UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE

INSTITUTO DO CÉREBRO

HOUSE SYMPOSIUM 2015

13 e 14 de novembro

IMIRÁ PLAZA HOTEL

NATAL

Friday 13.1. 2015

8.30 Opening

Sidarta Ribeiro (Director of the Brain Institute - UFRN)

9.00 – 12.00 Topic 1 - Coding and rhythms in sensory systems and hippocampus

Chair: Katarina Leão

9.00 SENSORIMOTOR RHYTHMS IN THE RAT
Diego Laplagne (D Laplagne group)

9.30 OLM INTERNEURONS PROMOTE THETA ACTIVITY IN THE VENTRAL HIPPOCAMPUS
Richardson Leão (R Leão group)

10.00 - 10.30 COFFEE BREAK

10.30 VISUAL CONTEXT IS REFLECTED DIFFERENTLY IN V1 FIRING RATES AND PHASE RELATIONS
Sergio Conde (K Schmidt group)

11.00 GAMMA-NO-GAMMA: THIS IS THE QUESTION
Sergio Neuenschwander (S Neuenschwander group)

11.30 LACK OF EVIDENCE FOR CROSS-FREQUENCY PHASE-PHASE COUPLING BETWEEN THETA AND GAMMA OSCILLATIONS IN THE HIPPOCAMPUS
Robson Scheffer-Teixeira (A Tort group)

12.00 - 13.30 Lunch break

13.30 - 15.00 Poster session 1 (presenting even numbers)

15.00 - 16.00 Topic 2 - Animal models of mental disorders

Chair: Fernanda Palhano

15.00 HIPPOCAMPUS - PREFRONTAL CORTEX COORDINATION DURING SOCIAL INTERACTION AND MEMORY EXTINCTION IN THE VPA MODEL OF AUTISM
João Bacelo (R Pereira group)

15.30 MESOLIMBIC ACTIVITY AFTER SOCIAL DEFEAT: A BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDY
Aron de Miranda Henriques-Alves (C Queiroz group)

16.00 – 17.30 Poster session 2 (presenting odd numbers)

Saturday 14.11.2015

8.30 - 11.30 Topic 3 - Neurological diseases

Chair: Lia Bevilaqua

8.30 NEUROPLASTICITY: UNDERSTANDING CORTICAL DEVELOPMENT AND DESIGNING REHABILITATIVE THERAPIES
Antonio Pereira (A Pereira group)

9.00 IS THERE A LINK BETWEEN CANCER AND NEUROLOGICAL DISEASES?
Sandro de Souza (S De Souza group)

9.30 ACTIGRAPHY AND CHRONOTYPE CHARACTERIZATIONS TO UNDERSTANDING POOR SLEEP QUALITY AND ACTIVITY-REST CYCLE DISTURBANCES IN FIBROMYALGIA SYNDROME

Kathiane de Santos Santana (M B Sousa group)

10.00 – 10.30 COFFEE BREAK

Topic 4 - Mental states

Chair: Katia-Simone Rocha

10.30 PSYCHOBIOLOGICAL AND CLINICAL RESEARCH WITH AYAHUASCA
Draulio Araújo (D Araujo group)

11.00 COMPUTATIONAL PSYCHOLOGY: HOW COMPUTATIONAL APPROACHES CAN HELP TO UNDERSTAND DIFFERENT STATES OF CONSCIOUSNESS
Natalia Mota (S Ribeiro group)

11.30 - 12.30 Poster session 3 (presenting odd numbers)

12.30 - 14.00 Lunch break

14.00 - 15.30 Topic 5 – Transgenic approaches and cell transplants

Chair: Andressa Radiske

14.00 IN VIVO AND IN VITRO DIRECT LINEAGE-REPROGRAMMING OF DISTINCT ASTROGLIAL POPULATION INTO NEURONS
Malek Chouchane (M Costa group)

14.30 SPECIFIC NEURONAL POPULATIONS IN THE DORSAL COCHLEAR NUCLEUS THAT RESPOND TO SOUND CAN BE CONTROLLED USING OPTOGENETIC AND CHEMOGENETIC PROTEINS
Thawann Malfatti (K Leão group)

15.00 TRANSGENIC ZEBRA FINCHES: A GENETIC APPROACH TO INVESTIGATE THE BIOLOGICAL BASES OF VOCAL COMMUNICATION
Tarciso Velho (T Velho group)

15.30 - 16.30 Poster session 4 (presenting even numbers)

16.30 - 18.00 Discussion and cocktail

ORAL PRESENTATIONS

Friday 13.11.15

Topic I - Coding and rhythms in sensory systems and hippocampus

9.00 Sensorimotor rhythms in the rat

Diego A. Laplagne

Laboratory Of Behavioral Neurophysiology, Brain Institute, UFRN.

During active exploration, rodents interact with the environment rhythmically. In 1964, Welker described a complex sensorimotor behavior of the rat he called sniffing composed of: "(a) protraction and retraction of the mystacial vibrissae, (b) protraction and retraction of the nose or tip of the snout, (c) head approach and withdrawal (or extension and retraction), and (d) rapid expiration and inspiration (polypnea)". During sniffing, all of these can synchronize at rates of 5-−11 Hz, resulting in a global motor rhythm. Neuronal activity across the brain is synchronous to this behavior: it emerges from the concerted activity of brainstem nuclei and, in turn, gates sensory information to the cortex. We have recently shown that ultrasonic vocalizations of the 50 kHz type are an integral component of this behavior and, as such, inherit its rhythmicity. Sniffing is thus not only relevant for sensation but also structures the output of social signals. We are now expanding our recordings to include other motor components of this behavior as well as neuronal activity across the brain to probe the existence of globally synchronized rhythms in the rat.

9.30 OLM interneurons promote theta activity the ventral hippocampus

Richardson N. Leao

Theta oscillations in the dorsal hippocampus are described as one of the most regular rhythms of the brain. This 4-12 Hz have been associated with multiple behaviors, especially with movement. On the other hand, theta activity in the ventral hippocampus has been implicated in emotions related behavior. It has been hypothesised that specific GABAergic interneuron subtypes play differential roles in driving hippocampal oscillations. Although different populations of hippocampal interneurons fire preferentially to specific phases of theta, phase-locking firing itself does not prove a causal role in theta generation. We found a specific subtype of oriens lacunosum-moleculare (OLM) interneurons expressing Chrna2 receptor differentially distributed along the dorso-ventral hippocampal axis. Using optogenetic tools in anesthetised and freely behaving animals, we found that activation of this population induce prominent theta activity in the ventral but not in the dorsal hippocampus. Interestingly, the induced theta rhythm was not correlated with animals' movements. In treadmill experiments, we found that rhythmical optical stimulation of OLM neurons can either increase or decrease the coherence between dorsal and ventral hippocampus depending on the position of the optical fibre within the dorsoventral axis of the hippocampus. Taken together, our results provide the first evidence of a single morphologically defined cell population that in a network including pyramidal cells causally drives ventral hippocampal theta oscillations.

10.30 VISUAL CONTEXT IS REFLECTED DIFFERENTLY IN FIRING RATES AND PHASE RELATIONS OF NEURONAL POPULATIONS IN V1

Sergio A. Conde Ocazionez, Tiago S. Altavini, Thomas Wunderle and Kerstin E. Schmidt

The activity of neurons in early visual areas can be modulated by stimulation outside their classical receptive field (CRF), presumably involving feedback and long-range lateral connections. Contextual influences are revealed already in the activity of early visual areas but their neuronal correlate is commonly defined based on the activity of single neurons (i.e. firing rate, oscillatory firing). Thus, it remains understudied whether stimulation outside the CRF is also reflected in the coordinated activity of populations of neurons (assembly activity and local field potential –LFP).

For studying context-dependent modulation of unit spiking and assembly activity, we recorded single units and LFPs from V1 of anesthetized cats (n=4) and identified groups of neurons forming assemblies. We stimulated with moving natural scenes in two conditions: i) a foreground patch covering all CRFs and moving in opposite or orthogonal direction to its background, ii) the corresponding homogenous whole-field (WF). We analyzed both firing rates of single neurons, and number of assembly activations. In order to evaluate the integration of the activity from a larger area, we estimated the phase locking of those events with low gamma (LG). Finally, we explored the influence of lateral connections through the corpus callosum (CC) by reversibly deactivating the contralateral visual areas (CC).

29% of all single units (n= 480) responded significantly different to Patch as opposed to WF conditions. Of those, 11.6% indicated response facilitation during Patch stimulation and the remaining 88.4% pointed to response suppression. 35% of all single units varied also their phase locking value depending on the stimulus condition, but at the same time, only 31% of those units showed significant variation of firing rates. 30 out of 70 detected assemblies were activated differently by WF and Patch conditions, all being suppressed by Patch. Compared to single units, much more assemblies (61.4%) showed different phase locking for WF as opposed to Patch conditions. Only 15% had significant variations of both number of activations and phase locking. CC deactivation resulted in a decrease of both firing rate and assembly activations and reduced differences between WF and Patch conditions. Interestingly, phase locking was highly affected by CC cooling during both stimulus conditions for single unit but not for assembly activity.

Our results indicate that different neuronal subpopulations can signal context-dependent modulation in their spatial and temporal response patterns independently. Further, lateral connections, i.e. through the corpus callosum, contribute to both, single unit and assembly activity, but apparently only to the low gamma phase relation of single cells but not of assemblies. Possibly, timing of assembly activations is structured within local intra-hemispheric circuits.

11.00 GAMMA-NO-GAMMA: THIS IS THE QUESTION

Sergio Neuenschwander

Vislab, Instituto do Cérebro - UFRN

Gamma oscillations have been implicated in various cognitive processes. In the visual system, gamma has been associated with feature encoding, perceptual

binding and attention. So far, most of the evidence has been derived from analysis of responses to artificial stimuli, such as bars, gratings and plaids. A crucial step in understanding how gamma contributes mechanistically to visual processing, however, is to study responses in more natural conditions, such as during free viewing of natural scenes and movies (Brunet et al., TICS, 2014). A few recent studies in the primary visual cortex of monkeys and humans led to diverging conclusions. In humans, gamma was absent from electrocorticographic responses (ECoG) to natural images and visual noise (Hermes et al., Cerebral Cortex, 2014). Similarly, temporal analysis of spiking activity in V1 of capuchin monkeys revealed strong beta but no gamma components in responses to colored pictures (Ito et al., Cerebral Cortex, 2011). An auto-spectral analysis of ECoG signals in the macaque showed, on the contrary, surprisingly strong gamma responses to colored and gray-scale images (Brunet et al., Cerebral Cortex, 2013). In order to clarify these controversies, here we recorded in V1 of capuchin monkeys spiking and local field potential responses to artificial and natural pictures and movies during both maintained fixation (over 2000 msec) and free viewing. In addition, we adopted a radical instance of natural seeing by creating a stage, where the monkeys could freely observe other monkeys, humans or real objects, while we recorded activity from the visual cortex.

Our preliminary results confirmed previous findings in the macaque (Lima et al., Cerebral Cortex, 2010). In general, artificial stimuli capable of activating the cortex strongly and selectively evoked stable, limit-cycle gamma oscillations (oscillation frequency 50 - 60 Hz, 20 recording sessions). Capuchins were not different from macaques in this respect. It is likely that gamma has not been observed before in the capuchin (Ito et al., 2011) just because all observations were made during free viewing of natural images. Our experiments showed that, during maintained fixation, gamma responses to optimal artificial stimuli can be indeed very strong and regular. In contrast, gamma was absent from responses to natural movies and free viewing of natural images while beta was very strong. Similar results were obtained so far for responses to real world scenes.

