P3/p1-234

PDF:

Functional connectome changes in an acute ketamine model of schizophrenia in rat.

Fedor, Flóra Zsófia[1,2,5]; Cserpán, Dorottya[3]; Molnár, Laura [2,4]; Hetényi, Dorottya [2,4]; Juhász, Gábor [5]; Fekete, Zoltán[6]; Somogyvári, Zoltán[3]; Borhegyi, Zsolt [2,5]

1, University of Pannonia, 8200 Veszprém, Egyetem st. 10., HUNGARY
2, MTA-TKI, MTA-ELTE-NAP B-Opto-Neuropharmacology Group, 1117 Budapest, Pázmány Péter sétány 1/c HUNGARY
3, MTA Wigner RCP Theoretical Neuroscience and Complex Systems Research Group,1121 Budapest, Konkoly-Thege Miklós st. 29-33. HUNGARY
4, Eötvös Loránd University, Faculty of Science, 1117 Budapest, Pázmány Péter sétány 1/c HUNGARY
5, Eötvös Loránd University, Laboratory of Proteomics, Institute of Biology, 1117 Budapest HUNGARY
6, MTA EK NAP B, Research Group for Implantable Microsystems Budapest HUNGARY

(correspondence to: zsborhegyi@gmail.com)

Foil matrix electrode on the dorsal cortex of the rat

M1 – Primary motor cortex
M2 – Secondary motor cortex
S1J – Primary somatosensory cortex, jaw region
S1FL – Primary somatosensory cortex, forelimb region
S1ULp – Primary somatosensory cortex, upper lip region
S1Sh – Primary somatosensory cortex, shoulder region
S1Tr – Primary somatosensory cortex, trunk region
S1BF – Primary somatosensory cortex, barrel field
S1 – Primary somatosensory cortex
MPtA – medial parietal association cortex
LPtA – lateral parietal association cortex
PtP – Parietal cortex, posterior area
V2 – secondary visual cortex
V1 – primary visual cortex
A – auditory areas
RSD – retrosplenial dysgranular cortex

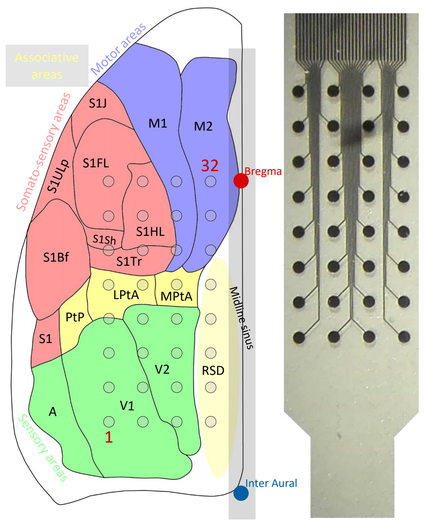

Fig. 1: A 32 channel 4 by 8 grid electrode alignment is shown on the dorsal surface of the rat. A photograph of the foil electrode is shown on the right while its schematic position used in all of our experiments can be seen in the middle. On the drawing colors label different functional areas, while the names of individual cortical structures shown by short version of their names are listed on the left. Midline sinus and skull reference point are also indicated. The channel numbering and position applies to all of our experiments. (The cortical surface map is based on the original reconstruction of Eszter Papp.)

The spectral comparison of normal EEG with ketamine induced EEG.

