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RESUMOS

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Sumário

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I Congresso Cepid Brainn

Palestras

Trabalhos/Neurotecnologia

Trabalhos/Neuroeducação

Trabalhos/Neurociência

Editorial

This issue brings the abstracts of the works presented at the 1st BRAINN Congress, held at the University of Campinas (Unicamp), Campinas, SP, on April 14th and 15th of this year. Brainn stands for "Brazilian Institute of Neuroscience and Neurotechnology". It is an initiative financed by Fapesp (São Paulo State Research Foundation) within its Cepid program (Cepid is the acronym, in Portuguese, for Research, Innovation and Dissemination Centers). The Brainn initiative aims at developing new technological products, such as neural probes, brain-computer interfaces, novel neuroimaging (acquisition and analysis) techniques, bioinformatics analysis tools, to serve as diagnostic, prognostic and treatment solutions for epilepsy and stroke, while keeping in mind important neurobiological questions, and disseminating knowledge in Neuroscience and Neurotechnology to the community. Brainn is thus composed of a highly multidisciplinary team coming from universities and research centers of São Paulo, as well as from other Brazilian regions and from some international partners (for more information, please visit www.brainn.org.br). The abstracts are divided in three categories: Neuroscience, Neurotechnology and Neuroeducation. The Neuroscience set includes genetic studies of the molecular mechanisms of epilepsy; studies using animal models of epilepsy; neuroimaging studies of cortical thickness in different diseases; functional neuroimaging studies to estimate connectivity among different brain regions; neuropsychological studies to assess memory impairment, depression, and other psychiatric disorders in epilepsy; assessment of novel techniques for studying and/or monitoring the brain, such as the use of near-infrared spectroscopy in the surgical room or in brain-computer interfaces, the use of texture analysis for classification of white matter lesions, and the use of MR spectroscopy to evaluate GABA variations. The Neurotechnology set brings works describing the development of novel software packages for EEG-fMRI data analysis, for neuroimaging data visualization and segmentation, for sequencing data quality control; also works describing the development of new hardware for brain assessment such as micro neural probes, a near-infrared spectroscopy system, and brain-computer interfaces; and the potential use of virtual reality as an aid tool for motor rehabilitation and neural plasticity. Since Neuroscience knowledge dissemination is a must in this initiative, two works in the area of Neuroeducation describe a task to teach Neuroscience to adolescents and another initiative to bring information on epilepsy (through the Out of the Shadows campaign) to school teachers and students of a distant community in Maranhão State. Although all works presented in the Congress were of high quality, three were selected as best works of the conference. These were the work of Spagnol et al., related to the Out of the Shadows campaign; the work of Herrera et al., that used a complex network model to assess the difference in brain connectivity from going from resting state to a language task; and the work of Lima et al., who assessed different commercial kits for microRNAs extraction. We, from the Brainn initiative, expect that all the works presented here bring useful information to the readers and show some of the things we intend to achieve with this project.

Programação

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Dr. Romis Faissol Attux, DCA, FEEC

Friday, April 11th, 2014

08:00AM to 05:00PM - First meeting on Neuroscience and Inclusive Education
5:00 PM - Book Launch "TECLA SAPIENS"
08:00AM to 05:00PM - Neuroscience Museum Visit

Saturday, April 12th, 2014
Pre - Congress Courses

08:00M to 12:00PM - Introduction to Computation Biology and Bioinformatics "RNA-Seq on Neurogenetics Data" - Dr. Benilton Carvalho
08:00AM to 12:00PM - Diffusion MRI: from concepts to applications – Dr Leticia Rittner
02:00PM to 06:00PM - Molecular Biology Applied to Neuroscience - Dr.André Vieira
02:00PM to 06:00PM - State of the Art in Artificial Consciousness - Dr.Ricardo Gudwin
02:00PM to 06:00PM - Computational Modeling of Human Memory - Dr.Ricardo Gudwin

Monday, April 14th, 2014

Neurotechnology Session

08:30AM to 9:00AM - Welcome ceremony
09:00AM to 10:00AM - Dr. Bruce Pike (keynote lecture)
10:00AM to 10:30AM - Guilherme Beltramini (Brainn experiments with fMRI and EEG-fMRI)
10:30AM to 11:00AM - Coffee break
11:00AM to 11:20AM - Dr. Rickson Mesquita (Measuring blood flow with optics)
11:20AM to 11:40AM - Dr. Roberto Panepucci (Microfabrication of Neural Probes)
11:40AM to 12:00PM - Medical Imaging – Dr. Jorge V.L. Silva, Dr. Wu Shin Ting, Dr. Roberto Lotufo, Dr. Leticia Rittner
12:00 PM to 2:00 PM - Poster Session

Lunch
2:00 to 4:30 PM - Mini-symposium on brain-computer interfaces, rehabilitation technologies and artificial consciousness
2:00 to 2:10 PM - Investigation of BCI paradigms using fMRI – Gabriela Castellano
2:10 to 2:20 PM - Applications of functional NIRS to brain control – Rickson Mesquita
2:20 to 2:50 PM - Feature extraction and classification of brain signals for BCI applications I – Diogo Soriano
2:50 to 3:10 PM - Feature extraction and classification of brain signals for BCI applications II – Romis Attux
3:10 to 3:30 PM - Coclustering for high level knowledge of brain function – Fernando Von Zuben
3:30 to 4:00 PM - Smart Environments for Accessibility – Eleri Cardozo
4:00 How Big Data and High Performance Computing is transforming Healthcare and Life Sciences
4:00 to 4:20 PM - Conscious and unconscious processes in artificial behavior generation – Ricardo Gudwin
4:20 to 4:30 PM - Virtual reality for motor rehabilitation – Alexandre Brandão
4:30 to 5:00 PM - Intel speaker (How Big Data and High Performance Computing is transforming Healthcare and Life Sciences)
5:00 to 6:30 PM - Meet the experts

Tuesday, April 15th, 201

Neuroscience and Neuroeducation Session

Roundtable - Neurogenetics
Moderators: Dr. Danyella Dogini and Dr.Cristiane Rocha

09:00AM to 9:15AM - Molecular biology applied to neuroscience – Dr. André Vieira
09:15 am to 9 : 30AM - Gene discovery in epilepsy , malformations of cortical development and stroke using next generation sequencing technology - Dr. Rodrigo Secolin
09.30AM to 9 : 45AM - The laboratory of computation biology and bioinformatics and the Brazilian Institute of Neuroscience and Neurotechnology (Brainn) – Dr. Benilton Carvalho
09:45AM to 9 : 55AM - Studying epilepsy by genetic manipulation in Zebra fish model – Dr. Marina Coelho Gonsales
09:55AM to 10:05AM - Applying next generation sequencing technologies in clinical practice : a demonstration project using genomic medicine in the Brazilian national health system (SUS) – Dr. Joana Prota
10:05AM to 10:30AM - Discussion
10:30AM to 11:00AM - Coffee break
11:00AM to 12:00PM - Dr. Ley Sander (The Changing Face of Epilepsy)
12:00PM to Closing - Prof. Fernando Cendes

Lunch

02:00PM to 05:00PM - Adjourn followed by a closed meeting of the Advisory Committee

Palestras

Journal of Epilepsy and Clinical Neurophysiology

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I Congresso Cepid Brainn

FEATURE EXTRACTION AND CLASSIFICATION OF BRAIN SIGNALS FOR BCI APPLICATIONS – PART II

Romis Attux[1], Thiago B. S. Costa[1], Sarah N. de Carvalho[1, 2], Diogo C. Soriano[3]
[1]FEEC/Unicamp, [2]ICEA/UFOP, [3]Ufabc

INTRODUCTION: We will present the initial results of a comparative analysis of methodologies to perform feature extraction and classification in SSVEP-based BCIs. The tests included two distinct classification structures (linear discriminant [1] / extreme learning machine [2]), two optimization criteria [1] (least squares / Fisher discriminant) and two feature extraction strategies (power spectrum estimation based on a filter bank / autoregressive modeling [3]).

MATERIALS AND METHODS: The EEG signals were acquired and amplified using a g.tec [4] 16-channel dry active electrode system, and were processed within the MATLAB environment. The stimulation system was composed of 3 LEDs programmed to oscillate at different frequencies (15Hz, 18Hz and 21Hz in this work). The experimental setup is shown in Fig. 1.

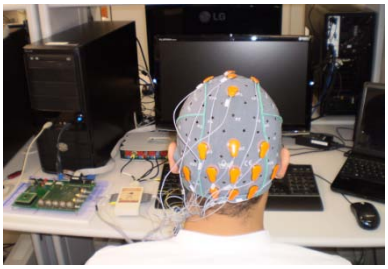

Figure 1: Experimental Setup

The acquisition process was carried out twice, generating a total of 9 minutes of EGG data sampled at 256 Hz. Windows of 125ms were used in the feature extraction / classification stages.

RESULTS: The most consistent results so far were obtained for a system based on spectral features and linear classification based on least squares. Table 1 summarizes the results for two trials (S1 and S2).
Table 1: Experimental Results

	S1			S2		
f(Hz)	15	18	21	15	18	21
C-V(%)	96,67	91,67	90,00	0	22,50	43,33

The classifier was trained with data acquired in the S1 trial, and two validation sets were built, the first with different data from the S1 trial and the other with data from the S2 trial (the hit ratios were obtained using these sets). The system had a good performance with data from the same trial and experienced a significant degradation in the case of different data.

CONCLUSION: This work made a preliminary analysis of methods for feature extraction and classification in SSVEP-BCI online systems. The results are encouraging, but these are practical issues to be dealt with before the system can be tested in a real-time configuration.

ACKNOWLEDGEMENTS: The authors thank Fapesp, Finep and CNPq for the financial support.

REFERENCES: [1] R. O. Duda, P. E. Hart, D. G. Stork, *Pattern Classification*, Wiley, 2000. [2] G. Huang, Q. Zhu, C. Siew, "Extreme Learning Machine: Theory and Applications", Neurocomputing, Vol. 70, Nos. 1 – 3, pp. 489 – 501, 2006. [3] S. Haykin, *Adaptive Filter Theory*, Prentice Hall, 1996. [4] http://www.gtec.at/, visited in 04/21/2014.

BRAINN EXPERIMENTS WITH FMRI AND EEG-FMRI

G. C. Beltramini[1]
[1]Neurophysics Group, IFGW, Unicamp

INTRODUCTION: FMRI (functional magnetic resonance imaging) is a flexible neuroimaging technique that is employed to study BOLD (blood oxygenation level dependent) signal changes related to tasks and functional connectivity during resting state [1]. Both types of experiments can be performed in healthy subjects and in patients to assess the effect of the disease. Other techniques that measure different aspects of brain activity can be combined with fMRI, such as near-infrared spectroscopy (NIRS) and electroencephalography (EEG). The Brainn group uses fMRI, NIRS and simultaneous EEG-fMRI in several projects involving healthy subjects and the following pathological conditions: autism, Parkinson's disease, Alzheimer's disease (AD), sensory neuronopathies, stroke and epilepsy. In this talk, a general overview of the experiments with fMRI performed by members of the Brainn group will be presented.

MATERIALS AND METHODS: All images were acquired with a 3T MRI scanner (Philips Achieva, Best, The Netherlands) at the Unicamp hospital. FMRI studies with stroke and epilepsy patients were performed using simultaneous EEG. An in-house-made fMRI cycling ergometer was used for the project that evaluates cerebral dynamics whilst exercising in healthy volunteers. While most of the studies include a resting state scan, a variety of experimental paradigms have been used in the task fMRI for each project. Scripts were written to analyze the task-related fMRI experiments (SAfE: Straightforward Analysis of fMRI and EEG-fMRI) and the resting state experiments (UF^{2}C: User Friendly Functional Connectivity).

RESULTS: In general, significant differences between patients and controls have been observed. For example, functional connectivity in mild AD patients and controls differed during rest in the default mode network, language network and executive function network. For the project that uses the cycling ergometer, different negative BOLD changes were seen depending on the exercise intensity. Moreover, the EEG-fMRI experiments showed positive and widespread negative BOLD changes related to ictal and interictal activity in stroke and epilepsy patients.

DISCUSSION: The differences seen in patients relative to controls result probably from a complex interaction between the disease and functional and structural changes. These alterations can affect not only specific brain regions but also distinct brain networks throughout the brain. In some cases structural lesions are not seen, but experiments using fMRI can show changes in brain function in comparison with healthy individuals, since widespread functional networks are affected.

CONCLUSION: The works conducted by the Brainn group help to advance our knowledge on how the brain is affected by various diseases. This could lead to the discovery of potential biomarkers and new treatment approaches, and bring us one step closer to understanding how the brain works.

REFERENCES: [1] Gusnard DA & Raichle ME, Nature Reviews Neuroscience 2: 685-694, 2001.

VIRTUAL REALITY FOR MOTOR REHABILITATION

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INTRODUCTION: Virtual Reality (VR) systems have been used in science as tools for teaching and learning, technical training and entertainment. In the field of neurofunctional rehabilitation, VR gives a real opportunity to complement conventional therapy on people who live with physical and cognitive limitations. Here we present a project, which is still at its initial stage, whose goal is to use VR to aid the improvement of motor and cognitive function in patients who have suffered a stroke.

MATERIALS AND METHODS: A set of VR applications, called Gestures [1], aimed at stimulating Human-Computer Interaction (HCI) through the movement of the lower and upper limbs, including trunk rotation, will be used. These applications were developed at LaVIIC – Ufscar, using a gesture recognition sensor (Microsoft Kinect) that scans the user's body and creates spatial coordinates from their joints [2]. The Kinect device consists of several electronic components (RGB camera, engine tilt, depth InfraRed sensor, microphone array) that allow it to capture gestures in real time; it has an USB 2.0 standard connection interface that allows it to be used in the development of computer applications.

RESULTS: The GestureChair application controls a free version of the game PacMan with hand movements (up, down, right or left) from a sitting position. If the user does not move fast enough, the software does not interpret any movement, preventing recognition of undesirable gestures. It was initially directed to spinal cord injury patients that can control the upper limbs [1]. The GestureChess application controls a virtual chess game from hand movements and requires simultaneous motor and cognitive activities [1]. It has potential to optimize skills related to the balance of the elderly, and to increase the cognitive reserve in adults, youngsters and children. A decrease of this reserve may be associated with the incidence of neurodegenerative diseases such as Alzheimer's and Parkinson's. The GestureMaps application controls the geographical exploration of the Google StreetView tool, where the user simulates the movement of walking. It was initially directed to people suffering from spatial disorientation [1]. The GesturePuzzle application requires the user to organize the parts of a virtual puzzle where the user can freely explore the shoulder joint in all planes of motion [1].

DISCUSSION/CONCLUSION: VR has been used as a means of rehabilitation and physical assessment in general [3-5], more intensively so over the last few years. The aim of the project is to use these VR tools in parallel with standard physiotherapy sessions, and investigate whether this improves patients' recovery. We also intend to carry out brain plasticity monitoring using electroencephalography (EEG) and functional Near-InfraRed Spectroscopy (fNIRS). For long-term prospects, we are studying the possibility of adding a Brain-Computer Interface (BCI) system based in motor imaging to control the applications, since there is some evidence that the best way of restoring brain motor function consists in inducing brain activity that in turn will induce brain plasticity [6].

REFERENCES: [1] Brandão AF et al., Tendências e Técnicas em Realidade Virtual e Aumentada, 4:1-16, 2014; [2] Shotton, J et al., Microsoft Research Cambridge & Xbox Incubation, 2011; [3] De Mauro A et al., 10th IEEE Int Conf Inf Tech Appl Biomed (ITAB), 2010; [4] Li S et al., 1st Int Worksh ETCS 2: 455-458, 2009; [5] Paolini G et al., IEEE Trans Neural Syst Rehabil Eng 99: 1, 2013; [6] Daly JJ et al., The Lancet Neurol 8: 1032-1043, 2008.

SMART ENVIRONMENTS FOR ACCESSIBILITY

E. Cardozo[1], L. Olivi[1], R. Souza[1], E. Rohmer[1], F. Pinho[1], P. Pinheiro[1], E. Guimarães[2]
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INTRODUCTION: This short paper addresses the research being conducted on the subject of smart environments for accessibility. An architecture for smart environments based on the paradigm of "Internet of Things" is being developed. This architecture allows an assistive vehicle (e.g., a robotized wheelchair or scooter) to interact with the environment in order to search for services useful in the current context. Examples of such services include monitoring services, communication services, navigation services, and rescue services. These services can be invoked with or without the intermediation of the person being assisted. The core of the architecture is a new generation of low cost processors that connect a diversified family of sensors and communicate via standard Internet protocols.

MATERIALS AND METHODS: We started with the development of navigation algorithms for assistive robots. The algorithm employs the paradigm of shared control [1] where the responsibility for the navigation is shared between the assisted person and the vehicle's control software. Our novel approach to shared control is to interpret the issued commands (e.g., from a Brain-Computer Interface) according to the actual context. Example of contextual variables includes the states of the environment, the vehicle, and the assisted person. These states are assessed through sensors. Body sensors are employed to estimate the state of the assisted person, e.g., his/her heart frequency and skin conductivity. Environmental sensors are deployed in order to supply the level of occupancy, the position of landmarks, and navigation routes. Sensors on the vehicle include rangefinders, compass, and odometers. The next step is to design an architecture for smart environment where this multitude of sensors supply useful services to the assistive robots. The environment must provide a "continuous" network in the sense that the resources and services are accessed in a straightforward and location independent way.

RESULTS: At the present, we are starting the prototyping an architecture of smart environment for accessibility. Some design decisions were already established such as the use of an IPv6 (Internet Protocol version 6) network, the adoption of the REST (Representational State Transfer) service interaction model, data representation based on JSON (JavaScript Object Model), and the ability to integrate different wireless sensor network technologies into the environment. An implementation of a portion of this architecture that runs on the assistive robots was already concluded [2]. The extension of this implementation that integrates body and environmental sensors is under way.

DISCUSSION: The results obtained until now lead us to conclude that smart environments bring advantages in terms of providing quality of life to disabled and aged persons. Instead of concentrating all the sensoring and processing functions on a battery-powered assistive robot, a significant portion of these functions can be provided by the environment without restrictions of energy, processing power, and network connectivity.

CONCLUSION: The activities reported here are conducted in the scope of the Brasilian Research Institute for Neurosciences and Neurotechnology (Brainn). This line of research in the Brainn aims to provide technologies for assisting and rehabilitating persons with severe locomotion restrictions such as those victims of stroke, multiple sclerosis, and severe spinal cord damage.

REFERENCES: [1] Olivi LR et al., Shared Control for Assistive Mobile Robots Based on Vector Fields. The 10th International Conference on Ubiquitous Robots and Ambient Intelligence, Jeju, Korea, 2013; [2] Souza, RS et al., A RESTful Platform for Networked Robotics. The 10th International Conference on Ubiquitous Robots and Ambient Intelligence, Jeju, Korea, 2013.

BRAINN AND THE BCB LABORATORY

Benilton S Carvalho[1], Iscia Lopes-Cendes[1]
[1]Department of Medical Genetics, FCM, Unicamp

INTRODUCTION: After identifying questions of interest and designing appropriately experiments to answer such questions, biomedical researchers make heavy use of high-throughput technologies like next-generation sequencing of DNA and RNA molecules. These technologies generate a vast amount of data, which can only be analyzed through the use of the latest computational and statistical methodologies. In this presentation, we advocate that a team of experts in (bio)statistics and computational biology is essential to streamline research on multidisciplinary biomedical projects.

MATERIALS AND METHODS: The Brazilian Institute of Neuroscience and Neurotechnology, Brainn, is an initiative to improve our understanding of the mechanisms that lead and follow epilepsies and stroke, and to promote the development of methods associated to prevention, treatment and rehabilitation from such diseases. A multidisciplinary environment must be set to achieve such goals, allowing researchers with different expertise to interact synergically. We introduce the Bioinformatics and Computational Biology Laboratory (BCB Lab), which is being established to assist collaborators by offering expertise in statistics, biostatistics, bioinformatics and computational biology.

RESULTS: The contributions that the BCB Lab can provide to the efforts made by BRAINN are manifold. Well-trained and experienced professionals comprise our team. Our fields of expertise are used frequently in similar initiatives and we foresee that this integration will facilitate the achievements of the goals set by Brainn.

DISCUSSION: The Brainn initiative is characterized by its multidisciplinarity. Improving our comprehension regarding epilepsies and stroke requires that teams of medical doctors, engineers, physicists, statisticians, computational biologists and other experts work together. The complex nature of the phenotypes of interest requires not only proper experiment design, but also the use of advanced statistical strategies for data analysis. Scientists from other fields can learn the techniques used for such tasks, but contributions from our team of experts can streamline research.

CONCLUSION: Setting up the BCB Lab is one step to establish a local reference for expertise in statistics, biostatistics, bioinformatics and computational biology. Within a multidisciplinary initiative, it is essential to keep collaborators in their areas of competence to maximize the potential for relevant findings. In that context, the BCB Lab is capable of providing high-quality support to collaborators in the aforementioned fields.

