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A PRELIMINARY STUDY ON UNSUPERVISED MACHINE LEARNING APPLIED TO DECOMPOSITION OF HIGH-DENSITY MYOELECTRIC SIGNAL

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Introduction: Classical blind source separation algorithms have been used to decompose high-density (HD) electromyogram (EMG) into its constituent motor unit (MU) spike trains [1,2]. However, these algorithms cannot completely solve the decomposition problem of non-stationary signals. Some recent algorithms try to disentangle these limitations by including a supervised deep learning algorithm in the data pipeline for online decomposition [3], but they still need *a priori* information provided by classical unsupervised algorithms. We used, in this study, an approach based on autoencoders - an unsupervised machine learning approach - to address the problem of decomposing HD EMG in more general experimental conditions. **Methods:** The architecture of the new decomposition algorithm is illustrated in Figure 1A. Basically, it has the same structure of an autoencoder, in which the MU spike trains (Z) are obtained through additional cost functions to computation of mean squared error. The cost functions assume the signal is sparse and there is no repeated MU spike trains. We compared the performance of the new algorithm with the ICA-based algorithm by Negro et al. [2]. The algorithms were compared using synthetic data generated by a multiscale model of the neuromuscular system coupled to a biophysical model of HD EMG generation. The full model encompasses 120 MUs and 64 HD EMG channels, but here we evaluated a reduced version with 10 channels and 15 MUs. The number of sources detected, the rate of agreement (RoA) between estimated and original MU spike trains, and the computation time were used to assess the performance of each algorithm employed in the study. **Results:** The proposed algorithm detected eight (out of 15) sources with a median RoA of 94.75%, while the algorithm by [2] also detected eight sources but the median RoA was 99.66%. The computation time of our algorithm was 74 min, while the model by Negro et al. [2] ran in 3 min. **Conclusion**: In the reduced scenario, the proposed algorithm underperformed the classical decomposition method both in terms of RoA and computation time. Further tests should be carried out with experimental recordings and with the full neuromuscular model in more realistic conditions, for instance by including non-linearities, additive noise, and non-stationarities.

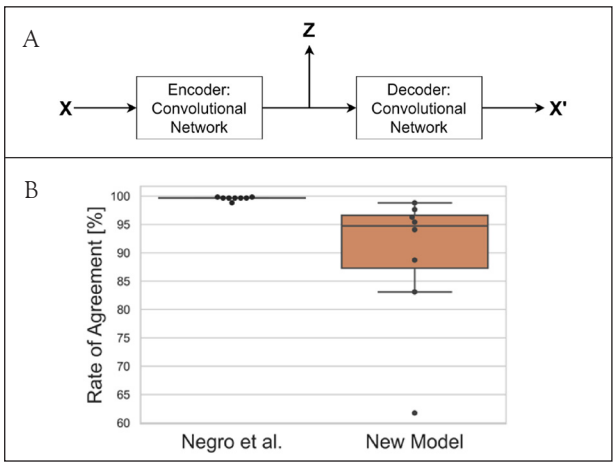

Figure 1. (A) Block diagram of the new decomposition algorithm, where X is the HD EMG data, Z is the estimated MU spike trains, and X' is the estimated HD EMG data. (B) Boxplots for the rate of agreement (RoA) of the estimated MU spike trains from the classical algorithm [2] and our proposal.

References: [1] Holobar et al., doi:10.1109/TSP.2007.896108; [2] Negro et al., doi: 10.1088/1741-2560/13/2/026027; [3] Clarke et al., doi: 10.1109/TBME.2020.3006508;

ANALYSIS OF BETWEEN-SESSION AND WITHIN-SESSION CALIBRATION OF A MI-BCI SYSTEM

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Introduction: In this study, we perform a comparative analysis of Motor Imagery (MI) Brain-Computer Interface (BCI) systems built with the between-session (session-to-session transfer) and within-session protocols. The analysis is based on two freely accessible datasets. **Materials and Methods:** To answer our research question, we used two datasets: Dataset 1 - BCI Competition IV dataset 2a [1], with 9 subjects and 2 data collection sessions, and Dataset 2 - OpenBMI dataset [2], with 54 subjects with 2 sessions, each with 2 trials of the MI paradigm. Each session was conducted on a different day, and the datasets were split into three sets: a training set, with data from the first day, a within-session validation set - with data also from the first day (without overlap with the training data) - and a between-session validation set, with data from the second day. For each subject, Independent Components Analysis (ICA) was applied and a classifier was fitted using the training set to predict left and right hand MI, and was then used to predict in the within-session and between-session validation sets. We used 6 ICA methods [3-7] and a baseline in the preprocessing step, followed by the extraction of the average power spectral density over the *mu* and *beta* bands (8 Hz to 13 Hz and 13 Hz to 25 Hz, respectively) using Welch's method. The features were selected using the incremental wrapper method [8]. **Results:** Figure 1 shows the average accuracy for each dataset considering the 7 different preprocessing methods for both setups: within-session and between-session. In Dataset 1, 4 ICA methods and the baseline had higher accuracy in the between-session setup. FastICA and Extended Infomax tied for the highest accuracy with 73.1%. In Dataset 2, ORICA (1) was the only method that performed slightly better in the between-session setup, and the Extended Infomax had the

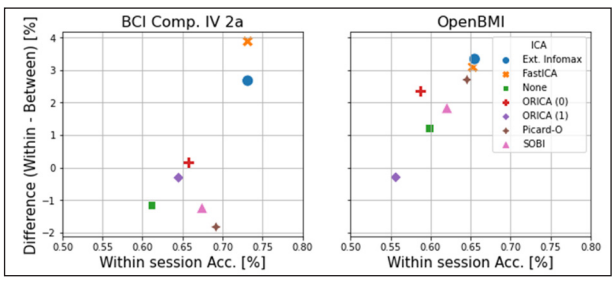

Figure 1. X-axis represents the within-session protocol average accuracy, and the Y-axis represents the average difference between the within-session and the between-session protocols.

highest accuracy, with 65.5%. **Discussion/Conclusion**: For both datasets, the Extended Infomax and FastICA had the highest discrepancy between test configurations. Picard-O was affected differently in each dataset, but maintained a top-3 accuracy. ORICA (1) had the lowest accuracy in Dataset 2, but was the only method with less than 1% accuracy difference when evaluating the BCI with data from a different day than it was trained with, which is desirable in many applications for consistent performance.

References: [1] BRUNNER, C. et al.. Graz Data Set A. Graz University of Technology, 2008. [2] LEE, M. et al.. GigaScience, v.8, n.5, 2019. [3] BELL, A. et al.. Neural Computation, 1995. [4] LEE, T. et al.. Neural Computation, 1999. [5] BELOUCHRANI, A. et al..IEEE Trans.on signal processing, 1997. [6] ABLIN, P. et al..ICASSP, 2017. [7] AKTHAR, M et al.. ISCAS, 2012. [8] KOHAVI, R. et al.. Artificial intelligence, 1997.

ANALYSIS OF DEEP LEARNING APPROACHES APPLIED TO BCI-SSVEP

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Introduction: The advent of deep learning (DL) and its successful applications [1] motivated the use of deep models in brain-computer interfaces (BCIs) to automate the feature extraction stage, and to potentially improve the performance. However, considering the paradigm based on steady-state visually evoked potentials (SSVEP), which naturally refers to signal information in the frequency domain, it seems questionable to use deep models whose learning starts from random feature extractors instead of taking advantage of the specific knowledge about the problem. Thus, the present study aimed at establishing a systematic comparison between the classical and the DL-based methodologies for building BCI-SSVEP systems. **Materials and Methods:** In this study, the Tsinghua BCI Lab public database containing electroencephalography (EEG) signals was used [2]. From this set, eight individuals and nine/thirteen electrodes located in the parietal and occipital regions of the cortex were selected. Furthermore, three scenarios were considered (with two, four and six frequencies), and four classic and four DL-based BCI models were constructed and assessed. While the first classic option, a BCI-CCA, uses the canonical correlation analysis (CCA) for feature extraction and a simple maximum criterion for classification, the remaining classic models employ the CAR filter for pre-processing and a support-vector machine (SVM) for classification, differing only in the technique chosen for feature extraction: (i) BCI-FFT-SVM, which takes the complete Fast Fourier Transform (FFT) of each electrode; (ii) BCI-FFT*-SVM, which explores the magnitudes of the FFT only at the stimulation frequencies and (iii) BCI-CCA-SVM, which adopts the CCA. The DL-based models were convolutional neural networks (CNNs) that process data in the time domain (BCI-CNN) [3], or in the frequency domain (BCI-Welch) [4]; in the latter case, the Welch's method is used to obtain the power spectral density, and two regularized deeper architectures were also tested (whose acronyms are complemented with Dropout and L1&L2). The hyperparameters were adjusted based on a grid search considering a cross-validation scheme with five folds. **Discussion/Conclusion**: According to Table 1, the BCI-CCA achieved the best average performance, followed by the BCI-FFT*-SVM analyzed only at the stimulus frequencies;

Results: **Table 1.** Average performance of BCI models with F1-*score* metric to each scenario.

Number of frequencies	CCA	FFT SVM	FFT* SVM	CCA SVM	CNN	Welch	Welch Dropout	Welch L1&L2
2	95.29%	74.13%	62.66%	72.16%	74.86%	46.88%	48.70%	53.13%
4	90.15%	77.06%	86.0%	72.14%	87.74%	78.27%	55.74%	60.57%
6	86.48%	64.01%	88.91%	69.86%	64.52%	79.25%	51.48%	56.27%

BCI-CNN achieved satisfactory results in most cases, but always inferior to BCI-CCA. The same was observed for the BCI-Welch. Although it was inferior to BCI-CNN in some cases, it performed better when compared to the deeper versions with four convolutional layers. Finally, the greater simplicity of the BCI-CCA emphasizes its suitability for the problem. **Acknowledgement:** The authors thank CNPq/PIBIC for the financial support.

Referencies: [1] I. Goodfellow, et al., ISBN: 9780262035613; [2] Wang, Y. et al., doi: 10.1109/TNSRE.2016.2627556; [3] Aznan, N. K. N., et al., doi: 10.1109/SMC.2018.00631; [4] Pereira, R. Master's thesis - FEEC/UNICAMP, https://hdl.handle.net/20.500.12733/3350, 2021.

ANALYSIS OF SYNAPTOSOMES IN BRAIN TISSUE FROM PATIENTS WITH PHARMACORESISTANT MESIAL TEMPORAL LOBE EPILEPSY

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Introduction: The synapses are electric and chemical communications among neurons, and synaptosomes are vesicles containing synaptic components [1]. The study of synaptosomes can give clues about synaptic transmission and its abnormalities since they contain all the machinery involved in releasing, reuptake, and storing neurotransmitters. In addition, synaptic proteins may be therapeutic targets in many neurological diseases [2]. Mesial temporal lobe epilepsy (MTLE) is the most common type of focal epilepsy in adults, with a high proportion of patients who do not respond to treatment with antiseizure medication [3]. This study aims to characterize the protein content of synaptosomes isolated from the tissue of patients with pharmacoresistant MTLE using mass spectrometry-based proteomics. **Materials and Methods:** We analyzed the synaptosomes isolated from brain tissue obtained from epilepsy surgery (hippocampus and anterior temporal lobe) of patients with pharmacoresistant MTLE (N=20 – 5 per group) and compared these with *post-mortem* control tissue (N=5). We divided patients into four groups classified according to disease duration determined at the time of epilepsy surgery, 10, 20, 30, and 40 years. We isolated the synaptosomes using the Syn-PER reagent (ThermoScientific), extracted and digested the proteins using the S-Trap columns (Protifi), and labeled them with TMT11-plex (ThermoScientific). Data were acquired using a ThermoScientificTM Orbitrap EclipseTM TribridTM coupled to a Dionex UPLC system (MacDonald Laboratory – University of Pittsburgh). *We used the* ProteomeDiscoverer and R software for bioinformatics analysis. We also performed a SynGO analysis to evaluate the different biological classes of proteins identified. **Results:** Overall, we identified 1,890 proteins, 7,521 peptides, and 18,601 PSMs (Peptide-spectrum match scoring). We found differences in the protein content of the synaptosome of the four groups of patients. Furthermore, a preliminary analysis showed that most proteins identified in the synaptosome of patients belong to the presynaptic fraction, followed by the post-synaptic and the synaptic membrane, respectively. **Discussion/Conclusion**: Our study explores for the first time the protein content of the synaptosome in brain tissue of patients with pharmacoresistant MTLE. As an initial finding, we identified differences in the proteomic content of these vesicles according to the duration of epilepsy. In addition, we expect to identify novel proteins that may play a role in disease mechanisms and pharmacoresistance.

References: [1] Goodman & Gilman's pharmacological basis of therapeutics. (McGraw-Hill, 2011). [2] Bai, F. & Witzmann, F. A. Synaptosome proteomics. Subcell. Biochem. 43, 77–98 (2007). [3] Blümcke, I., Beck, H., Lie, A. A. & Wiestler, O. D. Molecular neuropathology of human mesial temporal lobe epilepsy. Epilepsy Res. 36, 205–223 (1999).

ASSESSMENT OF MOTOR UNIT ACTIVITY IN PATIENTS WITH POST-COVID-19 SYNDROME

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Introduction: Some patients report symptoms persisting for weeks or months after acute coronavirus disease 2019 (COVID-19) infection, a condition called post-COVID-19 syndrome [1]. Among a wide range of long-term symptoms, persistent fatigue has been one of the most common complaints [2]. The concept of fatigue involves diverse aspects; however, muscular fatigue can be objectively measured as a reduction in the ability of a muscle to generate force [3]. Also, changes in motor unit activity are observed following fatiguing contractions [4]. Therefore, the present study aimed at identifying differences in motor unit activity during motor tasks between participants with post-COVID-19 syndrome and non-infected participants to explore neuromuscular aspects of long-lasting effects of COVID-19. **Materials and Methods:** Thirty-four participants were recruited and divided in two groups: COVID group (15 women, 2 men, 39±8 yrs.) and CONTROL group (14 women, 3 men, 41±9 yrs.). Participants had to perform an abduction force of the index finger of the dominant hand. The experimental protocol consisted of three blocks: pre-fatigue, post-fatigue, and post-rest. At each block, the maximum voluntary

contraction (MVC) was estimated, and three different tasks were performed: reaction time task (RT, at 20% MVC), trapezoidal force task (TRAP, at 20% MVC), and fatiguing contraction task (FT, at 40% MVC). Pre-fatigue block consisted of 2xMVC, 1xRT, 2xTRAP, and 1xFT. Post-fatigue block consisted of 1xMVC, directly after the previous FT task, 1xRT and 2xTRAP. There was a 30 min rest after the post-fatigue block, and the post-rest block consisted of 1xMVC, 1xRT, 2xTRAP and 1xFT. Surface electromyogram (sEMG) of the FDI (first dorsal interosseous) muscle was recorded and the software Neuromap (Delsys) was used for the decomposition of the sEMG signal. The decomposed signal was visually inspected, and intermittent motor units were excluded from the analysis. Here, the metrics of rate of force development (RFD), mean and maximal discharge rate (DR) of motor units in a 250 ms window from the onset of force were calculated for the RT task. An analysis of variance (ANOVA) was applied to the discharge rate data and non-parametric tests were applied to RFD data. The significance level was set to 0.05. **Results:** Significant differences were found between COVID and CONTROL groups in the mean discharge rate (12.85±0.44 Hz vs 15.23±0.48 Hz, p=0.010) and maximal discharge rate (14.95±0.66 Hz vs 18.85±0.80 Hz, p=0.014), with no significant difference between the blocks of the experiment. There was no interaction between experiment block and groups for these data. A significant difference was found in RFD data between groups only in the pre-fatigue block (251.70±35.74 %MVC/s vs 317.24±33.02 %MVC/s, p=0.026), but no significant difference was found in the post-fatigue and post-rest blocks, neither among blocks for this data. No significant correlation (assessed by Pearson's correlation coefficient) was found between mean DR and RFD or maximal DR and RFD. **Discussion/Conclusion**: Preliminary results obtained in this study suggest that there was a significant difference in motor unit activity between COVID and CONTROL groups in the RT task. Lower discharge rates and RFD values in the COVID group would be associated with alterations in the neuromuscular system of patients as a sequela of post-COVID-19. The present analysis needs some refinement, with possibly inclusion of new participants in the study to confirm the main findings.

References: [1] Sandler CX et al., doi: 10.1093/ofid/ofab440; [2] Lopez-Leon S et al., doi: 10.1038/s41598-021-95565-8; [3] Ortelli P et al., doi: 10.1016/j.jns.2020.117271; [4] Contessa P et al., doi: 10.1152/jn.00347.2016.

ASSESSMENT OF U-NET IN THE SEGMENTATION OF SHORT TRACTS USING CONVENTIONAL CLINICAL IMAGES

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Introduction: To study the structural connectivity in the white matter of the brain, the accuracy of tract segmentation strategies is extremely important [1]. The U-Net network is known for its capacity for image segmentation tasks, and its application in the segmentation part of specific tracts has shown excellent results for large tracts, but the same was not accomplished for short tracts [2]. The aim of this work was to evaluate the capacity of this network in predicting short tracts in data acquired with an imaging protocol used in the clinical practice. **Materials and Methods:** We used DWI and T1w images of 175 subjects from the Human Connectome Project (HCP) dataset and 30 healthy subjects from a 3T scanner in a local University Hospital following a standard clinical protocol imaging. For training it was used 100 subjects from HCP, 15 for validation and 90 for testing (60 from HCP and 30 from local university). Seven fiber bundles were evaluated, five short tracts: anterior commissure (AC), posterior commissure (PC), hippocampal commissure (HC), fornix (FX), uncinated fasciculus (UF); and 2 long fiber tracts: cortical spinal (CST) and inferior-occipital fasciculus (IFO). Similar to previous work [2], the three main directions per voxel obtained through the peak extraction function implemented on MRtrix3 [3] were used as input to the U-NET. The training was made with 100 epochs and separately for each tract. 3D-image was sliced in axial, coronal and sagittal, so that three 2D networks were trained, one for each orientation and the three final predictions were concatenated and averaged. Dice score was used to evaluate the accuracy prediction. **Results:** For the HCP test data, the Dice score obtained was satisfying, showing scores higher than 0.62 for short tracts, even for the ones with high curvature levels, like fornix and uncinate fasciculus (Table 1). Some differences between our work and previous one [2] is in the creation of reference segmentations, number of epochs, use of data augmentation and number of subjects in training and validation what can explain the variation between results. When applied to clinical data, we can observe a notable reduction in the accuracy of the predictions, that could be expected because of the high-quality data used in training. **Discussion/Conclusion**: In conclusion, the use of neural networks is a great resource to optimize the segmentation of specific tracts and bring greater reproducibility. The results for short length tracts, despite not reaching a Dice score as high as the large tracts, were satisfactory with a mean score of 0.63. However, despite presenting promising results, this approach should be used with caution in data acquired in other experimental conditions for both cases: long and short tracts.

Table 1. Mean (SD) Dice scores

	Short Tracts							Long Tracts			
	AC	HC	PC	FX L	FX R	UF L	UF R	CST L	CST R	IFO L	IFO R
Literature[2] (N=21)	0.66	-	-	0.66	0.63	0.78	0.66	0.84	0.84	0.82	0.83
HCP data (N=60)	0.61 (0.05)	0.60 (0.12)	0.63 (0.14)	0.62 (0.10)	0.62 (0.12)	0.64 (0.12)	0.70 (0.08)	0.75 (0.04)	0.83 (0.02)	0.66 (0.04)	0.63 (0.06)
Clinical data (N=30)	0.41 (0.10)	0.54 (0.12)	0.50 (0.12)	0.51 (0.12)	0.44 (0.14)	0.52 (0.10)	0.47 (0.06)	0.63 (0.07)	0.57 (0.07)	0.50 (0.09)	0.53 (0.06)

References: [1] Poulin, P. et al., doi:10.1016/j.mri.2019.04.013; [2] Wasserthal, J. et al., doi:10.1016/j.neuroimage.2018.07.070; [3] Tournier, J.-D. et al., doi:10.1016/j.neuroimage.2019.116137.

BASE-OF-SUPPORT AND SUPPORT-SURFACE CHANGES AND THEIR EFFECTS ON POSTURAL CONTROL OF CHILDREN WITH CEREBRAL PALSY: A SYSTEMATIC REVIEW

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Introduction: Deficits in postural control are commonly reported in children with cerebral palsy (CP).[1] Activities of daily living actively moves the center of the mass by changing configurations of the base-of-support, as well as requires stability maintenance in support-surface with different characteristics.[2] The neuromotor deficits in children with CP might create less adaptive motor responses compared to their typical peers when facing changes in environment information or in the task demands.[3] Aiming to describe postural response patterns of these children when facing changes in base-of-support configurations and support-surface characteristics, we systematically revised studies assessing the effects of manipulations in these components on postural control of children and adolescents with CP. **Materials and Methods:** Two independent reviewers conducted a systematic search in the following databases: PubMed, Web of Science, SCOPUS and Embase. The studies were only included if their objective was to assess the effects of activity and environmental-factor manipulation through the base-of-support and support-surface changes on postural control of children and adolescents with CP up to 18 years. The methodological quality of the studies was assessed using an adapted evaluation checklist, which was used in previous systematic reviews).[4] **Results:** Fifteen studies were included based on the eligibility criteria. Only four were classified as good methodological quality, 10 presented fair quality. The performance of sitting to standing movement with availability of hand support reduced postural oscillation, however, the configuration of the lower limbs during this movement did not modify postural oscillation in children with CP. As well, the base-of--support configuration at seated posture did not impact postural oscillation for none of tested children, but the posture with crossed legs induced a straighter postural alignment when compared to long sitting. The manipulation of the support-surface using anterior and posterior dislocation of the platform induced an oscillation of the body in the opposite direction in all participants. Nevertheless, children with CP showed lower tolerance to the surface-disturbance speed than their typical peers. Anterior tilt of the seat provided better stability, facilitating functional tasks using upper limbs, while posterior tilt increased postural sway. Moreover, surface malleability only increases postural stability when accompanied by visual withdraw. **Conclusion**: In conclusion, children with CP presented less adaptive postural adjustments for manipulations of base-of-support configuration and support-surface characteristics, than their typical pairs. For these children, characteristics of the support-surface might be used as a facilitator or as a restrictor of postural control. Moreover, the

availability of greater bases-of-support reduces the center of pressure sway and trunk displacement, increasing postural stability during sitting to standing movement in children with CP.

References: [1] Rosenbaum P et al., Dev Med Child Neurol Suppl, 109, 8-14; [2] Costa CSN et al., doi: 10.1016/j.humov.2018.11.003; [3] Cherng RJ et al., doi: 10.1016/j.ridd.2009.07.002; [4] Costa CSN et al., doi: 10.1016/j.ridd.2013.05.031.

BEHAVIORAL AND MESOLIMBIC ELECTROPHYSIOLOGICAL ALTERATIONS IN AN ANIMAL MODEL OF POSTICTAL PSYCHOSIS

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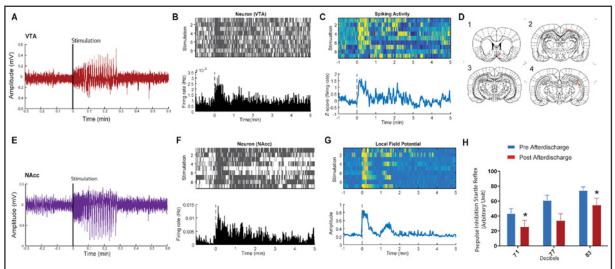
Introduction: Temporal lobe epilepsy is the most common type of focal epilepsy. It presents a high prevalence of psychosis, indicating that alterations on limbic circuits are essential for the development of psychiatric symptoms. We hypothesized that psychotic symptoms after an epileptic seizure are related to a hyperactivity of the hippocampus (HPC) -ventral tegmental area (VTA) pathway. We investigated if the HPC-VTA circuit was involved with psychotic-like behaviors in an experimental model of postictal psychosis induced by afterdischarges (AD). **Materials and Methods:** Adult male Sprague-Dawley rats (n=8) were chronically implanted with recording electrodes in the dorsal CA1 and stimulation electrodes in the ventral hippocampus. Once recovered from surgery, rats were tested in the prepulse inhibition of the acoustic startle (PPI). After 24 h, we established the stimulation threshold (minimum current to evoke at least a 5 s AD) induced by a 10 s, 50 Hz train stimulation (squared pulses of 0.1 ms duration, 150-350 μA, protocol used in all experiments). In the next day, AD was evoked, and rats were immediately reexposed to the PPI. In a second experiment, rats were chronically implanted with multielectrode arrays to record multi-unit activity in the Nacc and VTA during the hippocampal kindling protocol (10 stimulations for 3 consecutive days, n=1). All procedures were approved by the Committee on Ethics in the Use of Animals (102/2020). **Results:** Rats presented a reduction in PPI after AD (Student paired t-test and p-value FDR corrected for multiple comparisons, 71 dB: t(7)= 2.447, p=0,044; 77dB: t(7)= 2.875, p=0,06; 83dB: t(7)=2.589, p=0,045). In the second experiment, kindling generated stereotyped behaviors as wet-dog shakes, rearing, face-washes, and hyperlocomotion. We observed an increase in the firing rate of Nacc (n=9, 0.694 ± 0.095 Z-score) and VTA (n=3, 0.261±0,246 Z-score) neurons following ADs. **Conclusion**: Our preliminary findings indicate that the induction of ADs produced stereotyped behaviors and sensorimotor gating deficits, which were concomitant with increased activity of the VTA-Nacc pathway. These data suggest that the hyperactivity of the mesolimbic dopaminergic projections could be related to postictal behavioral abnormalities.



Legend: A: LFP trace on the VTA in red indicating the signal pre and post afterdischarge. B: Histogram of neuronal firings in VTA after stimulation. C: Standardized values for the neuronal activity in Nacc indicating an increase in firing rate. D: Structures that had a LFP record, such as the Hpcd (2) and spiking activity recording such as the Nacc (1) and VTA (3). The Hpcv was the kindling region (4). E: LFP trace on the Nacc in purple indicating the signal pre and post of afterdischarge. F: Histogram of neuronal firings in Nacc after stimulation. G: Standardized values for the Local field Potential (LFP) in Nacc. H: C: Sensorimotor gating deficit was observed after electrical stimulation in Hpcv in Sprague Dawley rats (300-320g).

Figure 1.

