Final scientific report
K-109790

In our proposal we aimed to uncover fundamental yet unknown aspects of subcortical modulation by focusing on the newly discovered, glutamatergic component of the ascending serotonergic system. We previously demonstrated that activation of glutamatergic neurons in the median raphe recruited a subset of interneurons in the hippocampus with very low latency, jitter and high success rate. Based on these findings, we hypothesized that glutamatergic neurons in the MR may be indispensable for initiating rapid switches of behaviors and for the correlated readjustment of modulation by the MR. To test our hypothesis, we planned to reveal the behavior-dependent activity of glutamatergic MR neurons. We aime to achieve our goal by combining high channel count electrophysiology to sample large number neurons in parallel, high speed tracking of the animals' movement in 3 dimensions to get behavioral data on neuronal timescale and optogenetics to identify neurons while being recorded.

During the grant period we have implemented the state-of-the-art combination of techniques that we proposed. Moreover, we have introduced patch clamp recording in head-fixed, behaving animals as a complementary method to study the inhibition/excitation dynamics in raphe neurons. By using the newly implemented tracking system, we have identified and characterized a novel operational state of the hippocampus that could be observed during rearing epochs while the animals were exploring the environment. We characterized vesicular glutamate transporter type 3-expressing neurons in the MR in anesthetized animals. In head-fixed behaving mice we have detected highly correlated activity of MR glutamatergic neurons with high frequency population transients (ripples) in the hippocampus. We could also show differential response of glutamatergic cells to salient versus neutral sensory stimuli. We also investigated the local GABAergic network of the MR since it is assumed to be crucial for determining its output. We observed large, often rhythmic membrane potential fluctuation and the occurrence of high frequency bursts in GABAergic neurons. Analysis of coupling to hippocampal patterns revealed both positively and negatively ripple-modulated inhibitory cells. By whole cell recording we could show massive hyperpolarizing transients in response to the optogenetic activation of the local GABAergic network. Finally, we also analyzed behavior-related population patterns in the MR and uncovered the transient augmentation of correlated activity by salient stimuli.

In summary, we have managed to assemble a cutting-edge combination of techniques by which the MR network could be studied in unprecedented detail. Our discoveries reveal the role of the major MR neuron types and their interactions in shaping the behavior-dependent modulation by the ascending serotonergic system.

Results in details

1. Hippocampal dynamics during rearing episodes (1)

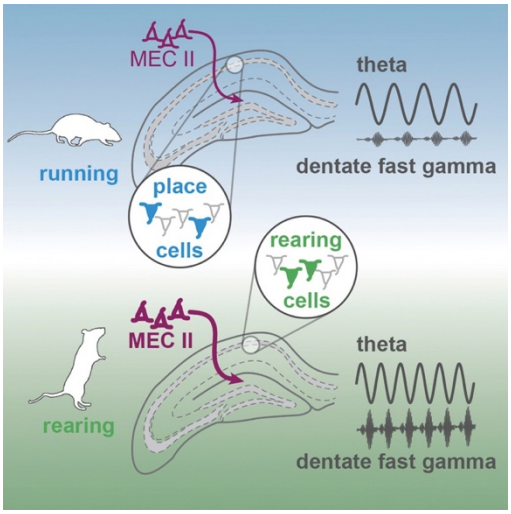

Figure 1. Rearing state vs. running during exploration. As shown on the cartoon, rearing episodes are characterized by increased theta frequency, strengthening of theta-coupled fast gamma in the dentate gyrus, activation of a subset of pyramidal cells (rearing cells) and the simultaneous decrease of place cell activity.

After setting up the 3D behavior tracking system, the first question we aimed to address was the operation of the hippocampus, the center of the brain's navigation system, during rearing. Sampling of distal cues during navigation is a key function of exploration that drives the animal to change its vantage point by rearing on its hind legs. By conventional (two dimensional) behavioral tracking rearing episodes cannot be reliably distinguished from momentary halts. Therefore, we turned to our system to reveal the electrophysiological underpinnings of rearing. We showed that the hippocampus is dominated by a different state compared to other phases of exploration. This state is characterized by elevated theta frequency accompanied by an augmented sink in the dentate gyrus and a dramatic increase of theta-fast gamma phase-amplitude coupling. We have also discovered that a subset of pyramidal cells increased their activity during rearing episodes whereas place cell firing was decreased if rearing occurred in a neuron's place field. Our findings (summarized in Figure 1) implied that the function of rearing can be both the collection of information from distal sources and the correction of the forming spatial map based on this information. Importantly, this study also served as a proof of concept of our behavioral tracking and highlighted the importance of high speed motion registration in 3 dimensions.

