Final scientific report of the "Élvonal" application of Dr Zoltan Nusser
Reporting period: 1st January 2018 – 30th September 2018

The applicant was awarded an ERC Advanced Grant in May 2018 on a topic that shows substantial overlap with that of the current "Élvonal" application, therefore the applicant prematurely terminated his "Élvonal" application on 30th September 2018, before his ERC grant started (1st October 2018) in agreement with his statement in the original application. Hereby, the applicant summarizes the research progress of the first 9 months of the grant period.

The main *hypothesis of the application was that quantitative molecular differences underlie the functional diversity of synapses* made by molecularly and morphologically homogeneous presynaptic neurons onto molecularly and morphologically homogeneous postsynaptic neurons. The *general aim* of the proposal was to identify the molecular fingerprints defining key biophysical properties of hippocampal glutamatergic synapses.

The following five *specific aims* were proposed to test the hypothesis:

a1 To identify the variability in the functional properties of homogeneous populations of hippocampal glutamatergic synapses.

a2 To develop a high-resolution method that allows quantitative molecular analysis of functionally characterized single hippocampal glutamatergic synapses.

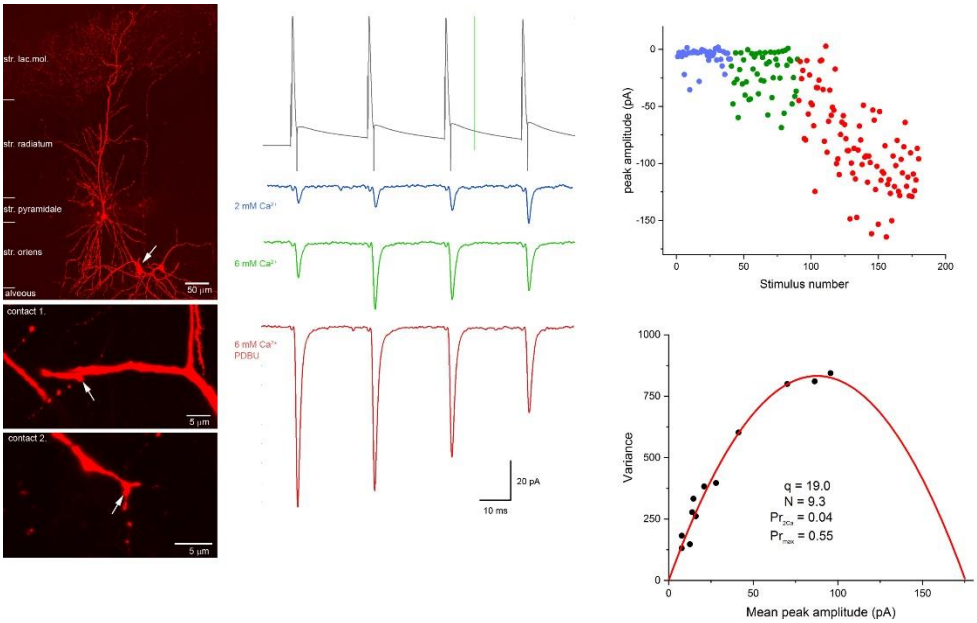
a3 To investigate the relationship between the biophysical properties of, and the amounts of synaptic molecules at, individual glutamatergic synapses.

a4 To reveal causal links between the quantity of different proteins and defined functional properties of these synapses.

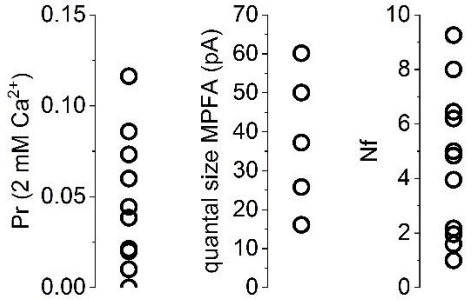
a5 To investigate the functional properties and the proteomics of synapses made by functionally characterized nerve cells in behaving animals.

During the reporting period, the research group of the applicant has made significant progress in the first two and the last specific aims. Here, a summary of the progress and achievements is presented.

a1) We have made a significant progress in accomplishing this specific aim. We have performed dual whole-cell patch-clamp recordings between hippocampal CA1 pyramidal cells (PCs) and oriens-lacunosum moleculare (O-LM) interneurons (INs) in acute brain slices of either FVBAnt mice or transgenic mice in which tdTomato is expressed under the promoter of the α2 nicotinic acetylcholine receptor (SHRNA2 mice in which the O-LM cells are specifically labelled). We applied a short train of presynaptic APs at 40 Hz and observed small amplitude, facilitating EPSCs between these cells, consistent with our previous data and those published by others. We have changed the concentration of the extracellular Ca^{2+} and applied a phorbol ester analogue (PDBU) to impose different release probability conditions to the synapses. We then calculated the mean and variance of the postsynaptic responses under each condition and fitted a parabola to the data to obtain the quantal size (q), the vesicle release probability (Pv) and the number of functional release sites (Nf; see figure below for one PC – O-LM cell connection.

