p. injection of LPS in OVX and sham-operated rats. However, while sham-operated animals had a reduction of the mechanical threshold from 45.13 ±1.04 g to 36.55 ± 2.20 g at 0.5 h after the i.c.v. injection of PGE_2, OVX female rats showed a similar response much later, from 45.00 ± 0.98 g to 35.78 ± 2.26 g 1.5 h after PGE_2. Regarding thermal hyperalgesia, the sham-operated group had a response 40% smaller to PGE_2, when compared to OVX female rats. The treatment of sham-operated animals with the EPAC inhibitor ESI-09 reduced both thermal and mechanical PGE_2-induced hyperalgesia by 62%, while no effect was observed in OVX animals. These results suggest that PGE_2-induced hyperalgesia observed during sickness syndrome is different in cycling and OVX female rats and that this difference may be related to the fact that in OVX female rats this response is not dependent on the AMPc-EPAC pathway. These differences may be related to the absence female sexual hormones in OVX rats. Acknowledgements and **Financial support**: CNPq. **License number of ethics committee**: #1075 #1155 **Financial support**: CNPq

05.029 Investigation of the involvement of the endocannabinoid system in the transcutaneous nervous electric stimulation-induced antinociception. Malta IHS[1], Oliveria HU[1], Santos RS[1], Pinho JP[2], Almeida AFS[2], Sorgi CA[3], Peti APF[3], Faccioli LH[3], Ferreira E[2], Souza GG[1] [1]Unifal – Fisioterapia, [2]UFMG – Patologia, [3] FCFRP-USP – Ciências Farmacêuticas - USP Ribeirão Preto

Introduction: This study aimed to investigate the participation of the endocannabinoid system in the Transcutaneous Electric Nervous Stimulation (TENS)-induced antinociception at peripheral and central levels. **Methods:** This study was approved by the Commission of Ethics in Animal Use (CEUA) of the Federal University of Alfenas, under the protocol number 557/2014. Male Swiss mice underwent an inflammatory pain model induced by intraplantar injection of Ehrlich tumor cells. Mechanical allodynia and the antinociceptive effect of TENS were measured by von Frey filament test. To investigate the involvement of the endocannabinoid system, AM251, which is an agonist to the cannabinoid receptor type 1 (CB_1), and MAFP, which is an inhibitor of FAAH, a endocannabinoid metabolizing enzyme, were administered through intraplantar (i.pl.), intrathecal (i.t.), and intra-dorsolateral periaqueductal gray matter (i.dlPAG) injections. Furthermore, liquid-chromatography/mass-spectrometry, western blot, and immunofluorescence assays were used to evaluate the anandamide (AEA) levels, the expression, and co-localization of cannabinoid CB_1 receptor expression after TENS application, respectively. **Results:** Results demonstrated that low and high frequency TENS reduced significantly the mechanical allodynia and this effect was reversed by AM251 and potentiated by MAFP at peripheral and central levels. In addition, low and high frequency TENS application increased the AEA levels and the cannabinoid CB_1 receptor expression in the paw, spinal cord dorsal horn, and dlPAG. **Conclusion:** This study concludes that the endocannabinoid system may be involved in the antinociceptive effect induced by low and high frequency TENS at peripheral and central levels. **License number of ethics committee**: 557/2014 **Financial support**: FAPEMIG

05.030 Evaluation of the antinociceptive effect of TX3-3 toxin isolated from spider poison *Phoneutria nigriventer* **in fibromyalgia model in mice**. Souza AH[1], Pedron C[2], Gomez MV[3] [1]Ulbra – Farmácia e Farmacologia, [2]Ulbra – Farmacologia, [3]UFMG – Biologia Celular e Molecular