INVESTIGATION OF BCI PARADIGMS USING FMRI

G. F. Slenes[1], E. L. Silva[2], G. C. Beltramini[2], F. O. Lima[1], L. M. Li[1], R. J. M. Covolan[2], G. Castellano[2]
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INTRODUCTION: In this work we present an investigation of two paradigms, or "thinking strategies", for brain-computer interface (BCI) systems, using fMRI. The first one consisted of opposing motor imagery (MI) to music imagery (MU), in an attempt to obtain easily differentiable brain signals (fMRI activation maps) that could perhaps guide EEG-based systems. In the second one, we investigated the effect of training on the fMRI activation map of 4 types of MI strategies (movement of right hand – RH, left hand – LH, feet – F, and tongue – T).

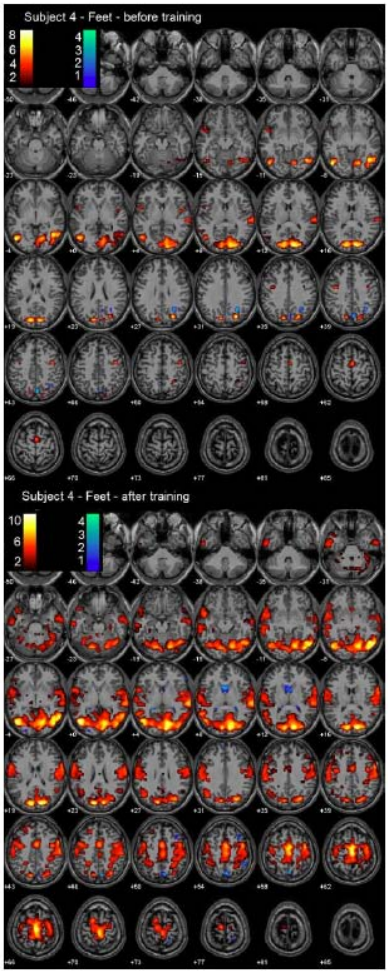

Figure 1. FMRI results for control group for MU x MI.

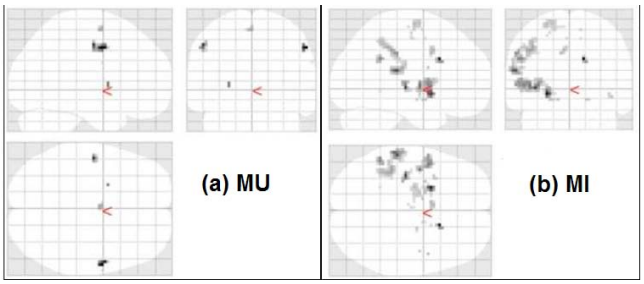

Figure 2. Activation map before (top) and after (bottom) training for MI-F for one subject.

MATERIALS AND METHODS: All images were acquired in a 3T Philips Achieva MR scanner. Subjects participating in the study were: 11 healthy subjects (mean age 27.9±4.6, 9 men) and 4 ischemic stroke patients (mean age 52.8±12.8, 2 men) in the 1st paradigm; and 10 healthy subjects (mean age 23.5±2.5, 8 men) in the 2nd one. The study was approved by the Ethics Committee of Unicamp and all subjects gave their consent. Instructions were given to subjects through a computer screen using the Eloquence system. FMRI data were analyzed the usual way with SPM8. The experiments were: **1st)** 21 blocks of 20s each, interleaving rest periods with either MU (singing the national anthem in thought) or MI (RH) periods. **2nd)** 16 MI blocks of 30s interleaved with 17 rest blocks of 16s each. MI blocks were 4 of each type: RH, LH, F, T. Two acquisitions were performed separated by a week interval. During this week, subjects were instructed to practice at home, with the same video used in the fMRI scan.

RESULTS: 1st) The control group showed specific and statistically differentiable activation patterns for MI versus MU (t-test, $p < 0.001$, Fig. 1). Stroke patients showed a very large variability, with no common activation pattern for either of the imagery tasks. **2nd)** The majority of subjects (6 out of 10) showed an increase of the BOLD response after training (Fig. 2), both in amplitude and spatial extent, for most MIs (all except LH).

DISCUSSION/CONCLUSION: 1st) Although patient variability was very large, tests were performed on a very small sample. Also, despite variability, results found for the control group are encouraging. General variability found is probably due to the different thought and emotional processes involved in MU. This experiment clearly showed the inherent difficulty in developing BCI systems for the main type of end users (i.e., disabled patients). **2nd)** Our findings corroborated results in literature that training is a fundamental part of an MI-based BCI system. Even minimal training (7 sessions), as performed in this experiment, could show differences at the fMRI BOLD level. Despite the encouraging results found for both paradigms, fMRI results do not guarantee good EEG classification results, and more tests need to be made using this technique.

STUDYING EPILEPSY BY GENETIC MANIPULATION IN ZEBRAFISH MODEL

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The zebrafish (*Danio rerio*) has been recognized as a promising animal model for the investigation of human diseases. Among the many advantages of this model are: organs functionally and morphologically similar to their human equivalents, easy visualization of early developmental processes and relatively low maintenance cost. Recent studies have shown successful applications of the zebrafish model in the studies of epilepsy, with experiments showing that seizure-like responses can be evoked in zebrafish as response to a common convulsant agent, pentylenetetrazole (PTZ). Zebrafish treated with PTZ display behavioral, electrographic, and molecular alterations similar to those seen in rodents, the main model for epilepsy so far. Therefore, the Zebrafish Laboratory of the Department of Medical Genetics - FCM/Unicamp has focused on using this animal model for molecular and functional studies in epilepsy. Our research group has already achieved promising results, as evidenced by the establishment of an efficient protocol for induction of seizures in adult fish using PTZ. One of our current research topics aims to investigate the role of neuroinflammation in seizures. Our results have shown a short up-regulation in the temporal transcript profile of interleukin-1b gene (*il1b*) after seizure in both immature and adult zebrafish brain. We also observed an age-dependent regulation of *il1b* in the developing zebrafish brain after seizure. In addition, ciclooxigenase-2b (*cox2b*) but not ciclooxigenase-2a (*cox2a*) mRNA levels were up-regulated after seizure in zebrafish larvae and no difference was found in adult zebrafish brain. Treatment with the non-steroidal anti-inflammatory drug indomethacin prior to the PTZ-induced seizure promoted a down-regulation of the *il1b* and *cox-2b* genes in zebrafish larvae as well as longer latency to reach seizure onset besides smaller number of seizure-like behavior when compared to those that had no indomethacin treatment. The laboratory is also pursuing the implementation of zebrafish genetic models to study candidate genes for epilepsy. One excellent candidate gene to initiate these studies is the neuronal voltage-gated sodium channel 1-subunit gene (*SCN1A*). This gene is considered one of the most clinically useful for molecular testing among the genes identified in different epileptic syndromes, with about 80% of the patients with a severe childhood epilepsy named Dravet syndrome harboring *SCN1A* mutations. Our strategy consists in blocking the expression of zebrafish *SCN1A* orthologous genes by injecting morpholino oligonucleotide (MO) into one-cell zebrafish embryos. After that, we aim to characterize the phenotype of larvae after MO injection. In addition, we intend to verify the existence of susceptibility to increased water temperature in order to simulate a situation frequently associated with mutations in *SCN1A*: the sensitivity to febrile seizures. To date, we have started to establish of a hyperthermia seizure-inducing protocol in young larvae that will that will serve as reference for comparisons with MO-injected larvae.

CONSCIOUS AND UNCONSCIOUS PROCESSES IN ARTIFICIAL BEHAVIOR GENERATION

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INTRODUCTION: There are two different meanings for the word "Neurotechnology". From one side, this word might mean the use of technology to help improving the field of Neurology in Medicine. A different meaning for the same term might include the many kinds of inspiration from neuroscience to the improvement of intelligent systems techniques. This contribution is pertained to this second meaning for the word "Neurotechnology". Among the many theories of consciousness, the Global Workspace Theory, from Bernard Baars investigates from the point of view of neuroscience, how conscious experiences can be correlated to specific brain signals, obtained through fMRI or other means, and also how other signals might be associated to some sort of unconscious processing happening in the brain. By using a constrastive analysis, he discriminated among conscious and unconscious phenomena which were unified in a theory having a remarkable characteristic: it can be simulated by means of a computational procedure, and potentially emulated by means of the design of parallel machines. Following some results found in the literature, from researchers trying to pursuit the same goal of creating new technologies from the work of Baars, in this work we investigate how conscious and unconscious processes might be interacting to each other in order to evolve complex behavior in an artificial creature.

MATERIALS AND METHODS: We rely mainly in Baars descriptions of his theory, and also on computational implementations of his theory provided by the group of Stan Franklin from University of Memphis, with his LIDA Framework. We disagree with some of the interpretations of Baars theory from Franklin's group, so we started our own development of a computational framework with our interpretation of some specific details of the theory. Our disagreement is related to the issue we want to investigate with this work, i.e. the interaction among conscious and unconscious processes.

RESULTS: This is the report of an ongoing research. At this point, we don't have final results, from the experimental point of view. But our results are computational specifications for the construction of algorithms, which might have a significant impact on the creation of new kinds of neurotechnology.

DISCUSSION: We propose that unconscious processes do not need access to the global workspace to be activated, and that the global workspace is just an interference point where conscious and unconscious processes are able to interact. Using this, unconsciou automated processes work as if in a kind of subsumption architecture. Nevertheless, conscious processes are able to interfere in these unconscious processes, resulting in a different behavior.

CONCLUSION: Due to this interference, conscious processes can be used to train and adapt new unconscious processes, leading to a kind of machine which is able to learn new kinds of behavior, incorporating this to its legacy automatic procedural behavior.

MICROFABRICATION OF NEURAL PROBES

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INTRODUCTION: Recording neural activity in-vivo is key to understanding the brain. Towards that goal, several research groups have developed micron-scale, needle-like, electrical probes that have one or more recording sites. These have originated from simple wire measurements, and progressed to ultra-small, micron-sized patch-clamp style microelectrodes. The application of microfabrication techniques, that originated in the microelectronics, to the production of miniaturized devices, the area of bio-MEMS, evolved and has been successfully applied to the production of neural probes. We will review some of the work done in leading laboratories and also present the current state of the development of Neural Probes within Brainn.

MATERIALS AND METHODS: Neural probes within Brainn initiative take advantage of a wide range of micro- and nano-scale fabrication technologies available locally at university labs and national labs. The process of producing neural probes starts with design of a microelectrode layout. This should be adequate to recording the region of interest with enough spatial resolution. The resulting design is produced with currently available CAD tools. In our project, photolithographic masks are produced through direct write lithography on a specialized laser tool. Currently we use the DWL66FS system at CTI, although other systems are available (at Unicamp's Lamult lab, and at LMF at Cnpem). These masks are used to generate the physical pattern on a substrate. The metal that makes up the microelectrodes can be deposited through several methods, available in many associated laboratories, such as thermal- or electron-beam- evaporation, sputtering, or electrochemical deposition. While these techniques are suitable for large scale production, methods that take advantage of industrial scale microelectronic foundries can be used as well, and are under consideration in our initiative.

RESULTS: Two approaches have been investigated, one based on silicon substrates, and another based on spin-on polymers. Silicon substrates typically are used to produce microelectronic components and integrated circuits, and have been used to produce very sophisticated neural probes in the past decade. Chemical etching of silicon allows very small, sharp and anisotropic structures to be produced at low costs and this is being evaluated by our group. A well known photosensitive proprietary resin known as SU-8 has shown good promise due to its potential for biocompatibility. This technique is very versatile and currently serves as our main process to investigate microelectrode materials, geometries and probe geometries.

DISCUSSION: Our current process has proved to be appropriate to generate novel devices and geometries. Currently, the characterization of the fabricated probes and microstructures is ongoing to allow optimization of probe materials and process. Packaging of the probes is required to allow connection of cables to these microscale devices, and is currently being developed.

CONCLUSION: Our work is enabling the microfabrication of neural probes using techniques described in the literature, focusing on scalable production methods. A platform is being established so that novel materials and geometries can easily be tried out. In addition, new functions such as sensing, optical excitation, microfluidic drug delivery, can be added to such a process, as others have demonstrated, establishing an innovation platform for future advances.

APPLYING NEXT GENERATION SEQUENCING TECHNOLOGIES IN CLINICAL PRACTICE: A DEMONSTRATION PROJECT USING GENOMIC MEDICINE IN THE BRAZILIAN NATIONAL HEALTH SYSTEM (SUS)

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After the advent of Next Generation Sequencing technologies, the decreasing costs of sequencing DNA have allowed the use of this technique not only as a research tool, but also as a diagnostic test for various clinical conditions. We present a demonstration project to evaluate the cost-effectiveness of the Whole Exome Sequencing as a diagnostic method for different clinical disorders compared to other molecular and cytogenetic tests in the context of the Brazilian National Health System.

WEB-BASED PLATFORM FOR MEDICAL IMAGING RESEARCH

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INTRODUCTION: The current scenario of Medical Imaging Research consists usually of processing large datasets using pre-existent scripts for batch processing and tools for interactive analysis and measurements. Such scenario presents several drawbacks. In the batch processing step, it is impossible to access the quality of intermediate results, hard to check if the parameters used were adequate for that dataset and file management must be done manually. Moreover, after the interactive analysis step, it is impossible to retrieve the sequence of operations performed interactively and very hard to check for errors when a suspect value appears in the analysis. Additionally, some problems arise from the fact that through all steps, spreadsheets and intermediate results have to be moved from one computer to another, and often have to be converted from one format to another.

MATERIALS AND METHODS: We propose a new paradigm, a web-based platform with appropriate environment for each Medical Imaging Research project. The access to this environment is controlled through a username and password. All dataset is accessible "within a click". Processing takes place in the cloud and all the tools are integrated in one environment. After running the processing pipeline, all pre-processing data, intermediate results, parameters and techniques are accessible through automatically generated web-pages. All the history of every single processing step, even manual interventions, is kept.

RESULTS: A prototype for segmenting and parcellating the corpus callosum (CC) was built and is being tested. The implemented pipeline performs: diffusion tensor imaging pre-processing, automatic midsagittal slice detection, automatic corpus callosum segmentation and parcellation and finally, computation of diffusion properties within each CC region.

DISCUSSION: Not only consolidated results are presented in a form of a table of computed values (for the whole dataset), but also the processing pipeline for each subject can be accessed individually, in single web pages containing all intermediate results (Fig. 1).

CONCLUSION: The web-based platform is a new paradigm that can solve several difficulties faced in a typical Medical Imaging Research scenario.

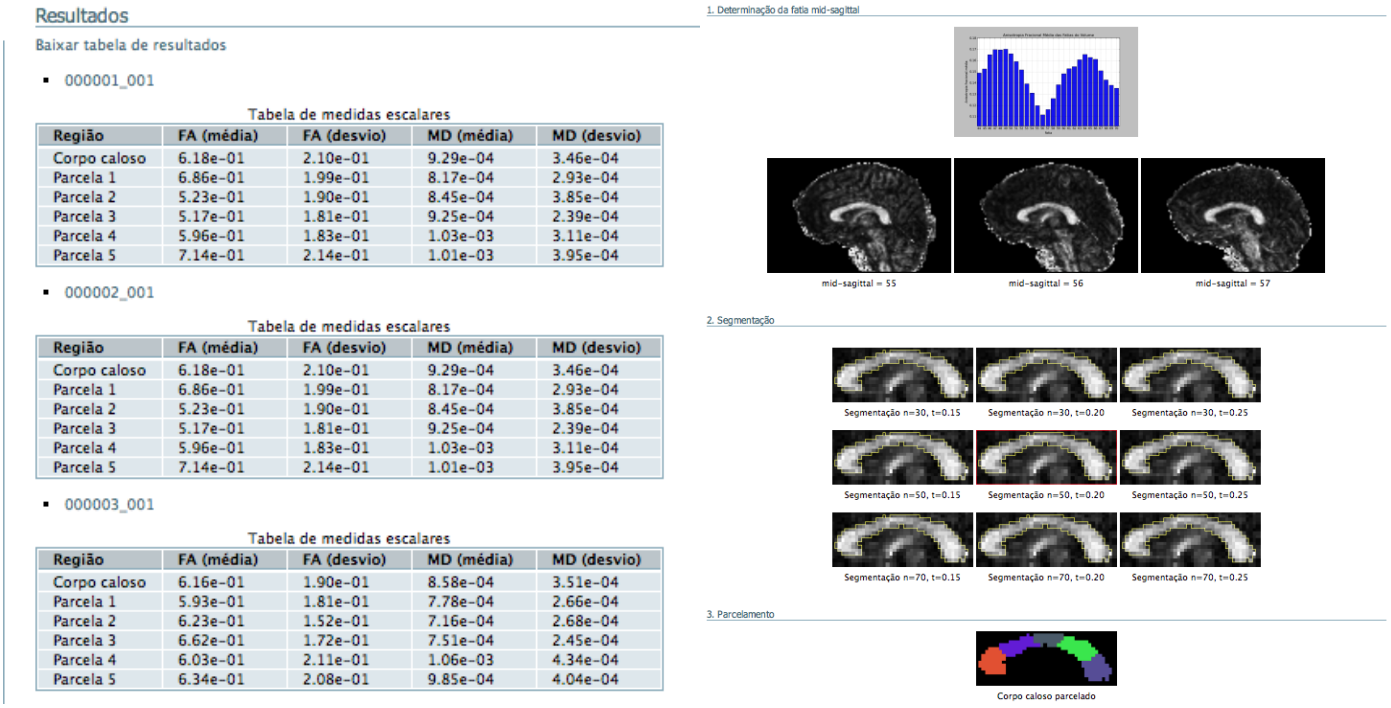

FIGURE 1 – Table with computed FA and MD for each region of CC (consolidated results) and one subject web page with intermediate results (midsagittal slice selection, segmentation and parcellation of CC).

THE CHANGING FACE OF EPILEPSY

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Epilepsy, the tendency for an individual to present with unprovoked seizures, is a common neurological condition affecting more than 60 million people worldwide. It is a major burden to the individual and to society as it carries considerable morbidity and premature mortality. Current antiepileptic treatment is still empirical and at best symptomatic. Epilepsy is always a symptom of a brain dysfunction and most likely there is always a genetic contribution albeit not always strictly inherited. Mutation in single genes are likely to be a relatively rare. Epigenetic changes, switches in gene function not related to changes in underlying DNA sequence, are in the other hand likely to be major contributors to the risk. Localised or organ-specific gene miscopying such as deletions, duplications are also likely to be important contributors. The cascade of events triggering epilepsy is likely to vary greatly among individuals and the understanding of the full process will led a paradigm shift away from the current empirical approach. Once the full epileptogenic process is fully understood, targeted and specific treatment may be developed which will revolutionise treatment of the condition.

GENE DISCOVERY IN EPILEPSY, MALFORMATIONS OF CORTICAL DEVELOPMENT AND STROKE USING NEXT GENERATION SEQUENCING TECHNOLOGY

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Introduction: New technology of DNA and RNA sequencing has allowed a deeper analysis in the whole genome of individuals, in order to identify putative causal variants for several diseases [1; 2]. In this presentation, we are focused on neurological complex diseases, including mesial temporal lobe epilepsy (MTLE), malformations of cortical development (MCD) and stroke. MTLE is a clinically well characterized syndrome, affecting approximately 2% of general population. Mesial temporal sclerosis, detected by magnetic resonance image, presents one of the main features of MTLE [3; 4]. Cerebral cortex malformations are syndromes related with abnormalities in cortical development stages, including cellular proliferation, migration and post-migration [5]. Periventricular nodular heteropia, lissencephaly, schizencephaly, polymicrogiria and focal cortical dysplasia are the most studied MCD [6]. Stroke is characterized by focal neurological loss of function, resulting in disruption of the blood supply to the brain. This syndrome is the third cause of death in developed countries, and the major cause of disability [7]. Despite some variants have been associates with these syndromes, until this moment none causal variant have been found. In addition, some issues without answer still remains, such as different variants found in familial affected individuals and non-familial ones; and affected individuals who do not present the associated variants already found. Therefore, in order to identify putative causal variants, our objective is to investigate these neurological complex diseases using next generation sequencing technology.

Materials and Methods: Until this moment, we have stored DNA samples from 398 MTLE individuals, 162 MCD individuals and 200 controls, collected in the Clinical Hospital at UNICAMP Clinical Hospital. In addition, we have also stored 909 stroke individuals and 1456 controls from Univille University (Joinville, SC). Next generation sequencing will be performed using HiSeq™ and MiSeq™ technology (Illumina, Inc., San Diego, CA, USA). Quality analysis and alignment of BAM files will be performed using from R and GATK bioinformatics tools [8].

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FEATURE EXTRACTION AND CLASSIFICATION OF BRAIN SIGNALS FOR BCI SYSTEMS I: SSVEP APPLICATIONS

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INTRODUCTION: This work aims to study the classification performance of Brain Computer Interface (BCI) systems with emphasis in different feature extraction techniques in the context of steady state visually evoked potential (SSVEP) paradigm [1]. Both classical spectral analysis – by means of the evaluation of power spectral density (PSD) [2] – and recurrence quantification analysis (RQA) [3] were used for feature extraction, being the best results attained by this last technique.

MATERIALS AND METHODS: the SSVEP paradigm concerns to a five command (one rest and 4 visual stimulated commands) SSVEP-BCI system based on LEDs flickering at 13, 18, 21 and 25 Hz. The whole interface used BCI2000 software and the signal processing stage was implemented in Matlab. Data were acquired at 128 Hz using 16 positions associated to classical SSVEP configuration and pre-processing just relied on the normalization of the time series. Feature selection was implemented using the Davies-Bouldin index and the classification was performed using linear discriminant analysis. Both PSD and RQA measures were used for 3 different healthy volunteers. All the experiments were performed in "off-line" operation.

