FINAL REPORT // ZÁRÓJELENTÉS

Mindenekelőtt szeretnénk egyértelművé tenni, hogy jelen projekt Négyessy László és Somogyvári Zoltán együttes vezetésével került végrehajtásra. A társvezetőségre vonatkozó kérelmünket a hivatal technikai okokra hivatkozva nem tudta figyelembe venni. A pályázattal kapcsolatos adminisztratív feladatok ellátását illetően ezzel a döntéssel egyetértünk és elfogadtuk. A pályázat támogatásával elvégzett munka azonban kettős projektvezetés eredménye, ezért kérjük figyelembe vételét az értékelés során!

First of all, we would like to make it clear that László Négyessy and Zoltán Somogyvári served as co-PIs of this project in spite that our request for the shared leadership was rejected. Accordingly, we accepted the reason of rejection that co-leadership is not compatible with the administrative tasks. However, in the evaluation please consider that the research supported by this proposal was made by the co-leadership of LN and SZ.

Pályázatunk fő célkitűzései a külföldi partnerektől kapott adatok újfajta, egyedi módszereken alapuló feldolgozására épültek. A célok megvalósítását nem akadályozta, de módosította a partnerektől származó adatok rendelkezésre állása. Az eredeti céloknak megfelelően új módszereket fejlesztettünk és kidolgoztuk a komplex Multimodal Mismatch Negativity (MMN) ingertől függő aktivitás többszempontú jellemzésének módját. Azonosítottuk a multiszenzoros integráció réteg-specifikus lépéseit egysejt aktivitás és LFP szinteken és implementáltuk a kanonikus kérgi interakciókat modellező hálózat serkentő és gátló neuronokból felépülő réteges modelljét. Koherencia elemzéssel vizsgáltuk a kérgi rétegek és áreák interakcióit a feladat elvégzése során. Továbbfejlesztettük az extracelluláris mérésből specifikus idegsejteken bementi áramokat azonosító módszerünket (single cell kernel Current Source Density method: skCSD). A kanonikus kérgi hálózat működésének fajspecificitását más együttműködésből származó humán adatokon vizsgáltuk. Kauzalitás elemzéssel ex vivo modellen azonosítottuk a LFP teljesítmény fontosságát az idegsejt aktivitás különböző állapotaiban. Emellett a kauzális interakciók minden formáját azonosítani képes módszert fejlesztünk, melynek validálása folyamatban van. A pályázat keretében eredetileg nem tervezett, de a céljainkkal egyező kutatásokat is elvégeztünk, melyek agykérgi áreák interakcióinak strukturális alapjait vizsgálták.

The major goal of our proposal was the analyses of electrophysiological data provided by the foreign collaborators by applying novel data analyses techniques. Achievement of our goals were somewhat modified by the availability of the appropriate data. In agreement with the aims of the proposal we developed methods and designed the analyses of the recordings obtained in our complex Multimodal Mismatch Negativity (MMN) task. We determined layer specific processing steps of multimodal integration at the single unit and LFP levels and implemented a large scale network model of laminar cortical interactions. Also, we studied the relationship of the activities of cortical layers and areas during the task presentation by way of analyzing coherencies. We further elaborated the technique identifying input currents on specific cortical neurons from extracellular recordings, which has been developed by our group, and formulated the single cell kernel Current Source Density method (skCSD). Species specific processing of the canonical cortical circuitry was investigated in human data obtained from other collaborations. We also discovered the importance of the power of LFP in different states of neuronal activities by identifying the causal relationship between signals obtained from recordings of different

spatiotemporal resolution (i.e. optical and electrophysiological) in vivo. Furthermore, our work on developing a new mathematical tool, which can identify all forms of causal interactions is at the phase of validation on experimental data. Finally, we complemented the original goals by studies on the structural correlates of interactions within and between cortical areas.

The major findings of this project was related to the 1) Neural correlates of Multimodal Mismatch Negativity (MMN), 2) New recording and data analyses techniques, 3) Modelling network dynamics and 4) Structural correlates of signal propagation and transmission in the cerebral cortex.