Introduction: Fibromyalgia is defined as a diffuse chronic muscleskeletic syndrome of non-inflammatory cause which physical examination indicates very painful spots when touched in pre-determined points (tender points) and by the presence of generalized body pain. The patients present day time fatigue and non-repairing sleep, among other clinical manifestations such as morning muscle and joint rigidity, paresthesia without characteristic neuropathic pattern, dry mouth, dizziness, tachycardia, tensional headaches. The pharmacological treatment induces better quality sleep as well as control of associate symptoms, such as depression and anxiety. The purified toxins of the poison *Phoneutria nigriventer* have been investigated in the treatment of several pain patterns. **Aim:** The aim of the present research was to adapt the proposed model for fibromyalgia in rats by Nagakura et. Al. 2009, in mice, using decreasing reserpine doses in order to test biogenic amines in the brains, assessing the antinociceptive effect of toxin Tx3-3 isolated from the poison of the *Phoneutria nigriventer* spider, as well as investigate the pharmacological action of the toxin Tx3-3 in depression and anxiety models in mice with fibromyalgia. And, finally, investigate the mechanism of action of the Tx3-3 through the neurotransmitters dosage in the animals' brains. **Methods:** Swiss male mice were utilized, Bioterio of The Lutheran University of Brazil (ULBRA), RS. The fibromyalgia model was achieved through the administration of a 0,25 mg/kg subcutaneous reserpine dose, for three days, once better response to the stimuli provoked was observed at such dose. On the fourth day, the animals developed nociception and depression, thus receiving different treatments. The groups were divided and then they received orally: saline, duloxetine, pregabalin and pramipexole. Other two groups received via intrathecal: PBS (phosphate buffer) which was the toxin dilution vehicle and the other group received the toxin Tx3-3. One hour after the drugs were administered, the mice were exposed to behavioral tests. The tests which were carried out were: mechanic allodyn, the hot plate test, the open field test and the induced swimming test. The dosage of monoamines in the animals' brains was also accomplished. All the experimental protocols approved by the Ethics Committee of Animals from Universidade Luterana do Brasil (CEUA, protocol: 2015-52P). **Results:** The applications of reserpine induced the decrease of the brain levels of the neurotransmitters serotonin (5-HT), dopamine (DE) and glutamate, therefore, the toxin Tx3-3 could revert such values. The toxin Tx3-3, provided an antinociceptive action in fibromyalgia in the test of thermic hyperalgesia and mechanic allodynia. It also demonstrated antidepressant activity in the induced swimming test, without presenting any motor alteration. The drugs duloxetine, pregabalin and pramipexole produced antinociceptive effect, either through mechanic stimulus or through thermic stimulus. **Conclusion:** The duloxetine didn't show antidepressant effects in the induced swimming test, but the pregabalin and the pramipexole did. This study suggests that the toxin Tx3-3 may have a future potential for the control of fibromyalgia. **License number of ethics committee**: 2015-52P **Financial support**: FAPEMIG, CAPES, CnPQ

05.031 Preemptive transcranial direct current stimulation induces analgesia and anti-inflammatory effect in rats submitted to a surgical pain model. Zancanaro M[1], Scarabelot V[2], de Souza A[3], Ströher R[4], de Oliveira C[1], Souza AH[5], Oliveira MS[6], Fregni F[7], Fregni F[7], Caumo W[1], Torres ILS[4] [1]UFRGS – Ciências Médicas, [2]UFRGS – Farmacologia da Dor e Neuromodulação, [3]Unilasalle – Ciências da Saúde, [4]UFRGS – Farmacologia e Terapêutica, [5]Ulbra – Ciências da Saúde, [6]UFSM – Bioquímica Toxicológica, [7]Spaulding Rehabilitation Hospital and Massachusetts General Hospital – Harvard University

Introduction: It is important to identify methods of central stimulation that can contribute with pain management. And, transcranial direct current stimulation (tDCS) is a noninvasive and low cost neuromodulatory technique, considering a non-pharmacological alternative for pain treatment. The aim of this study is to evaluate the effect of previous exposure to repeated tDCS in anti-nociceptive and anti-inflammatory response of rats submitted to surgical pain model. **Methods:** 56 adult male Wistar rats were divided in: control; drugs; surgery; drugs+sham tDCS; drugs+tDCS; surgery+Sham tDCS and surgery+tDCS. Bimodal tDCS was applied 0.5mA/20min/day/8days before surgical model adapted from Brennan. Mechanical allodynia (von Frey test) was evaluated at baseline, 30 and 60min, 24, 48 and 72hours after surgery. Myeloperoxidase(MPO) and N-acetyl-β-D-glucosaminidase(NAGase) activities were evaluated in the surgical site. IL-1β, IL-6 and IL-10 were evaluated in the spinal cord(SC), brainstem(BS), hippocampus(HC) and frontal cortex(FC). Statistical analysis: GEE/Bonferroni to nociceptive test and two-way ANOVA/SNK to biochemical data. **Results:** In mechanical allodynia there was an interaction between time x group (Waldχ2=2969.18; 36,P<0.05, n=8).Preemptive tDCS decreased NAGase and increased MPO levels in drug group and active tDCS ($F_{(6,21)}$= 11.935,P<0.05) and tDCS decreased NAGase levels in the operated animals ($F_{(6,21)}$=8.243,P<0.05). We found effect of surgery ($F_{(2,42)}$=16.61, and $F_{(2,42)}$=3.95P<0.05) and interaction surgery x tDCS ($F_{(2,42)}$= 6.28 and $F_{(2,42)}$=4.42,P<0.05) in HC and in FC, respectively, upon IL-1β levels; and effect of surgery ($F_{(2,42)}$=3.44 and$F_{(2,42)}$=5.36,P<0,05), and tDCS ($F_{(2,42)}$=3.88 and $F_{(2,42)}$=35.16, P<0,05) in BS and in SC, respectively; also interaction surgery x tDCS ($F_{(2,42)}$= 5,097,P<0,05) in BS. We observed effect of surgery ($F_{(2,42)}$=10.55 and $F_{(2,42)}$=11.48,P<0.05) and tDCS ($F_{(2,42)}$=3.11 and $F_{(2,42)}$=5.59,P<0.05) in HC and in FC, respectively, upon IL-6 levels; and tDCS effect ($F_{(2,40)}$= 9.42, P<0.05), and surgery x tDCS interaction ($F_{(2,40)}$= 5.23,P<0.05) in BS; surgery effect ($F_{(2,41)}$= 5.00, P<0.05) in SC. IL-10 levels showed effect of surgery ($F_{(2,42)}$=3.74, $F_{(2,42)}$=12.43, $F_{(2,42)}$=4.53,P<0,05) and tDCS ($F_{(2,42)}$=10.23, $F_{(2,42)}$=33.02, $F_{(2,42)}$=28.36,P>0.05) in HC, in BS and in SC, respectively; and interaction surgery x tDCS ($F_{(2,42)}$= 4.5 and $F_{(2,42)}$= 4.45,P>0.05) in HC and in SC, respectively. **Conclusions:** Preemptive tDCS was effective in controlling postoperative pain, and it alters inflammatory markers to post-operative evaluation in CNS. Also, tDCS contributes with tissue repair, avoiding the chronic inflammatory process and consequent fibrosis. **Keywords**: nociception; post-operative pain; rats; surgical model Brennan; tDCS. **License number of ethics committee**: HCPA - 160295 **Financial support**: CNPq; Graduate Research Group of Hospital de Clínicas de Porto Alegre, GPPG (I.L.S. Torres, grant 16-0295). CAPES. MCT/FINEP / COENG/2013.