RESULTS: As the main result, it was found that the threshold of the recurrence plot, chosen so as to yield a recurrence rate of 2.5%, defined the key discriminant feature, typically providing a mean classification error of less than 2% when information from 4 electrodes were used. Such classification performance was significantly better than that attained using spectral features for all subjects, which strongly indicates that RQA is an efficient feature extraction technique for BCI.

DISCUSSION: The RQA approach used here is close connected to techniques previously successfully used in EEG analysis to characterize its patterns by means of the evaluation of correlation dimension [4]. As a main drawback to the proposed recurrence-based approach, it can be mentioned the computational cost associated with the evaluation of an adaptive recurrence plot for this purpose, since BCI systems usually require a fast signal processing framework for real-time operation.

CONCLUSION: RQA seems to outline a powerful framework for signal processing in the BCI context, while its fully application still requires technological improvements in order to be run in real-time situations.

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3D INTERACTIVE VISUALIZATION FOR NEUROIMAGE EXPLORATION

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INTRODUCTION: Despite rapid evolving of neuroimage processing technologies, there is still a large number of challenging issues that perplex neurologists. Intended to help them to deepen the knowledge about the human brain, we aim at improving an interactive visualization of anatomical and functional neuroimages in native space. In the medical applications most interactive visualizations are limited to 2D or to normalized spaces. Our hypothesis is that, while the thinking processes of a neuro-expert are not describable as sequences of actions, an interactive 3D visualization system may allow her/im to guide a computer reaching plausible results more quickly. In addition, active voxels that promptly respond to a user action let a neuroscientist explore data of interest for better understanding, for formulating new hypotheses and for improving diagnostic workup and treatment. Therefore, we also conjecture that, through iterative visualization cycles, a problem may be better perceived and its solution may subsequently be systematized and translated into programs.

MATERIALS AND METHODS: Our main concern is how to bridge the medical doctor's goals and the displayed neuroimages, such that a physician has the feeling that s/he acts directly on the patient's brain and that the graphical displays form an extension of her/is cognitive process. We adopt a user-centered spiral design model, in which potential users are involved throughout the design process and a prototype is built for assessment at each stage [1]. We use the tools available in the open source GUI library, such as wxWidgets. The open source Grassroots DiCoM library is used for reading and parsing DiCoM medical files. All the rendering procedure runs on GPU. Medical image processing algorithms, such as histogram equalization, z-score computation and image filtering, are applied for enhancing the information embedded in the images. Currently, we conduct our experiments with CT, MR and PET images in the DiCoM format. The MR images were acquired in the Philips Achieva 3T scanner of our university teaching hospital and the CT and PET volumes in the Siemens s5vb20b multimodal imaging scanner.

RESULTS: In cooperation with Dr. C. L. Yasuda and Dr. F. Cendes, we developed a prototype that allows exploring laminar cortical structure in native space. By simply brushing a region of interest, cropping parallel to the scalp is automatically performed. Later, Dr. A. C. Coan and Dr. B. J. Amorim joined us. We designed and implemented an interactive environment for co-registrating MR and PET images to accurately locate a suspicious extra-temporal subtle epileptogenic lesion.

DISCUSSION/CONCLUSION: During the first CEPID meeting held in October 2013, Águas de São Pedro, we had an opportunity to discuss with Dr. R. C. Mesquita the application of our visualization system for precise placement of optodes on the scalp. These optodes are under development by the Medical Physics research group led by him. We also plan with Dr. Jorge Vicente Lopes da Silva to port our developed tools to the free software Invesalius maintained by his research staff in CTI and available at Portal de Software Público Brasileiro. These two contacts may open the application domain of our developed tools. Although at each development step new challenging problems come across, we still believe that it is possible to implement a clinically usable prototype in mid-term. It is therefore crucial to provide support for thorough medical assessment of our tools. In parallel, we aim to integrate the SPECT and DTI modalities for evaluating surgery›s safety margin. Depending on the outcomes we will plan further steps towards surgery planning and neuronavigation.

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CO-CLUSTERING FOR HIGH LEVEL KNOWLEDGE OF BRAIN FUNCTION

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INTRODUCTION: This work explores the great potential of co-clustering [3] techniques in the analysis of brain activity data. Co-clustering is a more powerful data mining technique than clustering because it simultaneously finds cluster structures over both objects and attributes in a data matrix. Speaking of brain activity, each row of the data matrix (object) may correspond to a distinct functional area and each column (attribute) may represent sequential activity patterns along time. With co-clustering, a single object/attribute can belong to none, one, or more than one co-cluster. Besides, co-clusters can be defined using coherence measures, thus being substantially more general than distance measures generally used in clustering. Co-clustering also has a strong connection with graph theory, being directly associated with a biclique in a bipartite graph. Some examples of the application of co-clustering techniques on brain activity data can be found in [4, 5]. Specifically, we highlight the features of a new family of co-clustering algorithms, called RIn-Close [6]. RIn-Close algorithms are the first ones able to efficiently enumerate all maximal co-clusters with (*i*) constant values on rows (CVR), (*ii*) constant value on columns (CVC), or (*iii*) coherent values (CHV) in numerical (integer or real-valued) datasets. Fig. 1 shows pictorial examples (with 6 objects and 4 attributes) of the patterns that we can find in a data matrix using co-clustering. Most of the existing works in the literature, including the ones that apply co-clustering to brain activity data, use heuristic-based co-clustering algorithms, with no guarantee of finding maximal co-clusters [6].

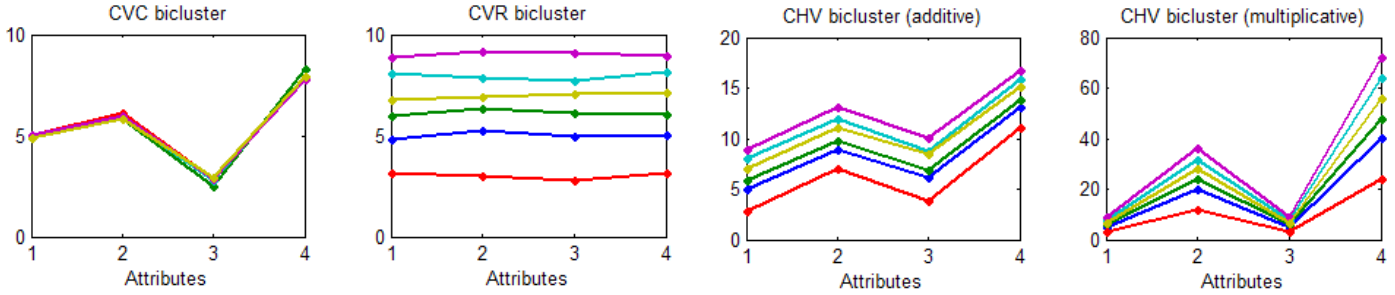

Figure 1: Examples of the kind of patterns that we can find in a data matrix using co-clustering.

MATERIALS AND METHODS: We used a public NIfTI-1 dataset[1] to perform our experiments, called *HAXBY8_R1*. Using the Kittipat's toolboxes [2], we converted it to a data matrix with 163,840 rows and 121 columns, where rows represent voxels and columns represent time stamps.

RESULTS: RIn-Close was capable of finding several perfect CVC, perturbed CVC and perfect CHV maximal co-clusters. Each maximal co-cluster corresponds to a set of voxels that has coherent behaviour across a subset of time stamps.

DISCUSSION/CONCLUSION: Each co-cluster may bring insights on mechanisms involved in brain function, thus supporting the identification of many neurological diseases [4]. The RIn-Close algorithms for co-clustering can be applied to reveal some aspects of the functional organization of the brain, potentially useful in the investigation of abnormal connectivity patterns in patients with neurological diseases.

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MOLECULAR BIOLOGY APPLIED TO NEUROSCIENCE

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The human nervous system is an extraordinarily complex structure composed of billions of neurons and thousands of kilometers of connections. This huge neuronal circuitry is assembled during development first through the processes of neuronal migration to specific places inside the nascent nervous system. Another crucial process is the extension and guidance of connections between neurons. All this events are the result of the molecular machinery operating inside cells, responsible for the detection and transduction of extracellular signals and for the dynamic remodeling of the cytoskeleton. Furthermore, connections between neurons can be modified, or reconfigured, during the life of the organism, for example in learning. The process of long-term potentiation, involved in learning, is mediated by a cascade of signaling and effectors molecules. This cascade, as others in biological systems, can be represented by a large network of interacting molecules. Each interaction in such networks was generally determined experimentally by hypothesis driven studies. A huge knowledge base of molecular interactions that take place inside cells was built, step by step, throughout decades of research.
New, faster and cheaper sequencing technology allows for large scale analysis of the transcriptome employing RNA sequencing. Since RNA molecules are intermediate products of gene expression, or as more recently shown, are also fine regulators of such gene expression, the sequencing of all this molecules in a tissue or condition makes possible the collection of large amounts of data regarding gene expression, gene editing, expression of regulatory RNA molecules among other events. One possible way to analyze these transcriptome datasets is the search for enriched groups of related genes, especially when one select the differentialy expressed genes among two different conditions. This may indicate biological processes responsible for the observed patterns of gene expression, consisting in a paradigm shift, since, biological hypothesis can be driven from large sets of data instead of testing previously selected hypothesis in the biological systems.
Some common forms of epilepsy affect mesial temporal lobe structures such as the hippocampus, and it is noteworthy that such structures and circuits are well conserved among humans and rodents. This fact reinforce feasibility of establishing experimental models of epilepsy. Currently in our laboratory, employing electrical stimulation of the hippocampus in rats, it is possible to induce epileptiform activity and also a cronic epileptic state.
We propose to compare the transcriptome of hippocampus structures of normal and epileptic rats in order to search for relevant biological processes responsible for functional and structural changes that take place in the hippocampus in such models and that may be relevant for epileptogenesis.

Trabalhos/Neurotecnologia

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J Epilepsy Clin Neurophysiol 2014; 20 (Suppl 2): 20-28

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STRAIGHTFORWARD ANALYSIS OF FMRI AND EEG-FMRI (SAFE): A TOOLBOX FOR SPM8

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INTRODUCTION: The clinical motivation and the most common use of simultaneous EEG-fMRI acquisitions occur in the field of epilepsy. Most frequently, the timing and type of epileptiform discharges obtained from the EEG are used as regressors in an fMRI paradigm to look for hemodynamic correlates of the epileptic activity. Since these discharges are spontaneous, the number and type of conditions may vary among patients and even among sessions. Thus, a specific fMRI paradigm must be created for each subject. Here we present a graphical interface which allows one to perform EEG-fMRI and fMRI analyses faster and less prone to human error.

METHODS: Using Microsoft Excel (2007), MATLAB (The MathWorks, Inc.) and SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8), a set of scripts in VBA (Visual Basic for Applications) and in MATLAB languages was written to analyze fMRI data recorded simultaneously with EEG. These scripts are specific for programs that have been largely used worldwide: BrainVision Analyzer 2 (BrainProducts GmbH, Munich, Germany) and SPM8.
First, an electroencephalographer analyzes the preprocessed EEG to identify the onset and duration of the epileptiform events. From this point to the final fMRI maps, very little user intervention is needed. After exporting the EEG events as an ASCII file and filling out a spreadsheet in MS Excel with the file and folder names, a macro creates a new and standardized spreadsheet with all necessary information for the fMRI analysis. Then, through a graphical user interface written in MATLAB called SAfE (Straightforward Analysis of fMRI and EEG-fMRI), the user chooses the options for the fMRI analysis. MATLAB and SPM8 read the spreadsheet and can automatically preprocess the data, create the design matrix, estimate the model, create contrasts (only F and T tests contrasting with the baseline) and save the results in images, charts and tables. All of this can be done for an arbitrary number of subjects, sessions and conditions.
If the user wants to perform an fMRI analysis without EEG, a spreadsheet must be filled in with the experiment paradigm according to a template, and the rest will be automatic.

RESULTS: In order to illustrate the usefulness of the program, we present results from an EEG-fMRI study of a left temporal lobe epilepsy patient whose EEG showed FIRDA (frontal intermittent rhythmic delta activity), slow waves and sharp waves. Using SAfE, we varied the time-to-peak of the gamma variate hemodynamic response from -9s to +9s [1] to find the hemodynamic function that would produce the statistical map with more activation (T-value added up for all suprathreshold voxels). We also grouped the fMRI maps of different times-to-peak to assess the temporal and spatial dynamic of the epileptiform activity [1].

DISCUSSION: The activation maps for FIRDA and slow waves were similar, while sharp waves presented hemodynamic changes in different regions. By shifting the time-to-peak of the hemodynamic response, positive BOLD changes were observed 3 s before the EEG event onset for all conditions, whereas negative BOLD signal was found around 3 to 5 s after the event onset. In FIRDA and slow waves, negative BOLD changes took place mostly in the default mode network.

CONCLUSION: Importing data from the EEG analysis software into the fMRI analysis package can be tedious and susceptible to error, the likelihood of which increases with the number of subjects, sessions and conditions. We wrote the program SAfE, which facilitates the task of generating functional maps from fMRI data recorded with or without EEG. Even a person with little training in fMRI analysis can use this interface very easily.

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VIRTUAL REALITY FOR MOTOR AND COGNITIVE STIMULATION

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INTRODUCTION: We present here a set of Virtual Reality (VR) motor gesture tools for neuromuscular stimulation. The idea was to provide users with non-conventional means of interacting with the VR environment, using motor gestures in a physically active an essentially playful way. A longer term goal is to apply these tools to rehabilitation of patients with motor disabilities such as those caused by stroke or some neuromuscular diseases.

MATERIALS AND METHODS: The set of applications was developed to be controlled using a gesture recognition sensor (Kinect – Microsoft), which scans the user's body using an infrared camera. The applications were written in the computer language Java and they run on Linux OS. The experiments and usability tests have been conducted at LaVIIC.

RESULTS: *GestureChair* – it was developed from a free version of the Pacman game and gives to the user the possibility of controlling the game using his hands (up, down, left or right). It was initially directed to spinal cord injury, which causes paraplegia and compromises the function of lower limbs but not the upper limbs, but in principle, it can be applied to any condition that partially disables upper limbs. *GestureChess* – it was developed to control a chess game, in which the first movement is a click that creates the origin of a spatial coordinate system. From this point it is possible to associate hand movements to pointer coordinates thus controlling the game. This is an application indicated to users who require intense cognitive stimulation related, in this case, to the calculation of probabilities required by chess game itself. Simultaneously, it is useful for upper limb stimulation. *GestureMaps* – it was developed for the exploration of geographic maps in Google Streetview. This application is controlled by body gestures, namely lower limb lifting (to simulate walking) and trunk rotation to the right or left (according to the desired direction). It was proposed having in mind elderly people who have spatial disorientation. *GesturePuzzle* – it was implemented as a virtual puzzle game where the user mounts an image from separate parts. The interaction with the VR environment occurs through natural movement of picking up and dropping the pieces where the user freely explores the shoulder joint in all planes of motion.

DISCUSSION: VR has been used for rehabilitation and physical assessment in general [1, 2], more intensively over the last few years. Chang et al. pointed to the use of the Kinect body tracking device in the rehabilitation of children with cerebral palsy and muscular atrophy [3]. Using a similar interface application, Ustinova et al. demonstrated that the use of gesture recognition increased the postural coordination of upper limbs in patients with traumatic brain injury [4].

CONCLUSION: The presented applications (*Gestures*) allow muscle recruitment at different speeds and ranges of motion, training of general motor coordination, and cognitive stimulation in situations of dual task performed in virtual environments, with gestural control. They can be turn into a cheaper alternative for additional physiotherapy sessions for neuromuscular disabled patients at the home environment.

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WEB-BASED PLATFORM FOR MEDICAL IMAGE PROCESSING AND ANALYSIS

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INTRODUCTION: This work presents the current ongoing development of a web-based platform suited for the deployment and publishing of tools, images and documents related to processing and analysis of medical images. The main purpose is to support interdisciplinary research from engineering and medical teams. The advantages are data and processing centralization, information security, availability, and support for managing experiments and studies.

MATERIALS AND METHODS: The platform is an extension of Adessowiki [1], which is a broader web-based collaborative platform for software development, research, and teaching. Currently, the tools for medical image processing and analysis must be written in Python/Numpy or C/C++ programming languages, in order to be published through the platform. Other software tools, like FSL 5.0 [2], can be integrated to the system.

RESULTS: The first prototype is a fully automated pipeline for segmentation and parcellation of the corpus callosum, which was adapted from a recent work by Freitas and collaborators [3]. The pipeline performs the preprocessing and generates the *Diffusion Tensor Images* (DTI). Then it finds the mid-sagittal slice, determines the corpus callosum region, and divides the corpus callosum area into five sections. The final product is a number of web pages that reports all the intermediary steps and final resulting images, together with microstructural measurement estimates for the corpus callosum area, and its sections, regarding scalar measures obtained from the DTI model.

DISCUSSION: The main differential aspect of this report is the ability to present the images related to all intermediary processing steps, serving as a document for quality and correctness control. Therefore, the user is able to assert any part of the pipeline and to repeat the processing with different parameters in situations where the automatically estimated ones were inadequate.

CONCLUSION: The proposed centralized web-based system offers several advantages for medical image analysis tasks, given the information sensibility and need for high computational power. There is no need to install any special software package in the user computer. Everything is accessible via a web-browser. The proposed approach avoids missing the processing parameters, usually an issue from interactive methods, and allows users to view the intermediate steps of batch processes, which are usually executed without visualization.

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LINEAR CLASSIFICATION APPLIED TO SSVEP-BCI SYSTEMS: PRELIMINARY RESULTS

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INTRODUCTION: In this study, the Steady-State Visually Evoked Potential (SSVEP) paradigm was employed in the context of a Brain Computer Interface (BCI) system [1]. In order to identify the brain patterns associated with SSVEP, two linear classification strategies were tested: the Least Squares Method (LSM) [2] and the Fisher Discriminant Analysis (FDA) [3]. The performance of each solution was evaluated offline with the aid of a cross-validation criterion.

MATERIALS AND METHODS: A subject was asked to focus his/her gaze, for one minute, at each light source. The evoked frequencies were 13, 18 and 21 Hz. The EEG signals were recorded at a sample rate of 256 Hz using a cap with 16 channels positioned according to the 10/20 system [1]. Data collection was performed for 3 sessions, giving rise to a database with 9 minutes.

EEG data was spatially filtered by a small Laplacian [4] for artifact removal. Next, three channels placed at the occipital zone (O1, O2 and Oz) were filtered by 3 bandpass filters centered at the evoke frequencies. The filtered signals were divided into windows of 0.5 or 1s, and the corresponding output power was calculated. The LSM and FDA classifiers were built to separate the three classes in a direct fashion.

RESULTS: The Table 1 presents our preliminary results in terms of rate of correct classification.

	LSM		FDA	
	0.5 s	1 s	0.5 s	1 s
13 Hz	57.7	61.0	55.0	57.2
18 Hz	48.7	46.3	60.2	61.5
21 Hz	70.0	70.8	70.2	71.5

Table 1 – Linear classifier performance

DISCUSSION: In Table 1, we can see that the two studied classifiers present similar performance, with a hit rate about 60%. The main difficulty was to process small windows of the EEG signal with the presence of artifacts. We note that classifiers perform differently for each frequency, so it would be possible to imagine an overall hit ratio as a potential adaptation criterion.

CONCLUSION: These preliminary results demonstrate that linear classifiers are potentially efficient to build an SSVEP-based BCI. Immediate next steps are: a) to improve the process of artifact removal; b) to test nonlinear classification paradigms.

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DEVELOPMENT OF A DIFFUSE CORRELATION SPECTROSCOPY SYSTEM

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INTRODUCTION: Blood flow plays an important role in brain physiology, since its change represents an indirect marker of metabolic consumption due to neuronal activation. Nowadays, there are few possible ways to measure blood flow continuously and noninvasively. Diffusion Correlation Spectroscopy (DCS) is a promising and relatively new optical technique that is capable of measuring relative blood flow (rBF) in the microvasculature of the deep tissues. Briefly, by shining light into the tissue and measuring the temporal autocorrelation function of the scattered intensity, it is possible to estimate the motion of scatterers in tissue (mostly, red blood cells). DCS does not provide a direct measurement of blood flow, but instead provides a blood flow index which has been shown to be highly correlated to blood flow [1]. Among several features of DCS over other techniques, it is cheap, non-invasive and portable, with high temporal (0.5 – 3s) and good spatial (~10mm) resolution [2]. The current work aimed to build and characterize a DCS module that can be used to assess perfusion in turbid medium, such as phantoms and in *vivo* measurements of biological tissue.

MATERIALS AND METHODS: The DCS module employs 4 four-channel Single Avalanche PhotoDiode arrays (PerkinElmer), a 16-channel correlator board (Correlator.com) and two high coherence lasers (CrystaLaser; 785 and 852nm). Three sensitive, low-noise power supplies feed the whole system, which is digitally controlled by a homemade software in LabVIEW. To characterize the DCS module, we first measured the stability of the output intensity of the lasers for ~15 hours and the dark count of each detector for ~3h. After that, we measured the stability of the system with a liquid phantom (diluted milk solution) for ~14h. Finally, we performed an arm occlusion test (two blocks of 30 s each) to evaluate the ability of the system to measure the rBF) in human muscle.

