

# **FIOCRUZ INSERM MEETING**

**9<sup>TH</sup> | 10<sup>TH</sup> DECEMBER 2015**

CELEBRATING 25 YEARS OF PARTNERSHIP

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**FIOCRUZ CAMPUS • RIO DE JANEIRO • BRAZIL**

New orientations of the cooperation:  
focus on neuroscience

**ABSTRACT BOOK**

# Program

## Fiocruz-Inserm Meeting: *Celebrating 25 Years of Cooperation*

9 - 10 December 2015

Focus on Neuroscience

### Wednesday 9 December

#### 9h45 – 10h | Opening Session

**10h – 10h15:** Historical Background on 25 years of Fiocruz-Inserm Cooperation (Wilson Savino)

**10h15 – 10h30:** French landscape of research in neurosciences (Etienne Hirsch)

**10h30 – 10h45:** New orientations of the cooperation: presentation of the future Fiocruz Institute of Neuroscience (Cecilia Hedin-Pereira)

#### 10h45 – 12h40 | Session 1 - Cellular & Molecular Neuroscience

10h45 – 12h25

Chairpersons: Wilson Savino, Gillian Butler-Browne

**Fiona Francis:** Cortical development and pathology

**Cecilia Hedin-Pereira:** Reelin as a regulator of Gabaergic neuron proliferation and migration in developing cerebral cortex

**Rosa Cossart:** A developmental backbone supporting adult hippocampal function?

**Carmem Gottfried:** Neurobiology of Autism Spectrum Disorder

12h25 – 12h40: Discussion

#### 12h40 – 15h15 | Poster session & lunch

#### 15h15 – 17h10 Session 2 | Physiology of Sensory & Motor Systems

Chairpersons: Cecilia Hedin-Pereira, Denis Pelisson

15h15 – 16h55

**Clément Lena:** Reciprocal connections between the cerebellum and other brain structures: a story of loops.

**Gillian Butler-Browne:** Toxic exosomes secreted by ALS muscle cells: role in ALS pathogenesis.

**Claudia Vargas:** Plasticity in the sensorimotor system after upper limb injury in humans.

**Sergio Neuenschwander:** Gamma or no gamma? This is the question.

16h55 -17h10: Discussion

**17h10 – 17h25 | Coffee break and selection of the best poster by the speakers**

**17h25 – 18h55 | Session 3 | Cognitive neuroscience and emotion**

Chairpersons: Clément Lena

17h25 – 18h40

**Denis Pelisson:** Neural Plasticity in Perception and Action: insights from eye movements.

**Sidarta Ribeiro:** Mechanisms of memory processing during sleep.

**Eliane Volchan:** Body and brain changes associated with invasion of peripersonal space in post-traumatic stress disorder

18h40 – 18h55 - Questions

**19h30 | Dinner**

**Thursday 10 December**

**8h30 – 10h | Session 4 – Computational neuroscience**

Chairperson: Sidarta Ribeiro

8h30- 9h45

**Viktor Jirsa:** Translational neuroscience: from large-scale brain modeling to patient prediction.

**Etienne Koechlin:** A computational approach to prefrontal executive function and human adaptive behavior.

**Adriano Tort:** Asymmetry of temporal and rate codes for space by hippocampal place cells.

9h45 – 10h: Discussion

**10h00 – 10h15 | Coffee Break**

**10h15 – 12h10 | Session 5 – Neuroinflammation and neurodegenerative Diseases**

Chairpersons: Etienne Hirsch, Carmem Gottfried

10h15 – 11h55

**Wilson Savino:** *In vitro* model of astrocyte-t cell interactions in HTLV-1 induced neural damage.

**Roland Liblau:** Neurons and T cells: Understanding this interaction for inflammatory neurological diseases

**Guillaume Dorothee:** Regulatory T cells in Alzheimer's disease: role in pathophysiology and therapeutic potential.

**Hugo Castro Faria Neto:** Cognitive impairment in cerebral malaria: mechanisms and new therapeutical approaches.

11h55 – 12h10: Discussion

**12h10 – 12h40 | Closing Ceremony & Award of the best poster**

## Abstracts by Invited Speakers

### **Cortical development and pathology**

**Fiona Francis**, Institut du Fer à Moulin, Inserm UMR-S 839, Université Pierre et Marie Curie, Paris

Cortical malformations are frequent causes of drug-resistant epilepsy and intellectual disability. More subtle abnormalities lead to certain epilepsies and neuropsychiatric disorders. These defects can arise through abnormal proliferation/function of progenitor cells, neuronal migration and/or connectivity. We use human genetics and mouse models as points of entry to query normal cortical development and physiopathology. For example, DCX, a microtubule-associated protein involved in neuronal migration is mutated in heterotopia, associated with mis-positioned neurons in the white matter, and severe brain gyral abnormalities such as lissencephaly. Dcx knockout mice are an excellent model to study aberrant connectivity and neuronal hyperexcitability related to migration defects during development, leading to epilepsy and behavioral abnormalities. We also recently found that Eml1 is mutated in severe heterotopia in mouse and man, and is involved in microtubule dynamics in cortical progenitor cells. In this case, perturbed progenitors are the most likely primary cause of the malformation, representing a novel mechanism for these disorders. Elucidating mechanisms regulating progenitors can also shed light on other cortical disorders, as well as neuron expansion during evolution. Using molecular and cellular biology, and studying mutant mice and patient mutations is revealing novel insights into the causes and consequences of cortical malformations and neuropsychiatric disorders, as well as normal mechanisms of brain development.

### **Reelin as a regulator of GABAergic neuron proliferation and migration in developing cerebral cortex**

**Cecilia Hedin-Pereira**, Program on Neuroscience, Oswaldo Cruz Foundation, Rio de Janeiro

The reelin gene was found to be involved in developmental disorders such as lissencephaly and autism spectrum disorder as well as psychiatric disorders as schizophrenia and bipolar disease. It codes for an extracellular matrix glycoprotein which during early development is synthesized in the marginal zone (MZ) at the cerebral cortex surface. Reeler mutants present a disruption of the typical inside-out layering of cerebral cortex and the mouse mutant displays a dysfunctional gait as well as other deficits. It has been proposed that reelin is a stop signal for glutamatergic neurons that migrate radially from the VZ. We have shown that the marginal zone, site of reelin production is in fact a novel neurogenic and gliogenic niche in the brain. We will discuss evidence that points reelin as a regulator of neurogenesis and migration of GABAergic neurons in cerebral cortex. Disruption in reelin signaling may cause an imbalance in GABA/Glutamate activity in the brain leading to several neural disorders such as epilepsy and schizophrenia.

**A developmental backbone supporting adult hippocampal function?"**

**Rosa Cossart**, Inmed, U 901, Marseille, France

Most adult cortical dynamics are dominated by a minority of highly active neurons distributed within a silent neuronal mass. If cortical spikes are sparse, spiking of single distinct neurons can impact on network dynamics and drive an animal's behavior. It is thus essential to understand whether this active and powerful minority is predetermined and if true to uncover the rules by which it is set during development. In this talk, I will present data supporting the possibility that birthdate is a critical determinant of neuronal network function into adulthood. More specifically, we reason that neurons that are born the earliest are primed to participate into adult network dynamics. This hypothesis is considerably fed by our past work aiming at understanding how cortical networks function and assemble during development. Hence, we have shown that an early birthdate: (1) specifies the specialization of GABA neurons with a hub function, that orchestrate perinatal network dynamics in the mouse hippocampus (Bonifazi et al. Science 2009) and develop into long-range projecting GABA neurons into adulthood (Picardo et al. Neuron 2011); (2) delineates a subtype of CA3 glutamate neuron with a "pacemaker" function in the absence of fast GABAergic transmission (Marissal et al. Nature Comm. 2012). I will first briefly present this set of published data.

To test the hypothesis that early born cells are primed to be recruited in the active minority of neurons in the adult hippocampus, we needed to probe microcircuit function *in vivo*, where the extensive and long-range connectivity of these cells is preserved. I will show how we have translated from the *in vitro* to the *in vivo* situation, our multidisciplinary method to investigate structure-dynamics relationship in cortical networks. Using this approach, I will present data showing that, in the absence of external landmarks, distance is encoded within the adult hippocampus in recurrent and self-circumscribed sequences of neuronal activation (Villette and Malvache et al., Neuron, 2015). These sequences integrate traveled distance and link sequential body movements to an internal distance template. These distance sequences are an excellent theoretical and experimental model to probe the involvement of early born cells in sparse dynamics because they repeatedly involve small subsets of neurons and because they almost represent default hippocampal dynamics in the absence of any external drive, which is probably more likely to be influenced by developmental programs. In the future, we will examine the recruitment of early born neurons in this sparse hippocampal network dynamics pattern.

**Neurobiology of Autism Spectrum Disorder**

**Carmem Gottfried**, Translational Research Group in Autism Spectrum Disorder, Clinical Hospital of Porto Alegre, Research Group in Neuroglial Plasticity, Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS.

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by difficulties in social communication and restricted repertoire of activities and interests. Moreover, it involves a complex interplay of both genetic and environmental risk factors, such as the prenatal exposure to the antiepileptic valproic acid (VPA). *In utero* exposure to VPA has been employed as a reliable model to improve the understanding of the neurobiology and behavioral impairments observed in human patients with ASD, including sociability, social preference and stereotypic behavior. In the past decade, studies indicated that

immunological factors may be involved in triggering ASD. In this context, important questions related to the etiology of ASD and neurodevelopmental alterations can be followed up through the use of animal models. Our group has been actively involved in the study of ASD, developing important preventive-reversive strategies. These studies contribute to the understanding of developmental alterations implicated in neural and behavioral impairments, highlighting the relationship between immune system and ASD triggering.

**Gamma or no gamma? This is the question.**

**Sergio Neuenschwander**, Brain Institute, Federal University of Rio Grande do Norte, Natal

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 weaken the notion that gamma is necessary for visual processing and raise questions on its role in neuronal communication.

**Reciprocal connections between the cerebellum and other brain structures: a story of loops.**

**Clément Lena**, Institut de Biologie de l'Ecole Normale Supérieure, Inserm U1024, Paris.

The cerebellum is a major brain device, which groups more than 50% of the brain neurons. While its circuitry and its computational properties have been extensively explored, understanding its function requires to analyze its interactions with the rest of the brain. We will present our recent work demonstrating that the cerebellum participates to brain computations by forming closed loops with other brain structures, and we will discuss the importance of these loops for the cerebellar operations.

**Toxic exosomes secreted by ALS muscle cells: role in ALS pathogenesis**

**Gillian Butler-Browne**, Institut de Myologie, UMRS 974, Université Pierre et Marie Curie, U974 - Inserm / UMR7215 - CNRS-AIM, Paris

**Background/Aim:** The potential involvement of exosome trafficking is implicated in ALS by the aggregation of lysosomally-directed proteins in the cytosol of patient cells (both sporadic and familial cases), and by mutations in genes involved in autophagy and multivesicular biogenesis pathways in familial cases. Exosomes are small vesicles shown to export functional proteins, mRNA and miRNA from different cell types including muscle cells. Several studies suggest an involvement of skeletal muscle in ALS. Our purpose was to determine whether exosome secretion is altered in ALS muscle cells, and could alter the intercellular communication between muscle and nerves.

**Methods:** To explore disruption of vesicle trafficking and secretion in ALS muscle, immunostains, western blots, and RTq-PCR were performed on samples from sporadic ALS patients and aged-matched healthy subjects (n=10/group). Transcriptomic analysis was carried out for secretome prediction and gene set enrichment. The effects of secreted exosomes from ALS and control muscle cells were tested by adding them to the culture medium of healthy myotubes and motoneurons.

**Results:** We observed a consistently striking and previously unnoticed accumulation of exosomal vesicles in patient myotubes, and confirmed this *in vivo* on muscle biopsies from sporadic ALS patients. *In silico* secretome prediction suggested a mechanistic basis of this, showing a significant enrichment in both endosomal and lysosomal compartments, indicating disruption of exosome genesis and secretion, which was consistent with gene set enrichment analysis showing disrupted vesicle trafficking. This phenomenon occurs independently of muscle denervation as oversecretion of exosomes was not observed from satellite cells obtained from denervated muscles (SMA-IV, SBMA or denervated human or murin muscles).

When secreted ALS muscle exosomes were added to the culture medium of healthy muscle cells, they induced myotubes atrophy (with smaller myonuclear domain), cellular stress (with an increased amount of blebs) and cell death (loss of nuclei associated with an increased in DNA damage).

**Conclusion:** We hypothesize that altered exosome secretion influences the intercellular communication between the muscle and its environment, including motoneurons. This phenomenon could be a key element in the disease progression.



Work financed by INSERM and Direction Générale de l'Organisation des Soins (DGOS), and by the Association Française contre les myopathies (AFM).

Le Gall Laura<sup>1</sup>, Duddy William<sup>1</sup>, Sylvain Roquevière<sup>2</sup>, Martinat Cécile<sup>2</sup>, Mariot Virginie<sup>1</sup>, Ouandaogo Gisèle<sup>1</sup>, Robelin Laura<sup>3</sup>, Ratti Francesca<sup>4</sup>, Mejat Alexandre<sup>4</sup>, Matthew Thorley<sup>1</sup>, Butler Browne Gillian<sup>1</sup>, Loeffler Jean Philippe<sup>3</sup>, Mouly Vincent<sup>1</sup>, Gonzales De Aguilar Jose-Luis<sup>3</sup>, Pradat Pierre François<sup>5</sup>, Duguez Stéphanie<sup>1</sup>

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### **Plasticity in the sensorimotor system after upper limb injury in humans**

**Claudia D. Vargas**, Deolindo Couto Institute of Neurology and Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, RJ.

Peripheral nerve injury has long been shown to produce reorganization in the brain. Knowledge of the forces that rule this phenomenon is however still scarce. In this talk I will present a set of results aiming at sensorimotor system reorganization in patients with peripheral nerve injury. Unveiling the mechanisms of plasticity shall contribute to enhance our predictive capacity about lesion outcomes and may also have a deep impact in treatment and rehabilitation.

**Financial support:** CNPq, CAPES, FAPERJ, FAPESP, FINEP.

### **Neural Plasticity in Perception and Action: insights from eye movements**

**Denis Pelisson**, Centre de recherche en neurosciences de Lyon, U1028

IMPACT team at the Lyon Neuroscience Research Center has a long-standing expertise in deciphering the neural processes which combine sensory information about our body and peri-personal space into cognitive representations and adequate goal-directed movements of various body parts (eye, head, upper limb, trunk). Recent research has specifically investigated the brain plasticity mechanisms which regulate perceptual and motor (upper-limb) performance over time, both while acquiring new skills and compensating for physiological or pathological changes of the neuro-muscular system. This research has also been successful in providing new solutions for the rehabilitation of sensory, motor or cognitive deficits consecutive to brain lesions (see review in Jacquin-Courtois et al 2013). In this talk, I will present recent human behavioral and physiological (fMRI, TMS) data illustrating how saccadic eye movements can provide insights into brain plasticity mechanisms regulating motor accuracy –and hence perceptual performance– over different time scales (minutes to days). These studies largely amend the classic view that such 'saccadic adaptation' mechanisms mainly involve cerebellar-dependent pure motor processes (Gerardin et al 2012; Panouillères et al, 2013, 2014; Lévy-Bencheton et al, 2013, 2014) and provide the first demonstration of their tight coupling with attentional processes (Habchi et al 2015, Gerardin et al 2015). The clinical perspectives of these findings will be discussed.

**Mechanisms of memory processing during sleep**

**Sidarta Ribeiro**, Brain Institute, Federal University of Rio Grande do Norte, Natal, Brazil.

Abstract: Sleep has been implicated with learning and memory in humans and several animal models. The mnemonic role of sleep has been hypothesized to stem from a non-Hebbian rescaling of synaptic weights (synaptic homeostasis hypothesis). Alternatively, sleep has been proposed to trigger a combination of non-Hebbian rescaling and Hebbian upscaling of synaptic weights in complementary circuits (synaptic embossing). This talk will review experimental and computational evidence regarding the synaptic embossing theory, including changes in neuronal activity, kinase phosphorylation and immediate-early gene expression. The role of sleep-dependent plasticity in memory corticalization and restructuring will be discussed.

**Body and brain changes associated with invasion of peripersonal space in post-traumatic stress disorder**

**Eliane Volchan**, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro.

Urban violence is an important and pervasive cause of human suffering. Violent events, such as armed robbery, are very frequent in certain cities, and these episodes increase the risk of post-traumatic stress disorder (PTSD). Assaultive trauma is characterized by forceful invasion of the peripersonal space, which is defined as a margin of safety around the body. The invasion of this margin of safety is often experienced as a threat to an individual's psychological or biological integrity and may lead to that individual's intense discomfort and anxiety. Neurobiology studies have suggested that the premotor cortex incorporates both a representation of peripersonal space and certain aspects of defensive motor functions.