BENCHMARKING BRAIN TISSUE DISSOCIATION FOR MULTIOMICS SINGLE-CELL SEQUENCING OF FOCAL CORTICAL DYSPLASIAS

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Introduction: Focal cortical dysplasias (FCDs) are a type of malformation of cortical development and one of the main causes of epilepsy in children [1]. According to the International League Against Epilepsy (ILAE) classification, both CFD type IIa and IIb lesions are characterized by disrupted cortical lamination and dysmorphic neurons [2]. The development of single-cell genomics allows high-throughput sequencing of individual cells in complex tissues, thus allowing to identify of populations of cell types and their molecular characteristics in heterogeneous tissues such as the cerebral cortex. In this project, we are applying single cell sequencing to determine the cell types involved in FCD lesions. **Materials and Methods:** Tissue was obtained from donors diagnosed with FCD type IIb (n=5) and FCD type IIa (n=3), aged between 3 and 40 years, from which dysplastic and control tissue was available. The nuclei extraction protocol for human brain tissue was obtained from the Jabado laboratory [3]. Briefly, frozen tissue was dounce-homogenized, incubated in lysis buffer containing 0.1% NP-40, followed by purification through 29% iodixanol (Sigma) for debris removal. Recovered nuclei were resuspended and counted. The integrity of the nuclei was evaluated under the microscope, and the nuclei were considered viable if they were round and had intact membranes. Library construction was performed using the Chromium equipment and the Multiome kit(10X Genomics), which allows simultaneous measurement of chromatin accessibility (ATAC) and gene expression (3' mRNA-seq) of individual nuclei. **Results:** We extensively optimized the original tissue dissociation protocol with the goal of increasing the concentration of nuclei for single cell analysis, while recovering high-quality nuclei and minimizing cell debris and aggregates. Parameters tested included lysis buffer duration and concentration, and centrifugation time. After several rounds of optimization, we were able to systematically recover suspensions with high concentration (3-3500 nuclei per microliter) and 80-90% nuclear integrity. Importantly, these optimizations allowed us to load up to 7-10,000 targeted recovery cores per sample, thereby using the optimal capacity of the Chromium equipment. We sequenced two FCD type IIb donors totaling 11,924 unique cells, including 8,943 cells derived from lesions and 2,981 cells from adjacent tissues, with an average of 1,838 genes detected per cell. Cluster analysis based on t-SNE found 8 major cell clusters, which will be further annotated using brain cell type markers to identify cell types in the disease. **Discussion/Conclusion:** Preparing a high-quality nuclei suspension is a limiting step in single cell experiments, especially with frozen archived tissues. Here, we determined the optimal parameters to obtain core extraction from cortical FCD tissues suitable for single cell library construction. This work will pave the way for the application of single-cell genomics to study the molecular and cellular landscape.

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BRAIN CONNECTIVITY CHANGES IN STROKE SURVIVORS DURING REHABILITATION

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Introduction: Studies have reported that functional connectivity (FC) of stroke survivors is usually reduced when compared to that of healthy subjects, and that rehabilitation procedures can lead to a strengthening of connections which is also correlated with motor gains [1]. Alongside with FC tracking with electroencephalography (EEG) or magnetic resonance imaging, new technologies that aim at more engaging and efficient treatments such as virtual reality (VR) and transcranial direct current stimulation (tDCS) are being used to assist and complement motor rehabilitation, respectively. In this context, this project has the main objective of studying FC alterations in chronic stroke survivors during a rehabilitation protocol that includes VR exercises and tDCS. **Materials and Methods:** Five subjects (2 female, 60-77 y, 8-54 months post-stroke), performed a 10-session full body rehabilitation protocol (30 minutes/session) with VR exercises during anodal tDCS (2 mA current) of the ipsilesional motor cortex (M1). The subjects were evaluated with clinical scales before and after the rehabilitation and with 60 seconds of resting-state EEG (g.tec g.USBamp, 16 channels) before and after the tDCS in sessions 1, 5 and 10. One subject attended only 9 sessions, and in this case the EEG was performed in sessions 1, 4 and 9. We evaluated the lagged-coherence in the alpha band (8-13 Hz) for 6 s epochs,

computed the degree of each epoch, and finally each electrode degree was averaged over the 10 epochs of each subject acquisition. To analyze the subject's evolution, the relative differences between the degree of acquisitions before tDCS in the last and first session and between the middle and the first sessions were computed. **Results:** The relative differences for the global (average over electrodes), ipsilesional M1 and contralesional M1 degrees are presented in Fig. 1. We can see an overall increase in the global and contralesional degrees, and a decrease in the ipsilesional degree over time. All subjects improved in the

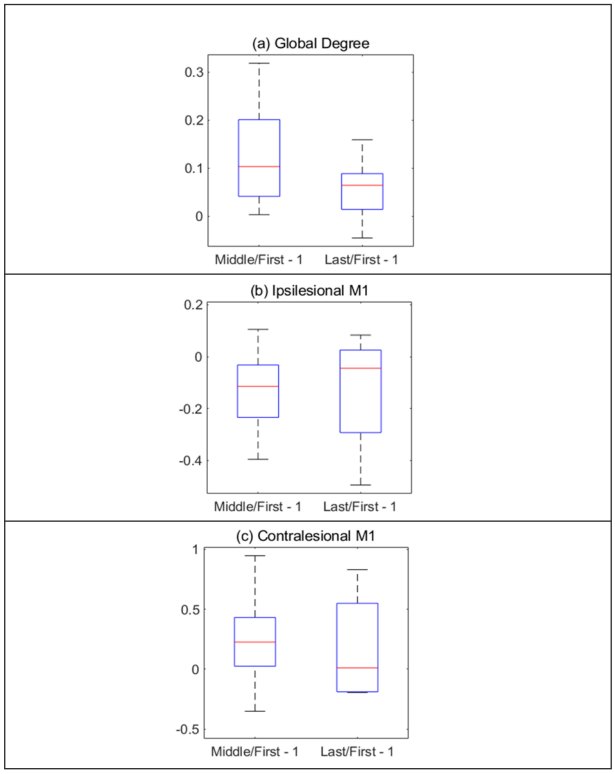

Figure 1. (a) Global , (b) ipsilesional M1 and (c) contralesional M1 relative degree differences.

BERG and upper extremity Fugl-Meyer scores after rehabilitation. **Discussion/Conclusion**: A global FC increase after rehabilitation as observed in this study has been reported in others [2], especially in the alpha band with tDCS [3], and its relation with improvements in clinical assessments is becoming more evident [1]. The degree alterations in the ipsilesional and contralesional M1 indicate local neuroplasticity that might be directly caused by the tDCS intervention. As future steps, we intend to include more subjects and a sham tDCS group in order to better understand the stimulation effects on brain FC and rehabilitation, correlate FC with the electromyography and range of motion measures and further explore its uses in prognostic. **Acknowledgement:** Work supported by FAPESP (grant 2020/16571-0)

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CHALLENGES IN AUTOMATED SEGMENTATION OF LUNG LOBES IN CT IMAGES OF DISEASED LUNGS

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Introduction: The main difficulty in performing automated lung lobe segmentation occurs in the presence of lung diseases, since they obscure or make part of the lobar fissures incomplete. In addition, variations in anatomy are frequent, especially in cases of lung disease such as cancer and COVID-19 [1]. In this work, the objective is to show the loss of performance in the segmentation of the lobes in lungs with incomplete lobar fissures when compared with lungs less affected by some lung disease, in which the fissures are probably better to be identified. **Materials and Methods:** In carrying out the experiments, the U-Net 2D-extended network [2] and the lungmask framework [3] were used. The first dataset was composed by 50 CT scans from LUNA16, a dataset for pulmonary nodules, with lung lobes manually segmented by Tang et al. [4]. The second dataset consisted of 8 CT scans of CoronaCases, a dataset with CT volumes of positive patients with COVID-19, with lung lobes manually segmented and provided by Balasubramaniam [5]. The metric used to evaluate the results was the dice [6]. **Results:** On CT scans with well-defined fissures, dice values were greater than 91% for all lobes. (Figure 1-A). The scans with well defined fissures were the ones that obtained the best segmentation in the test dataset, obtaining dice values of 96% (Fig.1-C). On CT images with incomplete fissures, especially on lung CT images with COVID-19 (Figure 1-B), the segmentation was poorly done and the dice values were relatively low (circled outliers in figure 1-A). **Discussion/Conclusion**: The lobar fissures that define the borders of the Right Middle Lobe are the most difficult to locate, resulting in a lower than expected dice value compared to CT images where the fissures are well defined. A solution to this problem is to train the network on a larger amount of data containing lung CT images with different pathologies such as cancer and COVID-19. An alternative to overcome the lack of annotated data is to develop a self-supervised approach.

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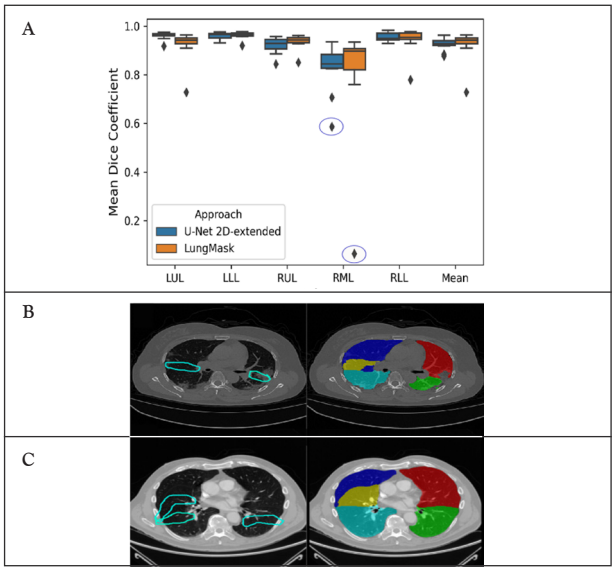

Figure 1. A- Mean Dice Coefficient. B- Incomplete Fissure in CT with COVID-19 (lungmask). C- Well defined fissure. Legend: Left Lower Lobe (LLL), Left Upper Lobe (LUL), Right Lower Lobe (RLL), Right Middle Lobe (RML), Right Upper Lobe (RUL) and Mean (Mean Dice Coefficient).

CHARACTERIZATION OF ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT PATIENTS USING BRAIN NETWORKS GENERATED FROM MAGNETIC RESONANCE TEXTURE ANALYSIS

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Introduction: Mild Cognitive Impairment (MCI) and early-stage Alzheimer's disease (AD) are brain pathologies characterized by memory loss, and are difficult to distinguish. However, while MCI is a condition associated with aging, AD can evolve into speech difficulties and impairment of motor and executive tasks. Therefore, it is important to find ways to accurately identify these patients [1]. Brain pathologies are often responsible for subtle changes in the brain tissues, i.e., techniques for perceiving such elusive alterations are required. Magnetic resonance texture analysis (MRTA) is a technique for extracting mathematical parameters that represent the underlying properties of an image. A previous study has shown that relevant information can be extracted from brain networks generated using MRTA parameters [2]. This study aims to determine whether it is possible to use these structural brain networks to

characterize and distinguish between healthy individuals and patients with MCI and AD. **Materials and Methods:** Structural brain images of 55 individuals (18 men) were acquired in a 3T scanner (Philips Achieva). Two MRI exams were performed on each individual, and they were classified as healthy individuals, patients with MCI, or patients with AD. MRTA was performed on these images using an isotropic 3D gray-level cooccurrence matrix (GLCM). Eight pairs of anatomical homologous (L and R) regions, commonly associated with AD, were extracted from the AAL atlas, and selected as ROIs: lobule VIII of the cerebellar hemisphere, middle frontal gyrus, hippocampus, middle occipital gyrus, postcentral gyrus, precentral gyrus, supplementary motor area, and thal amus. Using graph theory, the texture parameters extracted from the GLCM were used to generate individual brain networks, and the strength network parameter was then computed for each network. An ANCOVA with repeated measures statistical analysis was performed, using the network parameter as the dependent variable, the diagnostic as the independent variable, and sex, age, and years of education as covariables. **Results:** The ANCOVA revealed there is an effect of the diagnosis on the strength network parameter for the R thalamus [$F(2,49)=3.729$; $p<0.05$]. Bonferroni's post hoc showed there is a significant difference between MCI and AD (Figure 1). **Discussion/Conclusion**: This study has shown that structural brain networks have the potential to identify

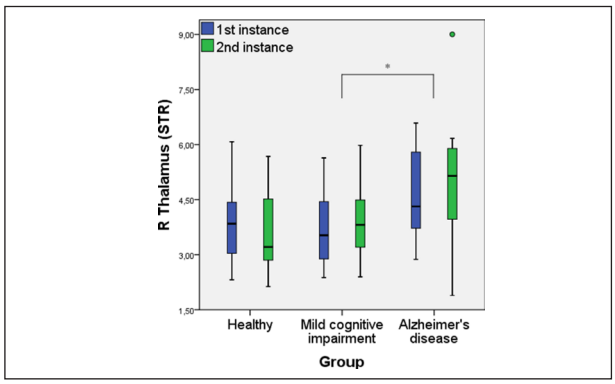

Figure 1. Box-plot for the R thalamus referring to the strength network parameter.

and distinguish brain pathologies in MR images. The next step is to apply this method to other anatomical regions. In this study, only the strength network parameter was used, but other parameters (e.g. eigenvector centrality) can also be extracted and analyzed. Future studies can aim at characterizing other brain pathologies, such as epilepsy.

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COMBINED EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION AND VIRTUAL REALITY TECHNOLOGIES FOR REHABILITATION OF ACTIVE JOINT RANGE OF MOTION IN STROKE SURVIVORS

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Introduction: Joint range of motion (ROM) impairments are common following stroke and are often associated with increased pain and reduced motor function [1]. In particular, loss of ROM in the paretic side joints of stroke survivors may develop early following stroke and aggravate with the lack of rehabilitation exercises [1, 2]. Both virtual reality (VR) and transcranial direct current stimulation (tDCS) technologies have been previously used independently for motor rehabilitation, but the combined effects of VR and tDCS for ROM improvements in stroke survivors are currently unknown. The aim of this study was to examine the time course of changes in active joint ROM of chronic stroke survivors following a rehabilitation program including VR exercises combined with tDCS. **Materials and Methods:** Three stroke survivors (2 female, 60-62 y, 23-54 months post-stroke) presenting hemiparesis in one side of the body participated in the study. They performed a structured 10-session full body rehabilitation program (30 minutes/session) consisting of VR exercises targeted for motor improvements in both paretic and nonparetic joint sides, while also receiving anodal tDCS (2 mA current, 30 s ramp up/down) on their ipsilesional motor cortex (M1). Bilateral shoulder abduction and hip flexion active joint ROM of participants were measured before and after sessions 1, 5 and 10 with a Kinect® v2 device system (KinesiOS/Tracker software), which allowed detecting their joint movements through a grid of infrared dots. The highest joint ROMs were recorded. **Results:** Joint ROM values tested before the start of sessions 1 (baseline), 5 and 10 are presented in Fig. 1. Most participants presented increases in shoulder and hip ROM from baseline throughout the rehabilitation program, which were more prominent in

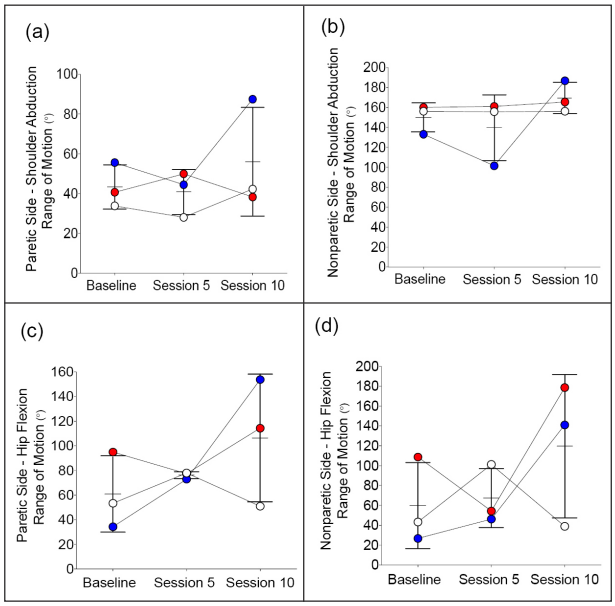

Figure 1. Mean ± SD of shoulder abduction (a and b) and hip flexion (c and d) ROM of the paretic and nonparetic sides, respectively. Circles and lines represent individual changes between sessions.

session 10. Participants also had ~3-52% decreases in hip flexion and shoulder abduction ROM from before to after session 1. However, ROM decreases in the same joint movements dropped to ~3-16% from before to after session 10, which may indicate that participants were more resistant to neuromuscular fatigue at the end of the program. **Discussion/Conclusion:** Increased active joint ROM of stroke survivors has been previously observed following 18 sessions of robot-guided rehabilitation exercises [3]. In contrast, our findings indicate that a rehabilitation program including the combination of both VR exercises and tDCS may be effective in increasing active joint ROM after only 10 sessions and seems to also result in less neuromuscular fatigue in the paretic joints of stroke survivors, which may be important for improving their functionality in daily activities. The next steps of our project will be to increase the sample size, include a control group with sham tDCS, and test the associations between electroencephalography (EEG), electromyography activity (EMG), ROM and clinical test measures in this population.

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COMPARATIVE STUDY OF STRUCTURAL BRAIN CHANGES CAUSED BY BILINGUAL EXPERIENCE IN HEALTHY ELDERLYS AND ALZHEIMER'S DISEASE PATIENTS VIA SYSTEMATIC REVIEW

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Introduction: There is evidence that bilingual patients would express the clinical symptoms of Alzheimer's disease (AD) up to 5 years later than their monolingual counterparts would. One possible explanation could be the construction of brain and cognitive reserves. To understand the impact of the bilingual experience on the brain, this study carried out a systematic review of studies that by using imaging techniques investigated differences in brain structure between bilingual and monolingual elderly people, whether they were

healthy or AD patients. **Materials and Methods:** This work used PRISMA protocol (*Preferred Reporting Items for Systematic Reviews and Meta Analyses*) for performing the systematic review. Two research questions were formulated using PICO methodology: (i) Are there structural differences between the brains of healthy bilingual elderly and healthy monolingual elderly people? (ii) Are there structural differences between the brain of bilingual elderly people with AD/MCI (Mild Cognitive Impairment) and monolingual elderly people with AD/MCI? In both cases, findings should be justified from the image analysis. To find the existing literature, a search query was applied in five databases: Virtual Health Library (*in Portuguese*), PsycINFO, PubMed, Scopus and Web of Science. **Results:** After the identification of studies from the primary survey, more than 400 papers were found and 20 of them were selected according to the inclusion and exclusion criteria. Three studies that analyzed the total volume of gray matter in the brain of healthy elderly people did not find differences between bilinguals and monolinguals [1,2,3]. Regarding the elderly diagnosed with MCI, the bilingual group had a lower total gray matter volume than the monolingual group [3]. In addition, greater cognitive decline and greater loss of parenchymal volume were observed in monolinguals compared to bilinguals. Such a loss was found in both gray and white matter volumes. Concerning the integrity of the white matter in regions of interest, the study [5] indicates that there are no differences in FA (*Fractional Anisotropy*) between the monolingual and bilingual groups when using *Propensity Score Matching*. **Discussion/Conclusion**: In the selected works, a great variation was observed in how the variable bilingualism is defined. Almost half of the selected works used simple questionnaires with self-report of bilingualism information, as LEAP-Q [4], LSBQ [5] and LBQ [6]. Only the Smirnov et al. work [7] used the MINT test (Multilingual Naming Test) to assess participants' proficiency in both the dominant and non-dominant languages. In addition, the analyzed subjects live in developed countries, which can bias the results. i.e., factors such as educational and socioeconomic level can influence the findings. Regarding the elderly diagnosed with MCI or AD, the influence of bilingualism seems to be greater. While there is a decrease in temporal and SMG (*Supramarginal Gyrus*) areas, frontal, parietal, cerebellar and ACC (*Anterior Cingulate Cortex)* areas show greater volume. This work also evaluated the functional changes in the brain caused by the bilingual experience, which we will present in future works.

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COMPARISON BETWEEN THREE FUNCTIONAL CONNECTIVITY METHODS FOR FEATURE EXTRACTION IN MOTOR IMAGERY BCIS

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Introduction: Motor imagery (MI) brain-computer interfaces (BCIs) aim at translating the neurophysiologic activity from movement imagination into commands for external devices. However, intra and inter-subject variability of brain responses poses a big challenge to the field, precluding BCIs adoption in clinical environments [1]. The aim of this work is to utilize graph metrics from motifs synchronization (MS), imaginary coherence (iCoh) and weighted phase-lag index (wPLI) functional connectivity (FC) methods applied to electroencephalography (EEG) signals, to classify left and right-hand MI tasks utilizing Support Vector Machines (SVMs) and Linear Discriminant Analysis (LDA). **Materials and Methods:** EEG signals (16 channels) were acquired from 1 healthy subject (18 years, man), right-handed, during 12 sessions performed on different days, over ~1 month. Each session was divided into 5 runs of 128 s, and each run was further divided into 8 grand blocks, consisting of rest (8 s, blank screen), preparation (2 s, cross as MI cue) and task (6 s, arrow) periods. During task blocks subjects should perform MI for the hand indicated by the arrow's direction. Task blocks were randomly assigned to left-hand or right-hand MI, with same number of blocks (4) per task per run. Pre-processing was performed with EEGLAB and consisted of: removing the 1st second of each run to eliminate a voltage spike from the equipment; re-referencing the signals to their common average; applying a 0.5 Hz high-pass filter to remove skin conduction artifacts; and using independent component analysis to remove eye blinking artifacts. The signal was then filtered in 2 frequency bands: α (8-12 Hz) and β (13-31 Hz). FC was estimated for each band and each second of task blocks with MS, wPLI, and iCoh. The following graph parameters were extracted from the FC matrices: strength, clustering coefficient, eigenvector centrality, local efficiency, modularity, transitivity, and rich club. The dataset was divided into train and test with the proportion 80/20. SVM and LDA were built using 5-fold cross validation splitting, with wrappers with sequential feature selector. All the combinations from the best 1 to 50 features (channel + graph metric) were tested and the largest mean accuracy was selected. **Results:** Table 1 shows mean accuracies for the best features' combination. The number of features for the best combinations were: 20 (5), 39 (19) and 38 (10) for SVM in the α (β) band, and 10 (41), 47 (20), 31 (45) for LDA in the α (β) band, for MS, iCoh and wPLI respectively. **Discussion/Conclusion**: In general,

Table 1. Mean accuracies of SVM and LDA classifiers with the best combination of graph metrics.

FC method	SVM Accuracy (%)		LDA Accuracy (%)	
	α (8-12Hz)	β (13-31Hz)	α (8-12Hz)	β (13-31Hz)
MS	55.3±1.4	55.7±0.3	52.3±2.2	54.1±1.4
iCoh	58.8±1.3	55.7±1.3	55.3±3.3	53.9±2.3
wPLI	56.8±1.6	55.5±2.1	55.2±3	53.3±1.2

SVM performed slightly better than LDA. Two of three best accuracies were in the α band, which agrees with previous studies on MI-BCIs [2]. The best results for SVM used less features for the β band; while this almost inverted for LDA. However, even the best results were poor for a real application. The next steps will be to increase the number of subjects and to explore new classifiers, graph features and FC methods.

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CORRELATION BETWEEN ACTIVATION MAPS AND MANUAL LACUNA SEGMENTATION WITH ENGEL SCALE VALUES IN PATIENTS WITH EPILEPSY – A PILOT STUDY

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Introduction: Epilepsy is a debilitating condition characterized by recurrent, unprovoked seizures, affecting 0.5-1% of the world population [1]. Resective surgery of the epileptogenic zone is the treatment of choice in focal epilepsies refractory to antiepileptic drugs. Nevertheless, the varied rates of good surgery outcome elicit uncertainties on the definition of the target regions to be removed [2]. In this pilot study, we investigated the validation of the overlap between activation maps and manual segmentation performed on the MRIcon in epilepsy cases and its relationship with surgical outcome. **Materials and Methods:** Thirty-five epilepsy patients (22 women) underwent a 3.0 T MRI scan (Philips Achieva) at Unicamp's Clinical Hospital. The anatomical postoperative T1 images were visually inspected for artifacts and then, the resective lacuna was manually segmented using MRIcron (version 1.0.20190902). To assess the correspondence between epileptiform activity on the EEG-fMRI and the surgical lacuna, we compared the activation maps to the manual segmentation using the Dice coefficient (DC). In addition, the DC values were assessed in light of Engel score, which is graded into I and II (free or rare post-surgery epileptiform activity) and III and IV (worthy or unworthy improvement in epileptiform activity). **Results:** We found a median DC of 0.0686 (range: 0.004 - 0.3936). Furthermore, there were 25 patients who had a DC value of zero (not considered in the median calculation), indicating no overlap between the activation maps and the manual segmentation. In addition, the Engel scores were tabulated as shown in the table below, excluded three patients whose data were not obtained. **Discussion:** In cases where the Dice coefficient is different from zero, the Engel scale values are considerably higher

Table 1. Correlation of Dice coefficient values and the Engel scale.

Dice / Engel	Ia	Ib	Ic	II	IIa	III	IV	IVa	IVb	Total
= 0	9	1	1	3	2	2	2	2	0	22
≠ 0	2	0	0	0	1	6	0	0	1	10
Total	11	1	1	3	3	8	2	2	1	32

than in cases where DC is equal to zero. In cases where DC = 0, 72.72% of the patients were classified as Engel ≤ 2, which means that the post-operative period passed without or with rare manifestations of an epileptogenic nature. On the other hand, in cases where DC ≠ 0, only 30% of patients had an Engel value ≤ 2, indicating that there was a greater recurrence of epileptogenic activity in these cases. **Conclusion:** The DC calculated (0.0686) is considerably lower than expected, which demonstrates a nonspecific relationship between the brain activity captured during epileptiform activity and the surgical lacuna. However, the inclusion of more patients is essential to confirm these results.

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DENTATE NUCLEI SEGMENTATION IN QUANTITATIVE SUSCEPTIBILITY MAPPING USING DEEP LEARNING

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Introduction: The dentate nucleus (DN), the largest of the cerebellar nuclei, is a structure buried deep within the cerebellar white matter and is rich in iron [1]. A reduced volume in DN is a common finding in spinocerebellar ataxia (SCA) and Friedreich's ataxia (FA), although occurring in different intensities. Recently, a growing number of clinical trials are in progress requiring sensitive outcome measures, and neuroimaging is a recognized developing field able to meet this demand. In this context, quantitative susceptibility mapping (QSM) stands out as a magnetic resonance imaging (MRI) technique for measuring local magnetic field distortions, providing an iron-sensitive contrast, thus enabling DN delineation. MRICloud [2] is the current leading solution based on a multi-atlas approach. Therefore, we propose a deep learning pipeline to target the cerebellar DN anatomical changes, thus providing a reliable neuroimaging biomarker. **Materials and Methods:** Our dataset consists of 86 QSM and T1 images acquired at different sites and MRI scanners. Although there is no access to which group – control or patient – each image is part of, we selected data from four centers. The Monash University team provided manual segmentation masks specifying left and right DN in all QSM images. Then, we designed a pipeline composed of a localization followed by a segmentation step. The localization model – a base 3D U-Net [3] – restricts the region of interest, cropping the original QSM to a predefined bounding box enclosing the cerebellum. For that, we used ACAPULCO [4] cerebellar prediction maps. Next, the 3D U-Net architecture with deep supervision (DS) and the nnU-Net [5] – state-of-the-art for medical segmentation – were selected for DN segmentation. Lastly, we calculated the Dice similarity coefficient (DSC) metric and visually inspected the predicted masks for performance assessment. **Results**: First, the localization network was trained (test DSC: 0.918±0.030), and a visual inspection revealed successful cropping on all images. The 3D U-Net with DS segmentation model achieved a DSC of 0.892±0.022 on the test set (left DN: 0.889±0.021, right DN: 0.895±0.023) following a training duration of 5 hours. We also trained two nnU-Net models with distinct input shapes, resulting in a DSC of 0.899±0.020 (left DN: 0.896±0.020, right DN: 0.901±0.021) on the smaller and 0.898±0.021 (left DN: 0.896±0.020, right DN: 0.900±0.021) on the larger shape. However, both nnU-Net models inverted the labels on two test samples such that the metrics reported overlooking these issues. The web-based tool MRICloud scored 0.742±0.067 (left DN: 0.725±0.074, right DN: 0.760±0.053). In figure 1, we present an example of our U-Net model prediction.

