PD-16 121248
Final report (2016. 12. 01 - 2019. 11. 30.)

In the following, I am going to summarize the achievements of the grant period in two main sections: first I will present the important new methodological development which provided the basis for the experimental results presented in the second section.

1) Development and implementation of new methods in the Laboratory
Head-fixed experimental arrangement for multichannel recording
During the grant period I have planned and constructed an air-supported spherical treadmill for head-fixed recordings. This new setup enabled us the simultaneous sampling of extracellular activity from the hippocampus and the median raphe by high channel count electrodes. The new head-fixed setup has been developed during the grant period in parallel with the experimental work. To this date four different behavioral stimuli (rewarding water drops, neutral LED flash, tone and aversive air puffs) can be delivered with arbitrary combinations. The entire system is based on an Arduino controller programmed by the grantee. In the current version we are able to record a total of 192 channels which allows us to collect large number of multiple single units from the median raphe and hippocampal region and (~ 20 – 30 and ~ 70, respectively) and to register their response to various stimuli.

Optogenetical tagging
The head-fixed setup enabled the parallel insertion of an optical fiber with the recording silicone probe into the target median raphe region. During the grant period optogenetic tagging was optimized for the median raphe vGAT, vGLUT3 and vGLUT2 neurons.
By using optogenetic tagging it was possible to dissect the brain and behavioral state- dependent activity of the heterogenous median raphe cell population.
Overall, these methodological developments introduced a new approach in studying subcortical modulation in our lab.

2) Experimental data
Results related to the glutamatergic median raphe
The main focus of the project was the investigation of the vGLUT3 glutamatergic cell population of the median raphe. However, during the research period, a yet uncharacterized, vGLUT2-expressing glutamatergic cell population was discovered in the median raphe. According to the anatomical results, this vGLUT2 population is connected with brain regions linked with aversive information processing. To functionally characterize the vGLUT2 neurons, we performed simultaneous recordings from the hippocampus and median raphe of vGLUT2-Cre mice expressing Channelrhodopsin2 in vGLUT2 median raphe neurons. vGLUT2 neurons were identified by optogenetic tagging. We delivered behavioral stimuli with different valence to the head-fixed awake animal.
We have found that vGLUT2 median raphe neurons can be activated by aversive (air puff) but not with rewarding stimuli (Figure 1). This data proved that the vGLUT2 median raphe cell population is involved in aversive information processing.

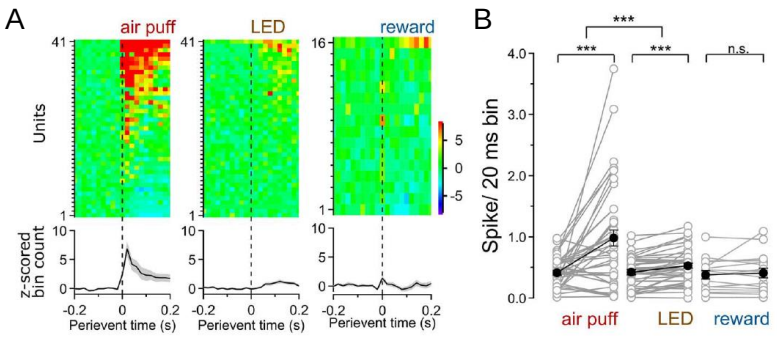

Figure 1. (modified Szőnyi et al. 2019, Science) Median raphe vGLUT2 neurons selectively respond to aversive stimuli. (A) Top matrices show the z-scored responses of individual vGluT2 neurons to different stimuli sorted by descending response magnitudes. Bottom plots show average response across all tagged units (from eight animals, mean ± SEM). (B) Paired plots of cells [same as those displayed in (A)] show the baseline versus stimulus-evoked firing activity of vGluT2 neurons (gray, individual tagged neurons; black, mean ± SEM). Air puffs evoked significantly larger responses than did LED flashes. Reward induced no significant population response.

We also characterized the coupling of identified vGLUT2 neurons to ongoing hippocampal oscillations. Median raphe vGLUT2 neurons did not show theta phase preference, however the firing rate of a subset of the neurons markedly decreased seconds before hippocampal sharp wave ripples.

The behavioral effect of the sustained activation of vGLUT2 neurons was also investigated. Prolonged optogenetic activation (10 s long 25 Hz) resulted in a rapid switch of brain states from large amplitude irregular activity (with sharp waves) to hippocampal theta oscillation. Behaviorally, sustained stimulation initiated movement of the animal. Further behavioral testing by our collaborators showed that population activation of median raphe vGLUT2 neurons resulted in place aversion. Chronic intermittent activity surges also led to anhedonia, a characteristic symptom of depressive behavior.