1) NEURAL CORRELATES OF MMN

We studied single neuronal correlates of mismatch negativity during multimodal processing in different cortical areas in an anesthetized mice. Extracellular potentials were recorded simultaneously in primary visual cortex (V1) and higher order area AL by means of two 32 channel, Michigan type microelectrode arrays in a multimodal mismatch negativity task combining specific auditory (2 pitches) and visual (2 orientations) stimuli (Fig. 1.1.a). An important part of our analysis was the distinction of "true" mismatch from response adaptation by comparing different stimulus presentations (Fig. 1.1.b). Our analyses show that neurons exhibit both repetition suppression as well as mismatch related "surprise" with a varying degree. Different auditory, visual and multimodal mismatch related single unit responses were identified. Furthermore, the analyses of the responses resulted in at least two major components with different time delays in the PSTH. A striking effect was that most V1 neurons were sensitive to the auditory mismatch in the anesthetized animal. Interestingly, a new type of neuronal response called conditional mismatch was revealed, which showed significant firing rate increase for auditory mismatch only when the preferred visual stimuli were presented (Fig. 1.2).

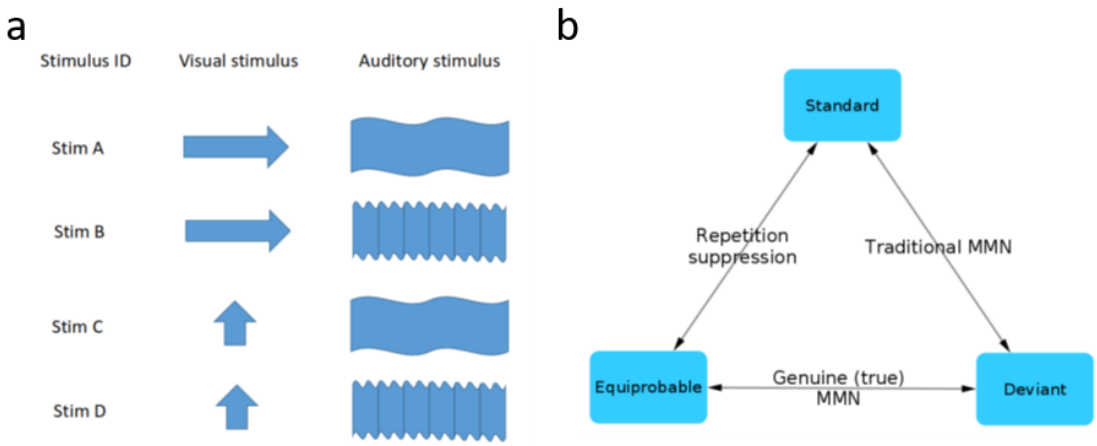

Figure 1.1. a) Individual stimuli combined in the audio-visual MMN paradigm. **b)** Stimulus comparisons used to determine different response types. Standard: single stimulus is presented, Deviant: an odd stimulus is intermixed with the standard stimulus, Equiprobable: different stimuli with the same

probability of occurrence are intermixed. The comparisons were extended by including different time windows resulting in the multiplication of the already large number of variables.