Overall, these results weakens the notion that gamma is necessary for visual processing and raises questions on its role in neuronal communication. So the debate on gamma or no-gamma continues…

11.30 Lack of evidence for cross-frequency phase-phase coupling between theta and gamma oscillations in the hippocampus

Robson Scheffer-Teixeira and Adriano BL Tort

Brain Institute, Federal University of Rio Grande do Norte, RN 59056-450, Brazil.

Brain rhythms of different frequencies may interact. There are several types of cross-frequency coupling, which are hypothesized to serve specific functions. In particular, phase-amplitude coupling between theta and multiple gamma sub-bands hallmarks the activity of hippocampal networks and is believed to take part in information routing. More recently, theta and gamma oscillations were also reported to exhibit reliable phase-phase coupling (Belluscio et al, 2012). Cross-frequency phase-phase coupling, also known as n:m phase-locking, occurs when is constant, where denotes the phase of oscillation A (B) accelerated m (n) times. For instance, a 1:5 phase-phase coupling between theta and gamma oscillations means that the instantaneous phase of theta when accelerated 5 times has constant difference to the instantaneous gamma phase; or, in other words, that 5 gamma cycles have fixed phase relationship to 1 theta cycle. The existence of n:m phase-locking suggests a mechanism of network communication that has long received theoretical support (Varela et al, 2001). However, while trying to reproduce Belluscio et al. (2012) findings, here we found that n:m phase-locking (1) is much lower than reported, (2) highly depends on epoch length, (3) does not statistically differ from chance (when proper surrogate methods are used), and that (4) filtered white-noise signals have similar n:m scores as actual data. Moreover, (5) the diagonal stripes in theta-gamma phase-phase histograms of actual data may be explained by theta harmonics. In all, these results point to lack of theta-gamma phase-phase coupling in the hippocampus, and suggest that studies investigating n:m phase-locking should rely on appropriate statistical controls and also rule out the possibility of contamination by harmonics, otherwise they could easily fall into analysis pitfalls.

Topic 2 – Animal models of mental disorder

15.00 Hippocampus - prefrontal cortex coordination during social interaction and memory extinction in the VPA model of autism

João Bacelo and Rodrigo Pereira

Brain Institute, Federal University of Rio Grande do Norte, Natal-RN, Brazil

15.30 MESOLIMBIC ACTIVITY AFTER SOCIAL DEFEAT: A BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDY

Aron de Miranda Henriques-Alves and Claudio M. Queiroz

Brain Institute, Federal University of Rio Grande do Norte, Natal-RN, Brazil.

Animals respond differently to stress. While some individuals are able to overcome the stressor (resilience), others may develop depression or post- traumatic stress disorder. Several lines of evidence suggest a link between behavioral phenotype and long-term plasticity in the classic brain reward circuitry after social defeat. Using in vitro preparations, some authors have described increased baseline firing rate and enhanced burst activity of dopaminergic neurons in the midbrain ventral tegmental area (VTA) in susceptible animals (but not controls or resilient animals). However, VTA neuronal activity in freely behaving animals during social interaction has been poorly characterized. In this talk we will show recent results which combined multi-site, multi-electrode electrophysiological recordings with detailed ethological observations to explore the relationship between VTA neuronal activity and social interaction in socially stressed mice. With that approach, we were able to show opposite modulation of DA and GABAergic neurons in VTA during social investigation in susceptible and resilient animals. We propose that after social defeat, susceptible animals fail to attribute reward value to social interactions, which could be associated with the expression of depression-like behavior in these animals.

SATURDAY 14.11.15

TOPIC 3 - NEUROLOGICAL DISEASE

8.30 Neuroplasticity: understanding cortical development and designing rehabilitative therapies

Antonio Pereira

Brain Institute, Federal University of Rio Grande do Norte, Natal-RN, Brazil.

Maturation of the brain is characterized by a drastic decrease in synaptic plasticity. Several cellular factors are associated with this outcome, including maturation of inhibitory GABAergic interneurons, which coordinate synaptic interactions among excitatory principal neurons and generate an oscillatory behavior responsible for facilitating the transfer of information among neuronal ensembles. A group of inhibitory interneurons which contain the calcium ligand protein parvalbumin (PV+) are characterized by fast-spiking electrical behavior and are involved with the generation of gamma-band frequencies. Recently, it was demonstrated that the power of gamma oscillations at rest is correlated with synaptic maturation in specific cortical regions. Our group is interested on understanding how structural and functional changes in inhibitory circuits are associated with cortical development and cognition in both animal and human models. Furthermore, we evaluate the effects of pharmacological manipulation of neural tissue on reopening critical periods of plasticity in cortical areas. This strategy could benefit the functional recovery of patients surviving brain injuries, suck as stroke.

9.00 Is there a link between cancer and neurological diseases?

Sandro de Souza

Brain Institute, Federal University of Rio Grande do Norte, Natal-RN, Brazil.

9.30 Actigraphy and chronotype characterizations to understanding poor sleep quality and activity-rest cycle disturbances in Fibromyalgia Syndrome

Kathiane dos Santos Santana, Ana Beatriz Moura Raulino, Eder Leandro da Silva Dantas, Maria Bernardete Cordeiro de Sousa

Brain Institute, Federal University of Rio Grande do Norte, Natal-RN, Brazil.

Fibromyalgia Syndrome (FM) is associated to musculoskeletal and widespread pain in addition to an extensive variety of symptoms and women have a higher prevalence of fibromyalgia than man (3,5% versus 0,5%). Sleep disturbances is one of the most prominent and concerning of fibromyalgia complaints. Then, our aim was evaluate sleep quality and activity-rest cycle in women with FM, using actigraphy and questionnaires for sleep and chronotype characterization. At the moment we have 12 women with FM (42.4 ± 11.1 years old) and 19 healthy women (41.8 ± 11.7 years old). To assess sleep quality and chronotype were used: (1) Morningness-eveningness questionnaire,(2) Epworth sleepiness scale, and (3) Pittsburgh sleep quality index. Moreover, 16 volunteers (10 with FM) wore an actigraph on the non-dominant wrist for 15 days for monitoring activity-rest cycle. This device was configured to record in 1-min epochs, which registers locomotor activity and it is useful as an unobtrusive and objective measure to assess the sleeping behavior. As parameters for actygraph, were collected Intradaily variability –IV, which provide information on rest activity rhythm fragmentation, and Interdaily variability – IS, which yields information about the rest activity rhythm synchronization with the light dark cycle. In addition, were registered the average on locomotor activity during day and night. As results, were founded that all volunteers with FM presented bad quality of sleep according to questionnaires that are not correlated to early or evening chronotypes. Furthermore, 42% of healthy women have bad quality of sleep, which was correlated with late chronotypes (r= -0,71). Related to actigraph data, was not possible to verify differences between the groups on average in locomotor activity during day and night (day: healthy- 3009,75, FM- 2885,27; night: healthy- 1830,31, FM- 2002,94). However, were founded that FM have high values for IV (t test, p=0,001), which could mean greater rhythm fragmentation and worse sleep efficiency. Also, volunteers with FM showed low values for IS compared to healthy women (t test, p= 0.02), indicating that the woman is not good synchronized with the 24-h light dark cycle. These preliminary data suggest that FM participants have more problems associated with sleep quality and rest activity cycle, which corroborate with other studies. Furthermore, we expect that this first study regarding IV and IS parameters in this syndrome could best characterize rest activity cycle and sleep disturbances in FM.

Topic 4 - Mental states

10.30 Psychobiological and clinical research with Ayahuasca

Draulio B. de Araujo

Brain Institute, Federal University of Rio Grande do Norte, Natal-RN, Brazil.

Ayahuasca is a psychedelic beverage used for ages by Amazonian Amerindians. It is most frequently prepared by the decoction of the bark and trunk of a shrub, Banisteriopsis caapi, scalded with leaves of the Psychotria viridis. From the neuropsychological perspective, it is known to induce complex mystical experiences, modulating the sensory, perceptual, cognitive, autonomic systems, and emotional processes. A renowned effect of Ayahuasca is the increased vividness of both perception and internally generated mental images, and a remarkable increase in introspection. This presentation will focus at our studies that used functional Magnetic Resonance Imaging to better understand the neural basis of these effects. Furthermore, we will present preliminary evidences from a set of studies that aim at testing the use of Ayahuasca as an alternative antidepressant.

11.00 Computational psychology: how computational approaches can help to understand different states of consciousness

Natália B Mota1, Mauro Copelli2 and Sidarta T. Ribeiro2

1Departamento de Física, UFPE; 2Lab Sono, Sonhos e Memória, Ice, UFRN Natal

What can speech analysis of memory reports (episodic memories, dreams) reveal about cognition? In this poster, we will discuss about computational speech analysis in 3 different questions about consciousness and cognition: Firstly, in elementary school children (1 year follow-up, n=76), learning reading and math during regular cognitive development; secondly, in teenagers during first episode psychosis (6 months follow-up, n=21) in cognitive decline,

and thirdly, in healthy adults woken up from 3 different stages of the sleep-wake cycle (EEG study about imagery reports during sleep transition). The structure of their reports is measured by graph attributes, and the semantic similarity between reports is measured by latent semantic analysis. Memory graphs are significantly related to cognitive development, and can predict the differential diagnosis between schizophrenia and bipolar disorder 6 months in advance.

TOPIC 5 – TRANSGENIC APPROACHES AND CELL TRANSPLANTS

14.00 IN VIVO AND IN VITRO DIRECT LINEAGE-REPROGRAMMING OF DISTINCT ASTROGLIAL POPULATION INTO NEURONS

Melek Chouchane and Marcos R. Costa

Recently, the field of cellular reprogramming has been revolutionized by works showing the potential to directly lineage-reprogram somatic cells into neurons upon overexpression of specific transcription factors. This technique offers a promising strategy to study the molecular mechanisms of neuronal specification, identify potential therapeutic targets for neurological diseases and eventually repair the central nervous system damaged by neurological conditions. Notably, studies with cortical astroglia revealed the high potential of these cells to reprogram into neurons using a single neuronal transcription factor. However, it remains unknown whether astroglia isolated from different regions of the central nervous system have the same neurogenic potential and if they will generate the same phenotype of induced neurons. In this study we investigate the potential to reprogram astroglial cells isolated from the post-natal cerebellum into functional neurons using the proneural transcription factors Neurogenin-2 (Neurog2) and Achaete scute homolog-1 (Ascl1). We also evaluated the capacity of astroglia isolated from the cerebral cortex or cerebellum and reprogrammed into induced neurons to integrate into a neurogenic (subventricular zone – SVZ) or non-neurogenic (cerebral cortex) milieu in vivo. We observed that cortical astroglia underwent a full process of neuronal reprogramming in the brain, independently of the region of trans-

plant and the transcription factor used. However, induced neurons behaved differently when transplanted in the SVZ or cerebral cortex. While astroglia overexpressing Ascl1 or Neurog2 reprogrammed into induced neurons migrated through the rostral migratory stream and integrated in the olfactory bulb (OB), astroglia overexpressing Neurog2 and transplanted in the cerebral cortex converted into spiny pyramidal neurons. Collectively, our results indicate that astroglial cells isolated from different regions undergo a full process of lineage reprogramming into induced neurons after transplantation in vivo. They also suggest that the region of transplant play instructive roles in the phenotypic specification of induced neurons.

14.30 SPECIFIC NEURONAL POPULATIONS IN THE DORSAL COCHLEAR NUCLEUS THAT RESPOND TO SOUND CAN BE CONTROLLED USING OPTOGENETIC AND CHEMOGENETIC PROTEINS

Malfatti, T.B., Hilscher, M.M., Leao, R.N., Leao, K.E.

Brain Institute, Federal University of Rio Grande do Norte, RN 59056-450, Brazil.

