Closing report on OTKA K83251

While we pursued one question: "How are hippocampal activity patterns generated during the interaction of excitatory and inhibitory neurons?" our findings can be presented in 3 methodically different but complementary parts.

A) Two quantitative anatomical studies (Takacs et al. 2012 and 2014) examined the external excitatory inhibitory inputs onto CA1 pyramidal cells and interneurons as well as inputs onto CA3 and CA1 pyramidal cells from PV and CB1/CCK containing basket cells.

We showed, that:
-while Schaffer collaterals primarily (~92%) innervate CA1 PC spines, local, recurrent collaterals of CA1 PCs dominantly (~60%) target inhibitory neurons in str. oriens.
-input from the entorhinal cortex targets primarily PCs (90~) in the apical dendritic layers, but its temporo-ammonic part shows somewhat higher inhibitory neuron selectivity (~20%) in str. oriens.
-these show that the 3 excitatory input of CA1 shows different extent of inhibitory neuron innervation and thus probably evoke feed-forward and feed-back inhibition to different extents
- a pyramidal cell body receives synapses from about 60 and 140 synaptic terminals in the CA1 and CA3 area, respectively. About 60 % of these terminals were PV positive, whereas 35–40 % of them were CB1 positive, that also expressed VGluT3.
-axon terminal volumes are similar, but CB1 boutons are more flat and the total volume of their mitochondria was smaller than that of PV-positive boutons. Both types of boutons frequently formed multiple release sites. PV-positive boutons possessed small, macular synapses; whereas the total synaptic area of CB1 boutons was larger and formed multiple irregular-shaped synapses.
- Our results represent the first quantitative measurement of the contribution of different cell types to the perisomatic innervation of pyramidal neurons, and may help to explain functional differences in their output properties, such as the speed of the IPSP and the presence or absence of asynchronous release.

B) Using paired IN-PC and IN-IN recordings in the CA3 area we examined the temporal properties of the inhibitory transmission of 3 classes of perisomatic inhibitory neurons (PV containing axo-axonic and basket cells and CCK containing basket cells). The paper is in prepartation (Kohus et al).
-we found characteristic differences in the amplitude, and speed of unitary IPSCs evoked by the 3 cell types both on PCs and INs. The transmission of PV cells is more reliable and precise than of CCK neurons
-we frequently observed PVBC-PVBC reciprocal connections and CCKBC-CCKBC connections but not PV to CCK or AAC to PVBC connections.
-we tested the transmission features with different in vivo observed firing patterns and at different frequences
-both in the case of PC as well as in IN targets the transmission of AACs and PVBCs show short term depression, while CCK transmission is facilitating and in some cells asynchronous transmitter release kicks in after many action potentials
-our measurements were done with the intention to provide data for neuronal network modellers on inhibitory transmission, therefore we fitted the observed short term plasticity of the different types of neurons to a model and its parameters can be used to calculate transmission properties for arbitrary sequence of spikes.
In summary we found that the transmission properties and short term plasticity of the different classes of perisomatic cells are characterestically distinct. The difference between transmission to PC or IN is not very different within a class.

C) We published a series of physiological and modelling papers (6) on the generation of hippocampal SWRs and on the difference between SWRs and interictal events. Three more papers are in preparation from materials presented as posters.

Hajos 2013
We recorded from a large set of CA3 area neurons during SWR activity, recorded their firing pattern in relation to SWRs. This was followed by recording of EPSCs and IPSCs in whole-cell configuration. The cells were anatomically identified and their input-output properties characterized. We found that different cell types show characteristic firing patterns and characteristic ratio of excitatory and inhibitory input in relation to SWRs, that influences their firing. PVBCs were firing multiple spikes associated with ripple oscillation troughs. AACs fired in the first half of SWRs, while CCK cells had low firing frewquency. PC fired very rarely.
-the dominant synaptic input to the pyramidal cell was inhibitory, whereas spiking interneurons received larger synaptic excitation than inhibition.
-the discharge of all interneurons was primarily determined by the magnitude and the timing of synaptic excitation.
-our data support the hypothesis that the active current sources restricted to stratum pyramidale during SWR soriginate from the synaptic output of parvalbumin-expressing basket cells.

Schlingloff 2014
We revealed the mechanisms important in the initiation, generation and termination of SWRs.
-SWRs are initiated through a combined stochastic/refractory mechanism. When sufficiently large number of pyramidal cells are active a buildup of activity starts in their recurrent collateral system.
-when activity reaches a threshold reciprocally connected PV containing inhibitory neurons start to generate ripple frequency oscillation and entrain pyramidal cell firing.
-SWRs terminate due to short-term depression of PV to pyramidal cell inibitory synapses.
-by showing that full SWRs can be evoked by optogenetically driving PV-positive neurons and inactivating their inhibition eliminates SWR associated LFP proves that PVBCs are necessary and sufficient for SWR generation
-SWR like activity can be evoked even in slices where quick excitatory transmission is blocked.

Karlocai 2014
We induced transmission from the SWR generating state to acute epileptic activity in in vitro slices by 1) increasing K+ concentration, or 2) partially eliminating inhibition using low concentration of GABA antagonist GABAzine, or 3) unblocking NMDA receptors by zero Mg2+, or 4) by application of 4-AP.
This way we could study the behavior of the network and of neurons during transition from the SWR state into the epileptic state and ask what is the difference in the generation of the two activity types.
-we showed that different hippocampal neurons change their firing patterns (typically massively increase their firing) during transition from the physiologically synchronous state (SWRs) into pathologically synchronous state (epileptiform events).
-we revealed an increase in cellular excitability and an increase in excitatory synaptic transmission together with a weakening of inhibitory transmission, that resulted in the transition to epilepsy.
-physiological SWRs proved to be a separate and different type of synchrony than pathological epileptic events, with distinct generation mechanisms.
-most importantly we revealed that the inhibition of fast spiking basket cells fails for several reasons during epilepsy: 1) the overexcited cells get into depolarization block and stop firing during the seizures, 2) their inhibition shows strong short-term depression by the middle of the epileptic episode and 3) while the strength of excitatory transmission increases the strength of inhibitory transmission decreases among conditions evoking epileptic events.