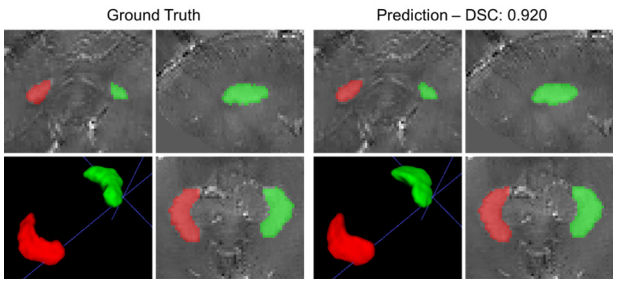

Figure 1. 3D U-Net with DS prediction on a test set sample.

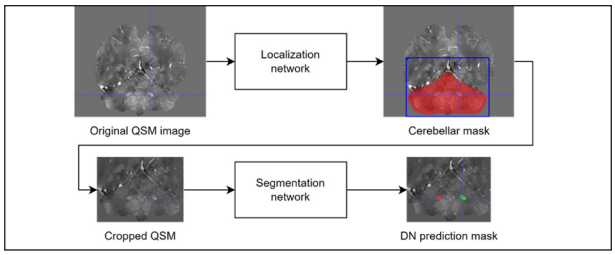

Figure 2. DN segmentation pipeline.

Discussion/Conclusion: In conclusion, our preliminary deep learning pipeline reveals promising results, encouraging our effort to provide a more precise automated quantitative assessment tool for DN cerebellar structure. Based on the data-centric approach, we are setting up a labeling protocol to guide segmentation mask refinement performed by expert neuroradiologists. Furthermore, to assess the model's applicability, we will undertake tests on external data from an independent site, which collection is already in course.

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DEVELOPMENTAL IMPAIRMENT IN CHILDREN EXPOSED TO SARS-COV-2 IN UTERO: A BRAZILIAN COHORT STUDY

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Introduction: The effects of in-utero exposure to maternal SARS-CoV-2 infection on the offspring's neurodevelopment are still unknown. **Materials and Methods:** We performed a prospective cohort of babies exposed to SARS-Cov-2 during pregnancy, and a control group of unexposed babies. Children's neurodevelopment was assessed using the guide for Monitoring Child Development in the IMCI context and the Ages & Stages Questionnaire (ASQ-3), at ages 4, 6, and 12 months. Maternal depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale (EPDS). **Results:** We followed 127 children for one year, 69 children in the COVID-19 exposed Group (EG), and 68 in the control group (CG). Maternal demographics were similar in the two groups, but prematurity was more prevalent in mothers infected with COVID-19 (21.7% vs. 8.8%, p= .036) and EPDS scores were also significantly higher among the EG (M= 11.00, SD=6.00 vs. M=8.68, SD=4.72, p= 0.04). Both groups had similar rates of cesarean delivery, Apgar scores, average birth weight, head circumference and length at birth. 20.3% of EG children and 5.9% of the CG received a diagnosis of neurodevelopmental delay within 12 months of life (p=0.013, RR= 3.44; 95% CI, 1.19- 9.95), with 10% of EG children presenting abnormalities at the cranial ultrasound. **Discussion/Conclusion**: COVID-19 exposure was associated with neurodevelopmental impairment. This study highlights the importance of specific guidelines in the follow-up of children exposed to in-utero SARS-CoV-2 in order to mitigate or prevent long-term effects on children's health.

Table 1. Maternal profile and children outcomes comparing babies with and without SARS-Cov-2 intra-uterine exposure.

	Exposed Group (N=69)	Control Group (N=68)	p[a]
Maternal Age, mean (SD), years	30.29 (6.58)	30.03 (7.08)	.824
Postpartum depression score,[b] mean (SD)	11.00 (6.00)	8.68 (8.68)	.040
Preterm birth (less than 37weeks), n(%)	15 (21.7%)	6 (8.8%)	.036
Birth weight, mean (SD), grams	3175.5 (523.98)	3207.03 (465.35)	.707
Apgar score 5° min < 7, n (%)	7 (10.1%)	1 (1.5%)	.063
Head circumference, mean (SD), cm	34.33 (1.75)	34.50 (1.72)	.567
Exclusive breastfeeding, n (%)	27 (39.1%)	27 (39.7%)	.945
Neurodevelopmental delay, n (%)	14 (20.3%)	4 (5.3%)	.013

SD,standard deviation; IQR, interquartile range. [a] Chi-Square test or Fisher's exact test was used for categorical variables and Student's t-test or Mann Whitney U test was used for continuous variables; [b] Edinburgh Postnatal Depression Scale (EPDS).

DIFFERENCES IN CELLULAR COMPOSITION BETWEEN FOCAL CORTICAL DYSPLASIA SUBTYPES UNRAVELED BY SINGLE-CELL DIGITAL CYTOMETRY AND IMMUNOHISTOCHEMISTRY

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Introduction: Focal Cortical Dysplasias (FCDs) are a type of malformation of cortical development and represent a major cause of intractable epilepsy in children, often requiring surgical resection of the affected brain tissue. Specifically, FCD type II can be subclassified into FCD IIa and IIb, both characterized by disrupted cortical lamination and dysmorphic neurons, with the latter also presenting balloon cells. Deconvolution of bulk gene expression data using single-nuclei RNA sequencing (snRNA-seq) signatures - also referred to as digital cytometry - has been shown to accurately estimate the cell type composition of healthy and diseased brain tissues. Here, we applied deconvolution algorithms with single-cell signatures to estimate cell type proportions in patients diagnosed with FCD types IIa and IIb. We then applied immunochemistry techniques to validate our findings. **Materials and Methods:** We grouped publicly available RNA-seq data into Dataset 1 (10 FCD IIa; 9 FCD IIb) [1,2] and Dataset 2 (11 FCD IIa; 21 FCD IIb) [3]. Raw data was uniformly processed using STAR for read alignment to the human genome hg38 and RSEM for transcript quantification using Gencode v.40, as implemented in the nf-core RNA-seq pipeline v. 3.8.1. Deconvolution with CIBERSORTx was performed using reference signatures of brain cell types extracted from snRNA-seq studies [4,5]. Immunostained slides (4 IIa; 4 IIb FCD patients – CEP #470/2003) were analysed using Aperio ScanScope and ImageJ, with ten representative subfields from each patient for identification of glial fibrillary acidic protein (GFAP; astrocyte marker). **Results:** We found a significant decrease of excitatory neurons (Wilcox rank-sum test, $P < 0.05$) and expansion of astrocytes ($P < 0.1$) in FCD IIb lesions when compared to FCD IIa, in both datasets and adjusting for sex and age covariates. In addition, by performing a detailed subpopulation analysis using 22 neuronal and non-neuronal cell types annotated from a multiple sclerosis snRNA-seq dataset containing 48,920 nuclei [5], we found that loss of excitatory pyramidal neurons, and astrocyte expansion (astrogliosis) are characteristics of FCD IIb ($P < 0.05$). Finally, we showed that FCD IIb lesions expressed a reactive astrocyte marker signature (GSEA, $P < 8x10^{-8}$), including higher levels of GFAP, nestin, vimentin, and SERPINA3, in line with the astrocyte expansion detected by deconvolution analysis. Immunohistochemistry analysis also showed a significant increase in GFAP-immunopositive area, favoring an expansion of the astroglial meshwork, in FCD IIb relative to FCD IIa ($P < 10^{-4}$). **Discussion/Conclusion**: In conclusion, FCD IIb lesions are characterized by loss of excitatory neurons, increased microglia activity, and remarkable astrocyte activation (astrogliosis), suggesting that neuroinflammation might play a role in this subtype. We next intend to extend immunochemistry analyses for markers of neuronal cells and activated microglia.

References: [1] Kobow K et al., doi:10.1111/epi.14934; [2] Assis-Mendonça GR et al., doi:10.1101/2022.08.23.22279011; [3] Zimmer TS et al., doi:10.1111/nan.12736; [4] Sutton GJ et al., doi:10.1038/s41467-022-28655-4; [5] Schirmer L et al., doi:10.1038/s41586-019-1404-z.

DOPAMINERGIC ACTIVATION MODULATES HIPPOCAMPAL-PREFRONTAL CORTEX OSCILLATORY SYNCHRONY

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Introduction: Interaction between brain networks is fundamental to the cognitive process. Oscillatory synchronization is proposed as a mechanism to coordinate neuronal activity from distant regions. One crucial aspect of elucidating brain function and its emergent properties is to understand how functional connectivity is modulated in different time scales according to environmental and physiological demands. Modulatory neurotransmitters such as dopamine have been implicated in changes in membrane excitability, modulation of oscillatory power, and coherence in crucial circuits for cognition, such as the hippocampus-prefrontal cortex (HPC-PFC) pathway. However, it is not known how dopaminergic activity modulates specifically the HPC-PFC oscillatory phase synchrony or which specific dopaminergic receptors are responsible for the effects. In this context, our objective was to investigate the influence of dopaminergic activity on HPC-PFC synchrony and to assess whether the observed effects are receptor-specific. **Materials and Methods:** Forty-two seven-week-old Sprague Dawley rats were anesthetized with urethane (1.2 g/kg) and implanted with stereotrodes in the HPC and PFC or a neural probe in the PFC. Different groups of rats were injected with dopaminergic receptors agonists apomorphine (i.p., in 0.75, 1.5, and 3 mg/kg doses), dopamine (i.c.v., in 100 and 500 nmol doses), SKF-38393 or quinpirole (i.c.v., in 1 and 10 μg doses) and their local field potential was recorded simultaneously. **Results:** We found that dopamine induces HPC-PFC theta phase synchrony dose-dependently. This effect is not reproduced by apomorphine nonspecific agonism at D_1/D_2 receptors or by SKF and quinpirole selective agonism at D_1 and D_2 receptors, respectively. We have also shown that a low dosage of apomorphine induces delta synchrony while a higher dosage shifts brain oscillations from delta to theta state. Additionally, we observed a late effect with peak activity between 30 and 40 minutes after dopamine 100 nmol, apomorphine 0.75 mg/kg, or quinpirole administration in which the HPC-PFC delta synchrony increased. **Conclusion**: Our results evidence the participation of dopaminergic neurotransmission in regulating HPC-PFC oscillatory synchrony, showing that dopamine induces theta phase synchrony, whereas activation of D_2 but not D_1 receptors induces delta synchrony. Our study contributes to understanding the dopaminergic role in the communication of brain networks.

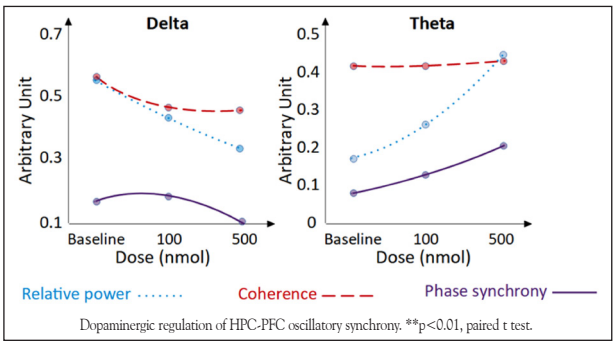

Figure 1. Dopamine dose-dependent effects on HPC-PFC slow oscillations synchrony.

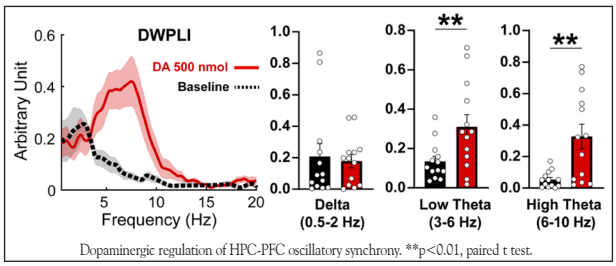

Figure 2. Dopamine induces theta phase synchrony, as shown by the debiased weighted phase lag index (DWPLI). Data shown as mean ± SEM.

ELECTROENCEPHALOGRAPHY ACTIVITY IN MOTOR-STROKE SURVIVORS DURING A TRANSCRANIAL DIRECT CURRENT STIMULATION AND VIRTUAL REALITY-BASED REHABILITATION TREATMENT

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Introduction: Brain-Computer Interfaces (BCIs) based on motor imagery (MI) have been used to aid in the motor rehabilitation of stroke survivors in the last decades. Electroencephalography (EEG) is one of the prefered techniques to perform such procedures, given its portability and high temporal resolution. During the performance of a MI task, an event-related desynchronization (ERD) of the *mu* rhythm – 8 to 12 Hz – in sensorimotor regions of the brain – mainly C3

and C4 – is generally observed [1]. Interestingly, in stroke patients, abnormalities in such responses may occur, but studies in this field are scarce [2]. Thus, the objective of this study was to use EEG to analyze ERDs for *mu* rhythms at ipsilesional sites in stroke survivors during a Virtual Reality (VR)-based rehabilitation program combined with transcranial direct current stimulation (tDCS), and further determine the treatment's evolution from this perspective. **Materials and Methods:** Four stroke survivors (1 female, 60-67 y, 8-26 months post-stroke) performed a 10-session full body VR-based rehabilitation exercise program. Participants also had anodal tDCS (2 mA current, 30 s ramp up/down) applied to their ipsilesional motor cortex (M1) throughout the duration of each session of the treatment (total: 30 min/session). EEG activity was recorded (g.tec's g.USBamp, sample rate of 256 Hz, 16 channels referenced to the mastoids) at three stages of the rehabilitation program: pre and post the 1st, 5th, and last (10th) sessions. The protocol consisted of a hand-MI task divided into eight segments of 8 s each (2 s cue, 6 s MI of right/left hands, 4x each, randomly ordered). During the task, an arrow showed the side of the movement, which should be imagined by the participants. EEG signals were analyzed to compare the power of the *mu* band during the MI task in relation to the resting-state period that preceded it. **Results:** Fig. 1 shows relative changes in *mu* band power for MI (task) compared to rest. **Discussion/Conclusion**: Pre-session analyses revealed that half of the patients (1 and 2) presented a more pronounced *mu* ERD in the last compared to the 1st session (Fig. 1), which might indicate a neuroplasticity effect of the intervention towards recovering motor-related

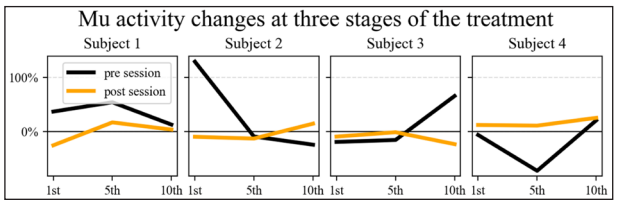

Figure 1. *Mu-rhythm* changes at sensorimotor regions of the ipsilesional side during MI of the contralateral hand.

brain activity [2]. In particular, for subject 4 ERD was more prominent in the 5th pre-session. Post-session data did not show any clear tendency; however, relative power changes indicated stability across sessions. This may be related to a tDCS within-session effect, which increases *mu* power at rest and task periods, minimizing relative changes. However, the inclusion of more subjects is necessary to investigate this issue. Further steps of this study will also be to examine other motor-related frequency bands [1].

References: [1] Pfurtscheller et al. (2005), 5th ed., Ch. 51 – ISBN 0781751268; [2] Tabernig et al. (2016) – doi:10.1088/1742-6596/705/1/012059

EFFECTS OF AEROBIC PHYSICAL EXERCISE IN QUALITY OF LIFE AND SEIZURE WORRY IN TEMPORAL LOBE EPILEPSY

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Introduction and Hypothesis: Aerobic physical exercise (APE) has been showing improvement of quality of life (QoL) and mood disorders in a series of chronic brain diseases [1]. A prior study from our group showed that people with temporal lobe epilepsy (TLE) with an active lifestyle present better QoL, but the influence of APE in the QoL of TLE is still unclear [2]. As drug-resistance is commonly associated with TLE, the possibility of seizures happening during the activities might prevent patients from benefiting from APE. **Objective:** We aimed to analyze the influence of APE in the QoL of TLE patients, also focusing on the seizure worry subitem of the QoL in epilepsy 31 (QOLIE-31) questionnaire. **Materials and Methods:** We selected and reviewed data from 51 individuals with TLE who participated in a 6-month APE program under the supervision of a board-certified physical trainer. TLE patients were divided into training (TLE-training) and control (TLE-control) groups. The TLE-training group went through an APE program of 6 months, while the TLE-control group was oriented to keep their routine. Both groups were assessed by the QOLIE-31 before and after the intervention (APE-time). A mixed-between subjects ANOVA was conducted to assess the impact of the APE intervention compared to TLE-control pre- and post-intervention on the QoL. We set $p<.05$ as statistically significant. **Results:** There was a significant interaction between intervention-groups and APE-time ($p=.0005$), showing that only patients on the TLE-training group presented with higher QoL after the intervention ($p=0.001$). As for seizure worry, we have not found any significant differences between groups ($p=0.50$). **Conclusion:** The APE improved QoL and did not increase the SW, which might be a feature of concern for both patients and healthcare practitioners. Further studies should focus on long term interventions to evaluate the long-term impact of APE in the QoL of TLE patients.

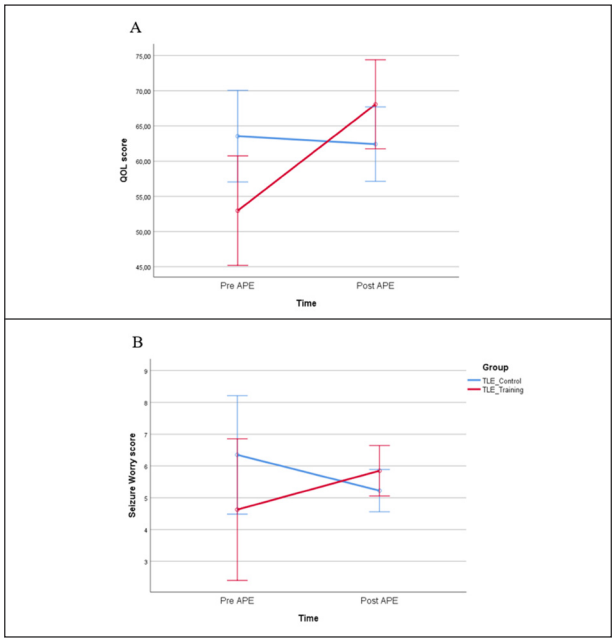

Figure 1. Line graphs showing the interactions and main effects of APE groups on the QoL (A) and seizure worry (B) scores pre and post APE. Error bars represent 95% confidence intervals, TLE: temporal lobe epilepsy, APE: physical aerobic training program.

References: [1] Volpato N et al., doi:10.1371/journal.pone.0181505; [2] Dauwan M et al., doi: https://doi.org/10.1007/s00415-019-09493-9.

EFFECTS OF VIRTUAL REALITY ON COGNITIVE AND PSYCHOLOGICAL CAPACITIES IN OLDER ADULTS: SYSTEMATIC REVIEW WITH META-ANALYSIS

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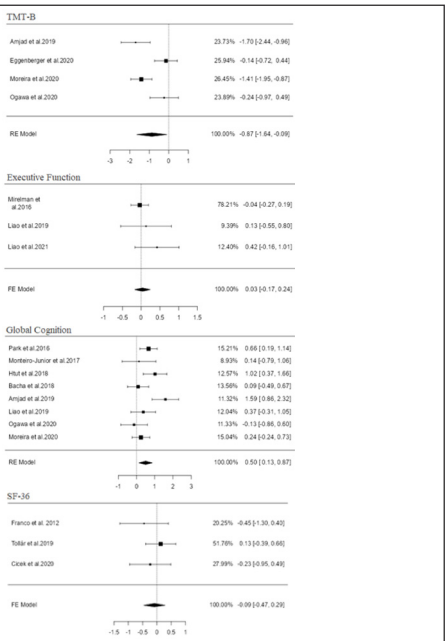
Introduction: Virtual reality (VR) associated with the regular practice of physical exercises (PE) has been shown to be an innovative method in the rehabilitation area, promoting better quality in lifestyle, with several scientific researchers published, highlighting these positive aspects. **Materials and Methods:** Our revision included articles published between 2012 and 2021, published in the electronic databases PubMed, SciELO, Cochrane Library and Web of Science. The search was conducted in English and divided into five parts: aging (aged, elder, older adults, ancient, old-aged); virtual reality (virtual reality, exergames); physical abilities (control, sedentary, stretching, coordination), cognitive abilities (executive function, cognitive function), and psychological abilities (well-being, quality of life, satisfaction). The inclusion criteria adopted were: sample population only of older adults (60 years and over), use of VR (immersive or non-immersive), experimental studies, control group practicing physical exercise or conventional rehabilitation, study investigating physical, cognitive, or psychological abilities. **Results:** We considered 23 articles eligible for this systematic review and 14 were included in the meta-analysis. The age ranged from 60 to 81 years, and the sample size ranged from 12 to 79 participants, divided into control and experimental groups. The interventions were carried out through conventional physical exercises (balance, strength, stretching) and virtual exercises

(wii-sports, walking simulation, cybercycling and games that exercised logic). **Discussion/Conclusion**: In the attention variable, we observed heterogeneity between groups, statistically significant difference between groups, favoring groups that did VR (p=0.028). For executive function, we did not observe heterogeneity between groups, neither difference between groups (p=0.194). For overall cognition, we found heterogeneity between groups (p=0.012) and statistically significant difference between groups, favoring VR (p=0.008). For quality of life (SF-36), we did not find heterogeneity between groups, neither statistically significant difference (p=0.657). The results showed that, when we compared VR with conventional exercises in the cognitive and emotional variables in this population, there are statistically significant differences in the attention and overall cognition of the participants who performed the VR intervention; however, we did not observe a statistically significant difference in the quality of life variable (Figure 1).



Abbreviations - TMT= Trail Making Test; CMD= crude mean differences; SF-36= Medical Outcomes Study 36 – Item Short-Form Health Survey; CI= confidence interval; RE= random effect; FE= fixed effect.

Figure 1. Comparison of virtual reality vs. conventional physical exercises in the cognitive performance and quality of life of older adults.

EKF SLAM-BASED AUTONOMOUS WHEELCHAIR WITH KNOWN AND UNKNOWN LANDMARKS

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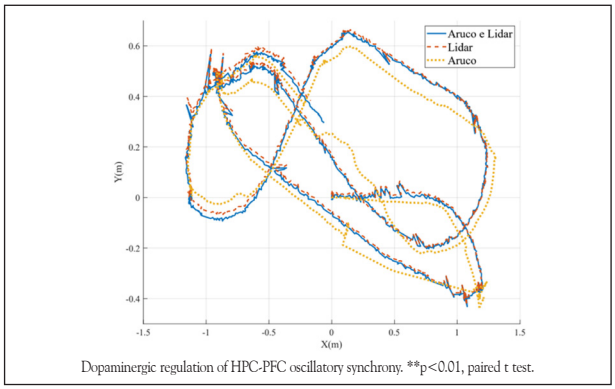
Introduction: Nowadays, more than one billion of people has disabilities, twenty percent of this number are considered to have a severe difficult and is expected to increase even more. In order to create new ways to make their life more comfortable and healthy, research groups are investing in the assistive technology [1]. One study object is the wheelchair, which people with high level of disability has difficulty to be independent. Some studies present control via brain pulses and head detection, however the safety and the comfort during the movement cannot be assured. For this it is proposed a shared control, releasing part of the control to the chair, and example task is the obstacle avoidance. However, to develop this feature, it is necessary to build a wheelchair as an autonomous robot and one important step it is the System of Localization and Mapping (SLAM) [2]. This work purposes the EKF based SLAM to be implemented on a smart wheelchair. **Materials and Methods:** Tests were conducted utilizing a standard robot in the robotics field, the P3DX that has a similar dynamic model to a wheelchair. The robot is equipped with a Lidar 2D (LMS111-10100), a Kinect camera and wheel speed sensors. A simple environment was built, simulating walls and ArUco markers were placed (Figure A). Lidar features are detect via Split and Merge, then the Extend Kalman Filter (EKF) SLAM method is implemented and the ArUco markers are used to help with the position accuracy. The algorithms were implemented in the Robotic Operation System (ROS) which facilitates the transference to the wheelchair. **Results:** The robot is released in the built environment and SLAM is running online. The system has two types of landmark detection, ArUco and Split and Merge, a graph (Figure B) presents the sole contribution of each method and when both methods are used together. It is important to emphasize, that the ArUco method, just has influence on the robot position. **Discussion/Conclusion**: It is possible to conclude that the EKF SLAM has great influence to give the wheelchair/robot the feature of localization and mapping. The Figure B, presents that the ArUco when is used sole, has its precision is decreased, due to the fact that when none marker is being detected, the odometry updates the position, increasing the error. The lidar by itself has a good result, but with the addition of the ArUco detection increases its accuracy, what can help the wheelchair to avoid obstacles and pass through tight spaces.



Dopaminergic regulation of HPC-PFC oscillatory synchrony. **p<0.01, paired t test.

Figure 1. Position throughout the test.

Dopaminergic regulation of HPC-PFC oscillatory synchrony. **p<0.01, paired t test.

Figure 2. P3DX in the Built Environment.

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EMPLOYMENT OF GENERATIVE ADVERSARIAL NETWORKS TO TRAIN AN SSVEP-BASED BCI

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Introduction: Brain-Computer Interface (BCI) allows communication with an external device via brain signals [1]. A paradigm often employed to develop a BCI is the Steady-State Visually Evoked Potential (SSVEP), which relates each command of an application with a specific visual stimulus that flickers at a well-defined frequency. The design of an SSVEP-based BCI involves brain signal acquisition, usually through electroencephalography (EEG), in order to calibrate the system and identify which visual stimulus the subject gazed at. Generative Adversarial Networks (GAN) [2] can be used to generate synthetic EEG-SSVEP signals and reduce the time and discomfort of this preliminary stage.

Materials and Methods: The dataset applied for GAN training consists of 16 EEG acquisitions of 12 s for two visual stimulations flickering at 10 and 12 Hz [3]. Only the signal from the electrode placed at Oz was employed. The signal has been windowed into 2 s and the GAN was trained for 400 epochs. The generator was composed of 6 dense layers (100, 256, 512, 1024, 512) and the discriminator of 7 dense layers (512, 1024, 512, 256, 1). The GAN generates EEG-SSVEP signals with 512 samples. The SSVEP-based BCI was designed using the magnitude of the FFT at 10 and 12 Hz as features and a linear classifier based on least squares [3]. **Results:** Figures 1 and 2 show the Fast Fourier Transform (FFT) of real and synthetic (generated by the GAN) EEG-SSVEP

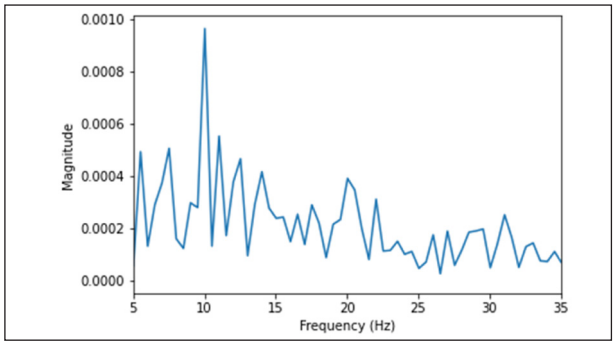

Figure 1. Real EEG-SSVEP at 10 Hz.