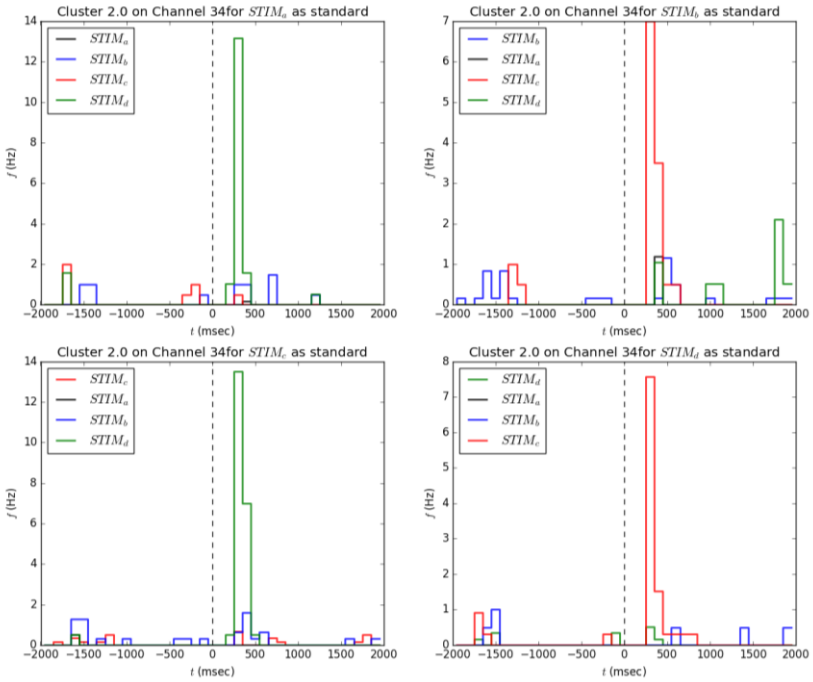

Figure 1.2. Event related spike histogram of a V1 unit. The cell is clearly sensitive to the auditory mismatch, but only in the presence of one of the visual stimuli (vertical) and not for the other.

We have also shown, that coherence is increased between and within cortical areas in specific frequency bands during auditory and bimodal mismatch detection (Fig. 1.3): In the primary visual cortex, V1, elevated coherence was observed in the 22-45Hz range, while in the antero-lateral (Al) area the coherence elevation was most pronounced in the 52-75Hz range. In contrast, between the two areas, the coherence was increased most in the 10-20Hz range and in lesser extent in the 22-45Hz interval.

These studies formed the basis of poster presentations (Somogvvári et al., 2017; Furuglyás et al., 2019) and an MSc thesis (http://cneuro.rmki.kfki.hu/sites/default/files/FK_diplomamunka.pdf). The analyses will be extended by including a larger dataset before publishing the results as a full research paper.

