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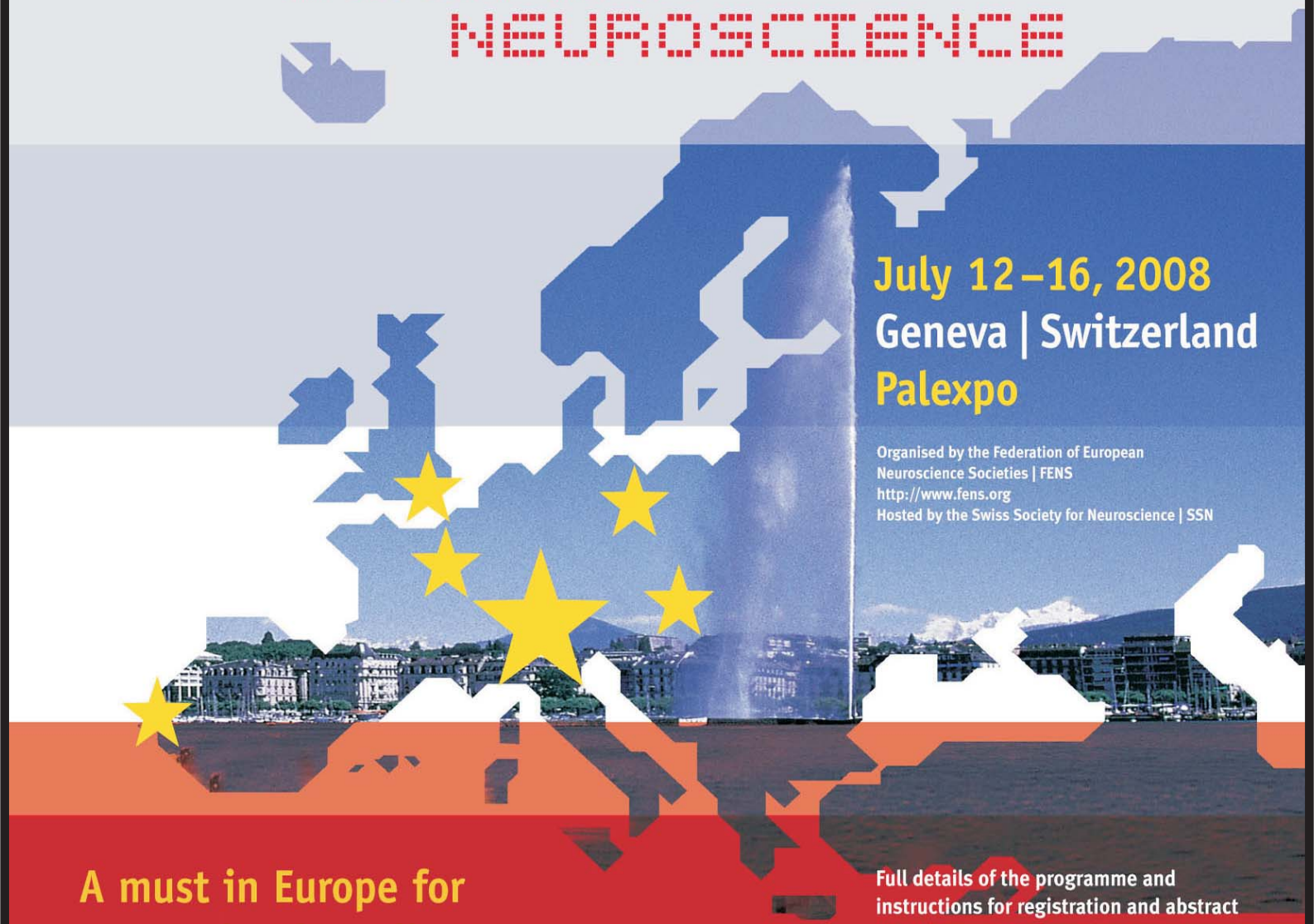

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Relation between the spread of epileptiform activity and neurogenesis in the epileptic hippocampus