Tinnitus is the perception of a sound in the absence of a corresponding physical stimulus. It is not clear yet what are the mechanisms involved in tinnitus and how it starts and/or becomes chronic. Due to the relationship between tinnitus and somatosensory trauma/stimuli, the dorsal cochlear nucleus (DCN), a region known to integrate somatosensory and auditory pathways, has been identified as a potential key structure in the generation of phantom sound perception. Here, we target specific neuronal populations in the DCN to investigate how this region may contribute to the generation of tinnitus signals that spread to other auditory areas. We examined the expression of optogenetic proteins (Channelrhodopsin 2 ChR2; and Archaerhodopsin 3 Arch3) and chemogenetic proteins (Designer Receptors Exclusively Activated by Designer Drugs DREADDs; more specifically Gqcoupled human M3 DREADD hM3Dq, and Gi coupled human M4 DREADD hM4Di), targeting neurons expressing Calmoduline Kinase II alpha (CaMKIIa) promoter in wildtype C57/Bl6 mice and neurons expressing nicotinic acetylcholine receptor subunit alpha2 promoter (ChRNA2) in ChRNA2Cre transgenic C57/Bl6 mice, using local virus injection, verified by fluorescence microscopy. Unit responses were differentiated based on their electro-physiological response to sound. We then investigated if firing of neurons expressing optogenetic or chemogenetic tools

can be controlled in vivo and if the same neurons also fire action potentials in response to precisely timed sound stimulation. Both in vivo and in vitro data show that neurons expressing ChR2 that responds to sound can respond to light stimulation with a stable and similar waveform. Also, preliminary in vivo data suggest that neurons expressing Arch3 that responds to sound decrease their firing rate using light stimulation. By applying these optogenetic and chemogenetic tools we aim to test tinnitus theories by producing an increased firing rate, trying to mimic tinnitus; or inhibiting increased spontaneous firing rate on animals with noise - or salicylate - induced tinnitus.

15.00 TRANSGENIC ZEBRA FINCHES: A GENETIC APPROACH TO INVESTIGATE THE BIOLOGICAL BASES OF VOCAL COMMUNICATION

Tarciso Velho

Brain Institute, Federal University of Rio Grande do Norte, Natal-RN, Brazil

Communication is a key function often affected in a number of neurodevelopmental disorders, including autism spectrum disorders (ASD). It has been hypothesized that the language impairments associated with ASD may be due to altered assembly of brain areas involved in vocal communication. In recent years a number of genes, including the transcription factor FoxP2 and its target CNTNAP2, have been linked to language specific impairments and to ASD in humans, suggesting that they could play a direct role in language deficits associated with ASD. However, the lack of adequate animal models to specifically study vocal learning and communication has prevented the dissection of genetic components involved in communication behaviors. To circumvent this problem and to address this question, we have developed new technologies to generate transgenic zebra finches, a songbird species. Vocal learning in zebra finches closely resembles speech acquisition in humans, making the best existing model for laboratory research. These genetically modified animals allow us to investigate the contribution of genes in which mutations are linked to language specific disorders to the formation and functioning of circuits involved in vocal learning and production. In addition, we also study the homeostatic mechanisms involved in the maintenance of the precise and stereotyped neuronal firing associated with singing. To address these questions, we use a combination of acute and chronic genetic manipulations to perturb activity of individual neurons or brain regions, coupled with calcium imaging in live behaving animals, and extensive behavioral analysis of vocal signals.

POSTERS

1. Patchy interhemispheric circuits account for a cardinal bias in ongoing activity within early visual areas

Tiago S. Altavini, Sergio A. Conde Ocazionez, Thomas Wunderle, David Eriksson, Kerstin E. Schmidt

Ongoing brain activity exhibits patterns resembling neural ensembles co-activated by stimulation or task performance. Such patterns have been attributed to the brain's functional architecture, e.g. patchy long-range connections. Here, we directly investigate the contribution of patchy connections between hemispheres to ongoing and evoked maps in cat area 18. We recorded voltage-sensitive dye imaging maps and spiking activity while manipulating interhemispheric input by reversibly deactivating corresponding contralateral areas. During deactivation, ongoing maps continued to be generated with similar frequency and quality as in the intact network but a baseline cardinal bias disappeared. Consistently, neurons preferring either horizontal (HN) or vertical (VN), as opposed to oblique contours, decreased their resting state activity, however, HN decreased their rates also when stimulated visually.

We conclude that structured ongoing maps are primarily generated by thalamo- and/or intracortical connectivity. However, long-range connections through the corpus callosum - in perpetuation of the long-range intracortical network – contribute to a cardinal bias, possibly, because they are stronger or more frequent between neurons preferring horizontal and/or cardinal contours. As those contours are easier perceived and appear more frequently in a natural environment, long-range connections might provide visual cortex with a grid for probabilistic grouping operations in a larger visual scene.

2. ELIMINATION OF EARLY BORN NEURONS AFFECTS THE SPECIFICATION OF LATE BORN NEURONS IN THE CEREBRAL CORTEX

Bruna Soares Landeira, Jéssica Alves de Medeiros Araújo and Marcos R. Costa

The cerebral cortex of mammals is histologically organized into different layers of excitatory neurons that have distinct patterns of connections with cortical or subcortical targets. During development, these cortical layers are established through an intricate combination of neuronal specification and migration in a radial pattern known as "inside-out": deep-layer neurons are generated prior to upper-layer neurons. In the last few decades, several genes encoding transcription factors involved in the sequential specification of neurons destined to different cortical layers have been identified. However, the influence of early-generated neurons in the specification of subsequent neuronal cohorts remains unclear. To investigate this possible influence, we induced the selective death of cortical neurons from layer V and VI before the generation of layer II, III and IV neurons. Next, we evaluate the effects of ablation of early born neurons on the phenotype of late born neurons by BrdU-chasing. Our data shows that one-day after ablation, layer VI neurons expressing the transcription factor TBR1 are newly generated while virtually no neuron expressing TBR1 was generated in the same age in control animals. This suggests that progenitors involved in the generation of neurons destined for superficial layers suffer interference from the selective death of neurons in deep layers, changing their specification. We also observed that while TBR1-positive neurons are located exclusively in deep cortical layers of control animals, many TBR1-positive neurons are misplaced in superficial layers of ablated animals, suggesting that the migration of cortical neurons could be controlled independently of neuronal phenotypes. Interestingly, this increase in the generation of Tbr1-expressing neurons was not observed when we induced neuronal cell death in vitro, suggesting that tissue organization is important for signaling between post-mitotic neurons and progenitors. Altogether, our data indicate the existence of a feedback mechanism of control between early-generated neurons and progenitors involved in the generation of granular and supra-granular cortical neurons. This mechanism could help to control the number of neurons in different layers and contribute to the establishment of different cortical areas.

3. OPTICAL MODULATION OF ORIENS LACUNOSUM-MOLECULARE (OLM) CELLS RESULTS IN SPECIFIC IMPAIRMENT OF MEMORY ACQUISITION IN AN OBJECT RECOGNITION TASK

Arthur S. C. França1, Samer Siwani2, Amilcar Reis2, Richardson Leão1, Adriano B. L. Tort1 and Klas Kullander2

1 UFRN / Brain Inst., Natal, Brazil; 2 Neurosci., Uppsala Univ., Uppsala, Sweden

Optogenetics has allowed the probing of specific populations of neurons in cognitive behaviors. In particular, the study of mnemonic process is one of the fields that have advanced with optogenetics. In the hippocampus, it has recently been shown that the cholinergic receptor alpha 2 subunit (Chrna2) is expressed in oriens lacunosum-moleculare (OLM) GABAergic interneurons. Here we used Chrna2/Cre mice to express ChR2 – a sodium channel sensitive to blue light – in hippocampal OLM cells. Chrna2/ChR2 and control animals were subjected to an object recognition task in which in they were exposed to 2 objects in open field in 2 10-min sessions (training and test). The sessions were separated by either 1 or 24h, referred to as short-term (STM) and long-term (LTM) memory tests, respectively. The main protocol consisted of applying 8-Hz blue light modulation during exploration of one of the objects in the training session, and comparing the preference ratio in the test session in which the lit object was placed along with a novel object. The preference ratio was defined as the time exploring the novel object divided by the time exploring the lit object. We found that while control animals recognized the novel object as expected, Chrna2/ChR2 animals were not able to recognize the novel object (preference ratio for the LTM test: control: 1.492 ± 0.1981 N=11 vs Chrna2/ChR2: 0.8908 ± 0.1042 N=7, p = 0.0369;STM test: control: 1.573 ± 0.2545 N=8 vs Chrna2/ChR2: 0.8877 ± 0.1467N=7, p = 0.0431). Importantly, both Chrna2/ChR2 and control mice were able to recognize the novel object in STM and LTM tests if the unlit object was placed in the open field in the test session. These results, together with results found for other variations of the main protocol, suggest that optical modulation of OLM cells results in a specific impairment in memory acquisition of the lit object. Our results support the hypothesis that OLM cells can act as a filter of the entorhinal cortex sensory input to CA1, thereby controlling the input of information necessary for memory acquisition.

4. Proteome Browser: Exposing The Proteome Complexity

José Eduardo Kroll1,2, Sandro J. de Souza1 and Gustavo A. de Souza3

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Next-generation sequencing (NGS) has been allowing deep characterization of transcriptomes through the identification of several alternative splicing variants. Moreover, in proteomics, NGS technologies have already proved to be useful in predicting new protein variants, which can be later validated by strategies such as mass spectrometry in tandem (MS/MS). Although advances in mass spectrometry technologies have been occurring, only a small portion of the human proteome is currently known. The newest orbitrap mass analyzer, for example, outputs a data of high-resolution, and it was used to compile the first Human Proteome project, which unfortunately targeted only highly known sequences (RefSeqs). Since available transcriptomic and proteomic data becomes more popular, there is an urge to integrate these data for acquiring more comprehensive results. Furthermore, strategies for gathering, organizing and representing these informations will be of great scientific interest. We propose, therefore, a wide integration of transcriptomics with proteomics using publicly available data, as like a Proteome Browser, a web tool that has the intention to help dissect the proteome complexity. The Proteome Browser will initially features MS/MS data from the Human Proteome Project, which comprehends 30 different organs/cell types and a total of 85 samples. The set of proteins, aimed to be validated, will be composed of entries from different sources, including Uniprot, Gencode, Splooce and the Illumina Human Body Map Project V2, which is composed of 16 NGS samples from 16 different human tissues. The Proteome Browser is being written in JavaScript, and it uses libraries such as JQuery and Raphaeljs for a more interactive and user-friendly interface. This project represents an advance in the merging of the different omics, and intends to expose the human inherent complexity as a mean to reach new insights.

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5. IMPAIRED TONOTOPY AND RECEPTIVE FIELDS IN THE PRIMARY AUDITORY CORTEX OF AN ANIMAL MODEL OF AUTISM

Anomal, RF; de Villers-Sidani E; Brandão JA; Diniz R; Costa, MR, and Romcy-Pereira RN

Autism is a neurodevelopmental disorder clinically characterized by deficits in communication, lack of social interaction and, repetitive behaviors with restricted interests. A number of studies have reported that sensory perception abnormalities are common in autistic individuals and might contribute to the complex behavioral symptoms of the disorder, including hearing impairments. In this context, we used an animal model of autism induced by valproic acid (VPA) to investigate the tonotopic organization and receptive fields of the primary auditory cortex (A1) and its local inhibitory circuitry, by counting its number or distribution of parvalbumin-positive cells.

In total, 30 Wistar rats were used for electrophysiology experiments (N=14) or histological analyses (N=16). We injected valproic acid (500 mg/Kg) in rats at E12.5. A1 of VPA and control rats were mapped from post-natal age 30 (P30) to P48. In total, 43 slices from 18 rats were used for histological analysis of parvalbumin-positive cells aging from P35 to P40. We quantified the total number of these cells in A1 of VPA (N=7) and control (N=9) animals.