Life-threatening urban violence events are a major trigger for defensive motor reactions in humans. Investigating structural brain alteration in PTSD victims of urban violence we found that, compared with traumatized controls, PTSD patients presented significantly reduced gray matter volume in the ventral premotor cortex. This may be associated with a disruption in the dynamical modulation of the safe space around the body in those patients. Tonic immobility, characterized by profound motor inhibition, is elicited under inescapable threat in many species.

To fully support the existence of tonic immobility in humans, participants exposed to violent crime listened to the script of their autobiographical trauma while biological correlates were recorded. Reports of script-induced immobility were associated with restricted area of body sway and were correlated with accelerated heart rate and diminished heart rate variability, implying that tonic immobility is preserved in humans as an involuntary defensive strategy and is a peritraumatic reaction for which cumulative clinical evidence had linked to the severity of the most disruptive sequelae of trauma exposure - PTSD.

**Translational neuroscience: from large-scale brain modeling to patient prediction**

**Viktor Jirsa**, Inserm U-1106, Institut de Neurosciences des Systèmes, Marseille

Seizures can occur spontaneously and in a recurrent manner, which defines epilepsy; or they can be induced in a normal brain under a variety of conditions in most neuronal networks and species from flies to humans. Such universality raises the possibility that invariant properties exist that characterize seizures under different physiological and pathological conditions. A new idea is to take advantage of invariances known from the theory of nonlinear dynamic systems and build canonical models that can be merged with realistic structural information obtained from non-invasive neuroimaging. This hybrid approach of phenomenological and biologically realistic modeling allows us to create personalized brain network models today.

Starting from first principles of the theory of slow-fast systems in nonlinear dynamics, we conceptualize seizure dynamics mathematically and establish a taxonomy of seizures based on seizure onset and offset bifurcations. We demonstrate that only five state variables linked by integral-differential equations are sufficient to describe the onset, time course and offset of ictal-like discharges as well as their recurrence. These state variables define the model system called the Epileptor, where two state variables are responsible for generating rapid discharges (fast time scale), two for spike and wave events (intermediate time scale) and one permittivity variable (slow time scale). The permittivity variable captures effects evolving on slow timescales, including extracellular ionic concentrations and energy metabolism, with time delays of up to seconds as observed clinically. We propose that normal and ictal activities coexist: a separatrix acts as a barrier (or seizure threshold) between these states. Seizure onset is reached upon the collision of normal brain trajectories with the separatrix. We show theoretically and experimentally how a system can be pushed toward seizure under a wide variety of conditions. Within our experimental model, the onset and offset of ictal-like discharges are well-defined mathematical events: a saddle-node and homoclinic bifurcation, respectively. These bifurcations necessitate a baseline shift at onset and a logarithmic scaling of interspike intervals at offset. These predictions were not only confirmed in our *in vitro* experiments, but also for focal seizures recorded in different syndromes, brain regions and species (humans and zebrafish).

Extending this generic approach rooted in nonlinear dynamics towards human brain networks, we reconstruct personalized connectivity matrices of human epileptic patients using Diffusion Tensor weighted Imaging (DTI). Subsets of brain regions generating seizures in patients with refractory partial epilepsy are referred to as the epileptogenic zone (EZ). During a seizure, paroxysmal activity is not restricted to the EZ, but may recruit other brain regions and propagate activity through large brain networks, which comprise brain regions that are not necessarily epileptogenic. The identification of the EZ is crucial for candidates for neurosurgery and requires unambiguous criteria that evaluate the degree of epileptogenicity of brain regions. Stability analyses of propagating waves provide a set of indices quantifying the degree of epileptogenicity and predict conditions, under which seizures propagate to nonepileptogenic brain regions, explaining the responses to intracerebral electric stimulation in epileptogenic and nonepileptogenic areas.

We demonstrate the predictive value of our seizure propagation model by validating it against empirical patient data. In conjunction, our results provide guidance in the presurgical evaluation of epileptogenicity based on electrographic signatures in intracerebral electroencephalograms.

**A computational approach to prefrontal executive function and human adaptive behavior**

**Etienne Koechlin**, Computational models of cognitive development (Unité Inserm 960, Laboratoire de Neurosciences Cognitives à l'Ecole Normale Supérieure), Paris.

I will present recent works from our lab combining computational modeling, experimental psychology and fMRI describing how the prefrontal cortex subserves decision-making and adaptive behavior. I will show how the ventromedial, dorsomedial, lateral and polar prefrontal regions along with the striatum forms an unified system combining planning, inferential and creative processes for efficient behavior in uncertain, variable and open-ended environments.

**Asymmetry of temporal and rate codes for space by hippocampal place cells**

**Adriano B.L. Tort**, Brain Institute, Federal University of Rio Grande do Norte, RN, Brazil

The rodent hippocampus plays a role in spatial memory and navigation. Some hippocampal neurons, called place cells, increase the firing rate when the animal is at a specific location of the environment, known as the 'place field' of the cell. As the animal crosses place fields, place cells form spike sequences coordinated by the hippocampal theta rhythm – a prominent field potential oscillation at 5-12 Hz – by emitting action potentials at varying phases of the theta cycle, a phenomenon known as 'phase precession'. Place field and phase precession are considered canonical examples of rate and temporal codes, respectively, in which the firing rate of the neuron and the exact spike timing relative to the theta cycle provide information about space. Whether temporal and rate coding are governed by independent or related mechanisms is currently unknown and the subject of wide debate. In this talk I will present novel results showing that the coupling of place cells to theta phase starts before major changes in firing rate (i.e., before entering the place field) and also precedes phase precession. In contrast, spike-phase coupling rapidly ceases as the animal leaves the place field. Thus, spike-phase coupling metrics reveal strong temporal coding asymmetry around the place field center.

Interestingly, the results further show that place cells are not coupled to theta phase at positions distant from the place field center, implying that the dynamics of place cell activity can be separated into three stages: phase coupling, phase precession and phase decoupling. In all, the results suggest independent mechanisms of temporal and rate coding by hippocampal place cells.

***In vitro* model of astrocyte-t cell interactions in HTLV-1 induced neural damage****Eduardo Samo-Gudo<sup>1,2</sup> and Wilson Savino<sup>1</sup>**<sup>1</sup>Laboratory on Thymus Research, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil;<sup>2</sup>Mozambican National Institute of Health, Maputo, Mozambique

Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic and slowly progressive neurodegenerative disease of the central nervous system (CNS). To date, the precise mechanisms by which HTLV-1 promotes these lesions remain poorly understood. Available data indicate that progression to HAM/TSP is characterized by the presence of an enhanced chronic immune response, accompanied by massive infiltration of mononuclear cells into the CNS, hypersecretion of pro-inflammatory cytokines and chemokines, as well as spontaneous proliferation T cells.

Although the lesions in the CNS have been primarily attributed to infiltrating lymphocytes, growing evidence points that glial cells, particularly astrocytes, also play role in this process.

We recently used a model of co-culture between human HTLV-1-infected and non-infected T-cell lines and astrocyte cell lines to mimic the *in vivo* T cell-astrocyte interactions.

We first observed that HTLV-1+ T cells adhere strongly to cultured astrocytes, and that co-cultures of HTLV-1 infected and astrocyte cell lines resulted in rapid syncytium formation, accompanied by severe morphological alterations and increased apoptotic cell death of astrocytes.

Additionally, cultures of astrocyte cell lines in the presence of supernatants harvested from HTLV-1-infected T cell cultures resulted in significant increase in the mRNA of CCL2, CXCL1, CXCL2, CXCL3, CXCL10, IL-13, IL-8, NFKB1, TLR4, TNF, MMP8 and VCAM1, as compared with the values obtained when we applied supernatants of non-infected T-cell lines. Lastly, soluble factors secreted by cultured astrocytes primed through interaction with infected T-cells further enhanced migratory responses, as compared to the effect seen when supernatants from the cultured astrocytes were primed with non-infected T cells.

Collectively, these data indicate that HTLV-1 infected T lymphocytes strongly interact with cultured astrocytes, leading to astrocyte damage together with modulation in the expression of a variety of cell-migration genes. Reactive astrocytes, in turn, may participate in the further recruitment of HTLV-1-infected T cells into central nervous system (CNS), thus amplifying and prolonging the immune damage of CNS.

**Financial support:** Fiocruz, CNPq, Capes, Faperj (Brazil), FOCEM (Mercosur)

**Neurons and T cells: understanding this interaction for inflammatory neurological diseases****Roland Liblau**, Centre de Physiopathologie de Toulouse Purpan, UPS-INSERM (UMR 1043), CNRS (UMR 5282), INRA (USC 1360), Toulouse

Central nervous system (CNS) inflammation occurs in a large number of neurological diseases. The type and magnitude of CNS inflammation, as well as the T-cell contribution, vary depending on the disease. Different animal models of neurological diseases have shown that T cells play an important role in CNS inflammation. Furthermore, recent studies of human neurological disorders have indicated a significant role for T cells in disease

pathology. Nevertheless, how individual T-cell subsets affect neuronal survival, damage and/or loss remains largely unclear. The processes by which T cells mediate deleterious effects within the CNS, with emphasis on the direct interaction between T cells and neurons, as occurs in multiple sclerosis, paraneoplastic cerebellar degeneration, and viral encephalitis begin to be understood. Therapeutic approaches targeting T cells and their mediators as treatment for neurological diseases may be envisioned.

### **Regulatory T cells in Alzheimer's disease: role in pathophysiology and therapeutic potential**

**Guillaume Dorotheé**, Inserm UMRS 938, Hôpital Saint-Antoine, Paris

Accumulating data emphasize the implication of cellular adaptive immunity in the pathophysiology of Alzheimer's disease, but the nature and role of implicated T cell populations remain poorly defined. Our studies in murine models suggest that regulatory T cells (Treg) critically control T cell responses to A $\beta$ , slow cognitive decline and modulate the microglial response to amyloid deposition. Our data also highlight the therapeutic potential of Treg-based immunomodulatory approaches.

### **Cognitive impairment in cerebral malaria: mechanisms and new therapeutical approaches**

**Hugo Castro Faria Neto**, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro

Infectious diseases are the most important cause of mortality in infants and young children, causing more than 13 million deaths per year, one in every two deaths in resource-poor countries. Among them malaria affects mainly patients with little or no background immunity - that is, children growing up in endemic areas, or travellers or migrants who come from areas without malaria, but are exposed to malaria later in life. Malaria is one of the major causes of premature death in the world population. In spite of the efforts to understand the pathophysiologic mechanisms of the disease, the morbid consequences of malaria are often overlooked in the face of high mortality rates, particularly in resource-poor countries. Neurologic impairment in severe cases of malaria may have a devastating impact on the development of the child and cause long-lasting cerebral impairment, and as a consequence, have profound societal influences in endemic areas and countries. Cognitive impairment is both frequent and persistent. Speech and language impairment – mainly in vocabulary, receptive and expressive speech, word finding, and phonology – is common in children with memory or attention deficits after cerebral malaria. The mechanisms that lead to cognitive dysfunction are not clear but neuroinflammation seems to play a fundamental role, suggesting the involvement of endothelial cells, microglia, leukocytes and platelets recruited from the circulation. Activation of endothelial cells and microglia may be a bridge connecting neuroinflammation and neuron toxicity that will deflagrate cognitive dysfunction. Astrocytes, the major glial cells in the brain may also be critically involved through their regulation of the blood-brain barrier integrity and neuronal functions (metabolism and synaptic strength regulation). Potential therapeutic interventions to this condition have not been investigated and are urgently needed. HMG-CoA reductase inhibitors (statins) and anti-oxidant drugs have been associated with pleiotropic anti-inflammatory effects. We investigated the pathophysiology of neurodysfunction and effect of treatment with statins and anti-oxidant drugs during experimental cerebral malaria and its possible therapeutic effect on cognitive impairment. *Plasmodium berghei* ANKA (PbA) infected mice displayed clear signs of CM and were treated with lovastatin or a combination of deferoxamine plus N-acetylcysteine in

addition to chloroquine. Intravital examination of pial vessels of infected animals showed a decrease in functional capillary density and an increase in the rolling and adhesion of leukocytes to the endothelium that were reversed by treatments. Brain levels of MDA, IL-1, TNF- $\alpha$ , MCP-1 and IL-12 levels were also increased in PbA-infected mice, but reduced after the treatments. Fifteen days post-infection cognitive dysfunction was detected by different memory and cognition tests in animals rescued from CM by chloroquine treatment, but cognitive dysfunction was absent in animals treated with either strategy. We collected evidence suggesting that excitotoxicity is the mechanism behind cognitive decline after cerebral malaria. In summary, neuroinflammation and excitotoxicity seems to be key players in cognitive decline after cerebral malaria. Therapeutic strategies targeting those events may have neuro-protective effects in malaria other severe infectious syndromes preventing cognitive impairment survivors.

## Abstracts presented as posters

### 01. Hsp90 complex proteins influence SVZ cell migration in a non-classical manner

Coelho, DM<sup>1</sup>; Miyakoshi, LM<sup>1</sup>; Nogaroli, L<sup>1</sup>; Lima FRS<sup>2</sup> Zanata, SM<sup>3</sup>, Martins, VR<sup>4</sup>; Hedin-Pereira, C<sup>1,5</sup>

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Chaperones and co-chaperones are proteins well-known to act in correct protein folding, protein degradation and other essential roles for protein maintenance. Although they have been found in extracellular space these proteins and their functions are usually restricted to intracellular environment. In this work we are trying to demonstrate how essential these proteins are in the migration of neuroblasts from the subventricular zone (SVZ). One specific goal is to investigate if they might influence the migratory behavior of these cells and whether Hsp90 complex proteins can play chaperone functions outside the cell environment.

In order to study the possible role of the proteins from this chaperone complex in neuroblast migration, we used a subventricular zone (SVZ) explant assay and visualized how migration is affected after treatment with antibodies and inhibitors through migratory halo and time-lapse videomicroscopy analysis. Moreover, conditioned medium analysis and cell surface (biotinylation) of dissociated SVZ cultures were performed to ensure extracellular presence of these proteins. Additionally, we also emphasize their extracellular presence using a live-labeling protocol.

Our results show that migratory halo perimeter and average speed were both decreased when Hsp90 and Sti1 were immunoblocked or when inhibitors to Hsp90 and Hsp70 were applied to the cultures. Furthermore we found presence of Sti1 and Hsp70 in the fraction of cell surface biotinylated proteins and also Sti1 in conditioned-medium. These results were complemented with live-immunolabelling data showing that Hsp90 binds to the cell surface.

Both immunoblocking and inhibitor data suggest that the chaperone Hsp90 and its co-chaperones have a functional role in SVZ cell migration. The presence of elements of the complex in the extracellular biotinylated fraction and in the conditioned medium support the hypothesis that these proteins might influence migratory behavior from the extracellular surface.

### 02. The absence of CCR2 changes synaptic structure in hippocampus and impaired the memory consolidation

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**Background:** The CCR2 is the receptor for CCL2/MCP-1. The CCR2 is expressed in monocytes, endothelial cells, activated neutrophils, microglial cells and astrocytes. Recently, some studies show the constitutive expression of CCR2 and CCL2/MCP-1 in places of the central nervous system, such as in hippocampus. How the hippocampus is an important place involved with the memory consolidation, we analyze the role of CCR2 in learning process and in memory consolidation.

**Methods:** CCR2 deficient mice and wild type were submitted to passive avoidance test and to water maze test, to examine the aversive memory and the contextual memory. The motor response was evaluated by open field test, and the reflex by pre-pulse inhibitory response. The animals were submitted to passive avoidance test of multiple trials, to analyze the learning curve and the retrieval of memory. The next step was to verify the expression of important proteins involved with memory consolidation by western blotting, such as the phosphorylated ERK and mature BDNF. And the last step was to verify if the CCR2 deficiency affect the synapsis in hippocampus, by the expression and co-localization of PSD95 and synaptophysin by immunohistochemistry and the expression of synaptophysin and PSD95 by western blotting.

**Results:** The absence of CCR2, impaired the memory consolidation of aversive memory and contextual memory. The CCR2 deficient mice required more trials to learn in passive avoidance test and the memory decay more fast, when we compared with wild type group. The expression of phosphorylated ERK was not increased 1 hour after the one trial in passive avoidance test in hippocampus of CCR2 deficient mice as well the expression of mature BDNF 12 hours after one trial in passive avoidance test. The animals CCR2<sup>-/-</sup> showed lower brain perfusion than C57BL6 mice. We observed that the deficiency of CCR2 affect the synapsis, with important decreased co-localization of PSD95 and synaptophysin in hippocampus and decreased expression of synaptophysin in hippocampus of CCR2 deficient mice.