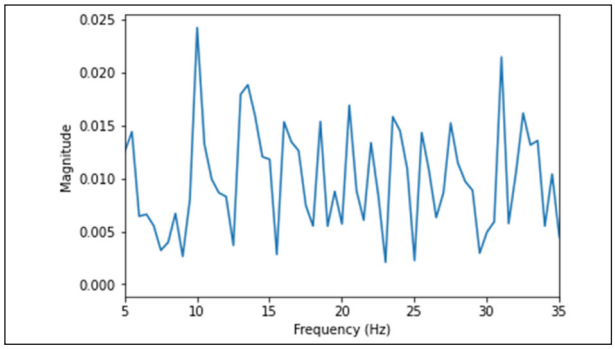

Figure 2. Synthetic EEG-SSVEP signal at 10 Hz.

signals, respectively. In both cases, a major intensity at 10 Hz is noted, although the synthetic signal presents a lower signal-to-noise ratio. Also, normalizations during GAN training generate a difference in the order of magnitude of the FFT. Our evaluation of the effectiveness of the synthetic EEG-SSVEP signals to train a BCI considered two scenarios: (1) using 100% real EEG-SSVEP data to train the BCI system and (2) employing 30% of synthetic data and 70% of real EEG-SSVEP data. Considering 20-cross-fold validation, the average performances for BCI were 84.5% (Scenario 1) and 88.5% (Scenario 2). **Discussion/Conclusion**: Techniques to simplify the training step of a BCI are important to provide feasible systems for disabled users. The application of GANs to train SSVEP-based BCI is a promising approach for this purpose. The EEG-SSVEP signals generated by the GAN in this study proved to be useful in the training process of an SSVEP-based BCI system. More tests with GANs and the use of pre-processing techniques in the synthetic signals could improve the results.

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ENHANCING SSVEP-BASED BCI PERFORMANCE WITH GAN-GENERATED EEG SIGNALS

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Introduction: Steady-State Visually Evoked Potential (SSVEP) is a widely used paradigm to develop Brain-Computer Interface (BCI) systems. The training and calibration stage for designing a BCI requires brain signals, which can take additional time to acquire. To address this issue, Generative Adversarial Networks (GANs) can generate synthetic brain signals to supplement BCI training. This study analyzes the performance of an SSVEP-based BCI trained with a combination of real and GAN-generated electroencephalography (EEG) signals. **Materials and Methods:** This study employed EEG signals [1] of Subject 21 collected from 9 channels: O1, O2, Oz, PO5, PO3, POz, P8, PO7 and P3. We used 6 trials of 5 s each considering 4 visual stimuli (8, 10, 12 and 15 Hz). A 1-s windowing was performed, generating 30 EEG signals for each frequency. The brain signals were filtered by the common average reference filter and features were the FFT magnitude at the 4 stimuli frequencies. A linear classifier based on the least squares method was used with a 20-80% training/testing split. For the GAN model, both the discriminator and generator employed dense layers with the following configuration: Generator: 8 (random noise dimension)2561286436 (ReLU). Discriminator: 36 (generated sample dimension) 1286436 (Softmax). 150 training epochs were conducted at a learning rate of 1e-4. The discriminator was trained on a 10:1 ratio for each epoch. The GAN operated in the feature space. **Results:** Table 1 presents the average accuracy and standard deviation considering 20 independent iterations with different partitions of data, without overlapping the data used to train

Table 1. SSVEP-based BCI performance (%) considering different combinations of real and synthetic EEG signals.

GAN-generated EEG by Frequency	0	2	3	5	7	10	20	24	1000	12	24	1000
Real EEG by Frequency	24	24	24	24	24	24	24	24	24	12	0	0
Average Accuracy Standard deviation	825 (baseline)	855	837	846	807	817	828	799	6612	5310	4815	608

the GAN and the BCI with the data used to evaluate the BCI performance. **Discussion/Conclusion**: The baseline performance of the SSVEP-based BCI was 82% and it increased slightly when synthetic EEG data is introduced to train the BCI. The best accuracy of 85% was obtained with 2 samples/ frequency of GAN-generated EEG signals. However, when 1000 synthetic samples were added to the training set along with 24 real EEG samples, the BCI performance dropped to 66%. Furthermore, when the number of real samples was reduced, the BCI performance was always lower than the baseline. Curiously, using just 1000 synthetic samples to train the BCI could achieve an average accuracy of 60% in discriminating the 4 classes, indicating that the EEG signals generated by GAN contain useful information for training SSVEP-based BCI. Thus, the results suggest that GAN-generated EEG signals can be useful for BCI training, although the introduction of a large number of synthetic samples can negatively impact BCI performance. Further investigations are necessary to determine the optimal balance between real and synthetic samples. **Acknowledgments:** The authors thank CNPq and UFOP for their financial support.

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EVALUATION OF THE SWIMMING AND TREADMILL RUNNING IN THE PREVENTION OF CHRONIC MUSCLE HYPERALGESIA IN C57BL/6 MICE

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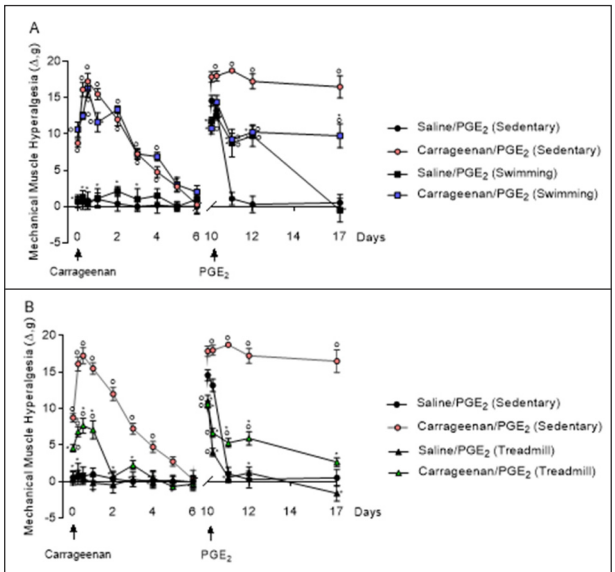
Introduction: Chronic pain is a complex disease. It causes great suffering for many people and has a high socio-economic impact worldwide [4]. Our research group demonstrated that regular swimming exercise prevented chronic muscle pain in Swiss mice [1]. Considering there are inherent differences in mouse strains that affect neuroinflammation [3], the aim of the present study was to evaluate whether swimming and/or treadmill running exercises would prevent chronic muscle pain in C57BL/6 mice. **Material and methods:** C57BL/6 male mice with 4 weeks old, obtained by CEMIB/UNICAMP (5973-1/2022), were used. The model of chronic muscle pain was induced by an injection of Carrageenan (Cg/100μg) in the right gastrocnemius muscle to induce an acute muscle hyperalgesia and 10 days later, in the same place, Prostaglandin E_2 (PGE_2/1μg) was injected at the same site to induce chronic muscle hyperalgesia. The acute and chronic muscle hyperalgesia were quantified by the Randall & Selitto test

(0-17 days). Regular swimming exercise was performed by sessions of 50 minutes, progressively increasing the load and lasting 3 weeks [2]. The treadmill running protocol consisted of 50-minute sessions, progressively increasing speed over the 3 weeks of exercise [5]. Statistical analysis was performed by Two Way ANOVA with Tukey's test, the significance level adopted was $p<0.05$. **Results:** The swimming exercise protocol did not prevent the acute mechanical muscle hyperalgesia but prevented the chronic one when compared to the sedentary group previously sensitized by the inflammatory insults ($p>0.05$, Two Way ANOVA). However, the swimming exercise protocol in the control saline group also induced muscle hyperalgesia in the chronic period when compared to the groups previously sensitized ($p>0.05$, Two Way ANOVA). The treadmill running protocol reduced the acute and chronic muscle hyperalgesia when compared to the sedentary group previously sensitized by the inflammatory insults ($p<0.05$, Two Way ANOVA, post Tukey's test). **Discussion and conclusion:** Our results show that only the treadmill running protocol was an efficient physical exercise protocol for the prevention of chronic muscle pain in C57BL/6 male mice.



A- Analyses of the swimming protocol in mechanical muscle hyperalgesia in C57BL/6 male mice. B- Analyses of the treadmill protocol in mechanical muscle hyperalgesia in C57BL/6 male mice. The symbols "*" indicate difference to sedentary Cg/PGE_2 ($p<0.05$, Two Way ANOVA, post Tukey's test) and "o" indicate difference to sedentary Saline/PGE_2 groups ($p<0.05$, Two Way ANOVA, post Tukey's test).

Figure 1. Analyses of the swimming and treadmill protocol in mechanical muscle hyperalgesia in C57BL/6 male mice.

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EXPLORING THE IMPACT OF ROLE-PLAYING GAMES IN THE CLASSROOM: A PEDAGOGICAL APPROACH PROPOSAL

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Introduction: Motivation is an important aspect of the learning process. To better understand teachers' roles in the academic organization of schools, and to increase student participation, and motivation, we developed a pedagogical approach incorporating some aspects of active learning methods, such as gamification, games, group work, problem-based learning, and flipped classrooms. We tested this approach using a hybrid learning context intervention in a high school History class during the COVID-19 pandemic. **Materials and Methods:** The intervention was divided into three phases: teacher training, teacher support for the preparation of the activities, and application of the pedagogical approach in the classroom context. We used gamification techniques including role-playing games and fictional narratives, where students played characters in a story, and the teacher was the game master. To conduct the learning context, Classcraft was used as a learning management system, Reddit for the forum discussions, Padlet for the posting reflections, Mindmeister for creating collaborative mind maps, Miro for group dynamics, and Kahoot! for the quizzes. After the intervention, a satisfaction questionnaire was applied to evaluate students' opinions on the impact of this pedagogical approach on their motivation and learning. **Results:** Of the 56 participants (age range of 16 to 18 years old), 37 answered the questionnaire; 95% of the students considered that the approach helped them learn the topics covered in the tasks; 90% reported that the approach met their expectations; and 100% had the opinion that the approach was appropriate for the History teaching class. Regarding motivation, 89% felt the approach increased their motivation to study the content, and 86% considered participating in another class using the proposed pedagogical approach. Regarding the use of the platforms adopted, only 13.5% of the students had difficulties in using them. **Discussion/Conclusion**: The acceptance of the pedagogical approach used was high. However, a small fraction of participants encountered difficulties in using the tools, demonstrating the challenge in the widespread use of technology, which is of utmost importance for digital literacy in modern societies. In the post-pandemic context, interactions between teachers and students can be mediated by various technologies, serving as learning tools in a hybrid environment, where part of the learning may take place remotely and the other part face-to-face. Our results suggest that the use of gamification enhanced the learning experience and was highly accepted by participants.

FULLY AUTOMATIC SEGMENTATION OF BRAIN LACUNAS IN EXTRA-TEMPORAL EPILEPSY USING A 3D DEEP LEARNING MODEL

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Introduction: Resective surgery is a common referral for pharmacoresistant focal epilepsies, yet we cannot accurately predict patient outcome. Chang and Englot [1] argue that the gross total volume of resection can help predict seizure freedom, and thus volumetric information about postoperative lacuna is relevant for statistical models. As the manual delineation of lacunae is tedious and time-consuming, we tested the performance of a deep learning model in this segmentation task in a cohort of extratemporal lobe epilepsy patients. **Materials and Methods:** Eighty-four postoperative T1-weighted MR images were retrospectively selected from UNICAMP and Cleveland Clinical Epilepsy Center databases. Five individuals manually segmented the areas of resection using MRIcron. We employed the 3D implementation of the nnU-Net method [2]—a self-configuring neural network based on the U-net architecture—to carry out the automatic volumetric segmentation for which we used a training set of 50 images. To evaluate its performance, we compared lacuna volumes from both approaches and the Dice coefficient (DC) using 34 images. Moreover, a sub-set of 58 images that were segmented by more than one rater was used to estimate the inter-rater DC. **Results:** nnU-Net

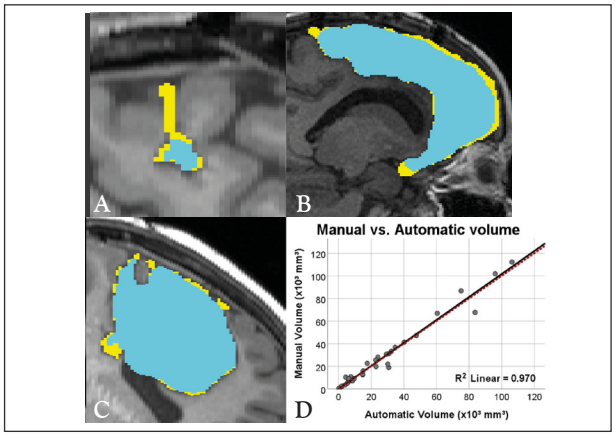

Figure 1. Sagittal view of the (A) worst (0.53), (B) median (0.91), and (C) best (0.96) Dice coefficient cases. Segmentation in yellow corresponds to manual and blue to automatic lacuna identification. (D) Manual vs. automatic lacuna volumetric data. The black line represents the linear fit and the red dashed line represents the y=x reference line.

used five cross-validation folds and 1000 epochs for each fold (default). We found a median DC of 0.90 (Q1-Q3: 0.83-0.94; range: 0.00-0.98) and of 0.91 (Q1-Q3: 0.87-0.93; range: 0.53-0.96) for the cross-validation and the test set, respectively. For images with more than two manual segmentations, we used the highest DC to yield a conservative inter-rater DC and obtained a median of 0.88 (Q1-Q3: 0.78-0.91; range: 0.00-0.97). The linear correlation between the manual and the automatic volumes was $r(32) = .98$ ($p<0.001$). The lowest, median and highest DC are shown in figure 1 along with the plot of the manual against the automatic lacuna volumetric data. **Discussion/Conclusion**: A recent study [3] implemented a 2D U-Net to automatically identify the resective lacuna segmentation in postsurgical temporal epilepsy MRI and obtained a median DC of 0.84 ± 0.08 and 0.74 ± 0.22 (median ± interquartile range) for the cross-validation and test set, respectively. In our study the 3D U-Net implementation from the nnU-Net method was able to segment the brain lacuna with a higher median DC (although using data from extratemporal patients), paving the way for more accurate characterization of brain lacunas.

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FUNCTIONAL STUDIES OF *SCN1A* GENE MUTATIONS ASSOCIATED WITH DRAVET SYNDROME

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Introduction: The *SCN1A* gene has recognized importance in the etiology of monogenic forms of epilepsies, having been linked mainly to Dravet Syndrome (DS) [1,2]SMEI. This gene encodes the α-1 subunit of the voltage-gated sodium channel (Nav1.1). This channel is a multiprotein complex that regulates sodium ion transport [3]. Thus, genetic variants in the *SCN1A* gene can potentially cause changes in the biophysical properties of Nav1.1, which in turn could cause changes in sodium ion flux, leading to neuronal hyperexcitability and epilepsy[4]. However, not all genetic variants have functional consequences; some are only normal genetic variations. Because of that, it may be challenging to establish the genetic diagnosis of patients with DS-associated variants in *SCNA1*. Indeed, the current gold standard for identifying causal variants linked to the phenotype is demonstrating loss of function. **Materials and Methods:** We studied three genetic variants identified in patients with DS investigated in our service for the genetic diagnosis of epilepsy. The wild-type forms of the α subunit, *SCN1A*, and the genes encoding the helper β subunits *SCN1B* and *SCN2B* were cloned by recombinant DNA technology. The mutations c.5177G>A, c.5329delG, and c.5434T>C in the *SCN1A* gene producing the mutant Nav1.1 proteins W1726X, V1777fsX1778, and W1812R, respectively, were obtained by site-directed mutagenesis from the plasmid: pCMV-SCN1A. Subsequently, the wild-type *SCN1A*, *SCN1B*, and *SCN2B* plasmids were cotransfected into the Hek-293T cell line, obtaining a wild-type Hek-293T cell line for the *SCN1A* gene. In addition, pCMV-*SCN1A*-mutants were cotransfected with wild-type *SCN1B* and *SCN2B* plasmids into the Hek-293T cell line, obtaining three mutant cell lines. We then used the Patch Clamp technique to characterize the biophysical properties of the mutant channels expressed in the Hek-293T cell. **Results:** The maximum current density point was: 107 pA/pF with a voltage of -10mV. The maximum inactivation was reached: -57.5 ± 1.4 mV. The recovery time from inactivation was 2.2 ± 1.1 ms. All of these biophysical properties of the wild-type sodium channel found in this investigation are similar to the properties of native human neuronal sodium channels described in other investigations. In the case of the Nav1.1 mutants W1726X, V1777fsX1778, and W1812R, the ionic current density (pA/pF) was significantly decreased to: -30±-1.2; without current; and -42±1.4; respectively. However, the other channel properties were not altered. **Discussion/Conclusion:** We successfully created a stable system to perform biophysical studies of the wild-type and mutant Nav1.1. Furthermore, we observed that in the three variants studied, there was a marked decrease in the current density compared to the wild type, indicating a loss of function of Nav1.1 in varying degrees. Thus, we conclude that, in all three patients, the genetic variants identified in *SCN1A* are related to the DS phenotype presented by the patients.

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HIGH TEMPORAL RESOLUTION DIRECTIONAL CONNECTIVITY FROM THE EEG OF EPILEPSY PATIENTS WITH THE MOTIF SYNCHRONIZATION TECHNIQUE

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Introduction: The evaluation of the interictal electroencephalogram (EEG) is an important step in the diagnosis and treatment of epilepsy, and identifying the epileptogenic focus (EF) is one of the main objectives of this type of analysis [1]. The "gold standard" for this assessment is visual inspection of the EEG by a neurologist looking for epileptiform events. Functional connectivity (FC) techniques have been used in an attempt to aid this assessment and help in understanding the brain dynamics behind these events. Our goal here was to explore the use of high-resolution temporal connectivity, with the motifs synchronization (MS) technique [2], to assert differences between the activity of brain hemispheres, as an indicator of EF laterality. **Materials and Methods:** EEG signals, obtained in EEG-fMRI sessions, from 10 patients with mesial temporal lobe epilepsy (3 with EF on the right, 6 on the left and 1 bilateral) were used. After removing artifacts, the sampling rate of these signals was reduced from 5000 Hz to 250 Hz. MS was used to generate a time-varying graph (TVG) of 80 ms epochs, with 50% overlap. For each epoch, the relative delay between temporal windows (0-12 ms) that generated the highest synchronization rate was chosen, thus defining directional graphs. A threshold of 90% was set for TVG binarization, to remove spurious connections. The hub metric [2] was used to measure the influence of a given node on the TVG. Finally, we compared the hub probability distributions of electrodes in left and right hemispheres to the EF for all subjects. **Results:** Fig. 1 shows the overall in-hubs distribution across all patients (A), which is more similar for both hemispheres than in (B)

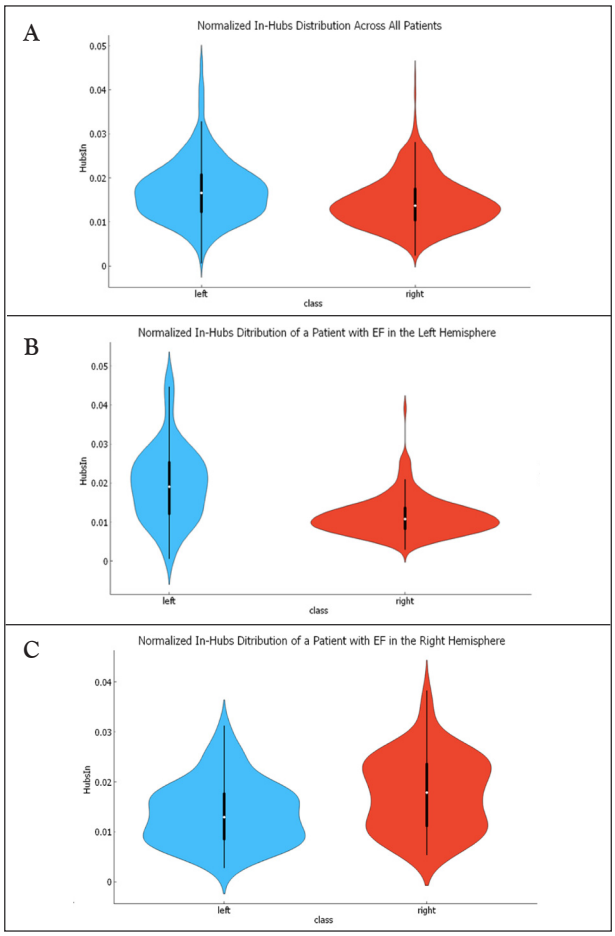

Figure 1. In-hub probability distributions: (A) across all patients; patient with (B) left EF and (C) right EF.

and (C), which are examples of distributions in which the EF corresponds to the hemisphere with the higher in-hub distribution. **Discussion/Conclusion:** Although there was a high variability from subject to subject, the results more often than not (in 7 out of 10 patients) showed higher in-hub distribution values for the hemisphere that corresponded with the EF. From this preliminary result we can infer that high temporal resolution directional connectivity may be used to extract information about EF location. Possibly, by increasing the number of patients in our database, refining our graph analysis and using graph neural networks (GNN) [3] we will be able to make an accurate spatial-temporal location of epileptiform events in the brain. **Acknowledgements:** We thank CAPES and FAPESP (grant 2013/07759-3) for financial support.

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IMPLEMENTATION OF A SELF-KNOWLEDGE DIGITAL JOURNEY TO ASSESS AND PROMOTE MENTAL HEALTH OF THE BRAZILIAN POPULATION IMPACTED BY THE PANDEMIC

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Introduction: The psychosocial impacts of a pandemic can last for several years, requiring large scale interventions to mitigate the psychological effects that are generated at a population level. In Brazil there is a lack of self-guided digital mental health interventions combining validated instruments and psychoeducation. With the current project we aim to evaluate the implementation of a free self-guided digital journey designed for psychological self-assessments and offering mental wellbeing guidance for the Brazilian population. **Materials and Methods:** A web-based self-knowledge digital journey within a mental health platform was developed (https://saudemental.idor.org/), which implemented online self-assessments using standardized mental health, personality, values and belief instruments via the open-access formR survey framework. The digital journey offers personalized feedbacks to users, which aims to increase self-knowledge, self-awareness, and engagement. The technical development process was accompanied by a proof of concept and a think-aloud protocol (n=18) to analyze the proposal suitability, identify areas for improvement and strengths. The interviews were recorded and analyzed thematically using the ATLAS-ti software. **Results:** The mental health platform hosting the digital journey proved capable of engaging an average of 20,000 people per month without extensive publicity. Interviewed users showed interest in the digital journey within the platform and reported strong identification with the customized feedbacks. Areas that were identified as needing improvement were a lack of spontaneity, low cultural diversity in content and features, a need to simplify and focus on practical applications within the feedback as well as need to be less academic and formal in style and content. The highlighted strengths included the use of animations, clear language, perceived reliability of the feedback received by the system, easy accessibility of the interface and the acknowledgement of the importance of the topic. Some of the feedback was immediately fed back into the design process and notable improvements in the accessibility and usability of the platform were noticeable. **Discussion/Conclusion**: The self-knowledge digital journey shows promise as an intervention to be implemented at large scale, being an innovative mental telehealth opportunity for the Brazilian population. Short and focused content, animations, and visual effects to retain attention were important engagement resources identified for the psychoeducational components of the intervention. We discuss the implications and applications of our experience in developing and applying this digital journey for other mental health interventions at scale in Brazil.

INVESTIGATION OF TRANSCRIPTOME ALTERATIONS (RNASEQ) IN NEURONAL POPULATION OF VENTRAL HIPPOCAMPUS IN MICE SUSCEPTIBLE AND RESILIENT TO CHRONIC SOCIAL DEFEAT STRESS

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Introduction: Depression is a common and recurrent mental disorder impairing the daily life and well-being of the individual. Social stress is already well established in the literature as a model of depression and, in this context, we can highlight the chronic social defeat stress model (CSDS). In this model, the social interaction test is used to assess social avoidance, considered a depressive-like behavior. Not all mice submitted to CSDS develop social avoidance behavior and are called resilient. The ventral hippocampus is a well studied structure in the context of depression, mainly because of its neuroplasticity and relation with other limbic system structures. In addition, the ventral hippocampal cell layers play different roles during behavior. Ventral CA1 inactivation increases avoidance of conflict cue behavior, while CA3 inactivation increases approach behavior to conflict cue. Furthermore, neurogenesis confers resilience to chronic stress by inhibiting granular cells from ventral dentate gyrus. Litterature shows different results depending on the specific hippocampal cell layer, making their separation very important. In this study we aimed to investigate the transcriptome alterations in distincts ventral hippocampal cell layers after CSDS. **Materials and Methods:** For the CSDS 12 week old C57/BL6 mice were submitted to 10 minute aggression sessions against 6 month old aggressor retired breeder Swiss mice for 10 consecutive days. One day after the last aggression session we performed the social interaction test and, after 24 hours, mice were euthanized and the brains were collected and fresh-frozen in isopentane. All brains were sliced in a cryostat, flushed with violet cresyl, and microdissected using a laser-capture microdissection apparatus to separate ventral dentate gyrus and ventral CA1 cell layers. **Results:** Following the social interaction test, we could classify 7 animals as resilient to CSDS showing social interaction ratio (SIR) average 3.02, and 11 animals showing social avoidance behavior (susceptible) with SIR average 1,03, in addition to 10 control mice, not submitted to CSDS protocol , with SIR average 2.32. Furthermore, 5 brains of each group were collected, processed, and microdissected, where we separated the ventral dentate gyrus and CA1 cell layers from the hippocampus for further analysis. **Discussion/Conclusion**: The CSDS protocol was successfully performed in the present study, once we could observe and classify the mice as presenting either susceptible or resilient phenotype. This is an ongoing study and the microdissected material from each animal will be submitted to transcriptome analysis of CA1, and Dentate Gyrus cell layers.

