UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE - UFRN

INSTITUTO DO CÉREBRO - ICe

HOUSE SYMPOSIUM 2021

CELEBRATING 10 YEARS OF ICE

Meet online at a virtual reality replica of our shiny new building (http://gather.town) and Zoom/YouTube!

NATAL-RN
May 27th - 28th, 2021

GREETINGS

Dear all,

We are happy to announce the 6th edition of our beloved House Symposium. This year the theme is "**10 years of ICe**". We will meet online on May 27th and 28th at a virtual reality replica of our shiny new building! (http://gather.town).

After a whole year of working remotely and limited social/work interactions we specially encourage all students to share and discuss your project & ideas with everyone through the flash talk and virtual poster sessions.

In the flash talk the students will give an extra-brief overview to interest viewers into visiting their poster. During the online poster session, students will have the opportunity to present in greater detail his/hers work to those attending their booth. For more specific instructions see "Instructions for Poster Formatting" at neuro.ufrn.br/housesymposium.

Let's all celebrate what really makes ICe great, the people.

Sincerely,

Organization Committee

VENUE

Meet online at a virtual reality replica of our shiny new building! (http://gather.town) and Zoom.

PROGRAM AT A GLANCE

May 27th

15:00 **– *Enter the Mindsphere! Guided tours of the ICe replica***

15:30 **– *10 years of ICe celebration***

15:35 **– *Music***

15:40 **– *Director Kerstin Schmidt addresses the ICe community***

15:55 **– *Slideshow* - *Warm Memories of ICe***

16:00 **– *Opening words House Symposium*** (Kerstin Schmidt/Sidarta Ribeiro)

16:15-16:45 **– *Student flash talks*** (virtual ICe auditorium)

17:00 – First 10 years of the Brain Institute (Kerstin Schmidt, Sidarta Ribeiro and Claudio Mello)

May 28th

08:30 **– *Breakfast*** (virtual ICe cafeteria/bar)

Keynote Speaker

09:00 **– "Social decision-making in rats"** (Cristina Márquez Vega)

10:00 **– *Mental Break*** (virtual ICe cafeteria/bar)

Talk Session 1 (virtual ICe auditorium)

10:30 **– "Physiological markers of noise-induced tinnitus in mice and possible targets for treatments"** (Katarina Leão)

11:00 **– "The Alzheimer's disease risk factor BIN1 regulates electrical activity in human neurons"** (Marcos Costa)

11:30 **– "Development and maturation of pathological high-frequency oscillations in epilepsy"** (Claudio Queiroz)

12:00-13:30 **– *Lunch Break***

Talk Session 2 (virtual ICe auditorium)

13:30 **– "Temporal relationships between respiratory rhythm and cortical oscillations"** (Adriano Tort)

14:00 **– "Visual small-world networks in individuals with and without psychosis"** (Lukas Galdino – Schmidt Group)

14:30 **– "*Studying the development of social behaviors in rat models of autism*"** (Rodrigo Pereira)

15:00 **– *Virtual Posters*** *(poster booths, virtual ICe patio)*

Talk Session 3 (virtual ICe auditorium)

16:30 **– "Inhibition behind psychedelics excitement"** (Richardson Leão)

17:00 **– "LSD as a cognitive enhancer"** (Sidarta Ribeiro)

17:30 **– "Ayahuasca and the pandemic"** (Draulio Araújo)

18:00 **– *BYOB social*** (virtual ICe cafeteria/bar)

ORAL PRESENTATION

Keynote Speaker

“Social decision-making in rats” Cristina Márquez Vega, Instituto de Neurociencias de Alicante

No man is an island, and all human behaviors are modulated by our social experience. How we take decisions in social contexts is a fundamental aspect of our daily lives, however, the underlying mechanisms are only starting to be addressed. We previously showed that rats display prosocial behaviours by providing food to conspecifics in foraging contexts. In this talk, we will focus on the mechanisms by which social context mediates this type of decision-making, with special interest on social status, gender and familiarity. Moreover, we will discuss how these types of decisions are conserved across different species and recent work on the identification of the neural circuits underlying the perception of emotional states of others.

Talk Session 1

T1. “Physiological markers of noise-induced tinnitus in mice and possible targets for treatments” Katarina Leão; Brain Institute, UFRN

Tinnitus, the perception of phantom sounds, often cause stress, anxiety and depression, and there is still no standardized treatment of the condition. One reason is the lack of physiological markers of tinnitus, making both research models and experimental treatments hard to validate. Here we have investigated if auditory event-related potentials are useful physiological markers of noise-induced tinnitus in mice. Our mouse model also tested if the cholinergic and endocannabinoid system regulates auditory event-related potentials, specifically related to auditory attention. In a second set of experiments we have explored correlations between noise-induced tinnitus and anxiety-related behavior, with anxiety as a potential physiological marker of tinnitus. Here we also tested if low dose cannabis extract can reduce noise-induced tinnitus and/or behavioral anxiety. Our results show that auditory event-related potentials, presented as paired-clicks, are larger in noise-exposed animals, and that noise-exposed animals to a lesser extent suppress repetitive auditory stimuli. We found that, compared to classic improvers of attention such as nicotine (1mg/kg), cannabis extract+nicotine have a strong synergistic effect in improving auditory filtering in noise-exposed animals compared to saline. This is due to a larger event-related potential to the first click stimuli ($p= 0.012$), and not due to larger suppression of the second click. For anxiety-related behavior we found noise-exposed animals to walk less in the open field test (>0.05) and be anxious in

the elevated plus maze compared to sham treated animals. We did not see any anxiolytic effect of cannabis extract (1mg/kg tetrahydrocannabinol, THC), rather an anxiogenic effect at this dose. In summary, increased auditory event-related potentials could be a translatable physiological marker of noise-induced tinnitus in patients, while anxiety is an additional physiological marker that can be examined in animal models of tinnitus. The potential benefits of cannabis extract to alleviate tinnitus remains inconclusive and different doses needs to be tested.

T2. "The Alzheimer's disease risk factor BIN1 regulates electrical activity in human neurons" Marcos Romualdo Costa; Brain Institute, UFRN

Alzheimer's disease (AD) is a multifactorial disease with a strong genetic background. Recent genomic wide association studies have identified several loci linked to an increased risk of AD. However, genes regulated by these variants and the pathophysiological mechanisms regulated by those genes remain largely elusive. In this work, we studied the role of Bridging Integrator 1 (BIN1), the second most important AD risk gene after APOE, in neurons generated from human induced pluripotent stem cells (hiPSCs). We showed that deletion of BIN1 is sufficient to cause neuronal hyperactivation, increased amyloid-beta production and Tau hyperphosphorylation, three pathological hallmarks observed in the brain of AD patients. We also showed that BIN1 is mostly expressed in hiPSC-derived glutamatergic neurons and that these cells express genetic signatures coupled with previous exposure to sustained electrical activity both in BIN1 heterozygous and knockout cerebral organoids. Together, our results reveal a role for BIN1 in the regulation of neuronal activity in humans and suggest that its implication in AD pathogenesis could be related to the neuronal hyperactivation and network dysfunctions observed in the AD brain.