Ute Häussler, Martin C. Müller, Ralph Meier, Carola Haas

Hippocampal sclerosis in mesial temporal lobe epilepsy (MTLE) is associated with the loss of neurons in the CA-region and the hilus, gliosis and pronounced granule cell dispersion (GCD). We have shown that GCD is not caused by increased neurogenesis but most likely by a displacement of adult neurons in TLE patients and in the intrahippocampal kainate model for MTLE in mice (Heinrich et al., 2006, JNS; Fahrner et al., 2007, Exp. Neurol.). In contrast, an increase in neurogenesis has been proposed to underlie the network changes in MTLE (Scharfman et al., 2000, JNS). Thus, we want to clarify whether excessively high excitatory activity during status epilepticus and recurrent epileptic seizures have a depressing or a stimulating effect on neurogenesis, separated from the direct effects of kainate. Therefore, we recorded epileptiform activity with implanted electrodes in the granule cell layer at several positions along the hippocampal septo-temporal axis of unilaterally kainate-injected mice. In parallel, we performed bromodeoxyuridin (BrdU) injections and doublecortin (DCX) stainings to characterize neurogenesis along this axis. We show that the loss of BrdU and DCX staining, as well as GCD in the dentate gyrus is limited to a focal area surrounding the injection site and that neurogenesis recovers at distance. Preliminary data even indicate increased number of DCX-positive cells in the subgranular zone of the ventral hippocampus compared to saline-injected controls, indicating increased neurogenesis. Accordingly, epileptic spikes are not limited to the area of strongest hippocampal sclerosis surrounding the injection site but spread along the whole length of the hippocampus already at the first days after injection until at least four weeks later. Thus, epileptiform activity does not disturb, but most likely stimulate neurogenesis which in turn may contribute to the development of epileptic seizures.

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Functional embedding of single neurons in dense neuronal networks

Steffen Kandler, Samora Okujeni, Jarno E. Mikkonen, Ulrich Egert

Interdependencies between architecture, activity dynamics, and computational properties in cortical networks are still poorly understood. Here, we investigate these relations in generic neuronal network cultures. Although the layered organization of the native cortex is not preserved in this preparation, basic properties are essentially similar but the complexity is reduced and thus more tractable. Moreover, the networks can be manipulated experimentally, i.e. they can be grown on patterned substrates, varied in density and size, or treated pharmacologically.

We record neuronal activity from dense networks (approx. 1000 cells per square mm) grown on 60-site microelectrode arrays (MEA) or glass coverslips. Our cultures display bursting activity that increasingly synchronizes with network maturation. We determine the local neuron-to-neuron connectivity in a range of up to 250µm and the embedding of individual neurons into the network activity by combining paired patch-clamp and extracellular MEA recordings. We analyze the distance-dependent connectivity for recorded neuron pairs and identify excitatory and inhibitory cells as well as single and reciprocal connections. Of all recorded neuron pairs 74% are connected; 68% of these make excitatory and 6% inhibitory connections; 19% of all pairs have reciprocal connections. Individual neurons typically participate in (multi-unit) network bursts only with few spikes and rarely fire long burst sequences. Based on DAB staining of patched neurons, we determine the local footprint of individual neurons within the network and derive a neuron class-specific distance function.

In summary, our work adds to the understanding how neurons integrate functionally and structurally into neuronal networks.

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Lithium Affects Cajal-Retzius Cells in Hippocampal Slice Cultures