INVOLVEMENT OF THE INFLAMMATORY CYTOKINE IL-1β IN THE DEVELOPMENT AND MAINTENANCE OF CHRONIC MUSCLE HYPERALGESIA

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Background and objectives: Recently, our research group demonstrated that there is an increase in muscle concentration of the inflammatory cytokine IL-1β during the acute phase of a mouse model of chronic muscle hyperalgesia [1]. Considering this increase may be related to the development of the chronic phase, we aimed to evaluate whether the local inhibition of the IL-1 receptor during the acute phase of muscle pain would prevent the development and/or the maintenance of chronic muscle pain. **Methodology:** C57BL/6 male mice with 4 weeks old, obtained from CEMIB/UNICAMP (5973-1/2022), were used. Acute muscle hyperalgesia was induced by an injection of Cg (100μg) into the right gastrocnemius muscle and, ten days later, an injection of Prostaglandin E_2 (1μg) was performed at the same site to induce the development of chronic muscle hyperalgesia. The inhibition of the IL-1 receptor was performed by local injection of the IL-1 receptor antagonist (IL-1ra), at the dose of (500ng), 5 minutes before the injection of Cg or 48h later. The muscle hyperalgesia was quantified by the Randall & Selitto test at 10-17 days after Cg. Statistical analysis was performed by ANOVA (One and Two Way) with Tukey's test, Area Under the Curve was used to assess the chronic period of muscle hyperalgesia, the significance level adopted was $p<0.05$. **Discussion and Results:** The administration of IL-1ra (500ng) before and after Cg administration reduced the development and maintenance of chronic muscle hyperalgesia when compared to the Cg group ($p<0.05$, ANOVA, post Tukey´s test). **Conclusion:** The results showed the local blockade of IL-1 receptors during the acute phase of an inflammation modulate the development and maintenance of chronic muscle pain. Together with our previous study [1], we suggest that an inflammatory insult in the muscle induces the release of IL-1β, which is related to the chronic muscle pain. **Figure 1:** Muscle IL-1ra administration reduce the development and maintenance of chronic muscle pain

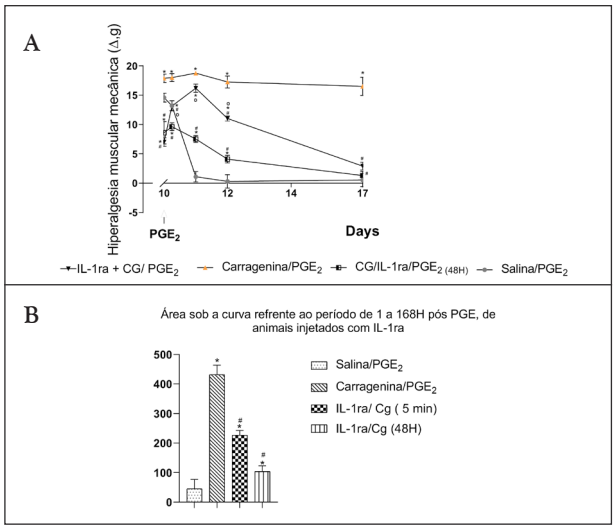

Figure 1. Timeline graph showing both groups that received IL-1ra before and after Cg reduced the development and maintenance of chronic muscle ($p<0.05$, Two Way ANOVA, post Tukey's test) compared to Carrageenan group. B. Area Under the Curve (AUC) graph ($p<0.05$, One Way ANOVA, post Tukey's test). The symbols "*" indicates difference to control saline group, "#" indicate difference to carrageenan group and "¤" indicate difference to IL-1ra (48h) group.

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IORT SYSTEM MODELING FOR ASSISTIVE HOMES

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Introduction: The Internet of Robotic Things (IoRT) is a new paradigm that relates robotics, internet of things, and cloud computing. IoRT provides smarter, more collaborative, heterogeneous, efficient, self-adaptive, context-aware, and even cheaper robotic networks [1],[2]. This work focuses on an IoRT-based smart home for the elderly or people with disabilities and thus achieve a better quality of life to them. Connectivity in this scenario must be divided into physical and psychological aspects. With this, the residency must meet four principles: Strengthen the individual's ability to establish connectivity; Reduce the difficulties and barriers of product and service aspects to establish connectivity; Increase opportunities to establish connectivity; Improve connectivity quality [3]. **Materials and Methods:** Two points to guide the modeling: It is essential that the system be modular, allowing the connection and use of different devices, according to the user's needs. System should be cognitive, with cloud support to run robust processes. **Results:** The modeling is developed in the bottom-up concept, initially structuring the relationship within the domestic environment until reaching the most remote services. It will be applied to manage common smart devices and assistive robots that help the user in their domestic routine. The home is understood as an ecosystem of interconnected devices that share resources with each other. The cloud will be used to work with database, digital twin, cognition, and other robust features. Digitization allows less local maintenance, agile updating of the logical part and better process management. Figure 1 presents the process description in 3 stages: residential, metropolitan network and cloud. In the house, there is the presence of a mediator. It guides local actions with a single command pattern. This allows the elderly or disabled person to have greater autonomy in the environment, as they will not need to learn how each device works, but only learn to use the mediator. **Discussion/Conclusion**: It is expected to provide simultaneously monitoring, services, and assistance. Thus, it will be possible to align health monitoring, domestic services, medical assistance, manipulation, mobility, telepresence, entertainment, monitoring, and telemetry. Regarding the mediator, it will be necessary to deepen the research to understand the limitations of the public, in order to define the best device. In addition to the smartphone, the possibility of using another type of gadget or even a virtual assistant is being studied.

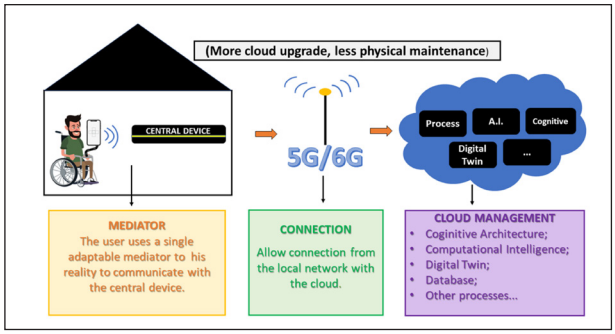

Figure 1. Process description.

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JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS: ROLE OF NR2 SUBUNIT AND CYTOKINES AS BIOMARKERS IN NEUROPSYCHIATRIC LUPUS

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Introduction: Juvenile-onset Systemic lupus erythematosus (SLE) is an autoimmune and inflammatory disease that can affect any organ or system, including the nervous system, referred to as neuropsychiatric SLE (NPSLE) recently revised by the American College of Rheumatology (ACR) [1-4]. While *N*-methyl-D-aspartate receptors (NMDAR) have been implicated as a mediator of neuronal damage in a number of neurologic disorders, cytokines has been long established in the pathogenesis of SLE, and Th1 and Th2 balance determined disease activity [5-8]. Since there is no exclusive biomarker able to predict the development of NPSLE, here we investigated the potential of cytokines and NMDA NR-2 subunit (NR-2) in this role. **Materials and Methods:** This was a cross-sectional study with the participation of 142 childhood-onset SLE patients followed up at the Pediatric Rheumatology Unit of UNICAMP. The study was approved by CEP (CAAE: 26428619.9.0000.5404). Demographic and clinical data were collected with the careful review of medical records. Patients were separated according with the presence or absence of NPM. The concentration of NR-2 and cytokines was determined in serum samples by ELISA and Multiplex, respectively. The SPSS was employed to perform statistical analyses. **Results:** There was no significant differences between the groups in relation to age, sex, race and level of schooling. Patients with MNP had lower age of SLE onset (13 vs. 14 years) and higher SLE duration (10 vs. 8 years). Although serum concentrations of NR-2 were similar between groups, IL-12 and IFN-gamma were significant higher in patients with NPM (Table 1). **Discussion/Conclusion**: There were higher levels of the Th1 cytokines IL-12 and IFN-gamma in the serum of patients with NPM, as seen before [9-11]. Therefore, serum cytokines could possibly be used as biomarkers for the cytokine-mediated inflammation in NPSLE patients.

Table 1. Cytokine and NMDA NR2 serum concentration from SLE patients with or without NPM.

	NPM present	NPM absent	p value
IL-4 (pg/mL)[a]	52.0 (0.6 – 2043.7)	49.2 (0.6 – 2075.5)	0.877
IL-6 (pg/mL)[a]	1.9 (0.1 – 139.2)	2.4 (0.1 – 107.2)	0.594
IL-10 (pg/mL)[a]	10.6 (0.3 – 81.2)	9.4 (0.3 – 110.6)	0.446
IL-12 (p70) (pg/mL)[a]	3.4 (0.1 – 12.5)	2.8 (0.1 – 9.9)	0.024
IFN-gamma (pg/mL)[a]	12.4 (0.2 – 140.7)	9.8 (0.2 – 118.2)	0.010
TNF (pg/mL)[a]	9.1 (0.1 – 43.8)	7.8 (0.1 – 37.9)	0.094
NR2 (ng/mL)[a]	9.2 (4.1 – 33.3)	8.89 (4.1 – 38.8)	0.441

NR2: NMDAR: N-metil-D-aspartate receptor subunit 2; NPM: Neuropsychiatric manifestations a. Medium (minimum – maximum), Mann-Whitney test was employed.

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MICE WITH HYPOFUNCTION OF NMDA RECEPTORS ON INTERNEURONS THAT EXPRESS PARVALBUMIN ARE MORE SUSCEPTIBLE TO STATUS EPILEPTICUS

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Introduction: GABAergic interneurons (IN) expressing parvalbumin (PV+) play a key role in generating gamma oscillations and processing information. These NI undergo a prolonged period of maturation and integration into cortical circuits during adolescence (period of high plasticity) and are critically involved in cognitive development. Its activity involves the participation of NMDA receptors and genetic mutations of these receptors are associated with several neurological development disorders, such as intellectual disability, epilepsy, or schizophrenia. In this sense, our hypothesis is that the dysfunction of NMDA receptors in PV IN increases the susceptibility to *status epilepticus* (SE), depending on the age at which the insult occurs, in addition to favoring the appearance of behavioral and cognitive impairment associated with morphological and functional alterations. **Materials and Methods:** Mice with ablation of the NR1 subunit in IN PV+ ($PV_{cre/+}NR1_{f/+}$) were used in a pilocarpine model. To assess the influence of age, SE was induced in animals 17 days after birth or 8-9 weeks. Morphological changes were followed by analysis of neuronal (NeuN), microglial (lba1) and astrocytic (GFAP) density, in addition to cell injury (Fluoro-Jade C). Data were analyzed according to distribution, and the value of $p < 0.05$ was used as a criterion for statistical significance. **Results:** Our data show that adult PV-Cre/NR1f/f mice are more susceptible to SE, presenting a higher latency ($p < 0,01$) and duration ($p < 0,01$) of the status in relation to the control animals. In these mice, the mortality rate is higher compared to control animals ($p< 0,05$). Among the control animals, only those that presented secondarily generalized seizures showed positive FJC labeling. Regarding $PV_{Cre}/NR1_{f/f}$, even animals with milder seizures (only Racine 4) showed positive FJC labeling. $PV_{Cre}/NR1_{f/f}$ mice show an increase in the number of GFAP+ cells relation to the control ($p = 0,02$), also PV+ cells in CA1 ($p = 0,02$) and dentate gyrus ($p < 0,001$). Regarding juvenile animals, 90% of animals with NMDA hypofunction in IV PV+ showed SE, while only 60% of controls. Status at P17 was also more intense (Racine 4) in these animals compared to controls (Racine 3). It was observed that there is no neuronal loss 48 hours after SE, though there is an increase in the number of GFAP+ cells in CA1 ($p = 0,03$). Our data also suggest that these animals show behavioral and cognitive changes in adulthood. Depressive, anxious, and psychotic behaviors were observed, in addition to impaired memory tasks. **Discussion/Conclusion**: The hypofunction of NMDA receptors in IN PV+ delays the onset of the seizure, however, makes the animals more susceptible, presenting more intense and lasting seizure. Young animals present behavioral changes and cognitive impairment in adult life.

MULTI-OMIC SINGLE-CELL ATLAS OF THE SCLEROTIC HIPPOCAMPUS OF PATIENTS WITH PHARMACORESISTANT MESIAL TEMPORAL LOBE EPILEPSY

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Introduction: Each cell type may be key to understanding whether a disruption in the epigenome landscape can contribute to disease. As such, single-cell studies may provide a unique perspective for investigating complex mechanisms involved in gene regulation in the context of disease. Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE+HS) is the most common type of focal epilepsy in adults and patients with MTLH+HS are frequently pharmacoresistant. MTLE+HS is a complex disorder with polygenic inheritance; thus, epigenetic regulation of gene expression may be involved in the mechanisms underlying the disease, as well in the response to pharmacological treatment, and surgical outcomes of patients with MTLE+HS. In this work, we investigate the genomic regulatory elements and gene expression patterns using multi-omic single-nucleus ATAC-seq and RNA-seq (snATAC and snRNA) analysis to characterize the cell composition of the sclerotic hippocampus of patients with pharmacoresistant MTLE+HS. **Materials and Methods:** We evaluated hippocampal tissue resected by epilepsy surgery from 16 adult patients with MTLE+HS type I. The single-nuclei dissociation from 25-50 mg of hippocampal tissue was performed using the Chromium Nuclei Isolation Kit (10X Genomics). The nuclei were counted, and their membrane integrity was evaluated using an AO/PI fluorescent dye. From the 16 samples, 32 multiome libraries (Single Cell Multiome ATAC + Gene Expression, 10X Genomics) were performed with a target recovery of 3,000-6,000 nuclei. Libraries sequencing was performed on Illumina NextSeq 2000 with a depth of 25,000 and 20,000 read pairs per nucleus for snATAC-seq and snRNA-seq, respectively. The fastq was generated with cellranger-arc mkfastq and the subsequent analysis was performed with cellranger-arc count and aggr. Data were visualized and analyzed using the Loupe Browser. **Results:** This is still ongoing work; and to date, we have been able to dissociate the nuclei efficiently with good recovery (>1,000/μl) and high integrity (>80%). All libraries (16 snATACseq and 16 snRNAseq) were successfully sequenced. We obtained an average and average of 4,700 cells/sample with about 3,700 high-quality ATAC fragments per cell and with an average of more than 1,000 differentially expressed genes (GEX) per sample. After applying quality control filters and aggregating all samples, we identified 75,445 cells with 2,909 average high-quality fragments per cell. Furthermore, we identified 16 clusters of different cell populations, with a median of 1,038 GEX/cell and 96,090 peaks, corresponding to genomic regulatory regions of open chromatin. The identification of different cell types, expression gene patterns, and regulatory elements is underway. **Discussions and Conclusions**: To our knowledge, this is the first study using snATAC-seq and snRNA-seq concomitantly to study MTLE+HS. We obtained high-quality sequencing data from more than 70,000 single-cells and at least 16 different cell sub-populations from the sclerotic hippocampi of patients with pharmacoresistant MTLE+HS. We believe that at completion of this work, we will generate original and important data contributing to a better understanding of the molecular processes leading to MTLE+HS.

NEURONAL AND GLIAL CELL CHANGES IN MESIAL TEMPORAL LOBE EPILEPSY WITH HIPPOCAMPAL SCLEROSIS UNRAVELED BY SINGLE-CELL BASED DIGITAL CYTOMETRY

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Introduction: Mesial Temporal Lobe Epilepsy (MTLE) is a type of pharmacoresistant focal epilepsy in adults, which may benefit from surgical resection of the affected region. MTLE often discloses hippocampal sclerosis (MTLE-HS), which involves neuronal loss and structural and functional alterations in glial cells. Digital cytometry applied to gene expression data (RNA-seq) has been used to infer cell type composition in healthy and diseased tissue - these computational estimates are highly correlated with cell type proportions derived from single-cell nuclei RNA-seq (snRNA-seq) [1] and immunohistochemistry studies [2], indicating that deconvolution algorithms accurately estimate brain tissue composition. Here, we applied digital cytometry based on single-cell signatures to estimate cell type composition of MTLE-HS hippocampus lesions. **Materials and Methods:** Bulk RNA-seq data obtained from public databases were grouped into Dataset 1 (n = 13) [3] and Dataset 2 (n = 16) [4]. Both datasets were uniformly processed with the RNA-seq nf-core pipeline v. 3.8.1 and gene quantification was performed with RSEM using the Gencode v.40 reference. The healthy control dataset with age- and area-matched samples (n=8) was obtained from the BrainSpan cohort [5]. We applied CIBERSORTx to analyze the RNA-seq data combined with reference signatures from major brain cell types derived from snRNA-seq studies [6,7]. **Results:** We found significant neuronal and glial cell changes in MTLE-HS when compared to healthy controls. Specifically, there was marked increase in astrocyte population indicating astrogliosis, which is a hallmark of hippocampal sclerosis. Moreover, neuronal loss found in MTLE-HS was more pronounced in inhibitory rather than excitatory neurons. A more detailed cellular analysis, employing a reference signature containing 24 different cell types derived from a snRNA-seq

study that sequenced 129,909 nuclei of the hippocampal axis [7], confirmed the expansion of astrocytes and decrease of hippocampal interneurons (which are mostly inhibitory [8]), as well as reduction in certain subtypes of pyramidal neurons. **Discussion/Conclusion**: In summary, our analysis of gene expression using digital cytometry revealed that MTLE-HS hippocampal lesions have significant changes in cell type composition compared to healthy tissue. These cellular changes, observed in two separate patient cohorts, involve reductions in both excitatory and inhibitory neurons, with a greater loss of inhibitory neurons, as well as several changes in glial cells. Taken together, these findings show that the MTLE-HS hippocampus has an imbalanced ratio of glial to neuronal cells.

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NEUROPROTECTIVE EFFECT OF ANNEXIN-A1-DERIVED PEPTIDE IN EXPERIMENTAL PARKINSON'S DISEASE INDUCED BY 6-OHDA IN MICE

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder of high incidence in the global population [1]. Recent studies demonstrate that neuroinflammation is a crucial condition of PD [2], prompting the search for more effective treatments with a focus on inflammation. In this context, we highlight annexin A1 (AnxA1) a calcium- and phospholipid-binding protein [3] with pro-resolutive capacity in neurodegenerative and neuroinflammatory conditions [4,5], however, its mechanisms of action have not been explored in PD. Thus, we evaluated the effect of the lack of AnxA1 protein and pharmacological treatment with its mimetic peptide Ac_{2-26} on an experimental mouse model of PD. **Materials and Methods:** Male C57Bl/6 wild-type (WT) and knockout ($AnxA1^{-/-}$) mice were distributed in two groups (n =12/group): PD+Saline and PD+Ac_{2-26}. Both groups underwent stereotactic surgery for intracerebral injection of 1 μL (6 $\mu g/\mu l$) of neurotoxin 6-hidroxydopamine (6-OHDA) into the right striatum (ipsilateral) and 1 μL of vehicle on the left (control; contralateral) [6]. The PD+Ac_{2-26} group was treated with Ac_{2-26} (100 μg/animal) intraperitoneally after the 6-OHDA infusion, followed by 6 more daily doses of peptide; PD+Saline group received sterile saline. Cylinder test was performed to assess sensory-motor function of mice on 0- , 7- and 14-days post-lesion. For the localization of dopaminergic neurons (tyrosine hydroxylase-TH marker) in the substantia nigra (SN), immunohistochemistry was performed. **Results:** Treatment with Ac_{2-26} improves the behavior of PD animals and prevents neuronal loss. In the cylinder test, WT and $AnxA1^{-/-}$ mice treated with Ac_{2-26} showed a marked increase in the proportion of left forepaw uses on days 7 and 14 compared with the saline groups (**$p < 0.01$ and *$p < 0.05$ respectively). To validate these findings, we quantified the number of TH-positive neurons. We observed that induction with 6-OHDA in WT and $AnxA1^{-/-}$ saline animals leads to a reduction in the percentage of TH-positive neurons, when comparing the right with the left sides (****$p < 0.0001$ and ***$p < 0.001$ respectively). In contrast, treatment with Ac_{2-26} prevented a reduction in the loss of TH-positive neurons in WT animals and even in AnxA1-deficient animals. **Discussion/Conclusion**: Our preliminary results indicate an important neuroprotective role of Ac_{2-26} in experimental PD, Thus, treatment with Ac_{2-26} could prevent the development of PD in affected patients. Future analysis will show the mechanisms of action of AnxA1 in modulating neuroinflammation.

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NOVEL HLA ALLELES IDENTIFIED IN BRAZILIAN PATIENTS WITH MESIAL TEMPORAL EPILEPSY AND HIPPOCAMPAL SCLEROSIS

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Introduction: Human Leucocyte Antigen genes (*HLA*), located at chromosome 6p21.3, have been implicated in the susceptibility to more than 100 diseases, especially those related to inflammatory, infectious, and autoimmune mechanisms. It has been demonstrated that inflammation is a biological process present in mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE+HS). Given that, we aim to sequence *HLA* genes in a cohort of well-characterized patients with MTLE+HS. **Materials and Methods:** The HLA region was sequenced in 310 patients with MTLE+HS and 301 healthy individuals from different regions of Brazil, most of whom were born in the southeast region of Brazil. We performed a comprehensive sequencing of 12 *HLA* loci *(-A, -B, -C, -DRB1, -DQB1, -DPB1, -DQA1, -DPA1, -DRB3, -DRB4, -DRB5, -*G) using NGSgo® panels (GenDx) and MiSeq Sequencer (Illumina). Data were analyzed with NGSengine v.2.16.2 software (GenDx) and IPD-IMGT/HLA database 3.38.0 as reference. Statistical analyses were calculated using the R studio. **Results:** We obtained results with eight-digit precision, but we restricted the reporting here to four-digits to compare with published data. Overall, the most common *HLA* alleles identified in Brazilian individuals were: *G*01:01*(0.76), *DPA1*01:03* (0.71), *DPB1*04:01* (0.30), *DRB4*01:01* (0.26), *DQB1*02:01* (0.25), *A*02:01* (0.23), *DQA1*05:01* (0.22), *DPA1*02:01* (0.21), *DQB1*05:01* (0.19), *DQA1*01:01* (0.18), *C*04:01* (0.17), *DQA1*01:02* (0.16), *DQA1*03:01* (0.15), *DPB1*02:01* (0.15), *DPB1*04:02* (0.15), *DRB1*07:01* (0.14), *DQA1*02:01* (0.13), *G*01:04* (0.12), *C*07:01* (0.12), *DQB1*03:01* (0.11), *C*06:02* (0.09), *A*01:01*(0.09), *G*01:03* (0.09), *C*07:02* (0.08), *A*03:01*(0.08), *DRB5*01:01* (0.08), *A*24:02* (0.08), *DQB1*06:02* (0.08), *DRB1*03:01*(0.07), *B*51:01* (0.07). Comparing patients and controls, we found a significant difference in allele frequency for *HLA-DQB1*02:01* (p=0.0222, 95%CI:1.059-1.847, OR:1.396), *HLA-DQB1*05:01* (p=0.0071, 95%CI:1.128-2.142, OR:1.557), and *HLA-DPB1*15:01* (p=0.0379, 95%CI:1.007-80.41, OR:7.123). Furthermore, we identified four new *HLA* alleles in patients with MTLE+HS (*C*07:1027, DQA1*03:03:08, G*01:41, G*01:05:02N*) and five in the control group (*A*02:1069, C*15:253Q, G*01:42, G*01:01:27,* and *G*01:38Q*). These allele names have been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in August 2022 and November 2022 upon our request in compliance with the policy stablished in the most recent Nomenclature Report for *HLA* alleles (Marsh et al. 2010), which states that *HLA* allele names will be assigned to new sequences as they are identified. The list of such new names will be published in the upcoming 2022 WHO Nomenclature Report. **Discussion/Conclusion**: Overall, our results may indicate a potential role for *HLA-DQB1*, and *HLA-DPB1* genes in MTLE+HS in the population studied. In addition, by investigating an ethnically diverse population, we were able to gain additional information about the variability of the human genome in the HLA region, including the identification and reporting of nine novel *HLA* alleles.

Reference: Marsh SGE, Albert ED, Bodmer WF, *et al.* Nomenclature for Factors of the HLA System, 2010. Tissue Antigens (2010) 75 291-455.

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PHOSPHOPROTEOMIC ANALYSIS OF BRAIN TISSUE FROM PATIENTS WITH PHARMACORESISTANT MESIAL TEMPORAL LOBE EPILEPSY

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Introduction: Using large-scale phosphoproteomics, it is possible to identify multiple phosphorylation sites in proteins obtained from normal and disease-related samples at distinct stages. Protein phosphorylation is widely known to regulate several essential functions, including brain development and normal neu-

ronal activity. Mesial temporal lobe epilepsy (MTLE) is responsible for around 40% of all epilepsies in adult patients, many of whom do not respond to treatment with antiseizure medication. Identifying differences in protein phosphorylation may lead to new insights into the underlying disease mechanisms and pharmacoresistance. In this study, we aim to determine the phosphoproteome of brain tissue obtained by surgery of patients with pharmacoresistant MTLE. **Materials and Methods:** We used state-of-the-art mass spectrometry-based proteomics, using a Thermo ScientificTM Orbitrap EclipseTM TribridTM to evaluate the amygdala, hippocampus, and temporal lobe of patients with MTLE divided into two groups: i) less than 20 years of disease duration (n=10), and ii) more than 40 years of disease at the time of epilepsy surgery (n=12). All surgical specimens had confirmed hippocampal sclerosis (HS) by histopathological examination. Data obtained were analyzed using the ProteomeDiscoverer 2.4 and R software. **Results:** Overall, the phosphoproteome was similar in the two groups of patients divided by disease duration. However, there were differences among the three structures analyzed, especially the loss of phosphorylation sites in the hippocampus. Furthermore, hippocampal dephosphorylation was observed in proteins involved in abnormal myelination, neurogenesis, and synaptic signaling in neurons and glial cells. Moreover, dephosphorylation of proteins with a relevant role in neurodegenerative diseases was found in the hippocampus, such as HERC1, TSC2, GAP43, DLG4, VDAC1, and Tau. Also, kinase activity analysis indicates the downregulation of three MAPK kinases of the Ras-Raf-MEK-ERK pathway in the hippocampus. Furthermore, correlation analysis revealed that phosphorylation in specific proteins correlated with disease duration, such as NEFM, CRYAB, ANLN, CNP, and HSPB1. **Discussion/Conclusion**: Although no major differences in the phosphoproteome of samples from patients with different disease duration were detected, our results indicate that subtle post-translational changes, such as phosphorylation in specific proteins, may be involved in the mechanisms leading to the progression of the lesion observed in patients with pharmacoresistant MTLE.

PREDICTION OF COGNITIVE SCORES AND GENDER CLASSIFICATION FROM DYNAMIC FUNCTIONAL CONNECTIVITY DERIVED FROM TASK-BASED MRI

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Introduction: There has been increased interest in understanding the neural substrates of general intelligence that could be used to account for its variability across individuals. Deep learning can be used to predict different cognitive measures, such as general (g) and fluid (gf) intelligence, and classify gender from neuroimaging data [3, 6], providing understanding of which information contributes more for predictions. We use a deep model that joins convolutional and recurrent layers to predict various cognitive values using dynamic functional connectivity (dFC) matrices derived from task-state functional magnetic resonance imaging. **Materials and Methods:** Neuroimaging, cognitive testing, and gender data from 1206 subjects were provided by the Human Connectome Project (HCP) [1]; after applying the filtering process, there was left a final sample of 874 individuals for analysis. We obtained general intelligence values through factor analysis using R's stats package [2]. After pre-processing, we used the average time series extracted by a 360-region parcellation to obtain the dFC matrices, which were constructed using the sliding-window method for Language and Working Memory (WM) tasks, respectively. We used the PyTorch platform and the predictive model [3] was a neural network with the following architecture: three 1D convolutional layers with filters of sizes 4, 8, 16 time windows; one concatenation layer combining features from the convolutional layers; one max-pooling layer, two LSTM (Long-Short Term Memory) network layers and one fully connected layer. In addition, we applied deconvolution layers on 100 random individuals to reconstruct the most important connections for the prediction. We used a 10-fold stratified cross-validation [4] based on cognitive metrics terciles and family identification, because multiple individuals in the dataset are genetically related. We removed the confounding effects of gender, age, laterality, total brain volume and reconstruction algorithm using multiple linear regression. The predictions were made from the task-state dFC matrices. We evaluated the statistical performance of the results with Pearson's correlation coefficient (r) for the prediction of intelligence and accuracy and the area under the ROC curve (AUC) for gender classification. **Results:** We could predict g with performances of $r = 0.486 \pm 0.082$ for Language task and 0.473 ± 0.087 for WM task. For gf, we obtained $r = 0.237 \pm 0.104$ (controlling for confounding variables) and $r = 0.361 \pm 0.087$ (with confounders) for Language task and $r = 0.334 \pm 0.092$ (controlling for confounding variables) and $r = 0.367 \pm 0.054$ (with confounders) for WM task. The performances for gender classification were of AUC = 0.871 and AUC = 0.823 for Language and WM tasks, respectively. With the weights obtained in the deconvolution process, we calculated the time-average and the average value between the deconvolutional layers to evaluate the existence of more relevant regions in the prediction of g and gf. Important FC features are widespread across the brain for both intelligence prediction tasks. When we neglected stratification and confounder removal, we only observed a significant decrease in prediction performance when using Language task data. **Discussion/Conclusion**: Functional MRI data in different task-states can successfully predict cognitive scores and classify gender. We show that task-based dFC has more predictive power than resting-state dFC when compared to the literature (Fan et al., 2020). Furthermore, we observed that removing confounders significantly reduces the prediction performance when using Language task data. The performances obtained using dFC were similar to those reported in the literature using static functional connectivity, when not regressing confounding variables [5]. No specific cortical network showed significant relevance in the prediction of g and gf, suggesting a homogeneous distribution of this complex construct.

