Summary

The goal of the **IDEGT: 125148 K_17** project was to better understand the signaling principles of hippocampal mossy fiber axon terminals as they are suitable models for the question how neuronal communication in cortical axons creates cognition and other neural functions. Specifically, the 125148 K_17 project focused on action potential signaling in the small mossy fiber terminals (sMFT) and compared them with the relatively well-known, but exceptionally large giant terminals (GMFT) of the same axons using direct patch clamp electrophysiology and voltage-sensitive dye imaging. However, the 125148 K_17 grant support had to be terminated, because our research group was granted a Consolidator ERC grant whose topic substantially overlaps with the OTKA_K support. Specifically, the aim of the awarded ERC grant is to provide insight into the fundamental signaling principles in various types of hippocampal axons, including the mossy fibers. In addition to the same overlapping questions, the two grants use the same experimental approaches. Because both funding agencies prohibit substantial overlap between ongoing grants and because of the larger scope of the ERC_CoG grant, I choose to continue this grant and terminate the OTKA_K grant support. Nevertheless, the aims of the 125148 K_17 project will be pursued - and hopefully achieved - with the help of the ERC_CoG grant. The ERC support started on 2018 April 1. Thus, the OTKA_K support was terminated four months after its start, on the previous day. NKFIH officers were notified in advance about the termination in advance.

The four month-long support period was sufficient to complete the initial goals of the 125148 K_17 project. Specifically, first using direct recordings from small and large mossy fiber terminals, we revealed that the recorded action potential shapes are similar in GMFTs and sMFTs in spite of their different structure and neuronal functions. Second, we created a computer model that simulate not only the recorded neuronal structures but also the recording instrument. This model allows for the isolation of biological signals from instrumental influence in distorted recordings, which is often the case with small recorded structures such as small axons. Third we established voltage-sensitive dye imaging from axons, which is a new method in our laboratory and allows for simultaneous measurement of action potentials in various locations along the axons without the influence of the recording pipette. During the four-month-long

period we performed proof-of-principle experiments necessary to show the reliability of this novel experimental approach. Albeit this short support period did not allow completion of publications, the results that were obtained with the help of this grant were instrumental for an ongoing project and will be included for papers that will be published in the future. Altogether, even this short period of support from the OTKA_K grant was essential for the large ERC_CoG project, which addresses the same question, the fundamental principles of axonal signaling, in a larger context.

Aim of the project was (copied from the original grant proposal): *Axons broadcast action potential activity to synaptic terminals that translate this digital signal to analogue postsynaptic responses in thousands of follower neurons. Albeit this digital/analog conversion constitutes practically half of the primary neuronal computation in the brain, its principles remain elusive. Our knowledge about the axonal signaling comes mostly from studies of axon terminals that have unusual morphology, such as the extremely large mossy fiber terminals (GMFT) of the dentate gyrus granule cells. However, similar insightful direct electrophysiology was not possible for small axons, which constitute the large majority of synapses of the CNS. As our preliminary data demonstrate, now we gain access to single small axon MFTs (sMFT) and directly investigate the properties of axonal signaling principles in these regularly sized axon terminals using patch clamp together with the support of novel imaging methods. Because the sMFTs and GMFTs formed on the same axons, we are able to examine how size-dependent axonal mechanisms contribute to their distinct physiological functions and whether axonal signaling is governed by the same principles in the small axon terminals as in the unusually large axonal structures. For this aim, we will directly compare the better known active and passive membrane properties of the GMFTs with those of the sMFTs. Furthermore, because the synaptic functions of the sMFTs and GMFTs are segregated, our results will be also important for determining the specific contribution of the sMFTs to hippocampal functions. Thus, this proposal will provide unprecedented insights into general axonal signaling principles and hippocampal functions.*