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Hippocampal slice cultures are an excellent model to gain insight into the mechanisms that govern the development of hippocampal cell and fiber layers (reviewed in Förster et al., 2006a). Most hippocampal dentate granule cells are born early postnatally, and thus migrate postnatally to their final positions. This migration process is controlled by Reelin, a protein which is secreted by Cajal-Retzius cells in the marginal zone of the dentate gyrus. An identified component of the Reelin signalling cascade is the enzyme glycogen synthase kinase (GSK)3 beta which has been shown to act on the cytoskeleton by modulating tau phosphorylation. Activity of GSK3ß is downregulated by application of pharmacological inhibitors, such as Lithium Chloride (LiCl). Lithium is also known as a drug for the treatment of schizophrenia, a neurological disorder that is thought to be causally related to developmental neuronal migration defects. Here, we were interested in the question whether LiCl may interfere with developmental processes in hippocampal slice cultures, known to be regulated by Reelin signalling. Different concentrations of LiCl were added to the incubation medium and the morphology of slice cultures was analyzed after 6 days in vitro. After treatment with high concentrations of LiCl we observed a loss of the proper arrangement of the dentate granule cell layer, suggesting that Lithium interferred with the proper positioning of dentate granule cells, a process that is known to be regulated by Reelin. Next, we studied the effects of Lithium on the morphology of Reelin secreting Cajal-Retzius cells by immunostaining them with an antibody against Calretinin. We found that LiCl induced neurite retraction of Cajal-Retzius cells in a dose dependent manner. Finally, we investigated by Western-Blotting whether LiCl treatment of cultures affects the Reelin content in the incubation medium. We found that the Reelin content decreased with increasing LiCl concentration. Surprisingly, these observations suggest that Lithium may interfere with hippocampal development upstream of the Reelin signalling cascade, possibly by directly acting on Reelin secreting Cajal-Retzius cells.

Bursting in cultured cortical networks with partial glutamatergic transmission blockage

Jarno E. Mikkonen, Steffen Kandler, Oliver Weihberger, Samora Okujeni, Heidi Teppola, Antti Pettinen, Marja-Leena Linne, Ulrich Egert

Synchronous spike bursts are relevant for plasticity and efficient information transfer within networks of the nervous system, but the mechanisms behind bursting are not fully understood. We have used microelectrode arrays (MEAs) to explore the dependence of synchronous bursts on underlying glutamatergic synaptic transmission. MEAs provide a simplified, accessible platform to explore broad-spectrum information processing and interactions in neuronal networks. Cortical neurons cultured on MEAs form a mature, spontaneously active network within 4 weeks in vitro. Since 90% of the electrical activity in MEAs resides in bursts, they provide an excellent model to identify excitatory components necessary for structuring bursts, and to estimate the impact of bursting on information transfer in neuronal networks. Here we decompose the bursting of cortical networks by applying increasing concentrations of glutamatergic AMPA and NMDA channel antagonists NBQX and APV. We show that both AMPA and NMDA channels promote bursting and that it is possible to identify their specific roles in shaping the bursts. Blocking AMPA channels compressed bursting into discontinuous periods of high network-wide bursting, or superbursting. This bursting had a binomial distribution of short and long bursts with longer bursts generally initiating the superburst. Burst intervals were also binomial corresponding to within and between superburst intervals. Although superbursts were tightly correlated across different electrodes, individual burst within the superburst had jittered onsets. Blocking NMDA channels abolished superbursting, reduced burst lengths dose-dependently and widened the distribution of burst intervals. Blocking both AMPA and NMDA channels abolished bursting. We conclude that NMDA channel activation promotes clustering of synchronized bursts, whereas AMPA channel activation supports randomly initiated, short bursts.

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Nonlinear interdependencies in epileptiform network dynamics revealed with the Finding Information in Neuronal Data (FIND) -Toolbox using distributed computing

Ralph Meier, Christian Garbers, Ute Häussler, Ulrich Egert, Ad Aertsen

Nonlinear multivariate analysis of neurophysiological data (Pereda et al. 2005) is computationally quite expensive. As a solution for this problem, we developed within the open source FIND-Toolbox (http://find.bccn.uni-freiburg.de) a Python based plug-in to distributed computing and to algorithms estimating nonlinear interdependencies. FIND supports the import of data in various proprietary formats and represents it as a unified data structure in HDF5, a uniquely suited technology suite for the management of very large and complex data collections. This approach allows both, the distribution of the recorded data on distributed computing clusters (using MPI) and the effortless collection and storage of the results. Using our open source software package, we studied the network activity dynamics causing the transition from normal brain activity into hypersynchronous epileptiform spiking in MesialTemporal Lobe Epilepsy (MTLE). We used the kainate mouse model, characterized by unilateral histological changes resembling hippocampal sclerosis in human MTLE. We prepared hippocampal slices and recorded population activity with 8x8 planar microelectrode arrays.