2. Divergent in vivo activity of serotonergic and non-serotonergic neurons in the median raphe region. (2)

In this study, we demonstrated that purely glutamatergic neurons, as marked by VGluT3-immunreactivity and serotonin-negativity (VGluT3+/5-HT–), fired faster, more variably and were permanently activated during sensory stimulation, as opposed to the transient response of the slow firing purely serotonergic (VGluT3–/5-HT+) subgroup. VGluT3+/5-HT– cells were also more active during hippocampal theta. In addition, the VGluT3–/5-HT– population, comprising putative GABAergic cells, resembled the firing of VGluT3+/5-HT– neurons but without any significant reaction to the sensory stimulus. Interestingly, the VGluT3+/5-HT+ group, spiking slower than the VGluT3+/5-HT– population, exhibited a mixed response (i.e. the initial transient activation was followed by a sustained elevation of firing). Phase coupling to hippocampal and prefrontal slow oscillations was found in VGluT3+/5-HT– neurons, also differentiating them from the VGluT3+/5-HT+ subpopulation. This study gave us the basic characteristics of the state-dependent firing pattern of VGluT3-expressing glutamatergic MR neurons.

3. Characterization of optically tagged glutamatergic neurons in the head-fixed behaving mice.

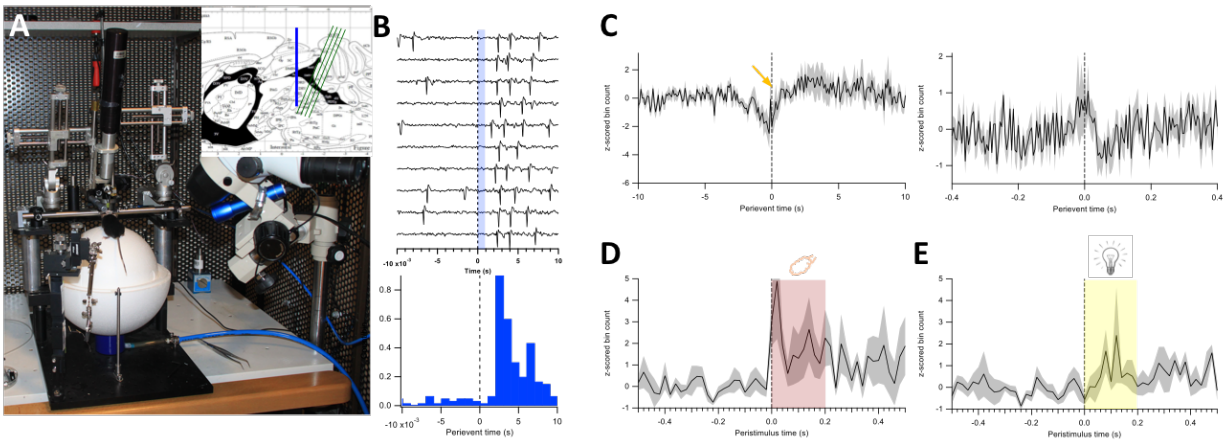

Figure 2. Characterization of Median Raphe glutamatergic neurons in head-fixed behaving mice. A. The recording apparatus. **B.** Optotagging of a glutamatergic (VGluT2) MR neuron: upper: spike raster, blue shaded area labels the 1 ms blue light pulse; lower: peri-stimulus time histogram showing the temporally focused entrainment of the tagged neuron. **C.** Left peri-event time histogram (PETH): a subgroup of VGluT3-expressing glutamatergic neurons decreased activity around ripples whereas another subgroup (right PETH) exhibited transient, rapid increase of activity. **D & E.** Differential effect of salient (aversive) versus neutral sensory stimuli: the former led to rapid activation in contrast to the slow ramp-up of firing by the latter.

Next, we developed a high throughput approach, head-fixed mice recorded by multielectrode arrays (silicone probes), to sample large number of neurons simultaneously in behaving mice for exploring behavior-coupled activity of individual neurons as well as emerging network patterns. We have recorded the neurons' response to both spontaneously occurring hippocampal population patterns and salient or neutral sensory stimuli. As demonstrated on Figure 2 we could successfully optotag glutamatergic (either VGluT2- or VGluT3-expressing, an example of the former is shown on panel B) MR neurons. A large proportion of VGluT3 neurons decreased firing starting multiple seconds before hippocampal high frequency population events (sharp wave ripples). In contrast to the inhibited group, some VGluT3 neurons was transiently facilitated during ripples. We could observe a striking differential response to salient versus neutral sensory stimuli: aversive air puffs caused a rapid, < 10 ms activation followed by sustained elevation of activity whereas the onset of response to neutral light stimulation was delayed by ~ 100 ms (Figure 2D and E). We plan to publish the results within a year.