Work plan for the first year of support in the application was (copied from the original grant proposal): *During the first year of the support we will purchase and install the new laser light source, mechanical shutter and the tools that are necessary to build them onto the existing microscope systems. These new devices are necessary for optimal signal-to-noise voltage-sensitiv dye imaging experiments from sMFTs (small mossy fiber terminals). We can start a number of crucial experiments, which require direct electrophyiology of sMFTs, independent of the new equipment acquisition . In the first year, we will collect data concerning the action potential properties in small and GMFTs (giant MFT) using direct whole bouton electrophysiology (as shown in FIG.1). The obtained AP shape properties will be necessary for the next experimental steps as detailed below. According to our experiences with the preliminary data, one experienced experimenter can obtain reliable action potential data from one or two sMFTs during each week. However, recording of more than one the GMFTs is feasible each day. Because the size of the sMFTs is in the same range as that of the majority of the cortical synapses, these experiments will promote our understanding of the general axonal signaling principles. One of the potential difficulties of these experiments derives from the small size of the recorded structure and the used pipettes. Because the biological voltage signals are potentially contaminated with instrumental distortions and the original kinetics of the fast neuronal events may be altered, we have to be cautious with the interpretation of the obtained signals. Using the newly acquired computer we will employ multi-compartmental simulations of complete segments of mossy fiber axons with and without a modelled pipette to isolate the biologically relevant*

Results of the supported period

1. We started a number of crucial experiments, which require direct electrophyiology of sMFTs, independent of the acquisition of new equipment. (We did not purchase equipment from the OTKA_K support). Specifically, we collected data concerning the action potential properties in small and GMFTs (giant MFT) using direct whole bouton electrophysiology (as shown in FIG.1). The recorded action potentials in GMFTs and sMFTs have surprisingly similar shapes. This is surprising, because there are substantial differences in biophysical properties of sMFTs and GMFTs mostly due to their different size. The passive membrane properties confirmed these differences, such as input resistance and membrane capacitance, yet, tha action potential shapes were similar. The functional consequences of the similar action potentials are not obvious and requires further investigations. Nevertheless, this results suggest that action potential shape does not contribute to the different synaptic properties of sMFTs and GMFTs. These results has been presented as part of posters at international conferences after the termination of the 125148 K_17 project.

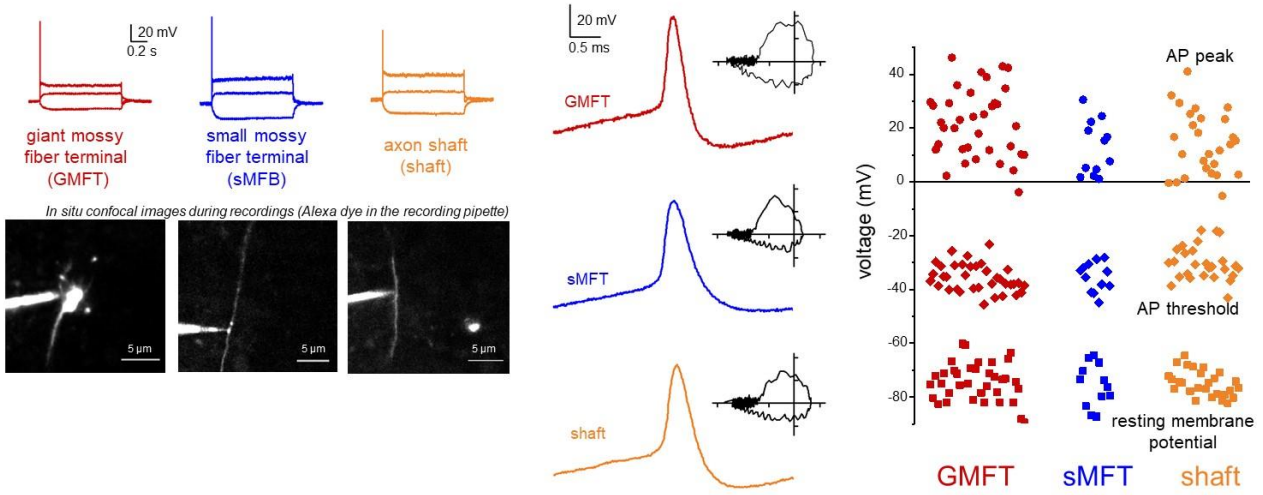

Figure 1.
Direct recordings from two different types of mossy fiber terminals and axons shaft. sMFTs and shaft were smaller than 2 m and were identified under improved DIC imaging in acute hippocampal slices. The action potential shapes and basic parameters were surprisingly similar, as quantified on the right graph.