The most sclerotic regions did not display epileptiform activity (EA). Thus, we elicited EA in histologically unchanged slices, distal from the kainate injection site.

We estimated the nonlinear interdependencies of layer specific hippocampal population activity from slices of epileptic mice, controls and pharmacologically treated control slices. We found significantly decreased values for several measures of nonlinear correlation in both, epileptic and treated slices, indicating a severe disturbance of network activity in regions that were histologically unchanged. This finding supports the idea of an epileptic focus that is much more complex with respect to network connectivity and spatial spread than previously assumed.

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Dendrite structure, connectivity and activity dynamics in cortical networks

Samora Okujeni, Steffen Kandler, Ulrich Egert

Connectivity is a critical parameter of neuronal communication and can amongst others be attributed to the size of axonal and dendritic fields. The homeostatic regulation of connectivity is crucial for the maintenance of stability in the activity dynamics of neuronal networks.

This work focuses on implications of dendrite architecture on connectivity and activity dynamics in cortical cell cultures grown on microelectrode arrays. These generic random networks display a self-regulated maturation process that is characterized by neurite outgrowth, synapse over-expression and pruning similar to the critical period found in the developing cortex. Within this scenario, the protein kinase C (PKC) is a key regulator of neuronal morphology, since it is involved in a variety of processes that guide the structural differentiation of neurons. Previous studies showed that inhibition of the PKC resulted in increased dendritic arborization in organotypic cerebellar slices.

Cultures were chronically treated with PKC inhibitors to increase neurite outgrowth and thus connectivity in the mature state. Immunohistochemical techniques combined with newly developed morphometrics revealed a significantly enhanced dendritic arborization (+30%) when PKC was blocked. Amplified immunoreactivity for the presynaptic protein synaptophysin furthermore indicated an increased synapse density. Electrophysiological recordings, however, revealed no significant changes in global level of activity, firing rates of single neurons or regularity of spiking. Functional changes that may result from increased dendritic fields are analyzed and considered in the framework of a homeostatic regulation of neuronal connectivity.

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Interplay of electrical stimulation efficacy and suppression of oscillatory bursting activity in neuronal networks *in vitro*

Oliver Weihberger, Jarno E. Mikkonen, Ulrich Egert

Processing and storage of information are fundamental features of neuronal systems. Dynamical interactions on a wide range of spatial and temporal scales are underlying those features and it is unclear by which mechanisms they are governed and how they depend on a specific environment. We aim to understand how neuronal networks realize the mechanisms underlying processing and storage of information, how they respond to incoming stimuli and how this knowledge can be used for a closed-loop interaction.

Cortical cell cultures grown on microelectrode arrays (MEAs) are generic neuronal networks accessible for recording and stimulation via multiple electrodes. During development, they undergo a transition in activity from uncorrelated single spikes to synchronized bursting and more complex burst patterns, called superbursts. We characterized the efficacy of electrical stimulation depending on the network's activity state: the response length increased with decreasing level of activity prior to stimulation, and stimulation at a fixed phase of the network's activity improved the reproducibility of responses. Stimulation during 10 - 20 second long superbursting periods resulted in the longest responses, but was ineffective just after the end of a superburst. We show that electrical stimulation in turn suppressed superbursting activity. This resulted in a more homogenous burst distribution and improved the reconstruction of input-signal properties from the recorded activity.

After electrical stimulation, several seconds elapsed until the network became active again. Consequently, repetitive stimulation led to refractory network states, setting an upper bound on the frequency of activity-inducing stimulation (approx. 0.2 Hz). The results indicate that the responses of this system of overlapping oscillations depend on the network state. Phase-coupled stimulation increases the predictability of the network's response.

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