In A1 of VPA rats, there was a significant enhancement in the fraction of recorded sites with multi and flat-peaked shape (Student's t-test, $p=0.0037$). The percentage of A1 sites tuned to high frequencies of sound (above 10 kHz) was significantly larger in VPA than in controls (ANOVA, $F=48.12$, $p=0.0001$). Tonotopic index was significantly greater in VPA rats when compared to controls (Student's t-test, $p=0.0004$). Recorded neurons in A1 of VPA rats showed significantly larger BW10 as compared to control animals (Student's t-test, $p<0.0001$). VPA animals also showed a higher number of responsive sites in A1 tuned to sound intensities above 50 dB (Student's t-test, $p=0.002$). The latency of neuronal responses to sound stimuli in VPA animals was significantly decreased in comparison to controls (Student's t-test, $p=0.0049$). We did not detect differences in the number of parvalbumin-positive interneurons in A1 of VPA and control rats.

Altogether, our findings show that neurophysiological impairments of hearing perception in this autism model occur independently of alterations in the number of parvalbumine-expressing interneurons.

6. Martinotti cells defined by Chrna2 coordinate layer V pyramidal cell activity

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GABAergic interneurons in the mammalian cortex possess a broad variety of morphological, neurochemical and electrophysiological properties. The somatostatin+ Martinotti cells (MCs), are the most prominent slow-inhibitory interneurons targeting distal dendrites of pyramidal cells (PCs). We identified the cholinergic nicotinic receptor alpha2 (Chrna2) as a marker to label layer V/VI MCs (MCsα2). We used a Chrna2-cre mouse line crossed with a fluorescent reporter (tdTomato) confirming that the layer V/VI MCsα2 exhibit the defining characteristic of a long axonal projection to layer I and extensive ramifications in layer IV. Immunohistochemistry showed that the vast majority of Tomato+ cells comprised a subpopulation of somatostatin+ interneurons. Whole-cell current- and voltage-clamp recordings were performed in the auditory cortex and confirmed that passive and active electrophysiological properties of MCα2 resemble the classical low-threshold spiking patterns of MCs. Recorded MCα2 usually exhibited spike frequency adaptation and burst discharge when depolarized from hyperpolarized potentials. In paired recordings, layer V PCs showed long inhibitory postsynaptic potential rise times and synaptic depression upon electrical stimulation of the presynaptic MCα2. Moreover, optogenetic manipulation of channelrhodopsin-activated MCsα2 demonstrated that layer V PC spiking can be controlled by MCsα2 via oscillatory inhibition of PC distal dendrites suggesting that MCsα2 may play a key role for PC network coordination.

7. SLC10A4 knockout mice show abnormal event-related potentials in response to an auditory paired-clicks gating test: implications for psychiatric disorders

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Slc10A4 is a vesicular monoaminergic and cholinergic associated transporter, and SLC10A4-knockouts (ko) show indications of a greater vulnerability to psycomimetic drugs making them a potential animal model for psychiatric diseases. Here, we use low dose ketamine (5mg/kg and 20mg/kg) to challenge animals and measure alterations in auditory event-related potential profiles to elucidate temporal components that could serve as markers for psychiatric disorders. Human electro-encephalogram (EEG) studies indicate an impaired sensory filtering capacity of auditory paired-clicks in schizophrenia and/or bipolar disorders. These event-related potentials seen in human EEGs in response to paired-clicks have distinct temporal peaks and can be semi-replicated by intrahippocampal electrode recordings in rats and mice. We compare auditory event-related potentials in freely moving mice chronically implanted with 16 channel intrahippocampal electrodes in SLC10A4 ko mice and control littermates. We found that intrahippocampal signal polarity and temporal acuity was strongly influenced by electrode placement within the layers of the hippocampus, which may explain the lack of consistency in previous studies that used single recording electrodes. We found a 'typical' response, with smaller amplitude to the second click, only in certain distinct layers for both controls and ko's following saline injections. In general, SLC10A4-ko's displayed a more complex waveform with extra peaks compared to control littermates. Low dose ketamine mildly affected event-related potentials in control animals but strongly alter the waveform in ko mice. Preliminary results from pre-pulse inhibition show SLC10A4 ko's to startle even following a low sound level prepulse, whereas controls do not. Taken together, these results indicate that SLC10A4-ko animals, with alterations in modulatory neurotransmission systems, are more sensitive to low dose ketamine as well as to startle tests than controls. Our results also indicate a role of Slc10A4 in psychiatric disorders and suggest that this transporter may be a target for psychotherapy.

8. Electrical activity modulates neuronal type choice by adult rodent brain progenitors

Sequerra E.B., Moura D.M.S., and Costa M.R.

One of the main issues in developmental neurobiology is to describe how a population of homogenous neural stem cells can generate the diversity of neuronal types encountered in the central nervous system. It was already described that spinal cord and neural crest-derived young neurons display a

pattern of spontaneous electrical activity that is important to their neurotransmitter specification. In the developing spinal cord, ventral young neurons display a higher frequency of calcium spikes than the dorsal ones. And, if electrical activity is manipulated, young neurons choose inhibitory phenotypes when activity is high and excitatory when it is low. In this work, we investigated whether a similar mechanism could operate during neuronal differentiation in the adult brain. In the adult rodent brain, new neurons are continuously generated in a region that surrounds the lateral ventricles, called subventricular zone (SVZ). These young neurons then migrate to the olfactory bulbs, where they differentiate into different classes of interneurons. Interestingly, these neurons are generated in segregated regions within the SVZ: while the latero-ventral part generates Calbindin+ periglomerular neurons (PG) and deep granular neurons (GN), the dorsal part generates TH+ PG neurons and superficial GN, and the medial part generates Calretinin+ PG and GN. However, it remains unclear whether electrical activity could be differently regulated in these compartments and contributes to the generation of those distinct classes of neurons. To test this possibility, we injected kainate on the medial-anterior striatum, close to the migratory pathway of all these neuroblasts to the olfactory bulb. Newly generated neurons migrating at the moment of the injection were identified using transgenic mice whose immature neurons express the fluorescent protein GFP or dTomato. Our preliminary results show that a larger population of neuroblasts differentiates into TH+ PG neurons at the expense of Calbindin+ PG phenotype after kainate injection. These observations suggest that electrical activity could be regulating fate acquisition in post mitotic neurons, intermediate progenitors or both. Surprisingly, we also observed differences in the generation of interneurons between left and right OBs, suggesting a possible lateralization in the distribution of new neuronal populations being generated.

9. Spike sorting with mixture models

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Action potentials recorded extracellularly may have different shapes. Correctly classifying spike waveforms into their underlying neuronal sources is a main challenge in in vivo electrophysiology. This process, called spike sorting, typi-

cally involves first reducing the dimension of the waveforms into a set of relevant features (e.g., height and width), and, second, using the extracted features to cluster the spike shapes into non-overlapping groups believed to correspond to different neurons. In this study, we explored the efficiency of applying Mixture Models (MM) to these two steps. MM estimate the probability function of random multimodal variables as a weighted sum of probability distributions of unimodal variables. In our case, we reasoned that waveform features that differ among neurons should exhibit multimodal distributions, thus likely to be detected by MM. We used MM (1) to identify waveform features with the greatest separability (based on the analysis of the estimated unimodal distributions), and (2) to perform unsupervised clustering of waveforms. Realistic simulations of extracellular spikes were used to test sorting performance with different feature extraction methods. We employed either principal component analysis (PCA) or wavelet decomposition and selected features using two different criteria: the variance, as in standard PCA, or a separability measure extracted from the MM approach. We found that employing wavelet decomposition followed by the MM separability metric yields the best sorting. The results further show that this MM-based framework outperforms the widely used Klustakwik (Harris et al. 2000), and has similar performance to the newer EtoS algorithm (Takekawa et al. 2012). Finally, we provide a friendly graphical user interface in MATLAB implementing our algorithm, which also allows for manual adjustment of the automatic results.

10. SPATIAL COGNITION IN HIGH ABILITY CHILDREN: SEARCHING FOR A PHYSIOLOGICAL MARKER USING ELETROENCEFALOGRAPHY

Anomal, Renata; Brandão, Daniel; Hazin, I, and Pereira, Antônio

Individuals with high intellectual abilities (IHA) have significantly superior ability in some area of knowledge. Despite of the creative and academic potential of IHA subjects, conventional educational system does not have necessary resources, both material and human, to provide the academic challenges of children with this cognitive profile. This project aims to identify physiological markers to improve the identification of visuospatial high abilities using electroencephalogram in IHA children and adolescents. Volunteers selected for the study will be adolescents (N = 15), aged from 13 to 18, presenting visuospatial high abilitiies, from "Talento Metropole" program (Digital Metropolis Institute of the Federal University of Rio Grande do Norte). Here, we will present initial results of our pilot experiments. Two subjects (from 18 to 30 yeras) performed a visuospatial cognition task, such as mental rotation test

of Metzler- Shepard, during a eletroencephalographic recording (64 channel system). We have been analyzed the ERP (event-related potential) of these subjects. This work is expected to obtain functional tools for the identification of individuals with high visuospatial skills, and to develop new school tatics to improving visuospatial skills.

11. A respiration-coupled rhythm in the rat hippocampus independent of theta and cortical slow oscillations

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During slow-wave sleep and deep anesthesia, the rat hippocampus displays a slow oscillation (SO; <1.5 Hz) that follows up-and-down state transitions in the neocortex (Wolansky et al, 2006). There has been some debate as to whether this local field potential (LFP) rhythm is entrained by respiratory inputs (Lockmann and Belchior, 2014). For instance, in ketamine-anesthetized rats, Fontanini et al. (2003) reported oscillations of similar frequency in the piriform cortex of rats, which were locked to the respiratory cycle. However, Vizcko et al. (2014) convincingly demonstrated that hippocampal and neocortical SO recorded from rats during either urethane or ketamine anesthesia, as well as during natural slow-wave sleep, were not coupled to respiration. In the mouse hippocampus, however, Yanovsky et al. (2014) have recently described a slow oscillation entrained by nasal respiration under urethane anesthesia; this rhythm, dubbed hippocampal-respiration rhythm (HRR), was most prominent in the dentate gyrus, and could be distinguished from simultaneously occurring theta waves (Yanovsky et al., 2014). Therefore, whether slow frequency oscillations in the rat hippocampus couple to respiration or not is at issue. Here we have concomitantly recorded respiration activity along with hippocampal, neocortical and olfactory bulb (OB) LFPs in rats anesthetized with urethane. During the course of anesthesia, time-resolved spectral analyses showed that hippocampal LFPs transitioned between activity states characterized by the emergence of different oscillations. By jointly analyzing multisite LFP recordings and respiratory cycles, we could distinguish 3 types of oscillatory activity: SO, HRR and theta. Moreover, we could find time periods in which all three oscillations co-existed. While theta oscillations tended to be

faster (>3 Hz) than HRR and SO, these latter two were typically of similar peak frequency (~1 Hz). Our results therefore solve the apparent contradictions among previous studies (Fontanini et al., 2003; Vizcko et al., 2014; Yanovsky et al., 2014) by demonstrating that the rat hippocampus can produce two types of slow oscillations <1.5 Hz: one that is entrained to the respiration cycle (HRR), and another that phase-locks to neocortical up-and-down transitions (SO). In all, the results suggest caution when referring to "slow oscillations" in the rodent hippocampus, as there may be different oscillatory activities that can overlap in time of occurrence as well as in peak frequency. Since they synchronize with different brain circuits, however, we postulate that each activity pattern plays unique roles in information processing.