T3. "Development and maturation of pathological high-frequency oscillations in epilepsy" Claudio Queiroz; Brain Institute, UFRN

High-frequency oscillations (HFO) are transient and periodic activity recorded in the local field potentials (LFP) of mammals. One typical example of HFO is the ripples, a brief (less than 50 ms duration) oscillation (frequency: 80-140 Hz) recorded in the pyramidal layers of CA1 and CA3, together with a negative transient (the sharp-wave) in the radiatum of CA1. It is believed their occurrence permeates the communication between the hippocampus and cortical areas, as the retrosplenial cortex, and it is linked to learning and memory. Conversely, fast-ripples are mainly observed in pathological tissue. Their central frequency spans higher above the ripples (from 250 Hz up to 750 Hz), and they result from abnormal network reorganization after an initial insult. Although their occurrence has been associated with seizure generation and epilepsy, it is unclear how pathological fast-ripples turn out into existence. One proposal suggests that they are new electrophysiological features that characterize the epileptic tissue. Another hypothesis proposes they

result from the hijacking of the ripple generation network. In this talk, we will review existing literature and present unpublished data obtained from animal models of epilepsy that support one of the two theories.

Talk Session 2

T4. “Temporal relationships between respiratory rhythm and cortical oscillations" Adriano Tort; Brain Institute, UFRN

The study of the interactions between respiration and brain activity has been focused on phase-entrainment relations, in which cortical networks oscillate phase-locked to breathing cycles. In this talk, I will present the recent discovery of new and much broader interactions that link breathing frequency to different patterns of oscillatory brain activity. Specifically, I will show that the instantaneous breathing frequency strongly correlates with the instantaneous frequency and amplitude of theta and gamma oscillations, two major network patterns associated with cognitive functions. Interestingly, changes in breathing frequency follow theta, suggesting a central drive, while in contrast, gamma activity follows changes in breathing frequency, suggesting the role of a reafferent signal. These results reveal new mechanisms by which nasal breathing patterns may relate to brain functions.

T5. “Visual small-world networks in individuals with and without psychosis” Lucas Galdino (Schmidt Group); Brain Institute, UFRN

Circuits in the brain can be described on several levels of complexity applying graph theory and a measure of global connectivity known as small-worldness. A small-world network allows approximating ideal brain structures as those, which would exhibit both, local segregation and global integration as expressed by higher clustering coefficients and lower characteristic path lengths. Typically, this network may maintain a considerable level of clustering among its nodes while some emergent edges integrate non-expected nodes, increasing the randomness of the complex network, and decreasing the average path length between all pairs of nodes.
In the last decade, a functional dysconnectivity syndrome that affects brain connectivity in a generalized way has been attributed to underlie the symptoms of schizophrenia. Despite the detailed description of anatomical and functional impairments that reflect that global disconnectivity, little is known about how sensory stimulation modulates network parameters in schizophrenia, such as small-worldness between different nodes engaged in visual processing. In order to address this question, we applied graph theory algorithms to EEGs recorded during visual stimulation and classified the functional network parameters of 13 patients with schizophrenia (SCZ) and 13 healthy controls (HC) (data from a collaboration with the UFPB). In detail, we measured the amplitude of visual-evoked potentials, and the number of nodes, edges, mean degree centrality, clustering coefficient, characteristic

path length, and small-worldness from inter-electrode coherences in the alpha (8-13 Hz) and low-gamma (36-55 Hz) bands. As expected from previous studies, patients presented smaller peak amplitude of evoked-potentials than HC. Interestingly, in contrast to the controls, SCZ did not change their small worldness index during visual stimulation. This implies that schizophrenia-related dysconnectivity has an impact on the ability of the low-gamma network to react to new sensory input. In addition, these results provide evidence about a possible electrophysiological signature of the global deficits revealed by the application of graph theory onto the EEG in schizophrenia.
The calculation of the small-worldness metrics allows comparing networks in health and disease. In an outlook, we apply the same metrics to compare the connectome of primary visual cortex data of carnivores and rodents (Ferreiro et al., 2021).

T6. "Studying the development of social behaviors in rat models of autism" Rodrigo Romcy-Pereira; Brain Institute, UFRN

The exchange and interpretation of signals from conspecifics in the social group are essential for the survival of mammals. In rats, the elaboration of appropriate (adaptive) responses begins during the postnatal period when the pup's innate responses are modulated by early social interactions with the dam. These early social interactions build up during juvenile stages and get more complex and dependent on the context. Gradually, neural circuits involved in motor, cognitive and emotional behaviors are refined by recursive feedback with behavioral experience until adulthood. In my talk, I will present recent findings from the literature and some results from my lab on social behavior development in rodents with focus on autism research.

Talk Session 3

T7. "Inhibition behind psychedelics excitement" Richardson Leão; Brain Institute, UFRN

Statistically, hallucinogens are the most powerful adjuvants in mood disorder managements. However, the stigma associated with these compounds impedes their adoption in clinical practice. A full understanding of the cellular processes involved in the antidepressant effect of psychedelics could reduce skepticism and show that these are not only party drugs. Our lab uses mice to investigate the cellular and network action of DMT and other hallucinogens. We showed that single doses of the hallucinogen 5-MeO-DMT doubles adult neurogenesis and accelerates the development of newborn neurons for weeks. Single photon imaging shows that during the 5-MeO-DMT 'experience', the hippocampus is silenced by increased GABAergic interneuron activity. I also found that N,N-DMT (but not other 5HT2a receptor agonists) acts postsynaptic in very specific hippocampal cell populations. Our results demonstrate that the cellular effects of magic mushrooms are still magic.

T8. "LSD as a cognitive enhancer" Sidarta Ribeiro; Brain Institute, UFRN

The therapeutic use of classical psychedelic substances such as d-lysergic acid diethylamide (LSD) surged in recent years, particularly in depressive disorders. Evidence supports that these positive effects stem from their ability to promote neurogenesis and neural plasticity, directly impacting plasticity-dependent cognitive processes, such as memory. Using a wide range of methods, we explored the sustained effects of LSD in processes and mechanisms related to memory and brain plasticity. The results show that LSD affects proteins associated with plasticity in human brain organoids, increases novelty preference in rodents, dependent on dose and age, and improves visual memory consolidation and recall in humans. Furthermore, our computational model of synaptic connectivity across hippocampus and prefrontal cortex provides a link between changes in neural plasticity and the changes in memory processes observed in rodents and humans. These results and mechanisms position LSD as a major promise as a cognitive enhancer.

T9. "Ayahuasca and the pandemic" Draulio Araújo; Brain Institute, UFRN

We'll discuss the associations between ayahuasca and the pandemic.

POSTER SESSION

P01 - Effects of anesthesia over the psychedelic drug reduction on anxiety measured by open field tests.

Lima da Cruz, R. V*.; Brisa, E.; Leão R. N.

The psychedelics are molecules that still classified as class A by UN, which effort maximum penalty for possession and use. They have been banned without a scientific reason, on the contrary, back there was promising evidence that such compounds could be useful to treat a variety of conditions ranging from depression to addiction with minor side effects compared to classical drugs prescript to treat the same pathology, the banishment was completely political. Nowadays, the interest in the subject increased, we are now at the dawn of the psychedelic research trying to further collect data about the physiological effects, embracing potential benefits and/or harms. Global research points toward a strong beneficial effect to mood and addiction disorders, but we are not much closer to understand its underlying mechanisms over consciousness than our predecessors during the late 60's. On previous findings from our lab, we did find that a single dose of 5-MeO-DMT one week prior to the test, was enough to create a reduction on overall anxiety measured by open field right after an acute stressor been applied, following the same trend, we now aim to answer if the animal needs to be conscious during the "trip" to show such reduction. In this frame, we tested if 20mg/kg of 5-MeO-DMT and 0.13mg/kg i.p of bicicline can influence the anxiogenic effect of an acute immobilization (10 minutes) right before an open field test. We are now analyzing the data acquired, our objective which such experiment is to see if the anxiolytic effect can be dissociated from the active experience of a psychedelic, thus further potentiating future psychedelic assisted psychotherapy strategies.