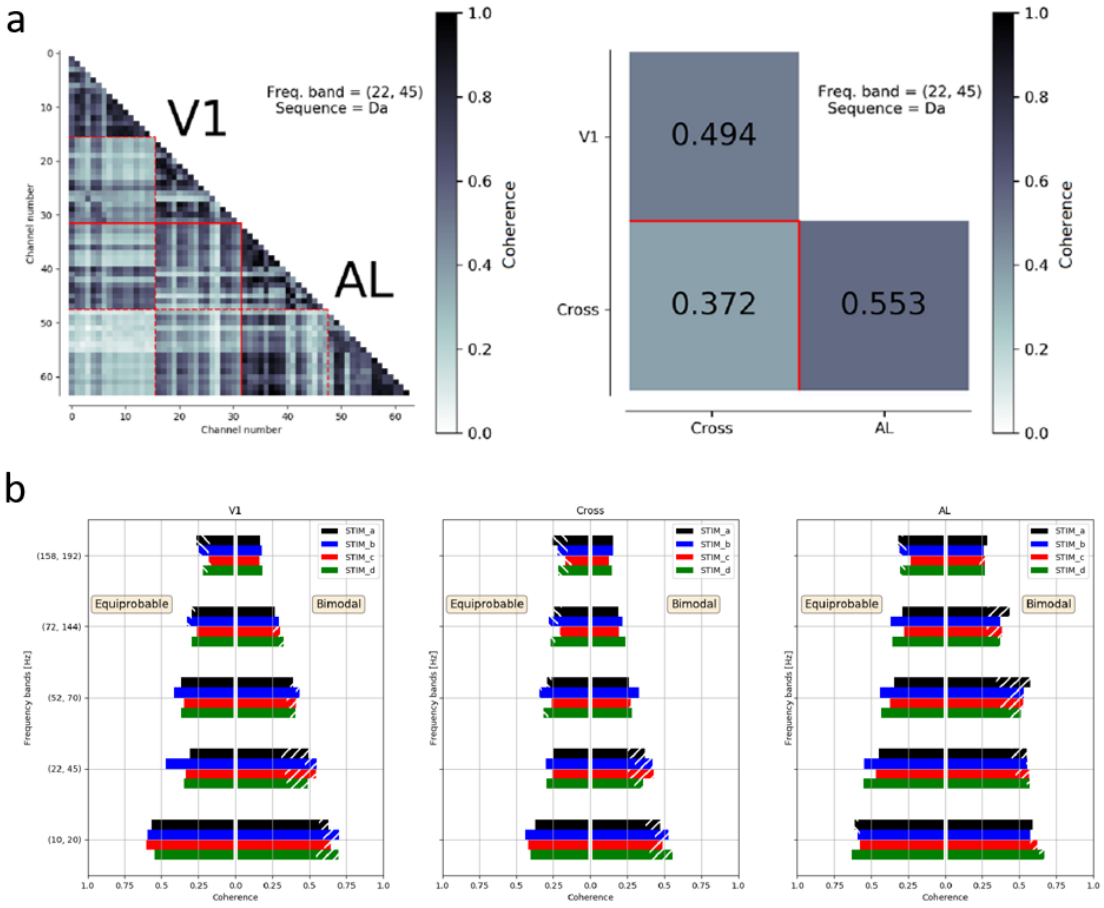

Figure 1.3. a) *Left*: Coherency map between all the channels. Values have been averaged in the annotated frequency band (from 22 to 45 Hz) for every channel during stimulus A embedded in the D standard session (bimodal mismatch). *Right*: Spatially averaged values. Averaging was done within the cortices (V1, AL) and between them (Cross) resulting in total coherence measures for them. **b)** Comparison of the total coherences in bimodal surprise enhancement within the V1 (right), AL (left) and between them (middle). Coherences in different frequency bands are compared between deviant (right bars) and equiprobable (left bars) stimulus sequences. Different colors mean the stimuli, and hatching indicates the discrepancy.

2) NEW RECORDING AND DATA ANALYSES TECHNIQUES

skCSD: single-neuron kernel current source method (skCSD) - We have developed a constrained inverse solution using kernel method to solve the Poisson inverse problem on the known, complex, branching morphology of a neuron called single-neuron kernel current source method (skCSD). We have shown, that inclusion of the known morphology largely enhanced precision of the inverse solution and applied the skCSD method to the first available experimental data, where both the extracellular potential were measured by a 1D multi-electrode array and the morphology of the cell (a CA1 pyramidal neuron) were reconstructed. The current source density distribution and the spreading of the currents along the morphology were determined during the averaged action potential. This first application was included into the paper about the method as a proof of concept. The final full length paper were published in the journal

eLIFE. The scripts for the analysis, written in R, were tested and made publicly available as an open source program package (Cserpán et al. 2017).

Parallel recording of optical and electrical signals - Cortical functional micro-structures, such as orientation selectivity columns and pinwheeles in the primary visual cortex were generally assessed by in vivo (blood dependent) intrinsic optical signal (IOS) imaging. Application of a new transparent cortical electrode array made possible the determination of those functional patterns based on the electrical activity and its comparison to the parallel optical imaging. Thus, we calculated the orientation preference map through electric imaging, based on the local field potential recordings and compared to the orientation preference maps calculated from the parallel intrinsic optical imaging, recorded through the transparent electrode array. Based on the comparison of the orientation maps obtained from optical an electrical recordings, the observed structural similarity raises the possibility to construct a unified imaging system which unifies the high spatial resolution of the optical imaging and the high temporal resolution of the electric imaging method. The results has been published in Sensors & Actuators: B. Chemical (Zátonyi et al. 2018).