**Conclusions:** The absence of CCR2 changes the structure of synapsis, decreasing the pre-synaptic protein synaptophysin in hippocampus. This data can explain the cognitive dysfunction in CCR2 deficient mice associated with lower expression of phosphorylated ERK and of mature BDNF.

### **03. Is the visuo-spatial information encoded in the human parietal p300 wave?**

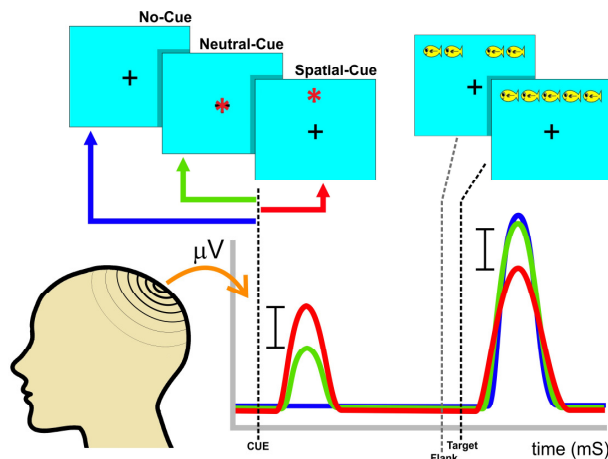
Dimitri Marques Abramov, Monique Pontes, Adailton Tadeu Pontes, Juliana Vieira, Carla Quero Cunha, Paulo Ricardo Galhanone, Leonardo Costa deAzevedo, Vladimir V. Lazarev

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**Background:** The P300 component of event related potentials (ERPs) reflects different aspects of subjective stimulus evaluation and decision making, independently of stimulus modality and physical parameters. Since this component has its maximum amplitude in the parietal scalp areas which relate to visuospatial processing, we suggested that the parietal P300 can be particularly modulated by visuospatial information.

**Objectives:** To evaluate a putative dependence of the midfrontal and midparietal P300 upon visuospatial information in 20 typically developing boys, aged 10–13 years, using the Attentional Network Test (ANT): a cued two-choice task estimating the alertness, spatial attention and executive control in equiprobable cue conditions (no cue, central neutral cue predicting the moment of target appearance, and spatial cues predicting the moment and location of target).

**Results:** The parietal neutral cue P300 had lower amplitude than the spatial cue one, while for the target ERP, on the contrary, the neutral cue condition P300 was larger than that of the spatial one. In the Fz, the late ERPs for both cue and target stimuli did not depend upon the cue condition. These results suggest that in the parietal cortex, the visuospatial information about the target seems to be encoded in the spatial cue P300 and contributes to the following 'decision making' process thus reducing an information processing "load" on the target ERP. This reduction is apparently reflected in lower target P300. The parietal ERPs of the spatial cue condition correlated with the individuals' executive I.Q. The parietal target P300 showed significant correlation with the corresponding frontal cue components only for the neutral cue condition, suggesting frontal control (attention) over parietal networks as a compensation of visuospatial information deficit when the target position is not predicted.



**Conclusions:** Our findings represent the P300 as a resultant of complex integrative interaction between frontal and parietal systems and discriminate its visuospatial and attentional components.

**Figure:** Reducing effect of spatial (red) cue P300 on the following target P300 in the parietal cortex.

*In-house grant from PIP.*

#### 04. Sympathetic inhibition improves cerebral blood flow and endothelial dysfunction in rats with metabolic syndrome.

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<sup>b</sup>Laboratoire de Neurobiologie et Pharmacologie Cardiovasculaire, Université de Strasbourg, France.

**Background:** Cardiovascular and metabolic risk factors that characterize the metabolic syndrome (MS) are accompanied by sympathetic hyperactivity.

**Objective:** Investigate the effects of a chronic oral treatment using centrally-acting sympatho-inhibitory drugs, clonidine or a selective I1 imidazolinine agonist LNP599 on the

brain microvascular alterations in rats under long-term high-fat diet.

**Methods:** Sixteen male Wistar rats were maintained under normal diet (CON, n = 10) or high-fat diet (HFD, n = 30) during 20 weeks. Thereafter, the HFD group was treated with different centrally-acting drugs, clonidine (HFD+CLO, 0.1 mg/kg), LNP599, a new pyrroline compound selective for I1-imidazoline receptors (HFD+LNP599, 20 mg/kg) or vehicle (HFD) by gavage. Systolic blood pressure (SBP) was analyzed by photo-plethysmography during the long-term treatment and the cerebrocortical perfusion was evaluated by Laser Speckle contrast Imaging (Perimed). Brain functional capillary density and endothelial-leukocyte interactions were assessed by intravital microscopy.

**Results:** Chronic treatment with both clonidine and LNP599 lowered SBP to control values and increased microvascular blood flow (HFD+CLO: 211 APU and HFD+LNP599: 266 APU, arbitrary perfusion units,  $p < 0.05$ ) when compared to HFD group (163 APU). Endothelial function was restored in both treated groups. HFD group presented a marked brain functional capillary rarefaction ( $117 \pm 11$  capillaries/mm.) which was reversed to control group values by CLO and LNP599 treatment ( $326 \pm 51$  and  $378 \pm 31$  capillaries/mm., respectively  $p < 0.05$ ). Clonidine and LNP599 treatments reduced the rolling of leukocytes (HFD+CLO:  $4 \pm 0.5$  cells/min; or HFD+LNP599:  $4.3 \pm 1$  cells/min; vs. HFD group:  $12 \pm 0.5$  cells/min,  $p < 0.05$ ) in brain venules.

**Conclusions:** The sympathetic inhibition improves brain capillary density and blood perfusion impaired by HFD. These results also highlight the role of central sympathetic activity on endothelial dysfunction and brain inflammation in metabolic syndrome.

**Financial support:** CNPq, Faperj.

#### **05. Reelin modulation of cerebral cortex layer 1 progenitor cells in postnatal neurosphere assay and adult rats after ischemia-induced stroke**

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Reelin is a glycoprotein secreted by Cajal-Retzius cells in the beginning of cerebral cortex development. Its main function is related to cell-cell signaling important for the lamination of this structure. Other functions have been reported in postnatal and adult ages, as its capacity to increase dendritic spine density, regulate dendritic and axonal growth, increase synaptogenesis and neurotransmission. Reelin has been implicated as a regulatory factor in neuroblast proliferation and migration in well known proliferative niches in the adult brain: Subventricular zone (SVZ) and Subgranular zone (SGZ). It has been reported recently that the reeler mutant presents less doublecortin positive cells in the penumbra zone. Our group demonstrated the existence of another proliferative niche in postnatal age, the layer I of the cerebral cortex, which has been confirmed to be persistent in rodent adult brain after stroke-induced lesion. Layer I was shown to be the source of new GABAergic neurons to the lesion. Based on this evidence, we questioned if reelin could modulate proliferation in postnatal layer 1 *in vitro* as well as in the adult layer 1 after a global cerebral ischemia. For the *in vitro* experiments, we employed the neurosphere assay. The upper third of postnatal (0-8 days) mouse cerebral cortex was dissected and their cells isolated. They were treated with medium containing EGF 20ng/mL, FGF2 10ng/mL with Reelin 0,5µg/mL or without Reelin. Reelin

conditioned medium was obtained from HEK293 cells transfected with the full length Reelin gene. After seven days in culture, neurospheres were photographed for measurements of neurosphere number and area, then dissociated and their total cells counted. For the global ischemia paradigm, we transiently occluded both common carotid arteries of the Wistar adult rats 4-6 months old for 10 minutes after which reperfusion was permitted and survivals of 3 and 7 days were given followed by perfusion and criosectioning. Immunohistochemistry was performed for reelin expression in layer 1 and reelin positive cells counted.

*In vitro* results show that layer I neurospheres treated with Reelin conditioned medium were  $\pm 26\%$  larger in size and increased in  $\pm 60\%$  the total number of cells from layer I compared to control. In the ischemia paradigm, we found a difference in number of GFAP (glial fibrillary acid protein) positive cells in layer 1 which was shown to be more expressed in ischemia-induced animals than controls. This data is correlated with the reactive astrogliosis and glial scar formation associated with morphological changes generated after stroke, confirming the effectiveness of the procedure. However, no difference in the number of reelin positive cells was found between ischemic and control animals three days after lesion.

With this data we can conclude that reelin modulates the progenitors of the postnatal layer 1 in mice increasing their proliferation. However, at least in the time window studied, we did not find this modulation in rat adult layer 1. So this effect may be unique to the postnatal period, when reelin is still secreted by Cajal-Retzius cells.

**06. Preventive strategy of autistic-like features in animal model induced by prenatal exposure to valproic acid: Possible role of excitatory/inhibitory balance.**

Victorio Bambini-Junior<sup>1,2</sup>, Mellanie Fontes-Dutra<sup>1,3</sup>, Gustavo Della Flora Nunes<sup>1,3</sup>, Mauro Mozael Hirsch<sup>1,3</sup>, Walquiria Souza Nunes<sup>1,3</sup>, Gabriela Zanotto Staeve<sup>1,3</sup>, Guilherme Bauer-Negrini<sup>1,3</sup>, Julio Santos Terra Machado<sup>1,3</sup>, Gustavo Brum Schwingel<sup>1,3</sup>, Daniella Arêas Mendes da Cruz<sup>1,2</sup>, Wilson Savino<sup>1,2</sup>, Carmem Gottfried<sup>1,3</sup>

<sup>1</sup>Translational Research Group in Autism Spectrum Disorder (GETTEA); <sup>2</sup>Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil; <sup>3</sup>Research Group in Neuroglial Plasticity, Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

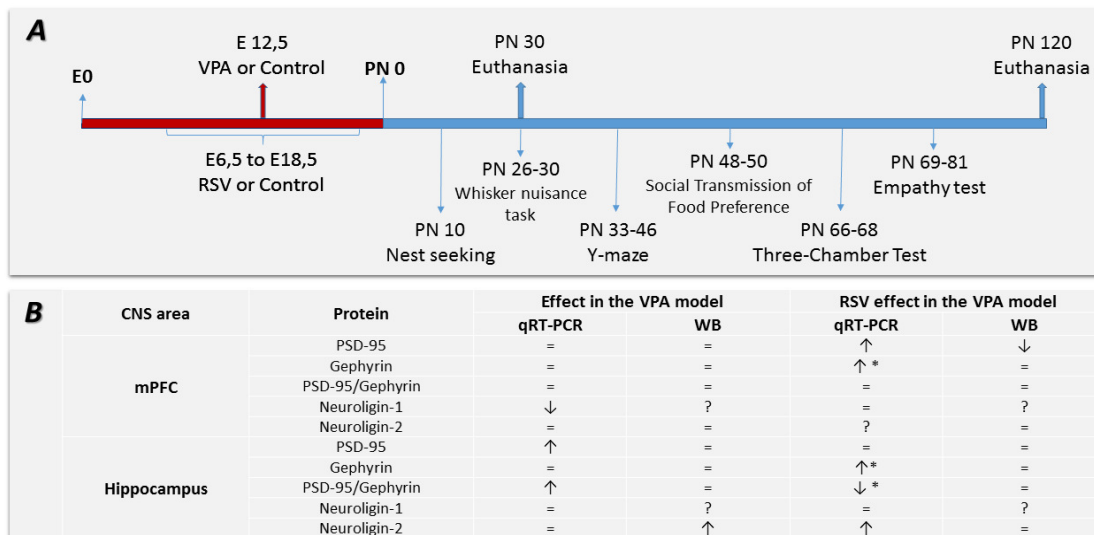
**Background:** Autism spectrum disorder (ASD) is a neurodevelopmental disability characterized by sociability impairments accompanied by communication deficits and stereotyped behavioral patterns. Although ASD etiology is still not known, there is growing evidence that an imbalance between excitation and inhibition is a prominent characteristic at the neuronal circuitry level. One of the known risk factors for ASD is maternal use of valproic acid (VPA) during gestation. Based on this observation, VPA has been commonly used to generate ASD-like condition in rodents. Resveratrol (RSV) is a polyphenol with neuroprotective, antioxidant and anti-inflammatory effects and its employment during the rodent gestation may represent an interesting strategy to clarify aspects involved in the pathophysiology of ASD.

**Objectives:** To investigate the influence of prenatal RSV treatment on a set of behaviors of interest in ASD. In addition, we assessed the influence of this treatment on mRNA and protein expression levels of excitatory (PSD-95 and neuroligin-1) and inhibitory (gephyrin and neuroligin-2) synaptic proteins in medial prefrontal cortex (mPFC) and hippocampus.

**Results:** Prenatal administration of resveratrol prevented VPA-induced impairments in social (by three-chamber test), communication (by social transmission food preference) and sensory behaviors (by nest seeking and whisker nuisance tests). In the empathy test rats exposed to VPA presented a delay to release a trapped conspecific. Related to protein expression, the hippocampal PSD-95/Gephyrin ratio decreased in VPA+RSV group, compared to VPA. In addition, RSV increased gene expression of gephyrin in both mPFC and hippocampus.

**Conclusions:** The present study demonstrates a prenatal intervention able to prevent behavioral alterations induced by VPA in rats. Also, we identified possible mechanisms by which RSV could diminish neuronal excitability. These data highlights RSV as an important strategy in the study of autistic-like behaviors in VPA model, as well as an important tool for seeking etiological targets and physiopathology studies in ASD.

**Financial support:** The present study and the people working under this project receive financial support from Fiocruz (Oswaldo Cruz Foundation), FAPERJ (Carlos Chagas Filho Foundation to Research in the State of Rio de Janeiro), CAPES (Coordination for the Improvement of Higher Education Personnel), CNPq (National Counsel of Technological and Scientific Development), HCPA (Clinical Hospital of Porto Alegre) and UFRGS (Federal University of Rio Grande do Sul)



Graphical abstract, containing the experimental timeline and the major expression (genic and proteic) findings of the present work. (A) The timeline above specifies the prenatal treatments with RSV, VPA and their controls. Moreover, it shows the moments the behavioral tasks are performed. (B) qRT-PCR: Real-Time Quantitative Reverse Transcription PCR. WB: Western blotting. \*  $p < 0.05$ . Other arrows stand for statistical trends  $p < 0.15$ .

### 07. Effect of treatment with resveratrol on schizophrenia like-behavior associated with maternal immune activation (MIA).

Thatiane R. Pombo<sup>1,2</sup>, Nathalia S.B. de Moura<sup>1,2</sup>, Frederico R. Ferreira<sup>2</sup>

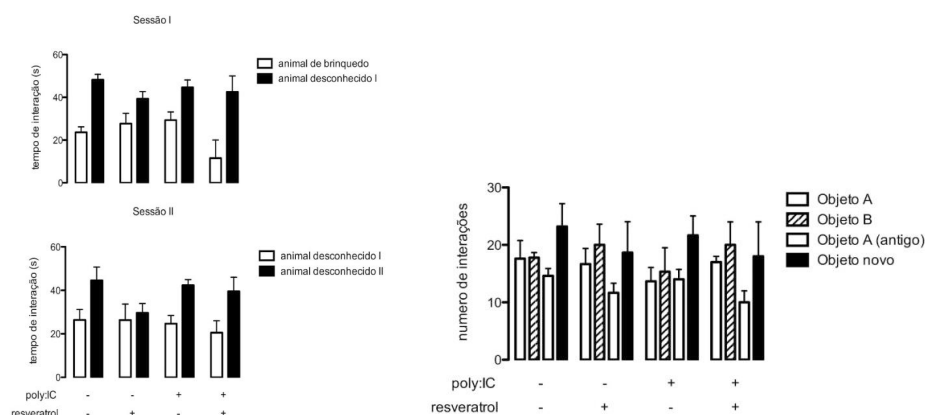
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**Background:** Schizophrenia (EZ) is a neuropsychiatric disorder characterized by positive and negative symptoms such as psychosis with loss of contact with reality, leading to delusions, hallucinations and behavioral changes. Several literature evidences support the hypothesis related to genetic and environmental interplaying on etiology of disease. Infections and immunogenic challenging during pregnancy or in the early stages of development/maturation of the central nervous system can occupy an important role in the etiology of EZ. Maternal immune activation (MIA) by viral infection or Poly I:C injection affects the neuronal proliferation, differentiation and gene expression, impacting the brain's development and favoring the progress of the disease. The association between gestational stress and increased risk of neurodevelopmental disorders in the offspring has been found in studies with infectious agents. IFN- $\gamma$ , a cytokine that possesses antiviral responses, is released to interfere on survival and proliferation of the infectious agent. It is known that antipsychotics have antioxidant and anti-inflammatory properties, increasing the neuro-immune hypothesis for the EZ, and the potential use of anti-inflammatory agents and antioxidants in treatment or even prevention of alterations induced by immunogenic insults during pregnancy. Resveratrol, a natural compound derived from grapes and wine, has anti-inflammatory and antioxidant property, presenting potential effect in the treatment of neurodegenerative diseases.