4. Characterization of the local inhibitory network of the MR by the combination of whole cell patch clamp recording and optogenetic manipulation of MR GABAergic neurons in head-fixed, behaving mice.

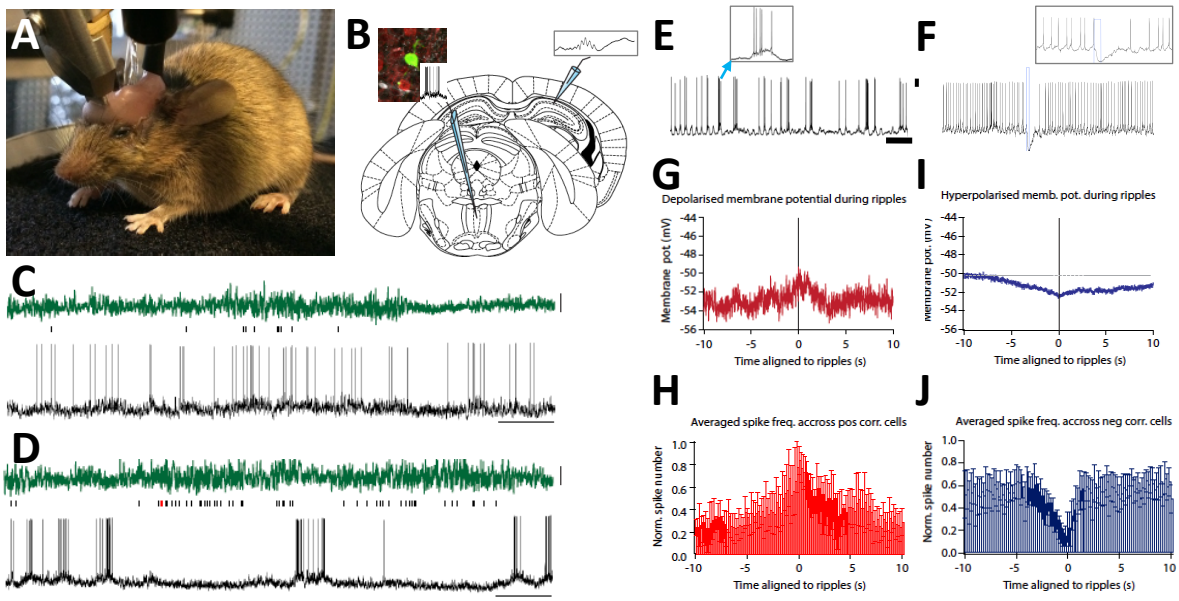

Figure 3. Characterization of the local inhibitory network in the Median Raphe in head-fixed behaving mice. A. The recording apparatus. **B.** Position of the patch and LFP recording pipettes. The left insert shows a recorded and labeled neuron with a short recording segment; right insert shows a hippocampal ripple. **C.** A GABAergic MR neuron that increased its activity during ripples (black vertical marks under the LFP trace) as opposed to another neuron (**D**) that was silent during the ripple state. C&D horizontal scale = 6 sec, vertical for LFP = 0.5 mV. **E.** GABAergic neurons were characterized by large subthreshold membrane potential fluctuations and occasional high frequency (up to 600 Hz intra-burst F) bursts (see insert). Horizontal scale = 1 sec, vertical = 10 mV **F.** Synchronous activation of GABAergic neurons triggered large hyperpolarization and intermittent pausing of activity in a non-GABAergic MR neuron. Inset duration = 1 sec. **G.** Ripple-triggered membrane potential average shows depolarization of activated cells in contrast to the ripple-coupled hyperpolarization of suppressed cells (**I**). **H & J.** Peri-ripple firing histograms show the increase of activity of some versus decrease of other GABAergic neurons.

