Detailed scientific report of OTKA PD 77864

In vitro electrophysiological investigation of synchronous population activity in rodent and human hippocampus. (Szinkron populációs aktivitás elektrofiziológiai vizsgálata patkány és emberi hippocampusban in vitro.)

Present project aimed to investigate synchronous population activity (SPA) emerging in human and rodent cortical slice preparations.

Background

Epilepsy is one of the most common neurological disorders in humans, and it is thought to be related to hyperactivity of neuronal circuits (Morrell, 1997). Particularly remarkable characteristic of epileptic cortices is their capacity to generate paroxysmal activity: seizures and interictal spikes. Paroxysmal events are commonly characterized by the hypersynchronous and wide spread activation of neuronal circuits. Pharmacological treatment is efficient in the majority of the cases, but a significant percentage falls into the therapy resistant category (Morrell, 1997). Lobectomy might provide a good solution in these cases. Surgically removed brain tissue from therapy resistant epileptic patients provides an excellent opportunity to examine neuronal activity in vitro at network and single cell levels, as well as to study morphological modifications. Spontaneous interictal-like activity could be observed in vitro in human hippocampal (our data: (Wittner et al., 2009), (Cohen et al., 2002; Wozny et al., 2005; Huberfeld et al., 2007) and neocortical (Kohling et al., 1998; Pallud et al., 2014) slice preparations, in a physiological perfusion solution. These synchronous events consist of a field potential transient superimposed with enhanced high frequency oscillations and cellular activity. Similar to these population bursts, sharp-wave ripples (SPW-R) have been described in the rodent hippocampus, in vitro (Papatheodoropoulos and Kostopoulos, 2002; Maier et al., 2003; Behrens et al., 2005; Nimmrich et al., 2005; Wu et al., 2005). SPW-Rs in vivo occur during slow wave sleep and behavioural immobility and are thought to have an important role in memory formation. The similarity of in vitro SPW-Rs in control rodent slices and interictal-like events in human epileptic tissue is considerable in many aspects. The frequency (0.5-5 Hz), the waveform were very

similar, and both glutamatergic excitatory and GABAergic inhibitory signalling are involved in these rhythmogenesis (Kohling et al., 1998; Cohen et al., 2002; Maier et al., 2003; Wu et al., 2005).

Scientific results

1. SPW-Rs in the rat hippocampus in vitro

Hippocampal sharp wave-ripples (SPW-Rs) occur during behavioural immobility and slow wave sleep, and are considered to play a crucial role in the formation of memory traces. We investigated the cellular and network properties of SPW-Rs with simultaneous laminar multielectrode and intracellular recordings in a rat hippocampal slice model, using physiological bathing medium. We used a 24 channel laminar microelectrode – covering the entire width of the examined hippocampal subregion – to record the local field potential gradient (LFPg). Simultaneous intracellular recordings were made to characterise the behaviour of pyramidal cells during SPW-Rs.

Spontaneous SPW-Rs were generated in the dentate gyrus (DG), CA3 and CA1 regions. These events were characterised by a LFPg transient, increased fast oscillatory activity and increased multiple unit activity (MUA). Two types of SPW-Rs were distinguished in the CA3 region based on their different LFPg and current source density (CSD) pattern. Type 1 (T1) displayed negative LFPg transient in the pyramidal cell layer, and the associated CSD sink was confined to the proximal dendrites. Type 2 (T2) SPW-Rs were characterised by positive LFPg transient in the cell layer, and showed CSD sinks involving both the apical and basal dendrites. T1 SPW-R was observed in the CA3 region in 35% of the cases (n=51/147), T2 in 55% (n=81/147), and simultaneous T1+T2 SPW-R in 10% of the cases (n=15/147). In both types of SPW-Rs, consistent with the somatic CSD source, only a small subset of CA3 pyramidal cells fired (n=9/43 cells), most pyramidal cells were hyperpolarized, while most interneurons increased firing rate before the LFPg peak (n=53/71 interneurons). Different neuronal populations, with different proportions of pyramidal cells and distinct subsets of interneurons were activated during T1 and T2 SPW-Rs. Several different interneuronal firing patterns have been described during SPW-R activity. Interneuron firing pattern varied according to the location and to the type of the SPW-R. About two-thirds of the interneurons showed

different firing pattern to the two types of SPW-Rs, observed during T1+T2 SPW-R activity (n=10/17 cells). Pharmacological experiments revealed the importance of both glutamatergic and GABAergic signalling in the generation of SPW-Rs. Decreasing the activity of perisomatic interneurons by bath application of the mu opioid receptor agonist DAMGO resulted in the dramatic decrease of recurring frequency of both types of SPW-Rs.

Activation of specific inhibitory cell subsets – with the possible leading role of perisomatic interneurons – seems to be crucial to synchronize distinct ensembles of CA3 pyramidal cells finally resulting in the expression of different SPW-R activities. This suggests that the hippocampus can generate dynamic changes in its activity stemming from the same excitatory and inhibitory circuits, and so, might provide the cellular and network basis for an input-specific and activity-dependent information transmission.

2. Interictal-like activity in the human epileptic hippocampus

The dentate gyrus, the CA2 region and the subiculum of the human hippocampal formation are resistant to the cell loss associated with temporal lobe epilepsy. The subiculum, but not the dentate gyrus, generates interictal-like activity in tissue slices from epileptic patients. We asked whether a similar population activity is generated in the CA2 region and examined the electrophysiological and neuroanatomical characteristics of human epileptic CA2 neurons that may be involved.