RESULTS: The output intensity of the lasers was very stable, with standard deviation of 0.21% and 0.26% for the 785 and 852nm, respectively. The mean dark count across all detectors over the ~3h was 320 counts per second. The intensity measured with the liquid phantom had a relative standard deviation of 2.2%. Lastly, the rBF measured during the arm occlusion test showed a decrease of 100% in all trials, as expected by physiology and demonstrated in previous literature.

DISCUSSION: The results from our first tests indicate that the system is quite stable, being able to measure small changes in blood flow as 2.2%. The arm occlusion experiment results suggest that the DCS system is indeed measuring blood flow. The magnitude of the measured variations is in agreement with the literature and expected physiolgy.

CONCLUSION: In this work we have built a stable DCS module and have shown that the system works as expected at the bench. We were able to successfully reproduce arm occlusion tests as previously done in literature. In the near future, we plan to translate the bench setup into a real box in order to have a portable device, and to further validate the technique as a promising blood flow monitor with controlled liquid phantoms experiments before proceeding to *in vivo* measurements.

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TECHNOLOGY OF ELECTROACTIVE AND BIOCOMPATIBLE MATERIALS FOR NEURAL PROBES

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INTRODUCTION: Neural probes are a type of invasive technology able to perform recording or stimulus measurements *in-vivo* on neuron cells. There is still a need to overcome long-term stability and biocompatibility effects. Towards that goal, several types of emerging materials targeted for structure and functionality in neurodevices, such as polymers, metals, oxides, among others have been investigated [1,2]. This project aims to create a platform to study the electrochemical properties of microelectrode arrays and thin films, validating the process initially with materials already studied such as Au, Pt and TixNy [2]. We intend to develop neural probes whose mechanical structure is based on the polymer called SU-8, which is an excellent candidate due to its biocompatible properties and microfabrication process [1].

MATERIALS AND METHODS: The Microfabrication of neurodevices was conducted in collaboration between Center for Information Technology Renato Archer (CTI) and Center for Semiconductor Components (CCS). The first models of neural probes with SU-8 metallized with TiN (Sputtering) and Au-Ni (electrodeposition) were performed. Using the techniques of electron beam and vacuum evaporation of Au-Cr, Au-Ti and Pt-Ti films was deposited on Si and glass substrates to study the general morphology metal by AFM. Moreover we want study possible eutectic effects in the SU-8 deposited on substrates such as glass and silicon. Characterization will be done with Cyclic Voltammetry (CV) and Electrochemical impedance spectroscopy (EIS) techniques on thin films and subsequently with microelectrodes for different diameters. For CV measurements we are doing a platform with Labview Ni USB 6251 for acquisition of analog data and triangular pulse generation in the system, however CV measurements will be made, also with a commercial potentiostat. For EIS measurements will be made by means of an impedance analyzer.

RESULTS: So far we have managed to fabricate some models of neural probes based on polymer SU-8. We successfully produced microelectrodes with metals such as Au-Ni and ceramic TiN (see Figure). We have developed a process for creating arbitrary 2D microelectrodes, and a process that should allow new materials to be investigated as microelectrodes.

DISCUSSION: With these results, we next intend to study the electrochemical properties of microeletrodos TiN, Au and Pt by measures of CV and EIF, and morphological part with AFM measurements. We seek to understand in greater depth the charge density effects and processes of mass transport in electrodes. In addition to SU-8 demonstrated its feasibility as a biocompatible material for neurodevices.

CONCLUSION: Understanding the mechanisms underlying the electrochemical behavior of new microeletrodes it is of great importance for development of future long-term neural probes. SU-8 was shown to be an adequate platform for investigating novel materials for such neural probes.

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BIOMEMS-BASED NEUROTECHNOLOGY

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INTRODUCTION: Microelectromechanical systems (MEMS) refers both to devices at the micro scale and to the microfabrication techniques originated from the semiconductor industry. They often consist in elements with certain physical or chemical properties which allow them to perform specific tasks. In particular, biological or biomedical applications of this technology (BioMEMS) have been already demonstrated suitable to a wide variety of issues in medicine and pharmaceutical research [1,2]. In fact, BioMEMS are being established as a key technology leading to the deployment of neuroengineering and neurotechnology. Implantable biomedical devices have great potential to the development of novel therapies, diagnostic methods and to improve the quality of life of several patients with neurological diseases and spinal cord injuries [2]. In this context, neural probes have been seen as an important instrument to neuroscience, allowing the study of neuronal activity with minimal invasiveness. This work aims the study and establishment of the current processes of design, fabrication and characterization of BioMEMS-based polymer neural probes to record and stimulate neuronal activity.

MATERIALS AND METHODS: The neural probes layout was designed using the currently available CAD tools. They were fabricated using well established surface micromachining methods (lithography, deposition, etching). Polymer SU-8 was chosen to serve as structural material, due to its relative flexibility and the feasibility to enhance the device biocompatibility [3]. Different conductive materials will be tested and compared as microelectrodes, such as Au, TiN, Pt and Ti. Electrochemical experiments will be conducted to characterize the sensing capability of each. Finally, *in vivo* tests will be performed in order to define the practical functionality of the device.

RESULTS: Different probe designs and lithographic masks were produced. A specific microfabrication process was defined in order to obtain SU-8 neural probes with enhanced stimulating and recording capabilities. Furthermore, prototypes of these devices were produced, with 240 μm in width and ≈ 5 mm in length, containing eight microelectrodes, using either Au or TiN. Lastly, mechanical test were performed to insure the mechanical stability of the probe during cortex insertion.

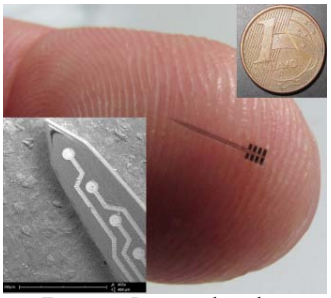

Figure 1: Projected probes

DISCUSSION: The probe/mask design methodology has proved to be appropriate to generate novel devices and geometries. Currently, the production steps are being optimized in order to develop and standardize an effective and inexpensive method of producing neural probes. The prototypes elucidated the need to modify the current design, in order to improve the handling and to facilitate subsequent packaging. After that, new designs will be made to fulfill neuroscientist's specific needs.

CONCLUSION: The present work is confirming the microfabrication viability of SU-8 based neural probes already documented in the literature, using low cost and low time consuming methods [3]. Small devices with narrow probes and microelectrodes were successfully projected and obtained. Besides, it's demonstrated the inherent potential of BioMEMS application in the neurotechnology and neuroengineering areas.

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RESTING-STATE CONNECTIVITY OF PATIENTS WITH CAROTID STENOSIS USING NIRS

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INTRODUCTION: Near-infrared spectroscopy (NIRS) is an optical technique that employs near-infrared light to probe deep tissue noninvasively and continuously. In this work we demonstrate how NIRS can be applied to analyze cerebral connectivity during the resting-state. In particular, we aimed to find differences in properties of connectivity maps in patients with asymptomatic carotid stenosis when compared to controls. We investigated the performance of the method by using two different types of datasets provided by NIRS, exploring optical and physiological information.

MATERIALS AND METHODS: We employed a CW6 instrument (TechEn, Inc., Milford, MA, USA) with 2 wavelengths (690 and 830 nm) and an optical geometry with 28 source-detector pairs (seeds). We recorded data for 5 minutes during the resting-state from 19 subjects, who were divided in four groups: Group 1, for patients with total occlusion in one artery and < 50% (i.e., normal) in the other (N = 4); Group 2, with total occlusion in one artery and 75-90% occlusion in the other (N = 3); Group 3, with 75-90% occlusion in one of the arteries and < 50% in the other (N = 9); Control, with < 50% occlusion in both arteries (N = 2). After acquisition, intensity data were band-pass filtered (0.008 - 0.09 Hz) and converted to changes in optical density before estimating hemoglobin concentration changes by using the modified Beer-Lambert law. For each seed time-course, we computed the Pearson's correlation coefficient with the temporal time-course of the other source-detector pairs, and built a correlation matrix (28x28). Adjacent matrices were built by varying the correlation threshold from 0.2 to 0.95.

RESULTS: In all cases, the mean degree of connected links decreases as the threshold increases. For correlation thresholds in the range from 0.50 to 0.85, hemoglobin concentrations show different patterns between controls/group 1 and groups 2/3. The mean number of correlations decreased by 14-35% in patients of groups 2 and 3, when compared to controls and group 1. We see no statistical difference between the groups when the light intensities are used as information to compute the correlation coefficients.

DISCUSSION: Although data acquisition is still ongoing, these preliminary data suggest that patients of group 1 present resting-state properties that are similar to controls. The resting-state properties of patients in the intermediate groups significantly differ from controls. This result appears to be connected to the number of collateral vessels opened; patients in group 1 have at least two collateral vessels opened, while this number is never over one vessel for patients of group 2 and 3.

CONCLUSION: NIRS-based connectivity maps during the resting-state have potential to distinguish physiological states in patients with carotid stenosis. Patients with consolidated occlusion tend to show similarities with controls, a fact that can be understood by the opening of collateral vessels in order to reestablish cerebral flow. In addition, we found that direct information from light intensity does not provide detailed connectivity maps, possibly due to mixing of hemoglobin. From our data, it appears that HbO and HbT are the best contrasts to differ the groups studied. We expect to collect more data to strengthen our argument very soon.

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DETERMINATION OF ABSOLUTE OPTICAL PROPERTIES FOR BRAIN, BREAST AND MUSCLE WITH DIFFUSE OPTICAL SPECTROSCOPY

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INTRODUCTION: Diffuse optical spectroscopy (DOS), commonly called Near-Infrared Spectroscopy (NIRS), is a suitable technique to probe biological tissue in vivo. In the near-infrared region of the spectrum, tissue absorption is dominated by hemoglobin, since water absorption is relatively low [1]. In addition, light propagation is governed by scattering processes, which enables light to travel deeper into most tissues. By measuring the scattered light from tissue, it is possible to determine tissue absorption, which can be related to concentrations of oxy-hemoglobin (HbO2) and deoxy-hemoglobin (HbR) [2]. In this work, we employed the DOS technique in the frequency domain which provides information about the phase-shift and the amplitude of the scattered wave to determine the absolute optical properties of biological tissue (brain, breast, muscle).

MATERIALS AND METHODS: We used a DOS system in the frequency domain for data acquisition. The equipment employs 4 photomultipliers and 32 light sources in 4 wavelengths, all with a modulation frequency of 110 MHz. From the diffusion equation for the light fluency in turbid media, and by considering the medium as uniform and semi-infinite, along with the approximation that source-detector distance is larger than scattering length, both the phase-shift and the logarithm of the amplitude depend linearly on distance. The slopes of the relationships depend on the optical properties of tissue [2]. In order to validate this technique, we used optical phantoms with predefined optical properties, whose values were found with an error of approximately 3% relative to the predefined values.

RESULTS: Both the phase-shift and the logarithm of amplitude presented a linear behavior for all three tissues analyzed. From the slopes we calculated tissue absorption to find HbO_2 and HbR concentrations. Total hemoglobin concentration and tissue blood oxygen saturation were calculated. The average results are presented in Table 1.

Tissue	HbO_2 (μM)	HbR (μM)	TCH (μM)	StO_2 (%)
Brain	39(5)	14(4)	53	73
Breast	18(2)	4(1)	22	82
Muscle	81(15)	13(4)	94	86

Table 1: Average oxy, deoxy and total hemoglobin concentrations, and tissue blood oxygen saturation, across all subjects for brain, breast and muscle.

DISCUSSION/CONCLUSION: Our results indicate that, by simultaneously measuring the amplitude and the phase-shift of scattered wave in turbid media, it is possible to estimate the absolute optical properties. However, it is important to calibrate the system beforehand in order to account for a coupling factor between the fiber and the medium. By analyzing the behavior of the amplitude and phase-shift, we conclude that the diffuse propagation and semi-infinite medium approximations appear to be appropriate in all the three biological tissues.

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A NOVEL TECHNIQUE TO SEGMENT BRAIN DTI DATA INTO THREE MAIN TISSUES USING INTRA AND INTER VOXEL FEATURES

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INTRODUCTION: This work proposes the use of an Artificial Neural Network (ANN) to segment the human brain in Diffusion Tensor Image (DTI) data. The goal is to segment the brain in White Matter (WM), Gray Matter (GM) and Cerebral Spinal Fluid (CSF) using both intra and inter voxel features. Although well studied on T1 images, this problem is still a challenge in DTI data.

MATERIALS AND METHODS: The DTI data were acquired from a Philips 3T MR Imaging DD 005 using a single-shot EPI technique with voxel size = 2x2x2 mm, interpolated to 1x1x2 mm, acquisition matrix = 128x128, FOV = 256x256 mm, 70 slices, no gap, TR = 8500 ms, TE = 61 ms, flip angle = 90°, 32 gradient directions, b = 1000 s/mm2, at FCM/Unicamp. The ANN used was a Two-Layer Perceptron feed-forward, with seventeen inputs, three neurons in the hidden layer and three neurons in the output layer. The experiments were performed with the Matlab Neural Network ToolboxTM. The ANN was trained from four human brains data by the Scaled Conjugate Gradient Back-Propagation algorithm. From the tensor data, ten intravoxel features and seven intervoxel features were extracted. To prove the robustness of the technique, three different models were used on the training process: (A) Threshold using Mean Diffusivity (MD) map to separate CSF from non-CSF and Fractional Anisotropy (FA) map to separate WM from non-WM [1]; (B) FAST-FSL 2-channels input into three classes using MD and FA maps; and (C) The same as (2), but using λ3 and λ1 eigenvalues maps [2]. The segmentation accuracy result was measured with the Volume Overlap (VO) and Volume Agreement (VA) metrics.

RESULTS: The following table presents the VO and VA values between the ANN result and the segmentation result model for each class.

Training models	Volume Overlap (%)			Volume Agreement (%)		
	CSF	GM	WM	CSF	GM	WM
(A) Threshold	95.8	99.2	99.8	95.8	99.3	99.9
(B) FAST MD / FA	93.1	88.3	84.5	94.6	88.4	84.6
(C) FAST λ_3 / λ_1	96.4	94.6	93.9	97.3	96.9	98.8

DISCUSSION: Model (B) has a small rate of true negative about WM, leading the ANN to have high error rate in that class. The results for model (C) are better than for model (B), since the average of true positive and negative rate are higher [2]. Model (A) has higher VA and VO values than (B) and (C) against manual segmentation [3]. In the table, the highest values are also of model (A), which confirms that the coherence of the segmentation model influence directly in the ANN learning.

CONCLUSION: The results confirm the effectiveness of the proposed segmentation technique. The present method shows itself powerful and suitable, since the training model can be changed every time a new segmentation method is proposed. The weak point is: the technique is based on voxelwise processing, thus global anatomical information is ignored.

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A TOOL FOR HIGH THROUGHPUT SEQUENCING DATA QUALITY CONTROL

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INTRODUCTION: Next Generation Sequencing has become the standard tool in biomedical sciences to investigate the association of genetic and epigenetic markers and phenotypes of interest. With this approach, millions of small fragments (about 100 bases) are simultaneously sequenced. These sequenced fragments are also known as reads and the sequencing process assigns quality scores to every sequenced base. Lower quality scores are often associated to issues during the base-calling process and the inclusion of such bases in the downstream analyses may negatively affect the results [1]. Therefore, identifying such reads is one important task in the data analysis workflow.

MATERIALS AND METHODS: The Rqc package has been used to analyze the quality of the data produced by our group and also tested with public data from other research groups. We compared the results with those produced by FastQC [2] and both produced similar outcomes.

RESULTS: We developed the Rqc package, based on the R statistical environment, to address this issue. In summary, the package is an optimized tool designed for quality control and assessment of high-throughput sequencing data. The quality control step uses raw data delivered as FastQ files. We use statistical procedures to describe and assess the quality of the reads through data summarization, GC content distribution across base position, distribution of read lengths and sequence content analysis. We use high resolution graphical representations to show these results to the final user.

DISCUSSION: Rqc fully supports the FastQ standard format (compressed or not), it is independent from technology, despite the differences between sequencing technologies. The package can handle multiple input files using high-performance computing solutions. It produces an easy to view report, which can be modified and distributed (web format, HTML).

CONCLUSION: Rqc allows for greater flexibility, including its capability to be transparently incorporated to pipelines within the R/Bioconductor ecosystem [3]. This is a work in progress and we are currently investigating additional functionalities to be added to the software.

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DEVELOPMENT OF A FEEDBACK INTERFACE FOR MOTOR IMAGING BASED BRAIN-COMPUTER INTERFACES: PRELIMINARY RESULTS

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INTRODUCTION: Recent studies have shown that the use of feedback interfaces for BCI (brain-computer interface) systems can be quite useful to new users. Feedback can improve the user's performance, helping her/him to understand how to gain control of her/his neural impulses, which are the primal interest for defining the user's intentions. This work consisted on the development of a feedback interface for motor imaging (MI) based BCI systems, in order to evaluate the effect of the use of such a method in the BCI performance. In a MI-based BCI system, the main tool to understand the user's intentions is the amplitude of the brain signals in the motor cortex.

MATERIALS AND METHODS: The interface was developed using the MATLAB software. It consists of a black arrow on a white background, that points to the directions "right" or "left" at random times, alternating its orientation after 30 to 45 seconds. While this time passes, the arrow can move across the screen in either direction, depending on which command the program receives. Ideally, the arrow should move in the direction it points to, but that will depend on whether the user correctly modulates her/his brain signals; so if the arrow points right and moves left, the user knows her/his thinking strategy is wrong. Currently, the input consists of a random number generated in the interval [-1, 1]. If it is positive, the arrow moves to the right; if it is negative, the arrow moves to the left and, if it is zero, the arrow does not move. Its movement is based on a coordinate system positioned on the screen, but set as 'not visible'. The program is set into a loop in which every interaction can change the horizontal position coordinate of the arrow, and the intensity of this change is proportional to the number. Every second is counted and when the time reaches its final value, the arrow disappears and it is set to the center of the screen, ensuring the process to occur over again.

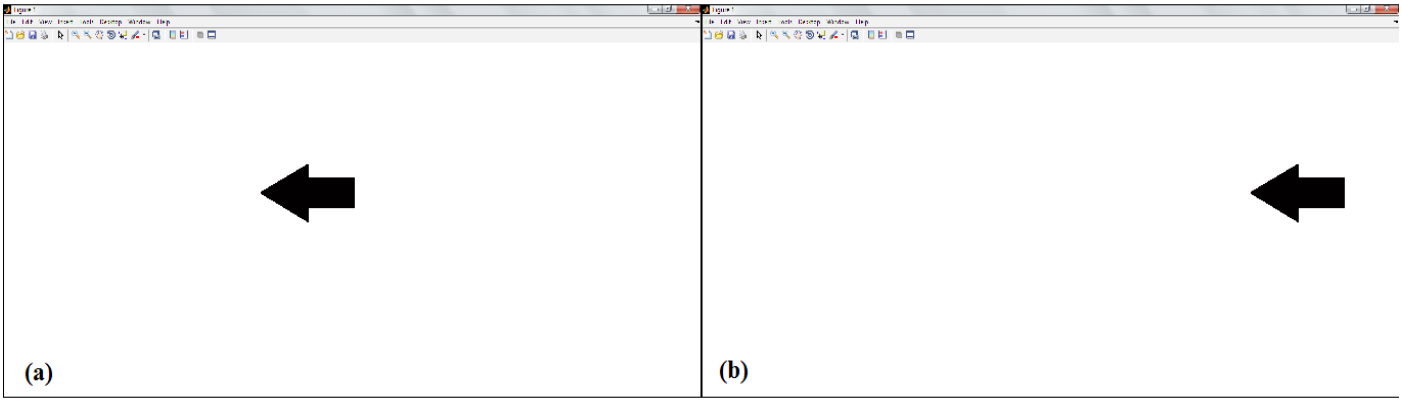

Figure 1. Interface showing arrow at its center (a) and later moving to the right (b).

RESULTS: Figs. 1(a) and 1(b) show the interface in progress with a positive answer (arrow going to the right). It can be noticed that the direction of the movement is to the right even though the arrow points to the left, which is not a problem, since there should not be any restriction of that kind.

DISCUSSION: So far, the interface has been running on simulated answers. In the future, the answer will come from the user's brain signals. Positioning electrodes on the motor cortex, it will be possible to acquire data from the brain using the Electroencephalography (EEG) technique. The user will think of the movement of the right or left hand, depending on which direction will be shown by the arrow in the interface. Then, a classification algorithm will analyze the brain signals and interpret the user's intentions.

CONCLUSION: We developed a feedback interface for MI-BCI systems. Currently, the classification algorithm is under development and the interface's script has been adjusted in order to work together with the algorithm. It is expected that, soon, it will be possible to interpret data collected from the user's brain with minimal error.