Besides glutamatergic neurons, we started to explore the role of local inhibition in setting the output of the MR. Previous results implied that local GABAergic neurons would form a feed-forward inhibitory network capable of collecting and gating the inputs of the MR. We deployed the patch clamp technique in head-fixed awake mice both for recording the membrane potential dynamics of GABAergic neurons and for uncovering the dynamics of inhibitory and excitatory potentials in the local targets i.e. glutamategic and serotonergic neurons, of the feed-forward inhibitory network. We observed much larger, oftentimes rhythmic membrane potential fluctuations of GABAergic neurons (Figure 3E) accompanied by high (~ 600 Hz) frequency bursts. Analysis of ripple-coupled activity uncovered positively as well as negatively correlated cells. Optogenetic activation of GABAergic neurons triggered massive (> 10 mV) hyperpolarizing potentials and the temporary cessation of spiking of non-GABAergic MR neurons. The results are expected to be published in a year.

5. Emergence of behavior-dependent population patterns in the MR.

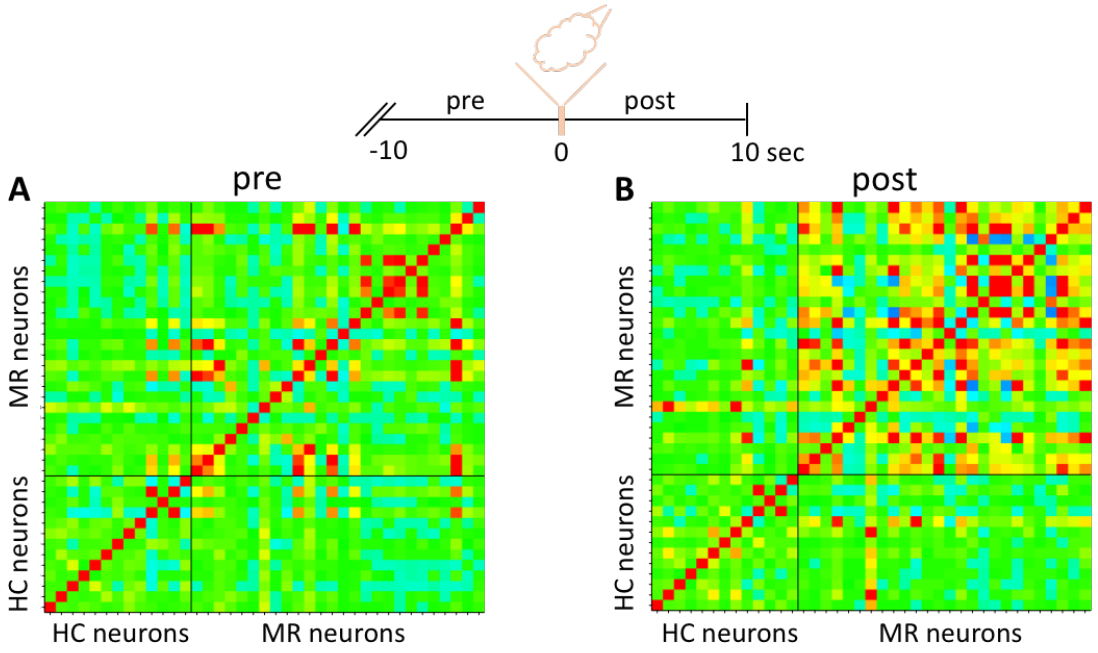

Figure 4. Salient stimuli induce the transient reorganization of correlation pattern of neuronal activity in the MR. A. Correlation between each pair of neurons recorded in the MR (n = 26) and the hippocampus (13) during a control period 10 seconds before delivering an air puff. Every cell in the matrix corresponds to a correlation value in a neuron pair (741 pairs) with warmer colors marking higher correlations. **B.** A dramatic increase of the number of correlating pairs was detected in the 10 second post-air puff period.

The application of high density multielectrode arrays (silicone probes) let us record multiple neurons in parallel from a geometrically well-defined volume (determined by the arrangement of recording sites) with minimal damage. We have found a significant reorganization of the MR network following an aversive stimulus (air puff). The number of co-active neurons, indicated by higher than control correlation, increased dramatically (Figure 4). We will investigate the role of the major MR neuron types in the reorganization process, the temporal dynamics of co-firing and long-lasting changes of correlation patterns probably reflecting plasticity processes.

Publications from the grant-supported topics

1. Domonkos A, Nikitidou Ledri L, Laszlovszky T, Cserep C, Borhegyi Z, Papp E, Nyiri G, Freund TF, Varga V. (2016) Divergent in vivo activity of non-serotonergic and serotonergic VGluT3-neurones in the median raphe region. *J. Physiol.* 594: 3775-3790.

2. Barth AM, Domonkos A, Fernandez-Ruiz A, Freund TF, Varga V. (2018) Hippocampal network dynamics during rearing episodes. *Cell Reports* 23: 1706-1715.