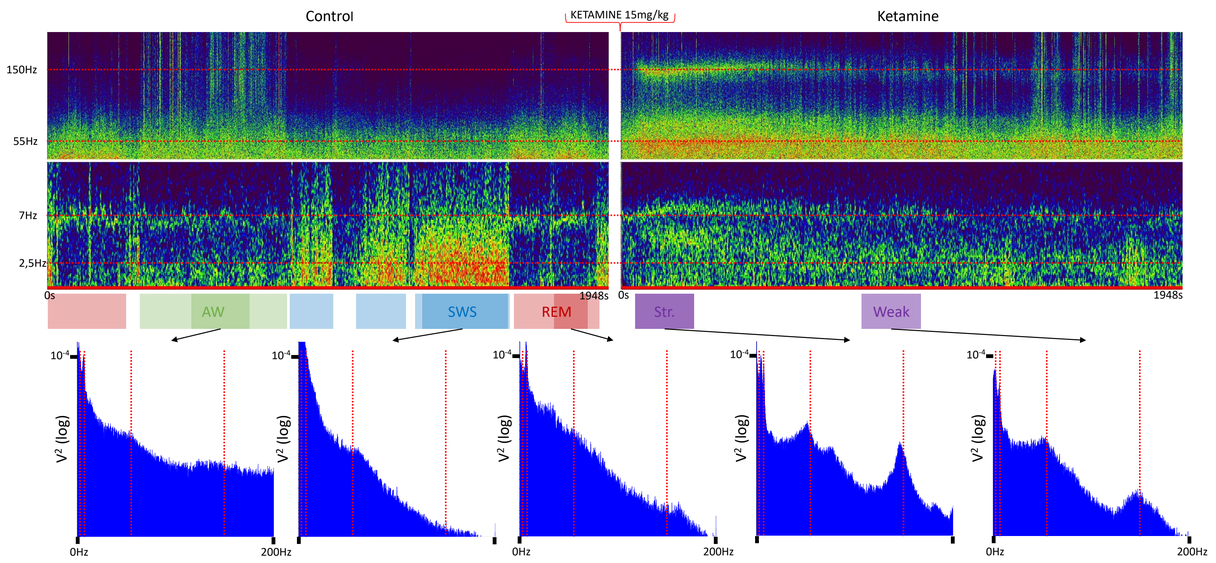

Fig. 2: Sonograms of control and ketamine injected (15mg/kg) state are shown on top left and right panes. Dashed red lines are labeling the middle of those EEG frequency ranges that showed clear disturbances in response to ketamine. Below the Control sonogram color codec areas labels the identified behavioral states (Green – Awake; Blue – Slow wave sleep; Red – REM sleep), and darker areas to indicate the parts used in analyses. Purple areas are labeling the time ranges after ketamine injection also used in analyses (Str. – Strong (early) ketamine effect; Weak (late) ketamine effect). The bottom shows the power density spectra of the labeled time ranges on logarithmic scale and the dashed red lines labels the same frequencies than on the sonograms. The sonograms were made with Spike 2 (upper two: top dB – 26, range dB – 24, block size – 8192; lower two: top dB – 42, range dB – 24, block size - 32768).

Effect of ketamine on the clustered coherence maps on the cortical surface

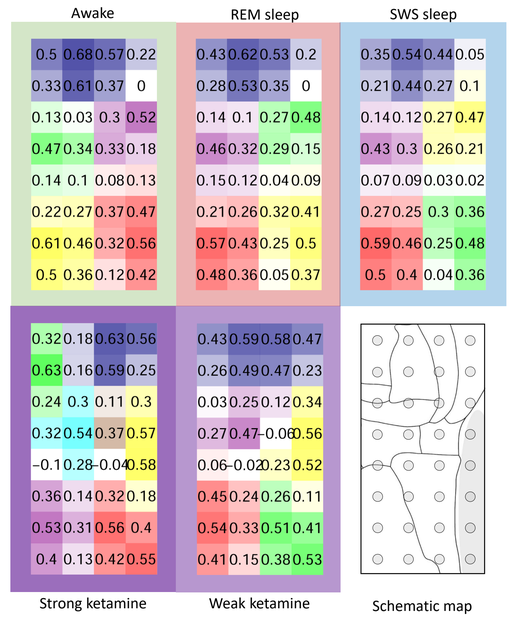

Fig. 3: The clustered coherence maps, despite the different color assignment shows great stability in its structure, following the anatomical/functional structure of the cortex. The cluster quality was highest at 5 clusters in all cases except the Strong (early) ketamine effect where 7 cluster can be verified. Colored borders indicate the EEG segments labeled in Fig. 2.