12. Effects of Ayahuasca on emotional regulation process

Fernanda Palhano Xavier de Fontes, Morgana Menezes Novaes and Dráulio Barros de Araújo

The psychedelic beverage Ayahuasca has been traditionally used by indigenous populations at Amazon region for religious and healing purposes. This tea combines the hallucinogenic tryptamine N,N- dimethyltryptamine (DMT) with reversible inhibitors of monoamine oxidase (iMAO) acting on 5-TH and sigma-1 receptors. The observed effects are diverse, including alterations in the sensory, perceptual, cognitive and emotional systems, as well as physical effects, as nausea and vomiting. Our group already showed that Ayahuasca selectively modulates frontal, temporal and occipital brain networks associated with intention, memory and vision, including the primary visual cortex (de Araujo et al., 2012). In addition, we found that the ingestion of Ayahuasca has an acute effect on the Default Mode Network (DMN), diminishing its activity and connectivity (Palhano-Fontes et al., 2015).

In this work, we have used functional Magnetic Resonance Imaging (fMRI) to investigate the effects of Ayahuasca on the emotional regulation. A group of 40 healthy volunteers performed an emotional regulation task, before and 24 hours after receiving a single dose of Ayahuasca or placebo. Based on the effects of Ayahuasca and subjects's self-reports of feelings of calm and tranquility after Ayahuasca ingestion, we hypothesize that the group taking Ayahuasca will present a modulation in brain regions known to be part of the emotional network, particularly an increase in prefrontal medial cortex and anterior

cingulate cortex activity and a decrease in amygdala and insular cortex activity, when compared with the placebo group.

13. Report of the OLM modulatory activity at mice CA1 using Arclight

Rafael Vitor Lima da Cruz and Richardson Naves Leão

Cortical circuits are generally comprised by a relatively homogeneous population of principal cells (PC) and diverse populations of interneurons that target specific PC compartments. In the hippocampus for example, there are axo-axonic interneurons targeting PC initial segment, basket cells that provide inhibition to PC perisomatic region and there are several interneuron populations that target dendritic domains of PCs. Oriens Lacunosum-Moleculare (OLM) cells are a population of dendritic- inhibiting interneurons with soma/dendrites located at the stratum oriens and axon terminals targetting distal PC dendrites at the stratum lacunosum moleculare of the hippocampus cornus ammonis. OLM cells gate the information flow between Entorhinal cortex and CA1. There has been also suggestions that OLM cells participate in the generation of theta oscillations. However, this latter role is elusive as electrophysiological recording techniques cannot sample for a large number of cells from a single population. We propose using genetically-encoded voltage indicators (GEVIs) to monitor OLM cell activity during theta oscillations. GEVI can target a genetically defined cell population and use fluorescence to register changes in membrane potential. This optical method, when associated to electrophysiology recordings, will allow the assessment of OLM activity in relation to the ongoing local field potential. We hope that our results will shed light in the role of specific interneuron populations in the generation of brain rhythms.

14. Visual Perception and Imagery Adaptation effects modulated by Ayahuasca

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Ayahuasca is a psychedelic tea used by Amerindians for centuries mainly for religious and medicinal purposes. Its composition contains the serotoninergic agonist, N,N-dimethyltryptamine (DMT), and monoamine oxidase inhibitors (MAOi), such as harmile and harmaline. Ayahuasca ingestion causes profound sensory, cognitive and affective changes. Among the ones related to the visual system, we can highlight the increase of brightness and sharpness of objects, vibrations of the visual field and vivid mental imagery, sometimes referred to as "seeings". Functional Magnetic Resonance Imaging (fMRI) studies have demonstrated a great superposition in the brain areas involved in visual mental imagery and visual perception. However, seeing something is quite different from imagining it, at least during ordinary awake states. On the other hand, a recent fMRI study conducted by our group showed that the signal of parts of the visual cortex, during periods of visual imagery with eyes closed, was similar in magnitude to periods of visual perception with eyes open, when individuals were under the effect of Ayahuasca. Therefore, the main goal of this study is to use electroencephalography (EEG) in order to detect neural differences between perceived and imagined stimuli before and after Ayahuasca intake, and better understand changes of neural processing of visual imagery and perception during the effects of Ayahuasca. Frequent Ayahuasca users performed a visual adaptation protocol before and after Ayahuasca or placebo ingestion. This protocol is capable of evaluating the neuronal activation of a test stimulus that is preceded by different adaptor stimuli. If the adaptor stimuli produce similar effects on the test stimulus, the underlying neural representation of both adaptor stimuli is similar. Hence, we used face and object adaptors, either perceived or imagined, and analyzed the N170/VPP complex, typically much larger for faces than for objects. Our results show that perceived and imagined adaptors affected the N170/VPP complex, although perceived adaptors suppressed the amplitude of the N170/VPP, whereas imagined adaptors enhanced it. Interestingly, the imagery adaptation effect decreased after Ayahuasca intake, so as to become more alike the perceptual adaption effect. This result corroborates with our previous fMRI findings where signal of the visual cortex during periods of visual imagery was statistically indistinguishable in magnitude to periods of visual perception during the effects of Ayahuasca. This study was approved by the University of Rio Grande do Norte Ethics Committee (N° CAAE: 21128113.7.0000.5537).

15. Joint characterization place and time cells in rat hippocampus

Rodrigo Pavão, Hindiael Aeraf Belchior, Alan Michel Bezerra Furtunato, Howard Eichenbaum, Adriano Bretanha Lopes Tort

Neurons from the hippocampal formation fire selectively to different aspects of the environment. In particular, "place cells" fire to specific positions of the space, and can also predict trajectories in spatial decision-making. More recently, different groups described the "time cells", neurons that fire during specific time intervals when position and behavior are constant, and that can also predict trajectories. However, it is not clear to which extent space, time and future trajectories are coded by the same cells. Our objective is to fill these gaps by jointly characterizing place and time cells in the rat hippocampus.

We trained rats to an alternation protocol on a figure-8-shaped maze with a 15-s delay period on a treadmill in the center section. We performed recordings with 24 tetrodes implanted in CA1 of the dorsal hippocampus of six rats during >10 experimental sessions each. This protocol permits to select time and place cells, and also their relation to future trajectories.

From the 1108 sorted neurons, 526 exhibited selective activity only to space ("strict place cell"), 15 only to time ("strict time cell"), and 213 to both space and time ("time-place cell"). Firing in the place field (and not in the time field) of 10 strict place cells and 3 time-place cells predicts trajectories. Strict place cells and time-place cells had similar peak firing rate and amount of place information. This is also valid to strict time cells and time-place cells coding time information. Interestingly, for strict time cells, most of the fields concentrated at the start and stop of the treadmill run, while for time-place cells the fields concentrated only at the start of the treadmill run.

Our results suggest that time-place cells and strict place cells code spatial information similarly, and, analogously, that time information coding is similar in time-place cells and strict time cells. We also found that only the time fields of the strict time cells code for the expected end of the treadmill run, suggesting that strict time cells but not time-place cells predict temporal contingencies of the environment. Moreover, we found that place fields but not time fields encode future trajectories, although this result should be interpreted with caution given the much lower number of time cells than place cells in our sample. In all, these findings support that hippocampal neurons encode a complex interaction between position and behavior structured in time.

16. Effects of Pranayama on Emotional Regulation

Morgana Menezes Novaes, Fernanda Palhano Xavier de Fontes, Danilo Forghieri Santaella e Dráulio Barros de Araújo

Yoga is a practice that originated in India, millennia ago, that can promote physical and mental well-being. Among the various yoga practices there is the Pranayama that are breathing exercises of yoga which can be an adjuvant treatment for the negative effects of stress and anxiety. Based on this, our study aims to evaluate changes promoted by the practice of Bhastrika Pranayama for anxiety and emotional regulation through functional magnetic resonance imaging (fMRI). For this, 30 young healthy adults were initially assessed by the IDATE scale to verify anxiety levels and by examination of functional Magnetic Resonance Imaging. After that, the subjects were randomly assigned into two groups (control and training). The first participated in control activities (crossword, puzzle, domino, game cards and checkers) and the second practiced Bhastrika Pranayama. The individuals were followed 3 times a week for 4 weeks. Each intervention lasted 30 minutes. Then all subjects were assessed again. The data are being analyzed and our hypotheses are that the trained group by Bhastrika Pranayama will have: decreased anxiety, less involvement of the insula and amygdala and greater engagement of brain structures that are involved in cognitive control, as the dorsolateral prefrontal cortex compared to control group.

17. COORDINATION OF RESPIRATORY, TACTILE AND VOCAL RHYTHMS DURING RAT EXPLORATORY BEHAVIOR

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When actively exploring the environment, rats exhibit several rhythmic sensorimotor behaviors with frequencies in the theta range (5-10 Hz). These include sniffing (active fast respiration), whisking (movement of the facial vibrissae), and ultrasonic vocalizations. Synchronizations between each pair of these behaviors have been observed. Vibrissae protraction-retraction can be associated with the exhalation-inhalation phase of breathing. Constriction of the larynx for vocal production, in turn, is locked to the exhalation phase of sniffing. Accordingly, vocalizations were observed to synchronize with the protraction phase of whisking. These and other observations point to an interaction of rhythm and pattern generators in the brainstem controlling the coordination of oral and facial movements. To better understand the hierarchies in these circuits we are simultaneously recording these motor rhythms during free social exploration, which is known to involve all three of them. For this purpose, eight electrodes are inserted surgically to acquire bilateral EMG signals from muscles controlling the protraction and retraction of the whiskers

and a cannula is implanted through the nasal bone to record the respiratory cycle. After one week recovery and habituation, two rats (one implanted and one naive) are placed across a gap where they can explore each other. These interactions are filmed with a high speed camera (250 Hz) to capture whisker movements and ultrasonic vocalizations are recorded from and overhanging microphone.. We are using frequency and phase analysis to validate the recorded signals. Both sniffing and whisking occur in bouts at theta frequencies. Furthermore, we observed the expected anti-phase relationship between the EMGs from muscles controlling whisker protraction and retraction as well as their tight synchrony with the sniffing cycle. We are conducting further recording and analysis to fully caracterize the interplay between these three sensorimotor rhythms involved in exploration and social interactions. This research is part of a major project which intends to study global synchronization amongst sensory, motor and brain rhythms.

18. ROLE OF RAT ULTRASONIC VOCALIZATIONS IN SOCIAL LOCOMOTIVE BEHAVIOR

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Rat ultrasonic vocalizations of fundamental frequency in the 30-90 kHz range known as '50 kHz' USVs are related to appetitive contexts, however, how these vocalizations participate in their social behavior is not completely understood. Previous data from our lab shows that the USVs are tightly synchronized with locomotion of the emitting rat. Here, we aim to study the role of USVs in social locomotive behavior of Long Evans rats, by devocalizing one of the rats and evaluating the locomotive behavior of the other. Using this approach we can assess if rats can use the USVs to track each other in space. Each animal will participate in two spatial interaction records: one with a control animal of the opposite, and the other with a devocalized animal of the opposed sex. Metrics of spatial correlation, such as average distance between the two animals will be analyzed for each record. Two-way ANOVA contrast will be calculated: 1) male condition (control or devocalized); 2) female condition (control or devocalized). Greater distance records with devocalized animals would prove decrease in spatial interaction. Lower spatial correlations in sessions including devocalized animals would support that ultrasonic vocalizations play a role in the coordination of movement between rats.

19. Do fast retinal oscillations play a role in vision? A study in the awake cat

Giovanne Rosso and Sergio Neuenschwander

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Recent experimental and theoretical work link neuronal oscillations to various cognitive processes, such as perceptual binding, attention and memory. In the retina, responses are often associated with fast high-amplitude oscillations, which are transmitted to the lateral geniculate and to the cortex. A series of experiments in the cat have shown that fast retinal oscillations may be important for binding of contiguous contours or surfaces and encoding of stimulus size.

A limitation in these studies, however, was that most of them were made in the anesthetized and paralyzed cat. Only a few early studies have been made in the non-anesthetized but still paralyzed cat and visual stimuli were often limited to whole-field flashes, far from natural vision conditions. Moreover, recently we made the surprising observation that fast retinal oscillations depend strongly on halothane (and isoflurane) anesthesia. It became imperative to verify whether oscillatory activity is also present in the awake cat, under naturalistic conditions, such as during free-viewing of a visual scene. This is the main goal of the present study.