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DEVELOPMENT OF A MONTE CARLO ALGORITHM FOR DIFFUSE CORRELATION SPECTROSCOPY

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INTRODUCTION: Monte Carlo methods are computational algorithms that use random variables for calculating real physical quantities. In particular, Monte Carlo methods have been recently used for modeling the propagation of light through the biological tissue [1, 2]. By modeling light as photon packets, it is possible to simulate random photon interactions with the particles in the medium and therefore compute photon paths, given a medium geometry. When the particle dynamics is considered, the physical quantity of interest is temporal the electric field autocorrelation function [5]. The temporal electric field autocorrelation function depends on the mean-squared displacement of the scattering particles. In this work, we implement a Monte Carlo algorithm taking into account the dynamics of scattering particles. We validated the algorithm by comparing the results obtained in the simulation with the diffusion approximation for different medium geometries, both in the steady state and in the time resolved case [3]. We also show how the temporal autocorrelation function can be obtained from the simulation.

MATERIALS AND METHODS: We used the Monte Carlo code created by Boas et al. [1]. Briefly, the algorithm simulates the photon density in a 3D volume with given optical proprieties (). The code generates the pathlength of each photon package from an exponential distribution. A new direction scattering is calculated; the azimuthal and polar angles are respectively generated from a uniform distribution and a Henyey-Greenstein phase function. The process continues until the photon escapes or it has traveled longer than a predefined period (approximately 10 ns), since the probability of photon detection is very small. The intensity can be estimated by the photon density fluence at each voxel. We modified the algorithm so that the pathlength of each photon could also be stored. With the photon random walk step information, we built a homemade script that can estimate the temporal autocorrelation function for the electric field, given the mean-squared displacement of the scattering particles.

RESULTS: We found good agreement between the results obtained with the modified Monte Carlo code and the analytical solution of the diffusion equation for a semi-infinite homogenous medium. The code and the analytical solution did not match for regions where the diffusion equation is not valid. We also valeted the code for two-layer medium. From the photon random walk step information, the temporal electric field autocorrelation function was also successfully validated against the analytical solution for a semi-infinite medium.

DISCUSSION: We found the Monte Carlo approach permits the study of light propagation in more complex media, such as heterogeneous media. This is fundamentally important for the study of biological tissue. In the future, we want to use the code to study the dynamic properties of the medium in more complicated geometries (e.g., two-layer and semi-sphere) which better suit the features of different organs geometries. We also plan to solve the inverse problem (i.e., to guess the optical proprieties of the medium by comparing the results with experimental data) for both DOS (Diffuse optical spectroscopy) and DCS (diffuse correlation spectroscopy).

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INTERACTIVE MULTIMODAL VISUALIZATION FOR 3D NEUROIMAGE EXPLORATION

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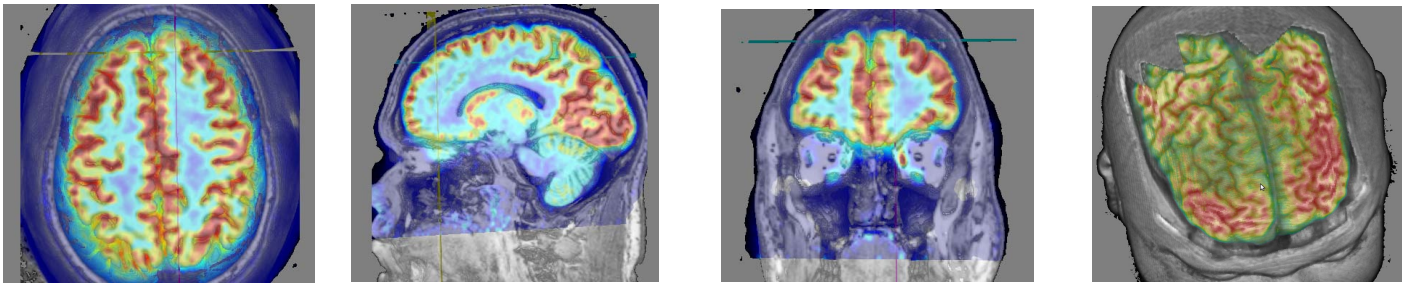
INTRODUCTION: Approximately 30% of patients with focal epilepsies have seizures resistant to antiepileptic drugs and surgical treatment is the best option for seizure control in these cases. The precise localization of epileptogenic focus and the brain structural abnormalities related to it are essential for both surgical planning and good postoperative outcome. However, in some patients the brain abnormalities related to the epileptic focus are subtle and difficult to be detected in the conventional visual analysis of the MRI studies. This work presents a 3D interactive multimodal environment which allows the co-registration of different structural and functional neuroimaging and enables a neuroscientist to intuitively visualize the cortical lamination.

MATERIALS AND METHODS: We implemented our 3D interactive visualization environment on top of open source libraries. Mutual information based registration algorithm is applied for co-registering multimodal neuroimages [1] and interactive curvilinear reformatting algorithm has been implemented for providing laminar cutting of the brain's cortical structures [2]. All the rendering procedure runs on GPU (graphics processing unit). We have conducted our exepriments with CT (computed tomography), MR (magnetic ressonance) and PET (positron emission tomography) images. The MR images were acquired in the Philips Achieva 3T scanner and the CT and PET volumes in the Siemens s5vb20b multimodal imaging scanner. With our analysis environment a physician, based on clinical and EEG data, forms a vague search query to get some regions of interest on the first try, and interactively refines the search query by following the domain-specific knowledge to get information from complementary modalities until a suspicious lesion pops out.

RESULTS: The images illustrate the outcomes of our proposed tool. There is a concordance between 2D and curvilinear reformatted views: a suspicious lesion in the right frontal lobe. Note the cortical surface in grayscale is underlying the regions colored in accordance with brain metabolism. Rainbow color palette is employed: red indicates a higher cell activity, while blue shows decreased activity or none at all.



DISCUSSION: To our knowledge, the existing multimodal visualization systems are not suitable for exploring laminar cortical structure. Preliminary medical assessment indicates that our proposed visual exploration may be useful for locating a suspicious extra-temporal subtle epileptogenic lesion. **Conclusion:** For assessing the reliability of our technique we plan to do intracranial EEG (electroencephalography) recordings. We also plan to further investigate the potential of the presented visualization technology for surgical prognosis and to improve the user interface.

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Trabalhos / Neuroeducação

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I Congresso Cepid Brainn

INITIATIVES IN NEURO-EDUCATION AS A TOOL TO BRING ADOLESCENTS' ATTENTION TO NEUROSCIENCE

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INTRODUCTION: If regular science learning represents a great effort at school, when it comes to neuroscience this learning becomes a challenge. Learning neuroscience might sound a difficult and unattainable task to students. Although, we believe that childhood and adolescence are critical in order to break myths about the brain and attract people to neuroscience research. The main question we addressed was how many facts and curiosities about the brain catch adolescents attention to neuroscience.

METHODS: We gave short term lectures to one hundred and fifty eight adolescents attending the *Ciência e Arte nas Férias* program at Unicamp about the following major topics: Neurochemistry, Anxiety, Music, Motricity and Memory. These topics were then developed around specific facts involving the brain: "our brain is in love – neurochemistry of passion", "stress and anxiety with the brain going nuts", "what music has to do with our brain?", "our brain is the one who runs the movement" and "memory – our brain can learn, memorize and then?". We focused on curiosities and subjects which could be easily associated to day-by-day issues. The students were divided into five groups and attended the five different lectures at the same day. At the end of each day, the students were then invited to participate in a quiz to test their comprehension of the classes. The quiz consisted of questions presented in a playful way always making references to the topics studied. Two groups also filled an anonymous Likert scale to assess: lecture content, presentation, motivation, didactic materials (slideshow, videos, etc), curiosities presented and the quiz. The Likert scale comprised four levels of quality: Below Average/Average/Above Average/Excellent.

RESULTS: A total of forty students answered the scale. None of the issues were considered Below Average or Average. Regarding the issues content, motivation and curiosities, 97.5% of students rated it as Excellent (2.5% above average). Presentation was classified as Excellent by 92.5% (7.5% Above average). Didactic materials and the quiz were rated as Excellent by 90 and 87.5% of the students (10 and 12.5% Above average), respectively.

DISCUSSION: Our results showed that the majority of students approved the methodology chosen and perceived it as a good experience. We have preliminary data that point out effective ways to teach neuroscience so that it may attract adolescent's attention.

CONCLUSION: Here we show that short lectures focusing on facts and curiosities about the brain are an interesting tool in learning neuroscience.

'EPILEPSY OUT OF SHADOWS': RONDON PROJECT AND ASPE PROMOTE WORKSHOPS IN THE COUNTRYSIDE OF MARANHÃO STATE, BRAZIL

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INTRODUCTION: Epilepsy is the most common chronic neurological disease worldwide. Apart from its psychological, physical and economic challenges, it also reveals barriers related to the family, school and society, especially due to stigma and lack of knowledge. In Brazil, there are approximately three million people with epilepsy, adding 300 new cases per day. Since 2002, the non-governmental organization called ASPE (Healthcare for Patients with Epilepsy) is the official executor of the World Health Organization Global Campaign named 'Epilepsy Out of the Shadows', aiming at increasing epilepsy awareness in Brazil. This paper presents the workshops sponsored by ASPE in January 2014, under the Operation 'Velho Monge' of the Rondon Project, organized by the Ministry of Defense in the State of Maranhão. These also aimed at promoting an awareness on epilepsy, through a research about people's perception, which laid the foundations to construct the knowledge within a group and empower multipliers.

MATERIALS AND METHODS: Activities were planned as two workshops of two hours each, performed in the same format with one-week interval. In partnership with the Education Secretary, seven schools were visited as a strategy to disseminate these activities, directly inviting approximately 35 teachers. Workshops were structured to encourage a group construction of knowledge on Epilepsy, approaching it to teach about the disease, treatment and procedures to attend a person during a seizure, demystifying misconceptions to reduce prejudice. First, each participant was asked to write in a piece of paper his/her concept of Epilepsy. Followed by a discussion and based on participants' prior knowledge, definitions about the theme were introduced, using slides, videos and testimonials of people with epilepsy as teaching resources. To conclude, two games were performed: first, participants received prizes (souveniers from ASPE) when answering questions about Epilepsy. Second, participants were asked about changes in their concept of Epilepsy and thoughts about the discussion while holding a line connecting those who spoke.

RESULTS: Workshops were attended by 70 participants, including 26 teachers, 24 students and 8 healthcare professionals. Their perception unveiled that previous knowledge on epilepsy was interspersed with incorrect information and stigma, according to concepts written when asked to describe what "epilepsy" meant to them. Furthermore, five among 53 replies were simple copies of information on epilepsy obtained in leaflets, showing that knowledge had to be reconstructed, not transmitted.

CONCLUSION: Based on the support of ASPE, in partnership with the City Education Secretary, along with participants from the Rondon Project, these workshops represented a group effort to propagate about Epilepsy awareness in a distant community in Maranhão State. The dissemination strategy was crucial to reach the widest audience of teachers and students. Therefore, the group discussion and games allowed many questions on Epilepsy to arise and be demystified. Participants demonstrated certainty replicating information about this disease and treatment. More important, they showed ability to explain and teach others. The last game created a physical connection among all participants, evoking a sense of support to disseminate Epilepsy awareness in their community. Thus, they committed to be multipliers, to expand epilepsy awareness within their family, friends and society through the networks established during these workshops.

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IDENTIFICATION OF A MOLECULAR MECHANISM LEADING TO FAILURE IN NEUROGLIAL DIFFERENTIATION IN FOCAL CORTICAL DYSPLASIAS (FCDS) OFFERS CLUES TO BRAIN DEVELOPMENT AND EPILEPTOGENESIS

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INTRODUCTION: The formation of the cerebral cortex involves complex steps, requiring tightly regulated molecular mechanisms for the efficient control of gene expression. Some of the molecular pathways that control gene expression are performed by microRNAs (miRNAs), a class of non-coding RNAs that regulate gene expression at the post-transcriptional level. FCD is characterized by a spectrum of abnormalities in the development of the laminar structure of the human cerebral cortex usually associated with cell abnormalities, giant/dysmorphic neurons and balloon cells and severe drug-resistant epilepsy. The mechanisms involved in the pathogenesis of type II FCD are not completely understood. In addition, it is unclear how abnormal cortical development can contribute to severe seizure generation in cortical dysplastic tissue. Our main objective was to determine whether abnormal miRNA regulation could be present in type II FCD. In addition, we aimed to identify potential miRNA target-genes abnormally expressed.

MATERIALS AND METHODS: We studied cortical tissue from 17 patients with FCD type II (9 patients with type IIA FCD and 8 with type IIB FCD) who underwent selective resection of the cortical structures for treatment of clinically refractory seizures. Control samples (n=20) were obtained from autopsies of individuals whose cause of death were other than central nervous system diseases. Total RNA was isolated with RecoverAll TM kit (Ambion) and RNA integrity was assessed by Agilent RNA Pico Chip Kit and Bio-Analyzer 2100. MiRNA expression profile was assessed by Affymetrix GeneChip platform miRNA array. Background correction, summarization and normalization were performed by RMA function. MiRNA expression was analyzed using RankProd (FDR $p < 0.05$). Bioinformatics algorithms were used for identification of miRNA-regulated genes and their putative function. Quantitative PCR (qPCR) (TaqMan™ - Life Technologies) and in situ hybridization (ISH) (Exiqon) and laser capture microdissection (LCM) were used to validate results of miRNAs screening experiments and to access expression and localization of target-genes.

RESULTS: Microarray analysis revealed 39 miRNAs which were downregulated and only one miRNA overexpressed. Decreased expression of three miRNAs was validated by qPCR: hsa-miR-31, hsa-miR34a and hsa-let-7f. In addition, overexpression of five times of *NEUROG2* gene, a possible target-gene regulated by hsa-miR-31, was observed in type II FCD. Furthermore, when analyzing specific regions by LCM, we found that *NEUROG2* was also overexpressed in white-matter as compared to control.

DISCUSSION/CONCLUSION: Our results indicate that the three miRNAs, confirmed to be downregulated, act as tumor suppressor genes and may lead to the abnormal histopathological features seen in type II FCD. *NEUROG2*, in cerebral cortex, has a specific temporo-spatial expression pattern, since it is exclusively detected in the ventricular zone, where the precursor-cells are located only during neurogenesis. It has been demonstrated that down-regulation of *NEUROG2* is a key step in the transition phase between inhibition of neurogenesis and induction of early gliogenesis. Therefore, our results support the hypothesis of failure in neuronal differentiation in FCDs, since the transition from neurogenesis to gliogenesis may be hampered by the abnormally increased expression of *NEUROG2* which was found in our study. Therewith, probably seizure generation is the consequence of incomplete cellular maturation and this immature brain tissue could explain a high excitability in patients with type II FCD.

FEASIBILITY OF DIFFUSE OPTICAL SPECTROSCOPY IN THE SURGICAL ROOM: A PILOT STUDY

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INTRODUCTION: Diffuse optical spectroscopy (DOS) is a portable technique able to probe cortical tissue in the human brain. DOS can monitor vascular physiological information, such as the oxygenation levels, continuously, non-invasively and with low cost. Its features make DOS a great potential monitor to situations where portability is required, such as in the surgical room[1]. In this pilot study, we attempted to verify the feasibility of DOS as a hemodynamic monitor during a long-term surgical procedure of carotid endarterectomy. In particular, we evaluated the role of a frequency-domain DOS (FD-DOS) system to provide information of absolute oxygenation levels during the period of surgery[2].

MATERIALS AND METHODS: We employed a commercial FD-DOS system (Imagent, ISS Inc., USA) for data acquisition. The system was composed of one photomultiplier tube as detector and four diode lasers as light sources with a modulation frequency of 110 MHz. Each source had two different wavelengths (690 and 840 nm). After the patient was positioned in the surgery room, the optical probe was positioned on the prefrontal cortex, in the ipsilateral side of the stenosis. The patient was kept sedated throughout the surgery, and all intervention procedures were monitored by DOS.

RESULTS: Hemodynamic levels of oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (HbR) were relatively constant throughout the surgery, with tissue oxygen saturation varying from 65% to 68%. During the clamping of the carotid, however, we observed a marked and instantaneous decrease of 20% in HbO_2, and an 25% increase of HbR in the brain region monitored. An inverse response was observed during unclamping: an increase (decrease) of 44% (30%) in HbO_2 (HbR) concentration.

DISCUSSION/CONCLUSION: Our results indicate that the DOS system is very stable and capable of measuring changes in oxygen levels during surgery. The use of frequency-domain allows for absolute oxygenation in tissue, which can provide the surgeon with reference, ideal standards of oxygenation levels to be achieved. Although more information is needed to establish DOS as a standard monitor in the surgery room, this pilot study shows that DOS is a promising technique able to provide reliable hemodynamic information during surgical procedures in order to prevent risk of complications. More experiments are ongoing.

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EVALUATION OF CYCLOOXIGANASE-2 MRNA EXPRESSION AFTER PTZ-INDUCED SEIZURE IN ADULT AND LARVAE ZEBRAFISH BRAIN

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INTRODUCTION: Epilepsy is a common neurological disorder characterized by recurrent spontaneous seizures that afflicts nearly 50 million people worldwide[1]. Cyclooxygenase-2 (COX-2) is a key enzyme that converts arachidonic acid into prostaglandins, which are a potent mediator of inflammation[2,3,4]. It has been reported that COX-2 mRNA levels are upregulated after inflammatory stimulation[2,3,4]. Because COX-2 is induced after seizure, it has been suggested that this enzyme can play a role in epilepsy[2,3,4]. Zebrafish is now acceptable as a suitable model for seizure studies. Since there are two functional *cox2* genes in the zebrafish brain[5] (*cox2a* and *cox2b*), we have investigated the expression of bothgenes after pentylenetetrazole (PTZ)-induced seizure.

MATERIALS AND METHODS: Adult zebrafish and larvae were maintained according to standard procedures[6] and all experiments were approved by animal ethical committee/Unicamp. Adult and 7 days post-fertilization larvae (dpf) were separated in seizure (SG) and control (CG) groups. Animals from SG were individually exposed to PTZ 15mM and animals from CG were handled in PTZ-free water. Seizure-like behavior stages where analyzed based on previous works[7,8]. A total of five samples were used for each group and age. Each larvae sample was composed by pooling 20 heads and for each adult sample were used a pool of two brains. At 0.05h after seizure, animals were anesthetized and their heads/brains were collected for total RNA extraction. RT-qPCR amplifications were carried out in triplicates with *ef1* as endogenous controls using TaqMan™ System. The relative quantification (RQ) was calculated by the equation RQ=2 –ΔΔCT. The latency between animal PTZ exposure and the first seizure behavior were calculated and presented as mean ± Standard Error of Mean (SEM). Statistical analysis was performed by Mann-Whitney test ($p<0.050$).

RESULTS: In seven dpf larvae, *cox-2b* mRNA was increased while *cox-2a* was detected at similar levels to the control group. The mean ± SEM of *cox-2a* and *cox-2b* mRNA levels in seven dpf larvae were: (i) *cox-2a*: $CG_{0.05h}$ 1.2 ± 0.06; $SG_{0.05h}$ 1.3 ± 0.11 (p = 0.27); (ii) *cox-2b*: $CG_{0.05h}$ 0.93 ± 0.02; $SG_{0.05h}$ 1.73 ± 0.18 (p= 0.004). In adult zebrafish, there was no difference in either *cox-2a* or *cox-2b* mRNA levels compared to control group (figure 5). The mean ± SEM of *cox-2a* and *cox-2b* mRNA levels in seven dpf larvae were: (i) *cox-2a*: $CG_{0.05h}$ 0.75 ± 0.07; $SG_{0.05h}$ 0.63 ± 0.03 (p = 0.15); (ii) *cox-2b*: $CG_{0.05h}$ 1.07 ± 0.08; $SG_{0.05h}$ 1.29 ± 0.17 (p = 0.21).

DISCUSSION/CONCLUSION: Our results showed that *cox2a* and *cox2b* genes have differential mRNA expression response after PTZ-induced seizure. *Cox2b*, but not *cox2a* mRNA level, is upregulatedafter seizure in zebrafish larvae and no differences are found in adult zebrafish brain. There is a structural difference between *cox-2a* and *cox-2b* genes suggesting that zebrafish *cox2b* is more similar than is *cox-2a* from mammalian *Cox-2*, which can explain our results. This is the first study investigating the *cox-2* response after seizure-induced in zebrafish. We showed that zebrafish have a similar response after seizures to that observed in rodent models, supporting evidence that this little fish is a valuable model for further studies of inflammation and seizures.

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COMPARISONS ON WHOLE EXOME CAPTURING COVERAGE AND EFFICIENCY AMONG DIFFERENT POPULATIONS AND TIME-POINTS

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INTRODUCTION: The coding region of the genome corresponds to less than 2% of its entirety and is known as exome. This portion of the human genome concentrates most of the pathologic mutations causing disease in humans. For example, when performing a whole genome sequencing of one individual we expect to find approximately three million variants. If we focus on coding regions, this number drops to less than twenty thousand. In this context, exome sequencing seems to be a cost-effective strategy for high performance molecular diagnosis applied to genomic medicine, with direct applications on neurogenetics. However, for a best interpretation of this approach it is important to determine whether ethnic differences in the population sequenced can affect sensitivity and specificity of the method, as we are analyzing patients from the Brazilian population with a mixed ethnic background.

MATERIALS AND METHODS: We selected 120 individuals from the 1000 Genomes Consortium in order to investigate the impact of ethnicity in capture and sequencing parameters obtained by exome sequencing using next generation sequencing technology. These exomes were captured, sequenced and aligned at three different time points. For each one of those temporal subsets of data there are 40 individuals, ten of each of the four considered populations (ACB – African Caribbean from Barbados; GBR – British in England and Scotland; YRI – Yoruba in Nigeria; JPT – Japanese from Tokyo). We are investigating the mean coverage for each exon within the human exome as well as capturing efficiency and coverage of specific genes considered to be of clinical relevance by the American College of Medical Genetics and Genomics [1].