Hippocampal slices were prepared from postoperative temporal lobe tissue derived from epileptic patients. Field potentials and multiunit activity were recorded in vitro using multiple extracellular microelectrodes. Pyramidal cells were characterised in intracellular records and were filled with biocytin for subsequent anatomy. Fluorescent immunostaining was made on fixed tissue against the chloride-cation cotransporters NKCC1 and KCC2. Light and electron microscopy was used to examine the parvalbumin-positive perisomatic inhibitory network.

In 15 of 20 slices, the hippocampal CA2 region generated a spontaneous interictal like activity, independently from the population events in the subiculum. Most CA2 pyramidal cells fired spontaneously. All cells fired single action potentials and burst firing was evoked in three cells. Spontaneous EPSPs were recorded in all cells, but IPSPs were detected in only 27% of the cells. Two thirds of CA2 neurons showed depolarizing responses during interictal-like events, while the others were inhibited, according to the

current sink in the cell body layer. Two biocytin-filled cells both showed a pyramidal-like morphology with axons projecting to the CA2 and CA3 regions. Expression of NKCC1 and KCC2 was reduced in some cells of the epileptic CA2 region, but not to an extent corresponding to the proportion of cells in which IPSPs were absent. Numbers of parvalbumin-positive inhibitory cells and axons were shown to be decreased in the epileptic tissue. Electron microscopy showed the preservation of somatic inhibitory input of CA2 cells, and confirmed the loss of parvalbumin from the interneurons rather than their death. An extra excitatory input (partly coming from sprouted mossy fibers) was demonstrated to innervate CA2 cell bodies.

Our results show that the CA2 region of the sclerotic human hippocampus can generate an independent epileptiform activity. Inhibitory and excitatory signalling were functional but modified in epileptic CA2 pyramidal cells. Overexcitation and the altered functional properties of perisomatic inhibitory network, rather than a modified chloride homeostasis may account for the perturbed GABAergic signalling and the generation of interictal-like activity in the human epileptic CA2 region.

3. Spontaneous population activity in the human neocortex

Spontaneous synchronous population activity (SPA) can be detected by electrophysiological methods in cortical slices of epileptic patients, maintained in physiological medium in vitro. In order to gain additional spatial information about the network mechanisms involved in the SPA generation, we combined electrophysiological studies with two-photon imaging and anatomy.

Neocortical slices prepared from postoperative tissue of epileptic (n=4) and tumor(n=3) patients were maintained in a dual perfusion chamber in physiological incubation medium. SPA was recorded with a 24 channel extracellular linear microelectrode covering all neocortical layers, in 10 of 22 human neocortical slices. After identifying the electrophysiologically active regions of the slice, bolus loading of neuronal and glial markers was applied on the tissue.

SPA related Ca^{2+} transients were detected in a large population of neighboring neurons with two-photon microscopy, simultaneously with extracellular SPA recordings. Based on their response rate we divided neurons in four subcategories. Beside silent cells we defined occasionally responding cells (showing Ca^{2+} signals during <20% of the SPA events), non-reliably responding cells (20-40% response rate) and

reliably responding cells (Ca^{2+} responses to >40% of the SPA events). With this method, we identified 22 silent cell (68%), 4 occasionally (13%), 1 non-reliably (3%) and 5 reliably responding (16%) cells in the tumor tissue. The distribution of the responding cells was considerably different in epileptic tissue: we found 19 silent cells (35%), 20 occasionally (36%), 11 non-reliably (20%) and 5 reliably (9%) responding cells. Simultaneously with SPA and Ca^{2+} signal recordings we performed intracellular whole-cell patch-clamp recordings (n=7 neurons). First in the literature, we described the somatic and dendritic Ca^{2+} responses of both human interneurons (n=4) and pyramidal cells (n=3), together with their somatic electrophysiological activity. The Ca^{2+} signals of human neurons were comparable to those found in animal tissue. The intracellularly recorded cells were filled for subsequent anatomy. The cells were reconstructed in three dimensions and examined with light- and transmission electron microscopy. We found the signs of considerable photodamage of intracellularly filled neurons at electron microscopic level, which was invisible at the light microscope.

Combining high spatial resolution two-photon Ca^{2+} imaging techniques and high temporal resolution extra- and intracellular electrophysiology with cellular anatomy may permit a deeper understanding of the structural and functional properties of the human neocortex.

Changes in the personnel, infrastructure, its effect on the research project

Two breaks of about one year were necessary during the project, due to the birth of the children of the principal investigator (2009, 2011). A third prolongation of one year had been asked due to the move of the host institute from its original place to the new Q2 building of the Hungarian Academy of Sciences (2013). All these changes have been approved by the OTKA committee.

Changes in the personnel had little effect on the course of the present project. The two undergraduate students marked in the application (Péter Váci, Zoltán Jakab) quit their scientific career in 2010. One PhD student (Katharina Hofer) and two undergraduate students (Csilla Szabó, Bálint Kerekes) joined the laboratory in 2012, and three PhD students started to work in 2014 (Edit Győri, Domonkos Meszéna, Márton Csernai). The project objectives proposed in the grant application have been all

accomplished during the course of the project. We described the network and cellular properties of both rat and human cortical slices, which results have been published in scientific peer-reviewed journals (Wittner et al., 2009; Kerekes et al., 2014; Hofer et al., 2015). Additional articles using data derived from the same epileptic patients have been also published with the aid of the present OTKA fund, describing the cellular and network features of slow wave activity (Csercsa et al., 2010), and of the K-complexes (Cash et al., 2009), as well as the propagation pattern of slow wave activity (Hangya et al., 2011) in the human cortex. We published further two methodological papers describing multiple channel microelectrodes in rats (Grand et al., 2010; Dombovári et al., 2014).

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