Opto-electric causality in vitro - Causal relationship between local field potential (LFP) and intrinsic optical signal (IOS) in evoked epileptiform activity was investigated in vitro based on parallel IOS and LFP recordings. As far as we know, this work was the first conclusive application of the Sugihara's new causality method, the cross-convergent mapping (CCM) (Ye et al., 2015) in neuroscience. As CCM is the first causality analysis method which can reliably detect the circular connection, we were in the position of investigating the question, whether only the evoked epileptic activity causes the intrinsic optical signal IOS, or there is a feedback mechanism as well, whereby the ion concentration changes measured by the IOS influence the termination or the renewal of the epileptic activity. We found only unidirectional causal drive from the electric towards the optical signal, and this work also demonstrated several phenomena which are instructive for further investigation. We found, that the correlation was small between the LFP and the IOS at the time of the actual causal effect and the peaks of the cross correlation function did not reflect the actual causal dependency in this case. Instead, the temporal derivative of the IOS was correlated with the LFP power at the time delay of the causal peak. Based on these observations, a simple model have been set up to describe the dependency of the IOS on the LFP power and IOS was reconstructed, based on the LFP signal. Besides, our results suggest that it is possible to calculate causality between two data series with drastically different time scales and provides useful know-how for application of causality analysis for any field of science. The results have been published in the Scientific Reports (Benkő et al. 2019).

Dimensional causality - We have developed the Dimensional Causality (DC) analysis method devised to detect and quantify the probability of all possible types of causal relationships between two time series: independence, direct or circular causal connection, and the existence of a hidden common cause. To our best knowledge, no single method existed before, which can detect and distinguish all these possible causal relationships, based on time series observations from deterministic dynamical systems. To detect these relations between two time series, Takens' embedding theorem is used to reconstruct the attractors of the underlying systems. The new method is based on the subadditivity of the system's attractor dimensions, where the key is the dimension of the joint attractor of the two systems. We showed that the relations between the joint and individual dimensions unequivocally determine the causal relations between the dynamical systems. The probability of the different causal relations is obtained via Bayesian inference. We validated our method on simulated examples of 'classic' chaotic dynamical systems, such as nonlinearly coupled logistic functions, coupled Lorentz-systems and Hindmarsh-Rose models. Besides

the tests on simulated dynamical systems, the EEG recordings during photostimulation as a part of the standard epileptic investigation protocol, provided us a good opportunity to test the applicability of our method on real-world data. DC method confirmed our hypothesis about the increase of common cause probability between the two hemispheres during flashing light stimulation suggesting the applicability of the method in clinical scenarios. The manuscript summarizing the results of this study is under revision and is published in preprint form on arxive.org (Benkő et al. 2018; https://arxiv.org/abs/1808.10806).

Localization of epileptic focus by DC - The significance of our DC method was demonstrated by applying it to electrocorticographic (ECoG) data recorded by subdural grid electrodes during presurgical investigation. Examining four distinct areas of epileptic activity in the patient's brain, the possible focus of epileptic seizure was identified; an area which drives the others, meanwhile the existence of a common drive is identified between the driven areas. These results suggest that common causes revealed by DC method contains relevant information for the diagnosis of epileptic patients in various clinical settings. The results has been published as a chapter in Springer Series in Bio-/Neuroinformatics and as a preprint (Benkő et al. 2017, 2018).

Signal processing software for electrophysiology - A user friendly graphical interface was developed for exploring the data recorded by subdural grid electrodes. The interface makes easy to calculate the kCSD, create power maps on different frequency regimes based both on LFP and CSD as well as segmenting the electrodes by coherence based clustering. The methods were applied to determine the role of cortical areas in ketamine induced oscillations recorded by a transparent cortical surface electrode grid parallel to intrinsic optical signal measurement. The methodology and preliminary findings were presented on a conference and it is already submitted to a methodological journal (Cserpán 2017; Fedor et al. under revision).

Novel anomaly detection method for time series - We have introduced a new anomaly concept called "unicorn" or unique event, and developed a new, model-independent, unsupervised algorithm to detect the unicorns. The Temporal Outlier Factor (TOF) is introduced to measure the uniqueness of events in continuous data sets from dynamic systems. The concept of unique events differs significantly from traditional outliers in many aspects: while repetitive outliers are no longer unique events, a unique event is not necessarily outlier in either pointwise or collective sense; it does not necessarily fall out from the distribution of normal activity. The performance of our algorithm was examined in recognizing unique events on different types of simulated data sets with anomalies and it was compared with the standard Local Outlier Factor (LOF). TOF had superior performance compared to LOF even in recognizing traditional outliers and it also recognized unique events that LOF did not. Benefits of the unicorn concept and the new detection method were illustrated by example data sets from very different scientific fields. (Benkő et al. 2020, under revision; published also as a preprint on arxiv.org).