RESULTS: A distance-based approach, such as principal component analysis, shows patterns on exomic coverage that are closely related to time progression and therefore protocol advancements added to new versions of the whole exome capture kits.

DISCUSSION: With those results in mind, we consider that the exome capture experiment is invariable on populations' differences, but it is highly dependent on time progression and advancements made by the exome capture kits' manufacturers.

CONCLUSION: The present work confirms that there are significant differences on whole exome capturing coverage and efficiency among different time-points, a strong evidence that advancements made on the whole exome capture phase have a huge impact on coverage and efficiency. Another point of concern is that the integration of different whole exome capture kits or even different versions of the same kit may be a challenging.

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USER FRIENDLY FUNCTIONAL CONNECTIVITY (UF²C): REALISTIC TEMPORAL COUPLING OF COOPERATING BRAIN AREAS

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INTRODUCTION: Functional connectivity (FC) studies can reveal brain interactional patterns without cause-effect meaning, identifying varied functionally cooperative networks related to distinct brain states. Resting state (RS) FC investigates the inter-regional correlations between spontaneous fluctuations in the BOLD signal in the absence of an experimental paradigm or any other explicit stimulus [1]. Among all functional networks, the Default Mode Network (DMN) is undoubtedly the most studied [2]. Standard analysis uses the whole voxels time series (TS) to estimate a correlation map, however a constellation of different transitory mental states can happen during these acquisitions and it may generate misleading DMN scores. This study aims to verify the effectiveness of the sectioned TS methodology to identify the influence of a confounder mental task during the RS acquisitions, presenting a novel computational tool for this purpose.

MATERIALS AND METHODS: Nine subjects had their images acquired in a 3T scanner (Philips Achieva): i) echo-planar image, voxel=3x3x3mm³, TE=30ms, TR=2s, 40 slices, 180 dynamics ii T1-WI, voxel=1x1x1mm³, TE=3.2 ms TR=7 ms, 180 slices. During the RS acquisition, the volunteers were instructed to close their eyes and do not fixate in a specific thought. After 3 min, the volunteers were audibly instructed (a tone) to open their eyes and think they are doing a physical activity (40 sec) and then instructed to back to rest. Images processing and analysis were performed using UF²C toolbox and included: dynamics realignment, tissue segmentation, normalization, smoothing, temporal regressions (for movement and tissues global signals) and band-pass filtering (0.008-0.1 Hz). The correlations were estimated using as reference a TS extracted from a ROI positioned on the posterior cingulated cortex. The correlations were performed by dividing each TS into six **(Fig.1)** parts, resulting in six statistical maps (and six average r-score values) for each subject.

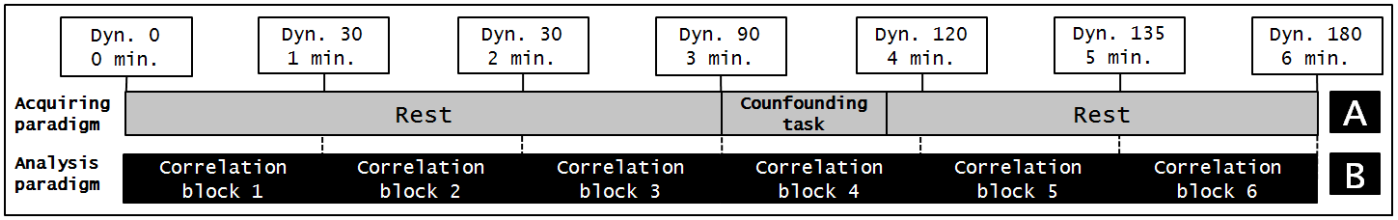

Fig.1: Experiment design. In "A" the acquiring paradigm and in "B" the block analysis layout.

RESULTS: We found significant differences (T-test p=0.01) comparing the block 4 r-scores with the average of the other blocks. Also z-tests were performed individually to compare the block 4 with the other blocks showing significant differences (p<0.02) in 87% of the cases (seven subjects).

DISCUSSION/CONCLUSION: We showed that mental tasks could causes abrupt changes in DMN intraconnectivity. This demonstrates the necessity of methods to controls for erroneous volunteer mental behaviors during the acquisitions. Despite the low number of subjects acquired, UF²C has shown to be efficient to find and separates the confounder block individually and should to be used as a data quality test. In summary, UF²C is a straightforward toolbox and enables standardized and reliable studies of FC. Regard the influences of possible confounder mental tasks during "resting state" acquisitions, the sectioned TS methodology could provide an option to control the data quality, verifying temporally the BOLD coupling between interactive brain areas.

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DIFFERENCES IN FMRI IMAGES RELATED TO PROPRIOCEPTION MAY BE FOUND BETWEEN SENSORY NEURONOPATHY PATIENTS AND CONTROL SUBJECTS: A PILOT STUDY

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INTRODUCTION: This work consisted on analyses of fMRI images to assess patients with sensory neuronopathy. This disease affects the peripheral nervous system, damaging sensory neurons' cell bodies, which are responsible for carrying vibration and proprioception information from the body to the brain. As the disease develops, the difficulties to control movements also progress, which is markedly reflected in gait impairment. In this pilot study, we propose a task protocol using the fMRI technique, and aim to verify whether this protocol is able to differentiate proprioception abilities of patients and controls.

MATERIALS AND METHODS: All images were acquired in a 3T scanner (Philips Achieva). During the exam, subjects were required to keep their elbows close to their trunks, with the forearms raised, forming an 'L' shape with each arm. In this position, they also needed to point both index fingers facing each other. Every time they heard the command 'Start', they should move repetitively, in and outward, both forearms in order to push their index fingers against each other. Repetitions should cease when they heard the 'Stop' command. The whole protocol was repeated twice: first, with their eyes closed, and, then, looking at their hands. Five patients (48.6±7.8 years old; 3 men) and 7 controls (26.7±3.8 years old; 3 men) underwent the examination. The images were analyzed with Matlab using the SPM 8 toolbox. We performed two-sample t tests between groups (patients and controls) comparing both conditions (eyes open and closed). We compared images with no correction for multiple comparisons and with a p-value of 0.001.

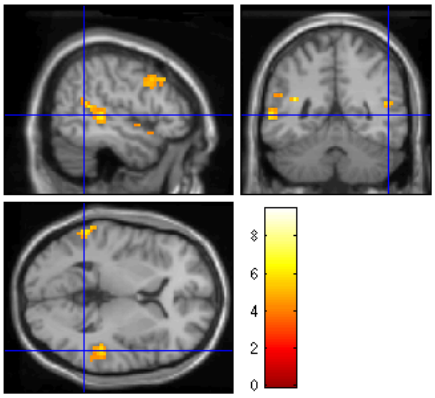

Figure 1. Areas related to the task execution during the examination.

RESULTS: We found significant differences in bilateral medial and superior temporal gyri and medial frontal gyrus as can be seen in Figure 1.

DISCUSSION: Although temporal areas revealed by this study are traditionally related to auditory and even visual activities, it is possible that the vestibular area, known for its association with proprioception, is also included in the highlighted region. The frontal medial gyrus is involved in movement planning, forming with other structures the premotor cortex.

CONCLUSION: The present work suggests that it may be possible to differentiate, using fMRI, proprioception abilities of control subjects and patients with sensory neuronopathies. Nevertheless, it is not a conclusive finding, since the amount of subjects enrolled in each group was quite small.

DISSECTING THE MOLECULAR MECHANISMS INVOLVED IN MESIAL LOBE TEMPORAL EPILEPSY BY LOOKING INTO THE TRANSCRIPTION PROFILE OF HUMAN DENTATE GYRUS

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INTRODUCTION: Mesial temporal sclerosis (MTS) is the most frequent pathologic abnormality found in patients with refractory mesial temporal lobe epilepsy (MTLE). However, it is still not clear how MTS is related to the underlying molecular and cellular mechanisms involved in the epileptogenic changes leading to MTLE. Aiming to investigate further these mechanisms we accessed gene expression profile in the *dentate gyrus* of patients with MTLE in comparison with normal tissue. We performed laser-capture microdissection of the *dentate gyrus* of three patients with familial MTLE and three patients with sporadic MTLE.

MATERIALS AND METHODS: We used control samples (n=2) from autopsy. To establish transcript profiles we performed microarray analysis using the Human Genome U133 Plus 2.0 array (Affymetrix™). Data was acquired using the GeneChip Scanner 3000 (Affymetrix™) and results were analyzed using DNA-Chip Analyzer (dChip). Statistical analysis was performed by RankProd with expression fold change ≥ 2.0 and p≤0.01 for a significant difference between groups.

RESULTS: We found a total of 103 genes with significant expression changes (up- and downregulation) in patients with familial-MTLE; whereas, 56 genes were differently expressed in patients with sporadic MTLE. When patient with familial and sporadic MTLE were compared we found 18 genes differently expressed. In familial MTLE some of the upregulated genes were: *KCNT2* (Potassium channel, subfamily T, member 2) and *PDZ* domain, which is involved with protein binding. By contrast, genes upregulated in sporadic MTLE were: *NTS* (neurotensin) which is involved in the immune response induced activation of *IL-8* and *BNC2* (basonuclin 2) involved in metal ion binding.

CONCLUSION: We found a significant different gene expression profile in patients with familial and sporadic MTLE, indicating the mechanism underlying these two conditions is different. In addition, based on function of the genes differentially expressed we can start to appreciate the main molecular and cellular mechanisms which are abnormal in MTS.

SEQUENCE VARIANT IN THE *ITGA9* GENE AS A RISK FACTOR FOR DEPRESSION IN PATIENTS WITH MESIAL TEMPORAL LOBE EPILEPSY

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INTRODUCTION: Mesial temporal lobe epilepsy (MTLE) is the most common type of temporal lobe epilepsy in adults [1]. Most patients with MTLE manifest major depression symptoms [2]. Recently, studies show that brain-derived neurotrophic factor (BDNF) levels may be related to major depression [3]. We have previously found an association between a single nucleotide polymorphism (SNP) rs166508 and bipolar disorder [3]. This SNP is located within the integrin alpha 9 gene (*ITGA9*) and the rs166508*AA genotype is associated with increased expression of *ITGA9* transcripts [3]. Since α9β1 integrin has been reported as a BDNF receptor, one hypothesis is that increased expression of *ITGA9* leads to an increased availability of the α9β1 integrin on the cell membrane, which may lead to increased BDNF binding, thus, decreasing its availability (Figure 1 (a); (b)) [3]. Indeed, decreased BDNF levels have been observed consistently in the serum of patients with bipolar disorder, major depression and epilepsy [3]. To evaluate whether the SNP rs166508 is associated with increased risk of depression in patients with MTLE.

MATERIALS AND METHODS: We analyzed DNA sample from peripheral blood of 146 patients with MTLE diagnosed according to the ILAE criteria, including 70 with depression and 76 without depression, classified according to DSM-IV criteria. The SNP rs166508 was genotyped using a 7500 Real-Time PCR System (Applied Biosystem) (Figure 2). Hardy-Weinberg disequilibrium and genotype frequencies were estimated using HAPLOVIEW software. Genetic association analysis was performed using logistic regression in R software. Statistical power was evaluated by the GPOWER software using the following parameters: two-tail; Odds Ratio = 1.5; significance level $\alpha = 0.05$; power $1-\beta = 0.8$.

RESULTS: Allele frequencies observed for rs166508*G and rs166508*A were 52.7% and 47.3%, respectively. In addition, rs166508 did not show Hardy-Weinberg disequilibrium (p=0.995). Logistic regression results did not show statistically significant association for rs166508*A allele with the presence of depression (p = 0.969).

DISCUSSION: However, statistical power analysis revealed that we would need 308 individuals with MTLE, including 154 with depression and 154 without depression to be able to detect an association using the parameters described above.

CONCLUSION: To date, our results could not confirm association between SNP rs166508 and major depression in patients with MTLE. However, power analysis clearly shows that our study is underpowered to identify differences in allele frequencies even if they exist. We have already collected additional samples (total of 322 patients with MTLE) and are waiting for the neuropsychological evaluation to be finished in order to increase the number of samples analyzed.

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BRAIN REGULATION OF PERCEIVED EXERCISE INTENSITY

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INTRODUCTION: The brain plays a crucial role in the regulation of exercise intensity, however, little is known about the modulation of the active brain areas involved. Ratings of perceived exertion (RPE) have been indicated to represent a conscious awareness of the applied effort and to set exercise tolerance levels in several intensities. The purpose of this work was to describe the activity modulation of the brain areas involved at different perceived cycling exercise intensities.

MATERIALS AND METHODS: Nineteen healthy adult males (weight 79.7 ± 10.5 kg, height 177 ± 9.1 cm, 27.4 ± 4.8 years; physical activity 2.3 ± 1.3 days/week) participated in this study. The participants performed an intermittent (30 sec cycling / 30 sec rest) incremental load test on an adapted cycling ergometer coupled to a Magnetic Resonance Imaging scanner (MRI). Special care was taken to optimize the subjects' comfort in this atypical supine cycling position and to avoid head motion. Perceived exertion was reported at the end of each cycling period. The initial load was set at 50 Watts and was increased by 25 Watts after every four cycling periods performed until the participants reached a RPE of "17 - Very hard" on the Borg scale. Blood oxygen level dependent (BOLD) imaging was performed while the participants completed this stochastic cycling protocol, acquired by 3T MRI scanner (Philips Achieva, Netherlands).

RESULTS: We The cycling periods were associated with brain activation in the pre- and post-central gyrus, superior temporal gyrus, insular cortex and cerebellum ($p<0.001$). The decrease activation areas related to the cycling periods were fusiform gyrus, frontal superior gyrus, medial frontal gyrus, parahippocampal gyrus, anterior cingulated cortex and precuneus ($p<0.001$). Single areas modulation analyses has shown different patterns of increased and decreased brain activity of the areas involved during cycling at different levels of RPE ($p<0.05$).

CONCLUSION: The present study illustrates brain regulation of the active areas involved during cycling exercise of increasing exercise intensities. Activation of regions associated with motor control, and deactivation of cognitive-related areas might indicate an integrative approach of the brain to regulate exercise performance and to set exercise tolerance through RPE.

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TUBB3 GENE AND COPY NUMBER VARIATION ANALYSES IN PATIENTS WITH POLYMICROGYRIA

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INTRODUCTION: Polymicrogyria (PMG) is a malformation of cerebral cortex development characterized by an excessive number of small gyri and abnormal lamination. The severity of PMG symptoms is directly associated to the extension of the malformation and brain region affected. *AFF2*, *TUBA1A*, *TUBB2B*, *TUBA8*, *SRPX2* and *WDR62 genes* have been described to be associated with different forms of PMG. Previous Sanger sequencing analysis carried out by our group in these genes, failed to identify deleterious variants in a large group of patients with PMG. Recently, mutations in *TUBB3, a* gene encoding a protein involved with spindle pole organization, have been implicated to the molecular etiology of PMG. In addition, genomic structural variants known as *Copy Number Variations* (CNV) have been associated with several neurological disorders ranging from psychiatric disorders to malformations of cerebral cortex.

OBJECTIVE: We aimed to search for deleterious variants in the *TUBB3* gene and pathogenic CNVs in patients with PMG.

MATERIALS AND METHODS: Mutation screening was performed by polymerase chain reaction (PCR) and Sanger sequencing of the coding region and intron/exon boundaries of *TUBB3* gene in a cohort of 27 patients with PMG. Sequencing reactions were carried-out in an ABI3500XLgenetic analyzer (Life Technologies Corporation). Missense variants were evaluated by mutation prediction software's such as SIFT, Polyphen and SNP&Go. Patients with no deleterious variants in *TUBB3* gene were submitted to CNV screening with the SNP-array CytoScan HD(Affymetrix). Analyses of CNVs were performed with Chromosome Suite (Affymetrix) software (Affymetrix). To assess the clinical significance of our insertions/deletions findings, we searched all CNVs found in the *Database of Genomic Variants* (DGV) and *The International Standards for Cytogenomic Arrays Consortium* (ISCA).

RESULTS: *Sanger* sequencing identified only neutral variants in *TUBB3* gene. However, we detected a total of 11 rare CNVs among the 27 patients, including eight gains and four losses. The average size of CNVs found was 277kb, ranging from 113kb to 669kb. Each CNV contains approximately four genes. Potentially pathogenic CNVs, according to DGV and ISCA databases, contained genes involved with N-methyl-D-aspartate (NMDA) receptors (*GRIN3A*), cell division (*HAUS7*), centrosome (*PCTN*), DNA replication (*MCM3AP*), regulation of dopaminergic signal transduction (*PPP3C*),collagen (*COL6A1, COL6A2*)and axon guidance (*DCC*).

DISCUSSION: *TUBB3* gene is not involved with the etiology of PMG in our cohort of patients. We have shown that SNP-array is a powerful tool to identify genetic abnormalities in patients with PMG. In addition, it can point to new candidate genes potentially involved in normal and abnormal cortical development.

CONCLUSION: These results suggested new hypotheses, which can improved diagnosis and contribute to a better understanding of the etiology of PMGs.

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GENE EXPRESSION ANALYSIS USING BIOINFORMATICS METHODS IN RNA-SEQ EXPERIMENTS

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INTRODUCTION: High-throughput sequencing technologies allowed biomedical researchers to advance greatly in their studies by granting them access to molecular signatures with accuracies that were never seen before. Current technologies produce hundreds of millions of sequenced fragments, often referred to as *reads*, which can be used to characterize in detail macromolecules like DNA and RNA.

MATERIALS AND METHODS: In this study, we use RNA sequencing technologies (RNA-Seq) to better understand gene expression patterns of Mesial Temporal Lobe Epilepsy (MTLE) through the use of bioinformatics tools on tissue obtained from animal models. We used an analysis pipeline comprised of TopHat (for alignment), Cuffdiff (for differential expression) and CummeRbund (for data visualization) to investigate the differential gene expression between control and pilocarpine-treated animals.

RESULTS: We identified 19 differentially expressed genes, among them Neurod6 gene that had a 4-fold increase in expression and Sv2b gene with a 6-fold increase.

CONCLUSION: Both genes are related to pathways involved in neurologic diseases ("neuronitis disease"), which has been related, among others to nocturnal frontal lobe epilepsy.

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DEPRESSIVE DISORDERS IN PATIENTS WITH REFRACTORY MESIAL TEMPORAL LOBE EPILEPSY: UPDATED RESULTS

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INTRODUCTION: Objectives: To assess depressive disorders in patients with temporal lobe epilepsy (TLE), refractory to antiepileptic drugs (AEDs).

PATIENTS AND METHODS: We performed a cross-sectional study, interviewing and collecting information from records of patients who sought treatment at the Epilepsy Clinic of the HC-UNICAMP. The population consisted of adults older than 24 years of age followed at UNICAMP, diagnosed with refractory TLE, in appropriate use of AEDs and lack of established mental retardation, dementia or language problems. Patients underwent a semi-structured psychiatric interview, which gave diagnosis according to the International Classification of Diseases (CID-10) - WHO. We applied the following instruments: (1) Mini International Neuropsychiatric Interview (MINI) and (2) the Beck Depression Inventory (BDI).

RESULTS: There were 40 patients aged 24-60 years. Thirty-one of these (77.5%) had depressive disorders: 14 (45.2%) with dysthymia, 11 (35.5%) with recurrent depressive disorder and 6 (19.3%) with bipolar disorder who had depression at the time of evaluation. Two (5%) had mixed anxiety disorder and depression. The other 7 patients (15%) showed signs of depression and anxiety, without imposing a diagnosis of depression, one of them with organic anxiety disorder. Only 8 of the 31 patients (25.8%) had received prior satisfactory antidepressant treatment.

DISCUSSION: The duration of epilepsy tended to be higher in patients with depressive disorder ($p = 0.10$). There was no association between depression and seizure frequency.

CONCLUSIONS: This study confirms that depressive disorder is common and underdiagnosed in patients with TLE refractory to AEDs. The duration of epilepsy had a tendency to be higher in depressed patients. There was no association between depression and seizure frequency.

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EVIDENCE OF DECREASE OF THE NETWORK MEAN DEGREE WHEN HEALTHY VOLUNTEERS GO FROM RESTING STATE TO LANGUAGE TASK

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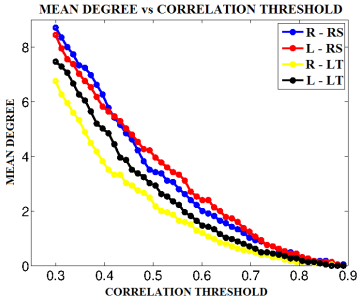
INTRODUCTION: The purpose of this work was to find differences in brain networks when two states of the brain are compared. For this, functional networks were built [1,2] using preprocessed and low-frequency filtered fMRI data. One session was done during resting state (RS) and another during a language task (LT). Two hypotheses were tested: 1) Existence of network metrics that reflect differences for these two functional networks; 2) Existence of network metrics for LT that reflect hemisphere dominance for language, if this dominance exists.