Chiovini 2014
We contributed to this study by supporting with neuronal modelling how ripple locked Ca2+ spikes are generated in PV-inhibitory neurons of the CA1 area.

Gulyas Freund 2014
We summarized our findings on the difference between SWRs and IIEs in an invited review. Both type of activity starts with a stochastic initiation of activity that is followed by a buildup of excitation. However while during SWRs the PVBCs are engaged and can control PC firing, due to the pathologically strong excitability and excitatory transmission, PVBCs are overexcited during epileptic events and their already weakened inhibition is interrupted, because the cells get into depolarization block. At this point PCs start an uncontrolled pseudo synchronous firing that results in pathological HFOs. We suggested that epileptic events are degenerate SWRs that highjack elements of the SWR generation mechanisms.

Friedrich 2014
To build cell-type-specific models of hippocampal neurons based on our experiments, we developed a software tool for the automatic optimization of the parameters of neuronal models. The program provides a convenient graphical interface which guides the user through the steps of the optimization process. It interfaces with the actual simulator software, supports several common experimental protocols, implements a variety of measures for the comparison of simulation results and target data, and offers several efficient algorithms to search for the optimal parameters. The software has been used to fit both highly simplified and realistic models of hippocampal neurons to physiological recordings. We presented the work in abstract form (a tool for all scopes).

Gulyas et al. (in preparation)
We explored how the same network can generate different activity patterns. The activity of the in vitro hippocampal slice can be shifted among SWRs, gamma oscillation and epileptic activity. We measured how cholinergic activation (evoking gamma) or high K (inducing epilepsy) changes cellular excitability and synaptic transmission parameters.
-we found that an increase in excitability and a decrease in inhibitory and excitatory transmission causes the shift of dynamics from the SWR generating state to the gamma generating state.
-when excitability is increased, without a drop in excitatory transmission the result is epileptic activity.
-thus we can explain the generation of all three activity patterns within the same framework: depending on the location of the system in the 3 dimensional parameter space (axes are: excitability, excitatory and inhibitory transmission) the initiation rate of transient high activity events, such as a SWR, a gamma cycle or an epileptic event as well as the level of activity buildup (that depends on the ratio of E and I transmission)will be different.
-while we observed that SWRs and gamma oscillation and SWRs and epileptic events are separated by a featureless transition state, gamma oscillation can be continuously morphed into epileptic activity, explaining the neurological observations that epileptic events are often associated with gamma oscillations.

Besides the experiments, and based on the data we developed a large-scale model of the hippocampal CA3 region to explore the mechanisms of SWR, gamma and epilepsy generation. We found that our model based on measured cellular and synaptic parameters could faithfully reproduce the experimentally observed activity types when excitability and synaptic transmission parameters

were changed accordingly. This supports our conclusions concerning the role of the above parameters in shaping network dynamics.

Gulyas at al in preparation
Following the Schlingloff and Karlocai papers that implicitly suggested that physiological and pathological high frequency oscillations are of different origin. Here we explicitly prove by locally inactivating AP generation and somatic inhibitory currents that while the LFP during SWRs are generated by periodic inhibitory potentials and not APs, the pathologic HFOs observed during epileptic events can be eliminated by blocking AP generation but do not change following the elimination of perisomatic inhibition. While subsequent SWRs are stereotypic and of similar structure on nearby electrodes epileptic bursts do not correlate on nearby electrodes because the PCs fire only in pseudo-synchrony, that is not coordinated by inhibition. The observation emphasizes the diagnostic value of recording EEG at high frequency when locating epileptic foci. The presence of pathological HFO is an indication of the collapse of inhibition in the epileptic focus and can help to locate the focus.

Kali et al in preparation
We extended our network model of area CA3 to examine the effects of non-random connectivity established during prior learning. When recurrent excitatory connections were learned from simulated place cell activity via spike-timing-dependent synaptic plasticity, activity sequences were replayed during subsequent spontaneous sharp wave events, in agreement with in vivo observations. We also found that the detailed structure of synaptic weights had a major effect on the large-scale population dynamics, and networks with learned weights captured the average behavior of the real network much better than networks with randomly shuffled weights did. The results of our collaboration with the Renyi Institute using graph theoretical analysis of the connectivity rules of the random and trained network are under preparation.

The most important conclusions of our work:

-excitatory input pathways to CA1 activate inhibition to different extent
-different perisomatic inhibitory cell populations have distinct terminal structure, and transmission properties
-the short term plasticity of synaptic transmission is an extremely important element of the generation of dynamics during different network states
-PVBCs are crucial in the generation of SWRs by generating high frequency oscillation through their reciprocal connectivity.
-their several-fold failure is also importatnt in the generation of epileptic events
-interictal and other epileptiform events are degenerate SWRs, in both case the activity starts through a combined stochastic/refractory buildup mechanism, but during pathological activity inhibition fails and uncontrolled PC firing generates pathological high frequency oscillations.
-physiological and pathological high frequency oscillations are of different origin. Ripples during SWRs are generated by periodic PVBC inhibitory potentials, while pathologic HFOs observed during epileptic events are pseudo-synchronous action potentials of PCs firing bursts due to the collapse of inhibition of PVBCs