Based on this data we hypothesized that the newly discovered vGLUT2 neurons are key players in the processing of aversive stimuli, and their activity facilitates the appearance of hippocampal oscillation patterns linked to information acquisition. The above results can significantly contribute to a better understanding of the mechanism of neuropsychiatric disorders like major depression and anxiety disorders, and highlights that the glutamatergic component can stand behind pathological states previously linked to purely serotonin.

The other glutamatergic median raphe component, the vGLUT3 neurons are also in our research focus. We used the same experimental approach as for the investigation of vGLUT2 neurons however, the optogenetic tagging of vGLUT3 neurons are more challenging, because of their more restricted spatial distribution within the median raphe (these neurons are concentrated in a narrow, 100-200 micrometer wide region in the midline). To facilitate the identification of vGLUT3 neurons we increased the recording channel number to 64 in the median raphe. We found that the identified vGLUT3 neurons are weakly activated by aversive or neutral stimuli. Sustained activation of the vGLUT3 neurons initiated movement and hippocampal theta oscillation, similarly to vGLUT2 neurons. We have found that a subgroup of vGLUT3 neurons showed theta phase preference but none of the neurons in our sample exhibited slow ripple coupling activity as was the case with vGLUT2 neurons.

We are proceeding with the data collection related to the vGLUT3 neurons, but we already identified important differences between the two glutamatergic median raphe cell populations as highlighted above.

The largest cell population of the median raphe is composed of GABAergic neurons but the literature is relatively scarce about their physiology and function. In the grant period we collected large number of data about the median raphe GABAergic neurons. We utilized Archaerhodopsin to optogenetically identify them. Our data revealed heterogeneous hippocampal oscillation-associated activity patterns in this population. A subgroup of identified GABAergic neurons showed clear phase coupling to hippocampal theta oscillation. Most of these GABAergic neurons were also characterized by fast, ripple-coupled activity. Interestingly, the preferred theta phase was correlated with the sign of fast ripple coupling (negative or positive, Figure 2).

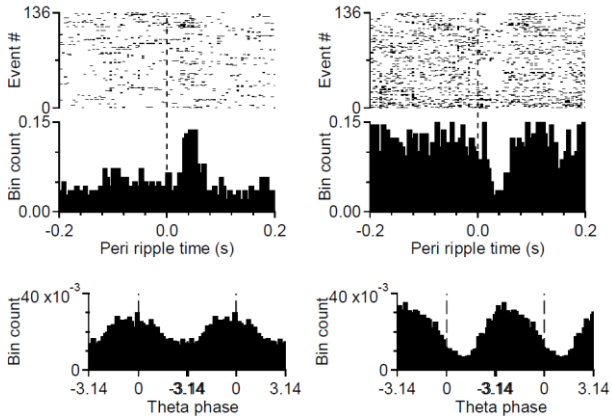

Figure 2. A subgroup of median raphe GABAergic neurons exhibited hippocampal oscillation coupled activity. Top: perievent histograms of 2 identified GABAergic units with increased (left) or reduced activity following hippocampal ripples. Bottom: corresponding theta phase couplings.

The GABAergic responses to aversive behavioral stimuli were heterogeneous (no response, increased or decreased activity). Anatomical data from our collaborators corroborated the heterogeneity of GABAergic neurons: they are endowed with different afferent and efferent connectivity pattern indicating complex local processing.
We are proceeding with the analysis of the median raphe GABAergic neurons but also working on to develop a more specific behavioral testing paradigm (focusing on positive and negative reward prediction errors) to dissect the observed anatomical and functional heterogeneity.

Other projects during the grant period
Hippocampal activity patterns during rearing behavior
During the grant period we completed and published a study investigating hippocampal oscillatory patterns and characteristic unit activity linked to rearing behavior. The project, by investigating hippocampal activity patterns, loosely connected to the main objective of the grant i.e. hippocampus - median raphe connection. In this study we discovered that during rearing behavior the hippocampus switches to a different operational mode. This mode is accompanied by elevated theta oscillation frequency, paralleled by a dramatic increase of the theta-high gamma coupling in the dentate gyrus probably owing to increased amount of multisensory information flow into the hippocampus via the dentate gyrus. We found that place cells' activity ceased during rearing and we discovered that a subgroup of putative pyramidal cells, the so called rearing-ON cells fired at the time of rearing events.

Muscarinic effect of clozapine N-oxide
In a collaboration, we focused on clozapine-N-oxide (CNO), the central compound of pharmacogenetics. It was uncovered that CNO in vitro decreases hippocampal ripple amplitude and increases ripple occurrence through muscarinic receptors. In vivo, CNO was peripherally

retroconverted to clozapine which can cross the blood brain barrier. We showed that in vivo, following CNO intraperitoneal injection, hippocampal ripple parameters were not altered. These results indicate that probably CNO itself may have the “nonspecific” muscarinic-type effect on ripples. The results are in manuscript format and under submission for publication.