Detailed scientific report of OTKA K119443 (October 2016 – September 2021)

Investigation of cortical synchronous population activity in patients with epilepsy or tumour.

During the 5 years of the project, we concentrated on several different subprojects to describe the special characteristics of oscillatory activities occurring in the human brain. Altogether, we published ten articles concerning the topic of the project (list of publication is at the end), nine in international scientific journals, one in a Hungarian journal. Furthermore, two PhD students defended their theses during this period, from work done within this project.

BACKGROUND

Epilepsy is one of the most common neurological disorders in humans and is often associated with serious cognitive deficits. The epileptic cortex can generate paroxysmal activity characterised by the hypersynchronous and wide-spread neuronal firing: seizures and interictal spikes between seizures. Interictal spikes recorded from the scalp of humans are considered to be hallmarks of epileptic activity. They consist of high-amplitude, fast EEG transients, or spikes, followed by a slow wave of duration several hundreds of milliseconds (de Curtis and Avanzini, 2001). Spontaneous interictal-like activity can be recorded in slices of epileptic human neocortex (Kohling et al., 1998; Pallud et al., 2014) and hippocampus (Cohen et al., 2002; Wozny et al., 2005; Huberfeld et al., 2007; Wittner et al., 2009), perfused with a physiological solution. The waveform of interictal-like events recorded from slices of human hippocampal and neocortical tissue from epileptic patients shows some similarities to interictal spikes recorded in vivo (Cohen et al., 2002), when band-pass filtered as the EEG (1-100 Hz). However, our preliminary data suggested that a synchronous population activity, similar to previously described interictal-like events, is also generated in slices derived from patients with tumour but without epilepsy. We aimed to reveal the characteristics and generation mechanisms of these synchronous population events and examine their relationship to epilepsy. Furthermore, as we use similar linear microelectrode and recording setup for in vivo and in vitro recordings, we could also relate our experimental results to the findings derived from living, freely moving patients. We thus focussed on other relevant synchronous activities of the human cortex, such as alpha rhythms, sleep spindles and epilepsy-related high frequency oscillations. Understanding the synchrony initiation mechanisms within the human cortical neuronal populations can help us to better understand the functioning of the human brain during physiological and pathological conditions. Furthermore, describing the mechanisms specific to humans can reveal the characteristics of the human disease and might help to develop new therapeutic approaches and more realistic animal or pharmacological models.

RESULTS

1. Synchronous population activity and interictal epileptic discharge in the human neocortex

We investigated the spontaneously occurring synchronous population activity (SPA) and interictal epileptic discharge (IED) in human neocortical slice preparations derived from epileptic and non-

epileptic tumour patients. In our first study published in this topic, in the **Journal of Physiology (Tóth et al., 2018)** we described that the human neocortex could generate two different types of synchronies: SPA and IED. IED was generated only in epileptic tissue, whereas SPA was found both in epileptic and non-epileptic tissue. The electrophysiological properties, such as recurrence frequency, local field potential and multiple unit activity amplitudes, high frequency oscillations of IEDs were different from those of SPAs. SPAs occurring in epileptic and non-epileptic tissues were similar, however, the epileptic hyperexcitability could be demonstrated in the fine details. We showed with intracellular recordings, that more cells were depolarized during SPA in epileptic than in tumour tissue. Furthermore, the density of excitatory synapses was significantly higher in epileptic vs. in non-epileptic tissue, while no difference could be shown in the density of inhibitory synapses. We concluded that since both non-epileptic and epileptic tissue can generate SPA, this type of synchronous activity might not be pathological. In contrast, in vitro spontaneously occurring IEDs resemble to interictal spikes are considered to be epileptiform events.