2. It became clear that the small size of the recorded structures limit these direct recording experiments not only because of the difficulty of performing the actual recordings (the size of sMFTs is in the range of 1-2 µm), but also because the recorded signals from these small structures are inherently distorted. Specifically, the capacitance of the sMFTs is typically 0.5-1.5 pF, which is in the range of the uncompensated capacitance of the recording pipette and amplifier circuit, even in case of the best possible recording circumstances. Therefore, my colleagues came up with a solution that removes instrumental distortion from these recordings and obtains isolated biological signals. With the leading role of János Brunner, the postdoctoral researcher who received his salary from the 125148 K_17 they measured and reproduced the electrical behavior of the individual components of the amplifier circuit and pipette. The simulations were validated by several measurements of the isolated circuit elements. These simulations reproduce not only the typical recording artefacts but also the distortions that they introduce. When these simulations ran together with the precise reconstruction of the recorded biological structure, it is possible to isolate the biologically relevant signals. In short, it is possible to see how action potentials of small axons would look if the recording instruments (and their distortions) are not present. The progress of this modelling study was faster than anticipated and the experimental part is already concluded. The first version of the manuscript about the model is already completed and it will be submitted for consideration as a paper in 2020.

3. One of the goals of the proposal was to establish voltage-sensitive dye imaging from individual axonal structures. The first step for this goal was to establish the optimal experimental conditions, such as optimal dye concentration, duration of the dye loading and the determination of the potential toxicity of the VSD imaging. For this aim we used somatic dye loading during the supported period because somatic recordings are much more reliable compared to axonal recordings. Thus, we were able to test multiple parameters and measure a known phenomenon to validate the accuracy and efficacy of this new imaging methods. Somatic loading was sufficient to load JPW1114 dye into the proximal axon and image them at high imaging rates for this initial aim. An example is shown on *Figure 2*. These experiments were done by the PI with the technical assistance of Dóra Kókay. We continued the development of VSD imaging after the termination of the K_17 support using other funding sources.