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QUANTIFIED T2-MAPS PROVIDE COMPLEMENTARY FINDINGS ON VOXEL-BASED ANALYSIS APPLIED TO EPILEPSY SUB-SYNDROMES

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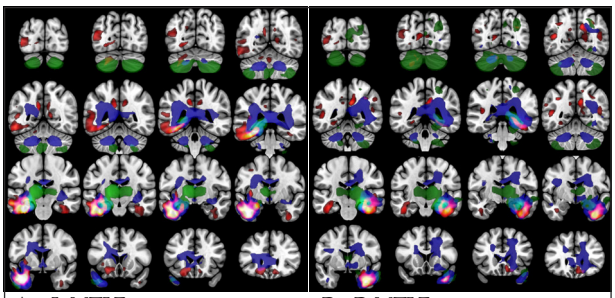
Introduction: This study evaluated patients with left (L) and right (R) mesial temporal lobe epilepsy (MTLE), the most prevalent epilepsy subtype, commonly associated with hippocampal sclerosis. Voxel-based neuroimaging techniques can classify, and improve diseases sub-classifications by localizing regions of alterations regarding a reference sample. Here, we implemented a routine to standardize quantified T2-maps estimated from multi-echo T2-weighted images (WI), evaluating its potential to provide complementary voxel-wise information about the intrinsic structural pattern of alterations of L- and R-MTLE patients.

Materials and Methods: All the 322 subjects included (129 controls; 105 L-MTLE; 79 R-MTLE) were scanned with a 3T MR device and 32 ch. head coil. The imaging protocol was based on a 3D-T1WI (isotropic voxels of 1 mm^3, TR of 7 ms, TE of 3.2 ms, 180 slices, matrix of 240x240) and a coronal T2WI multi-echo image (voxels of 0.41x0.41x3 mm^3, TR of 3300 ms, TEs of 6, 12, 18, 24, and 30 ms, 36 slices, gap of 2 mm, matrix of 176x176). T2-maps were estimated using single exponential fit, voxel-wisely. The T1WI and the T2-maps were both preprocessed using CAT-12 toolbox. The images were corrected for bias-field inhomogeneities, segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid, spatially normalized using the DARTEL algorithm, modulated to account for normalization effects on local



A – L-MTLE B - R-MTLE

Legend: Combined results. In hot scale, T2 analysis; in green, GM alterations; in blue, WM alterations.

Figure 1.

volumes and smoothed. Group differences were accessed using three ANOVA tests for T1-GM, T1-WM and T2 maps ($p<0.05$; FWE corrected). **Results:** All three modalities showed global maxima on the ipsilateral hippocampi or close surrounding tissues. Twenty-one per cent of the altered areas were exclusively revealed by the T2-map analysis for L-MTLE and 11% for R-MTLE. On L-MTLE, the T2 analysis exclusively found alterations on the lateral-posterior regions of the ipsilateral temporal lobe, contralateral hippocampus and bilateral basal frontal lobe. For the R-MTLE group, the T2 analysis showed ipsilateral inferior temporal gyrus and contralateral hippocampus, besides posterior and anterior cingulum. **Discussion/Conclusion**: The preprocessing pipeline has proven stable and capable of segmenting and normalizing the quantified T2 maps. The results suggested that voxel-wise analysis based on quantified T2-maps could identify clinically coherent structural alteration patterns that are complementary to established modalities such as T1WI voxel-based morphometry.

REFRAMING DISORDERS OF CONSCIOUSNESS THROUGH MULTIMODAL NEUROIMAGING

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Introduction: Despite our best medical advances, the mechanisms of the brain dynamics that underlie the induction and maintenance of disorders of consciousness (DoC) remain poorly understood. Differential diagnosis of DoC using behavioral signs of awareness is challenging and unreliable, resulting in a misdiagnosis of approximately 40% [1]. New methods are required to complement bedside diagnosis and provide more information about the patient's underlying brain state. Recent studies have successfully used functional neuroimaging techniques to identify and study areas in the brain with preserved function in patients diagnosed as vegetative. On this basis, these techniques have been used to deepen our understanding of how brain activity and connectivity are affected in DoC and explored as means for detecting and measuring covert conscious awareness in patients [2]-[4]. The aim of this work was to perform a review of the main works assessing DoCs, particularly those using functional neuroimaging. **Methods:** Over 60 articles (published between 2000 and 2022) were chosen and reviewed based on their overall impact, relevance to the field and significance to the advancements in the clinical use of functional neuroimaging in DoC in three main sections: (1) a medical literature review to describe and classify the disorders and identify the current knowledge gaps and clinical challenges surrounding DoC; (2) a review of a variety of neuroimaging techniques and methods of analysis used in clinical trials to fill in these gaps and address these challenges; and lastly (3) a summary of the latest proposed clinical pipelines and clinical trials platforms to approach diagnosis, prognosis and treatment of DoC. **Results:** Reviewed articles report that the high rates of misdiagnose, low neuroprognostic accuracy and scarcity in effective treatment approaches can be attributed to the lack of: (1) clear and reliable behavioral evidences of consciousness in patients with severe DoC; (2) an established and standardized framework that integrates neurobehavioral and multimodal neuroimaging assessments of consciousness levels; and (3) large-scale randomized controlled trials that use functional neuroimaging to optimize and measure the therapeutic effects of different treatment approaches. To address these gaps, this work explored suggestions of a precision medicine approach in which DoC patients undergo functional neuroimaging protocols to identify and assess covert consciousness levels [5], reviewed efforts to establish a golden standard clinical pipeline to identify, manage and treat DoC patients [6] and proposals of a connectome-based clinical trial platform for developing and testing targeted therapies that promote early recovery of consciousness [7]. **Discussion/Conclusion**: In conclusion, rigorous clinical trial designs and international collaborations will be essential for advancing the field of DoC research and treatment. In this work, it was shown that a systematic and personalized neuroimaging-assisted multimodal approach will be key for the improvement of care, development of new therapies and ultimately, for improving the lives of patients with DoC.

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SMARTVEST: ASSISTIVE TECHNOLOGY FOR PERCEPTION AND POSTURAL CORRECTION OF PEOPLE WITH STROKE

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Introduction: People Individuals with functional limitations due to stroke have short- and long-term difficulties in perception and postural control. This study aimed to develop and evaluate the Smartvest assistive technology (AT) for people with hemiparesis and rehabilitation professionals, assisting in postural perception and control. **Materials and Methods**: After interviewing physiotherapists to elicit requirements, we developed the Smartvest AT iteratively and conducted tests with therapists and patients. We evaluated user satisfaction via the Quebec User Evaluation of Satisfaction with Assistive Technology (QUEST 2.0)[1], compared the AT's perception of trunk movement amplitude (flexion/extension, rotation, and lateral flexion) to a kinematic analysis device (Vicon® Gait Plug-in system), and conducted a study on the therapeutic viability of the device in the rehabilitation process. The study involved 40 individuals with hemiparesis resulting from stroke and 20 rehabilitation professionals with at least one year in stroke rehabilitation. Ninety percent of the patients reported they experience difficulties in controlling one side of their body. Patients used Smartvest in a walking circuit that started at the patient's chair and ended at a fridge where they had to serve themselves a glass of water: the task comprised getting up from the chair, walking forwards, backward, and sideways, walking to the fridge, going back the same way, and sitting on the chair. **Results**: Smartvest comprises a vest and a smartphone application (Postural) providing guiding instructions using multimodal feedback: audio messages (e.g., move left) and alerts via vibration and screen color change (blue and red). The therapist initially calibrates the application for the best posture the patient achieves. The app provides messages only after the patient exceeds the tolerance period for incorrect posture. Results from kinematic analysis comparison of trunk range of motion register differences less than 10 degrees. Therapists considered Smartvest a viable resource for the rehabilitation and facilitation goals (100%), also evaluating comfort (65%), ease of use (60%), and effectiveness (45%) for people with stroke. When performing the circuit during rehabilitation sessions, patients with stroke receiving the Smartvest feedback achieved 3.13 times more correct postures with fewer trunk displacement errors (1.75 times). Regarding each participant's performance, the average percentage of correct postures for the total movement of each individual was 19% of correct postures without Smartvest feedback and 26% with Smartvest feedback. **Discussion**: Our investigation demonstrates that Smartvest interprets trunk movements per the baseline and that patients receiving Smartvest feedback perform better regarding correct posture. They executed more movements to achieve the correct posture and maintained the correct posture for longer. This result is significant in rehabilitation aiming proprioception and motor learning. In conclusion Postural motivated users to seek motor solutions (trunk displacements) to receive positive feedback, helping to perceive posture. People with hemiparesis resulting from a stroke have difficulty automating these functions, even during rehabilitation.

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STUDY OF HIPPOCAMPUS-PREFRONTAL CORTEX CONNECTIVITY DURING A WORKING MEMORY TASK IN RATS SUBJECTED TO *STATUS EPILEPTICUS* DURING DEVELOPMENT

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Introduction: *Status Epilepticus* (SE) during childhood is associated with development of epilepsy and cognitive impairments. Experimental studies show that SE during early-life do not present the neuropathological findings associated with temporal lobe epilepsy (TLE). However, they describe behavioral alterations and memory impairments . Understanding how epileptogenic processes occur and compromise cognition throughout development is essential to guide clinical interventions. Therefore, we hypothesized that these deficits result from changes in hippocampal-cortical-thalamic connectivity, specifically in the synchrony of oscillatory field activity involving these neural circuits. In this context, our objective was to investigate the relationship between performance

in a working memory task and the synchrony between the structures of the medial prefrontal cortex, dorsal hippocampus and middle dorsal thalamus of rats that underwent SE during development. **Materials and Methods:** We used 25 Sprague Dawley male rats divided into two experimental groups: the control group (CTRL, N=13) and the experimental group with SE induced by pilocarpine (PILO, N=12) . Animal Use Ethics Committee (CEUA) approved the project, 16/2020 protocol. Rats were submitted to an SE protocol 12 days after birth (P12), starting the experiment at P11 with lithium chloride treatment (LICl, 127 mg/kg, , i.p.), and 18-20 hours later, we administered methylscopolamine (1 mg/kg, s.c.) to all animals. After 30 minutes, saline (0.9% NaCl, s.c.) was injected in the CTRL group, and pilocarpine (80 mg/kg, s.c.) in the PILO group. SE2 was interrupted after 2 hours with diazepam (5 mg/kg, i.p.) administration. After 50 days, we initiated the spatial working memory protocol in a customized T-maze. Each animal was trained to find a reward (a drop of 20% weight/volume sucrose solution), until reaching 70% of correct answers per day for 3 days. Next, the trained animals underwent stereotaxic surgery for the implantation of stereotrodes in the prefrontal cortex (PFC), middle dorsal thalamus (MD), and dorsal hippocampus (DH). After seven days of recovery, rats were re-trained until criteria was once again reached. Once this criterion was achieved, the animals were trained and submitted to the random delay test using intervals of 30, 60, and 90 seconds, with 12 attempts daily, for ten days in the paradigm *delayed non-match-to-sample*. **Results:** Rats submitted to SE presentless correct choices in the T-maze compared to the CTRL group (Two-way repeated measures treatment × delay interaction: $F(1,23) = 11.44$, $p=0.0026$). This t difference is greater in the delays of 60 seconds (Sidak's multiple comparisons *post-hoc*, t-test: $t(69) = 2.95$, $p=0.0129$), and 90 seconds (Sidak's multiple comparisons *post-hoc*, t-test: $t(69)=2.61$; $p=0.0323$). We managed to record high-quality multisite local field potentials throughout the task, which will allow the investigation of the neurophysiological bases of these cognitive deficits potentially linked to impairments of PFC-MD-DH network activity. Analyzes of electrophysiological signals are still in progress. **Conclusion**: In conclusion, we found that seizures during earlier life generates impairments in the working memory, implying cognitive deficits in adulthood. We developed an opportunity to understand how seizures during development alter cognitive processing and lead to comorbidities. We are now analyzing the electrophysiological data to understand the functional role of PFC-MD-DH in the working memory deficits during epileptogenesis.

SUCCESSFUL NEURONAL NUCLEI ISOLATION FROM FLASH-FROZEN BRAIN TISSUE OF PATIENTS WITH MESIAL TEMPORAL LOBE EPILEPSY WITH HIPPOCAMPAL SCLEROSIS

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Introduction: Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE+HS) is one of the most frequent and severe types of epilepsy. HS is characterized by neuronal death and gliosis at the hippocampus and dentate gyrus segments. Currently, there is great appreciation for the diversity of cell composition and the regulatory elements have different dynamic properties in each cell of the body. Every cell may be the key to understanding how a disruption in the epigenome landscape can contribute to disease development. Single-cell omics techniques allow for the analysis at an individual cell level and mapping different cell populations. However, nuclei isolation is especially difficult in surgical tissue presenting cell loss. Also, lipid-rich tissue like the brain may impose additional problems. In this context, we developed an original experimental protocol for nuclei isolation from frozen tissue of patients with pharmacoresistant MTLE+HS treated by epilepsy surgery. **Materials and Methods:** We used three samples of the flash-frozen sclerotic hippocampus: two samples weighing 50mg; and one with 75mg. The protocol followed the steps: (a) mechanical lysis with twenty pumps of each pestle at a 2mL ounce; (b) chemical lysis with incubating the macerated tissue in time lyses tested; (c) mechanical lysis passing eight times the homogenate into a syringe and 23G needle; (d) filter the sample in 40μm and 30μm filter; (e) centrifugation for 5min, at 500g and 4 °C; (f) remove cell clumps and debris with optiprep in 50% and 29% solutions; (g) centrifugation for 30min, at 10.100g and 4 °C; (h) resuspension at a final volume of 25uL and counting the viable and inviable nuclei with trypan blue at Neubauer chamber. Then, we proceeded with one hippocampus at a time and performed three combinations: (i) 50mg of tissue, 500uL of lysis buffer, and five minutes of incubation; (ii) 50mg of tissue, 500uL of lysis buffer, and ten minutes of incubation and (iii) 75mg of tissue, 750uL of lysis buffer and ten minutes of incubation. **Results:** We determined that the best quality and nuclei counting was obtained with the combination of 50mg of tissue, 500uL of lysis buffer, and ten minutes of incubation: 2.9x10e6 nuclei/mL of viable nuclei and 0.35x10e6 nuclei/mL of inviable (89% viability). We also observed the presence of clumps and cell debris; however, this did not impair single-nuclei multi-omic sequencing experiments. In addition, we determined that the isolated neuronal nuclei are suitable for multi-omic single-nucleus Assay for Transposase-Accessible Chromatin using Sequencing (snATAC-Seq) and single-nucleus Sequencing of RNA (snRNA-seq) analysis. **Discussion/ Conclusion**: Although the protocol has a core skeleton established by Maitra et al. 2021 [1], we added modifications that improved the final output. Thus, we obtained a final concentration of 2.900 nuclei/μL that is satisfactory to initiate single-nuclei multi-omic sequencing, in which the minimum required concentration is 160 nuclei/uL. We also obtained excellent quality material regarding membrane nucleus stability and the number of clumps and debris. To our knowledge, this is the first nuclei isolation protocol specially designed and optimized for the surgical tissue of patients with MTLE+HS. We expect that by using this protocol, we and others may generate new information about the underlying mechanisms leading to cell death in MTLE+HS.

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THE USE OF TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) FOR MOTOR FUNCTION REHABILITATION AND GENERAL HEALTH IMPROVEMENTS OF STROKE PATIENTS: AN OBSERVATIONAL STUDY

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Introduction: Transcranial Direct-Current Stimulation (tDCS) is a non-invasive technique used to stimulate the cerebral cortex with low-intensity continuous electrical current elicited by two electrodes that are positioned on the scalp. This technique promotes spontaneous alterations in the cortical activity, classified as either anodic or cathodic, which may result in increased or decreased excitability of the underlying cortex, respectively, and elicit changes in neural activity via depolarization or hyperpolarization of membrane potentials [1]. However, the effects of tDCS as a complementary therapy to the motor recovery and general health improvements of stroke survivors are not fully understood. Therefore, an observational investigation on the therapeutic use of tDCS along with virtual reality (VR), aiming for non-invasive modulation of the brain during therapy, may be necessary. The aim of this study was to explore the tDCS technique, understand its physical and physiological principles, learn how to manipulate it in practice, apply it to stroke patients during their participation in our project involving VR and tDCS daily sessions at the PhINeR Lab, and track participants' motor and general health improvements through observational reports throughout the rehabilitation program. **Materials and Methods:** A Microestim NKL tDCS equipment was used to deliver electrical current of 2 mA (maximum impedance: 40 kΩ) in 4 chronic stroke survivors (61 to 77 years old). Anodic stimulation was performed in positions C3 or C4 (primary motor cortex) in the contralateral side of the subjects' motor lesion, and the therapy had a duration of 30 minutes, occurring concomitantly while participants performed upper and lower limb VR exercises, during 10 sessions carried out over 2 weeks. Different measurements were performed before, after and during the protocol period to compare the effectiveness of the therapy, namely electroencephalography, electromyography, clinical and functional tests. Also, daily reports about the improvements of the participants were recorded in a notebook. **Results:** Through initial observations, we noticed that the level of cognitive difficulty, sedentary lifestyle and psychological, occupational and physiotherapeutic follow-up of the patients had a great influence on their daily session performance during the VR protocol combined with tDCS. Nevertheless, most patients reported some improvement: 2 in coordination and motor strength activities, 1 in balance, 2 in chronic pain (headache and leg pain),

and 1 patient reported improvement in their eyesight. **Discussion/Conclusion**: The stroke survivors included in the current study showed overall motor and general health improvements after therapy with tDCS and VR, although the results indicated that between-subject variability existed. In the literature, the heterogeneity of volunteers can be one of the biggest challenges to achieve more definitive conclusions on the effects of tDCS [2]. Also, the lack of a control group (sham tDCS) in our study limits a complete understanding on the specific effects of tDCS on the findings observed. Therefore, in the future we intend to recruit more patients in an attempt to get a more homogeneous population, and also have a sham tDCS group. This study was also successful in translating the knowledge acquired from previous tDCS studies to set up our own protocol to be used in research and applied settings in stroke patients. Finally, the observed results allow a positive perspective for increments of the research in the future.

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TRANSCRANIAL DIRECT CURRENT STIMULATION COMBINED WITH VIRTUAL REALITY REHABILITATION EXERCISES FOR IMPROVEMENTS IN THE LEVEL OF MUSCLE ACTIVITY AND FUNCTIONALITY IN STROKE SURVIVORS

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Introduction: Abnormal levels of electromyographic (EMG) activity are often reported in the paretic muscles of chronic stroke survivors [1, 2], which seem to result from altered motor unit (MU) pool recruitment following hemispheric stroke and lead to reduced muscle function [2]. However, less is known about the effects of advanced rehabilitation technologies on muscle activity and functionality in this population. The aim of this study was to examine changes in EMG activity and functional performance in chronic stroke survivors throughout a rehabilitation program including virtual reality (VR) exercises combined with transcranial direct current stimulation (tDCS) technologies. **Materials and Methods:** Four stroke survivors (1 female, 60-67 y, 8-26 months post-stroke) performed a 10-session full body rehabilitation program (30 minutes/session) consisting of VR exercises, while also receiving anodal tDCS (2 mA current, 30 s ramp up/down) on the ipsilesional motor cortex (M1). Surface EMG activity (root mean square calculation over a 250 ms period) was recorded from participants' paretic deltoid and rectus femoris muscles during shoulder abduction and flexion, hip flexion and knee extension maximal voluntary isometric contractions (MVICs) before the start of sessions 1, 5 and 10 by a Neuro-EMG-Micro-4 system. In order to also examine the acute influence of tDCS on EMG activity, all participants repeated this same sequence of MVICs two times before the start of session 1 (without and with the use of tDCS, respectively). In addition, timed up and go (TUG) and 10m walk functional tests were assessed at sessions 1 and 10. **Results:** EMG values of sessions 1 (baseline), 5 and 10 are presented in Fig. 1. Mean values indicated overall increases in EMG activity of the paretic muscles throughout the rehabilitation program, but individual data demonstrated that these changes were more prominent for shoulder abduction and flexion for some participants. EMG activity was also greater during shoulder abduction (~14%) and flexion (~5%), and knee extension (~3%) when participants received tDCS compared to not receiving tDCS in the baseline session. Improvements in the time to complete TUG (~4%) and 10m walk (~15%) tests also occurred at the end of the rehabilitation program (session 10) compared to baseline. **Discussion/Conclusion:** Low or altered EMG levels have been previously found in patients with chronic hemiparesis, leading to inefficient force production [2, 3]. Our rehabilitation protocol seemed effective in increasing stroke survivors' overall paretic muscle activity, alongside functional performance. However, the between-session variability found in EMG activity indicates that some alteration in MU recruitment patterns may have persisted in some individuals. Our findings also suggest that tDCS may acutely increase corticospinal excitability, leading to increased EMG activity in most muscles. Our future steps will be to increase the sample size, include a sham tDCS group, and explore associations between EMG, cortical, and performance outcomes in stroke survivors.

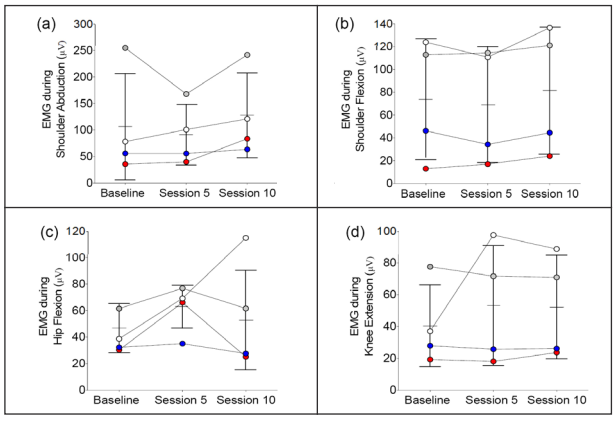

Figure 1. Mean ± SD EMG activity (without tDCS) during shoulder abduction (a) and flexion (b), hip flexion (c) and knee extension (d). Circles and lines represent individual changes between sessions.

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TRANSCRIPTOMIC ANALYSIS OF PERIRHINAL(PER) AND LATERAL ENTORHINAL CORTEX (LEC) OF MALE MICE

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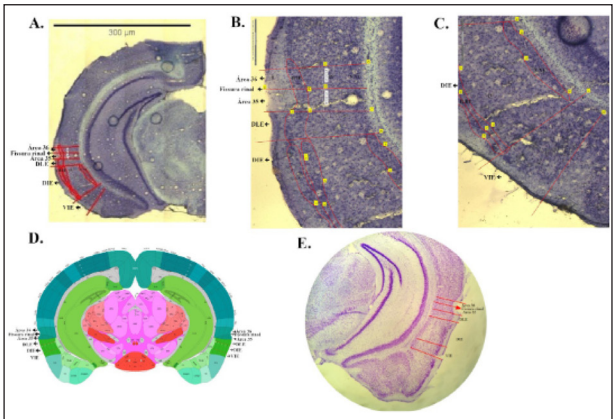
Introduction: The perirhinal cortex (PER) plays an important role in acquiring knowledge about the recognition of memory and identification of novel and familiar objects. PER comprises two horizontal bands areas 35 and 36, that have different cytoarchitectures and connections. The lateral entorhinal cortex (LEC) is important for processing visual and spatial information, receiving direct inputs from PER, and being one of the most major cortical inputs to the hippocampus. The LEC is subdivided into dorsolateral (DLE) and ventral intermediate (VIE) subregions, which have different cytoarchitectures and connections. The PER projects to the LEC which in turn projects to the hippocampus. This study aims to investigate the biological processes and molecular components of 35 and 36 areas of PER, DLE, and VIE of LEC. **Materials and Methods:** We utilized 10 male C57BL/6 mice (CEMIB-UNICAMP), in which five animals were separated for RNA-sequencing, three for further validation and two for reference slice production. The brain tissue will be coronally sliced to preserve



Legend: Images of perfused (histology group), frozen (sequencing group), and atlas (https://atlas.brain-map.org/) referring to bregma -3.28, indicating the regions by black arrows of interest: Areas 36 and 35 constituent of the perirhinal cortex (PER), lateral entorhinal cortex dorsolateral (DLE), intermediate dorsal lateral entorhinal cortex (DIE) and lateral entorhinal cortex intermediate ventral (VIE) in black arrows, and the rhinal fissure in red arrow. A, B, and C. Figures referring to the frozen slide (sequencing group), which were captured by LCM (ZEISS) at 5x. The red lines indicate the boundaries of the regions above and the white dashed lines indicate the width measurements of areas 35 and 36 of the PER. D. Modified figure referring to the online atlas (https://atlas.brain-map.org/) in the region of bregma -3.28, the previously mentioned regions are indicated by black arrows. E. Figure referring to the perfused slice from the validation group, captured by microscopy (LEICA) at 5x. The images show the regions indicated by the black arrows, the rhinal fissure by the red arrow. and the boundaries between these regions by the red lines.

Figure 1.

35/36 areas of PER, DLE, and VIE of LEC structures. Then, the brains were submitted to Laser Capture Microdissection (LCM) (Zeiss) to select 35/35 PER and DLE/ VIE of LEC regions using a surgical microscope (Zeiss). The total RNA will be extracted followed by RNA sequencing and bioinformatic analysis. **Results:** The preliminary results demonstrated the identification of 35/36 areas of PER, DLE, and VIE subregions of LEC, in fixed, frozen slices and mouse brain atlas (https://portal.brain-map.org/) according to the bregma -1.77, -2.38 e -4.04. **Discussion/Conclusion**: Our preliminary results demonstrated the importance of morphology and anatomical location to correctly identify and separate the regions using LCM.