P02 - 5-methoxy-N, N-dimethyltryptamine (5-MeO-DMT) lasting effects on dentate gyrus electrophysiological profile and plasticity-related gene expression

Margareth Nogueira, Daiane Golbert, Richardson Leão

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Serotonergic psychedelics are getting attention due to growing evidence pointing to their therapeutic capabilities against anxiety and mood disorders, often with a single

dose. This lasting effect is possibly due to neuroprotective properties and structural plasticity. Here, we sought to verify the effect of 5-methoxy-N, N-dimethyltryptamine (5-MeO-DMT) on firing patterns, membrane properties, and calcium transients in the hippocampus 5 days after treatment. In addition, we evaluate the mRNA expression of genes involved in synaptic plasticity and neuronal activity specifically at Dentate Gyrus (DG) and ventral CA1 through a laser capture microdissection (LCM) approach. We found that 5-MeO-DMT leads to a more excitable pattern of membrane properties, such as input resistance, resting membrane potential, besides a higher voltage peak amplitude and afterhyperpolarization. On the other hand, we identified a lower firing rate in dentate gyrus granule cells (GC) from treated mice. Still, we found a higher relative expression of TRIP8B gene in CA1, but no significant differences in relative gene expression of plasticity-related genes after 5 days of treatment, although 5-Meo-DMT modulates differentially DG and vCA1 regions.

P03 - Dentate Spikes in Young Mice are Indicators of the Major Genetic Risk Factor of Late-Onset Alzheimer's Disease

Rodrigo MM Santiago*, Adriano BL Tort

UFRN, Brain Institute

Alzheimer's disease (AD) corresponds to a progressive loss of memory and other cognitive functions caused by neurodegeneration, primarily affecting the entorhinal cortex (EC) and hippocampus. Among the three forms of the apolipoprotein gene likely to be found in humans, the allele ε4 (apoE4) is the major genetic risk factor of late-onset AD, and mice with human apoE4 knocked-in (apoE4-KI) recapitulate age- and sex-dependent memory deficits. Analyzing hippocampal electrophysiological data of apoE4-KI and apoE3-KI (control) mice with machine learning techniques, we find that features of the dentate spike (DS) waveforms predict the genotype at a young age. This result reflects pathophysiological alterations in the EC-dentate gyrus network, which, once successfully correlated with behavioral outcomes, can prove to be potential biomarkers to an early diagnosis of AD.

P04 - Respiratory coupled rhythms and oscillatory patterns of anxiety-like behavior in the elevated plus-maze test and retest

Izabela Lima Paiva; Marina Pádua-Reis, Diana Aline Nôga, Clara Hartmann, Martina Blunder, Adriano B. L. Tort.

UFRN, Brain Institute

Anxiety is a complex emotional state. It involves overvaluation of potential threats, sustained worry, a noticeable change in breathing and altered brain activity. Recent studies have found that the respiratory flow captured by the nasal epithelium entrains brain oscillations at the same frequency as breathing, modulating the communication of anxiety-related brain areas. Recording breathing is essential for disambiguating the respiration-coupled rhythm (RR, 0.2 to 14 Hz) from other brain oscillation patterns. Specifically, theta (4 to 12 Hz) and RR can only be confidently distinguished during exploratory behavior by analyzing the local field potential (LFP) power spectrum and the LFP coherence to respiration. Interestingly, several studies have pointed to theta synchrony as a mechanism for communication between the medial prefrontal cortex and the hippocampus during anxiety-like behavior in rodents, but they did not consider the RR in their analyses. With this project, we want to investigate if an anxiogenic experience can increase hippocampal–prefrontal synchrony through RR modulation. For testing this, we will perform respiration and LFP recordings in mice during the test and retest in the elevated plus-maze (EPM).

P05 - Cyclic alternation of quiet and active sleep states in the octopus

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Previous observations suggest the existence of ‘Active sleep’ in cephalopods. To investigate in detail the behavioral structure of cephalopod sleep, we video-recorded four adult specimens of Octopus insularis and quantified their distinct states and transitions. Changes in skin color and texture and movements of eyes and mantle were assessed using automated image processing tools, and arousal threshold was

measured using sensory stimulation. Two distinct states unresponsive to stimulation occurred in tandem. The first was a 'Quiet sleep' state with uniformly pale skin, closed pupils, and long episode durations (median 415.2 s). The second was an 'Active sleep' state with dynamic skin patterns of color and texture, rapid eye movements, and short episode durations (median 40.8 s). 'Active sleep' was periodic (60% of recurrences between 26 and 39 min) and occurred mostly after 'Quiet sleep' (82% of transitions). These results suggest that cephalopods have an ultradian sleep cycle analogous to that of amniotes.

P06 - Low gamma spike-field coherences in the primary visual cortex of agouti

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Neural oscillations in the low gamma band (30-49 Hz) have been related to visual processing. Coherence in the low gamma band is an established measure to study neural synchronicity between different cortical regions but can also be applied to study the coordination of spiking activity and the local field potential as signature of functional cortical layout and local connectivity. Previously, we described that spike-field phase locking in the gamma band is tuned to orientation in cat, but not rodent primary visual cortex. Since cats exhibit periodic columns of orientation-selective neurons in pinwheel-like structures, strong and tuned interactions between spikes and local field potentials of the same electrode could reflect that orderly columnar layout when stimulating with salient oriented visual stimuli. Although all visual rodents investigated so far possess orientation-selective neurons they express no regular maps as cats or primates, but if at all, mini-columns interspersed with "salt-and-pepper" regions. This hypothesis was also supported by recent findings of our group for the largest rodent examined on orientation selectivity so far, the agouti. As cats, agoutis respond to gratings, though of lower spatial frequency and with a bias for horizontal orientations. However, neuronal responses to textures with less or no orientation component remain to be explored. In the present project, we examine whether spike-field coherences and frequencies evoked by textures of different saliency and containing less or no orientation component differ in a species-specific manner, which could be explained by the supposedly different functional layouts. Although coherences in the low gamma band occur more frequent, more vigorously and with higher oscillation frequencies in cats than agoutis, we observe significant gamma coherence in both. Moreover, and species-independent, "classical" grating stimuli always evoke higher spike-field coherences than random dot textures or bars. Our result indicates that both layout

types, columnar and interspersed networks, can express coherent low gamma oscillatory activity especially when challenged with optimally oriented gratings.