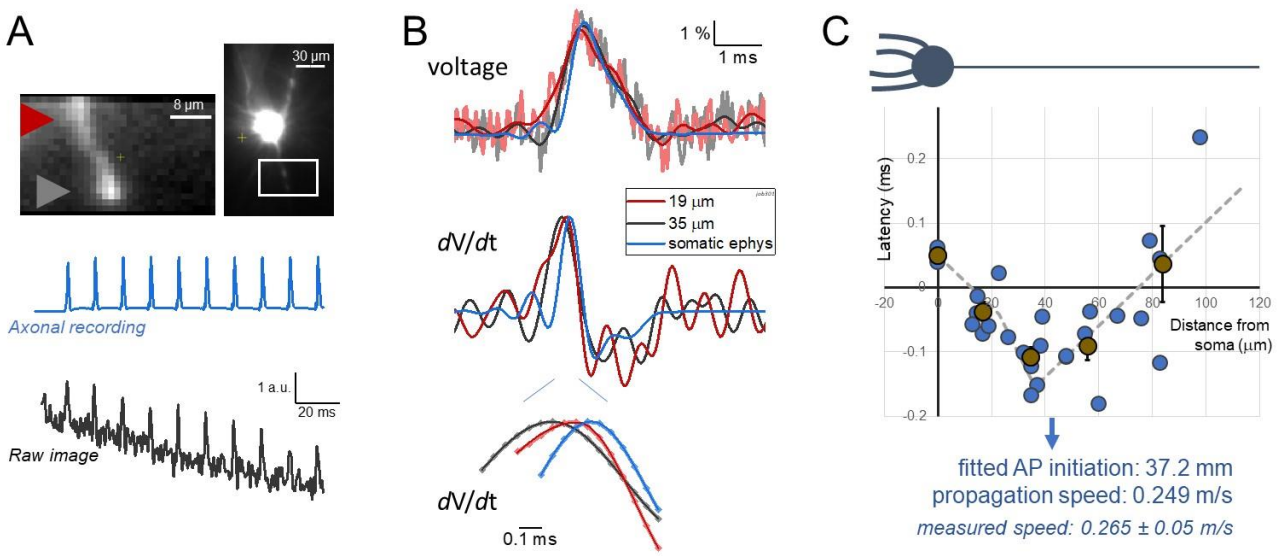

Figure 2.
Voltage-sensitive dye imaging revealed proximal initiation of action potentials in the axon initial segments of dentate gyrus granule cells. **A**. Voltage-sensitive dye JPW1114 (80 µM) was loaded in granule cells within an acute hippocampal slice using somatic patching. After a 20-40 minute loading period the axons were imaged at 20kHz imaging rate (black trace from the representative cell) while action potentials were evoked from the soma (blue traces). **B.** Average action potentials along the axon initial segment. The first derivative of the imaged voltage signals allowed the precise measurement of the propagation of the spikes. **C.** Summary data from 12 granule cells. Latency values are shown relative to the somatically recorded spikes. The data shows that action potentials of granule cells are initiated between 35-40 µm from the soma and propagates at approximately 0.25-0.27 m/s speed.

Altogether, this short support was essential for a substantial development of the methodological repetoar of the research group allowing us to address previously unattained fundamental questions about the neuronal signaling principles.

Use of resources

We did not use the K_17 support to purchase the ***equipment*** that were planned in the proposal, including laser light source and optical components. Nevertheless, these equipment became available from other funding sources. We were able to complete the experiments that were planned during the initial months of the K_17 support because these not yet needed the new equipment components. A modification has been requested and granted (on 2018.01.24.) for the allocations and differences in the budget.

The support provided salaries for a postdoctoral researcher (who was involved in the generation of result #1 and #2) and a technician who helped the project in several technical aspects. Altogether, 1 698 573 has

been spend on salaries and related salary costs. Other group members were also contributed to the project, but their salaries came from different sources.

Consumable costs: For the experiments showed above we needed to use experimental animals. Furthermore, some of the equipment that were necessary for the experiments are part of the institute imaging facility, which charges with user charges. A computer maintenance cost also incurred during the supported period that was necessary for the sufficient network and computational capacity of the modelling experiments.

Publications: Albeit we could complete a publication within this short support period, the results that were obtained with the help of this grant were instrumental for an ongoing project and will be included for papers that will be published in the future. One of these manuscript (modelling the recording instruments) is almost complete and will be submitted in the first half of 2020.

Összefoglaló

A project célja a hippocampalis moharost axon terminálisokban zajló jelfeldolgozási folyamatok jobb megértése, mivel az axonok működését - bár közismerten alapvető szerepűek az idegi jelfeldolgozásban - kevéssé érjük, mivel közvetlen vizsgálatuk technikailag nehéz volt. A projekt azt a konkrét kérdést tette fel, hogyan működnek az akciós potenciálok az apró méretű moharost terminálisokban (sMFT) a már jobban ismert, de különlegesen nagy méretű moharost terminálisokhoz (GMFT) képest. Ehhez közvetlen patch clamp elektrofiziológiai és feszültség-érzékeny festék imaging módszereket hívtunk segítségül. Azonban az OTKA támogatást idő elött le kellett zárni, mivel a kutatócsoportunk elnyert egy ERC Consolidator grant-ot, amelynek a témája jelentősen átfed az OTKA_K támogatással. Ezért, a 125148 K_17 project 4 hónap támogatási időszak után lezárult. Mindenesetre a project céljai meg fognak valósulni az ERC_CoG támogatásával. A rövid négy hónapos támogatási időszak elég volt ahhoz, hogy a 125148 K_17 project kezdeti céljait teljesítsük. Közvetlen patch clamp elvezetésekkel kimutattuk, hogy a GMFT- és sMFT akciós potenciálja meglepően hasonlóak. Továbbá létrehoztunk egy olyan computeres szimulációt, amely lehetővé teszi a biológiai jelek izolálását olyan elvezetésekből is, ahol a mérő apparátus elkerülhetetlen hibákkal szennyezi az elvezetett jeleket. Így egy eddig megoldatlan - a saját kísérletink esetén különösen súlyos - problémára tudunk megoldással szolgálni. Harmadrészt, adaptáltunk egy új feszültség-érzékeny festékeken alapuló mérési eljárást az axon különböző pontjain történő akciós potenciál mérésekhez. Az elvégzett kísérleteink igazolták ezen új módszer megbízhatóságát.