MATERIALS AND METHODS: Twelve healthy subjects (mean age 35 ± 10, 6 men) participated in this study. The study was approved by the Ethics Committee of UNICAMP and all subjects signed an informed consent. Volunteers took part in two fMRI sessions (one RS and one LT). Four functional networks, corresponding to regions in each hemisphere (left/right) for each session (RS/LT) were built, using undirected and unweighted graphs. For each network the following parameters were computed [3]: 1) Mean degree; 2) Cluster coefficient; 3) Characteristic path length. Left/right networks for LT were compared to each other, and this result was compared to the corresponding fMRI activation result (computed using a general linear model with SPM8), to see if the dominance shown in fMRI activation maps reflected somehow in network parameters.



RESULTS: Fig. 1 shows the behavior of network mean degree as a function of the correlation threshold for one volunteer. It shows that the number of connections for the RS networks of the two hemispheres are higher, when compared with the LT networks. These results hold for 7 of the 12 volunteers ($p < 0.05$). Also in Fig. 1, the mean degree of the LT networks shows a left hemisphere dominance. Qualitatively, this behavior seems to hold for 6 of the 12 volunteers, but only for 3 results were significant ($p < 0.05$). Standard fMRI analysis showed left hemisphere dominance for 8 volunteers.

DISCUSSION/CONCLUSION: Mean network degree decrease in the LT compared to the RS session seems to reflect the well-known synchronization of neurons at rest and desynchronization while performing a task, which shows as an amplitude decrease of certain frequency bands of the EEG signal [4]. In the present case, when the brain performs the task, the correlation among different regions decrease, and so does the number of nodes in the corresponding networks. We also found that 6 out of 8 volunteers that showed left hemisphere dominance for LT with standard fMRI analysis could also be detected comparing the mean network degree of the corresponding left and right hemisphere networks. In short, the present results show differences between the two studied functional networks for the majority of subjects, with the number of connections for the slow frequencies

BOLD signal networks decreasing when the brain performs a LT. Also, the mean degree of the network may give some evidence of brain hemisphere dominance for language. Given the fact that the brain regions used for building the networks were arbitrary, this work shows that this approach is useful to extract global information from brain fMRI data.

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ALTERED CORTICAL THICKNESS AND ITS CORRELATION WITH UPDRS SCALE IN PARKINSON DISEASE

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INTRODUCTION: Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. As has been well described, the subcortical areas are the first affected; however, the progression to cortical involvement and how it correlates with disease severity are still unclear.

MATERIALS AND METHODS: 56 patients with PD (age 59.52±9.90) according to the UK Parkinson's Disease Society Brain Bank criteria were compared with 27 healthy controls (HC) (age 57.77±10.06). None of the patients had family history of PD. Anatomical T1-weighted MR images were obtained on a 3T scanner. We used the civet pipeline and the SurfStat toolbox on Matlab to process and analyze the images. We used the AAL template. We divided patients into 3 groups according to disease severity measured by H&Y scale and compared each group with the HC group. We also correlated patients' cortical thickness (CT) with H&Y, NMS, UPDRS, UPDRS III and SCOPA scores.

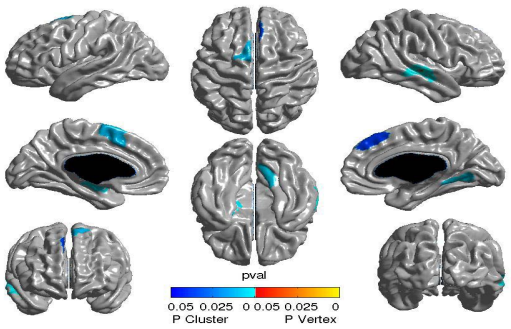

Figure 1. Correlation areas between cortical thickness and UPDRS scores (p<0.05).

RESULTS: Mean disease duration was 8.4±6.5 years. Mean UPDRS and UPDRS part 3 scores were 36.00±18.52 and 17.00±8.3, respectively. There was a correlation between patients CT and UPDRS scores in the right superior temporal gyrus (p<0.05) (Fig. 1). The comparison between group 1 and HC revealed decreased cortical thickness in the left superior temporal gyrus, left gyrus rectus and left olfactory cortex (p<0.05, local maxima 5.4). Regarding group 2, the areas with lower CT were right postcentral gyrus, right supplementary motor area and right inferior frontal gyrus (p<0.05, local maxima 4.8). For group 3 significantly lower CT was found in the left inferior frontal gyrus, left precentral and postcentral gyrus, left supplementary motor area, left inferior frontal gyrus, left gyrus rectus, right temporal pole, right fusiform gyrus, right middle temporal gyrus, and right occipital gyrus (p<0.05, local maxima 5).

DISCUSSION/ CONCLUSION: Our results show that there is an increase in cortical thinning across PD stages; this possibly reflects disease progression as previously suggested by Braak's hypothesis of PD pathological progression. Progression of PD has been well demonstrated using other neuroimaging methods and the CT measure is also an useful tool to assess disease stages.

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ETIOLOGY-BASED CLASSIFICATION OF BRAIN WHITE MATTER HYPERINTENSITIES THROUGH TEXTURE ANALYSIS

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INTRODUCTION: The improvement of the quality of magnetic resonance imaging (MRI) allowed the observation of more subtle or smaller abnormalities. Among incidental findings, white matter hyperintensities (WMH) are frequently observed and its prevalence increases with age [1]. Underlying pathology of WMH may be diverse, but they can be classified predominantly in ischemic and demyelinating. This paper proposes a computerized method that aims to distinguish tissue containing WMH with different etiology (i.e. demyelinating or ischemic) by combining texture analysis [2,3] and SVM classifier [4].

MATERIALS AND METHODS: Our image database was formed by T2-weighted MRI and it was obtained in the axial plane (3 mm thick, flip angle 120 degrees, repetition time 6800 ms, echo time 129 ms) at the Clinical Hospital of the Department of Medicine of University of Campinas (UNICAMP). Regions of interest (ROI) were manually extracted in the 2D slices of the MRI and noted based on clinical data of the patients. It was extracted 64 ROIs of WMH with ischemic etiology from 4 patients with stroke, and 143 ROIs representing WMH with demyelinating etiology from 50 patients with multiple sclerosis.

RESULTS: Experiments explored different texture attributes extracted by using different approaches: histogram, run length matrix, co-occurrence matrix and gradient in order to analyze their texture discrimination. We found that the most discriminating texture attributes were extracted from the histogram and the co-occurrence matrix. Besides, the SVM classifier achieved an accuracy rate of 86.95% when using the texture attributes to distinguish WMH with different etiology, demyelinating or ischemic. The algorithm was developed on Adessowiki [5], a collaborative environment for development and documentation of scientific computing algorithms.

DISCUSSION: This result indicated that the extracted texture attributes were representative, making possible to distinguished WMH with different etiology (ischemic or demyelinating) by using a SVM classifier. This task would be complex to be visually assessed.

CONCLUSION: The present work confirmed that it is possible to classify WMH on MRI according to their etiology by combining the texture analysis with the use of a classifier. In future works, we intend to eliminate the manual ROI extraction step by developing a semi-automatic method for WMH segmentation. We are also planning to increase the image database in order to validate the method and to analyze its robustness.

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NON-INVASIVE BIOMARKERS IN THE PLASMA OF PATIENTS WITH EPILEPSY: ASSESSING AN IMPROVED PROTOCOL FOR MICRORNAS EXTRACTION

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INTRODUCTION: One of the main challenges in the modern medicine is treating central nervous system (CNS) diseases because of the complexity of their pathomechanisms and the difficulty of achieving an accurate diagnosis at an early stage such as in Focal Cortical Dysplasia (FCD), a malformation of the cerebral cortex who is often associated with intractable epilepsy, whose surgical resection of abnormal tissue is frequently performed in order to achieve better seizure control. Nevertheless, surgery indication may be delayed due to a long investigation with medication treatment. Therefore, the identification of biomarkers in FCD could potentially improve the efficiency of diagnosis which in turn could lead to early treatment. One potential candidate for biomarkers is circulating microRNAs (miRNAs), small noncoding RNAs present in extracellular human body fluids including plasma or serum. It is well known that changes of miRNAs levels in circulation reflect changes in diseased tissues. In addition, miRNAs have demonstrated to have high stability in plasma, to be strongly associated with specific disease states, can be noninvasively and easily quantifiable. However, there are limitations for this potential biomarker, such as the small amount of circulation miRNAs combined with the large concentration of proteins, which make extracting miRNA from serum or plasma technically challenging. Therefore, the utility of those stable molecules as key biomarker requires a highly reliable and reproducible protocol for RNA purification. Obtaining an extraction of high quality is essential for the following steps as qPCR and data analysis. Therewith, our aim was to identify the most suitable method for extracting miRNAs from human plasma samples.

MATERIALS AND METHODS: We tested four different commercial kits/reagents to identify which one offers more effectiveness in clinical samples preparation: mirVana PARIS kit (Ambion), TRIzol-LS (Life Technologies), miRNeasy Serum/Plasma Kit (Qiagen,), and QIAamp Circulating Nucleic Acid (Qiagen). To date, blood samples were collected from 36 patients from Clinics Hospital from UNICAMP (17 patients with FCD and intractable epilepsy; 19 patients with mesial temporal lobe epilepsy (MTLE)) and 17 healthy control subjects. All donors provided written informed consent. All blood samples were initially centrifuged at 1500 g for 10 minutes to separate the plasma and blood cells. The supernatant was carefully removed and centrifuged again at 12000 g for 10 minutes at 4°C. The collected plasma was used for the subsequent step of isolating RNA. RNA purity and concentration were evaluated by spectrophotometry. To identify if a specific miRNAs species may be useful as biomarkers of pharmacoresistance in patients with FCD and MTLE we used miRNA quantification performed by quantitative PCR (qPCR) using TaqMan (Life Technologies) assays: hsa-miR-23a, hsa-miR-31 and hsa-miR-134, that are strongly involved in epileptogenesis. The differential miRNA expression will be analyzed using Wilcoxon test.

RESULTS AND CONCLUSION: Our preliminary results indicate that in terms of quantity and purity, the different extraction methods gave rise mainly to poor 260/280 and A260/230 ratio. In addition, we observed that extraction with TRizol-LS produced a higher concentration of RNA. We also observed that Ct values obtained using miRVana PARIS kit are the most suitable; it exhibited better stable values in triplicate sample, which resulted in a better standardization. Therefore this commercial kit was chosen among all tested kits.

MTLE PATIENTS WITHOUT HIPPOCAMPAL SCLEROSIS HAVE MEMORY IMPAIRMENT

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INTRODUCTION: Mesial temporal lobe (MTLE) epilepsy is frequently associated with hippocampal sclerosis (HS), however there are patients called "negative MRI" that do not have MRI signs of HS nor other lesions on MRI visual analysis [1], but also have cognitive complaints. Then we compared the cognitive performance of MTLE patients with HS and recurrent seizures (HS-r) or without recurrent seizures (HS-nr) and negative MRI patients with recurrent seizures (nHS-r).

MATERIALS AND METHODS: We evaluated 71 patients (3 of them were left hand dominant and one ambidextrous) divided into 5 groups: right HS-r (n=14), left HS-r (n=19); right HS-nr (n=10), left HS-nr (n=16) and nHS-r (n=12). We considered up to 3 dyscognitive seizures per year as nonrecurring. There were 7 patients from nHS-r group who had the EEG lateralized to the left. All patients underwent the same neuropsychological battery comprising Rey auditory verbal learning test (RAVLT)-coding, delayed recall and recognition, verbal and nonverbal memory subtests from Wechsler memory scale-revised, Stroop test-congruent and incongruent tasks, semantic and phonological verbal fluency, Boston naming test, vocabulary and block design subtests from WAIS-R to estimate the IQ and the Edinburgh inventory. The statistical analysis was carried out using the software SPSS21®.

RESULTS: There were no differences between groups related to age [F=0,999; p=0,414], educational [F=1,355; p=0,259], age at onset [F=0,925; p=0,455] and disease duration [F=0,875; p=0,484]. However, we found difference between groups related to the RAVLT- delayed recall [F=3,083; p=0,022] showing that right HS-r had better performance than nHS-r (Tukey=0,014) and RAVLT- recognition [F=4,976; p=0,001] showing that nHS-r had worse performance than right HS-r (Tukey=0.002)> right HS-nr (Tukey=0.017)> left HS-r (Tukey=0.004). There was also difference between left HS-r and nHS-r (Tukey=0.013) related to verbal learning [F=3.258; p=0,017] showing that left HS-r had better scores.

DISCUSSION: The results showed there were differences between nHS-r and all groups except with left HS-nr regarding episodic memory and learning. The tests in which we found statistical significance were the RAVLT that are good to predict verbal memory impairment related to temporal lobe, but are not so sensible to find deficits related to hippocampal dysfunction. So this result demonstrates that negative MRI patients present impairment on temporal lobe even still we could not find any lesion so far. However we also found that the same group had learning difficulty related to verbal paired that is critically dependent on mesial temporal structures [2], although the nHS-r patients did not have hippocampal lesion according to the visual and volumetric analysis. Importantly, pathology in patients with TLE is not confined to the hippocampus, but frequently encompasses parahippocampal structures ipsilateral to the seizure focus and the most of our nHS-r patients had EEG lateralized to left [3]. In addition, there is evidence suggesting that medial and lateral aspects of the temporal lobe are diferentially involved in the control of verbal memory.

CONCLUSION: Although nHS-r patients have negative MRI they presented cognitive impairments related to episodic memory that is probably because of temporal lobe damage and other mesial structures not related to the hippocampus.

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GENE EXPRESSION ANALYSIS IN GENETIC ANIMAL MODELS OF EPILEPSY

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INTRODUCTION: Wistar audiogenic rat (WAR) is a genetic epilepsy model susceptible to audiogenic seizures, after high-intensity sound stimulation. Another genetic model we have recently identified is the generalized epilepsy with absence seizures (GEAS) rat. The aim of this study was to determine the molecular pathways involved in the susceptibility to seizures of these two strains using gene expression analysis.

MATERIALS AND METHODS: We obtained total RNA from five susceptible WAR (hippocampus and corpora quadrigemina), and five control Wistar, as well as from hippocampus of three GEAS rats and three control Wistar. Gene expression analysis was performed using the microarray technology, and analyzed in R environment using the Affy and RankProd packages from Bioconductor, as well as the MetaCore® platform to identify molecular networks, gene ontology categories and gene interactions. Genes with differential expression and a possible biological role in epileptogenesis were validated by qRT-PCR.

RESULTS: The genetic profile obtained from the microarray analysis showed a total of 1624 differentially expressed transcripts in the corpora quadrigemina of WARs and 1351 genes differentially expressed in the hippocampus compared with controls, with 616 upregulated and 1008 downregulated in corpora quadrigemina and 660 upregulated and 691 downregulated in the hippocampus of WARs. Enriched gene ontology categories identified in WAR were involved in oxidative phosphorylation, neurophysiological process GABA-A receptor life cycle. The genes validated by qRT-PCR were *Grin1*, *Nedd8*, *Il18* and *Slc1a3*. In GEAS rats the genetic profile obtained from the microarray analysis showed a total of 2307 differentially expressed transcripts in the hippocampus and 2282 genes differentially expressed in the somatosensory cortex compared with controls, with 1039 upregulated and 1268 downregulated in hippocampus and 991 upregulated and 1291 downregulated in the somatosensory cortex. The top enriched gene ontology categories included: oxidative phosphorylation, LRRK2 in neurons in Parkinson's disease. The genes validated by qRT-PCR were *Grin1*, *Gabbr1* and *Slc6a1*.

CONCLUSION: This study may help to clarify the underlying molecular mechanism that leads to the predisposition to seizures in these animals. Our results indicate the possibility that an abnormal energy metabolism exist in the central nervous system of both models.

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PSYCHIATRIC DISORDERS IN FOCAL EPILEPSIES

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INTRODUCTION: Psychiatric disorders (PD) are often found in patients with epilepsy (KANNER, 2013). These comorbidities appear to be the product of a complex interaction between the effects of antiepileptic drugs (AEDs), neurobiological changes associated with epileptic seizures, subjective experiences and social vulnerability caused by psychosocial impact of epilepsy (OLIVEIRA et al., 2010). The objective of this study was to evaluate the occurrence of PD in focal epilepsies and analyze the clinical and demographic data concerning the presence of these comorbidities.

MATERIALS AND METHODS: We evaluated 105 patients [62 women and 43 men, aged between 18 and 66 years old, mean (SD) 44.7 (11.4)], with focal epilepsies classified as mesial temporal lobe epilepsy (87) and extra temporal lobe epilepsy (18). The following psychological tests were applied: Structured Clinical Interview for DSM-IV (SCID-I), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) and Neurological Disorders Depression Inventory for Epilepsy (NDDI-E). IBM SPSS20 software was used for the statistical analysis.

RESULTS: We observed PD in 45 (42.9%) patients, according to the results of the SCID-I, and of these, 23 (51.1%) had no prior diagnosis of these comorbidities. The group of patients with PD presented a higher monthly seizure frequency ($p > 0.001$) and suicidal ideation ($p > 0.001$). Women showed a higher incidence of PD than men ($p > 0.001$). The scores of psychological tests applied were higher in patients with PD (BDI $p > 0.001$; BAI $p > 0.001$ and NDDI-E $p > 0.001$) and in women, considering the gender comparison (BDI $p = 0.002$, BAI $p = 0.001$ and NDDI-E $p = 0.003$).

DISCUSSION: The occurrence of PD is significantly higher in focal epilepsies when compared with the incidence in the general population (DALMAGRO et al., 2012). Our data indicate that these comorbidities are still underdiagnosed and undertreated. PD patients had a higher monthly seizure frequency in addition to high scores on the BDI, BAI and NDDI-E tests. Women also had higher scores on the tests, suggesting a higher susceptibility to PD.

CONCLUSION: The investigation of PD in patients with epilepsy needs more attention, especially for patients at risk (women and refractory patients) to provide more specific and appropriate treatment when necessary.

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ANALYSIS OF GABA VARIATION DURING VISUAL STIMULATION USING LCMODEL

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INTRODUCTION: Gamma-aminobutyric acid (GABA) is the chief inhibitory neurotransmitter of the central nervous system, and it has been extensively studied in recent magnetic resonance spectroscopy (MRS) experiments. It has been shown that resting GABA concentration in the visual cortex is inversely correlated with the BOLD magnitude response to a visual stimulus [1]. On the other hand, several works have demonstrated a dependency between the BOLD signal and the flickering frequency of visual stimuli, with the BOLD response peaking at 8Hz (e.g. [2]). In the present work we performed a functional MRS (fMRS) experiment to evaluate GABA concentrations in the visual cortex of individuals subjected to visual stimuli flickering at 4Hz, 8Hz and 16Hz. We expected a decrease of GABA for the 8Hz stimulus compared to the other ones.

MATERIALS AND METHODS: The paradigm used consisted in the application of a visual stimulus (radial checkerboard) flickering at 4Hz, 8Hz and 16Hz, distributed in 7 blocks (4min and 15 spectra each): rest-4Hz-rest-8Hz-rest-16Hz-rest. Sixteen healthy subjects (mean age 26 ± 8 years, 13 men) participated; all gave their consent (the work was approved by the ethics committee of Unicamp). Data were acquired in a 3T Philips Achieva scanner with the MEGA-PRESS pulse sequence of spectral editing, with a selective pulse in the GABA peak at 1,9ppm, which resulted in a "clean" (no overlaps) GABA peak at 3ppm. Data processing were done using Matlab scripts that corrected frequency and averaged the data. The quantification was done using the LCModel (*Linear Combination of Model spectra*) software, which is a user-independent method that fits the data with a linear combination of spectra from the metabolites of interest.

RESULTS: Three subjects were discarded due to low spectral quality. After analysis with LCModel, subjects were grouped following the initial hypothesis of decreased GABA response with a maximum at the 8Hz stimulus. The results showed that the minority of the subjects (6 out of 13) had a response that follows this hypothesis (Fig. 1).

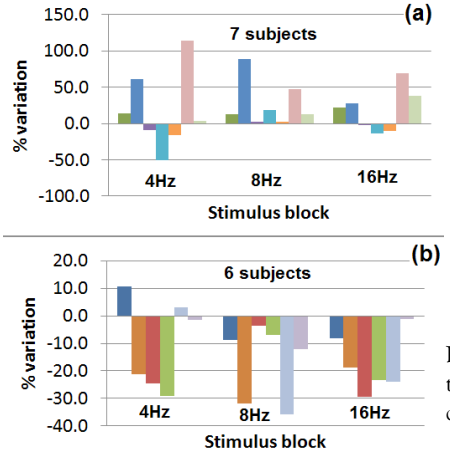

Figure 1. Percentage GABA variation with respect to the 1st rest block for each stimulus. (a) Increase and (b) decrease at 8Hz block.

DISCUSSION: Although the initial hypothesis is based on several studies, the variation of GABA involves more complex mechanisms that often exhibit variable behavior in the same individual, and even more so among individuals who do not always follow that proposed by our hypothesis. Such variability, together with the intrinsic problems related to the MRS technique, which has a low sensitivity, may have caused the discrepancy between the results and the assumption.

CONCLUSION: The results are not conclusive yet. This analysis with LCModel is one way to observe the variation of GABA. A new analysis will be done with the Gannet (GABA Analysis Toolkit) software in order to compare results and thus get a more concrete conclusion.