P07. Testing the inheritance of the behavior of leaving water in an auto fertilizing simultaneous hermaphrodite fish

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The Genus Kryptolebias possess the two only know species of self-fertilizing vertebrates in which individuals have both male and female germ cells at the same time (simultaneous hermaphroditism). This form of reproduction produces populations of these species that have a low level of heterozygosity and are, therefore, an interesting model for studying phenotypic plasticity. To establish a colony of Kryptolebias hermaphroditus in the laboratory, we analyzed gonads of individuals fixed in formaldehyde to check at which size they start producing eggs. We observed that only animals with 20mm long had eggs. Subsequently, in December of 2018, we collected 48 K. hermaphroditus longer than 20mm from the Centro Tecnológico de Aquicultura from UFRN, in Extremoz, RN. All animals collected were conditioned in plastic containers at FishLab (Department of Physiology, UFRN) where they remained for acclimatization. Currently, there are 20 captured K. hermaphroditus, 3 K. hermaphroditus from the first generation (F1), and 6 K. hermaphroditus from the F2 in the bioterium. During the entire period in the bioterium, oviposition was observed and the embryonic development of K. hermaphroditus eggs was monitored and compared with the stages described for K. marmoratus. The oviposition of the F1 (by captured animals) occurred between June and September, showing seasonality in the second semester of the year. In the second generation of K. hermaphroditus born in the lab (F2), oviposition occurred from September to December, showing a dislocation in the egg-laying period and also a greater number of eggs per individual. One of the plastic responses of K. hermaphroditus is the ability to leave the water to the terrestrial environment. After acclimatizing the wild specimens, experiments were carried out measuring the time of emersion for 10 minutes in an arena build with 3 brackish water wells separated by a dry area. The first hypothesis tested was that the size of K. hermaphroditus influences the time spent outside the water. The result showed high variability, however, there is a positive link between the size and time of emersion. The second hypothesis tested was that the emersion behavior can be influenced by the chemical signals released in the water by a conspecific or a predator (15 individuals in each group). The two treatments did not alter the time of terrestrial exposure of captured K. hermaphroditus nor the latency to get out of the water. Our preliminary

observations suggest that the F2 animals do not leave the water. Since it seems to not be an innate behavior, in this project we will 1- test if they can be motivated to get out of the water by a prey (ants), 2- if they can get a detour outside of water to reach a reward (Artemias), the reward is occult in the detour (cognition test), or 3- if the laboratory-born animals can learn with a model (captured animal) to leave the water.

P08. Olfactory epithelium as a possible pathway for ZIKV to enter the brain: An unexpected infection from the amniotic fluid?h

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Patients who were born with microcephaly caused by the Zika virus (ZIKV) have a greater chance of developing malformations of anterior structures of the central nervous system (CNS). The cortex and basal ganglia are examples of regions where the change occurs often while caudal regions, like the cerebellum and brain stem, are only affected in a minority of cases. The pattern of malformation can be directly related by viral tropism to the anterior brain or by the site of ZIKV entry into the CNS. Several studies show that the virus can break through the placental barrier and infect amniotic fluid. As the olfactory epithelium (OE) is in direct contact with the amniotic fluid, we hypothesize that this is a possible route of entry. In this project, we are testing the hypothesis that ZIKV enters the brain through the OE. We used injection into the amniotic fluid to trace the virus infection timeline. In addition, we want to inhibit/ reduce the entry of ZIKV into the brain by ablating the OE. Throughout the experiment, we used a ZIKV strain isolated in Pernambuco in 2015. The mice are from the C57BL strain, acquired through the animal bioterium of the Brain Institute of the Universidade Federal do Rio Grande do Norte. To control the biological cycle of the mice, we maintain a 12-hour light and dark cycle. We inject ZIKV in the amniotic fluid on embryonic day E13 (during prosencephalon neurogenesis). We checked infection through immunohistochemistry 1-day post-infection (dpi), 3dpi, 6dpi, and 12dpi using an antibody that recognizes the envelope protein (4G2). At 1dpi, the presence of ZIKV in the OE was not observed with the injection of the virus in the amniotic fluid. From 3dpi we found cells labeled with markers of the virus. At 3dpi and 6dpi the infection in the OE and olfactory bulb (OB) increased, while the anterior and caudal regions of the brain remained. At 12dpi a great reduction in the presence of the virus was observed in the OE and the posterior region of the brain. The pattern of ZIKV expression in the brain suggests that the virus is spreading through differentiating neurons and their processes. To test the effect of the ablation of OE on the brain ZIKV infection, we are using postnatal P3 mice. ZnSO4 injection (0.17M) is

administered in the right nostril (approximately 5µl) and saline solution in the control group. The next day, the virus is injected into the right nostril of both groups. Our preliminary tests show that the unilateral injection of ZnSO4 kills most of the EO and its projections for the OB. Bilateral injections of ZnSO4 in postnatal mice lead to high mortality. Also, we observed that the intranasal injection of ZIKV in P3 mice is sufficient to infect the OE after 6dpi. With the results we have achieved so far, it is possible to affirm that ZIKV infects OE and this is a possible pathway for the virus to enter the brain. We were able to successfully perform OE ablation and, in the future, we want to establish causation of brain infection through the OE.

Key words: cortical plate, flavivírus, intrautero, retina

P09. In vitro effects of high-THC cannabis extract and repetitive transcranial magnetic stimulation (rTMS) onto activity and membrane properties of Layer 5 Type A Pyramidal Neurons and Chrna2-Martinotti cells (MCα2) along the rostrocaudal axis of the primary auditory cortex of adult C57BL/6J mice.

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Acoustic noise overexposure can cause tinnitus and hyperacusis, a hypersensitivity to sound. In animals with behaviorally assessed tinnitus, increased spontaneous firing rate, synchrony and bursting activity in several auditory regions such as the primary auditory cortex (A1) and dorsal cochlear nucleus are thought to underlie tinnitus perception. Also, in A1 Chrna2-Martinotti Cells (MCα2) inhibitory interneurons can synchronize via distal dendrite targeting burst-firing Type A Layer 5 pyramidal neurons (L5 Corticofugal PNs), which have corticocollicular response gain increased in noise-exposed mice and a dendritic tree that extensively branches in layer 1. Using in vitro calcium imaging and whole-cell patch clamp, we want to investigate if either high-THC cannabis extract, a putative modulator of the oscillations-related I(h) current, and repetitive transcranial magnetic stimulation (rTMS), which likely targets layer 1 dendrites, can affect synchronization and membrane properties of MCα2 and Type A L5 PNs along the rostrocaudal axis of the primary auditory cortex of adult C57BL/6J mice.

P10. Investigation of the relationship between the use of pesticides and the occurrence of CNS congenital malformations at Rio Grande do Norte state

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Insecticides are substances used for insect control in agriculture and they act by modifying these animals' cell membrane properties until their death. To quantify the potential harm of these substances to humans, it is essential to consider not only effects on adults but also lethal and non-lethal effects on embryos, as they are more susceptible to environmental agents. Previous studies from our group have shown that neural plate cells display calcium transients and that its inhibition leads to neural tube defects (NTDs). Since insecticides can change the cell membrane potential, they may operate during the nervous system formation altering the usual neurulation process through interfering with the electrical activity in neural plate cells. Given that exposure to agrochemicals was identified as a risk factor for NTDs, it is relevant to analyze the occurrence of physiological changes in the developing organism to measure the impact of these compounds on public health. In this project, we intend to start to determine a chain of causation between different types of insecticide exposure and the origin of NTDs by investigating this relation through space and time in the state of Rio Grande do Norte. To test this hypothesis, we are going to cross-check the data between (i) spatial distribution of usage of agrochemicals (Agrochemical Atlas, Larissa Bombardi, and IBGE's agricultural census), (ii) the amount of pesticide used compared to agrochemicals in each location (IBGE/IDIARN) and (iii) the rate of congenital malformations of CNS per live birth through DATASUS; considering each Rio Grande do Norte's city/microregion. Moreover, we are going to dissect the temporal distribution of births of CNS malformations to detect regions where there was an increase during the last ten years. Our preliminary data show that some municipalities have a rate of birth of spina bifida 3 to 4 times higher than what is expected for the general population (1 in every 1000 births). By comparing two neighbor municipalities, Limoeiro (CE) and Apodi (RN), we observed that the high agrochemical use in Limoeiro in the last ten years is associated with an increase in the rate of spina bifida births. In the current project, we intend to produce a map of the impact of the use of pesticides on the development of CNS malformations in our state.