Spectral density maps of the cortical surface

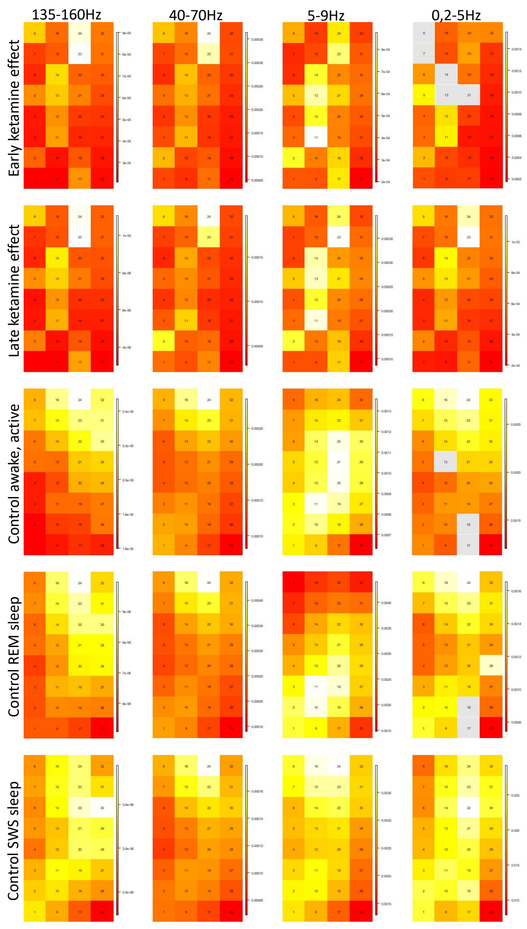

Fig. 4: The power spectrum values of EEG frequency bands (columns) were calculated and color-coded to show their distribution over the cortical surface in different brain states and ketamine of the rat. While the gradual power changes are dominating the drug free control brain states (bottom 3 rows), they show rather fragmented, mosaic like distribution is seen when ketamine is involved (top two rows). Individual color scales are shown on the right of all maps.

Modulating effect of slow EEG (<5Hz) on high frequency oscillation (~150Hz)

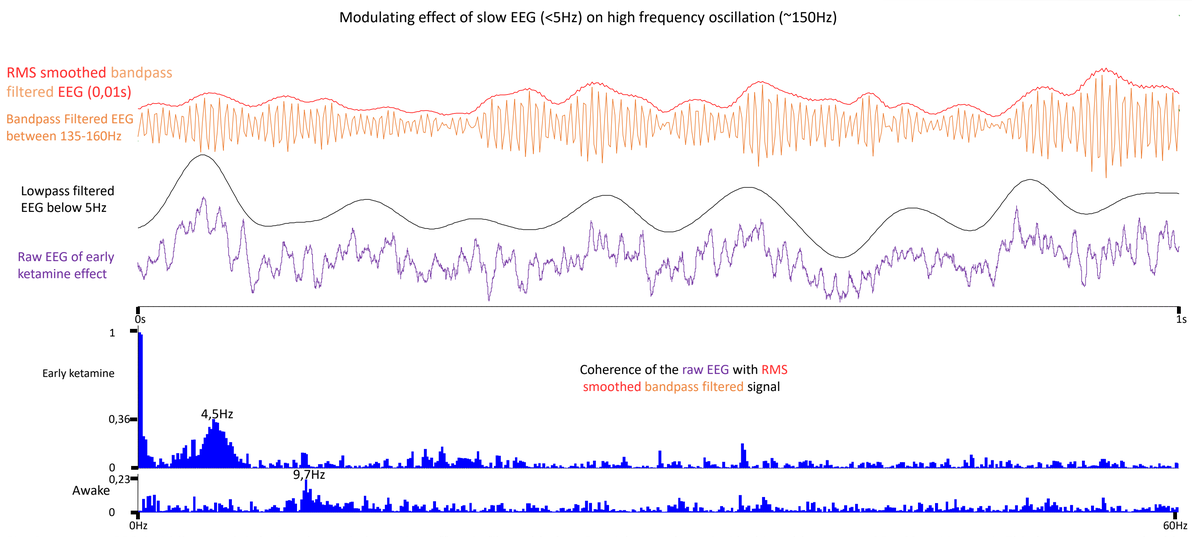

Fig. 5: A 1s raw EEG (middle, purple) during the early ketamine effect is filtered below 5Hz (shown above in Black) and between 135-160Hz (Orange). This later was smoothed by root mean square function (Spike2, 0,01s, Red). The coherence between the smoothed high frequency oscillation and the original EEG is shown below the raw EEG in blue, between 0-60Hz. As a reference the coherence of the same process from a 1s normal, awake EEG is shown below.

Traveling of flash light evoked response is altered by Ketamine

Fig. 6: Visually evoked potentials from 32 channels (0,2Hz, 1ms wide LED flash behind contralateral eye bulb) has been averaged from control as well as ketamine injected state of a rat. Top chart shows responses from 8 example channel (their position is shown on the satellite image), the control in blue and the ketamine effected in red, overlapped.
Below, KCSD plots of 4-4 time points (5ms wide) is shown, and the corresponding clustered coherence ma is shown on right. Note the differences in the spreading of the VEP on the KCSD, and the delicate differences on the coherence map.