**Objectives:** This study aimed to investigate the effects of treatment with resveratrol on schizophrenia like-behavior associated with immunogenic challenging during pregnancy.

#### Results:



**Conclusions:** Test model developed correctly but has the necessity to investigate the inoculum (poly I:C) and drug (resveratrol) effects in different strains and the necessity of repetition of the tests in order to investigate other results.

**Financial support:** CNPq, IOC – Instituto Oswaldo Cruz, FAPERJ

**08. Behavioral features and cellular dynamics in a novel animal model of depression****Schitine CS<sup>1</sup>, Nunciato AC<sup>1</sup>, Oliveira C<sup>1</sup>, Nogaroli L<sup>1</sup>, Hedin-Pereira C<sup>1,2</sup>.**<sup>1</sup>Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro,<sup>2</sup>Neuroscience Program, Oswaldo Cruz Foundation, Rio de Janeiro.

Major depression is the most common neuropsychiatric disorder, but little is known about its neuropathophysiology. There are many possible causes for depression and, among them are genetic, social, psychological and biochemical components. However, deregulation of the hypothalamic-pituitary-adrenal axis has been described as a major trigger for depression. It is also known that neurogenesis is decreased in the hippocampus during depression.

We asked if subventricular zone neurogenesis and oligodendrogenesis could be affected in this paradigm. The paradigm of depression used in this study mimics chronic stress by administering exogenous corticosterone in drinking solution associated with social isolation for 30 days in adult Swiss mice.

Two experimental groups were employed: control group, received filtered water or vehicle and were maintained in collective cages and CORTICO/ISO group, which received 0,25mg/ml corticosterone in drinking solution and were maintained socially isolated. After this period, we observed significant differences between the two groups as to their behavioral profile as well as cellular and morphological features. CORTICO/ISO group showed a decrease in food ( $F= 34,8$ ,  $p < 0, 0001$ ), drink consumption ( $F= 2,250$ ,  $p=0, 0478$ ) and body weight ( $F= 5,167$ ,  $p < 0,0001$ ) in comparison to the control group. Their behavioral profile was investigated by the application of the sucrose preference test and the forced swimming test. Animals submitted to corticosterone and isolation showed an increase in immobility ( $t= 3.013$ ,  $p= 0, 0003$ ) and decrease of sucrose preference ( $t= 4.873$ ,  $p < 0.0001$ ) comparing to animals in control condition. The behavioral features in depressive-like animals were associated with changes in cellular dynamics. We verified a reduction in proliferative oligodendrocyte progenitors cells ( $p= 0, 0006$ ) and an increase in proliferation levels of progenitor cells ( $p= 0, 0113$ ) in the subventricular zone. However, preliminary data have shown that withdrawal of corticosterone in CORTICO/ISO group for an additional 30-day period reverted the weight loss and the depressive-like behavior measured by sucrose preference test and forced swimming test.

Thus, these data indicate that our model of chronic stress is efficient in inducing the depressive phenotype, leading to behavioral alterations, are restricted to the treatment period. Furthermore, our data show a deficit in the capacity for oligodendrocyte turnover suggested by the decrease in oligodendrocyte progenitors in white matter tracts.

**09. On the diagnosis of attention deficit hyperactivity disorder (ADHD) through the posner's attentional network test (ant) and event-related potentials**

Dimitri Marques Abramov, Adailton Tadeu Pontes, Carla Quero Cunha, Monique Castro Pontes, Juliana Vieira, Paulo Ricardo Galhanone, Saint Clair Gomes Junior(\*), Leonardo C. deAzevedo, Vladimir V. Lazarev

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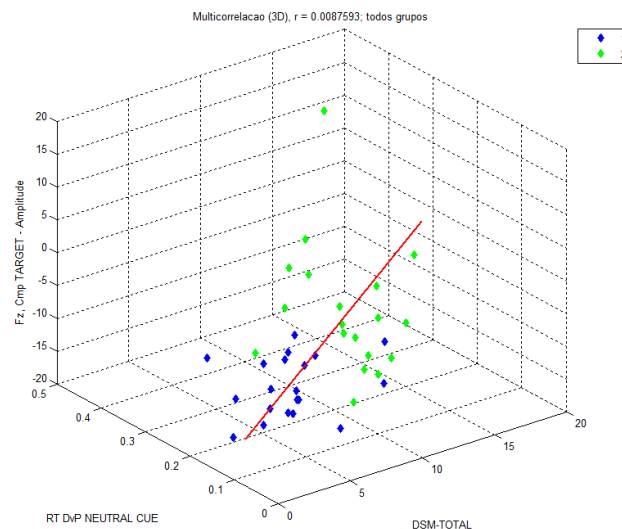
**Background:** The nosology of ADHD is controversial, and the diagnosis is based on subjective estimation according to the DSM criteria through clinical assessments, structured interviews, etc. However, there are evidences that in ADHD, the P300 component of event related potentials (ERPs) is affected and may serve as one of neurophysiological markers. We estimated ERPs during performance of the Posner's Attentional Network Test (ANT) (according to Kratz et al 2011) under No-Cue, Neutral-Cue and Spatial-Cue conditions, correlating the results with the scores of the DSM-IV (the predictor variable, that determines the groups), in 11-13 years old boys with (n=20) and without (n=19) diagnosis of ADHD.

**Objectives:** To study the neurophysiology of ADHD as related to behavioral performance and clinical characteristics, discussing if DSM may be a valid screening tool for the ADHD diagnosis, possible biomarkers, and the dimensionality of this mental phenomenology.

**Results:** ADHD boys showed: smaller amplitudes of the frontal ERPs, without any change in the parietal P300, for both Cues and Target and larger *intraindividual* variability of reaction time (IVRT) mainly in the Neutral-Cue Condition. The DSM-IV scores correlated (Pearson's method) with several measurable parameters. There were significant correlations between clinical, behavioral and neurophysiological variables, for each group many coefficients increasing when ADHD and control subjects were analyzed together. We selected behavioral (IVRT in the Neutral-Cue condition) and neurophysiological (amplitude of the Target ERP at Fz) variables that showed statistically significant differences between the groups and higher correlation coefficients between them (at least when the groups were merged), for cluster analysis (hierarchical method), which did not reveal new groups.

**Conclusions:** (1) The DSM criteria seem to be an adequate predictor for quantifying attention and hyperactivity, correlating with various behavioral and neurophysiological variables; (2) the selected physiological and behavioral variables show a pattern of continuous variation, suggesting that Attention Deficit and Hyperactivity could be a dimensional condition, i.e., quantitative variation of normality

**Figure:** 3D-correlogram of the variables: DSM-total score (predictor) x IVRT in the Neutral-Cue condition x Amplitude of Target ERP at Fz. Blue: Controls, Green: ADHD.





### 10. A preliminary model for the cerebral neurodynamics in the attention deficit and hyperactivity disorder (ADHD)

Dimitri Marques Abramov, Adailton Tadeu Pontes, Carla Quero Cunha, Monique Castro Pontes, Juliana Vieira, Paulo Ricardo Galhanone, Leonardo Costa deAzevedo, Vladimir V. Lazarev

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**Background:** The physiopathology of ADHD is unknown; however there is an agreement that the functioning of the frontal lobes, with their key role in the attentional control, is somehow impaired. It is also well known that cognitive processes reflected in parietal P300 component of the event related potentials (ERPs), can detect these impairments.

**Objectives:** To study functional relationships of the midfrontal region (Fz) with other brain areas by correlating clinical features with the results of Attentional Network Test (ANT) and ERPs and EEG spectra (by wavelets) during its performance in 20 boys with and 20 without (control group) diagnosis of ADHD. ANT is a cued two-choice task for estimating the alertness, spatial attention and executive control.

**Results:** The ADHD group showed (a) smaller Cue and Target ERPs in Fz, (b) smaller Voltage Variation between cue and target presentations (VV) in Fz, F8 (anterior right temporal) and T4 (right temporal) and (c) larger earlyTarget ERPs in C3 (left central) than the controls. To study correlations between ERPs, both ADHD and control subjects were analyzed in a same group. The Cue-Fz ERPs showed significant correlation with Target-Pz (midparietal), and VV-Fz with early Target-C3 -C4 ERPs, suggesting that the frontal cue processing somehow modulates the central and parietal activity related to the target response. The VV-F8 correlated with VV-Fz, VV-T4 and VV-Pz, (while these components did not correlate among them) The gamma amplitude spectra in posterior regions were correlated with IQ, and in all the other sites with Cue-Pz ERP, mainly in ADHD. Delta and theta amplitude spectra of all derivations correlated with Target-Fz ERPs only in the control and with Target-Pz in both groups. The VVs showed a complex patterns of correlations with the wavelet spectra.

**Conclusions:** The results reinforce the role of frontal cortex in cognitive and motor control, apparently showing its impairment in ADHD, with alteration in interhemispheric asymmetry involving a putative integrative site at the anterior right temporal region F8. The EEG gamma and theta characteristics seem to reflect certain neurophysiological alterations in ADHD.

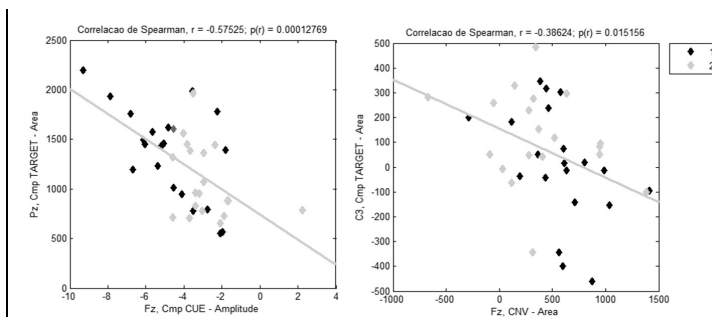


Figure: scatter plots (correlograms: black - controls, grey - ADHD) showing the relations among cue-related components from Fz and Target-related components from C3-C4 and Pz.

### 11. Improvement in performance in the attention network test in children with attention deficit hyperactivity disorder

Carla Quero Cunha, Adailton Tadeu Pontes, Dimitri Marques Abramov, Monique Castro Pontes, Juliana Vieira, Paulo Ricardo Galhanone, Vladimir V. Lazarev, Leonardo Costa deAzevedo

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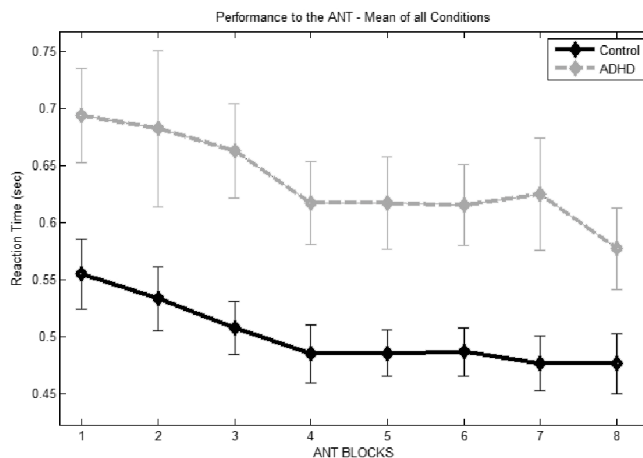
**Background:** Attention Deficit Hyperactivity Disorder (ADHD) is usually included in the category of learning disabilities. It was shown that attention is provided by different neural subsystems that can be studied by means of the Attention Network Test (ANT). This test permits estimating efficiency of the three different neural networks related to vigilance, orienting and executive function (Fan et al., 2002).

**Objectives:** To study the dynamics in reaction time (RT) along the ANT trials in the search for skill learning patterns of attentional performance in 21 boys with and 20 boys without (TD) ADHD diagnosis according to DSM-IV, aged 9-13 years.

**Results:** Taking into account the conditions of cue and target-flanks congruence, ADHD patients always reacted significantly slower than controls in all the conditions, without any difference in the error rates (as was also shown by other authors). The RT differences between the groups per block are presented in the Figure (mean of all trials for all conditions ± standard error of mean). The arrow points to the block 4 where the RT begins to be different from that of the block 1 in both groups. The dynamics of the RT changes along the blocks were very similar in both ADHD and TD boys, who improved the attentional task performance in the same way. The same pattern of performance improvement were observed for any condition of cue or target

**Conclusions:** The ADHD and TD groups demonstrated the same learning performance, although the attentional performance in the former was worse than in the latter. This suggests that cognitive alterations in ADHD patients do not affect the acquisition of a new skill that is related to attention. (in house grant – PIP)

**Figure:** Dynamics of group average reaction time (with standard error of mean) along 8 ANT blocks for controls and ADHD patients.



**12. Alzheimer - associated A $\beta$  oligomers impact the central nervous system to induce peripheral metabolic deregulation**

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**Background:** Alzheimer's disease (AD) and diabetes are chronic degenerative diseases increasing in prevalence in aging populations worldwide. Although clinical and epidemiological studies have linked AD to diabetes, with each disease increasing the risk of developing the other, why AD patients present increased probability of developing diabetes is unknown.

**Objectives:** We hypothesized that AD-associated amyloid- $\beta$  oligomers (A $\beta$ Os) could impact brain regions responsible for metabolic control and therefore represent a key pathogenic link between AD and deregulated peripheral glucose homeostasis.

**Results:** Intracerebroventricular (icv) infusion of A $\beta$ Os in mice triggered peripheral glucose intolerance, a phenomenon further verified in two transgenic mouse models of AD. Systemically injected A $\beta$ Os failed to induce glucose intolerance, suggesting A $\beta$ Os target brain regions involved in peripheral metabolic control. Accordingly, we show that A $\beta$ Os affected hypothalamic neurons in culture, inducing eukaryotic translation initiation factor 2 $\alpha$  phosphorylation (eIF2 $\alpha$ -P). A $\beta$ Os further induced eIF2 $\alpha$ -P and activated proinflammatory IKK $\beta$ /NF- $\kappa$ B signaling in the hypothalamus of mice and macaques. A $\beta$ Os failed to trigger peripheral glucose intolerance in tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) receptor 1 knockout mice. Pharmacological inhibition of brain inflammation and endoplasmic reticulum stress prevented glucose intolerance in mice, indicating that A $\beta$ Os act via a central route to affect peripheral glucose homeostasis.

**Conclusions:** Our findings establish that icv-injected A $\beta$ Os trigger inflammation in the hypothalamus and reveal novel shared molecular mechanisms between hypothalamic dysfunction in metabolic disorders and AD. Our discovery that A $\beta$ Os instigate hypothalamic deregulation and cause peripheral glucose intolerance draws attention to a brain structure that has been largely ignored to date in the study of Alzheimer's pathogenesis.

**Financial support:** Human Frontiers Science Program (HFSP), National Institute for Translational Neuroscience (INNT/Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP2012/12202-4), Canadian Institutes for Health Research (CIHR) and Canada Research Chair Program.

**13. Striving for the best treatment for pediatric acute demyelinating syndromes: results from cohort analysis**

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**Introduction:** The 2013 International Pediatric MS Study Group report brings awareness to the difficulties in managing the diagnostic work-up and treatment of Pediatric Acute Demyelinating Syndromes (ADS), and highlights their differences from adult onset forms and the need for pediatric guidelines. Taking into account that pediatric ADS course with increased inflammatory response, frequent relapses, long term disabilities and quality of life losses, a specialized outpatient clinic to follow-up children with ADS was created in our hospital. Cohort analysis of these patients and work-up/therapeutic strategies are described in this study.

**Methodology:** Prospective cohort analysis from a Pediatric ADS clinic population over the past year.

**Results:** Eighteen patients (8 boys), mean age of 7.3yo (range: 1.8-13yo), at their first episode of ADS were seen in clinic, and protocols were created to be used through their differential diagnosis, relapses and remission phases. Five presented with multiple sclerosis (MS), 7 with Acute Disseminated Encephalomyelitis (ADEM), 3 with Neuromyelitis Optica (NMO), and 3 with Clinically Isolated Syndromes. Seven patients presented ADS symptoms prior to temporal-spatial dissemination diagnosis. Serum from all patients were tested for inflammatory biomarkers: IgG index calculation, oligoclonal Bands, lymphocyte counts, cytokines (IFN-gamma, TNF-alpha, IL-17, IL-4 and IL-10). Currently, five patients with MS are treated with subcutaneous Interferon beta-1A, and a fourth with Glatiramer acetate due to prior treatment failure. NMO is treated with Azathioprine or Glatiramer acetate. Immunotherapy/cyclophosphamide treatment is used for ADEM with good response.