P11. The effect of low dose Cannabis extract in noise-induced tinnitus perception

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Tinnitus is an auditory condition characterised by phantom sounds described as ringing or hissing sounds. Chronic bothersome tinnitus often leads to stress, anxiety and even depression and there is a lack of standardised treatments, . Recently the endocannabinoid system has gathered attention as it might modulate behaviour and

synaptic activity. However, literature is still inconclusive about beneficial or detrimental effects of Cannabis treatment for tinnitus. Here we used a model of noise- induced tinnitus to investigate firstly, if tinnitus is altered by low dose Cannabis extract (2.44 mg/kg; THC, 1.0 mg/kg), and secondly, if anxiety is related to tinnitus-like behaviour and if it is affected by Cannabis. We used the gap prepulse inhibition of the acoustic startle (GPIAS) to assess tinnitus perception and the open field (OF) and elevated plus maze (EPM) to evaluate anxiety behaviour. Our preliminary results show that we can detect tinnitus-like behaviour when subdividing noise-exposed mice (NE) into Exposed Tinnitus mice (ET) and Exposed No Tinnitus mice (ENT), but Cannabis treatment had no effect on tinnitus-like behaviour. Moreover, we investigated if anxiety-like behaviour was associated with noise exposure or tinnitus perception, and found that Cannabis treatment made NE mice enter the centre less ($p = 0.0492$) and decreased locomotion ($p = 0.0416$). In EPM, sham mice showed more arm entries independently of treatment (vehicle, $p = 0.0416$; Cannabis, $p = 0.0047$) while NE entered less in open arms after Cannabis ($p = 0.0026$). Also, NE spent more time in closed arms and less time in open arms after Cannabis (closed, $p = 0.0043$; open, $p = 0.0272$). Subdivision of NE mice revealed, for OF, that Cannabis reduced centre entries for ET ($p = 0.0317$) while reduced time in centre for ENT ($p = 0.0272$). For EPM, ET showed less entries into arms (closed, $p = 0.0256$; open, $p = 0.022$) and spent more time in closed arms ($p = 0.0094$) after Cannabis. In summary this work shows that anxiety tests can be an addition to GPIAS test to evaluate tinnitus and therapeutical effects. However, future studies will test lower doses for potential anxiolytic effects of THC, in treating tinnitus-related stress and anxiety.

P12. Effects of Kainic Acid in neuroblasts onto neuronal differentiation

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Adult subventricular zone (SVZ) cells give rise to the different olfactory bulb (OB) neuronal subtypes. It has been suggested that the fate of these neurons is determined within the SVZ niche. In order to test the plasticity of neuroblasts in migration, we here inject kainic acid (KA; 50nL of 20mM solution) close to their migratory pathway (rostral migratory stream; RMS). Three lineages are being used for testing general (Dcx-CreERT2), or specific neuroblast populations (Chrna2-Cre and Emx1-Cre) when crossed with lox-GFP mice. Mice Dcx-CreERT2 received three daily doses of tamoxifen before surgery and were injected with KA or PBS. Injection of kainic acid generates an epileptic seizure after surgery. To analyze whether the effect of the epileptic seizure can affect both hemispheres, we will use the contralateral side as a control of the KA over the RMS. Sections were cut with 30µm in a cryostat and stained with markers of each subtype of neuron analyzed, along

with anti-GFP antibody. Cells were counted manually at each layer of the OB. KA Injected Dcx-CreERT2 animals have the glial tube surrounding the migratory neurons disorganized, after 36 hours. Some neuroblasts ectopically migrate to the surrounding parenchyma. Multiple neuroblasts maintain their migration to the olfactory bulb, where they differentiate into neurons. We observed a shift in the proportions of different OB neuronal subtypes after 30 days. The proportion of granular cells is increased in KA-treated animals while the fraction of Tyrosine Hydroxylase (TH+) neurons in the glomerular layer is decreased in KA-injected animals. These data suggest that KA injection is sufficient to change the distribution of neuronal types generated by SVZ neuroblasts. We are describing the normal differentiation of Chrna2 and Emx1 populations in the OB. By analyzing the mRNA expression pattern for Chrna2 in the Allen Brain Atlas, we observe that first neurons appear in P4 animals. The number of neurons peak in P28, in P56 the number of cells decreases. In Chrna2-Cre animals, the inner plexiform layer and the mitral layer had the highest percentage of cells. There are no Chrna2+ cells in the region of the RMS. In the EMX1-Cre animals, we observed rare co-labeled of GFP+/Calb+ cells in adult animals and others co-labeled such as GFP+/Calretin+, GFP+/TH+, GFP+/GABA+ were observed. GFP+ granular neurons are all superficial. Further investigation on the role of KA in the differentiation of these specific populations will be fundamental for establishing the plasticity of neuroblasts. Ethics Committee - N °: 012/2016, CEUA-UFRN.

P13. Analysis of microchimerism between mother and embryo in the origin of congenital ZIKV syndrome

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Microchimerism is a biological phenomenon that happens in the woman's and embryo's bodies and is characterized by a bidirectional exchange of cells between mother and child. Even years after birth, those cells can still be found in the woman's blood and tissues. During pregnancy though, the maternal immune system is tolerogenic and does not respond against the presence of embryonic cells. Nonetheless, some events, like infections, may result in a disruption in the mother's immune tolerance to the fetus giving rise to the production of reactive antibodies against fetal antigens and the migration of maternal immune cells to the embryo. Since 2015, multiple patients that experience gestational Zika Virus (ZIKV) infection develop central nervous system (CNS) malformations, like microcephalia. In this project, we aim to analyze if ZIKV induces higher levels of microchimeric cells in both individuals and if the presence of Zika is capable of disrupting maternal immune tolerance activating cellular responses against the embryo's CNS. In the first aim, we will test if embryonic cells, in special neural cells, are reaching the maternal blood.

For achieving this purpose, we will cross β-actin-eGFP+ males and wild-type females (C57BL/6J). During corticogenesis, we will inoculate ZIKV in the amniotic fluid. To analyze the presence of microchimeric cells of offspring, flow cytometry, and immunocytochemistry will be executed on the mother's blood to identify eGFP+ cells and their markers. In the second aim, we will characterize if the maternal immune cells reach the embryonic brain. We will test so by crossing a heterozygous β -actin-eGFP female a wild-type male, followed also by the injection of ZIKV in the amniotic fluid, decapitation of the embryos, production of brain slices, and immunohistochemistry to identify maternal cells in the brain and their cell type markers. By combining to this last experiment the use of a second transgenic line, Dcx-Cre-ERT2 x lox-tomato, we will produce animals in which the invading maternal cells are green (β -actin-eGFP) and the young neurons and neuroblasts are red (Dcx-Cre-ERT2). The purpose of this cross is to detect, quantify the interaction of maternal immune cells with embryonic neurons in brain slices submitted to time-lapse microscopy. We expect, to detect and quantify, in aim 1, eGFP+ embryo cells circulating in maternal blood, as well as to identify how many of them originated in the CNS. In aim 2, we will detect and characterize the presence of maternal immunological reactive cells in the embryo's CNS, describing the cellular mechanisms related to the presentation of antigens that leads to a disruption in maternal immune tolerance. Since the high number of infections caused by ZIKV in Brazil, several families have been affected, mostly the poor ones. Precisely, understanding the neurobiology under the host-parasite relationship is a way to provide a perspective on treatments, prevention of new cases avoiding future outbreaks and public health breakdowns.