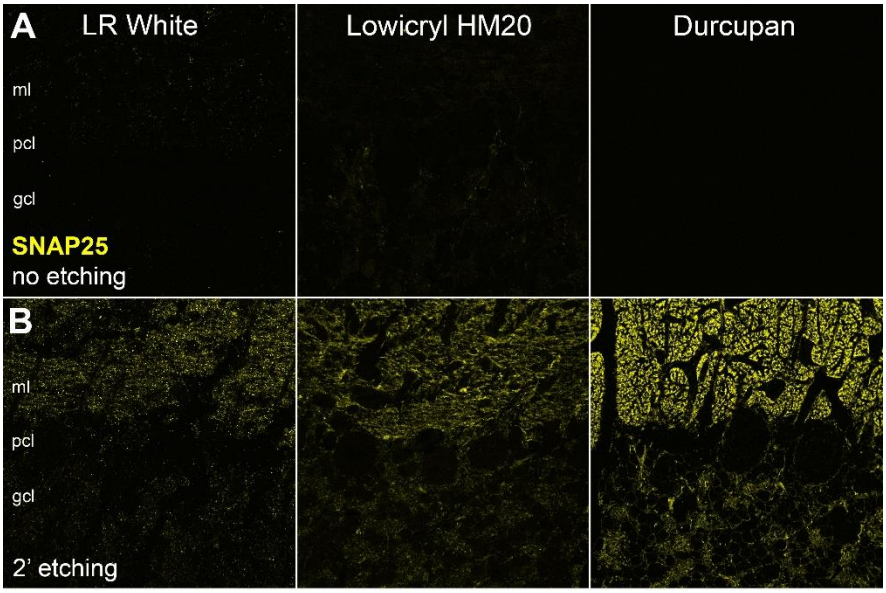


For the connection shown above, we obtained a q of 19 pA, an Nf of ~9 and the Pv at 2 mM Ca^{2+} was only 0.04. From the anatomical analysis of the connected pair, we determined that there were only 2 synaptic junctions between the presynaptic axon and the postsynaptic dendrite, indicating that there were 4-5 functional release sites per presynaptic active zone. We successfully determined quantal parameters in 11 pairs in which the postsynaptic cells were morphologically identified as O-LM INs and found remarkable variability among these quantal parameters (see figure below). The Nf varies from 1 to 9, the Pv from 0 to 0.12 and the q from 15 to 62 pA. These results provide clear evidence that our 1st hypothesis was correct; namely the biophysical properties of synapses made by morphologically and molecularly homogeneous pre- and postsynaptic cells are highly heterogeneous.



We have also conducted experiments in which we have examined the EPSCs made by CA1 PCs onto parvalbumin expressing, fast spiking basket cells and found remarkable heterogeneities in the amplitude and short-term plasticity of the EPSCs. The quantal analysis of these connections is under progress.

a2) We have made a substantial progress in our second specific aim. We are in the final stage of completing a study in which we have developed a novel, sensitive, quantitative postembedding light-microscopic immunolocalization technique. The most important aspect of our method is the use of conventional epoxy resins (e.g. Durcupan) instead of the widely used acrylic resins (e.g. LR White and Lowicryl HM20) for postembedding localization. Our major finding is that etching the epoxy resin-embedded tissue with Na-ethanolate transforms the tissue into a highly immunogenic material, which allows the immunolocalization of dozens of synaptic proteins with a much higher sensitivity than that obtained on acrylic resins (see figure below).



In addition, we have developed an antigen-retrieval method, based on SDS treatment at 80C that further increases the sensitivity of the reactions. In order to be able to localize many synaptic proteins in a single synapse, we had to develop an elution method with which a set of primary and secondary antibodies can be removed (eluted) from the labelled tissue following the imaging step and a new set of primary and secondary antibodies can be applied to visualize new proteins. These staining-elution steps can be repeated 4-5 times, allowing the visualization of a dozen of synaptic molecules. By carefully selecting the most relevant proteins, we could quantitatively determine the amount of these proteins and compare them to the biophysical properties of the synapses. Our aim is to correlate the biophysical properties of individual synapses with the amounts of a large number (n) of proteins in a multi-dimensional (n+1) space and ask which protein or proteins show the strongest correlation with a given functional property. We have presented a poster on the FENS Forum in Berlin 2018 of our results and we are now preparing a manuscript for publication.

a5) We have made two major achievement regarding our fifth specific aims. We have implemented hippocampal two-photon Ca^{2+} imaging of GCaMP6f-expressing neurons in awake head-restrained mice. This required the implantation of an 'imaging window' through the neocortex above the dorsal hippocampus. This has been published first by Dombeck et al. and later by many other laboratories and we have successfully implemented this technique in our laboratory. In parallel with this development, we have also implemented a spatial navigation task for head-restrained mice. The mice are navigating in a virtual environment in two different linear mazes. The animals are rewarded in the reward zones with a drop of water and the reward zones are located in different positions in the two linear mazes. We have successfully trained two cohorts of mice (2x10 mice), which performed around 90% accuracy after approximately 5-7 days of training. Now we are performing two-photon Ca^{2+} imaging of hippocampal CA1 PCs while the animals navigate in these virtual linear mazes.

We have also implemented the spike inference method of Deneux et al. (2016, Nat Comm) to transfer the fluorescence [Ca^{2+}] transients into action potential trains. All spike inference methods so far failed to achieve accurate estimation of spike timing due to many reasons, but the most obvious one is the widely different expression levels of the genetically encoded Ca^{2+} sensors (e.g. GCaMP6) following transgenic or viral expression. We have made two fundamentally important progress in this field and achieved a previously unprecedented spike inference accuracy of ~5%. We have determined the expression level of GCaMP6f after the imaging session and subselected cells with similarly low expression levels. By doing

this, we could significantly reduce the error rate. In addition, we developed an iteration method of determining the unitary, single AP-evoked fluorescent Ca^{2+} transient amplitude from the average unitary Ca^{2+} transient amplitude. By estimating the unitary amplitude of Ca^{2+} transients in each individual cell and using it for the consecutive spike inference, the mean error rate was further reduced. We have written a MS (Éltes et al., 2018, EJN, under revision) from these data, which is now submitted to the European Journal of Neuroscience for publication. The support of the 'Élvonal' application is acknowledged.