**Conclusion:** This preliminary cohort analysis describes our current work-up and therapeutic protocols for ADS at our hospital.

**Financial support:** PIP/IFF-Fiocruz

**14. The role of Tryptophan-kynurenine metabolism in *Mycobacterium tuberculosis*-infected macrophages and its potential involvement in depressive disorder**

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Tryptophan-kynurenine pathway has been related to chronic infection, inflammation and neurological diseases such as depression. Indoleamine 2,3-dioxygenase-1 (IDO-1) expression increases tryptophan-kynurenine pathway activation in mycobacterial infections in

humans and mice. In *Mycobacterium tuberculosis* infection, IDO regulates tryptophan availability which may influence the immune system via the tryptophan depletion effects.

Dysregulation of kynurenine pathway can lead to immune system activation and accumulation of potentially neurotoxic compounds and has been associated with depression disorders. Here, we evaluated the activation of tryptophan-kynurenine pathway for *M. tuberculosis* infection in human primary macrophages. Also, the potential immunomodulatory role of minocycline, which is a tetracycline family antibiotic widely used in alternative treatment for bacterial infections, including leprosy. In addition, minocycline has also shown great efficacy in treating depressive disorders and neurodegenerative diseases.

Our data showed that *M. tuberculosis* infected macrophages are able to upregulate the tryptophan-kynurenine pathway activation by significantly increase the secretion of kynurenine. Interestingly, minocycline was able to reverse this effect in infected cells, proving to be an interesting therapeutic tool in regulating the activation of this pathway in *M. tuberculosis* infection.

Finally, peripheral macrophages activation in chronic infection tuberculosis suggests that kynurenine metabolism modulation appears to be an important aspect in the development of depression in TB patients due to a chronic immune response and antibiotic minocycline may be a promising therapeutic target for treating both diseases.

**Financial Support:** FAPERJ

### **15. Effect of cannabinoid hemoglobin-derived peptides (RVD-hemopressin and VD-hemopressin) in the postnatal mice subventricular zone neurogenesis**

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Neural stem cells (NSCs) are present in the embryonic brain and persist in the subventricular zone (SVZ) of postnatal and adult brain. These cells continue to generate neurons and glial cells throughout life. Therefore, SVZ progenitors could be a source for replacement of neurons and glial cells after brain injury. Evidence indicates that endocannabinoid signaling plays a role in the modulation of neurogenesis. Hemopressin (Hp), a nine-residue peptide derived from the  $\alpha$  chain of hemoglobin, acts as an inverse agonist of type 1 cannabinoid receptor (CB1) (Heimann *et al.*, 2007). Moreover, two N-terminally extended peptides: RVD-hemopressin (RVD-Hp) and VD-hemopressin (VD-Hp) were found as agonists of these receptors (Gomes *et al.*, 2009). These findings led to the notion that cannabinoid receptor activity can also be modulated by peptides and not only lipids. Little is known about the role of RVD-Hp or VD-Hp in neurogenesis. We therefore aimed to study their effect on proliferation and differentiation in neonatal C57/BL6 mouse neurosphere cultures derived from SVZ stem/progenitor cells. SVZ cells were obtained from 1 to 4 postnatal day C57/BL6 mice. Cells were plated on nonadherent dishes for 6 to 8 days to generate neurospheres. To investigate the effects of RVD-Hp and VD-Hp on cell proliferation, neurosphere cultures were plated and exposed to 10  $\mu$ M 5-bromo-2-deoxyuridine (BrdU) for 4 hours prior to the end of the 48 hour treatment with cannabinoid peptides. Our data indicate that RVD-Hp and VD-Hp treatment for 48h increase the number of BrdU positive cells in neurospheres derived from SVZ. The effects of 1  $\mu$ M RVD-Hp or VD-Hp on cell differentiation was assessed in SVZ

neurosphere cultures exposed for 8 days. After treatment, cells were processed for immunocytochemistry using neuronal markers. Our findings show that 8 day RVD-Hp and VD-Hp increased the number of NeuN-positive cells in SVZ neurospheres cultures. In order to evaluate neuronal functionality in SVZ cultures, intracellular  $Ca^{2+}$  variations were monitored upon KCl and histamine stimulation. Former protocols show that KCl promotes  $Ca^{2+}$  influx in neuronal cells while histamine (His) triggers an increase in  $Ca^{2+}$  intracellular levels in immature SVZ cells. SVZ neurospheres treated with 1 $\mu$ M RVD-Hp or 1 $\mu$ M VD-Hp for 7 days in culture showed an increase in the number of neuron-like cells (His-KCl ratio below 0.8) when processed for a calcium imaging protocol. Altogether, our data demonstrate that RVD-Hp or VD-Hp increased proliferation of SVZ cells. The cannabinoids peptides also induced an increase in functional neuronal differentiation. In conclusion, our results highlight a role for RVD-Hp and VD-Hp in the regulation of NSC proliferation and cell fate in SVZ suggesting these peptides may represent a pro-neuronal signal and a possible future strategy for CNS repair.

**Support:** CNPq, Capes, FAPERJ

#### **16. The stress in the classroom: a correlational study between childhood stress and the school performance**

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**Background:** The stress is a state of tension in the body, which is forced to mobilize its entirety to face a new situation; it is part of the adaptive system. Stress is the body's reaction to psychological and physical components, caused by psycho-physiological changes that occur, when a person is faced with a situation that somehow, either, or too difficult or very exciting. These physiological changes suffered by the organism can evolve into four phases: alert phase, resistance phase, Phase Near-exhaustion and exhaustion phase. Stressed children will be stressed adult.

**Objective:** Based on this, the aim of this work is evaluate the level of stress in children of the first cycle of basic education correlating with your school performance. **Results:** This study was conducted in two elementary schools (private and public school) at the Rio de Janeiro city. We evaluated 75 children aged between 8-12 years of both sexes. For the data collection we used to *Escala de Stress Infantil* (ESI) by Lipp and Lucarelli. The psychological test is used to establish the existence or not of the stress in children, it has 35 items on Likert scale grouped into four factors: physical reactions, psychological reactions, psychological reactions with depressive components and psychophysiological reactions. Our results showed that 37 (49.3%) stressed children in both schools, distributed in different phases: 17 (46 %) alert, 11 (29.7%) resistance, 6 (16.2 %) near-exhaustion and 3 (8.1%) exhaustion. In relation to school performance, 86% stressed children present school grades more than 6.0.

**Conclusion:** We conclude that the stress not directly influenced the school performance of children analyzed. The work will evaluate other factors.

**17. Leprosy Neuropathy Evaluated by NCS Is Independent of the Patient's Infectious State**

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**Background:** Leprosy causes nerve injury, which mimic various clinical and neurophysiological conditions, rendering it an excellent model of peripheral neuropathy.

**Objective:** Aiming to facilitate early detection and treatment, we looked to identify the particular electrophysiological characteristics of leprosy nerve lesions that would clearly distinguish them from those of other diseases. **Results:** A retrospective study including 822 nerve conduction studies (NCS) of 509 patients was developed to appraise the electrophysiological pattern of leprosy neuropathy. At all stages evaluated (before, during and after multidrug therapy, NCS alterations were similar regarding extension, topography, severity, degree of damage, and type of lesion. Acute events may occur even after a long period of time after release from treatment. Sensory impairment on NCS (92-96%) was more frequent than motor (70-77%). The former showed axonal lesions while the latter showed a demyelinating pattern. No typical pattern of nerve lesion was observed even in the same patient. While the different types of nerve lesions (axonal, demyelination, and mixed) were observed in different proportions in both the sensory and motor nerves, the same type of lesion predominated during the three periods evaluated. A mosaic like polyneuropathy was observed in all periods evaluated, suggesting the aggregation of successive nerve fiber injuries and regenerative processes. Variable degrees of nerve involvement produced asymmetric neuropathy in this group of patients.

**Conclusions:** Once the axonal loss is installed, nerve function is little affected by inflammatory, immune and/or bacterial events, and chronic neuropathy is established, leading to the well-known sequelae of leprosy occurring at any time before and after leprosy diagnosis.

### Main nerve conduction alterations found at the three periods of nerve conduction study (NCS) evaluation

NCS alteration		<i>At diagnosis (n=200)</i>	<i>During MDT (n=201)</i>	<i>Post RFT (n=329)</i>
Median number of nerves altered (IQR)	<b>Sensory</b>	4 (2-6)	3 (2-6)	4 (2-6)
	<b>Motor</b>	2 (1-3)	1 (1-3)	2 (1-3)
Total number of nerves impaired (%)	<b>Sensory</b>	733/1840 (39.8%)	738/1824 (40.5%)	1344/2912 (46.2%)
	<b>Motor</b>	333/1380 (24.1%)	286/1368 (20.9%)	500/2184 (22.9%)
All nerves with no conduction elicited	<b>Sensory</b>	20 (10%)	13 (6.5%)	31 (9.4%)
	<b>Motor</b>	1 (0.4%)	1 (0.4%)	4 (1%)
Predominant type of lesion	<b>Sensory</b>	Axonal (56.0%)	Axonal (56.2%)	Axonal (56.2%)
	<b>Motor</b>	Demyelination (68%)	Demyelination (64.2%)	Demyelination (61.4%)
<b>Conduction block</b>		49 (24.5%)	46 (22.9%)	76 (23.1%)

IQR=interquartile range, MDT=multidrug therapy, RFT=release from treatment

**Financial support:** Instituto Oswaldo Cruz/ Fundação Oswaldo Cruz

### 18. Role of autophagy in neural pure leprosy neuropathy

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Neural pure leprosy neuropathy is a well-accepted clinical entity that is followed by extensive axonal damage, aberrant demyelination, severe nerve inflammation and disturbed Schwann cell and macrophage responses. Of note, extensive axonal demyelination is not only observed in leprosy neuropathy patients but also in other peripheral diseases. Whether or not demyelination is caused by either chronic inflammatory reaction and/or Schwann cell responses is still under intense debate and investigation. Previous data from our laboratory have shown the involvement of pro- and anti-inflammatory cytokines in the immunopathogenesis of nerve injury *in vitro* and *in vivo*. However, recent reports have shed light on the potential role of autophagy mediating myelin breakdown and clearance after nerve damage in mice. Taking into consideration the involvement of autophagy in nerve injury, we evaluated, in the present study, the enrollment of autophagy during neural pure leprosy neuropathy. We obtained nerve biopsies from controls (n=10, diagnosed with other neuropathies) and patients (n=8, diagnosed with neural pure leprosy) and evaluated the pattern expression of autophagy-related (ATG) genes. Our preliminary data shows that nerve biopsies from patients with leprosy neuropathy have augmented expression of mRNA for the following genes *ATG5*, *ATG7*, *BECLIN1/ATG6* and *LC3B/ATG8* as compared with control non-leprosy nerves. Analysis *in vitro* using Schwann cell lineage ST88-14 demonstrated that



*Mycobacterium leprae* stimulation increases autophagosome formation, but hampers the autophagic flux. rIFN- $\gamma$  exposure was able to targets *M. leprae* to autolysosomes. In conclusion, our data suggests an important involvement of specific key autophagy genes in the immunopathogenesis of neural pure leprosy neurophaty.

**Financial Support:** CNPq, FAPERJ

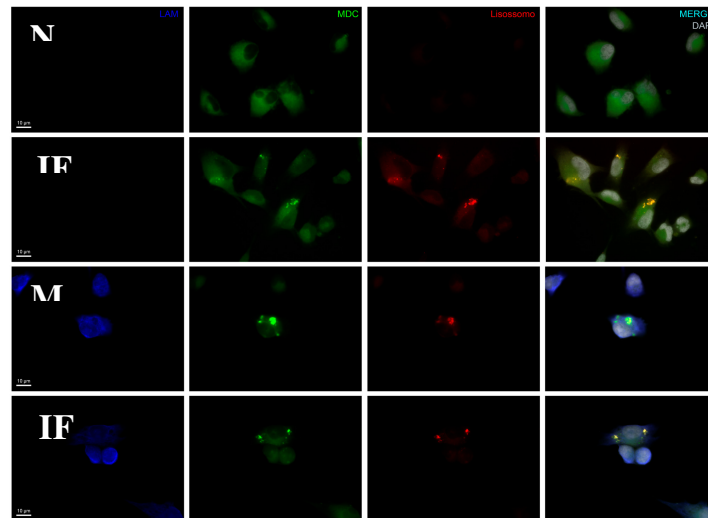


Figure 1. *Mycobacterium lepra*-induced autophagy is hampered by rIFN- $\gamma$  treatment in ST8814 Schwann cell line *in vitro*.

### 19. Biological relevance of the purinergic signalling in leprosy pathogenesis: a possible pathway involved in the leprosy nerve damage.

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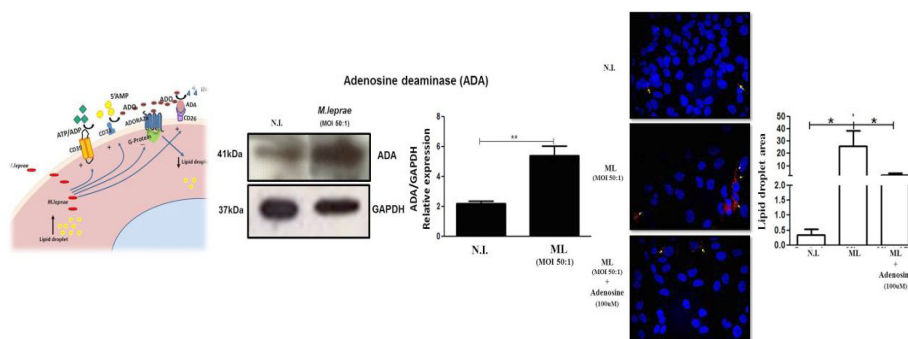
**Background:** Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (ML), which evokes a strong inflammatory response and leads to nerve damage due to its tropism towards Schwann cells(SC). Two important aspects that favour the establishment of infection and survival of ML are: lipid droplet accumulation and the capacity of ML to induce rapid demyelination after extracellular binding to myelinated SC. Furthermore, Rambukkana in 2013 showed that ML infection leads to reprogramming of SC by downregulating lineage/differentiation-associated genes and upregulating genes mostly of mesoderm development, conducting to SC desdiferenciación and thus promoting dissemination of infection. Recently, the purinergic receptor P2Y2 was shown to participate of SC myelination, in addition, this receptor as well as adenosine nucleoside are important to lipid metabolism. This receptor of the purinergic signaling system plays an important role by modulating inflammatory and immune responses via extracellular adenine nucleotides and their derived nucleoside adenosine. In addition purinergic signaling appears to play important roles in neurodegeneration, neuroprotection and neuroregeneration.

**Objective:** Our objective is therefore to evaluate the participation of the purinergic signaling pathway in leprosy pathogenesis by analyzing the influence of ML infection on different components of this pathway and its impact on lipid bodies biogenesis in SC.

**Results:** Our results have shown that ML infection increase the activity and the expression of important purinergic ectoenzymes (CD39, CD73 and adenosine deaminase) involved in the adenosine turnover and that this nucleoside is able to decrease the lipid droplet accumulation induced by ML infection. We have also observed the involvement of A2a, P2X7 and potentially of the P2Y2 receptors in this phenomenon.

**Conclusion:** Our results bring new insights into the involvement of the purinergic pathway in nerve damage, which can lead to potential targets for novel leprosy treatment and a better comprehension of neurodegeneration.

**Financial Support:** Ministério da Saúde-Fiocruz/CNPq/Faperj



## 20. Responsiveness to *Mycobacterium leprae* (ML) antigens in pure neural leprosy

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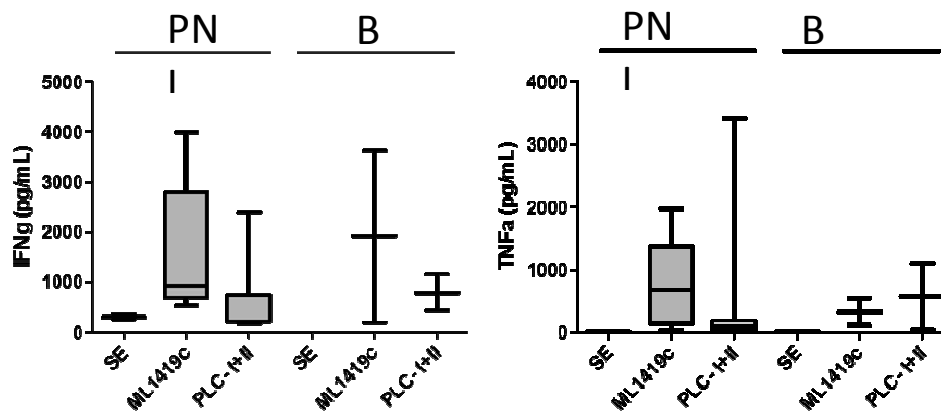
**Background:** *Mycobacterium leprae* (ML) is an obligate intracellular pathogen that induces inflammatory lesions in the skin and peripheral nerves. Detection of leprosy skin lesions, acid-fast bacilli and peripheral neuropathy are major clues in the final diagnosis of the disease. But only peripheral neuropathy, without skin lesion, is found in pure neural leprosy (PNL). The investigation of the pattern of immune and inflammatory responses of these individuals to ML-specific antigens can provide relevant tools for differentiating PNL from non-infectious peripheral neuropathies, and a better understanding of the mechanisms involved in the pathology of peripheral nerve lesions.