P14. Early auditory experiences shape discrimination of vocalizations in zebra finches

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Songbirds are considered one of the best animal models to investigate the neurobiological basis of vocal learning. As in humans, these animals have distinct phases for vocal learning that are influenced by social and motor experiences. More specifically, social deprivation in songbirds can generate deficits in the individual's vocal production, whereas singing prevention delays vocal maturation. However, it is not completely clear whether limited experience during development can affect the animal's auditory discrimination abilities, a well-known phenomenon in humans. To address this question, we manipulate social and motor practice experiences in zebra finches, the most widely used songbird species. We first confirmed that our manipulation altered vocal learning without grossly affecting development. More

precisely, socially deprived animals showed abnormal songs, typical of animals lacking a song model. In contrast, juvenile animals where singing practice was limited continued to produce immature vocalizations as adults. Notably, behavioral experiments demonstrated that control animals, i.e., animals with normal social experiences, showed a lower performance in auditory discrimination tasks compared to isolated and juvenile animals. Conversely, singing-limited birds did not differ from controls. These results suggest that early development auditory experiences may influence the discriminatory capacity in adult zebra finches, while vocal practice appears to have little influence in the performance of adult birds. That is, birds that are exposed to normal social experiences during postnatal development may have diminished ability to discriminate conspecific songs. Such interpretation is in agreement with the increased specificity observed in auditory neurons, and would parallel the perceptual losses observed during speech acquisition in humans.

P15. Hippocampal-Prefrontal Interactions during Spatial Decision-Making

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The hippocampus has been linked to memory encoding and spatial navigation, while the prefrontal cortex is associated with cognitive functions such as decision-making. These regions are hypothesized to communicate in tasks that demand both spatial navigation and decision-making processes. However, the electrophysiological signatures underlying this communication remain to be better elucidated. To investigate the dynamics of the hippocampal-prefrontal interactions, we have analyzed their local field potentials and spiking activity recorded from rats performing a spatial alternation task in an 8-shaped maze. We found that the phase coherence of theta peaked around the choice point area of the maze. Moreover, Granger causality revealed a hippocampus->prefrontal cortex directionality of information flow at theta frequency, peaking at starting areas of the maze, and on the reverse direction at delta frequency, peaking near the turn onset. Additionally, the patterns of phase-amplitude cross-frequency coupling within and between the regions also showed spatial selectivity, and a new method revealed that hippocampal theta and prefrontal delta modulated not only gamma amplitude but also inter-regional gamma synchrony. Lastly, we found that the theta rhythm dynamically modulated neurons in both regions, with the highest modulation at the choice area; interestingly, prefrontal cortex neurons were more strongly modulated by the hippocampal theta rhythm than by their local field rhythm. In all, our results reveal maximum electrophysiological interactions between the hippocampus and the prefrontal cortex near the decision-making period of the spatial alternation task, corroborating the hypothesis that a dynamic interplay between these regions takes place during spatial decisions.

P16. Slow oscillations and dream content reflect the adaptation during sleep to a predatory virtual competition

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Despite the study of the functions of dreams extends throughout human history, they remain as a mystery, raising multiple theories to explain its purpose. One of these is the Threat Simulation Theory. It postulates that the dream is a defense mechanism preserved along the evolution of animals for its ability to simulate threats, preparing the animal in advance for the occurrence of real events. On the other hand, one of the most understood functions of sleep is the consolidation of memories. Together, the mechanisms of dreams allow us to imagine future threats and the mechanisms of sleep make it easier for us to remember this training when faced with real situations. In fact, evidence points out that dreams are simulations specialized in threatening events. On this basis, the use of videogames may prove to be a valuable tool to expand the understanding of dreams and sleep as an adaptive mechanism to threats. Experiments were performed with 14 pairs of human participants, who came simultaneously to the laboratory and had their brain activity recorded by electroencephalography (EEG). During registration the pair played a realistic electronic game for 45 minutes, then slept for 2 hours and then played again for another 45 minutes. In the game, one of the participants acted as prey and the other as a predator. The prey could hit the opponent with punches only, while the predator could hit with punches and a firearm. Another goal for the prey is the collection of items on the environment, as an analogy to food gathering. Dream reports were evaluated by 4 raters according to how much they think the participant dreamed and the pertinence of 6 dream properties. The sleep block was scored following an automatic method. Spectral power and slow wave parameters were also measured. Multiple comparisons were corrected by permutation tests. The results indicate that dream is more likely to occur for preys (Chi-squared test; $p = 0.018$). Also the more the dream is related to the game, the bigger is the increase in performance on the second game for preys (Spearman correlation; uncorrected; $p = 0.0168$). The gain in game scores also correlated positively with the proportion of sleep time on the stage N3 (Spearman correlation; $p = 0.0064$) and the power in delta oscillations on central electrodes C1, Cz and C2 (Spearman correlation; $p = 0.001$) for the preys. None of these effects were observed for the participants with the predator roles. These results corroborate the Threat Simulation Theory, as the benefits of dream and sleep only occurred for the participant under threat.

P17. Postnatal development of social behaviors in rat model of autism

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Prenatal epigenetic dysregulation by histone deacetylase inhibitors can induce impairments in the expression of postanal social behaviors and dysfunctions in neural circuits connecting limbic and prefrontal cortical areas. As such, these animals may exhibit autistic-like features and can be considered as animal models of autism. In these animals, behavior alterations are most commonly described in adults, while little is known about the postnatal establishment of their behavioral repertoire particularly during the first three postnatal weeks of life. Therefore, the aim of this study was to quantify a set of pairwise social behaviors and the pattern of ultrasonic vocalization induced by maternal separation of animals prenatally treated with valproic acid (VPA) and saline-treated controls. For this, VPA and control rats were generated from a single intraperitoneal injection in pregnant Wistar females on the gestational day E12.5 (500mg / kg VPA, i.p.;0.15M saline solution). During the social interaction experiment, pairs of P7 – P21 rats (nControl=44; nVPA=46) were subjected to single sessions of free social interaction carried out on a linear acrylic platform for 3 minutes. Each trial was recorded in video by a high-resolution camera, which was subsequently analyzed by an experimenter with no knowledge of the groups. The number of events, onset latency and duration of crawling, walking, self-grooming, allogrooming, undercrossing, mounting, proactivity, exploratory behavior, as well as the kinked tail phenotype of VPA rats were evaluated. Ultrasonic vocalizations (USV) were recorded from each pup during maternal separation at the ages P11, P14 and P18 (nControl=26; nVPA=25). Briefly, each pup was carefully separated from its dam and positioned in a cage with bedding that was placed inside an anechoic chamber maintained at 23ºC and monitored by a video camera. A digital ultrasonic microphone (250 kHz sampling rate, 16 bits A/C) recorded 5 minutes of ultrasonic vocalizations from each animal. Each sound file was stored in a hard disk in .wav format for offline analysis. USVs were analyzed in three steps: (1) USV detection and (2) USV curation, which were done in a semi-automatic manner by a software developed in our lab. In order to study the dynamics of USV features along the ages, we (3) extracted USV features using the DeepSqueak software. USV classification was carried out using a neural network previously trained on a database of adult rat USVs. Our results show that the emergence and expression of social behavior components occur gradually in both control and VPA animals. However, when paired by age, we observe that (1) VPA pups have greater locomotor activity; (2) reduced social interaction; (3) reduced allogrooming; (4) similar rates of self-grooming in all the ages; and (5) subtle differences in the number of undercrossings and mount. USV emission pattern in VPA neonates was reduced compared to controls in all three ages analyzed P11, P14 and P18, but particularly at P18. This pattern was more evident in males. Finally, no significant correlation was

observed between the phenotypic feature of VPA animals, a tail curvature, and the behavioral traits measured.

