FK-18 129019
Closing report (2018. 12. 01 - 2023. 11. 30.)

During the grant period we have implemented novel experimental methods, analysis procedures and the grant contributed to multiple scientific works culminating in 6 published scientific papers (among them: Nature Communications, PLOS Biology, Science) and opening up further promising research areas. The grantee is first author in 2 and coauthor in 3 of the 6 published works. In 1 publication the method developed by the grantee was used and therefore the grant was mentioned in the acknowledgements (Nature Communications. 2023 doi: 10.1038/s41467-023-41746-0).

1. Hippocampal code for aversive events

Using our novel head-fixed experimental arrangement developed by the grantee we have investigated the hippocampal coding of aversive experiences. We have found pyramidal cells with aversive stimuli linked activity, defined as air puff cells. Their responses did not depend on stimulus location, it followed the aversive event if it was relocated to a different site. We have also found that spatial aspects are shaping their activity even if the responses were location independent. Our data showed that if the aversive stimulus occurred inside a place cells' place field it resulted mostly activity reduction while the firing enhancement was more frequent if the stimulus occurred outside the place field ("conjunctive" place cells). Air puff cells on average contacted postsynaptically twice as much interneurons as other pyramidal cells highlighting that they represent functionally distinct population of hippocampal pyramidal cells. We have implemented a novel independent component analysis based assembly detection algorythm and discovered that air puff cells were preferentially coactive, forming functional neuronal assemblies, „air puff assemblies". These air puff assemblies were active mostly during immobile periods coincident with hippocampal sharp wave ripple activity. The reactivation strength of air puff assemblies were gradually enhanced between consecutive air puff stimulus epochs. Strikingly in about half of the sessions the air puff assemblies were already present even before the first air puff stimulus.

The above detailed scientific results were accepted and published in Nature Communications.

2.Median raphe vGLUT2 neurons in fear memory recall

At the beginning of the grant period we have published (Science) in a collaborative work the discovery of the median raphe vGLUT2 neurons and their crucial role in fear memory aquisition.

In the current project our goal is to clarify the role of the newly discovered vGLUT2 neurons in the recall of past fearful events. We have implemented an auditory cued fear conditioning paradigm in head-fixed experimental arrangement and performed optogenetical tagging to identify median raphe vGLUT2 units. The summarized electrophysiological data indicated that the median raphe vGLUT2 neurons enhanced their firing activity during fear memory recall. The magnitude of this firing enhancement showed gradual reduction, „extinction" upon repeated presentation of auditory stimuli parallel with with the pupil responses.

Overall these data provide strong evidence for the involvement of the vGLUT2 neurons in fear memory recall.

The combined optogenetic electrophysiolgoical data is part of a large project using further behavioral experiments and anatomical investigations. The manuscript is under preparation.

3. Functional connection between the GABAergic median raphe and the hippocampus

During the reported period we have published our study investigating the GABAergic cell population of the median raphe.

According to our data most of the optogenetically identified GABAergic median raphe neurons showed phase coupling to hippocampal theta oscillation. Large part of the theta coupled GABAergic neurons increased or decreased their activity during hippocampal ripple oscillation. Interestingly GABAergic neurons with enhanced ripple linked activity preferentially fired at the ascending phase of theta while neurons with reduced ripple linked activity preferentially fired at the descending theta phase. Our results point to the tight functional connection between the GABAergic median raphe and the hippocampus.

4. Optimal recording channel geometry for multichannel electrophysiological recordings
In this collaborative work we have investigated the geometry of optimal channel arrangement to achieve the highest unit number per one recording channel. Our results are published during the grant period.

5. Brainstem nucleus incertus shapes dentate gyrus engram cell selection during fear memory processes
In this recently published work we have shown that brainstem nucleus incertus through interneuronal activation is able to allocate dentate gyrus engram cells. Reactivation of the nucleus incertus and its dentate gyrus interneuronal target results in engram reactivation and fear memory recall. The grantee performed the in vivo head-fixed electrophysiological experiments proving the functional connection between the anterior cingulate cortex – nucleus incertus – dentate gyrus.
The findings were published in PLOS Biology.

6.Implementation of one-photon calcium imaging
To investigate large scale neuronal activity during fear memory processes I have assembled and tested (first time in Hungary) the miniscope develeoped by researchers at the University of California, Los Angeles. The miniscope is a miniature microscope fixed on top of the mouse head suitable to record the calcium activity of hundreds of neurons simultaneously. The method would present a continuation of the granted project and also complementing the acute head-fixed experimental arrangement by allowing freely moving chronic recordings.

7.During the reported period I have supervised the diploma works of two students. Their work focused on assembly analysis and one-photon microscopy. They have successfully defended their diploma. I am supervising the work of another student performing median raphe related experiments.

8. During the grant period I gave several presentations to students (ELTE, Semmelweis University) about in vivo electrophysiology, optogenetics and the neurobiological basics of emotions, navigation and memory processes.