Simultaneous multiple-electrode recordings were made from the lateral geniculate nucleus (LGN) and the retina of anesthetized cats (N= 3) and from the LGN of an awake cat (N= 1). Comparisons were made for responses to natural movies and flashed stationary light stimuli. To test specifically the role of retinal oscillations in encoding stimulus size we designed a protocol made of a light circle of varying size along the trial. Our present results based on single-cells extend our previous findings in the anesthetized cat, which were restricted to an autocorrelation analysis of LGN multi-unit responses. Both ON- and OFF-responses to varying size stimuli show that coherent oscillations appear only after the stimulus attained a minimum size of about 5° (depending on the contrast level), suggesting that oscillations in the retina are rather limited in

encoding subtle changes in stimulus size. Recordings obtained directly from eye showed that oscillations in the retina, as in the LGN, are highly correlated with the concentrations level of halothane. Notably, in a series of sessions we were able to record LGN responses in an awake cat, which was subsequently anesthetized with halothane, keeping the same recording site. Oscillations were completely absent in the awake condition and appeared strong as usual during the halothane anesthesia.

Overall these results weaken substantially the notion that fast retinal oscillations are meaningful for vision.

20. Morphological alterations in immature and mature hippocampal neurons following kainic acid injection

Moura DMS, Queiroz CM, and Costa MR

In the mammal adult brain, newly generated neurons are constantly added to the dentate gyrus in the hippocampus. Many conditions are known to affect the proliferation, survival and integration of these newborn granule neurons, but the cellular and molecular mechanisms involved in their regulation are not completely understood. Temporal lobe epilepsy is the most common form of epilepsy and its focus is located in the medial temporal lobe structures as hippocampus and amygdala. Among other characteristics, it is associated with a variety of granular cells abnormalities such as ectopic location, abnormal basal dendrites and mossy fiber sprouting. In this work, we set to investigate whether old and newly generated neurons contribute to the cellular alterations observed in the dentate gyrus after epileptical induction. Using transgenic mice, we induced the expression of the green fluorescent protein (GFP) in cohorts of cells generated before (mature cells) or after (immature cells) intrahippocampal kainic acid injection and analyzed the fates of cells 7 weeks later. During the kainate administration the animal was recorded with electrodes in bilateral hippocampus under the isoflurane anesthesia and on its following awakening phase and onset of SE. Behavior analysis was observed in all animals to characterize seizures intensity. The histological analysis showed granular cell dispersion and CA1/CA3 cell death as previously described in the model. In the mature cells group, GFP labeled cells in the ipsilateral side of the kainate injection showed bigger cell size, and morphological alterations. These effects were not observed in the contralateral side. However, in the immature cells group, we observed a dramatic reduction in the number of neurons in

the ipsilateral side. Interestingly, the number of GFP+ cells were increased, but most of the cells adopted a glial morphology and expressed the astrocytic protein GFAP. Finally, the number of GFP+ neurons was increased in the contralateral side, suggesting that epileptical activity and direct kainic acid injection may affect hippocampal neurogenesis differently. Together, our preliminary data suggest that both mature and immature neurons are affected by kainic acid injection. Moreover, they also indicate that a population of progenitor cells within the dentate gyrus can switch their fate and generate astrocytes at the expense of neurons after kainate injection.

21. Optothermal effects in the brain triggered by optogenetic stimulation

Helton Maia Peixoto, Rossana Cruz e Richardson N Leao

Optogenetics is revolutionizing neuroscience but an often-neglected effect of light stimulation of the brain is the generation of heat. Biological tissues are optically inhomogeneous and with refractive indexes higher than that of air. Hence, part of the radiation is reflected at the tissue-air interface, while the remaining part is transmitted to the tissue. Absorbed light can be converted to heat, radiated in the form of fluorescence and/or consumed in photobiochemical reactions. Depending on the light energy, a large amount of tissue can be damaged. The time-dependent heat generated in a tissue via interaction with pulsed or intensity-modulated optical radiation can be described by the bio-heat equation, where changes in tissue temperature over time can be expressed for a certain distance through the tissue. Bio-heat models that also include blood perfusion show that this effect modifies the heat transfer within the tissue. Furthermore, laser radiation increases the stored energy from its initial state and, as a result, it diffuses the heat away from the radiated area in proportion to the temperature gradients generated within the tissue. The main objective of this work is to investigate the optothermal effect of a visible light laser beam incident upon the brain of rodent animals. For all the simulations performed we assumed that the reflection and absorption coefficients were constant, the laser beam had a Gaussian profile and was distributed in a two-dimensional plane. We simulated heat transfer using a simplified bio-heat model including blood perfusion and metabolism in COMSOL Multiphysics commercial software. Laser heating was simulated considering two stationary conditions: continuous mode and pulsed mode. We used parameters for heat transferred obtained from rodent tissue. We have found that at relatively mild intensity (40mW) laser radiation considerably increases the temperature at

the surrounding tissue. We show that the optothermal effect depends on several factors such as the optical and thermal properties of the neural tissue and of the surrounding media, as well as the laser beam size and shape. These results indicate that laser intensities used in optogenetics experiments may affect neurons not by exciting opsins but by generating heat.

22. Characterization of proliferative behavior by neural progenitors in the postnatal SVZ

Cunha-Pereira C., Sequerra E.B. and Costa M.R.

The subventricular zone (SVZ) is a stem cell niche that surrounds the lateral ventricles throughout the whole life of vertebrates. SVZ progenitors originate new neurons that migrate to and differentiate in the ipsilateral olfactory bulb. The neuroblasts that leave the ventricle area form a migratory route called rostral migratory stream (RMS), which also contains glial progenitors and neural stem cells. In this study, we are analyzing the in vitro behavior of SVZ and RMS cells isolated from postnatal mice. Our aim is to describe patterns of cell division, differentiation and death of different types of progenitors/post mitotic cells in these regions. To do that, we use time-lapse video microscopy to image the behavior of cultured cells up to 7 days. These videos are then used to create lineage trees classified by their cellular phenotype - neuronal, glial or mixed, and by their division speed - when the first division occurs before 36 hours they were classified as fast-dividing and after as slow-dividing. We also quantify the trees undergoing apoptosis of 50% or more of the clone. Up to now, we analyzed 188 clones: 63% of the trees were neuronal, 32% glial and 5% mixed. Virtually, all neuronal clones were derived from rapid cycling progenitors while glial cell clones derived mostly (88%) from slow-dividing progenitors. The mixed clones were in its majority, 78%, derived from fast clones. 30% of fast clones are apoptotic while only 4% of slow clones are. The preliminary data presented here suggest live imaging can prospectively identify neuronal and glial progenitors by their proliferative behavior. Thus, this system may be useful to isolate individual neuronal and glial progenitors and compare their gene-expression pattern.

23. Frequency following response in musicians with absolute pitch ability

Raphael Bender Leite, Sérgio Mota Rolim, Claudio Marcos Queiroz

Pitch is one of the primary auditory sensations that allows animals to sort sounds on a scale ranging from low to high. Some people are very accurate to identify and nominate the pitch of a particular sound without any external

reference, an ability named Absolute Pitch (AP). Previous studies on AP have focused on the structural and functional plasticity in cortical structures and it has been suggested that AP possessors have a larger left planum temporale than non-AP. This structural change is positively correlated with the subject's performance in a pitch-naming task. Additionally, in an interval-judgment task, no activity within the right inferior frontal cortex was observed in AP as compared to non-AP subjects. Taken together, these data suggest that AP ability relies on the activation of various cortical structures; however, no study has evaluated the role of subcortical structures in this ability. Herein, we investigate whether auditory brainstem processing is different in musicians with and without AP ability and our working hypothesis states that auditory brainstem responses of AP musicians have better-defined spectral representation of the f0 and its partials. For that, we performed a computer-based pitch discrimination test in self-defined population of AP musicians - musicians with similar age and music education were included as control group. Reaction time and mean absolute deviation (MAD), i.e., the average distance (in semitones, ST) of subject's response and the assigned pitch, were computed. Individuals were classified as AP or non-AP by comparing their MAD with a random distribution (confidence of 95%). Frequency Following Response (FFR) was used to quantify sustained phase-locking activity in the brainstem to a complex flute sound (f0: G2 - 98Hz). Phase-locking values (PLV) for selected frequencies (f0 and f1) were used to compare the responses between AP and non-AP groups. As expected, MAD was smaller in AP than in non-AP musicians (mean±std: 0.2±0.3 and 2.4±0.7 ST, respectively) and AP musicians react faster than non-AP (3.6±1.8 and 6.4±2.9 s, respectively). Concerning the FFR, AP group showed higher PLV at f0 in comparison to non-AP (0.085±0.048 and 0.050±0.028, respectively). Interestingly, PLV for f1 was smaller in AP group in comparison with non-AP (0.021±0.006 and 0.028±0.007, respectively). Finally, we have observed a negative correlation between the PLV at f0 and the reaction time for all volunteers (R^2 = 0.21). These results revealed that tonal representation at low levels of the auditory pathway is altered in AP musicians. Enhanced PLV of the f0 observed in AP suggests increased phase consistency along trials of the brainstem neural responses in musicians with the ability. Further studies are necessary to unveil the role of consistent brainstem activation in shaping cortical networks responsible for pitch perception and/or labeling.

24. Differential firing of neurons recorded in the hippocampus and related cortical structures during naturally sleepwake cycle

Bruna del Vechio Koike, Kelly Soares Farias, Francesca Billwiller, Pierre-Herve Luppi, Claudio Marcos Queiroz

A number of studies reported that unit activity in the cortex and hippocampus is different during waking and slow wave (NREM) sleep (SWS). Only a few studies studied the firing rate of neurons during paradoxical (REM) sleep (PS). It seems nevertheless crucial to examine such activity in particular when considering that it has been shown that memory consolidation involving the hippocampus and related limbic structures is impaired following PS deprivation. To this aim, we implanted in the hippocampus and the retrosplenial cortex of rats, customized multi-electrodes arrays movable by a nano-drive. Recordings were made in freely moving rats (n=16) to evaluate the modifications of the firing rates across all vigilance states. Our preliminary results show that multi-unit (n=76) discharges rates of neurons of the retrosplenial cortex decrease by 50% during SWS and in contrast increase of 30% during PS in comparison to waking periods. The activity of putative inhibitory interneurons (PIN, short peak-to-peak duration; n=37) was more markedly modulated by sleep than that of putative excitatory neurons (PEN, long peak-to-peak duration; n=57). Interestingly, activity of PIN neurons in the hippocampus was phase-locked with theta oscillations during both waking and PS. Nevertheless, we found out that the increased theta frequency occurring during PS compared to waking was not sufficient to explain the increased firing rate observed during this state. Our results corroborate and expand previous observations that neuronal activity in limbic structures is modified by sleep. We are now performing automatic PS deprivation to determine whether the firing rate of neurons is modified during the PS episodes following specific PS deprivation. Future experiments are also planned to unveil whether exposing animals to cognitive tasks with different emotional valences can modify state-dependent modification of firing rate.