**Objective:** To investigate the response of patients with PNL to ML-specific antigens. To evaluate the potential of a short-term, whole blood assay for diagnostic support in PNL.

**Results:** Patients under follow-up at the Souza Araújo Outpatient Unit, with diagnostic indication of PNL, were also submitted to nerve biopsy and neurological evaluation (n=7; 0-4 months of multidrug therapy for leprosy; all female from the State of Rio de Janeiro; 25-68 yrs old). The diagnosis of PNL included histopathology of the nerve lesion. In 4 out of 7PNL patients, qPCR for presence of *ML*-specific DNA was positive in the biopsy. Acid-fast bacilli were not detected in the biopsies or lymph of these patients. Two untreated borderline tuberculoid leprosy (BT) patients were also included as positive controls for responsiveness to *ML* antigens (1 male, 18 yrs; 1 female 43 yrs). Following stimulation of heparinized venous blood (1mL, 24hrs, 37°C) with a *ML*-specific recombinant protein (ML1419c) and a pool of *ML*-specific peptides, levels of biomarkers in response to *ML*-specific stimuli were evaluated in the plasmas, using a multiplex cytometry assay.

**Conclusions:** High levels of IFN-gamma, TNF (Figure) and IL-17 among other biomarkers were induced in response to *ML* antigens in PNL demonstrating a response comparable to the tuberculoid forms of leprosy in the PNL patients.

**Financial support:** FAPERJ, Secretaria de Vigilância Sanitária (SVS)-Ministério da Saúde.



### 21. Fermentative Metabolism Modulation of Schwann Cells Infected by *Mycobacterium Leprae*

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**Background:** Leprosy is caused by *Mycobacterium leprae*, which primarily parasitizes tissue macrophages in the dermis and the Schwann cells of peripheral nerves. In many countries, leprosy has been successfully eliminated; however, it still remains a major public health problem in Brazil, India and Africa. The bacteria develop during its genome reduction evolution a variety of mechanisms to avoid or circumvent host immune responses and survival within these cells. Actually, it is believed that nerve injury results from predominantly

active infection of *M. leprae* in the neural parenchyma, which generates polymorphonuclear cells recruitment and subsequent neuritis. Lactate is a product of aerobic glycolysis that plays important roles in the axonal metabolism, which is released by Schwann cell into the extracellular space, taken up by nearby neurons or axons, and oxidatively metabolized, representing an important energy source.

**Objectives:** To assess the lactate production by Schwann cells infected by *M. leprae* and its implication in the neuropathology associated with the disease.

**Results:** In the present study it was observed that Schwann cells infected with *M. leprae* showed a two-fold reduction in lactate production and over expression of the genes corresponding to monocarboxylate transporter MCT1, MCT2 and MCT4. Concomitantly, we observed an increase of glucose 6-P dehydrogenase (G6PD) enzyme expression in infected cells, the step-rate enzyme of the pentose phosphate pathway oxidative phase. When using 6-ANAM, inhibitor of G6PD enzyme, we successfully recovered lactate release by infected Schwann cells, concomitantly with a drop in *M. leprae* viability.

**Conclusions:** Reducing the level of lactate released by infected Schwann cells can represent a new mechanism of axonal insult related with Leprosy. Inhibition of pentose phosphate pathway could provide therapeutic effects on leprosy, combined with the

## **22. Genome-wide SNP association with leprosy susceptibility in a group of household contacts**

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**Background:** Leprosy is a complex and multifactorial disease that can progress towards neural degeneration. Household contacts from patients are heavily exposed and have the highest chance to develop disease. The bacilli exhibits low variability that combined with the broad clinical spectrum and the polarized cellular response towards *M. leprae* suggests that the human host controls the myriad of host responses. Several genetic polymorphisms located in immune response genes have been consistently associated with disease susceptibility.

**Objectives:** We performed a genome-wide association study evaluating household contacts of leprosy patients that developed the disease, i.e., cases (N=67) and contacts that did not develop disease i.e. controls (N=319) in a follow up of at least two years after confirmed diagnosis of the leprosy index case. After DNA extraction, genotyping was performed with Illumina GT HumanCore-12 BeadChip (240.000 polymorphisms). A preliminary logistic regression test was used to determine significance values of the polymorphisms. As inclusion criteria for further analysis SNPs were selected if they had association values of Odds Ratio higher than 2 or lower than 0.5 and significance levels < 0.001. After selecting top associated SNPs we mapped the genes where the polymorphisms were located and performed gene enrichment analysis and evaluated SNP association through logistic regression using GO/Reactome and R environment respectively. Finally adjustment for multiple comparisons was performed using FDR.

**Results:** A total of 240.000 markers passed quality control of raw genome data. Based on the top markers, enrichment analysis of associated SNPs pointed towards genes that represented biological processes of neuron generation, regulation of neuromuscular junction and neurological system process. Of the polymorphisms located in this specific set of genes, 17 were significantly associated either with susceptibility or protection towards disease development in the sample of household contacts.

**Conclusions:** We determined a group of markers that can be used as leprosy progression markers between household contacts. Further validation is required for either genetic replication in larger cohorts and also functional evaluation of the associated genes.

**Financial support:** FAPESP, FAPERJ, CNPq, CAPES (PAPD/RJ)

### **23. The possible value of skin biopsy for pure neural leprosy diagnosis.**

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**Background:** Pure neural leprosy (PNL), characterized by peripheral neuropathy in the absence of dermatological alterations, is diagnosed based on the clinic-epidemiological data and the nerve biopsy results. However, histological findings are nonspecific and *Mycobacterium leprae* detection is rare. Criteria for the diagnosis of the cutaneous forms of leprosy are well established for skin biopsies. Among other features, nerve fiber destruction and/or the presence of inflammatory infiltrate surrounding them suggest indeterminate or tuberculoid leprosy. Therefore, alterations of the nerve fibers present in most of the skin biopsies samples of the affected dermatome could be useful as a diagnostic tool for PNL.

**Objectives:** To evaluate the value of skin biopsy for the diagnosis of PNL using histopathology analysis.

**Results:** A total of 178 biopsy samples (nerves and skin samples, 89 each) obtained from 89 PNL patients diagnosed during 2004-2014 at the Souza Araújo Outpatient Clinic were examined. Histological examination using hematoxylin & eosin, Gomori trichrome and Wade stains were used to assess the presence of cutaneous nerves (observed in 75/89 of the samples) and acid-fast bacilli (positive in 6/75 of the samples). In 75 skin biopsy samples, 256 nerve fibers were found, 119 (47%) of which had alterations. The parameters used to define probable leprosy were: perineural and endoneural mononuclear inflammatory infiltrate, (21/75 and 10/75, respectively), epithelioid granuloma (9/75), and fibrosis (23/75). Histopathological alterations commonly present in nerve biopsies of other peripheral neuropathies were observed in the PNL skin biopsies: Perineural thickening (16/75, 21%), Schwann cell proliferation (10/75, 13%), and microfasciculation (2/75, 3%). No alterations were observed in 25/75 (33%) samples. Contingence concordance was evaluated in 47 skin and nerve biopsy samples where at least one of the selected histopathologic parameters was observed (The Table).

**Conclusions:** These preliminary results suggest that skin biopsy may be a useful tool to aid PNL diagnosis.

**Table:** Comparison between nerve and skin biopsies (47 samples each) findings regarding the parameters selected to define probable leprosy. The biopsies were obtained from 47 patients with pure neural leprosy diagnosed at the Souza Araújo Outpatient Clinics.

Parameters	Total concordance (Presence or absence in both skin and nerve samples)	Total discordance (Presence or absence in either skin or nerve samples)
Positive for acid-fast bacilli	34 (72%)	13 (28%)
Mononuclear inflammatory infiltrate	19 (40%)	28 (60%)
Epithelioid granuloma	23 (49%)	24 (51%)
Fibrosis	26 (55%)	21 (45%)

**Financial support:** CNPq, FAPERJ

#### **24. Leprosy ulnar neuropathy evaluation by nerve conduction study.**

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**Background:** ulnar neuropathy is caused by Leprosy and focal compressions. But it may also be found in other diseases, rendering the differential diagnosis a challenge. The nerve conduction studies (NCS) are used for the objective and early evaluation of neuropathy. In addition, they allow inferring the physiopathological process.

**Objective:** to establish NCS characteristics in order to differentiate leprosy from non-leprosy ulnar neuropathy.

**Results:** NCS from patients diagnosed with leprosy (n=93) were compared with those from patients diagnosed with other diseases (n=177). Age older than 60 years influenced sensory parameters, specially producing amplitude reduction. The main electrophysiologic characteristics identified for leprosy ulnar neuropathy were: absence of sensory conduction, bilateral motor conduction alteration and higher frequency of demyelination over axonal lesion in the motor ulnar nerve conduction. The motor NCS parameters used to define demyelination, among them, conduction velocity, were different between the groups, except for the elbow segment. In addition, conduction block and, specially, temporal dispersion, on their own, observed at the forearm and elbow segments, were also characteristic of leprosy ulnar neuropathy. Regarding the cutaneous and pure neural forms of leprosy, neuropathic lesion was predominant in similar proportion in both of them. Besides this type of lesion, in the sensory NCS, only axonal lesion was observed. In conclusion, from the electrophysiologic point of view, leprosy ulnar neuropathy is unique, independent from being of cutaneous or pure neural forms of the disease, in spite of the latter being more severe.

**Conclusions:** NCS was a useful tool for differentiating leprosy from non-leprosy ulnar neuropathy, mainly of the motor nerve conduction, by means of the isolated and combined analysis of the various NCS components.

**Financial support:** CNPq, Fiotec

**25. Clinical and neurophysiological features of leprosy patients with neuropathic pain**

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Nerve pain is a frequent symptom in leprosy patients. It can be shown as neuropathic pain (NP) or nociceptive pain in leprosy reaction, as neuritis. Paucity data to correct diagnosis results in common prescriptive errors when neuritis confuse with NP and vice versa.

The present study identified demographic, clinical and neurophysiological features of 42 patients with leprosy neuropathy presenting criteria for NP. Patients were selected, asking about the presence of pain during routine evaluation. Data analysis about pain characteristics, DN4 (Douleur Neuropathique 4 Questionnaire) scale score and Hamilton depression scale score, as well as clinical and neurophysiological exams, such as electromyography, quantitative sensory testing, laser-doppler flowmetry and contact heat evoked potential stimulator, were used to characterize these patients. Burning is the most common word for pain description, in 25 (60 %) patients. Despite of normal neural conduction, 14 (33 %) patients had NP. In the early stages of the disease, even before leprosy diagnosis, 19 (45 %) patients have already had NP and treatment did not prevent the occurrence of pain in 15 (36 %) patients.

Leprosy reactions are considered risk factors to NP and occurred in 32 (76 %) cases. Knowledge of characteristics that differ pain in acute neuritis and exacerbation of chronic NP in leprosy reactions is essential to avoid wrong treatment of pain and excessive use of steroid and its side effects.

**Financial support:** FAPERJ; FIOTEC.

**26. *Mycobacterium leprae* hooked to Schwann cell metabolism leads to leprosy neuropathy**

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Axons are huge cellular structures that demand an enormous bioenergetics metabolism for maintaining its physiological properties. This impressive task is accomplished by the combined delivery of energy cargos from neuronal cell body and by axon-associated glial cells. While reports have shown that metabolic provisions provided by oligodendrocytes seem to be essential for central nervous system axonal support, it remains unclear whether

or not Schwann cells, the wrapping peripheral nervous system glia, plays a role for axonal integrity during developing and neurodegenerative disease. Our laboratory is keenly interested in the understanding of Schwann cells metabolism and how the infection by *Mycobacterium leprae* alters this energy consumption/production balance. We recently demonstrated that Schwann cell infection by *Mycobacterium leprae* led to modulation in glycolysis and lactate production *in vitro* and *in vivo*. Taking into consideration that axon loss is a common hallmark observed in leprosy neuropathy, we aimed to explore, in the present study, the expression of key metabolic regulators involved in energy production. We also addressed changes in axonal and Schwann cell mitochondrial profiles morphology. Our preliminary data suggests important alterations in the expression profile of key genes involved in glucose and lactate production, including LDHA, G6PD, Malic enzyme, GSR, among others, in nerve biopsies obtained from patients suffering from leprosy neuropathy when compared to nerves harvested from patients undergoing non-leprosy neuropathies. In addition, by comparing mitochondrial morphology in leprosy and non-leprosy nerve tissue, we observed a 77%-fold increase in mitochondria area that were localized within myelinated and non-myelinated axons in the leprosy group. In sum, our preliminary results points to the notion that *Mycobacterium leprae* infected Schwann cells suffer an energy metabolism modulation and this feature might be linked to the extensive axonal degeneration seen in patients diagnosed with leprosy neuropathy.

**Financial support:** CNPq / Faperj

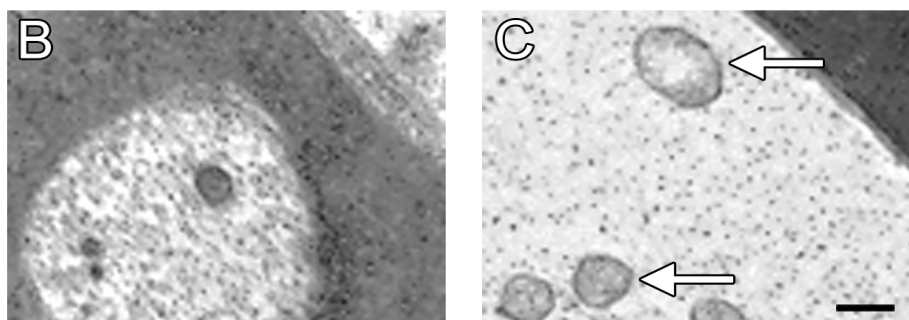


Fig.1. Ultrathin sections obtained from nerve biopsies harvested from non-leprosy patients (B) and leprosy neuropathy individuals (C) showing the marked swelling in mitochondrial profiles localized within a myelinated axon (arrows).

### 27. Post-MDT leprosy neuropathy: differentially diagnosing reactional neuritis and relapses.

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In leprosy, aggravation of newly appearing nerve damage emerging after MDT poses difficulty to differential diagnosis, between reactional neuritis and relapse. In addition, a neuropathy other than leprosy can not be ruled out. The confirmation of a relapse requires retreatment, however, the criteria for relapse diagnosis with this presentation have not been clearly established in the literature yet.



The objective of this study was to ascertain the role of the nerve biopsy in the differential diagnosis of the post-MDT leprosy neuropathy based on histological alterations across the selected groups.

We examined 50 post-MDT nerve biopsy samples (23 reactional neuritis and 27 relapse samples). The nerve sampling was guided by a careful clinical evaluation and the finding of neuroelectrophysiological alterations, such as disturbances of conduction velocity and/or action potential amplitude. Relapse diagnosis was suspected in face of persistence of the symptoms more than 5 years after treatment, refractoriness to anti-reactional treatment, aggravation of the impairment of neurological function. Using these criteria, reactional neuritis was assigned to the nerve sample whenever AFB was absent and relapse, when AFB was found in the nerve. The presence x absence of each histopathological alteration was attributed to each nerve sample examined. The following histological alterations were more frequently found in the relapse than in the reactional neuritis group: perineurial infiltrate ( $p < 0.05$  and ); foamy macrophages ( $p < 0.00000$ ); epineurial, perineurial and endoneurial fibrosis ( $p < 0.02$ ); perineurial hyperplasia ( $p < 0.04$ ), reduction in the number of myelinated nerve fibers in both paraffin ( $p < 0.004$ ) and epon-embedded sections ( $p < 0.03$ ). No significant differences were found in respect of epithelioid granuloma, remyelination, axonal a regeneration of nerve fibers across the groups. The differences in the frequency of alteration across the samples could reflect distinct pathobiological courses of reactional neuritis versus relapse, however a more in depth knowledge of biology of *M leprae* infection is required to interpret these results.

We could conclude that nerve biopsy is useful as a tool for the differential diagnosis of post-MDT peripheral neuropathy given that detection of acid-fast bacilli in the samples may favor the decision to relapse; however this decision should be strongly supported by clinical and neuroelectrophysiological data.