State dependency of causal interactions between brain areas

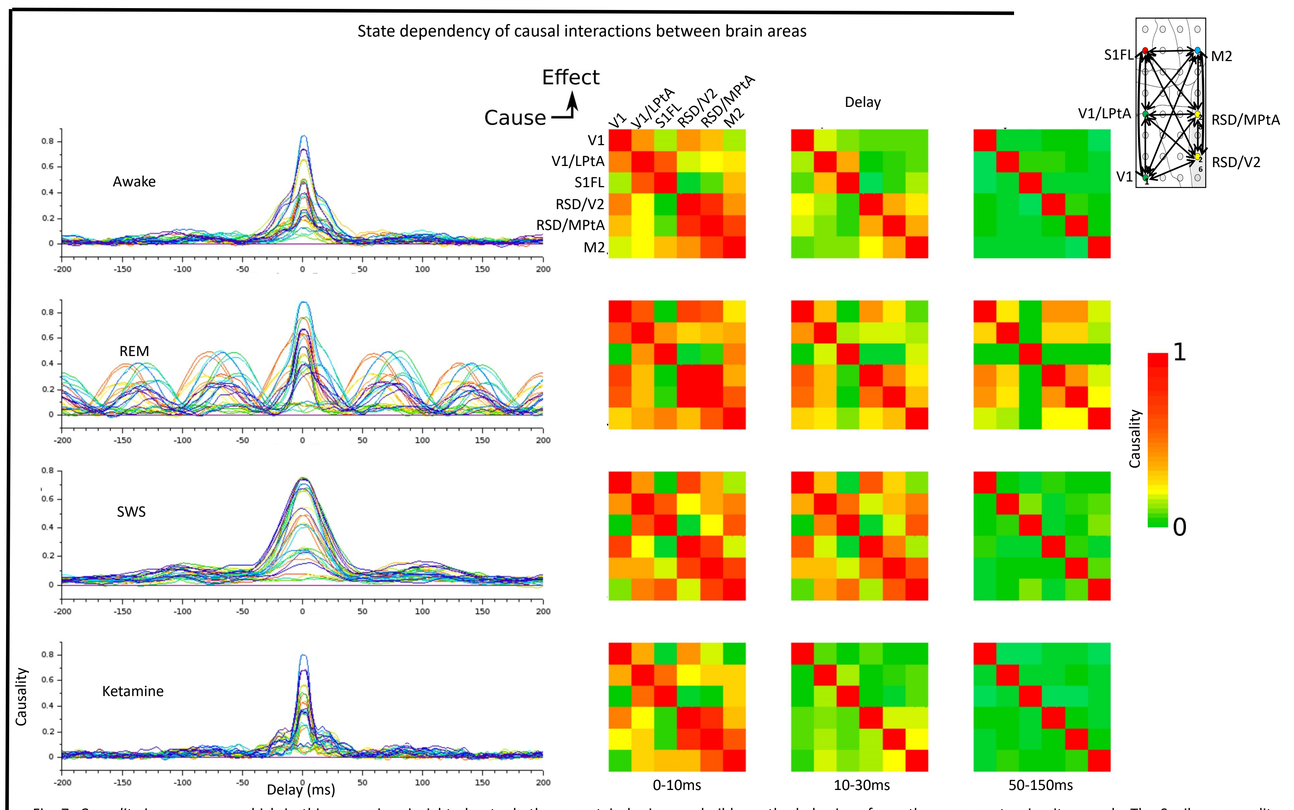

Fig. 7: *Causality* is a measure, which in this case, gives insight about whether a certain brain area builds on the behavior of an other area, or turning it around. The Sugihara causality [Sugihara, George, et al. "Detecting causality in complex ecosystems." science338.6106 (2012): 496-500.] has a value close to 1, when one part of an interacting system is a strong cause of the other. For the causality analysis the kCSD time series from 6 locations , which were the representatives of their cluster, were used. As causality is a directed measure this means 36 relationships. On the left part of the figure the causality values with certain time delays for each pairs having different colors are shown in case of awake, REM, SWS and ketamine protocols. Based on the peaks on this figure 3 time windows of interest were selected: 0-10, 10-30 and 50-150ms. The maximal causality values between the 6 areas (V1,V1/LPtA, S1FL,RSD/V2,RSD/MPtA,M2) are visible, where On the right side of the figure the maximal causality values within these time frames are presented as color coded images. At first sight the figures seem to be symmetric meaning circular relationship, but there are exceptions as well. In case of the ketamine paradigm causal interactions vanish with narrow time delays.