3) MODELLING NETWORK DYNAMICS

According to the aims of the proposal we applied a comprehensive modelling framework of the laminar and areal interaction in the cortical network. We implemented a two layer, Wilson-Cowan neural field model to better understand structure-function relationships in hierarchical cortical dynamics by studying the formation of causal functional links in a weighted anatomical network of the primate cerebral cortex (Fig. 3.1). This model was published by Mejias et al. (2016) and our implementation fully reproduced their

observations. Structure-function relationship was studied by correlating three measures: 1) convergence degree (CD), a topological measure of signal flow, 2) an anatomical index of cortical hierarchy and 3) dynamical dependence by computing spectral Granger causalities between areas. To obtain a biologically relevant CD a modified version of the shortest path structure of the weighted graph was introduced, which favors robustness in the expense of the winner-take-all approach (Fig. 3.2). CD exposed a densely connected component of higher-order areas resembling the rich club of the network that was not seen in the anatomical hierarchy (Fig. 3.3). Due to computational limitations structure-function relationship was studied in a subnetwork of eight areas, which was studied previously both experimentally as well as theoretically by other groups (Bastos et al., 2015; Mejias et al., 2016). Remarkably, CD significantly correlated with the empirical index of anatomical hierarchy as well as with the causal relationship of areal activities (Fig. 3.4). This is the first study showing the close correspondence of network topology and dynamics in a weighted and hierarchical model of the large-scale cortical network. These findings provide insight into large scale cortical dynamics. The study formed the basis of an MSc thesis (http://cneuro.rmki.kfki.hu/sites/default/files/thesis_vb_final.pdf). These promising preliminary findings are under further elaboration and hopefully will result in the submission of a full research paper.

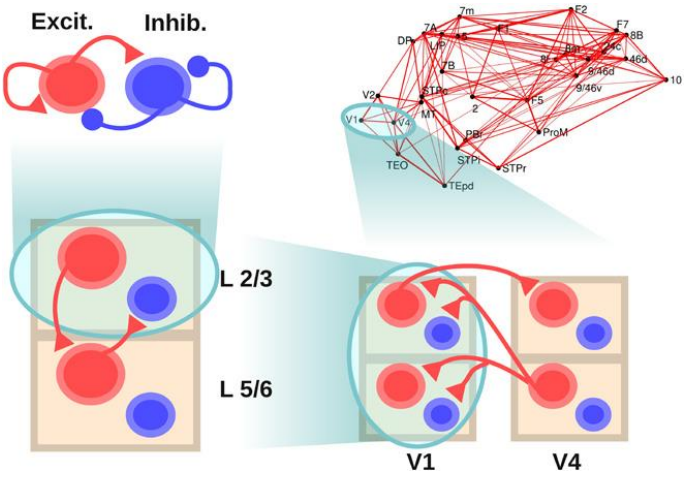

Figure 3.1. Scheme of the large-scale model. The scheme shows the four levels considered: a within-layer local microcircuit consisting of an excitatory (Excit.; in red) and an inhibitory (Inhib.; in blue) population (upper left), a laminar circuit with two laminar modules (corresponding to supra- and infragranular layers, lower left), an interareal circuit with laminar-specific projections (lower right), and a large-scale network of 29 cortical areas based on macaque anatomical connectivity (upper right). Each level is anatomically constrained, and its dynamics provide insight into different electrophysiological observations in macaques. Only the connections at each level not shown at a lower level are plotted, for clarity. From Mejias et al. (2016).

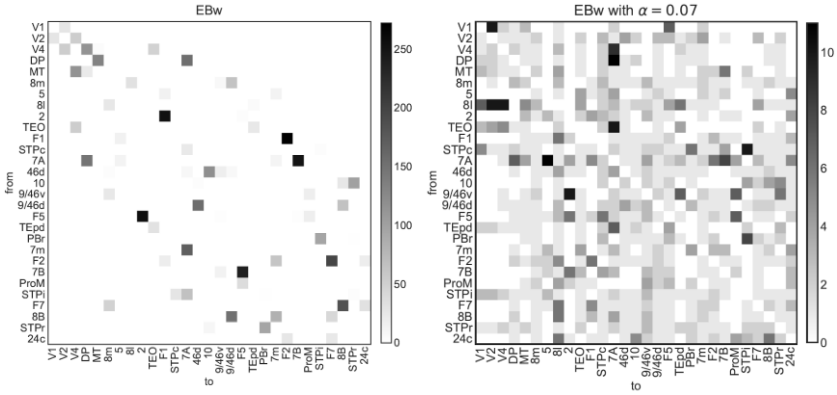

Figure 3.2. Results of the modified shortest path detection method. Traditional computation finds only a few edges, many of them with very high values (left). The modified version results in a more realistic rich EB structure (right).

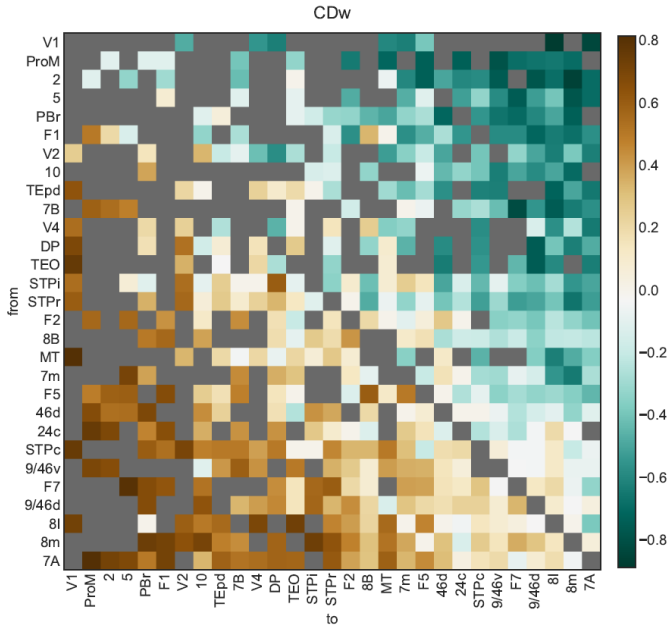

Figure 3.3. CD values for the weighted 29×29 graph, reordered by the CD flow, revealing the rich-club of higher order associational areas in the lower left corner. Note an almost perfect gradient, perpendicular to the diagonal, moving from extremely convergent (dark brown) to extremely divergent (dark blue) edges, with the zero CD edges mostly aligning in the middle, along the diagonal.

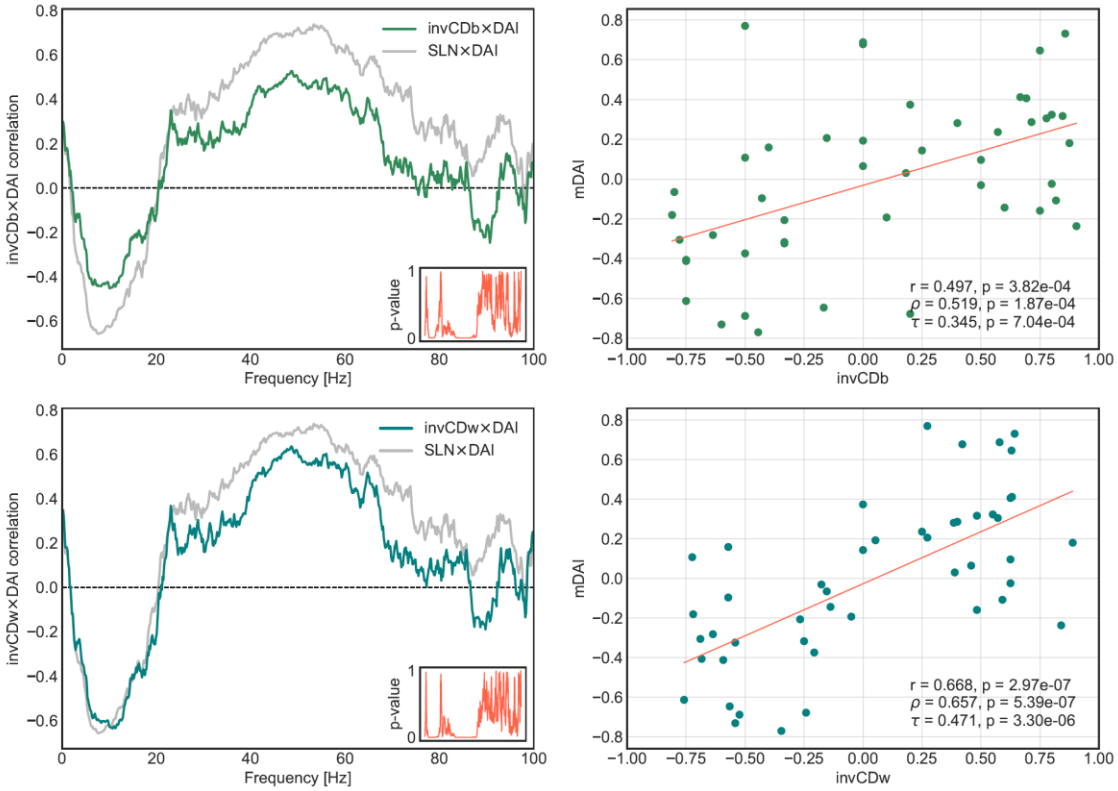

Figure 3.4. Relationship between the DAI (an index of directed interactions computed on the basis of spectral Granger causality) and the inverse binary CD (top) and the inverse weighted CD (bottom) for the 8×8 subgraph. *Left*: Spearman rank correlations for the invCDs and the DAI as functions of frequency. The insets show the p-values; there is a significant negative correlation in the alpha-band and a significant positive correlation in the gamma-band for both invCDs. Remarkably, the weighted CD shows almost the

exact level of correlation in the alpha-band (for feedback connections), falling short only in the gamma-band (for feedforward connections). *Right*: correlation between the invCDs and the mDAI. The legends show the correlation coefficients and their corresponding p-values in the same manner. All correlations are significant ($p < 10-4$ for the binary and $p < 10-6$ for the weighted case).

4) STRUCTURAL CORRELATES OF SIGNAL PROPAGATION AND TRANSMISSION IN THE CEREBRAL CORTEX

While it was not included in the aims we performed studies on the structural correlates of cortical signal transmission within the hierarchical circuitry (including long range intrinsic and inter-areal connections) of areas 3b and 1 of the squirrel monkey with the support of the grant.

At the level of synapse, we applied 3D electron microscopic reconstructions and quantitative comparisons to study ultrastructural properties of synapses relevant to signal transmission dynamics in the hierarchical circuitry of the primate somatosensory cortex. Quantitative analyses of the 3D ultrastructure of synapses indicated that cortical pathways form two kinds of synaptic contacts: one with structural properties permitting signal transmission at high fidelity, and another with morphological properties of the modulatory type. These findings are crucial in understanding the dynamics of interactions of the different hierarchically organized cortical pathways. The results have been published in the Eur J Neurosci (Ashaber et al. 2020).

At the population level, we began studying the structural correlates of activity propagation and dissemination of information in the somatosensory cortex by way of 3D axonal reconstructions. We aimed to shed light on the importance of the modular organization of signal transmission in the cerebral cortex. Based on several morphometric measures our preliminary findings indicate that axon arborization patches compared to out-patch regions, are specific sites of convergence of efferents formed by neuronal populations with shared functional representation. Axonal patches are sites (modules) of intense axonal and bouton convergence, which could play fundamental role in cortical communication dynamics and supports the significance of population activities. The results have been presented in the form of posters in international conferences (Mir et al. 2020a,b).

LITERATURE

Bastos, AM et al. 2015, Neuron, 85, 390–401.
Mejias, JF et al., 2016, Science Advances, 2 (11) e1601335.
Ye, H et al., 2015, Sci. Reports 5, 14750.