2. Initiation mechanisms of SPA and IED

In our further study we investigated the participation of excitatory and inhibitory cells in the generation of SPA and IED. This article is accepted in the journal **Scientific Reports (Hofer et al., 2022)**. First, we demonstrated with intraoperative electrocorticography (ECoG) that interictal spikes occur in vivo mostly in epileptic patients, and rarely in tumour patients. We correlated the occurrence of interictal spikes in the anaesthetised patients with the generation of SPA in vitro and could demonstrate that SPA can occur in tumour patients who did not show any spiking activity in the resected neocortical area. With this analysis we showed that SPAs are indeed non-epileptiform activities, as supposed in our first paper (Tóth et al., 2018). In this study we performed in vivo chronic recordings as well in the neocortex of epileptic patients and could identify similar electrophysiological events to in vitro occurring SPAs and IEDs. Both types of synchronous activities showed similar waveform in the depth of the neocortex in vivo and in vitro. Derived from in vitro recordings, we clustered hundreds of cells both from epileptic and tumour tissues, and divided the cells into excitatory and inhibitory groups, based on their action potential waveform and discharge properties. We found that both inhibitory and excitatory cells fire during both SPAs and IEDs. While SPAs are constituted of a symmetrical build up and decline of their firing, IEDs are asymmetrical: excitatory cells tend to fire at the beginning, and inhibitory cells at the end of the events. We could also show that the bursting behaviour has an important role in the generation of SPA. Although the overall burstiness of all cells was significantly lower in epileptic tissue, the firing of the intrinsically bursting pyramidal cells was considerably linked to the ascending and peak phases of the IEDs. This could not be showed in case of SPAs, where regular firing pyramidal cells and inhibitory interneurons were showed to fire heavily during the peak of the events. We concluded that SPA is the result of a balanced firing of excitatory and inhibitory cells, while IEDs are possibly initiated most probably by bursting pyramidal cells.

3. Disinhibition induced epileptic activity in the human neocortex

In our next study we investigated the epileptiform events induced by the blockade of the GABAergic inhibition. The use of the GABAa receptor blocker bicucculline induced the appearance of both IEDs and seizures. This work was published in the **Journal of Physiology in 2019 (Kandrács et al., 2019)**. We showed that both epileptic and non-epileptic human neocortical tissue generated both IEDs and seizures, however, several different forms of IEDs appeared in the epileptic tissue, whereas they

were uniform in the non-epileptic tissue. We differentiated between spatially and temporally simple and complex IEDs. All different variants of these IEDs, as well as spatially more restricted IEDs appeared in epileptic samples, while in slices derived from tumour patients only generated spatially and temporally simple IEDs. Seizures were several to tens of seconds long epileptic events, which appeared at significantly higher probability in epileptic than in non-epileptic tissue. With cell clustering methods we showed that SPA can be initiated by either excitatory or inhibitory cells, with a slightly higher overall firing of excitatory cells. In contrast, disinhibition induced IEDs and seizures were mainly initiated by the heavy firing of inhibitory cells. In all induced epileptic events bursting pyramidal cells always followed the firing of other cell types. This contrasts to the finding of our other paper, i.e., bursting pyramidal neurons initiate spontaneously occurring IEDs, and draw attention to the cautious use of pharmacological models to the description of the human disease. We also concluded that the epileptic human neocortical tissue has "hubs" able to generate epileptiform events, which might be the result of the epileptic reorganisation.

4. Mathematical model to investigate neuronal firing

Closed-loop neurostimulation is a newly developing method to interrupt or even prevent the generation of epileptic seizures. The successful application of this technique requires a reliable seizure prediction, which could be based on the detection of single cells. However, when clustering single neurons in extracellular recordings we faced the problem how the AP shape changes when an epileptic seizure arises. At the onset of seizures, a large population of neurons start to fire with a high frequency, therefore, the shape of the single units becomes uncertain, since it is masked by a multitude of other cells' APs. We developed a mathematical model to investigate the firing pattern of individual neurons. We implemented and extended the results of Ozaki concerning the maximum likelihood estimation of Hawkes processes with exponential response function. We tested this algorithm on real dataset, and verified its accuracy using a large, simulated dataset. We published an article about this topic in the Hungarian journal **Alkalmazott Matematikai Lapok (Perczel et al., 2019)**.

5. Propagation of alpha rhythms in the human neocortex

During the five years of the project, we also analysed the data obtained with the previously implanted intracortical linear microelectrodes. These electrodes and the recording system is very similar to the one used in vitro experiments. We analysed alpha rhythms in humans during quiet wakefulness. We found that alpha rhythm both in visual and somatosensory cortex propagates from higher-order to lower-order areas. In posterior cortex, alpha propagates from higher-order anterosuperior areas towards the occipital pole, whereas alpha in somatosensory cortex propagates from associative regions towards primary cortex. Furthermore, alpha is dominated by transmembrane currents and cell firing in the supragranular cortical layers. Together, these results suggest that the alpha rhythm likely reflects short-range supragranular feedback which propagates from higher to lower-order cortex and from cortex to thalamus. These physiological insights suggest how alpha could mediate feedback throughout the thalamocortical system. We published one paper describing this project in collaboration with the laboratory of Prof. Eric Halgren (UCSD, San Diego, USA) and Sydney S. Cash (MGH, Harvard Medical School, Boston, MA, USA) in the **Journal of Neuroscience (Hagler et al., 2018)**.

6. Sleep spindles in the human neocortex

Furthermore, we recorded sleep spindles (10-16 Hz oscillations) in human NREM sleep, from different cortical layers, with the same intracortical linear microelectrodes. Two patterns of spindles were distinguished based on their spatial generation mechanisms. Spindles mainly appeared in the supragranular and granular layers, and rarely invaded the infragranular layers. They were confined either to supragranular or granular layers, but in about half of the cases they spread to both layers. Current density analysis demonstrated that the supragranular spindles were generated by supragranular pyramidal cells, whereas granular spindles were initiated by infragranular pyramidal cells. We verified the location of the microelectrode within the neocortex with post hoc histology. We published our paper describing this project in collaboration with the laboratory of Prof. Eric Halgren (UCSD, San Diego, USA) in the journal **Proc Natl Acad Sci U S A (Halgren at al., 2019).** We further analysed the laminar profile of the sleep spindles. We made simultaneous ECoG and linear microelectrode recordings. Many spindles detected on the microelectrode occurred only in one layer and were absent from the ECoG, but with increasing amplitude simultaneous detection in other layers and on the ECoG became more likely. ECoG spindles were in contrast usually accompanied by microelectrode spindles. We could not show compelling evidence that different spindle types are associated with different laminar profiles. We concluded that spindles are generated in cortical and thalamic circuits with similar cortical innervation patterns. We published this study in the journal **NeuroImage (Ujma et al., 2021)**.

7. Perisomatic inhibition and its role in epilepsy and in synchrony generation

The perisomatic inhibition was further analysed in the human epileptic hippocampus during this period, in collaboration with Dr. Zsófia Maglóczky (MTA KOKI). Our earlier work has been completed with new, more detailed electron microscopic analysis of the parvalbumin-containing neurons located in the human hippocampal CA1 and CA2 region. Furthermore, another population of basket cells, the cannabinoid receptor containing interneurons were investigated to complete the picture about the perisomatic inhibition. These results were published in **BioMed Research International (Wittner and Maglóczky, 2017)**. We completed this research with the electrophysiological and pharmacological investigation of the parvalbumin and cannabinoid receptor-containing interneurons in the human epileptic and non-epileptic neocortex. We found that although these cells participate in the generation of SPA, they do not have any leading role in the initiation of the synchronous events. In line with the pharmacological studies, we investigated how the two perisomatic basket cell types innervate layer 2/3 pyramidal cells, the neuron group which predominantly participates in SPAs. In an electron microscopic study, we analysed the synaptic coverage of the pyramidal cell bodies in regions generating and non-generating SPA both in non-epileptic and in epileptic human tissues. We could not demonstrate any difference between regions generating and lacking SPA, neither between epileptic and non-epileptic tissue. The only difference we found was that axon terminals of parvalbumin-positive basket cells became smaller in number but larger in regions generating SPA in the epileptic tissue. This study was published in 2021, in the **International Journal of Molecular Sciences (Tóth et al., 2021)**.

8. Electrically evoked ripple activity in the human hippocampus

In this project we aimed to examine the laminar distribution of high frequency oscillations in the hippocampus evoked by single pulse electrical stimulation. We published this study in 2021, in

Epilepsy Research (Tóth et al., 2021). Intraoperative recordings were made from anaesthetized epileptic patients from the hippocampus with the aid of our laminar microelectrode, while stimulating the parahippocampal gyrus of the temporal neocortex. Single pulse stimulation evoked high frequency oscillations in all examined hippocampal regions, i.e., cornu Ammonis 2-3 (CA2-3), dentate gyrus and subiculum. The evoked high frequency oscillations were not uniform, but rather the combination of ripples, fast ripples, sharp transients and multiple unit activity. The occurrence of fast ripples showed a relationship with the degree of hippocampal sclerosis: they were generated with lower probability in the CA2-3 region and higher probability in the subiculum if the sclerosis was severe. We concluded that the subiculum was active, producing high frequency oscillations in parallel with the cell loss in the hippocampus, which emphasizes the role of this region in the generation of epileptic activity.

PUBLICATIONS

Conference talk:

Wittner L (2020) Physiological and pathological synchronies in the human neocortex, in vitro. **IBRO Workshop**, 29-30 January 2020, Szeged, Hungary

PhD Thesis

Hofer K (2017) Investigation of cortical synchronous activity of neuronal populations, in vitro. PhD dissertation, Pázmány Péter Catholic University, Faculty of Information Technology ad Bionics, Doctoral School, 13th of December 2017. Supervisors: Lucia Wittner and István Ulbert

Kandrács Á (2020) Spontaneously occurring and disinhibition-induced synchronous activities in the human neocortex, in vitro. PhD dissertation, Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Doctoral School, 21st of September 2020. Supervisor: Lucia Wittner

Articles:

Wittner L and Maglóczky Z (2017) Synaptic Reorganization of the Perisomatic Inhibitory Network in Hippocampi of Temporal Lobe Epileptic Patients. **BioMed Research International** Volume 2017, Article ID 7154295, 13 pages https://doi.org/10.1155/2017/7154295

Hagler DJ Jr, Ulbert I, Wittner L, Erőss L, Madsen JR, Devinsky O, Doyle W, Fabó D, Cash SS, Halgren E. Heterogeneous Origins of Human Sleep Spindles in Different Cortical Layers. **J Neurosci**. 2018 Mar 21;38(12):3013-3025.

Tóth K*, Hofer KT*, Kandrács Á, Entz L, Bagó A, Erőss L, Jordán Z, Nagy G, Sólyom A, Fabó D, Ulbert I and Wittner L (2017) Hyperexcitability of the network contributes to synchronization processes in the human epileptic neocortex. **J Physiol** (London) 2018 Jan 15;596(2):317-342.

Halgren M, Ulbert I, Bastuji H, Fabó D, Erőss L, Rey M, Devinsky O, Doyle WK, Mak-McCully R, Halgren E, Wittner L, Chauvel P, Heit G, Eskandar E, Mandell A, Cash SS (2019) The generation and propagation of the human alpha rhythm. **Proc Natl Acad Sci U S A**. 2019 Nov 19;116(47):23772-23782. doi: 10.1073/pnas.1913092116. Epub 2019 Nov 4.

Perczel Gy, Erőss L, Fabó D, Gerencsér L, Hajnal B, Szabó Cs, Vágó Zs, Wittner L: Epilepsziás agyi hálózatok egysejt-aktivitásának jellemzése Hawkes-folyamatok segítségével, **ALKALMAZOTT MATEMATIKAI LAPOK** 36: (2) pp. 213-224., 2019

Kandrács Á, Hofer KT, Tóth K, Tóth EZ, Entz L, Bagó AG, Erőss L, Jordán Z, Nagy G, Fabó D, Ulbert I, Wittner L. (2019) Presence of synchrony-generating hubs in the human epileptic neocortex. **J Physiol.** 2019 Dec;597(23):5639-5670. doi: 10.1113/JP278499.

Tóth EZ, Szabó FG, Kandrács Á, Molnár NO, Nagy G, Bagó AG, Erőss L, Fabó D, Hajnal B, Rácz B, Wittner L, Ulbert I, Tóth K (2021) Perisomatic inhibition and its relation to epilepsy and to synchrony generation in the human neocortex. **Int J Mol Sci** 2021 Dec 24;23(1):202. doi: 10.3390/ijms23010202

Ujma PP, Hajnal B, Bódizs R, Gombos F, Erőss L, Wittner L, Halgren E, Cash SS, Ulbert I, Fabó D (2021) The laminar profile of sleep spindles in humans. **NeuroImage** 226 (2021) 117587. https://doi.org/10.1016/j.neuroimage.2020.117587

Tóth E*, Bokodi V*, Somogyvári Z, Maglóczky Z, Wittner L, Ulbert I, Erőss L, Fabó D (2021) Laminar distribution of electrically evoked hippocampal short latency ripple activity highlights the importance of the subiculum in vivo in human epilepsy, an intraoperative study. **Epilepsy Research** 169 (2021) 106509 https://doi.org/10.1016/j.eplepsyres.2020.106509

Hofer KT, Kandrács Á, Tóth K, Hajnal B, Bokodi V, Tóth EZ, Erőss L, Entz L, Bagó AG, Fabó D, Ulbert I, Wittner L (2022) Bursting of excitatory cells is linked to interictal epileptic discharge generation in humans. **Scientific reports** 2022 (accepted)