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LONGITUDINAL MEMORY PERFORMANCE IN PATIENTS WITH MESIAL TEMPORAL LOBE EPILEPSY AND HIPPOCAMPAL ATROPHY

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INTRODUCTION: Mesial Temporal Lobe Epilepsy (MTLE) associated with hippocampal atrophy (HA) is a syndrome highly refractory to clinical treatment. However, a subgroup of MTLE patients with good response to antiepileptic drugs has been described[(1)]. The presence of HA in this subgroup becomes a common feature with refractory MTLE patients, allowing us to investigate the influence of seizure frequency on memory, as an isolated factor. To investigate the relationship between seizure frequency and memory impairment, we performed longitudinal memory assessment in MTLE patients with frequent seizures, MTLE patients with infrequent seizures and MTLE patients who underwent surgical treatment.

MATERIALS AND METHODS: We performed two neuropsychological assessment in 17 MTLE patients with frequent seizures (9 with right HA and 8 with left HA), 15 MTLE patients with infrequent seizures (5 with right HA and 10 with left HA) and 15 MTLE patients who underwent surgical treatment (11 with right HA and 4 with left HA). Frequent seizures were considered as, at least, one dyscognitive seizure per month. Infrequent seizures were considered as three or less dyscognitive seizures per year and no event evolving to a bilateral convulsive seizure. Neuropsychological assessment included: subtests from Wechsler Adult Intelligence Scale-Revised to estimate Intelligence Quotient (IQ); subtests from Wechsler Memory Scale-Revised to evaluate verbal and visual memory and Rey Auditory Verbal Learning Test. According to the sample distribution, we used *paired t test* or *Wilcoxon test* to analyze differences on memory performance between the first and the second neuropsychological assessment.

RESULTS: There were no differences between the first and the second neuropsychological assessment in the MTLE group with frequent seizures. The mean interval between evaluations was 23.17±8.18 months. The MTLE group with infrequent seizures showed better longitudinal scores in estimated IQ ($p=0.03$), general memory ($p=0.009$), visual memory ($p=0.005$) and Visual Reproduction I subtest ($p=0.003$). The mean interval between evaluations was 25.53±7.61 months. The MTLE group that underwent surgical treatment showed better longitudinal scores in Logical Memory II subtest ($p=0.01$). We carried out post-surgical neuropsychological assessment for at least 6 months after the surgical treatment. The mean interval between evaluations was 27.26±8.15 months.

DISCUSSION/CONCLUSION: As initial hypotheses, no significant differences on memory performance between the first and the second evaluation were expected, because regardless HA being considered a progressive disorder[(2, 3)], neuronal loss in hippocampus may be occurring slowly and a longer follow-up can be necessary to observe significant changes in memory impairment. Therefore, better scores observed in some subtests may not exactly represent that patient's memory is better. These findings can be justified, in part, by familiarity/learning by neuropsychological assessment repetition, however all patients underwent this same bias which suggests that the high seizure frequency could decrease the ability of familiarity/learning with the second neuropsychological assessment. Nevertheless, the 'protective' effect of low seizure frequency on memory remains difficult to ascertain. In short, the MTLE group with infrequent seizures showed more better longitudinal scores than the other groups, suggesting the high seizure frequency is probably related to the decrease of ability of familiarity/learning with the second neuropsychological assessment.

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THE DIFFERENTIAL PATTERN OF CEREBELLAR ATROPHY IN PATIENTS WITH TREMOR-PREDOMINANT AND BRADIKINETIC-RIGID-PREDOMINANT PARKINSON'S DISEASE

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INTRODUCTION: Many authors consider the basal ganglia-thalamo-cortical loop as the tremor generator in Parkinson's Disease (PD), as a consequence of the dopamine depletion in the nigrostriatal neurons. However, recent studies have addressed that the cerebellum and its pathways are also modified in PD, suggesting an unclear cerebellar involvement in tremor presentation in PD. The objective of this work is to evaluate: (1) the cerebellar grey matter (GM) of patients with PD; (2) the cerebellar GM of a Tremor-predominant group of PD patients (PDT); and correlate with (3) the cerebellar GM of a Bradikinetic-Rigid-predominant group of PD patients (PDBR).

MATERIALS AND METHODS: Our sample contained 66 PD patients, however for the first analysis - all PD patients versus healthy controls (HC) - we had to exclude six patients due to unusual covariance in the homogeneity test performed. So, in the first analysis we evaluated 60 PD patients (mean age 57.78±10.00) and 80 HC (mean age 57.10±9.47). Then, we divided the 66 PD patients into two groups (45 PDT (mean age 61.50±17.68) and 21 PDBR (mean age 56.50±10.60) and compared each group to the HC group. We acquired T1 weighted MR images at a 3T scanner. We used

the SUIT tool from SPM for a more detailed evaluation of the cerebellar GM, and performed a VBM analysis. Statistics were done with SPM 8/ DARTEL p=0.001, uncorrected and k=100 voxels.

RESULTS: In the first analysis we detected GM atrophy in the left lobules Crus I and VIIb.

DISCUSSION: Most of the previous whole brain VBM analysis did not show cerebellar GM changes. However, when analyzing the PDT only, we detected significant cerebellar GM decrease. Therefore, the cerebellum is morphologically changed in the PD patients that manifest tremor. The cerebellum has emerged as a key structure in both the primary pathology of the disease, but also as compensatory mechanisms in PD. The cerebelo-thalamo-cortical (CTC) circuit has been pointed out as the responsible for maintaining the tremor in PD. It remains unclear, however, whether the CTC is the trigger or just the amplitude and frequency regulator of PD tremor.

CONCLUSION: Even though we observed cerebellar changes in the whole PD group vs the control group, the cerebellar grey matter atrophy observed seemed to be driven by the tremor-predominant group. These findings confirm a possible cerebellar role in the genesis of resting tremor in PD.

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PERFORMANCE OF SLEEP DEPRIVED MICE ON NOVEL OBJECT RECOGNITION TASK

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INTRODUCTION: Many studies already have shown that sleep deprivation affects animal's performance on memory tasks. However, inherent factors in experimental protocols can induce changes in several physiological systems and could interfere with the analysis of the specific effects of the lack of sleep (*e.g.*, aversive stimuli and physical effort required on memory tasks, and long periods of sleep deprivation). Thus, the aim of our study is to evaluate the memory of mice through a not aversive memory task after 12h of sleep deprivation.

MATERIALS AND METHODS: C57BL/6J male mice (3 months, 25-30g) were distributed in 2 groups: control (CT, n=12), and sleep deprived (SD, n=12). Memory was evaluated through the novel object recognition task (NORT). In the training session, each animal was placed in a square arena (75x75x37cm) containing 2 different objects. Exploration was allowed for 5min and then the animal returned to its homecage. This procedure was repeated 5 times in a row, with 15-min intervals between each exposure. Test was performed 24h after training, in which animals were exposed for 5min to a new pair of objects: an identical copy (familiar object), and a new object. On the behavior analysis we compared the total exploration time of objects on training, and the exploration rates between sessions, for both groups. If memory for object recognition is intact, it is expected that animals spend more time exploring the new object [1]. Sleep deprivation by the modified multiple platforms method was performed immediately after training, and lasted 12h. Mice of SD group were placed in small platforms inside a water tank. When the animal on top of the platform reaches the paradoxal sleep, it falls on water due to the muscle atonia, and then wakes up. After sleep deprivation, the animals of SD group returned to their homecages, while the CT group remained there for the entire period (CEP 0657/11).

RESULTS: We observed no significant difference between the total exploration time of objects on training for both groups (one-way ANOVA, CT: $F_{1,22}$=0,643 p=0,431; SD: $F_{1,22}$=0,00001 p=0,998). However, in the comparison of exploration rates (time spent exploring the new object by time spent exploring the new object plus average time for exploration of the familiar object) between sessions the CT group showed significant higher values on test when compared with training, while the SD group had similar taxes on both sessions (repeated measures one-way ANOVA: CT: $F_{1,11}$=6,824 p=0,024; SD: $F_{1,11}$=0,533 p=0,480).

DISCUSSION: NORT is a task used to assess exploratory behavior, and also to evaluate learning and memory. In the training session, both groups CT and SD explored the two objects equally, and there was no preference. In the test session of our study, while CT group spent more time exploring the new object, the exploration of both objects is similar in SD group. The preference for the exploration of the new object is regarded as an indication that a representation of the familiar object exists in the animal's memory [1].We can suggest that CT group consolidated their memory, but PS group had consolidation impairment caused by sleep deprivation.

CONCLUSION: These results indicate that 12h of SD after acquiring a not aversive task may cause consolidation memory impairment.

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MRI-BASED TECHNIQUES SHOW AXONAL DAMAGE SIGNATURE IN SPG4-HSP

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INTRODUCTION: Hereditary spastic paraplegias (HSP) linked to the locus SPG4 are genetically and clinically heterogeneous neurodegenerative disease, responsible for 35-45% of all cases of HSP. There are few MRI-based studies, which investigated the cerebral damage in this disease, and most of them have a heterogeneous cohort and not reliable results. The objective of this study is to determine the signature of SPG4-HSP using a multi-modal neuroimage approach and to look for possible correlates of such damages.

MATERIALS AND METHODS: Eleven patients (mean age 46.0 ± 15.0 years, 8 men) with SPG4-HSP, with molecular confirmation, and 23 matched healthy controls (mean age 51.4 ± 14.1 years, 17 men) were enrolled. All subjects underwent MRI scans in a 3T Philips device. For VBM, Freesurfer and Spineseg analyses, we used volumetric T1 images of the brain. The analyses of spin-echo DTI images were performed in FSL (TBSS analysis) and in ExploreDTI (tractography analysis). Correlations were then carried out with these results and with the clinical data.

RESULTS: Cerebral cortical analysis using FreeSurfer did not showed any cortical thinning. Nevertheless, VBM showed significant results for grey matter (GM) at the right superior temporal and fusiform gyrus and cuneus. The white matter (WM) analysis showed significant results at putamen and lentiform nucleus. TBSS analysis identified a significant difference at fractional anisotropy (FA) in left cortical spinal tract (CST) with some findings in the right hemisphere. However, the tractography, performed with ExploreDTI, revealed reduced FA in both CSTs and also in the averaged FA of both CSTs. Finally, we found significant reduced area without flattening of spinal cord (SC), which correlated with the reduction of right CST's FA ($r = 0.637$, $p = 0.048$).

DISCUSSION: Our WM analyses corroborate the probable physiopathology of SPG4, since the abnormalities were restricted to the cortical spinal tract, suggesting functional impairment of the longest axonal nerve fibers. Furthermore, an area decrease was demonstrated but no flattening of the spinal cord was found. This suggests involvement of the cortical spinal tract and the anterior horn, with relative preservation of the posterior portions. The GM analysis in these patients confirms that the classic form of this illness does not present damages in the cortical mantle, assuming that cortical thickness measurement is more reliable and sensitive to alterations in cerebral cortex than VBM analysis. Therefore, we attribute the dementia reports in patients with SPG4-HSP to the mutation variability or subcortical structures dysfunction.

CONCLUSION: The SPG4-HSP, in its pure form, is characterized by axonal damage at the cortical spinal tract, with spinal cord atrophy, without cortical damage. In addition, phenotypic variability is an important characteristic in this illness, mostly in MRI-based studies.

DISTRIBUTION OF TEXTURE PARAMETERS OF MR IMAGES FOR CONTROL SUBJECTS: PRELIMINARY RESULTS

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INTRODUCTION: This work consisted on applying the texture analysis (TA) technique to brain magnetic resonance images (MRI) of healthy subjects, in order to assist in the validation and consolidation of this technique, thus providing greater subsidies for the use of TA in pathological situations.

MATERIALS AND METHODS: Twenty-five healthy subjects (mean age 28 ± 4, 36% men) participated in this study. The study was approved by the local ethics committee. T1-weighted high-resolution MRI were acquired in a 3T Philips Achieva scanner. The images were analyzed using the co-occurrence matrix (COM) [1], implemented in the MaZda software [2]. COM allows the extraction of statistical parameters of an image by analyzing the spatial pixel distribution, such as: Angular second moment, Contrast, Correlation, Sum of squares, Inverse difference moment, Sum average, Sum variance, Sum entropy, Entropy, Difference variance and Difference entropy. Three regions were analyzed in this work: Medulla oblongata, Corpus callosum and Pons. COMs for distances 1 to 5 pixels and 4 directions (0º, 45º, 90º, 135º), totalling 20 matrices, were computed for each region. The 11 parameters cited were extracted from each matrix, and averaged over directions (leaving 5 matrices/region). Then, histograms were computed for every parameter (for every COM distance) to analyze their distribution.

Table 1. Mean and standard deviation of texture parameters (distance 1).

	Pons		Medulla oblongata		Corpus callosum	
	Mean	StD	Mean	StD	Mean	StD
AngScMom	0,0002	0,0003	0,00007	0,00006	0,0005	0,0003
Contrast	0,03	0,01	0,06	0,01	0,004	0,004
Correlat	0,0010	0,0003	0,0013	0,0003	0,0003	0,0003
SumOfSqs	0,03	0,01	0,04	0,01	0,003	0,004
InvDfMom	0,0010	0,0004	0,0012	0,0002	0,0009	0,0003
SumAverg	0,29	0,05	0,44	0,06	0,15	0,04
SumVarnc	0,08	0,04	0,12	0,03	0,01	0,01
SumEntrp	0,0028	0,0008	0,0050	0,0007	0,0008	0,0006
Entropy	0,004	0,001	0,008	0,001	0,0010	0,0008
DifVarnc	0,014	0,005	0,022	0,006	0,002	0,002
DifEntrp	0,0020	0,0005	0,0035	0,0005	0,0006	0,0004

RESULTS: Table 1 shows results for distance 1. For each parameter, the mean and standard deviation, given by their histograms, were calculated.

DISCUSSION: Histograms did not present any specific shape, but data were not too scattered, only for the Corpus callosum region, whose standard deviation is comparable to its mean value.

CONCLUSION: Results presented here are preliminary. In the future, we intend to calculate more histogram parameters (such as higher order moments), and mainly, to increase our subjects sample. With a larger sample, we will separate results in subgroups by age and sex, to find out whether texture changes with these variables. Later on we also intend to increase the number of analyzed regions. We expect to have standard values for healthy subjects that could be used to establish comparisons with values from patients suffering from different neurological diseases.

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FUNCTIONAL MEASUREMENTS OF NEAR-INFRARED SPECTROSCOPY IN HUMAN ADULTS: CONSEQUENCES TO BRAIN-COMPUTER INTERFACES

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INTRODUCTION: The ability to observe the functional activation of the brain has grown rapidly in the last years. Among several methods, there is near-infrared spectroscopy (NIRS), a technique that uses the principles of photon diffusion and allows the monitoring of brain activity through the hemodynamic changes from the incidence of light upon the scalp. This work aimed to develop functional activation experiments whose paradigms have potential applications to Brain-Computer Interface (BCI).

MATERIALS AND METHODS: Data were acquired with a NIRS instrument (NIRScout, NIRx, Inc.) that consists of 16 sources at 2 wavelengths (760 and 850 nm) and 32 detectors, allowing for a total of 64 source-detector pairs (channels). We performed two specific functional tasks: 1) motor task, in which the subject was asked to open and close its hand, and; 2) a cognitive task involving verbal fluency. Ten subjects were recruited for the protocol. The channels were positioned in order to cover a large area of the head, including the middle, inferior and superior frontal gyrus; superior and medium temporal gyrus; precentral and postcentral gyrus; supramarginal and parietal gyrus.

RESULTS: During the motor activation of the left hand, we observed a significant variation of oxy (HbO), de-oxy (HbR) and total hemoglobin (HbT) concentrations on the right hemisphere, contralateral to the stimulated hand. The mean HbT and HbO increases were about 0.9 µmol and 1.1 µmol, respectively. HbR was shown to decrease about 0.3 µmol. For the verbal fluency task, we observed a negative change of 0.12 µmol in HbO in the left hemisphere. This result was expected, since the analyzed person was right-handed. We also measured a variation of 0.02 to 0.13 µmol of HbO concentration in the medium frontal and the precentral gyrus of the right hemisphere.

DISCUSSION/CONCLUSION: Our results suggest an increase of HbO and HbT, and a decrease of HbR, after the start of a motor task, in the contralateral side of the stimulation. We also observed a function lateralization during the language task, involving Broca's and Wernicke's areas. These results match what is expected from neurophysiology, and agree with results of functional Magnetic Resonance Imaging previously published in the literature.

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APPLICATION OF LASER MICRODISSECTION OF HIPPOCAMPUS SUBFIELDS FOR RNASEQ ANALYSIS IN ANIMAL MODELS OF MESIAL TEMPORAL LOBE EPILEPSY WITHOUT *STATUS EPILEPTICUS*

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INTRODUCTION: A recently developed animal model based on long electrical stimulation of the perforant pathway (PP) in rats is capable of inducing hippocampal damage that more closely resembles that found in patients with mesial temporal lobe epilepsy (MTLE). Although this model has been well characterized, it remains unclear how different hippocampus subfields are affected. RNAseq based transcriptome analysis offers the possibility of profiling global gene expression, and laser microdissection allows for analysis of discrete cell populations. Therefore, the aim of the present study is to establish a protocol for precise RNA extraction from different hippocampus subfields.

MATERIALS AND METHODS: For surgery, six male adult Wistar rats were anesthetized with Isofluorane. Electrodes were implanted bilaterally in the dentate gyrus and in PP. After one week three rats were stimulated at 2 Hz with continuous paired 20V/0.1ms square pulses, with 40ms interpulse interval, intercalated with 10 seconds trains of 20Hz/20V/0.1ms square pulses applied at every minute. The above protocol was applied once for 30 minutes in two consecutive days, and was used for 8 hours in the third day. Control animals had electrodes implanted but were not stimulated. Fifteen days following stimulation rats were anesthetized with isoflurane, quickly euthanized by decapitation and the brain was quickly removed and frozen at –60°C. Serial frozen sections (60µm) were produced in a cryostat (Leica) and mounted in PEN covered glass slides (Zeiss). Slides were Nissl stained, dehydrated and the CA1, CA2, CA3 and the dentate gyrus from dorsal and ventral hippocampus were laser microdissected using Zeiss PALM LCM. RNA was extracted from microdissected samples.

RESULTS: Stimulated rats presented extensive pyramidal layer lesions; however, we were able to obtain more than 500ng of high quality RNA (RIN > 7) from each microdissected region.

DISCUSSION: Drastic changes in the morphology and cell composition of a brain region complicate the interpretation of transcriptome data applied to the study of epileptogenesis. Extensive neuronal loss and immune cells infiltration, as observed in the pyramidal layer of the present data, would hinder the possibility of analyzing gene expression mechanisms taking place in neurons. On the other hand, transcriptome analysis of relatively preserved regions, like the dentate gyrus, may indicate neuronal mechanisms involved in the process of epileptogenesis. Therefore, laser capture microdissection, as applied in the present study, is crucial for separating hippocampus sub-regions from which transcriptome analysis would be more advantageous.

CONCLUSION: The present protocol is adequate for the analysis of different hippocampus subfields by next generation sequencing.

PHYSICAL ACTIVITY HABITS, CARDIORESPIRATORY FUNCTION AND BODY MASS INDEX IN PATIENTS WITH REFRACTORY TEMPORAL LOBE EPILEPSY

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INTRODUCTION: Refractory Temporal Lobe Epilepsy (TLE) is characterized as focal epilepsy with spontaneous recurrent seizures on the temporal lobe, and without complete control of seizures by the antiepileptic drugs. The uncontrollable frequency of seizures and the risk for injury can lead patients to be discouraged from practicing sports and exercising. Sedentary life style can lead to poor physical capacity, overweight and lower quality of life. The purpose of this work was to measure the habitual physical activity, cardiorespiratory function (CF) and body mass index (BMI) in people with refractory TLE and to identify if this population needs a physical activity program to improve their physical conditions.

MATERIALS AND METHODS: We recruited 40 randomized patients, with mean age of 42 years old (ranging from 20 to 58 years old, SD = 10 years), 26 women and 14 men from Outpatient Clinic of Epilepsy of Clinical Hospital at Unicamp. We applied the International Physical Activity Questionnaires (IPAQ). So, they were submitted to maximal treadmill stress test by incremental protocol for assessing CF, and the BMI was calculated by weight and height. For the analysis, we used the Chi-square to evaluate the CF between those who practiced leisure physical activity and those who did not.

RESULTS: Nine out of all patients (22%) reported the weekly practice of leisure physical activity (LPA); however, there was no difference in CF among patients who were engaged in this physical activity and those who were not. 37% out of all patients presented low CF by the maximal treadmill stress testing, and 73% presented regular cardiopulmonary capacity. No patient was rated with good capacity, and no patient had seizure during the test. 37,5% of the volunteers were considered healthy according to BMI classification; other 42,5% were classified overweighted and 20% were considered obese level 1 and 2.

DISCUSSION: No patient had seizure during the maximal treadmill stress test. People with refractory TLE can practice intense physical activities. However we didn't observe difference in CF between the group that reported practice LPA and the group that did not practice LPA. No patient was rated as good CF and most of them were considered overweighted. These data show that people with epilepsy had not practiced physical activity in an ideal zone target to avoid suffering the risks of a sedentary lifestyle.

CONCLUSION: The TLE patients need to be encouraged to practice physical activity. Therefore, this population needs a physical activity plan accompanied by physical education professionals to improve their physical condition, their quality of life and even can decrease risk factors for other comorbidities.

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