### **28. Analysis of oxidative stress in Schwann cells during infection with *Mycobacterium leprae***

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**Background:** Leprosy is still endemic in many underdevelopment countries like Brazil, which occupies the second place in leprosy new cases reported worldwide. The bacillus is the only known bacteria capable of infect the peripheral nervous system, mainly affecting macrophages and Schwann cells, leading to segmental demyelination and axonal death. In parallel with this project, our group observed increased activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD), besides producing pentoses, CO<sub>2</sub> and NADPH, this route is important for the protection of cells against oxidative stress and in the regeneration of glutathione system. Cells exposed to oxidative stress feature damage to lipids, proteins and DNA, as well as the depletion of ATP levels, causing failure in cellular functioning and it can cause necrosis. Glutathione reductase represents the main defense against free radicals, which has been identified as an important metabolic insult among leprosy patients.

**Objectives:** This study aims a systematic analysis of the redox potential, the free radicals generation and cellular defenses during *M. leprae* infection.

**Results:** Our data showed that *M. leprae* infection is not able to induce oxidative stress, on the opposite, the infection protect Schwann cells from hydrogen peroxide insult. This effect is probably attributed by increase of pentose phosphate pathway and glutathione system, due to the fact that was avoided by G6PD inhibitor 6ANAM. . The protein carbonyl amount determined among leprosy patient's sera demonstrates that besides the *in vitro* infection confer protection; these patients are suffering oxidative stress.

**Conclusions:** *M. leprae* is able to decrease the formation of reactive oxygen species in Schwann cells *in vitro*, but leprosy patients presents high levels of protein carbonyl in sera, indicating that *in vivo* the oxidative stress is stronger than the anti-oxidants defenses, probably due to the immune system response.

### **29. *Mycobacterium leprae* Modulates Glucose Uptake and Metabolism in The Host Cell**

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Leprosy is a chronic neurodegenerative disease caused by *Mycobacterium leprae* (ML), an intracellular pathogen able to infect Schwann cells (SC) of the peripheral nervous system. These cells are responsible for myelination and release of metabolites to axons and signal transduction in peripheral nerves. The host-pathogen interaction in leprosy showed a role for immune-mediated demyelination that leads to nerve injury, nonetheless other pathways are suggested to participate in the neural lesion, *i.e.*, lipid and energetic metabolism (glycolysis and respiratory chain).

Thus, we performed an *in vitro* study to assess metabolism in the human SC cell line ST8814 infected with ML and evaluated glucose uptake and cleavage; mitochondrial electrical potential; consumption of oxygen and lipid biosynthesis.

We demonstrated that infected cells increased by 2-fold the uptake of 2-NBDG accompanied by increased expression of the *slca2a1* gene which encodes important glucose transporter (GLUT-1). Moreover, we found an increase in glucose 6-P dehydrogenase (G6PD) expression and activity, which is a key enzyme in the pentose pathway. The fluorescence of TMRM suggested a reduction of mitochondrial electrical potential and decrease in basal oxygen consumption in ML-infected SC, indicating mitochondrial dysfunction. Interestingly, ATP citrate lyase, a key enzyme between glucose metabolism and biosynthesis of lipids, showed a 3-fold increased activity. Finally, the use of a G6PD inhibitor (6-ANAM) was able to reverse metabolism modulation observed during infection.

These results allow a better understanding of the mechanisms that generate the success of ML as well as the observed nerve damage. In addition, since ML causes subversion of SC glucose metabolism, the pentose pathway could be a new target to control leprosy. Also, there are common patterns associating leprosy and other infectious or non-infectious

neurodegenerative diseases such as parkinson's, diabetes and it is likely that regulation of this pathway help to create insights for treatment of these pathologies

**Financial support:** CNPq, FAPERJ, CAPES (Brazil)

### **30. Neonatal breast overfeeding is neuroprotective in an infant rat model of pneumococcal meningitis**

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**Background:** Bacterial meningitis (BM) causes cortical necrosis and hippocampal apoptosis leading to permanent neurological sequelae. Microglia-mediated inflammation has been identified as the primary mediator of the neuronal dysfunction observed in BM. It is noteworthy that obesity may cause cognitive decline, a process involving excessive microglia activation. Considering that overweight can activate the inflammasome and *prime* the immune system, we hypothesized that neonatal overweight influences neuroinflammation and hippocampal neuron loss in BM.

**Objective:** We assessed the effect of neonatal overweight on neuronal apoptosis in the hippocampal dentate gyrus in an infant rat model of pneumococcal meningitis.

**Results:** Overweight was successfully induced by manipulating the litter size (four pups/dam, overfeeding; or 10 pups/dam, normal feeding). At the 11<sup>th</sup> day after birth, the overfed animals were 34.8% fatter than the controls ( $P < 0.001$ ). The 11-day old rats were intracisternally infected with 10  $\mu$ L of a suspension containing  $\sim 3 \times 10^6$  c.f.u. *S. pneumoniae*, or saline. Quantitative culture of cerebrospinal fluid (CSF) at 18 hours p.i. showed no difference in bacterial titer between overfed and normal fed animals. All animals were then treated with ceftriaxone (100mg/kg s.c.). Twenty-four hours p.i., animals were euthanized, the right brain hemisphere was post-fixed in 4% paraformaldehyde and the apoptotic cells in the granular layer of the dentate gyrus were counted in Nissl-stained microscope slides. Hippocampi were removed from the left hemispheres and dissected into two halves, for further RNA and protein analysis. Overfed infant rats had 50% less apoptotic cells in their dentate gyrus than controls ( $P < 0.05$ ). There was no correlation between apoptotic cell counts and bacterial titer in the CSF.

**Conclusions:** Neonatal overfeeding is neuroprotective in our infant rat model of pneumococcal meningitis. We are now investigating molecular biomarkers of microglia and inflammasome activation, which could be underlying the neuroprotective effect of breast overfeeding.

**Financial support:** CNPq (400046/2013-0), FAPEMIG (CBB – APQ-0266114), and FioCruz.

**31. Decreased kynurenic acid: quinolinic acid ratio in the cerebrospinal fluid of children suggests a deleterious role for the kynurenine pathway in bacterial meningitis**

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**Background:** Acute bacterial meningitis (BM) causes excessive activation of N-methyl-D-aspartate (NMDA) receptors leading to cortical and hippocampal neuron death. As opposite, enteroviral meningitis is most often benign. The kynurenine (KYN) pathway is the major catabolic route of tryptophan (TRP) and some of its metabolites are neurotoxic while others can be neuroprotective. Most important, kynurenic acid (KYNA) is an antagonist of the NMDA receptors and protects neurons from excitotoxic brain damage in experimental BM, while quinolinic acid (QUINA), the last metabolite in the KYN pathway, is agonist of NMDA receptors.

**Objectives:** We aimed to shed light on the role of the KYN pathway in the pathophysiology of acute meningitis by assessing the levels and ratios of its key metabolites in cerebrospinal fluid (CSF) samples of infant patients (median age: 2 years-old; range: <1 to 11 years) with meningococcal (n = 7), pneumococcal (n = 8), and enteroviral meningitis (n = 10), and controls (n = 11).

**Results:** CSF concentrations of TRP, KYN, 3-hydroxykynurenine (3-OH-KYN), 3-hydroxyanthranilic acid (3-OH-AA), KYNA, xanthurenic acid (XA) and QUINA were measured simultaneously on an Ultra High Performance Liquid Chromatography system hyphenated to a high-resolution ESI-QTOF mass spectrometer. When compared to controls, TRP:KYN, and 3-OH-KYN:3-OH-AA ratios were decreased and KYN:3-OH-KYN were increased in pneumococcal and enteroviral meningitis, while KYN:KYNA and 3-OH-AA:QUINA were increased, and KYN:3-OH-AA ratios were decreased in the three forms of the disease. Only patients with BM showed decreased KYNA:QUINA ratio. There was no correlation between the ratios of any pair of KYN metabolites and the standard cytochemical parameters tested.

**Conclusion:** Infection of the CNS with meningococci, pneumococci and enteroviruses leads to a preferential up regulation of the neurotoxic branch of the KYN pathway within the intrathecal space, but only BM decreases KYNA:QUINA ratio, possible contributing to a neurotoxic condition.

**Financial support:** CNPq (400046/2013-0), FAPEMIG (CBB – APQ-0266114), and Fiocruz.

**32. Comparative proteomics of cerebrospinal fluid reveals a predictive model for differential diagnosis of pneumococcal, meningococcal, and enteroviral meningitis, and novel putative therapeutic targets**

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**Background:** Meningitis is the inflammation of the meninges in response to infection or chemical agents. While aseptic meningitis, most frequently caused by enteroviruses, is usually benign with a self-limiting course, bacterial meningitis remains associated with high morbidity and mortality rates, despite advances in antimicrobial therapy and intensive care. Fast and accurate differential diagnosis is crucial for assertive choice of the appropriate therapeutic approach for each form of meningitis.

**Objectives:** We used 2D-PAGE and mass spectrometry to identify the cerebrospinal fluid proteome specifically related to the host response to pneumococcal, meningococcal, and enteroviral meningitis. The disease-specific proteome signatures were inspected by pathway analysis.

**Results:** Unique cerebrospinal fluid proteome signatures were found to the three aetiological forms of meningitis investigated, and a qualitative predictive model with four protein markers was developed for the differential diagnosis of these diseases. Nevertheless, pathway analysis of the disease-specific proteomes unveiled that Kallikrein-kinin system may play a crucial role in the pathophysiological mechanisms leading to brain damage in bacterial meningitis. Proteins taking part in this cellular process are proposed as putative targets to novel adjunctive therapies.

**Conclusion:** Comparative proteomics of cerebrospinal fluid disclosed candidate biomarkers, which were combined in a qualitative and sequential predictive model with potential to improve the differential diagnosis of pneumococcal, meningococcal and enteroviral meningitis. Moreover, we present the first evidence of the possible implication of Kallikrein-kinin system in the pathophysiology of bacterial meningitis.

**Financial support:** CNPq (474602/2009-5), CAPES, FAPEMIG, and Fiocruz.

**33. Resveratrol acts anti-inflammatory and neuroprotective in an infant rat model of pneumococcal meningitis**

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**Background:** Resveratrol (RSV) has anti-inflammatory properties and cross the blood–brain barrier with a good safety profile. RSV up-regulates ApoA-I, a protein that inhibits the LPS-induced activation of IL-1 $\beta$  and TNF- $\alpha$  in serum, and up-regulates CCL2, CCL3 and IL-6 transcription in monocyte-derived macrophages. We have previously reported increased levels of APOA-I in the cerebrospinal fluid of patients with bacterial or enteroviral meningitis. Thus, we hypothesized that RSV is neuroprotective in pneumococcal meningitis by up-regulating ApoA-I in the brain.

**Objective:** We tested the anti-inflammatory and neuroprotective potential of RSV as adjuvant therapy in an infant rat model of pneumococcal meningitis. Also, we assessed the effects of adjuvant RSV on *ApoA-I* expression in the hippocampus.

**Results:** Eleven-day old rats were intracisternally infected with *S. pneumoniae* (~2x10<sup>6</sup> c.f.u.) or saline, and were orally administered with adjuvant RSV (50mg/kg) or vehicle (carboxymethyl cellulose, 10g/L) in pre- or post-treatment (three and 18h p.i.). At 18h p.i., infection was documented by quantitative culture of cerebrospinal fluid and all animals received ceftriaxone (100mg/kg s.c.). At 24h p.i. animals were euthanized and apoptotic cells were counted in the granular layer of the dentate gyrus in Nissl stained microscope slides from the right brain hemisphere. The hippocampus from left hemisphere was used for mRNA and protein analysis. RSV-treated infected rats had significantly lower apoptotic cell counts and hippocampal protein levels of CCL3, CCL2 and IL-1beta when compared to infected animals receiving placebo. *ApoA-I* transcription was not influenced by meningitis or adjuvant RSV, whereas APOA-I were increased > 28-fold by meningitis (P<0.001), but was not affected by RSV.

**Conclusion:** Adjuvant RSV is anti-inflammatory and neuroprotective in experimental pneumococcal meningitis. RSV neuroprotective effects in BM does not involve the up-regulation of APOA-I. We are now investigating alternative pathways that could elucidate the mode of action of RSV in in pneumococcal meningitis.

**Financial support:** CNPq (400046/2013-0), FAPEMIG (CBB – APQ-0266114), and Fiocruz.

**34. Adjuvant vitamin B<sub>12</sub> attenuates apoptosis in the granular layer of hippocampal dentate gyrus in an infant rat model of pneumococcal meningitis**

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**Background:** Perturbations in methyl bioavailability are associated with neurological disorders. The reduction of the methylation capacity is due to the accumulation of S-adenosylhomocysteine, an inhibitor of methyltransferases that uses S-adenosyl methionine as donor of methyl groups. Homocysteine (Hcy) can be remethylated in a process dependent of folic acid and vitamin B<sub>12</sub>, or be withdrawn from the methylation cycle by its conversion to cysteine, in a two-step transulfuration pathway that requires vitamin B<sub>6</sub>. Our group has shown that Hcy cerebrospinal fluid levels are significantly higher in children with acute bacterial meningitis than in children without infection in the central nervous system. Considering that vitamins B<sub>6</sub> and B<sub>12</sub> are essential cofactors of key enzymes in the Hcy metabolism, we hypothesized that adjuvant therapy with these vitamins are neuroprotective in pneumococcal meningitis.

**Objective:** We investigated the neuroprotective potential and the effect on Hcy homeostasis of adjuvant post-treatment with B<sub>6</sub> and B<sub>12</sub> in experimental pneumococcal meningitis.

**Results:** Eleven-day old rats were intracisternally infected with *S. pneumoniae* (~2x10<sup>6</sup> c.f.u.) or saline. Infected and sham-infected animals were post-treated with intramuscular B<sub>12</sub> (6.25mg/kg), B<sub>6</sub> (125mg/kg), or B<sub>6</sub>+B<sub>12</sub> (125mg/kg and 6.25mg/kg, respectively) at 3h and 18h post-infection. At 18 hours p.i., bacterial titers were documented by quantitative culture of cerebrospinal fluid and all animals received ceftriaxone (100mg/kg s.c.). At 24h p.i. animals were euthanized and apoptotic cells were counted in the granular layer of the dentate gyrus in Nissl stained microscope slides from the right brain hemisphere. The hippocampus from left hemisphere was removed for further analysis. Adjuvant B<sub>12</sub> reduced apoptosis in 40% ( $P<0.001$ ) compared to placebo. B<sub>6</sub>+B<sub>12</sub> had no effect, and all infected rats treated with B<sub>6</sub> died before 24h p.i.

**Conclusion:** Adjuvant B<sub>12</sub> in post-treatment was neuroprotective in our infant rat model of pneumococcal meningitis. We are now assessing the hippocampal levels Hcy metabolites.

**Financial support:** CNPq (400046/2013-0), FAPEMIG (CBB – APQ-0266114) and Fiocruz.

### **35. Inhibition of thromboxane synthase reverses cerebral ischemia in murine cerebral malaria by *Plasmodium berghei* ANKA**

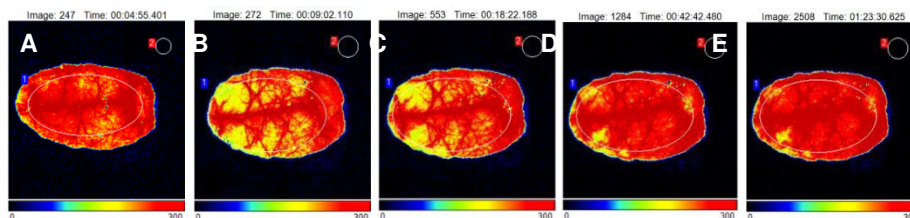
Aline da Silva Moreira<sup>1</sup>, Vanessa Estado de Freitas Almeida<sup>2</sup>, Cesare Bianco- Júnior<sup>1</sup>, Claudio Tadeu Daniel-Ribeiro<sup>1</sup>, Eduardo Vera Tibiriçá<sup>2</sup>, Leonardo José de Moura Carvalho<sup>1</sup>

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**Background:** Cerebral malaria (CM) is one of the most severe complications of *Plasmodium falciparum* infection, and responsible for the death mainly of children under 5 years of age. The mainstay treatment for CM is intravenous artesunate, nevertheless 15-20% of the patients receiving this drug still die and 5-30% of those who survive may show neurological sequelae. Therefore, it is necessary to understand the mechanisms of CM pathogenesis to develop adjuvant therapies. Vascular dysfunction characterized by low bioavailability of nitric oxide (NO), a key mediator of vascular tone, and by vasoconstriction leads to decreased cerebral blood flow with consequent ischemia and tissue hypoxia that ultimately leads to death in CM. However, other mechanisms may be involved in this dysfunction. Arachidonic acid (AA) metabolites are important regulators of physiological cerebral blood flow through its vasodilating and vasoconstrictive properties. In pathological conditions there is deregulation of the system, leading to a predominance of vasoconstrictor stimuli such as thromboxane A2 (TXA2) and to cerebral ischemia process.