P18. Sleep, dream content, and academic performance in students that will make a selective process to enter in higher education

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The elaboration of the dream storyline includes the combination of different fragments of memory. Some of the current dream theories consider dreams from a Darwinian perspective, in which dreams evolved as a safe behavior able to create a space for simulating possible future threats linked to the past waking state. According to this theory, dreams function as a probabilistic oracle since this cognitive anticipation could confer an advantage in terms of survival. Another theory based on neuropsychological mechanisms proposes that dreams would be self-regulating emotions such as fear. The more vivid and complex dreams happen during REM sleep, in which circuit modulations through gene expressions associated with waking events influence the formation of memories, which has consequences in learning processes. REM sleep happens mainly in the end of the night, and since adolescents tend to sleep later (phase delay) they are usually deprived of REM sleep and dreams. This phenomenon leads to a series of behavioral problems, which can influence students' performance. Objective: The present study proposes to analyze the relationship between sleep, dreams, and academic performance in young students. Methods: We will collect the dream reports of young students during the periods of school simulations and Enem (National High School Exam). Subsequently, we will classify the dreams into three groups: 1) Eve of the exam; 2) Previous to the exam, but not the day before, and 3) Control. Then, we will apply the following online questionnaires: 1) Sociodemographic; 2) Enem, pandemic, general habits and anxiety (STAI-S and STAI-T); 3) Pittsburgh Sleep Quality Index (IQSP); 4) Epworth sleepiness scale (ESE); 5) Munich chronotype (MCTQ) and 6) Academic motivation scale (EMA). The student's performance in the simulations for Enem will be collected from the data provided by the schools or directly with the students if they feel comfortable sharing. The reported dreams will go through a process of tokenization and elimination of stopwords. For structural analysis, we will use the SpeechGraphs (SG) software. The calculated attributes will be WC (word count), LCC (largest connected component), and LSC (largest strongly connected component). To analyze the emotional value of dream content, we will use a linguistic classifier, the Linguistic program Inquiry and Word Count (LIWC). We will also use the FastText method with the Python language, a classifier that uses supervised learning based on neural networks. With that, we will look for the semantic similarity between the

dream content and test words. Expected results: With the information related to sleep aspects (sleep quality, daytime sleepiness, chronotype, social jet lag), pandemic, anxiety, and motivation, we may understand how these cyclical manifestations behave in young students in the current context and the face of a significant event. We expect these aspects to contribute to the elucidation of the processing and consolidation of memory and learning in a brain undergoing high levels of synaptic plasticity and maturation.

P19. Cluster analysis of phosphoproteomic of rats exposed or not to novelty and following different sleep cycles

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Sleep is a physiological state important for memory consolidation. Sleep comprises two main stages: slow-wave sleep (SWS) and rapid-eye-movement sleep (REM). It is known that SWS and REM have major roles for the consolidation of newly acquired memories, but the mechanisms such as the phosphoproteomic differences between those sleep stages are still unclear.
Aim: Test if the phosphoproteomic profile of rats exposed to different experimental conditions (named in the study as: SWS-, SWS+, REM- and REM+) can be discriminated by data-driven analysis.
Methods: Twelve rats were used in the study, which were placed in 4 different groups (SWS-, SWS +, REM-, REM +), each group consisted of 3 individuals. The +/- indicates whether the animals were exposed (or not) to novelty and SWS/REM indicates differences in the sleep cycle of the animals during the study. Feature selection and dimensionality reduction, cluster analysis and surrogate control analyses was performed to achieve the aim of the study.
Results & Discussions: Through feature selection and dimensionality reduction were detected 51 proteins that allowed to cluster the animals according to its experimental conditions. This result was subjected to a surrogate analysis, thus validating its reliability through a statistical test. The study indicates that phosphoproteomic profile differs between SWS/REM sleep, suggesting that they have distinct and complementary roles in memories consolidation.

P20. Polygenic risk for schizophrenia: From Paleolithic to the Post Neolithic

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Schizophrenia is a serious psychiatric disorder with ~ 1% global prevalence and ~70% heritability. It interferes strongly with how the individual perceives the world, typically generating sociability difficulties with negative implications for the individual's fitness. Because schizophrenia is a psychiatric condition exclusive to our species, theories based on the comparison of neural and genomic connectomes have sought a connection between the schizophrenia phenotype and the evolution of the human brain. Thanks to large GWAS studies and the development of polygenic scores, the polygenic inheritance of psychiatric disorders, especially schizophrenia, has come to be better understood and gain clinical relevance in predicting phenotypes. A decade of accumulation of ancient DNA sequencing data now allows for the analysis of a reasonable number of samples, and that makes the study of the evolutionary history of schizophrenia possible. We sought to analyze the relationship between the frequency of possible schizophrenic phenotype based on the concept of polygenic risk and periods of major changes in a behavioral pattern throughout human history. The Polygenic Risk Score (PRS) sums the number of risk alleles among a set of SNPs associated with a particular phenotype, weighted by their effect sizes estimated from a certain cohort. We calculated it using a method that uses Empirical Bayes theory to improve the effectiveness of the prediction: Derive Polygenic Risk Score Based on Empirical Bayes Theory (EBPRS) implemented in R language. The training data resulted from the most important GWAS for SCZ composed of more than 108 loci and their respective weights, deposited in the Psychiatric Genomics Consortium. The test data we obtained from the "Allen Ancient DNA Resource (AADR): Downloadable genotypes of present-day and ancient DNA data" from David Reich Lab, Harvard University and consisted of 3589 complete genomes dating from 1100 to 90000 BCE, aligned in 1,233,013 positions of the reference genome version hg19; all publicly accessible. The data were grouped into five groups Early Upper Paleolithic (25,000 - 90,000 AP) (EUP), Late Upper Paleolithic (11,000 to 25,000 AP) (LUP), Mesolytic (8,500,000 to 11,000 AP), Neolithic (5,000 to 8,500 BP), and Post-Neolithic (1,000 to 5,000 BP, including the ages of copper and bronze, as well as later periods). We observed a significant decrease between the averages of polygenic risk scores between Early Paleolithic (90,000 - 25,000) and Late Paleolithic ($p = 0.027$) and subsequently a significant increase between the Neolithic (5,000 - 8,500) and Post Neolithic (3,200 - 1,000)) ($p = 9.3e-07$). The decrease in polygenic risks for schizophrenia between 90,000 and 25,000 suggests the occurrence of changes in selective pressures that resulted in the schizophrenia phenotype becoming less adaptive than an increase in the frequency of these markers in the post-Neolithic period, in turn, could be the result of a positive selection for the schizophrenia phenotype or even of intermediate phenotypes (schizotypy), perhaps related to the different context of life in larger societies. Returning to Karl Jaspers' concept of the Axial Age, there was a major change in human mentality in the period

from 800 to 200 CE, which, marked by an increase in the complexity of philosophical, political, religious, and artistic thought in Afro-Eurasia, could be related to positive selective pressure on the schizophrenia or schizotypy phenotypes. Alternatively, theories that point to warfare as an important selective pressure for the emergence and growth of societies from the Bronze Age onwards could favor the selection of the schizotypy phenotype contributing to the state of heightened belligerence. To explore whether selective pressure changes caused the patterns observed in the polygenic scores, we will test whether the observed distribution of polygenic scores among populations could have been plausibly generated under a neutral genetic drift model. Otherwise, we will consider it as a sign of directional selection.