25. Perspectives of cell therapy in temporal lobe epilepsy

Kelly Soares Farias, Malek Chouchane, Daniela Maria de Sousa, Marcos Romualdo Costa, Claudio Marcos Queiroz

Epilepsy is a high incident neurological disorder affecting 1% of the human population. The pathology can be classified in different types, and temporal lobe epilepsy (TLE) is the most common one and also the most refractory to antiepileptic drugs. Clinical and experimental data showed that TLE is characterized by extensive cell death (hippocampal sclerosis), synaptic reorganization (mossy fiber sprouting) and reactive gliosis in limbic structures, which leads to the unbalance between excitation and inhibition. Several studies suggest that cell transplantation, such as embryonic stem cells and mesenchymal cells, can be used as a promising therapeutic alternative to ameliorate the symptoms of TLE. However, the cell fate and the physiological mechanisms by which transplanted cells modify pathological network is still unknown. Here,

we hypothesized that astrocytes play a major role in the reestablishment of brain homeostasis in TLE, mainly through potassium and glutamate-related metabolism. To test this hypothesis, we transplanted green fluorescent protein-positive (GFP+) astrocytes in the hippocampus of pilocarpine-induced status epilepticus mice and recorded epileptiform activity in multiple brain areas. Up to now, we have identified 18 spontaneous seizures (13 and 5, in saline-treated [N=4] and astrocyte-treated [N=2] groups, respectively) in 621 hours of electrophysiological recordings (103±17 hours/animal). Preliminary analysis suggest that transplantation of astrocytes decreased the behavioral expression of seizures activity (Racine' scale: 5.7±0.5 vs 3.4±1.8, in saline- and astrocytes-treated mice, P<0.01) but no differences were found in seizure duration (44±2 vs 43±6 s) or seizure frequency (0.02±0.01 vs 0.03±0.02 seizures/hour, for saline-treated and astrocyte-treated groups, respectively). We are currently investigating whether this observations correlate with the integration of astrocytes in the pathological hippocampus. Although preliminary, our results put forward the potential therapeutical role of astrocyte-based cell therapy in TLE. Further experiments are needed to corroborate these initial observations.

26. Transcriptome analysis of the frontal cortex of a rodent model of autism

Araujo-Sousa C., Kroll, J.E., Sousa J.A.B.M., Linhares M.G., Freitas C.R., Souza S.J., Romcy-Pereira R.N.

Autism (ASD) is a heterogeneous group of brain disorders characterized by behavior and sensory impairments related to multiple etiologies. Neuroanatomical and neurophysiological abnormalities in the cortex have been recently described in autistic patients and are postulated to underlie some of the behavior symptoms. In rodents, prenatal exposure to valproic acid (VPA) reproduces autism-like phenotypes in the offspring with several physiological dysfunctions observed. However, it is still poorly understood how prenatal VPA affects post-natal gene expression in the cortex and the reported abnormal circuit functioning. Here, we present preliminary data on gene expression analysis of the frontal cortex of rats treated with VPA intra-uterus. VPA animals were generated by treating pregnant rats with 500mg/Kg i.p. VPA on E12.5 and 3 different litters were used in the experiments. We used (1) cDNA sequencing (RNAseq) to analyze the transcriptome of the frontal cortex of VPA and control animals at P15; and (2) qRT-PCR to evaluate the expression of c-MET proto-oncogene tyrosine kinase receptor (MET) and brain derived neurotrophic factor (BDNF) in the medial prefrontal cortex (mPFC) and

dorsal hippocampus (DH) of P1 and P15 rats – both genes have already been implicated in ASD and code for proteins involved in neuronal migration and circuitry maturation. Briefly, control and experimental RNA samples of the cortex (pools from 5-6 animals; RIN 8.6-8.7) were used to prepare bar-coded cDNA fragments for sequencing. From a total of 16,032 genes analyzed, 179 and 15 genes were differentially expressed in the frontal cortex of VPA rats at $p<0.05$ and $p<0.01$, respectively. Differential expression varied from -0.7 to +1.63 fold-change. Ontology analysis showed that differentially expressed genes (DEG) are involved in synaptogenesis, neuronal excitability, myelin organization, hormone signaling, and transcriptional control. Furthermore, our RNAseq results show that (1) DEG are significant enriched in genes associated to synaptic and myelin functions (Gene ontology); (2) 1.278 genes have specific splicing junctions in VPA animals; (3) several VPA-specific splice variants code for non-functional synaptic and myelin proteins. In addition, a preliminary expression analysis of the cortical development and neurotrophic genes (MET and BDNF) at age P1, using qRT-PCR, did not reveal any significant expression differences between VPA and control animals in the mPFC and DH. Experiments been conducted to validate and further evaluate the molecular cascades affected in the cortex following VPA exposure.

27. Post-natal sensory motor development in the VPA animal model of autism

Sierra, O. and Romcy-Pereira, R.N.

Sensory and motor disturbances are widely present in the majority of individuals with of autism spectrum disorder (ASD). Recent theories of ASD propose that these – often over-looked – sensorimotor disturbances are critical to understand their more characteristic symptoms, such as difficulties in communication and social interaction. From a developmental perspective, it is widely accepted that the adult mind emerges from early sensorimotor operations. Hence, the possibility remains that ASD mind develops as an alternative way to perceive and interact with the world. Analyses of retrospective videos of ASD children show that abnormalities in motor development are present before the ASD diagnosis. To test this hypothesis is necessary to carry out longitudinal studies, which in humans represent a big challenge. Alternatively, one can use a valid animal model of ASD to monitor the sensorimotor development. In the present study, we injected pregnant rats with valproic acid (VPA) to induce an autistic-like disorder in their offspring. From PD1-PD15, we monitored their sensory and motor development of VPA and control rats using an assay of behavioral tests. After PD15, we assessed social interaction

in pairs of siblings until PD21 in both groups. The aim of this study is to correlate sensorimotor development with social interaction capturing the developmental dynamics through a longitudinal design.

28. Inheritance of social behavior and atypical cortical inhibitory organization in the VPA model of autism

Brandão JA, Araujo-Sousa C, Soares AM, Fernandes BP, Costa MR and Romcy-Pereira RN

Cellular and synaptic dysfunctions in cortical neural circuits are commonly implicated in the expression of autistic-like behaviors, including sensory and social interaction deficits. Although its high degree of genetic inheritability, the action of environmental factors during pregnancy, such as prenatal exposure to valproic acid, significantly contributes to the expression of autistic phenotypes. However, little is known about the inheritance of autistic traits in environmentally derived models of autism. Here, we evaluated the behavior performance and interneuron distribution in the prefrontal cortex of a group of rats prenatally exposed to VPA (F1 generation) and their offspring (F2 generation). Our results show that VPA treatment in the F1 induced clear autistic-like behavioral abnormalities such as hyperlocomotion, stereotyped behavior and reduced social interaction with an unfamiliar mate. Cellular quantification revealed a significant decrease in the number of parvalbumin-positive interneurons in the anterior cingulate cortex and in the prelimbic division of the mPFC, suggesting a functional excitatory/inhibitory imbalance. Interestingly, the F2 generation also showed social deficits in the three-chamber test and altered number of parvalbumin-positive interneurons in the mPFC, but similar levels of locomotion and stereotypies as controls. No behavioral or cytoarchitectural differences were observed between males and females in our study. Together, our results indicate that non-adaptive social behaviors and atypical inhibitory cellular organization in the prefrontal cortex of rats prenatally exposed to VPA can be inherited by their offspring.

29. LEARNING OF NOVEL VOCAL USAGE BY THE COMMON MARMOSET

Thamiris Botelho Ribeiro Conceção, Hjalmar Turesson and Sidarta T. Ribeiro

Vocal learning is a key property of human speech. Comparative research has revealed that such learning is also present in songbirds, parrots, dolphins, and seals. In contrast, there is no reliable evidence for vocal learning in non-human primates. Indeed, there is much evidence that vocal repertoires among non-human primates are small, fixed and genetically determined; in particular, the learning of novel vocalizations has not been observed. Even learning a novel usage for pre-existent vocalizations is limited and appears to occur solely during development.

The capacity for learning new usages of vocalizations has been addressed by operant conditioning experiments. These studies have mostly been conducted with macaque monkeys rewarded for producing a particular vocalization, the coo call. Increases in call rate and sound intensity have been taken as evidence of learning. However, criticism has been made that these results are confounded and likely do not demonstrate learning of new vocal usage. Without training, macaques spontaneously produce coo calls in feeding related contexts, and the motivational state plays an important role in triggering these calls. It is possible that as subjects learn that they will receive food rewards in the experimental context, arousal increases, and as a result, the rate and intensity of food-related calls increase. Thus, relying on food to reinforce food-related calls is problematic and often produces ambiguous results.

Here we present results from experiments with the common marmoset showing operant conditioning of the phee call, a social contact call that is not related to food. Phee calls are multi-pulse calls, consisting of 1-7 pulses, most commonly 2-3. Using a vocal conditioning paradigm the subjects were rewarded, first for producing any phee call, and then, for producing phee calls with a set number of pulses. Daily training sessions lasted 15 min. After around 30 sessions the subjects showed significant changes in the phee rate. Once the call rate was stable, the second part was initiated where the subject were rewarded first, only for calls with 1-2 pulses, and then for calls with more than 2 pulses. Since the phee call is not food related, and reward was given for both fewer and more pulses the arousal related confounds affecting earlier studies should be avoided.

30. Orientation- and direction-selective visual responses in V1 of agouti

Dardo Nahuel Ferreiro, Sergio A. Conde Ocazionez, Luã Carlos de Souza, Fred Wolf and Kerstin E. Schmidt

Since the discovery of a columnar functional organization in visual cortex over 50 years ago, neural responses in early visual areas have been widely described for several mammals such as cats, monkeys, squirrels, rats and mice. For all rodents studied so far, there is no evidence of a columnar organization in V1, as compared to carnivore and primate cortices. Nevertheless, orientation selective neurons have been found in all of the mentioned species. This opens up the question whether the connectivity underlying the emergence of such cortical response properties follows a different blueprint in animals with salt and pepper as compared to those with columnar organization. Since the "salt-and-pepper" mammals investigated so far had smaller visual cortices than "columnar" mammals, theoretical studies also suggest that cortical organization could be determined by brain size rather than by phylogenetic differences.

In order to shed light onto these questions, we set out to compare the cortical functional architecture between carnivores and big rodents of similar visual cortex size. As a first step, our efforts were directed toward characterizing visual response properties in V1 of a big-brained rodent such as agouti (Dasyprocta aguti) as compared to the well-described properties of cats (Felis catus domestica). To this end, we performed electrophysiological recordings of multi-unit activity (MUA) and local field potentials (LFP) from up to 48 channels in both anesthetized cat and agouti visual cortex. Contrast reversing checkerboards stimuli were presented to study visual evoked potential (VEP) responses to several different temporal and spatial frequencies (0.04 – 2.56 cyc/deg at 2, 4 or 8 Hz), and drifting sinusoidal grating stimuli were used in order to study spiking responses to orientation and direction of movement.

Preliminary data from two agoutis indicate VEP amplitudes in the LFPs with cutoff spatial frequencies lower than for cat area 17 (~2.56 cyc/deg) but comparable to cat area 18 (0.32-0.64 cyc/deg). As for cats tested with the same protocol, amplitudes were higher for a temporal frequency of 2Hz than for 4Hz and 8Hz.

Analysis of MUA (spikes) to different grating stimuli revealed so far 46 orientation and 10 direction selective multi-units among the 90 visually responsive recording sites, especially at low to moderate spatial frequencies (<= 0.32 cyc/deg) at 4Hz. Currently, spike sorting is applied to identify the exact ratio of selective versus nonselective responsive units.

In conclusion, we were able to visually evoked LFP and MUA from anesthetized agouti V1, and observed selective responses to both orientation and direction of the stimuli as described for small rodents. In the future, optical imaging of intrinsic signals and spike sorting will help to describe the functional organization of V1 in detail, i.e. by determining the extent of clustering of neurons with similar responses into functional modules.