USING DIFFUSE OPTICAL METHODS TO ASSESS CRITICAL CLOSING PRESSURE (CRCP) AT THE BEDSIDE

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Introduction: The human brain is known for its autoregulation mechanisms that maintain cerebral blood flow (CBF) at appropriate values to prevent damage since high CBF can cause intracranial hemorrhage, while poor CBF will result in ischemia. CBF is driven by cerebral perfusion pressure (CPP), which can be clinically assessed as the difference between arterial blood pressure (ABP) and intracranial pressure (ICP), *i.e.*, CPP = ABP – ICP [1]. This relationship is more suitable if ICP is replaced by critical closing pressure (CrCP) since ICP does not take into account the wall tension in the vasculature. Briefly, CrCP is a threshold at which blood pressures below this value lead to brain vessel collapse or, in other words, the cessation of CBF. Thus, assessing CrCP is of clinical interest since it reflects the autoregulation ability of the brain. In this work, we explore the potential of combining Diffuse Optical Spectroscopy (DOS) and Diffuse Correlation Spectroscopy (DCS) to estimate CrCP as previously proposed in the literature [1]. We demonstrate the technique in a healthy population using more accurate and stable optical data analysis models than previous studies. **Materials and Methods:** We acquired five minutes of optical data in the prefrontal region of the cortex on 15 elderly participants (7 males, years old) at rest. DOS data was measured using a commercial system (Imagent, ISS), while DCS information was acquired with a homemade system [2]. Simultaneous ABP measurements were collected in the participant's finger with a commercial system (Finometer, Finapress). For data analysis, both DOS and DCS data were used as input in an optimized layered model [3] that considers tissue heterogeneity to assess the absorption and the effective diffusion coefficients, respectively, from which changes in CBF were derived. CrCP was estimated by comparing CBF and ABP in a Winkessel model [1], similar to previous research. **Results:** In the cohort we acquired data so far, DOS provided median (interquartile range) oxygen saturation (S_tO_2) of and total hemoglobin concentration, which reflects the blood volume, of. The median (interquartile range) ABP measured was . Combining all data measured, we obtained a median (interquartile range) CrCP of. **Discussion/Conclusion**: In this work, we combined two optical techniques, DOS and DCS, to assess CrCP noninvasively. By improving the data analysis model, we achieved more accurate physiological information. The method was demonstrated in healthy participants. In the future, we plan to expand the sampled cohort to create a database that can quantitatively assess loss of autoregulation after brain injury, as optical techniques can determine physiological parameters noninvasively.

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USING IN-SILICO METHODS TO EVALUATE MUTATION PATHOGENICITY IN GEFS+

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Introduction: $Na_V1.1$ channel is an integral membrane protein that mediates the voltage-dependent sodium ion permeability of excitable membranes, thus initiating and transmitting electrical impulses in the central nervous system. Nav1.1 is encoded by the *SCN1A* gene, which is linked to several forms of genetic epilepsy. Protein function is closely related to its structure and dynamics. Investigating the relationship between protein structure, dynamics and function has led to many developments in medicinal chemistry. Here, we present a multiscale approach to investigate several *SCN1A*-related variants identified in patients with a monogenic form of epilepsy, genetic epilepsy with febrile seizures plus (GEFS+). We aim to determine how these variants affect channel structure and dynamics, which in turn helps to understand how genetic variants lead to the functional impairment of the $Na_V1.1$ channel. **Materials and Methods:** So far, we have investigated five variants, R101W, R393H, R946C, R1639C, and K1654R, which have been identified in patients with GEFS+. $Na_V1.1$ model was generated using MODELLER 10.2. Residue conservations were computed using the ConSurf server. Essential site scanning analysis was carried out on ProDy 2.2.0. Protein dynamical network analysis was conducted on Bio3D 2.4.3. **Results:** In terms of structural properties stabilizing the protein, we found that the residue R954 forms a salt bridge to glutamic acid E951 present at the channel selectivity filter to Na^+ ions (DEKA ring), possibly playing a role in the selectivity for Na^+ ions during the cooperation between the selectivity DEKA and the vestibular EEDD rings. In addition, the residue R1639 is located in the S4 helix of domain DIV, while the K1654 is positioned at the end of the same helix, which is likely essential to the channel function. Except for K1654R, all mutations investigated disrupt salt bridges formed in the wild-type structure. The PD helices are largely conserved along the structures, as well as helices S3 and mainly S4 from VSD subdomains. Interestingly, all mutations studied in the $Na_V1.1$ are located in very conserved positions. Residues R393 and R954 were identified as essential residues to $Na_V1.1$ dynamics, with most of the affected residues above the top quartile threshold. These residues are inserted in a cluster of residues at the top of the pore, most involved in the selective filtering of Na^+ ions. Residues R101, R1639, and K1654 also presented influences in their neighboring regions, but to a smaller degree. It is remarkable, however, that the residues that most influence the global dynamics of $Na_V1.1$ are those in the selective filter of Na^+ ions and the intracellular N- and C-terminal portions. **Discussion/Conclusion**: We investigated five mutations of the $Na_V1.1$ channel that occur in highly conserved positions. All mutations involve a substitution that changes the net side-chain charge (except for K1654R). The residue R946 forms a salt bridge with the E951, an essential residue to the $Na_V1.1$ function (Na^+ ion selectivity). Moreover, the mutation R1639C removes a positive charge of the voltage-sensing domain IV helix S4. This helix acts as a sensor of the membrane potential variation, both in activation and inactivation of the channel. Taken together, these data indicate the deleterious potential of the observed mutations. We presented an efficient structure-based approach that effectively detects straightforwardly functional relevance of residues of biological interest. By collecting information on the structural changes caused by the mutations and understanding the essential dynamics of the $Na_V1.1$ channel, we could predict the deleterious effects of the mutations observed in the clinical study. In addition, we acquired novel information regarding the relationship between structural and functional aspects of the $Na_V1.1$ channel; thus, contributing to unraveling the mechanisms involved in monogenic epilepsies.

VOLUMETRIC CORPUS CALLOSUM SEGMENTATION INTEGRATED TO INCCSIGHT SOFTWARE FOR SUPPORTING DTI-BASED STUDIES

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Introduction: This study presents an extension of the inCCsight software to allow automated volumetric segmentation of the corpus callosum (CC) based on diffusion tensor imaging (DTI), a technique often used in neuroimaging studies. CC volumetric segmentation is essential for many analyzes of brain connectivity, but most available software does not provide this functionality for DTI images. The method proposed in this study presents a solution to this problem, providing users with the ability to segment the CC automatically, in addition to incorporating custom installments and integrating other segmentation and/or installment methods. The experimental results demonstrate that the proposed method is effective and accurate for segmenting the CC in DTI images, making it a valuable tool for brain connectivity analysis. **Materials and Methods:** InCCsight is a software that provides an automated DTI-based analysis pipeline for the Corpus Callosum, comprising: diffusion maps computation; midsagital slice selection; CC segmentation by two distinct methods; automated Quality Control (QC) of segmentation results; CC parcellation by five different methods;

and results visualization on a rich, comprehensive dashboard, including several tables and plots. Magnetic resonance images of subjects acquired at the Hospital das Clínicas (Unicamp) were used. For each subject, diffusion-weighted images (DWI) obtained using a 3T scanner were available. The data is anonymized, making direct identification impossible. All data is pre-processed using FSL for correction, registration of DWI volumes, calculation of the diffusion image (DTI) and its eigenvalues and eigenvectors for conversion to Nifti format. **Results:** The new version of inCCsight can be downloaded and installed in any operational system (Linux, Windows and macOS). The interface is user friendly and no longer requires the use of command line to choose input files. It is possible to use the "Previous study" button to repeat the last process carried out, avoiding a new load and execution time, as the tool stores the data of the last subjects rotated and can display them without processing them again. With the insertion of a volumetric method, a way of viewing the resulting 3D data was inserted, as it differs from the remaining methods. In this way, the interface has the possibility to change between the views for 2D Midsagittal Section Segmentation and 3D Volumetric Section Segmentation. As a result, the user can select any subject to visualize the rendered 3D segmentation and the mean FA, MD, AD and RD values inside the structure. **Discussion/Conclusion**: This work presents the first available DTI-based volumetric CC segmentation method incorporated into an open-source software. The software also encompasses the possibility of incorporating new segmentation and parcellation methods by the user at the click of a button, supporting collaborative and reproducible research. The main pendencies and problems of the previous version were solved, in addition to new features being included in the tool. The improvements in the interface provide the user with great ease of use and that requires low technical knowledge both to handle, as well as for maintenance or improvements. The project is still under development, available at: https://github.com/MICLab-Unicamp/inCCsight.

VOLUMETRIC SEGMENTATION OF THE CORPUS CALLOSUM: COMPARING 2D, EXTENDED-2D, AND 3D APPROACHES TO TRAIN THE MODEL ON ANISOMETRIC DIFFUSION MRI

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Introduction: Diffusion tensor imaging (DTI) is a technique that provides relevant information about structures with a high concentration of neural fibers, such as the Corpus Callosum (CC) [1]. Studying the CC on DTI requires its segmentation as a preprocessing step, but most automated methods and tools perform segmentation of this structure using T1-weighted images [2]. For this reason, we propose the segmentation of the CC directly on DTI, and to provide a robust method, we studied possible data pre-processing such as voxel resizing (through interpolation) and image cropping to remove the excess background (regions with zeros). We also explored the different possibilities for the CNN input channels - 2D, extended-2D, or 3D - in order to improve the performance of the CC segmentation model. **Materials and Methods:** The dataset contains T1 and DWI acquisitions from 122 subjects, split into training (57 subjects), validation (15 subjects), and testing (50 subjects). Segmentation masks of the training, validation, and test datasets were obtained manually delineated by an expert. The training was performed using Fractional Anisotropy (FA) as input channels. We trained 3D e 2D versions of a U-Net, without making any changes on voxel size and no image cropping. And we trained the 2D U-Net version using neighboring slices in a multi-channel approach (extended-2D). The evaluation metric used was the Dice Similarity Coefficient (Dice). **Results:** Fixed parameters for the three models include the ADAM optimizer with Dice loss, 500 for the number of epochs, and a learning rate of 0.0001. The patch size used by both 2D U-Net models was 128x56 in the sagittal view and a batch size of 32; for the 3D U-Net, the patch size was 112x160x56 and a batch size of 2. Evaluation of the models was done through volumetric Dice in the testing dataset. The best model was the 3D U-Net, with a mean Dice of 85,21%, while the 2D U-Net obtained a Dice of 84,07%, and the extended-2D, a Dice of 84,56%. **Discussion/Conclusion**: The 3D model trained with the anisometric and without crop data (Fig.1, left) obtained better results than the previously proposed model [3], also 3D on FA map, but trained with isometric and cropped data; a learning rate of 0.001 and a patch size of 88x64x88, obtaining a mean Dice of 83,34% in the same test set, but with data also isometric. This result suggests that changing the data is not a good practice because the voxel interpolation introduces noise in the image and can harm the model training. Furthermore, the comparison between models trained with different approaches (3D, 2D, and extended-2D) shows that volumetric information used by the 3D convolutions improved the results, as expected. However, the extended-2D approach (Fig.1, center) captures better the shape of the CC than the 2D (Fig.1, right) since it uses the neighborhood information. Despite the superiority of the 3D model, the computational costs of each model should be taken into consideration.

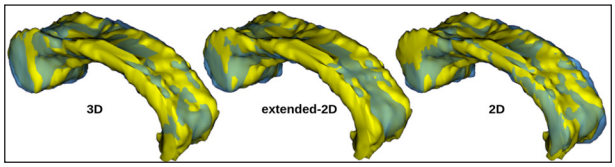

Figure 1. Volumetric results superimposed: manual mask (yellow) and model output (blue).

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ASSESSMENT OF THE FOCUS OF ATTENTION IN SPATIALIZED SOUND STIMULI USING P300

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Introduction and Hypothesis: According to [1], lack of attention is one of the leading causes of school failure among children. This problem may manifest in several diseases, including Central Auditory Processing Disorder (CAPD). According to [2], CAPD is best defined as a central processing disorder caused by deficits in rapid temporal processing and in the accurate representation of auditory space. This deficit may result in an inability to recognize binaural cues used by the CANS to separate target sounds during the listening process. A diagnosis of CAPD in children over six years can be obtained with the Listening in Spatialized Noise-Sentences (LiSN-S) test [3]. However, this test has the disadvantage that it requires a collection of equalized target sentences in the native language of the patient and is also susceptible to errors caused by the test operator. Human brain activity can be observed through electrophysiological procedures, such as Auditory Evoked Potentials (AEPs) [4-5]. The Long Latency Auditory Evoked Potentials (P300) are performed by focusing the individual's attention on stimuli [6]. We hypothesize that by paying attention to binaural stimuli that differ only in their perceived position, the P300 response will also be observable. If this hypothesis is confirmed, a second hypothesis will be tested that in patients with CAPD, these stimuli will not elicit a P300 response. **Objective:** This research aims to evaluate the possibility of identifying a central auditory processing deficit by electrophysiological procedures.

These are the specific objectives of the project:

- Evaluate neural activities when using a traditional sound stimulus.
- Evaluate neural activities when using a sound stimulus with spatial cues.
- Characterize abnormalities in the analysis of the P300 in individuals with disability in recognizing binaural cues.

Materials and Methods: The electroencephalogram (EEG) equipment will be OpenBCI Cyton. The evaluation will be done through the standard procedure for performing the P300 for auditory stimuli, using the 10-20 System [7] for electrode placement. 300 frequent (80%) and rare (20%) syllable stimuli will be randomly presented. The evaluation will initially be performed with the traditional P300, and subsequently, the stimuli will be altered, including spatial positioning, and will be conducted using binaural synthesis [8]. **Relevance:** With the method proposed by this research, it is expected that patients with Central Auditory Processing Disorder (CAPD) can have an early and reliable diagnosis. This evaluation will allow for starting treatment early and obtaining higher success rates.

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CROSS-DATASET MOTOR IMAGERY CLASSIFICATION WITH DEEP LEARNING AND RIEMANNIAN GEOMETRY

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Introduction and Hypothesis: Brain-Computer Interfaces (BCIs) are systems that allow the association of brain signal activity with external actions through the use of, usually, electroencephalography (EEG) to capture such activity and computational methods to process the data. In the context of device control, a classifier, based on a trial (a portion of the whole signal) extracted from some subject, is required to assign it a class before triggering an actuator. The registered signals are affected by noise and artifacts due to muscular, ocular, and other activities, limiting the use of traditional classification techniques. Current state-of-the-art approaches based on EEG use Deep Riemannian Networks (DRN), which combines Riemannian Geometry (RG) with Deep Neural Networks (DNN). However, these appear to under-utilize the full capabilities of the RG properties. We propose a DRN architecture, in which EEG signals are treated as trajectories over the manifold rather than single points, such that the progress of the signal (verified by a sliding window) and its functional connectivity have higher relevance. **Objective:** The main objective is to propose a novel DRN architecture that uses trajectory knowledge obtained from the EEG signals in the Riemannian Manifold. With a focus on the Motor Imagery paradigm and a cross-dataset perspective, the additional information provided by the trajectories should improve efficiency and reduce the amount of data necessary for calibration. **Materials and Methods:** This work shall use open-source EEG datasets, which can be easily obtained using python libraries, such as MOABB, pyRiemann and MNE, that also allows direct integration in the code. To obtain the trajectories, we intend to split each EEG trial into smaller segments, then obtain their associated covariance matrices. The geodesic of one segment to the next constructs the trajectory. The proposed architecture is shown in Figure 1, layer by layer, with temporal and spatial convolutions, construction of the trajectory and respective operations in the Riemannian manifold using, initially, the SPDNet.

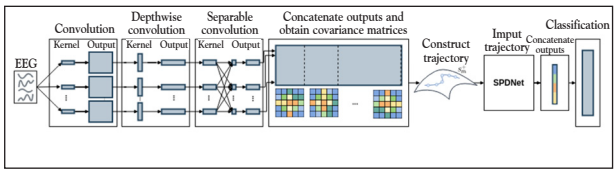

Figure 1. Each layer of the proposed novel DRN architecture.

Relevance: BCI systems have been used in a diverse set of applications, and many of those are in the medical area. Motor imagery, in particular, has been applied successfully to rehabilitation post-stroke. Therefore, building classifiers that have higher accuracy and are more robust to noise will enable even more relevant systems. Furthermore, the novelty of using trajectories in RG should bring together a deeper understanding of EEG signals and of the combination of RG and DNN.

DEEPCTSEG: A DEEP LEARNING TOOL FOR BRAIN COMPUTED TOMOGRAPHY (CT) SEGMENTATION

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Introduction and Hypothesis: Computed tomography (CT) is an essential non-invasive diagnostic imaging modality for evaluating the central nervous system. It is widely available world-wide, and it is the most common neuroimaging exam in Brazilian Unified Health System (SUS). Volumetric metrics derived from head CT scans can serve as an important proxy for the evaluation of brain changes and neurodegenerative diseases. CTseg (https://github.com/WCHN/CTseg) is an open-source tool based on the Statistical Parametric Mapping toolbox (SPM) that enables quantitative evaluation of brain parenchyma volume. Nonetheless, CTseg segmentation is computationally costly and time-consuming due to the spatial normalization procedure and has several program dependencies, hindering reproducibility and large-scale clinical implementation. **Objective:** To develop "DeepCTseg", a deep learning model for brain parenchyma segmentation based on head CT exams. **Materials and Methods:** Head CT data from a wide age range of healthy individuals will be segmented initially using a Docker Image of CTseg. This will yield masks containing gray matter, white matter, cerebrospinal fluid, soft tissue, and bone. These masks will be binarized and inspected visually for quality control. All the images will be rescaled to the model's input dimension and aligned to the LAS standard (256 x 256 x 256). In order to highlight the regions of interest, the original images will be windowed between -15 and 100 HU. To build up our model the multi-task 3D segmentation model, we will use a modified U-Net and VParNet architecture. Following the neuroradiologist evaluation, the model will be retrained to improve performance. The segmentation's accuracy will be measured using the Dice coefficients, Jaccard index, intersection over union, and Hausdorff distance. As a second step, we intend to refine our model so that it can accurately segment pathological cases, including brain atrophy and hydrocephalus. **Relevance:** Due to its widespread clinical application, we intend to develop a rapid and accurate method for brain parenchyma segmentation in head CT scans. In the future, the volume metrics extracted from these images may serve as a biomarker for large-scale screening of pathological cases, as well as aid the specialist physician to detect and monitor neurodegenerative patients.

INFLUENCE OF THE OFFSPRING SEX ON THE ENERGETIC BALANCE AND NEURONS IMMUNOREACTIVE TO THE MELANIN-CONCENTRATING HORMONE IN THE MEDIAL PREOPTIC AREA OF LACTATING RATS

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Introduction: Melanin-concentrating hormone (MCH) is a neuropeptide that regulates various motivated behaviors [1]. The ventromedial medial preoptic area (vmMPOA) is crucial in regulating maternal behavior and contains MCH-immunoreactive (-ir) neurons during lactation [2]. The number of MCH-ir neurons in the vmMPOA is proportional to the number of offspring [3]. As male and female pups require different maternal behaviors [4], we hypothesize that the sex of the litter quantitatively influences the number of MCH-ir neurons in the MPOA. Additionally, since prolactin and MCH levels are related [5], we aim to quantify prolactin levels in maternal plasma. **Materials and Methods:** We used 40 Long-Evans rats, 30 females, and 10 males, divided into five groups with six rats per group (n = 6 per experimental group). We employed the harem method to obtain the primiparous rats (three females and one male). The litter was standardized in postpartum day 1 (PPD1) with 10 pups with different amounts of each sex. We obtained primiparous rats using the harem method, and standardized the litter on postpartum day 1 (PPD1) with ten pups of varying sex ratios. The experimental groups include: primiparous lactating females with 10 male offspring per mother (GPM); primiparous lactating females with 10 female offspring per mother (GPF); primiparous lactating females with 5 male and 5 female pups per mother (GPC); primiparous lactating females with 7 male and 3 female offspring per mother (GPMM); and primiparous lactating females with 7 female and 3 male offspring per mother (GPMF). We monitored the feeding and drinking patterns of primiparous rats throughout lactation. On the 19th day of lactation, we performed euthanasia by transcardiac perfusion using formaldehyde with borax. The brains were collected, sectioned (40 μm) using a freezing microtome, and stored in an anti-freeze solution. Blood samples were collected to quantify plasma prolactin levels. We performed immunohistochemistry using the peroxidase method to quantify MCH-ir neurons in the MPOA, marked using Neurolucida® software. The number of neurons was standardized based on cytoarchitectonic references of the area counted. Statistical analysis was conducted with a significance level set at $p < 0.05$. **Preliminary results:** Blood and brain samples have been collected and some have already been stained and counted using microtomy and immunohistochemistry. Water and feed intake data have been analyzed but N values are incomplete (Figure 1). Our study provides significant insights into the mechanism of MCH in maternal behavior.

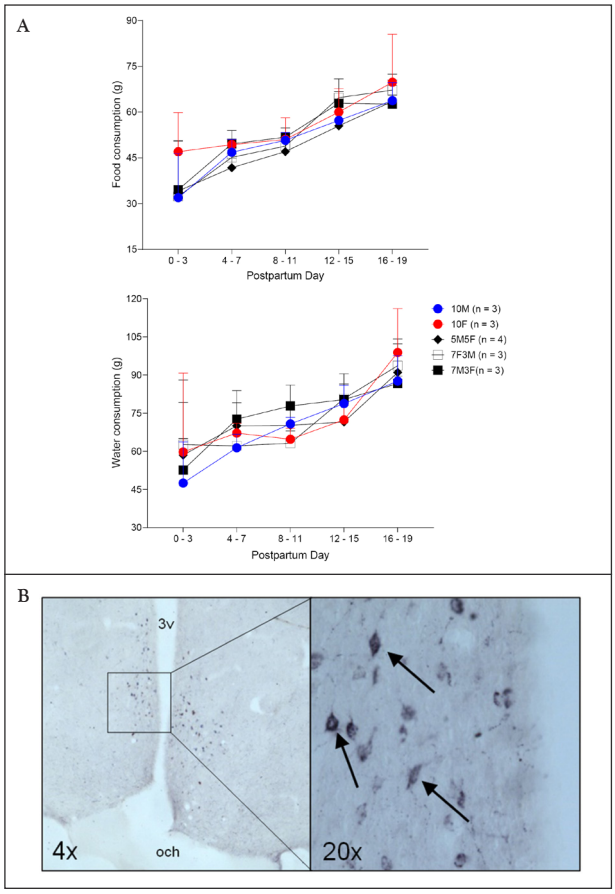

Figure 1. A – Food and water consumption during the lactation period. There has been no statistical difference between the groups so far; data as mean and standard deviation. B – Coronal section of the MPOA highlighting MCH-ir neurons (arrow); och = Optic chiasm; 3V = Third ventricle.

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EMPATHY AND AFFECTIVE MODULATION OF INTENTIONAL BINDING: DOES EMOTIONAL VALENCE MATTER FOR SENSE OF AGENCY?

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Introduction and Hypothesis: Sense of agency (SoA) is the feeling evoked when one's voluntary actions produce external consequences. It is evidenced by intentional binding – the subjective contraction of the time interval between an action and its consequence [1]. This effect can be measured by temporal binding paradigm tasks, in which observers must judge the time interval between either a voluntary action or an externally caused event and provoked external consequences [2]. As the outcome component of SoA often refers to a socially driven sensory-motor response, one might expect socioaffective stimuli to be important to the manifestation of the described phenomena. However, besides some experimental studies have shown that this effect might be modulated by the emotional valence of the consequence, some other recent findings disagree on whether or not emotionally positive consequences after an action lead to stronger temporal binding when compared to negative ones [3, 4]. This discrepancy in the literature might be due to an excessively subjective experimental task as well as covariates omitted from the protocols, that neglect socio-affective aspects such as empathy. Also, there is a lack of neuroimaging findings to improve our understanding of cortical signatures of SoA and the neural apparatus involved in temporal binding effects. Therefore, alternative methodologies and stimuli are welcome to shed some light on the aforementioned ambiguities currently present in the literature [5]. **Objective:** This study aims to clarify the influence of emotional valence on SoA, as evidenced by temporal binding, as well as its correlations with the individual trait of empathy and the cortical representation of valence. **Materials and Methods:** Our proposal is to adapt former experimental protocols that verified emotional modulation over SoA and intentional binding [3], introducing a two-interval forced choice (2IFC) method [6]. Moreover, we will investigate the correlation of the strength of the modulation by valence with the individual trait of empathy, measured through Interpersonal Reactivity Index (IRI) and with the score of a valence classifier trained on cortical activation as measured through functional near-infrared spectroscopy (fNIRS). Also, we intend to refine our data by using scores of depression and anxiety traits, obtained by the DASS-21 questionnaire, as another co-variable alongside the empathy trait measurements, looking for better goodness-of-fit in our model. If, using the new psychophysical procedure, the existence of the modulation of temporal binding by outcome valence gets confirmed, we expect to find evidence that volunteers with higher scores on empathic abilities show a higher modulation of outcome valence of a sense of agency. At the within-subject level, we expect the cortical signature of valence to correlate with temporal binding in the predicted direction. **Relevance**: We expect that our future findings may contribute to the development of better clinical strategies in interventions with patients with psychopathological conditions that cause damage to the recognition of the consequences of their actions at an emotional level. Also understanding how our perception is affected by our emotions is crucial to understand how affective states might influence even basic cognitive processes.

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NEURAL CORRELATES OF FREEZING OF GAIT IN A SAFE VIRTUAL REALITY-BASED SETUP IN INDIVIDUALS WITH PARKINSON'S DISEASE

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Introduction and Hypothesis: One of the most debilitating symptoms in individuals with Parkinson's disease (PD) is the Freezing of Gait (FoG). The management of the FoG is not efficient with the currently available medications, neurosurgery, and physical interventions, given the lack of pathophysiology knowledge of this complex phenomenon [1]. Therefore, an in-depth investigation of the dynamics of the postural circuitry in the brain in patients with FoG is fundamental to moving toward better understanding and efficient treatment possibilities. We hypothesis that freezers will perform worse in the LegoPress compared to non-freezers; Higher neural activity in the supplementary motor area and dorsolateral frontal cortex in prior periods and during FoG episodes compared to baseline (gait in and out of VR). **Objective:** We aim to build and validate a setup integrating VR and a robotic device, permitting us to investigate FoG in a safe seated position. To investigate whether biomechanical and neural freezing measurements in

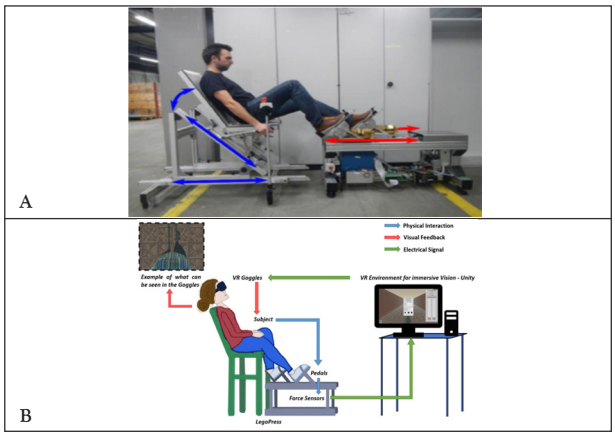

Figure 1. The LegoPress. A. The red arrows represent the free leg-press degrees of freedom for lower limb flexion-extension, while the blue ones are the possible predefined settings on the chair. B. The virtual avatar is controlled with flexion-extension forces exerted by the subject.

our setup (the LegoPress) correlate to objective and subjective measures of FoG in a standing position. Once we have tested and validated our setup as a useful tool to induce and unstruck FoG episodes, LegoPress can be used to improve our understanding of neural activity in the periods leading up to and during episodes of FoG. **Materials and Methods:** We will analyze 40 individuals with idiopathic Parkinson's disease, divided into two equal groups (with and without FoG). The subjects will do a task of walking in a virtual reality environment whose scenario will provide FoG episodes. Afterwards, they will do a task of alternating turns and walking with obstacles. We will measure neural activity using fNIRS and FoG times using inertial sensors. We will analyze neural activity of supplementary motor area and dorsolateral frontal cortex. **Relevance:** We intend to analyze whether freezing behavior observed during a virtual reality gait task may share similar neural substrates to freezing of gait. Such a relationship could offer a potential avenue for modeling the phenomenon of freezing of gait in Parkinson's disease, allowing for the exploration of the neural correlates of freezing.

