# Final report

**The general goal of the ERC_HU_15 grant** was to support preparation for the next ERC grant application. Fulfilling this expectation, I resubmitted my application to the 2017 call of the ERC Consolidator grant with a research plan revised based on the referee comments and our new results obtained during the ERC_HU_15 grant. My application was retained for funding in this call; the ERC project is expected to start in the summer of 2018. In my view, the new methodical advances, especially the verification of CaMPARI labeling, have made a significant contribution to the success of the ERC application.

## Results of the specific aims of the ERC_HU_15 grant:

### Aim 1: To elucidate the role of changes in synaptic and dendritic properties in the formation of hippocampal pyramidal cell ensembles by activity-dependent labeling.

To understand the synaptic and dendritic changes that accompany and facilitate the formation of functional ensembles in the hippocampus, our goal was to set up a new method enabling *in vivo* permanent labelling neurons that are active during a learning-related behavioral paradigm, to allow post-hoc targeted recordings in slices to compare these properties in labeled and unlabeled neurons. To label hippocampal neuronal populations involved in ensemble coding at a defined time point, we chose the recently developed CaMPARI technique. CaMPARI is a modified genetically encoded $Ca^{2+}$ sensor protein which undergoes irreversible green-to-red photoconversion when increased intracellular [$Ca^{2+}$] coincides with illumination with 405 nm light.

First we have optimized the expression of AAV-CAMPARI in hippocampal CA1/CA3 neurons by systematic testing of virus injection coordinates and virus concentrations. We obtained strong cytoplasmic expression of CaMPARI in hippocampal neurons, nicely delineating neuronal processes while excluded from the nucleus. The neurons appeared healthy, we did not detect signs of virus-induced cell damage or death.

Next we characterized activity-dependent labeling with CaMPARI in CA3 pyramidal cells in *in vitro* brain slices. We tested various cell activation approaches, and found electrical stimulation of mossy fibres to be the most effective, which triggered reversible, reliably repeatable somatic Ca2+ signals in some of the neurons, as detected by CaMPARI. After

identifying responsive and non-responsive cells, repeating the stimulus together with illumination by 405 nm light induced clearly detectable, permanent green-to-red photoconversion of CaMPARI, only in the responsive cells. Control experiments ruled out nonspecific photoconversion.

After *in vitro* characterization of CaMPARI in CA3 pyramidal neurons, we aimed at labelling active hippocampal ensemble neurons *in vivo* in behaving rats. We set up a spatial navigation paradigm (running multiple laps in a maze for reward) and implanted optic fibre to the CA3 area. After repeated illumination at a part of the maze, we prepared slices and could observe several putatively photoconverted CA3 and CA1 neurons. We began preliminary targeted recordings from these cells.

**Aim 2: To characterize cooperative synaptic interactions in local dendrites compartments of hippocampal CA1 pyramidal neurons.**

Recent studies demonstrated fine-scale subcellular organization of functionally similar synaptic inputs into small clusters (2-6 synapses) in hippocampal pyramidal cells and hotspots of excitatory synaptic activity in basal dendrites of CA1PCs *in vivo*. Our aim was to explore the cooperative interactions that may occur in spatiotemporally coordinated synapse clusters in CA1 pyramidal cell dendrites and the potential plasticity mechanisms generating spatially colocalized synaptic arrangements. At the time of application of the ERC_HU_15 grant, we had a manuscript under revision in which we dicovered that at distal segments of perisomatic dendrites of CA1 pyramidal cells, up to four spatially clustered coactivated inputs are able to cooperatively amplify NMDAR mediated $Ca^{2+}$ signals, even when somatic summation of EPSPs remains linear without evoking dendritic spikes. In contrast, proximally located clusters of four inputs did not express cooperative Ca2+ nonlinearity. We also showed that repetitive coactivation of four spatially clustered synapses at distal dendritic sites can induce postsynaptic long-term potentiation without backpropagating action potentials or local regenerative dendritic events. In contrast, no cooperative LTP was observed by four synapses at proximal dendritic sites. We planned to finish revision experiments, if required, during the timeframe of the ERC_HU_15 grant. The paper has been accepted and was published just before the start of the current grant (Weber et al., 2016 Nature Communications). We continued this research line addressing several further questions about the rules of cooperative synaptic plasticity in dendrites.

Using a combination of two-photon imaging, glutamate uncaging and electrophysiology in acute slices, we determined the minimal synchronous input pattern required for local cooperative LTP. Our results revealed that at distal segments of thin dendrites, synchronous activation of at least three synapses, located not more than 5 micrometers from each other, is sufficient to induce LTP. This result is consistent with our previous Ca2+ measurements showing substantial nonlinear spine Ca2+ influx beginning with this pattern. In contrast, at proximal dendritic segments even very large input clusters were unable to undergo cooperative LTP unless they evoked local or somatic spikes.

We next elucidated the role of local dendritic spikes in cooperative LTP. We found that at distal dendritic segments, the LTP of clustered inputs is not strengthened further by dendritic spikes over the level induced by nonregenerative form of LTP. However, LTP of spatially distributed inputs depends crucially on the ability of the pattern to evoke dendritic spikes. At proximal dendritic segments dendritic spikes are obligatory for the induction of LTP.

Interestingly, our results showed that when dendritic spikes were triggered during the LTP induction, the input specificity of the plasticity was compromised, as neighboring unstimulated synapses also became potentiated. This effect was local, NMDA receptor dependent, and did not require post-induction activity.

We have also used computational modelling in morphologically realistic CA1 pyramidal cell model to elucidate the impact of synaptic clusters on somatic membrane potential upon realistic input patterns. We found that clusters smaller than 10 inputs did not significantly facilitate somatic depolarization. This indicates that enhanced output is unlikely to be responsible for strengthening these clusters, and their generation is rather mediated by local plasticity mechanisms.

**Aim 3: To investigate the role of synaptic integration in environmental feature coding by CA3 hippocampal pyramidal neurons *in vivo*.**

Hippocampal CA3 pyramidal neurons fire action potentials preferentially at specific locations in a given environment („place cells”). It is generally assumed that place field selectivity of a given cell is determined by the synaptic input it receives, but very little is known about how the integration of inputs contributes to this function. We aimed to investigate the contribution of synaptic inputs and active dendritic integration to instructing place field selectivity of CA3PCs using *in vivo* patch-clamp recordings in awake head-fixed mice.

In the past 1.5 years we have fully set up and optimized our in vivo patch-clamp electrophysiology rig and the cranial window preparation and head fixation protocols. We achieved good quality whole-cell current-clamp recordings from several hippocampal (putative CA3 pyramidal) neurons in anaesthetized and awake head-fixed mice, along with concurrent LFP recordings. In several of these cells we observed signs of prominent dendritic regenerative plateau potentials, suggesting that active dendritic integration may play an important role in coding properties of these neurons. We have set up a virtual reality system on the rig for to study place coding by recorded neurons during spatial navigation in different environments.