31. STUDYING PSYCHOPATHOLOGIES AND EVOLUTION OF MIND THROUGH LITERATURE GRAPHS

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To better understand patients with psychopathologies, Mota et al. (2012, 2014) analyzed graph attributes of interviews obtained from schizophrenic, bipolar disorder I and non-psychotic control individuals. This study points the possibility of a quantitative psychiatric assessment, showing remarkable differences between schizophrenic and bipolar subjects in at least 10 graph attributes. Under classifier analysis, the attributes presented high sensibility and specificity. Also working on text analysis, Diuk et al (2012) applied the LSA (Latent Semantic Analysis) tool, to estimate the occurrence of the "introspection" concept inside a certain time period of human development. This work, based on Julian Jaynes ideas, found an increase in the levels of introspection concept in the transition from oral cultural tradition to the written tradition, during the so-called Axial Age (800 BC – 200 BC). The study used ancient scriptures from the judeo-christian and greco-roman cultures, and recent texts. The result had corroborated the Jayne's theory about the recent origin of human consciousness, through the breakdown of a mental functioning mode known as "bicameral". It is characterized by less introspection and recurrent intervention of "voices", which resembles psychotic behavior. Inspired on these ideas, our study seeks to apply graph analysis to literature, in two lines: 1) analysis of texts from writers previously diagnosed with psychosis, in modern literature; 2) analysis of Axial Age texts. For this, we obtained the digital version of books on the Internet public domain (in websites like Project Gutenberg, MIT Classics and Sacred Texts) and converted them to .txt version. We used the Speechgraphs software that calculates several attributes with graph theory basis. We also are constructing classifiers by Weka software. On our first study line, we found significant differences between schizophrenic and bipolar subjects, on the Edges attribute (12.65; $p<0.05$, N=34), and LSC attribute (10.39, $p<0.05$, N=34) on a 30 word-count window, which are consistent with the previous studies by Mota et al (2012, 2014). On the other strand, we studied 4 traditions of the ancient scriptures: Judeo-Christian (JC, N = 76), Greco-Roman (GR, N = 41), Zoroastrian (ZO, N = 18) and Hindu (HI, N = 38), on a 100 word-count window analysis. This assay showed a very interesting pattern of increase of connectivity, illustrated by Largest strongest connected component - LSC attribute (JC $R^2 = 0.37206$, HI $R^2 = 0.39883$, ZO $R^2 =$

0.47667, p<0.001,). In addition, decrease of recurrence on the speech, like in Repeated Edges – RE (JC R^2 = 0.27136, ZO R^2 = 0.45522, p<0.001) and Parallel Edges – PE attribute (JC R^2 = 0.27005, ZO R^2 = 0.47006, p<0.001). These results corroborate the hypothesis about patterns already found in psychotic patients, and also help to find new evidence about the hypothetical bicameral mind behavior. Next steps include use random graph technique to generate stronger controls.

32. Behavioral and electrophysiological study of memory corticalization

Almeida-Filho DG; Golbert DCF; Souza AC; Sousa BF; Gonzalez MC; Cammarota M; Ribeiro S

Memory is one of the most important cognitive functions, for it shapes our behavior, and thus defines who we are. Memory consolidation involves an early phase during which memories are labile, and a late phase during which memories are resistant to disruption. Since Milner's pioneering investigation of patient H.M., who suffered bilateral hippocampal lesion, we know that structures from the medial temporal lobe, especially the hippocampus, are involved in the acquisition of new memories. This type of lesion causes an anterograde interruption of declarative memory acquisition as well as a retrograde memory deficit, with recent memories being more impaired than remote ones. This initial observation was supported by plenty of subsequent evidence of progressive disengagement of the hippocampus in the process of memory recall, with a corresponding engagement of neocortical structures. Since 2002, our group has produced molecular and electrophysiological data that point to the role of sleep in memory corticalization. To address this issue, we are currently investigating the electrophysiological correlates of memory corticalization in rats exposed to contextual fear conditioning, a well-known quantitative task for long-term recall, in which the fear memory correlate is the freezing behavior. We tested rats at different time-windows after training (2, 14, 28 or 42 days; n=8 per group). We also performed a memory recovery protocol in which the animals, after being tested, were exposed to 3 consecutive extinction sessions across 3 days, and then received a mild shock at the end of the final extinction session, followed by a retest after a 48h time window. Our preliminary results show that there were no differences across the 2-days, 14-days and 28-days groups in the percentage of freezing behavior during the first 5 minutes of the test session. Animals in the 14-days group showed decreased extinction, and strong recovery compared to all groups. The 42-days group had the strongest memory recall of all groups, but also exhibited the most prominent extinction after the 3 extinction sessions. We

are now performing electrophysiological recordings in hippocampal and neocortical sites, before, during and after contextual fear conditioning and testing, to directly characterize memory corticalization. In the near future we intend to disturb memory corticalization by interfering with sleep, and blocking protein synthesis, neuronal activity or specific gene expression related to memory consolidation.

33. Optogenetic stimulation of medial septum in anesthetized and freely behaving rats

Souza, A.; Santos,V.; Maia,H.; Targino,J.H.; Queiroz,C.; Leão,K., Leão, R.; Ribeiro, S.

Theta rhythm consists of an electrophysiological hippocampal oscillation present in mammalian species (4-12 Hz with variations across species). This oscillation is present during active waking and is also prevalent in local field potentials (LFP) during rapid eye movement sleep (REM sleep). Several studies have shown that theta rhythm is important in cognitive tasks and that the medial septum is a key region for its occurrence. The septum sends cholinergic, GABAergic and glutamatergic projections to the hippocampus, which in turn projects axons to the septum. Besides the septum, other regions are involved in regulating theta rhythm, forming a complex network of interactions among brain areas that result in theta rhythm. Optogenetics is a recently developed method that has been widely used in various research areas. It allows us to manipulate the electrical activity of neurons through light stimulation. One of the existing techniques consists in using a viral vector to induce the neuronal expression of ion channels associated with the light-sensitive molecule rhodopsin (e.g. ChR2). Once infected, the neurons become sensitive to light of a particular wavelength. The present research aimed to perform luminous stimulation of the brain in anesthetized and freely behaving animals using chronically implanted electrodes and optical fibers in animals infected with a viral vector for ChR2 expression. Surgical viral injections were performed in the medial septum; histological results confirmed the expression of ChR2 by way of the presence of the eYFP reporter protein in the septum and also in hippocampal processes. Moreover, we performed acute experiments with luminous stimulation of the medial septum and LFP recordings of the septum and hippocampus of anesthetized animals. Action potentials were recorded in the septum. In these experiments we observed a significant increase in the firing rates of septal neurons during luminous stimulation (n = 300 trials). Furthermore, we found an early light-evoked response in the hippo-

campal LFP. Chronic experiments with luminous stimulation of the medial septum and hippocampus in freely behaving animals were also performed in combination with LFP recordings. We found that the luminous stimulation of the septum is able to induce theta rhythm in the hippocampus. Following up these preliminary results, we are now working to increase the size of our experimental groups. We are also preparing necessary controls using empty viral vectors, or the light-gated chloride pump halorhodopsin, in both acute and chronic experiments. We also intend to investigate the selective stimulation of specific neuronal populations in transgenic mice. Our final goal is to investigate the effect of septal stimulation during the object recognition task, as well as during subsequent episodes of slow wave and REM sleep.

34. Gain of transcription factor binding sites in humans occurs more frequently in genes with higher expression breadth

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The gain of transcription factor binding sites (TFBS) is believed to represent one of the major causes of biological innovation. Here we used strategies based on comparative genomics to identify 27,285 TFBS specific to the human genome (TFBS-HS), when compared to chimpanzee and gorilla genomes. These TFBS-HS are associated to 1,449 genes. A comparison of the expression pattern of these genes and the corresponding ortologs in chimpanzee and gorilla identified genes differentially expressed in human tissues. Genes associated with TFBS-HS were enriched in gene ontologies categories related to transcriptional regulation, signaling, differentiation/development and nervous system. Furthermore, genes associated with TFBS-HS presents a higher expression breadth when compared to genes in general. This biased distribution is due to a preferential gain of TFBS in genes with higher expression breadth rather than a shift in the expression pattern after the gain of TFBS.

Keywords: TFBS, Genes, regulation, expression, evolution.

35. Direct lineage-reprogramming of Müller glial cells into neurons

Ana Raquel Melo de Farias, Roberta Pereira de Melo, Marcos Romualdo Costa

In recent years a number of techniques have been described with a view to the possibility of replacement of lost cells in neurodegenerative diseases or acute central nervous system injuries. One of such techniques is the lineage-reprogramming of somatic cells into neurons, either directly or indirectly. In the present study, we evaluated the ability to reprogram the Müller glia cells into neurons through the use of two pro-neural transcription factors. The electrical transfection method using the Nucleofector was used to assess whether the transcription factors Neurogenina 2 (NEUROG2) and Achaete-scute homolog 1 (ASCL11 or Mash1) would be sufficient to induce Müller glia cells to differentiate into neurons. To assess neuronal phenotypes after nucleofection, we evaluate the expression of neuron-specific proteins, morphology and neural activity by immunocytochemistry, time-lapse video-microscopy and calcium imaging, respectively. We also observed that the addition of growth factors EGF (Epidermal Growth Factor) and FGF2 (Fibroblast Growth Factor Basic) during glia cell expansion increased the reprogramming efficiency. In conclusion, our work show that Müller glia cells can be reprogrammed into cells with neuronal morphology and activity, in particular, cultured in the presence of growth factors.

36. Autophagy regulates axonal degeneration after traumatic lesion in the central nervous system.

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Axonal degeneration is one of the initial steps in many neurological disorders and has been associated with increased autophagic activity. Although there is increasing data on the regulation of autophagy proteins in the neuronal soma after traumatic lesion, their characterization in the axon is scarce. Furthermore, the role of autophagy in axonal degeneration remains still unclear. Here, we examined the regulation of autophagy during axonal degeneration in a rat model of spinal cord injury (SCI) and the role of autophagy in axonal degeneration using in vitro and in vivo models. We showed by immunohistochemical evaluation of autophagy-related proteins and electron microscopy that autophagy is upregulated during axonal degeneration after SCI. Downregulation of autophagy by overexpression of a dominant negative form of ULK1 (ULK1.DN), a key autophagy protein, decreases axonal degeneration in cortical neurons in vitro. Overexpression of ULK1.DN in optic nerve axons attenu-

ates axonal degeneration within the first hours after optic nerve lesion. Moreover, inhibition of autophagy by overexpression of ULK1.DN protects rubrospinal tract axons from degeneration 7 days after SCI. In conclusion, we show that autophagy is increased early and for a sustained period in degenerating axons after SCI. Moreover, inhibition of autophagy stabilizes the axons after traumatic lesion in the central nervous system (CNS), indicating that autophagy might be an important executive step involved in axonal degeneration. Therefore, autophagy may represent a promising target for future therapeutic interventions in the treatment of axonal degeneration in traumatic CNS disorders.

37. Polymorphism in the oxytocin receptor gene and fibromyalgia: inspecting new perspectives

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Fibromyalgia (FM) is a disorder of unknown etiology characterized by chronic widespread pain and is commonly accompanied by a constellation of additional symptoms such as fatigue, stiffness, affective disturbance, anxiety and depression. Current evidences show potential genetic factors playing a role in the development and symptom severity of FM. An important candidate to evaluate the effects of genetic factor on clinical outcomes in patients with FM is an oxytocin receptor polymorphism (OXTR). Variations in the gene coding for the OXTR, in which a single nucleotide polymorphism (SNP) rs53576 (which involves a guanine (G) to adenine (A) substitution) has been associate with diminished prosocial behaviors and mood states. The aim of the present study was to correlate OXTR (rs53576) with symptoms in women with FM. We are currently collecting data, and partial results from thirty-nine subjects who met the 2010 American College of Rheumatology (ACR) criteria for FM are under genetic SNP characterization. SNP for 6930G>A (rs53576) is being analyzed by polymerase chain reaction (PRC). Association between the presence of polymorphism and symptoms of FM such as mood state and pain test are in progress.

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