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NEURAL STRESS CORRELATES INDUCED BY TIME-RESTRICTED CALCULATIONS MEASURED WITH FUNCTIONAL NEAR-INFRARED SPECTROSCOPY

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Introduction and Hypothesis: Anxiety disorders and depression are one of the main causes of incapacity worldwide [1]. Unproportional fear and concern caused by mental disorders impose many obstacles, which are often reflected on the patient's daily activities such as work, sleep, social meetings, or eating habits. In extreme cases, stressful situations induce physiological alterations and physical symptoms, such as changes in cardiac frequency, arterial blood pressure, or respiration rate [2,3]. Given its multifactorial characteristics, there is still a lack of clinical assessments to provide adequate information about the patient's mental health and, consequently, appropriate treatment. In this context, complementary methods to monitor patient lifestyle are valuable to improve diagnostics [4]. Functional Near-Infrared Spectroscopy (fNIRS) is a noninvasive and versatile neuroimaging technique that can be used to study the underlying aspects of the brain functional state using diffusive optics to measure changes in brain oxy- and deoxyhemoglobin concentrations. We hypothesized that stress induced by time-restricted mental calculations will evoke neural, systemic, and physiological changes. In this context, the aim of the present project is to quantitatively assess the effects of stress on the anxious brain and systemic physiology. **Objective:** This project aims to investigate the physiological and cognitive bases of anxiety disorders in performance situations. Specifically, our goal is to identify functional correlates associated with anxiety disorders by identifying biomarkers derived from brain activity and systemic physiology. **Materials and Methods:** All participants will fulfill the SIAS-6 and SPS-6 self-reporting questionnaire to assess anxiety levels and then will be studied in a controlled environment, in which stress will be induced by time-restricted mental calculations. Measurements will be performed with a NIRS system (NIRScout, NIRx Medical Systems) with a probe composed of 16 LED sources and 37 detectors (source-detector separation varied from 0.8 to 4 cm), symmetrically covering the frontal and parietal lobes, and a commercial physiological measurement system (Finometer, Finapress). The experimental protocol is designed in blocks that are composed of randomized 20 to 30 seconds of simple arithmetic followed by 30 to 45 seconds rest, repeated for 20 times. In the initial 10 blocks, the participants are free to perform the calculations without any time restriction; their average time resolution is evaluated and used as the time limit for the subsequent blocks. In sequence, the last 10 blocks are performed in the same manner but with time restriction. **Relevance:** In 2017, the World Health Organization estimated that 3.6% of the world population suffer from anxiety disorders, which emphasizes that the promotion of mental health is still a challenge in the current society. To this day, clinical interviews are still the gold-standard for mental disorders diagnostics. If successful, the proposed project will enable the quantitative assessment of anxious disorders as a complementary diagnostic tool, with great potential in improving patient's quality of life without any biological hazard.

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NEUROIMAGING MARKERS IN HEALTHY BRAIN AGING: A SYSTEMATIC REVIEW

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Introduction: Normal aging-related cognitive and functional decline has serious personal and societal effects. Magnetic Resonance Imaging (MRI) sequences, such as T1-weighted imaging, diffusion tensor imaging (DTI), and fluid-attenuated inversion recovery (FLAIR) can detect structural changes that occur in the normal aging brain, providing valuable information about volume atrophy, changes in white matter integrity and in cerebrospinal fluid spaces, which can aid in the early detection of age-related disabilities. **Objective:** (1) To evaluate biomarkers and their neural correlates assessed with T1, DTI and FLAIR in healthy elderly people. (2) To determine which MRI modalities provide the most reliable and promising biomarkers for evaluating the normal brain aging. **Methods:** A systematic review will be conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline. Embase, The Cochrane Library, Lilacs, Pubmed/MedLine, SciELO, Scopus and Web of Science databases will be searched for Portuguese, Spanish and English studies that investigated structural brain changes using MRI (T1, FLAIR, DTI) in a population aged 60 years and older. **Relevance:** This methodological review of MRI studies in normal brain aging aims to advance the knowledge in the field by highlighting potential gaps and biases in previous literature, as well as the challenges and opportunities. Ultimately, a deeper understanding of these MRI biomarkers can potentially be used to unveil the complex brain aging mechanisms and effectively intervene in this process to delay and treat age-related disabilities.

OCCUPATIONAL THERAPEUTIC ANALYSIS OF VIRTUAL REALITY AS A REHABILITATION RESOURCE AFTER STROKE

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Introduction and hypothesis: The healthcare area has mainly benefited from technological advances; virtual reality (VR) emerges as a complement and alternative to conventional therapies [1]. Damages to the central nervous system such as strokes are among the leading causes of neurological injuries. Their sequelae compromise individuals' independence, autonomy, and participation, directly affecting their occupational performance. In contrast to the high-cost tools, the KinesiOS application was developed for movement recognition for motor and neurofunctional evaluation and intervention, allowing the subject to integrate into the virtual rehabilitation program. Several tests have already been carried out to evaluate the effectiveness of this application, and it has been improved, but there is still a need-to-know aspect such as its use and effectiveness in adult neurological rehabilitation in relation to motor control, learned disuse of the hemi body and the impact on participation in daily activities. **Objective:** The present study intends to evaluate the usability and effectiveness of KinesiOS [2] in the rehabilitation process of people with motor sequelae in the upper limbs resulting from a stroke. **Materials and methods:** This is a non-randomized clinical trial. The recruitment of people will be carried out with the subjects referred to the outpatient neurology clinic of the University Hospital of the Federal University of São Carlos (HU-UFSCar) currently managed by EBSERH (from Portuguese, Empresa Brasileira de Serviços Hospitalares). The control group will comprise ten subjects with motor sequelae in the upper limb due to stroke and undergoing conventional rehabilitation treatment twice a week. The intervention group will comprise ten other subjects with motor sequelae of stroke in the upper limb that were not in rehabilitation treatment. They will undergo interventions using the application for 16 meetings with 30 minutes a frequency of twice a week. Subjects will undergo pre and post-treatment interventions through

a protocol composed of standardized tests, standardized questionnaires, and open questions to show changes in limb use skills and self-perception about the program used. Data will be analyzed through statistical tests to identify functional changes in both groups. **Relevance:** This study will contribute to the development of knowledge, considering the gaps in using VR, as a rehabilitation resource after a stroke. It is also intended to increase access to this resource and thus improve the evidence, and adjustments of KinesiOS studied at the Gleb Wataghin Physics Institute. The contribution of this project can also favour the research of other professionals involved in the rehabilitation process. This low-cost tool, in addition to allowing telerehabilitation, is also capable of increasingly promoting its reach in rehabilitation processes, mainly considering Brazil's fragility of access to these services.

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PRELIMINARY STUDY OF THE FORWARD-FORWARD ALGORITHM IN EEG CLASSIFICATION

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Introduction and Hypothesis: Brain-Computer Interfaces (BCIs) are computational systems that establish a direct control of a device through the decoding of brain signals by means of, for example, machine learning algorithms. Artificial Neural Networks (ANNs) are complex models that can be applied to a large variety of supervised and unsupervised machine learning tasks. Recently, the so-called Forward-Forward (FF) algorithm was proposed, landmarking a new paradigm for ANN training [1]. Although it can be considered a preliminary investigation on the subject, the FF algorithm has already shown promising results in some specific problems, being more biologically plausible than Backpropagation. Among the features described in its original work, it is emphasized that FF is a feasible alternative in machine problems that does not require neither a large volume of data nor very large models. Beyond that, FF can provide a fast integration to online systems due to its nature, where layers are updated "on the fly". Brain-Computer Interfaces are applications that could benefit from the usage of such an algorithm, since the response time is very relevant and data acquisition is a very expensive process. In that sense, we investigate the use of the FF algorithm in the classification of electroencephalogram (EEG) data, vastly used in BCI applications. The scope of this preliminary study compares the results obtained from a conventional ANN model optimized by the Backpropagation and an ANN model trained by the FF algorithm. We also investigate the aspects of Contrastive Learning that inspired the FF algorithm on the EEG domain, guided by different techniques to generate negative data required by the Negative Pass in FF. **Objective:** Provide an overview on deep learning models trained with FF algorithm in EEG classification tasks; Formalize feasible strategies to generate negative EEG data for Contrastive Learning-based models, and investigate the benefits of BCI systems relying on the FF algorithm; Define a comparative baseline between backpropagation and the novel method FF algorithm for further investigations in BCI applications. **Materials and Methods:** Our proposed experimental pipeline is based upon Wang *et al* BCI-SSVEP's benchmarking dataset, represented by 40 different labels, in which each label represents a frequency of interest. We will assess the performance of ANNs trained with backpropagation and the FF algorithm using different configurations of parameters (neurons and layers), and hyperparameters (activation functions, batch size, learning rate). We will evaluate the results using a 5-fold cross-validation process, adopting accuracy as the efficiency metric. **Relevance:** Despite the preliminary character of this novel training method for ANN models, we believe that the potential benefits suggested in the original work [1], such as the faster convergence with smaller datasets (which is convenient in the context of a single subject), are pertinent for the development of BCI systems. Therefore, by investigating the performance of this training algorithm over EEG data, we intend to validate these mentioned benefits and possibly find evidence for an alternative research path of classifier models for EEG signals in the scope of Brain-Computer Interfaces.

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QUANTIFICATION OF FUNCTIONAL CORRELATES OF NEUROPLASTICITY DURING LEARNING LOGICAL TASKS USING COGNITIVE TRAINING

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Introduction and Hypothesis: The acquisition of learning is associated with the control of neuroplasticity during the execution of several cognitive processes [1]. Longitudinal cognitive training triggers learning changes that can be quantified with neuroimaging techniques. Studies made with functional magnetic resonance imaging (fMRI) and functional near-infrared spectroscopy (fNIRS) techniques showed that sudoku-type tasks could induce neuroplasticity while the learning process occurs [2,3]. In particular, due to its portability, fNIRS has the advantage of studying such processes in natural learning environments. The technique enables the measurement of brain function through hemodynamics since fNIRS measurements are sensitive to changes in oxy and deoxy-hemoglobin concentrations over time. We hypothesized that the quantities estimated by fNIRS measurements during cognitive tasks are correlated with the neuroplasticity induced by learning and that a longitudinal study in this context can provide insights into how the acquisition of logical problem-solving occurs. **Objective:** This project aims to quantify the functional correlates of brain neuroplasticity during learning a task that requires logic to be completed. We will use the Sudoku game as the task to be learned. This activity will be carried out for ten sessions over twenty days, with a level of difficulty that gradually increases as learning occurs. Brain activity during the task will be measured using the fNIRS technique. **Materials and Methods:** We intend to recruit 20 healthy undergraduate participants, aged between 18 and 24 years old, who will have to learn the game by solving sudoku problems, guided by a commercial app over ten sessions held on different days. NIRS commercial system (NIRScout, NIRx) will be used to monitor brain functioning while participants play. Data from different source-detector pairs will be associated with nodes located in different brain regions, forming a complex network represented by a graph. From the networks, it is possible to extract local and global parameters that can be used to characterize aspects of the network topology. Also, functional analysis will provide the study on how brain activation changes throughout different sessions. **Relevance:** Among the cognitive processes of greatest interest are those associated with learning. The brain's ability to learn a stimulus occurs due to neuroplasticity, and it demands attention [1]. At the same time, retrieving what has been learned depends on memory, one of the central executive functions involved in learning [4]. Although these processes occur at the neural level, their functional correlates can be observed with neuroimaging techniques. Understanding the cognitive processes involved in learning can directly impact education and drive insights into the best conditions to promote learning [5].

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SKIN LESION ANALYSIS USING MULTISPECTRAL IMAGES CAPTURED BY A LOW-COST DEVICE

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Introduction and Hypothesis: The analysis and follow-up of skin lesions are important for the diagnosis and treatment of some diseases, such as lupus. However, this task can be laborious, subjective, and limited when done only visually. In this case, spectral imaging combined with computational methods proved interesting. From this type of image, additional information beyond the visible spectrum can be obtained, as well as the spectral signatures of the different regions of a sample, the latter being a characteristic curve of a material that can be used to identify it [1,2]. The hypothesis, then, is that multispectral imaging can be used for characterization and classification of different materials and organic tissues, allowing the development of a more automated and less subjective method of skin lesion analysis. Furthermore, since multispectral cameras are expensive and unviable for the Brazilian public health system, it is believed that a low-cost version could be built. **Objective:** The goal is to build

a low-cost multispectral camera from a microcontroller combined to a camera module and test it first on a simpler problem, such as the analysis of grains and seeds, and then in the analysis of skin lesions of lupus' patients. It is also in the scope of this project the proposal of an automated method of analysis of these images, through image processing algorithms and machine learning. **Materials and Methods**: To achieve the goals of the project, it will be necessary: a microcontroller, a camera module, LEDs of different wavelengths and a dark box with built-in lighting. Initially, the camera setup will be built and tested. After that, the multispectral images will be collected. Then, the acquired images will be processed and analyzed through the following steps: noise removal, calibration, feature extraction (spectral signature being one of them) and the training of a classifier. **Relevance:** Skin lesions occur in various diseases and health conditions. Lupus, for example, affects approximately sixty-five thousand Brazilians and skin lesions manifest in 80% of their cases, being one of the main criteria for its diagnosis. Besides that, skin lesion assessment is useful for evaluating the disease's progress and for adjusting its treatment [3]. In cases like this, an automated method of skin lesion analysis (quantification, characterization, and classification) is fundamental and could assist health professionals in their clinical decisions. Also, trying to achieve this at a low-cost is relevant, since it would make the new method feasible for Brazil's unified health system, whereof more than 70% of the Brazilian population relies on (data from 2019) [4]. Finally, the results of this project could be similarly useful in other applications, like in the analysis of other organic tissues or in the inspection of agricultural products.

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STUDIES ON CLASSIFICATION OF EEG DATA USING AUGMENTATION TECHNIQUES

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Introduction and Hypothesis: In the last years the studies and applications in BCI (Brain-Computer Interface) systems have experienced a significant growth, mainly because of the advance of deep learning techniques. BCI systems based on electroencephalogram (EEG) data can use different paradigms. In this study, the focus is on the motor imagery paradigm, which aims at recognizing a movement imagined by the BCI user. Most of the papers that achieved a significant performance in correctly classifying the movements, commonly make use of deep classifiers [1]. For a suitable training, these methods require a large volume of data, however, EEG data from user might not be available or, in most cases, there are only a few of them. In light of this, data augmentation techniques reveal to be a promising strategy to artificially generate novel information from previous EEG signal and provide a new dataset that allow the deep classifier to generalize the classification task and to improve accuracy. **Objective:** Based on data augmentation techniques in image classification tasks using CNN (convolutional neural networks), this study is interested in applying a variation of the CutOut [2] technique to EEG data, Basically, this method aims to remove random points from the data and generate new samples of reduced length in relation to the original data. **Materials and Methods:** The database used to carry out the experiments was the BCI Competition IV 2a, which contains data from motor imagery paradigm. There are 9 subjects, containing 4 classes of captured imagined movements. Data were preprocessed with a bandpass filter, removing frequencies below 4Hz and above 38Hz, and then normalized. The proposed variation of CutOut for EEG considers each experiment performed by each user and generating N variations of these experiments with an X number of missing points from the collected signal, such points are randomly removed in each of the experiments, hence N new bases are generated with X points less than the original sample. In the sequence such samples were used for EEGNet [3] training. In Figure 1 we can see the preliminary results that were obtained from an experiment comparing the proposed method in relation to the use of the original data with EEGNet. **Relevance:** Through this work, a new method for data augmentation is proposed, working in a similar way to the already known cutout method adapted to EEG data. Furthermore, the new samples generated by this method have small variations, making possible good generalization of classification and increase in accuracy of deep classifiers for BCI Systems.

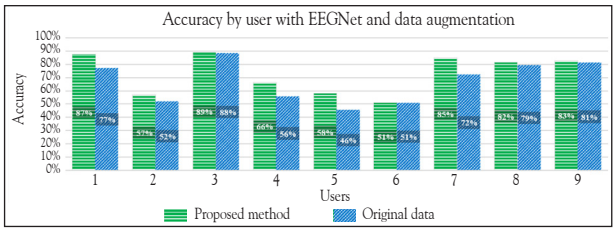

Figure 1. Preliminary results with the proposed method applied in EEGNET.

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STUDIES ON SOS-BASED NONLINEAR BLIND SOURCE SEPARATION APPLIED TO EEG DEEP CLASSIFIERS

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Introduction and Hypothesis: BCI (*Brain-Computer Interface*) systems based on electroencephalography (EEG) open up the possibility of establishing direct communication between the human brain and machines, allowing a device to be controlled by means of thoughts, (something useful, especially when considering people suffering from neuronal diseases). In order to be efficient, EEG signals need adequate preprocessing to allow the extraction of the relevant features. This is a very challenging task, considering that the signals are noisy and possibly present nonlinear behavior, given the characteristic response of the electrodes and the propagation of signals from different regions of the brain. In this way, the present work focuses on EEG data preprocessing, starting from the assumption that the EEG recordings are the result of a nonlinear mixture of neuronal signals. We will use a method of blind source separation in the nonlinear context (NLBSS) based on second-order statistics (SOS) [1], exploring the temporal structure of the signals. We believe that, from the extraction of the independent components, the training of an artificial neural network as a classifier [2] can be more efficient, possibly using smaller data windows. **Objective:** Based on the assumption that the EEG signals are the result of nonlinear mixing (such as Post-Nonlinear mixtures), we intend to use an NLBSS method as a preprocessing step for EEG classification by Deep Learning methods. The NLBSS technique, called A-SOBIPNL [1], uses SOS to extract temporal information and constructs a nonlinear separation function represented by a truncated Fourier series, allowing a wide variety of nonlinear functions to be covered. Once the EEG signals preprocessing step has been accomplished, data will be sent for classification through a convolutional network, such as EEGNet [3]. **Materials and Methods:** The EEG data used in this work refers to dataset 2a of BCI Competition IV [4], which is a database focused on the motor imagery paradigm, containing EEG data collected from 9 users with 4 classes: hand movement imagination data (right and left), feet and tongue. In the preprocessing step, we use a generalization of the A-SOBIPNL algorithm [1] to treat EEG data. This separates the potentially nonlinear mixtures, allowing each one to be represented by an independent component. On data classification, we will use the EEGNet, which is a compact convolutional network containing three main layers, where the first one works as a temporal convolution, filtering the frequencies; the second layer uses a depthwise convolution, working as a specific frequency spatial filter; and the last one is the separation convolution layer, which learns a temporal summary for each feature map individually, followed by a pointwise convolution, which optimally mixes the feature maps together. **Relevance:** The work contributes with a novel standpoint to represent EEG signals (from the nonlinear mixing model perspective), and contributes with a new application to the ASOBIPNL algorithm. In addition, it aims to reduce the amount of data used in the training of artificial neural networks.

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TASK-SPECIFIC TRAINING IN AN AUGMENTED REALITY CONTEXT (INTERACT PROTOCOL) FOR CHILDREN WITH SPASTIC CEREBRAL PALSY: A PROTOCOL FOR A RANDOMIZED CONTROLLED TRIAL

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Introduction: Children with cerebral palsy (CP) have disorders affecting static and dynamic postural control in high-complexity environments[1]. In this context, interventions with task-specific training and technological resources focusing on motor learning are recommended. Thus, augmented reality (AR) games may also be highlighted due to their multisensory and motivating stimuli. Otherwise, few studies explored this field. **Objective:** This study aims to present the INTERACT Protocol, which will verify the effect of training on postural control, capacity and motor performance, motivation, and satisfaction in motor performance, compared with conventional treatment of ambulant children with spastic CP. **Materials and Methods:** INTERACT Protocol is an acronym of an INdividualized and Task-specific with environment Enrichment using Real and Augmented realities with Active Training. Sixty-two children with spastic CP with Gross Motor Function Classification System levels I and II, aged between 6 and 12 years, will participate in the study. The primary outcomes will be postural control during sit-to-stand activity in different task contexts and during standing functional reach assessed by the force platform (BERTEC) and markerless kinematics cameras. Secondary outcomes will be mobility performance (Pediatric Assessment of Disability Inventory) and motor skills (Challenge Test). Motivation will be assessed with the Dimensions of Mastery Questionnaire, and acceptance, satisfaction, and adherence to the intervention using an online questionnaire. The intervention will last 12 weeks, with two individual sessions of 60 minutes per week and on alternate days, totaling 24 sessions and 24 hours of training. Children will perform task-specific training in an augmented reality context. The tasks will be based on pre-established goals chosen between three groups of activities (sit-to-stand, up and down stairs, and functional reaching), and children will have to assemble the puzzles in virtual reality while performing the tasks in the real world. The equipment[2] enables children to see their reflection on the screen, which facilitates the connection between virtual and real realities and represents augmented reality. The complexity of activities will gradually increase during the treatment with environmental enrichment. The control group will receive conventional training for the same period and volume as the children in the experimental group. The assessments will occur before and after 6 and 12 weeks after the beginning of the training and with a 4-month follow-up to verify the retention of the outcomes. **Relevance:** This study will contribute to developing knowledge about the benefits of augmented reality in children with CP and the association of this low-cost technology with task-specific training. In addition, the results will indicate how reliable this protocol might be and the best resource to be used in the clinical rehabilitation of children with CP.

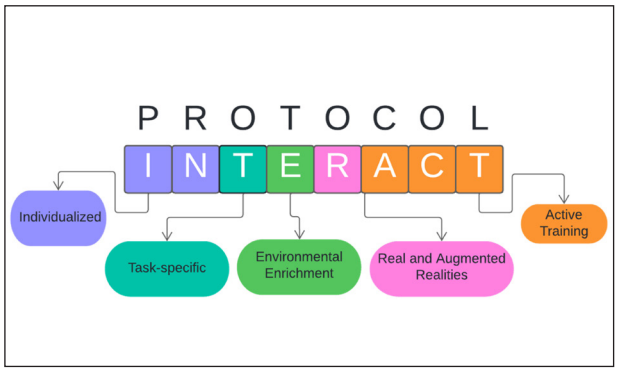

Figure 1. Acronym of the protocol.

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TISSUE EXPRESSION OF PROTEINS RELATED TO BRAIN DEVELOPMENT AND CONNECTIVITY IN FOCAL CORTICAL DYSPLASIA TYPE II

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Introduction: Focal cortical dysplasia (FCD) is a common cause of pharmacoresistant epilepsy [1]. According to the classification proposed by the International League Against Epilepsy (ILAE), FCD type II is characterized by dysmorphic neurons (IIa and IIb), which may be associated with balloon cells (IIb) [2]. Previous studies from our group by using RNA-sequencing of surgically removed specimens from individuals with histopathologically confirmed FCD type II have shown increased expression of lumican (*LUM*), neuroligin 3 (*NLGN3*), *RAB13*, semaphorin 3F (*SEMA3F*), tenascin C (*TNC*), and WNT inhibitory factor 1 (*WIF1*) genes in comparison with controls. Moreover, we verified decreased expression of chondroitin sulfate proteoglycan 5 (*CSPG5*) gene in patients with FCD type IIa. Specifically, LUM, SEMA3F, TNC, WIF1 and CSPG5 are proteins that play a role in cell proliferation, migration, and cortical organization during the cerebral development [3, 4, 5, 6, 7, 8, 9]. NLGN3 and RAB13 are involved in synaptic function [10, 11]. As far as we know, there are no previous reports on the expression and possible pathophysiological roles of the proteins encoded by such genes in FCD type II tissue. **Objective:** This study aims to investigate the expression and distribution of proteins (LUM, NLGN3, RAB13, SEMA3F, TNC, WIF1, and CSPG5 in FCD types IIa and IIb surgically removed tissues. **Materials and Methods:** Formalin-fixed and paraffin-embedded sections of FCD type IIa (n=4), type IIb (n=9) and control (autopsied patients without history of neurological disease; n=7) will be submitted to immunohistochemical reactions by using primary antibodies against LUM, NLGN3, RAB13, SEMA3F, TNC, WIF1, and CSPG5. All immunostained slides will be scanned by using an Aperio Scanscope CS2 (#23CS100) device. Qualitative assessment will be performed by describing nuclear and/or cytoplasmic expression in cells localized at the cortex and at the superficial and deep white matter. Thirty representative pictures (20X magnification) of each section will be randomly chosen for the quantification of marker in the ImageJ® software environment by using the IHC Profiler plugin, the Threshold tool and calculating the percentage of the immunostained area. Statistical analyses will be performed with the Kruskal-Wallis and the post hoc Dunn tests (GraphPad Prism software; version 8.0). Statistical significance is established at a $p < 0.05$. **Relevance:** Cellular pathways involved in the pathophysiology of chronic seizures due to FCD type II are still debatable in humans. The present study aims to contribute to this topic by complementing original results from our group regarding RNA-sequencing and providing data on the expression of proteins related to brain development and connectivity both in the lesion and the adjacent brain environments. **Funding:** FAPESP (2013/07559-3, 2016/50486-5, 2019/08259-0, 2020/12651-0) and FAEPEX – Unicamp (2037/19, 2428/20 and 2059/22).

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TRANSCRIPTOME ANALYSIS OF MICE HIPPOCAMPAL SUBREGIONS TREATED WITH DIFFERENT CBD DOSAGES

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Introduction and Hypothesis: Cannabidiol (CBD) is one of the most abundant compounds in the cannabis plant. CBD has already been explored as a therapeutic strategy showing anticonvulsant effects, neuroprotectors and antiepileptics under various conditions, both human and animal models. Previous investigations have already revealed effects of CBD administration,

however, we are the first to produce RNA-seq data for the effects of CBD in hippocampal subregions. Genes enrichment data after administration of 100 mg/kg CBD for 7 days indicates a reduction in the electron transport chain transcripts and ribosomal biogenesis. This study will examine whether these changes in gene expression persist in other hippocampal subregions (CA1, CA3, DG) after different CBD administration dosages." **Objective:** In order to understand the effect of CBD on the transcriptome of different hippocampal layers, we will be sequencing and quantifying messenger RNAs from the ventral and dorsal regions (CA1, CA3, DG subfields) after intraperitoneal administration of CBD (5 mg/kg, 20mg/kg and 100 mg/kg). After that, we will analyze which genes are differentially expressed, and how they may affect biological processes and pathways when compared to control animals. **Materials and Methods:** Adult mice will be separated into four experimental groups: control (saline), seven-day treatment with CBD in three different dosages (5 mg/kg, 20mg/kg and 100 mg/kg, 1 dose per day for 7 days). Finally, the animals will be euthanized 24h after the last administration of CBD and the brain tissues will be collected and sliced for microdissection to RNA extraction of the subfields (CA1, CA3 and DG) for transcriptome analysis. **Relevance:** Despite progress in obtaining medicinal cannabis in Brazil, restrictive policies continue to contribute to prejudice surrounding cannabis, impacting both research and society. Brazilian researchers face bureaucratic obstacles related to the failed war on drugs when trying to access cannabis compounds for scientific research, leading to significant delays in the development of cannabinoid knowledge. Nevertheless, the therapeutic effects of cannabis are well-established, but the specific genetic and cellular mechanisms activated by different CBD dosage ranges remain unclear. Thus, RNA-Seq results of this study aim to further clarify these mechanisms.