Summary of results

Ketamine induced disoriented behavior in rat is paralleled with widespread spectral changes in EEG. Beside the characteristic high frequency oscillation around 150Hz, other EEG bands are also affected.

The clustered coherence map follows the basic anatomical/functional map of the cortical surface in control and with fine changes in ketamine induced state as well, demonstrating high level of stability in different brainstates and resistivity to disturbances.

Interestingly, in the background of the stable coherence map, there is a disturbed frequency power distribution over the cortex, when ketamine is affecting the brain, as well as when the animal is in different states like awake, REM or SW sleeping.

A moderate slow frequency modulation of high frequency oscillation has been found during strong ketamine effect.

Visually evoked potential resulted in strikingly different activity pattern shown by the kCSD plots, and moderate changes in the clustered coherence map during ketamine in effect.

Causality analysis revealed interesting connections among the clusters defined by the clustered coherence map. REM sleep has causality in a wider time window then any other states, and the opposite is true for the ketamine influenced brain.

Conclusion

There is a highly disturbed frequency power distribution in the background of the fairly stable coherence map when ketamine is injected. This can influence synchronicity relations among brain areas causing the disorganized behavior. This may explain the same behavioral signs in decreased (schizophrenia) or increased (ketamine) gamma power states.

Supported by:
FIEK_16-1-2016-0005; MTA-ELTE-NAP-B 888810; KTIA_NAP-B-13-2-2015-0004
ERA-NET FLAG-ERA, Human Brain Project, NKFIH NN-118902: CANON project and National Research, Development and Innovation Office NKFIH under grant no. K-113147 and National Brain Research Program grant no. KTIA_13_NAP-A-IV/1,2,3,4,6.

Introduction

Schizophrenia is a chronic developmental connectome disease that has positive symptoms like delusions, hallucinations and negative symptoms like decreased emotional responsiveness, poverty of speech. Because of limitations of human experimentation it is a challenging task to establish animal models for studies of cellular and network changes behind such a human mental disorder of cognitive functions cannot observed in animals. The animal models of psychiatric disorders usually reliable models of the human disease in certain sense and very different in others. The already known cellular mechanism contributes to Schizophrenia is the decrease in number of NMDA receptors on fast spiking GABAergic cortical interneurons resulting decreased electric activity and reduced power of gamma oscillations. Reduction in gamma oscillation coupling has been observed in human Schizophrenia subjects and interpreted as electrophysiological marker of altered connectome. Since reduced NMDA mediated transmission is a hallmark of Schizophrenia the sustained application of an NMDA receptor antagonists as MK801 or ketamine application in subanaesthetic dose induce schizophrenia like symptoms in humans and has seemingly similar electrophysiological effects in rats (Krystal et al., 1994). So it is a model of human Schizophrenia in electrophysiological sense and could be investigated invasively in rodent models. Brain mapping studies on human Schizophrenia subjects revealed that connectivity map of the cortex indexed by gamma oscillation power distribution as well as phase coherence distribution changes as cognitive symptoms develop. EEG mapping uses 126 or more electrodes on a human brain but it is hard to make such a high resolution EEG study in rodents because of technical limitations. Recently we developed a thin-layer plastic foil electrode manufactured by MAMS technology allowed lithographic printing high density electrodes for rats. The novel electrode construction allowed a good contact of the platinum electrodes with the cortical surface and we printed a 32 electrode matrix in a 12x4 mm strip which covers the rat cortex from the primary visual cortex to the frontal one. The electrode arrangement allowed more than one electrodes on each functionally distinct cortical areas so we were able to make a rodent EEG mapping record similar to the human EEG mapping.

The presented study aims to uncover electrophysiological signs of connectivity changes in the rat cortex after administration of low doses Ketamine. Applying power spectra distribution analysis and phase coherence mapping we demonstrate the general and fundamental changes in the cortical connectome in parallel with the behavioral symptoms of sub-anesthetic dose of Ketamine with particular attention to the special and temporal distribution of a transient 150 Hz oscillation.