**Objective:** To determine the effect of pharmacological inhibition of TXA2 synthesis on cerebral ischemia in mice with experimental cerebral malaria (ECM) by *Plasmodium berghei* ANKA (PbA).

**Results:** On day 6 after infection, animals that developed ECM showed a significant decrease in cerebral blood flow compared to non-infected control animals. The administration of ozagrel, an inhibitor of thromboxane synthase, which prevents the generation of the vasoconstrictor metabolite thromboxane A2, increased cerebral blood flow in animals with ECM (**Fig. 1**). Similar effect was observed with administration of L-arginine, a precursor of NO.



**Figure 1:** Representative images of cerebral blood flow demonstrated by Laser Speckle Contrast Image System (LSCI). **A:** Before infection; **B:** D6 post infection; **C:** After intervention with saline; **D** and **E:** After intervention with ozagrel.

**Conclusions:** Ozagrel and L-arginine show a potential role as adjuvant therapy for CM.

**Financial support:** Fiocruz, CAPES, FAPERJ.



**36. Infection of mouse neurospheres by *Toxoplasma gondii*: an in vitro model to investigate changes in neurogenesis during congenital toxoplasmosis**

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*Toxoplasma gondii* is an obligate, intracellular parasite belonging to the phylum Apicomplexa and affects up to a third of the world's population. *T. gondii* is commonly transmitted to virtually any warm-blooded vertebrate through oocysts expelled by its definitive host (felines) or bradyzoite-containing tissue cysts residing in undercooked meat. The tachyzoite form of the parasite is characterized by rapid proliferation inside the host cell and can cause acute disease associated with tissue destruction. In immune competent hosts, inflammatory stimuli induces tachyzoites to differentiate into bradyzoites, which form a tissue cyst, that remain quiescent for long periods. *T. gondii* acute infection during pregnancy cause congenital toxoplasmosis resulting in severe damage to the Central Nervous System, including blindness and hydrocephaly.

To assess the impact of infection on neuro/gliogenesis and the fate of intracellular parasites, we infected murine neural progenitor cells (NPCs) with tachyzoite forms of the avirulent ME49 strain of *T. gondii*. NPCs are maintained undifferentiated as neurospheres and can spontaneously differentiate into neurons, astrocytes and oligodendrocytes upon removal of the mitogens present in the culture media. Cultures were analyzed for parasite load and specific genes expression at different times using qRT-PCR and Confocal and Electron Microscopy.

Our preliminary analysis showed that infected NPCs have altered levels of connexin43 and pannexin1, both described as playing important roles on neurogenesis. In addition, we verified the presence of bradyzoites after 96 hours of infection, indicative of spontaneous stage-conversion – without any additional treatment such as INF- $\gamma$ , as described in other cell systems.

This model has proven to be a valuable tool to investigate *in vitro* the mechanisms involved in the pathogenesis of congenital toxoplasmosis and elucidate how this infection alters the correct development of the CNS.

**Financial support:** CNPq, Fiocruz, Faperj

**37. Histopathology and viral detection in the central nervous system of mice infected with DENV2 by the intracerebral route**

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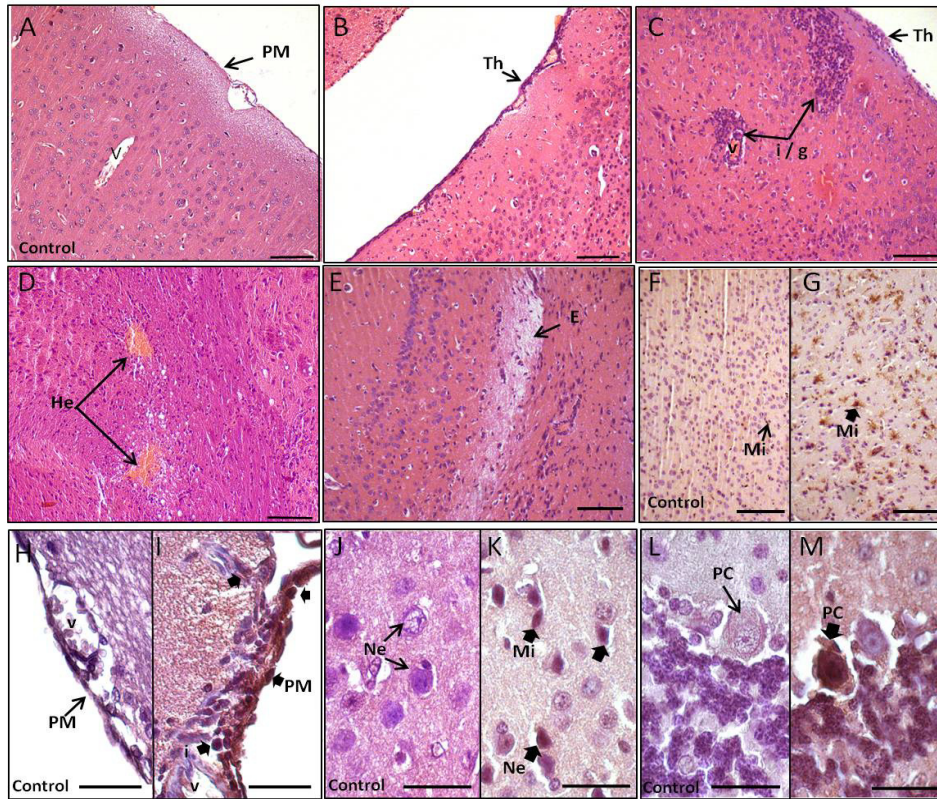
**Background:** One of a great difficulty to study dengue is the lack of an animal model that mimics the infection effects observed in humans. Mice inoculated by the intracerebral route (i.c.) with dengue virus (DENV) is a classical and one of the few immunocompetent murine model which leads to the manifestation of apparent infection signs. Morbidity, regarding neurological clinical signs, usually arises from the 7<sup>th</sup> day post infection (d.p.i.). In dengue human cases, in turn, involvement of the nervous system has been increasingly reported, indicating virus neurotropism.

**Objectives:** To evaluate histopathological effects in the brain and cerebellum of BALB/c mice inoculated by the i.c. route with a lethal dose of DENV2, with virus detection in these tissues.

**Results:** Hemorrhage and edema (fig. D, E) were detected in the nervous tissue of infected mice, mainly at the beginning of infection, while strong cellular response (reactive gliosis (fig. C), microglia hyperplasia and hypertrophy (fig. G), thickening of the *pia mater* (fig. B), mononuclear infiltrate in the neuropil and perivascular (fig. C)) was observed in latter time. High titers of infectious virus particles and viral RNA copies were detected in the nervous tissue by plaque assay and RT-PCR real time, respectively. Moreover, we verified the presence of the DENV2 NS3 antigen, a non-structural protein which detection indicates virus replication, in neurons (fig. K, M), microglia (fig. K), mononuclear infiltrate (figure I) and endothelial cells by immunohistochemistry assay. We also identified DENV2 in the serum, mainly between the 9<sup>th</sup> and 11<sup>th</sup> d.p.i.

**Conclusions:** Infected mice showed histopathological effects in the nervous tissue, similarly to human fatal cases, especially in late stages of infection. Furthermore, after the i.c. inoculation, infective virus particles were detected not only in the central nervous system but also in the circulation, suggesting that the DENV crossed the blood brain barrier allowing a peripheral infection.

**Financial support:** Fiocruz; IOC; FAPERJ; CNPq; PDTIS



**Figure: Histopathology and DENV2 replication detection in the nervous tissue of infected mice.** PM – pia mater; Th – thickening of the pia mater; i – mononuclear infiltrate; i/g – mononuclear infiltrate and reactive gliosis; He – hemorrhage; E – edema; Mi – microglia; Ne – neuron; PC – Purkinje cell. Large black arrow – NS3 detection. Bars = 100  $\mu$ m (A, B, C, D, E); 50  $\mu$ m (F, G); 20  $\mu$ m (H, I, J, K, L, M).

### **38. Sensitivity threshold sinusoidal electrical stimuli using Silver Spike Point electrode: A new perspective for neuropathies**

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*Oswaldo Cruz Foundation (Fiocruz)*

**Background:** Neuropathies can affect the sensory fibers differently, such as the nerve injury occurring as a consequence of diabetes mellitus and Hansen's disease. Therefore, assessing each kind of sensory fibers is an important contribution for the early diagnosis of a neuropathy. Sinusoidal electrical current can stimulate different types of sensory fibers depending on the frequency (3000 Hz stimulates A $\beta$  preferably, 250 Hz the A $\delta$ , A $\beta$  or C and 5 or 1 Hz the C). However, its selectivity is questionable, especially concerning to thinner fibers using the standard bipolar golden discs.

**Objectives:** The aim of this study is to investigate the selectivity of the stimulus for each type of fiber using Silver Spike Point (SSP) electrode. The sensitivity threshold (LS), cognitive response (RCg) and reaction time (RT) were evaluated in 30 volunteers using sinusoidal electrical stimulation at frequencies of 3.000, 250, 5 and 1 Hz.

**Results:** The RT showed a downward trend concerning to frequencies. There was no significant difference in LS between standard bipolar golden discs and SSP electrodes. On the hand, the latter showed higher selectivity in the RCg (86.4%) than standard electrodes (52.0%) for the thinner fibers, at frequencies of 5 and 1 Hz.

**Conclusions:** Silver Spike Point electrodes improved the assessment of thinner sensory fibers. Therefore, the use of such kind of electrode contributes for the study, diagnosis and evolution of neuropathy indicators. As next step of this study, the method will be assessed in patients from the Hansen's disease Laboratory of the Oswaldo Cruz Foundation (Fiocruz).

**Financial support:** CNPq, FINEP, CAPES and FAPERJ.

**Table:** Cognitive response using the Standard electrodes and SSP electrodes, in the frequency of 3.000, 250, 5 and 1 Hz (n=30).

Perception	3000 Hz	250 Hz	5 Hz	1Hz
<b>vibration</b>				
<b>pressure</b>	Standard: 76.4%	Standard: 35.2%	Standard: 11.6%	Standard: 0%
<b>pricking</b>	SSP: 59.2%	SSP: 27.2%	SSP: 0%	SSP: 0%
<b>contraction</b>				
<b>sting</b>				
<b>needle</b>	Standard: 11.7%	Standard: 35.2%	Standard: 58.8%	Standard: 52.0%
<b>burning</b>	SSP: 27.2%	SSP: 46.2%	SSP: 86.4%	SSP: 86.4%
<b>itch</b>				
<b>Reports of both groups</b>	Standard: 11.9%	Standard: 23.4%	Standard: 17.6%	Standard: 29.4%
	SSP: 13.6%	SSP: 20.1%	SSP: 13.6%	SSP: 13.6%
<b>Did not know how to describe</b>	Standard: 0%	Standard: 6.2%	Standard: 12%	Standard: 18.6%
	SSP: 0%	SSP: 6.5%	SSP: 0%	SSP: 0%

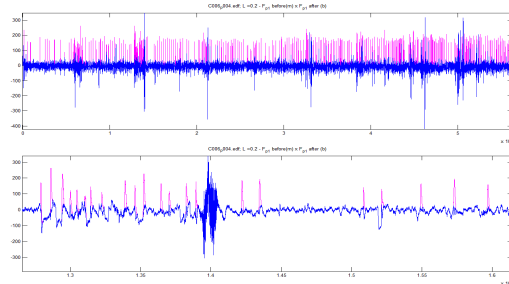
### 39. *Filter-blink*: a methodology for eliminating vertical electrooculogram artifact by statistical pattern detection.

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**Background:** Eye motion during the blink can be a significant artifact in the ERP analysis (mainly if ERP is time locked to blinks). Blinks produce a typical positive potential in the vertical electrooculogram (VEOG), with high amplitude and symmetrical pattern in frontopolar derivations and spreading with amplitude reduction towards posterior direction. Two methods are used to suppress the VEOGs. The more conventional and widespread one adopts linear regression to subtract the VEOG signal of each EEG channel. The second method, which uses Independent Component Analysis (ICA), is considered the most adequate but it removes, together with VEOGs, some EEG information, especially in setups with few channels.

**Objectives:** To develop a new statistical method for suppressing VEOGs, which (1) identifies the position of blinking in the frontopolar channels in time, (2) uses these positions to average EEG, resulting a 'blink template' for each channel; (3) subtracts, for each channel, the templates from respective EEG segments (when blinks happened), when the linear correlation index between the template and the segment is greater than 'r'.



**Results:** The method suppressed nearly 90% of the VEOGs in the frontopolar signals (see figure, blue after application). For  $r = 0.6$ , after suppression, the averages of the EEG epochs triggered by the blink positions ranged to zero volt for all channels. The filter did not alter the waveforms of the ERPs of interest. The remaining noise after suppression was non-linear.

**Conclusions:** The method is effective for VEOG suppression without distortion of the EEG.

#### 40. RELEVANCY AND RECENT EVOLUTION TRENDS IN BRAZILIAN NEUROSCIENCE RESEARCH NUMBERS: PRELIMINARY SURVEY USING THE BRAZILIAN CLINICAL TRIALS REGISTRY (ReBEC) DATABASE

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#### BACKGROUND

Given the projection of Neuroscience1 on the global research scene, a preliminary survey of Brazilian studies in this area is propitious - especially regarding those in the advanced stages, which knowledge is more consolidated for use as evidence.

The Brazilian Clinical Trials Registry (ReBEC) database's features make it relevant to strategic prospecting: contains studies already evaluated in ethical and methodological terms; it is public, free, open access, bilingual (English/Portuguese) and its data is exported to the International Clinical Trials Research Platform (WHO), contributing to the worldwide visibility of Brazilian research. It is the largest in Latin America & Caribbean region competing only with the Clinical Trials (CT.gov/EUA) for the preference of Brazilian researchers.

**OBJECTIVES**

To describe the frequency of studies in the neurosciences area between 01/2011 and 11/2015, in ReBEC's database.

**RESULTS**

General descriptors Mental and behavioral disorders and the Central Nervous System Diseases were used to search ReBEC's total database and the approved studies data. Among the approved studies, 13.78% have one of these descriptors, or both. The growth of TMC research is constant, although the volume is less than DSN. TMC studies vary in the period, with growth peaks between 2012 and 2015<sup>2</sup> (Figure 1).

**CONCLUSIONS**

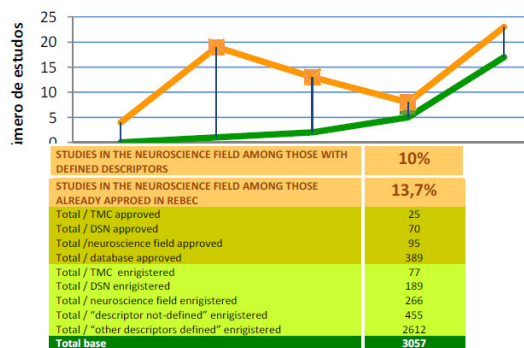
The positive growth in the number of studies in the Neuroscience knowledge area evidenced the opportunity to strengthen its potential with financial support, intra/inter-institutional collaboration -as well as knowledge transparency, using thematic repositories and ICTRP primary registries. Additionally, these are underreporting numbers, as Rebec only became mandatory from July 2012 and there are other studies awaiting approval. The creation of a Neuroscience National Institute in Brazil, allied with protocols' transparency, extends and gives visibility to this field in Brazil.

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<sup>1</sup> As described by Lent (2002), Neuroscience studies the physical realization of the information process in the animal and human nervous systems. It encompasses three major areas: neurophysiology, neuroanatomy and neuropsychology

<sup>2</sup> The registration in Rebec was the only one accepted by the Brazilian regulation between July 2012 and February 2015, which suggests that in this period the competition with ClinicalTrials.gov was much smaller and more studies registrations were made uniquely in the Brazilian database.

**Figura 1. Number of approved studies in ReBEC that use “Mental and Behavioural Disorders” and “Diseases of the Nervous System” ICD descriptors - from 2011 to 2015 Brasil**



TMC - Transtornos Mentais e Comportamentais/Mental and Behavioural Disorders; DSN – Doenças do Sistema Nervoso Central/ Diseases of the Nervous System; apr – approved studies.  
Elaborado pelos autores.

### DISPLAY

Digital or paper.

### FINANCIAL SUPPORT

Institutional (ReBEC/Fiocruz)

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