Keywords: PRS, schizophrenia, evolution, Axial Age

P21. Prenatal VPA-treated rats have a smaller USV syntax repertoire at postnatal day 14

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Autism is a heterogeneous developmental disorder in which neural circuits involved in social cognition and sensory processing are dysfunctional. Vocal production plays a central role in social bonding in many mammalian species. Although not considered a communication mode acquired by learning, rat ultrasonic vocalizations (USVs) are produced at all developmental stages (from pups to adulthood), in distinct social contexts, conveys distinct emotional states and seem to have defined neurochemical routes. Pup USVs, in particular, are the earliest forms of emissions and occur when pups are found isolated from their mothers. Rodents exposed to valproic acid (VPA) during embryonic development reproduce many of the behavioral and physiological alterations observed as autistic endophenotypes. The present study, aimed to analyze the dynamic of USV production in VPA-treated rats from P7 to P21. At age P7, P14 and P21 pups were put in an acoustic isolated box with red light and an ultrasonic microphone (Dodotronics; Ultramic250K) positioned 18 cm above. For 300s, USVs were recorded using the software Audacity, with a sampling rate of 250 kHz. USVs were analyzed in MATLAB. We used an entropy-based method for USVs identification. After that we clusterized and classified the USVs using DeepSqueak, a deep learning-based method. Our results shown that VPA pups emitted less USVs in at P14, but no difference was found in P07 or P21. We also analyzed the total time that pups expended vocalizing. No difference was observed between VPA and control. With DeepSqueak we classified the USVs in 11 types using a supervised learning method. We compared the relative frequency of occurrence for each type of

USV at the ages investigated. From our USV classification, we found that control animals exhibit more types of USVs than VPA animals at P14. Our result showed that VPA-treated animal emits fewer USVs and shows a less diverse vocabulary at P14 than control animals.

P22. ZENK regulates vocal learning and song duration in zebra finches

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Activity dependent gene expression is thought to represent the link between neuronal activity and memory formation. This response includes a series of activity-dependent genes that are thought to mediate the changes necessary for memory consolidation and maintenance. Among these genes, *zenk* (a.k.a. *egr1*) was one of the first examples of a behaviorally driven gene and has been linked to memory formation in rodents. Nonetheless, the role of *zenk* in vocal learning, the exact behavior in which it was initially discovered as activity-dependent, remains unknown. To investigate the precise contribution of this gene for the acquisition and maintenance of song memories in zebra finches (*Taeniopigygia guttata*), we developed a dominant negative ZENK to manipulate the transcriptional activity of the endogenous protein. Using this tool we found that normal ZENK activity is required for song maintenance in adults and song acquisition in juveniles. Downregulation of ZENK transcriptional activity using an alternate inhibitor, NAB1, confirms these results and provides support for the specificity of our manipulation. Moreover, our results show that ZENK blockade consistently leads to transient disruption of the song duration in manipulated birds compared to controls, suggesting that the maintenance of the *tempo* requires this activity dependent gene. Our findings provide for the first time a clear causal link between activity dependent genes and song learning and song stability. More importantly, they represent one of the first steps towards understanding the
molecular processes involved in the acquisition, consolidation and reconsolidation of vocal signals, a process that is also required for human speech.

Funding by: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

P23. Understanding respiratory brain rhythms across the rat behavioral space

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In 1942, work by Edgar Adrian showed that the influx of air in the olfactory epithelium of hedgehogs generates oscillations in the olfactory bulb. Recent work in rodents has shown that neuronal oscillations synchronized to the phase of the respiratory cycle occur not only at the olfactory bulb but also across distant brain areas, from prefrontal to visual cortices to subcortical areas, such as the hippocampus and the amygdala. Intriguingly, these widespread respiratory brain rhythms are not continuously present. Instead, the amplitude of the coupling between neuronal activity and respiration across brain regions changes dramatically with time.

We set out to understand how the emergence of respiratory rhythms relates to the cognitive and behavioral state of the rat. We recorded respiration (intranasal pressure) and distributed brain signals (anterior cingulate, prelimbic, medial orbital and parietal cortices and olfactory bulb) in 7 rats as they freely behaved in three different settings: social interaction, open field with object exploration and elevated plus maze. We simultaneously recorded depth and luminance video and ultrasonic vocalizations. This will allow us to monitor in detail the behavior of the rats and their transitions through phases of the sleep/wake cycle, and to ask when and how respiratory rhythms emerge in the brain.

P24. In isolation or in entourage: comparing the antiseizure activity of phytocannabinoids in a status epilepticus model

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The use of Cannabis-derived products as medicine increased worldwide, especially after demonstrating cannabidiol (CBD) antiseizure property in patients with difficult-to-treat epilepsy. On the other hand, there are still discussions whether combined cannabinoids (aka entourage effect) would provide better seizure control than compounds given in isolation. To test this hypothesis, we compared extracts obtained from two Cannabis strains in the pilocarpine-induced status epilepticus (SE) mice model: one with high content of tetrahydrocannabinol (XT-THC group; THC:CBD ratio=107:1) and another rich in CBD (XT-CBD group; CBD:THC ratio=10:1). Positive controls included diazepam (a clinical anti-SE medication) and pure (100%) CBD solution (ethic approval: 013/2016, CEUA/UFRN). We scored SE severity using the latency for first behavioral seizure, the duration of generalized seizures, and the Racine scale. We scored the general behavior (such as

ambulation) and autonomic responses such as piloerection and salivation) as a secondary outcome. Our results show that XT-THC (doses ranging from 0.47 to 472.50 mg/kg) modulates pilocarpine-induced behavioral seizures in a non-linear manner. While the highest dose of XT-THC (472.5 mg/kg, ip, n=6) decreased seizure severity in comparison to vehicle (inert corn oil solution, 10 mL/kg, ip, n=18; $p<0.05$, Mann-Whitney test), the dose of 4.72 mg/kg, ip, n=7) increased the rate of animals displaying rearing and falling, wild running and jumping ($p<0.05$, Mann-Whitney test). Importantly, seizure attenuation at the highest dose of XT-THC was similar to the one observed in the diazepam-treated group (5 mg/kg, i.p., n=6; $p=0.80$, Mann-Whitney test). Conversely, XT-CBD reduced generalized seizure duration and Racine scale in a dose-dependent manner (doses ranging from 0.47 to 212.6 mg/kg, ip, n=5-6; $p<0.05$, Kruskal-Wallis test). Surprisingly, pure CBD (20 mg/kg, ip, n=6) increased the latency for first behavioral seizure in comparison to vehicle ($p<0.05$, Kruskal-Wallis followed by Dunn's test) but did not reduce seizure severity. Although XT-CBD and XT-THC had the same absolute concentration of CBD, the presence of other phytocannabinoids clearly modified the anticonvulsant profile. Together, these results suggest that Cannabis antiseizure efficacy depends on the hidden synergism between traditional phytocannabinoids and other natural products present in the extracts. Future assays with isolated compounds (THC and CBD) and extracts with variable proportions of phytocannabinoids will be necessary to elucidate further the mechanism associated with the antiseizure effect of cannabinoids in this animal model.

Funding: Financiadora de Estudos e Projetos (FINEP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ).

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