Methods

Polymer electrode assembly: The polymer foil electrode is made of a platinum film covered by a polyimide foil (4 micron) and another 4 microns thick polyimide layer that has a passivating function. The leads from the electrode discs 15+270 nm thick titanium and platinum layer. The amount of material above the contacts and wiring were removed. The 32 contact sites are aligned in a 4*8 grid. The sites are 1mm from each other, and one contact is 300 micrometer in diameter.
Electrode implantation: Wistar rats (350–500 g) were used in this study. The animals were housed in groups of 2–4 per cage (diurnal cycle, lights on from 9:00 to 18:00), with ad libitum access to food pellets and water. Rats were anaesthetized with Isoflurane (0.5-1 %, Forane, Abbott), and mounted in a stereotaxic frame. The skull was exposed and cleaned, than the skull bone was thinned to provide a suitable surface for the electrode implantation. Three stabilizing screws were fixed around the thinned bone surface at anterior, posterior, and medial directions. Than the thin bone surface was folded up (toward the medial direction) and the electrode was positioned so that all of the contacts had direct contact with the brain surface. The 25th contact was positioned 0.5-1 mm lateral, and in line with the Bregma and the longitudinal axis of the assembly was parallel to the mid-sagittal line. After the proper positioning the bone was folded back to the top of the electrode to give protection and insulation to the assembly. To stabilize the bone surface, an UV-bound cement (TetricEvoFlow, IvoclarVivadent; Liechtenstein) was used to fix the bone surface above the foil electrode and the skull was covered by dentacrylic cement (GC America Inc.,USA) which also hold the sockets and covered the screws. A screw ground electrode was placed above the cerebellum. A 5 mm diameter red LED (Bright LED Hongkong Ltd.) as a light source was placed retrobulbally that had minimal contact with the tissue (Szabo-Salfay, Palhalmi et all., 2001). After surgery, antibiotics were given to the animal (2,5% Baytril, Bayer Hungária Ltd., Hungary). Animals were allowed to recover at least for 3 days after implantation.
Experiments: During the experiments, animals (n=11) were housed in 12-12 hours light-dark cycle using 500 lux light. Electrophysiological measurements were made in a closed Faraday cage in darkness (under 0.17 msd), using a 32 channel Amplipex KJE-1001 (Amplipex Ltd., Hungary) amplifier. The behavior of the animals was recorded with camera and their movements was monitored with a 3D accelerometer (Supertech). During the stimulation with LED the animal was exposed by a series of 0,2 Hz stimuli after 10 minutes of dark adaptation. Spike2 (Cambridge Electronics Design Limited, UK) program was used to give stimuli (1ms wide, 100 / session) via BioStim stimulator (SupertechLtd., Hungary). Ketamine (Calypsol, in 15 mg/kg or 50mg/kg dose) was injected intraperitoneally followed by the recording of the EEG for at least two hours. EEG recording was made on 36 channels (32 for the electrode, 1 for the stimulus events and 3 for the accelerometers) at 20 kHz sampling rate using AmpliRec (Amplipex Ltd.) program.
Analysis: The power map was calculated by averaging the squared amplitudes for each channel separately for a certain time window of the raw or the filtered signal.
kCSD and clustered coherence: In this case coherence [https://en.wikipedia.org/wiki/Coherence_(signal_processing)] can be interpreted as a measure, which informs about how much populations are co-working. Between two time series a value close to 1 means very similar patterns with a certain delay in time, a value close to 0 means negligible relationship. For the purpose of estimating relationships between populations, we calculated the kernel Current Source Density at the locations of the electrode, as it is a more direct measure of the underlying neural mechanisms [Potworowski, Jan, et al. "Kernel current source density method." Neural computation 24.2 (2012): 541-575.] . Calculating the coherence between all the kCSD values related to the population of neurons close to the certain electrodes resulted in a table informing about the similarities between the functional behavior of the neuron populations. By applying the partitioning around medoids clustering algorithm [Kaufman, Leonard, and Peter J. Rousseeuw. Finding groups in data: an introduction to cluster analysis. Vol. 344. John Wiley & Sons, 2009.] we aimed to identify the functionally cooperating brain areas. The reasonable number of clusters were selected by the average silhouette width measure. Global and local maxima values were considered as meaningful for indicating the number of optimal clusters with an expectation to be greater than 0.4. The resulted map of functional brain areas might be used for estimating the anatomical brain areas and show changes in electrophysiological behavior due to various brain states or drugs. The power map, kCSD, and clustered coherence were done by using BrainAreaR software made by Dorottya Cserpán (Poster number: P1/p3-103)

Dorottya Cserpán's poster: