Our first results were published in The Journal of Physiology (J Physiol. 2018 Jan 15;596(2):317-342. doi: 10.1113/JP275413).

> "Tóth K, Hofer KT, Kandrács Á, Entz L, Bagó A, Erőss L, Jordán Z, Nagy G, Sólyom A, Fabó D, Ulbert I, Wittner L. *Hyperexcitability of the network contributes to synchronization processes in the human epileptic neocortex.*"

We described the general characteristics of the spontaneously occurring population activity (SPA) in post-surgical epileptic and non-epileptic human neocortical slice preparations. We recorded the local field potential gradient (LFPg) with the aid of a 24 channel linear microelectrode placed perpendicular to the pial surface.

We showed that both type of samples generates this type of activity, although there were certain differences between them. In the epileptic samples, a higher percentage of slices exhibited population activity, with higher LFPg amplitudes. Antagonists of either the glutamatergic or the GABAergic system blocked the events in a reversible manner both in non-epileptic and epileptic samples. These results indicate that both glutamatergic and GABAergic signaling are necessary in the generation of SPAs. Pyramidal cells were intracellularly recorded in neocortical slices from epileptic and from non-epileptic patients, simultaneously with the extracellular linear recordings. We found that in the epileptic tissue significantly more intracellularly recorded neurons received depolarizing synaptic potentials during the population events and discharged more reliably during the events. Light and electron microscopic examinations showed slightly lower neuron densities and higher densities of excitatory synapses in the human epileptic neocortex. In addition, we described an interictal-like activity with a remarkably lower occurrence rate and higher LFPg amplitude. This type of activity was confined only to the epileptic samples and were characterized by hypersynchronous and considerably higher levels of excitation. Our results show that hyperexcitability characterizes the human epileptic neocortical network, and that it is closely related to the emergence of synchronies.

We examined the role of inhibition in the generation of the SPAs. In the aforementioned paper we showed, that a higher density of excitatory synapses was present in the epileptic tissue whereas the total neuronal density was lower. In a next set of experiments, we blocked the GABAergic inhibition by applying GABA(A) receptor antagonist bicuculline.

Results of these experiments were published in The Journal of Physiology (J Physiol. 2019 Sep 15. doi: 10.1113/JP278499).

> "Kandrács Á, Hofer KT, Tóth K, Tóth EZ, Entz L, Bagó AG, Erőss L, Jordán Z, Nagy G, Fabó D, Ulbert I, Wittner L. *Presence of synchrony-generating hubs in the human epileptic neocortex.*"

In summary, we found, that SPAs were suppressed both in non-epileptic and epileptic tissues, however spontaneous bicuculline-induced epileptiform interictal-like events and seizures emerged. Both the temporal and the spatial complexity of these events were higher in epileptic than in non-epileptic tissue. We found remarkable differences between the properties of SPAs, interictal-like events and seizures. The recurrence frequency was lower, while the

LFPg and MUA amplitudes and the high frequency power were higher in case of interictal-like events and seizures. We aimed to uncover the role of excitatory and inhibitory processes in synchrony generation by analyzing the activity of clustered single neurons during physiological (SPA) and epileptiform synchronies. About half of the clustered neurons participated with an elevated firing rate in physiological synchronies with a slight dominance of excitatory cells. In contrast, more than 90% of the neurons contributed to interictal-like spikes and seizures, and an intense and synchronous discharge of inhibitory neurons was associated to the start of these events.

These data suggest that a balanced excitation and inhibition characterized physiological synchronies, whereas disinhibition-induced epileptiform events were initiated mainly by non-synaptically synchronized inhibitory neurons. The differences between epileptic and non-epileptic tissue suggest the presence of excess excitation and pacemaker regions in the epileptic neocortex.

Two posters were presented of these results. One at the FENS Regional Meeting, Pécs, 20-23 September 2017 and one at the 11th FENS Forum of Neuroscience, Berlin, 7-11 July, 2018.

To further examine the possible structural basis of the hyperexcitability in the epileptic samples, the perisomatic innervation of pyramidal cells was examined and related to the generation of synchronies.

The cellular and axonal distribution of parvalbumin (PV)-positive axo-axonic- and fast-spiking basket cells was described, and the synaptic coverage of layer 3 (L3) pyramidal cells (length of synaptic active zones/soma perimeter) was studied at the electron microscopic level, in regions where SPA emerged, in vitro. The density of PV-positive cells decreased in the human epileptic neocortex compared to non-epileptic (54±19 vs 78±14 cell/mm^2 p=0.0080), while no considerable difference was seen in the axonal cloud.

The length of the synaptic active zones was measured in case of both labelled (PV-positive) and unlabeled axon terminals, innervating the perisomatic region of L3 pyramidal cells. These axon terminals were giving exclusively inhibitory synapses. The average length of the synaptic active zones of unlabeled terminals were found to be significantly higher in the epileptic samples, compared to non-epileptic (0.33±0.10 μm vs 0.26±0.09 μm, p=0.0000). Similar was found in the case of the PV-positive terminals (0.32±0.08 μm vs 0.27±0.10 μm, p=0.0081). The average length of the synaptic active zones of unlabeled and labelled terminals was similar both the non-epileptic- (0.26±0.09 μm and 0.27±0.10 μm, p=0.5314) and in the epileptic samples (0.33±0.10 μm and 0.32±0.08 μm, p=0.8397).

The perisomatic synaptic coverage of L3 pyramidal cells given by PV-positive axon terminals was significantly lower in the epileptic samples, compared to non-epileptic (0.26±0.34 vs 0,73±0.51, p=0.0002). However, the synaptic coverage provided by unlabeled terminals was higher in the epileptic specimens (1,06±0,75 vs 0.62±0.44, p=0.0048). In non-epileptic samples approximately same amount of the perisomatic synaptic coverage was provided by the PV-positive terminals and the unlabeled ones (0,73±0.51 vs 0.62±0.44, p=0.3621). In contrast, in the epileptic samples a significantly higher amount of the synaptic

coverage was provided by the unlabeled axon terminal (1.06±0.75 vs 0.26±0.34, p=0.0000). In a few trials, blocking PV-positive interneurons by the pharmacological agent DAMGO had no effect on the population activity. However, increasing the number of experiments is needed.

Examining the type 1 cannabinoid (CB1) receptor-expressing interneuron mediated perisomatic inhibition of neocortical pyramidal cells, we found that a higher amount of CB1 receptor-positive axon terminals contacted the perisomatic region of pyramidal cells in the epileptic samples at the electron microscopic level, compared to non-epileptic tissues. However, a detailed statistical analysis still has to be done. This finding corresponds to the aforementioned results, since majority of the PV-unlabeled axon terminals are supposed to be CB1 receptor-expressing ones.

These results were presented as an oral presentation at the 48th Annual Meeting of the Society for Neuroscience (San Diego, November 3-7, 2018) on one of the nanosymposium, titled “Epilepsy: Human studies”.

Three posters were presented of these results, one at the EMBO Workshop on Cortical Interneurons in Health and disease (Mallorca, Spain 2018.june17-20), one at the 11th FENS Forum of Neuroscience (Berlin, 7-11 July, 2018.) and one at the Magyar Tudomány Ünnepe (Budapest, 2018.11.22).

Since the cholinergic system is involved in the modulation of the perisomatic inhibitory cells, in another set of experiments we started to investigate the possible involvement of the cholinergic system in the different characteristics of the events generated by non-epileptic or epileptic neocortical slice preparations. We studied the effect of the non-selective muscarinerg acetylcholine receptor (mAChR) agonist, carbachol on the network activity by extracellular recordings. In non-epileptic samples carbachol decreased both the frequency and the amplitude of the SPAs to 75-80% and 68-90%, respectively. In contrast, in the epileptic samples carbachol almost blocked the population events. Both the frequency and the amplitude of SPAs showed a more intense decrease, to 5-30% and 18-32%, respectively. The effects of carbachol were reversible. Statistical evaluation still has to be done.

It is known that PV-positive perisomatic inhibitory basket cells carry the M2 mAChR receptors on their axon terminals. To study the role of these receptors (and subsequently this type of inhibitory cells) in the mediation of the effect of carbachol, we applied the specific M2 receptor antagonist AF-DX 116. In non-epileptic samples, AF-DX 116 could almost entirely reverse the effect of carbachol on the amplitude of the spontaneous events (from 70-80% to 98-101%). In epileptic samples, AF-DX 116 reversed the effect of carbachol to a lesser degree (from 25-30% to 75-90%). Statistical evaluation still has to be done.

We made experiments in rat brain slice preparations to assess the size of neuronal networks that can generate spontaneous synchronous activity. By applying laser pulses in the two photon microscope, we used a microsurgery method to modulate either the spontaneously occurring

synchronous population activity or the interictal-like activity induced by low magnesium bath. In most cases the microsurgical procedure led to a marked decrease in the LFP amplitude and recurrence frequency of the SPA and interictal-like activity and it eliminated the multiunit activity in the case of interictal-like events.

Our preliminary data was presented as a poster in the FENS Regional Meeting (Pécs, 20-23 September 2017).

We finished the development of an anatomical method required for the analysis of the in vitro data. It enables the automatic quantitative analysis of the amount of cell death near the extracellular laminar electrode as a function of the distance. We developed a home-written cell-counting routine in Matlab environment, which we validated in case of silicon and SU-8 polymer based electrodes.

Results of the silicon-based probes were published in two papers in journals "Biomedical Engineering / Biomedizinische Technik" and in "Scientific Reports".

> "Fiáth R, Hofer KT, Csikós V, Horváth D, Nánási T, Tóth K, Pothof F, Böhler C, Asplund M, Ruther P, Ulbert I. *Long-term recording performance and biocompatibility of chronically implanted cylindrically-shaped, polymer-based neural interfaces*. Biomed Tech (Berl). 2018 Jun 27;63(3):301-315. doi: 10.1515/bmt-2017-0154."

> "Richárd Fiáth, Adrienn Lilla Márton, Ferenc Mátyás, Domonkos Pinke, Gergely Márton, Kinga Tóth, István Ulbert. *Slow insertion of silicon probes improves the quality of acute neuronal recordings*. Sci Rep. 2019 Jan 14;9(1):111. doi: 10.1038/s41598-018-36816-z."

Manuscript of the SU-8 based probes has just been submitted to the journal "Biomaterials".

> "G. Márton, E.Z. Tóth, L. Wittner, R. Fiáth, D. Pinke, G. Orbán, D. Meszéna, I. Pál, E.L. Győri, Z. Bereczki, Á. Kandrács, K.T. Hofer, A. Pongrácz, I. Ulbert, K. Tóth. *The neural tissue around SU-8 implants: a quantitative in vivo biocompatibility study*"

Three posters were presented of these results. One at the FENS Regional Meeting (Pécs, 20-23 September 2017.), one at the 48th Annual Meeting of the Society for Neuroscience (San Diego, November 3-7, 2018.) and one at the Magyar Tudomány Ünnepe (Budapest, 2018.11.22.).