05.033 P2X7 receptors mediate the antinociceptive effects of semisynthetic compounds obtained from a benzyl-isothiocyanate isolated from *Moringa oleifera* **flowers in a pre-clinical model of temporomandibular joint pain in rats**. Chaves HV[1], Parente AC[1], Val DR[1], Paula IMB[1], Lopes FMLS[1], Assis EL[1], Gomes FIF[1], Barbosa FG[2], Almeida DKC[2], Mafezoli J[2], Silva MR[2], Clemente-Napimoga JT[3], Costa DVS[4], Brito GAC[4], Pinto VPT[1], Cristino Filho G[1], Bezerra MM[1] [1]UFC-Sobral – Ciências da Saúde, [2]UFC – Química, [3]São Leopoldo Mandic – Odontologia, [4]UFC – Morfologia

Introduction: Temporomandibular joint (TMJ) disorders cause pain and limit the quality of life. Mechanisms involved in this clinical condition, however, are not fully elucidated and the established therapeutic approach is not completely efficacious. Moringa oleifera possesses several therapeutic uses, among them antinociceptive and anti-inflammatory effects. Here, we aimed to evaluate the antinociceptive and anti-inflammatory efficacy as well as central and peripheral mechanisms of semisynthetic compounds obtained from a benzyl-isothiocyanate isolated from Moringa oleifera flowers (MC-D7 and MC-D9) in the rat TMJ. **Methods:** Male Wistar rats (180-240g) were pre-treated (v.o.) with saline solution (0.9%), MC-D7 (0,01; 0,1; 1µg/kg) or MC-D9 (0,01; 0,1; 1ηg/kg). After 60 minutes, rats received an intra-articular injection of saline solution (50 µL, 0.9%) or formalin (50 µL, 1.5 %) into the left TMJ. Nociceptive responses (orofacial rubbing and flinching) were quantified in second for 45 minutes. Afterward, animals were culled and periarticular tissues (PAT), trigeminal ganglia (TG), and subnucleus caudalis (SC) were harvested. It was investigated the role of cannabinoid system involvement through CB1 and CB2 antagonists, vascular permeability assay with Evans blue, IL-1β and IL-8 dosages in the PAT and GT (ELISA), protein expression of adhesion molecules ICAM-1 and CD55 in the PAT (Western blot), and protein expression of P2X7 and NF-Ƙβ in the SC (Western blot). **Results:** MC-D7 and MC-D9 presented antinociceptive and anti-inflammatory effects by reducing behavioral responses, vascular permeability in the PAT, cytokine levels in the PAT and TG, ICAM/CD55 in the PAT, and P2X7 and NF-Ƙβ in the SC, whilst the cannabinoid system did not take a role in the MC-D7 and MC-D9-mediated effects. **Conclusion:** MC-D7 and MC-D9 presented antinociceptive and anti-inflammatory effects through peripheral and central mechanisms, showing a therapeutic potential for the TMJ pain treatment. **License number of ethics committee**: 03/2015 **Financial support**: CNPQ, FUNCAP, INCT-IBISAB, CAPES