1st year (2017-09-01 - 2018-08-31)
Role of the inhibitory neurons in the pontine reticular formation in switching behavior

In the first year of the three-year research grant our aim was to describe the morphological and functional connection between the frontal cortex and the glycinergic neurons of the pontine reticular formation and determine the efficacy of information transmission between the two regions. For this we used the following animal strains, virus constructs and experimental procedures.

animal strain:
rbp4-cre//GlyT2-eGFP double transgenic mouse strain: in these animals cre and eGFP are expressed under the control of rbp4 and GlyT2 promoters respectively. Cre expressing cells are found in the layer 5 (L5) of frontal cortical areas and can be transfected by cre dependent virus constructs. eGFP expressing cells can be found in the rostral brainstem and show green fluorescence.

virus construct:
To transfect the cre expressing L5 pyramidal cells in the frontal cortex we used cre dependent virus containing the floxed genes of channelrhodopsin 2 (hChR2), a photo-sensitive cation channel and mCherry reporter molecule.

experiments:
Our aims were to

1. map the distribution of frontal cortical fibers around the glycinergic cells
   To label the brainstem projecting L5 pyramidal neurons of the frontal cortical regions we injected cre dependent virus construct (DIO-mCherry-hChR2) to the frontal cortex of rbp4-cre//GlyT2-eGFP mice and mapped the cortical fiber distribution in the rostral brainstem. We found that cortical fibers were uniformly distributed in the whole rostral brainstem, including the reticular formation or nucleus pontis oralis (PnO)

2. show the functional connection between frontal cortex and the glycinergic cells
   To show the functional connection between the frontal cortex and the glycinergic cells of the brainstem we recorded local field activity from the frontal cortex and individual neuronal activity from the brainstem. Recorded cells were post hoc identified.
   The brainstem projecting L5 pyramidal neurons were transfected by the aforementioned cre dependent virus construct. Photoactivation of these cells at 1, 10 and 20 Hz resulted in increased firing of the glycinergic neurons precisely locked to the stimulation. The evoked response probability was above 80% even at high frequency stimulation (n = 4).

   To temporarily suspend frontal cortical rhythmic oscillation we used pharmacological approaches. 2 molar KCl was dripped on the surface of the cortex resulting in reversible disruption of the cortical rhythmic oscillation. During these periods the rhythmic firing of the glycinergic cells became irregular and their firing rate decreased.

3. show the synaptic connection between the frontal cortex and the glycinergic cells (correlated light- and electron microscopy)

To show the synaptic connection between the frontal cortex and the glycinergic neurons we use double immunolabeling. The reporter molecule of the injected virus construct (mCherry) was visualized by DAB-Ni, the glycinergic cells were visualized by DAB. Electronmicroscopic examination revealed that the cortical terminals established synaptic connections with thick dendrites of the glycinergic neurons.

To show the morphofunctional connection between the frontal cortex and the glycinergic cells of the brainstem one recorded, responsive, labeled and post hoc identified glycinergic neuron and the cortical fibers around it were visualized by DAB and DAB-Ni respectively. A putative synaptic connection was found between a terminal with cortical origin and a spine-like dendritic structure of the labeled, responsive, GlyT2+ cell. The correlated light-electron microscopic validation of the synapse is in progress.

In summary we showed

1. that the L5 pyramidal neurons uniformly innervate the rostral brainstem and the glycinergic neurons of the PnO.
2. that the information transmission between the two regions is very effective
3. the morphological basis of this effective information transmission at the EM level (synaptic connection between the cortical terminals and the glycinergic cells)

Our results were presented at the following conferences:

Frontal motor cortical control of thalamus projecting inhibitory cells of the brainstem
(Thalamocortical Interactions Gordon Research Conferences and Gordon Research Seminars, 2018, Renaissance Tuscany Il Ciocco, Italy) – poster
Plattner V. M., Bősz E., Diana M. A., Giber K., Acsady L.

Cortical control of the inhibitory pathway from the brainstem to the thalamus
(Inhibition in the CNS Gordon Research Conferences and Gordon Research Seminars, 2017, Les Diablerets, Switzerland) - poster
Plattner V. M., Bősz E., Diana M. A., Giber K., Acsady L.

Motor cortical control of thalamus projecting inhibitory neurons in the brainstem
(Society for Neuroscience 2017, Washington DC, US) - poster
Plattner V. M., Bősz E., Diana M. A., Giber K., Acsady L.

Interim report 2018-09-01 - 2018-12-31
Role of the inhibitory neurons in the pontine reticular formation in switching behaviour

In the additional three months following the first year, we were focusing on:

1. developing a reliable method to quantify and statistically analyze the distribution of frontal cortical fibers around the glycinergic cells in the brainstem after virus injection to different cortical areas. (see point #1 in the 1st year report)
2. increasing the sample size to be able to reliably show the significant changes in the firing properties of the glycinergic cells following cortical photoactivation (see point #2 in the 1st year report)
3. collecting more data to precisely identify the dendritic domain of the glycinergic neurons targeted by cortical fibers (see point #3 in the 1st year report)
4. developing behaviour tests to decipher the role of the cortico-brainstem-IL thalamic circuit in motor planning and execution.

2nd year report (2019-01-01 - 2019-12-30)

The project was paused for 1 year

Closing report (2017-09-01 to 2019-12-31)

During the three-year research grant we had the following goals:

1. describe the morphological and functional connection between the frontal cortical regions and the glycinergic neurons of the brainstem pontine reticular formation
2. determine the efficacy of information transmission between the two regions. For this we used rbp4-cre//GlyT2-eGFP double transgenic mouse strain and cre dependent virus constructs containing the floxed genes of channelrhodopsin 2 (hChR2), a photo-sensitive cation channel and mCherry reporter molecule (see the first-year report for details)
3. developing behaviour tests to decipher the role of the cortico-brainstem-IL thalamic circuit in motor planning and execution.

After 1 year and 3 months the research was paused for a year due to a postdoctoral fellowship abroad and was not continued.

Our main results from the active research period are the following:

We showed,

1. that the L5 pyramidal neurons uniformly innervate the rostral brainstem and the glycinergic neurons of the PnO.
2. that the information transmission between the two regions is highly effective and reliable even at high stimulation frequencies
3. the morphological basis of this effective information transmission at the EM level (synaptic connection between the cortical terminals and the glycinergic cells)
4. the activation of the glycinergic pathway in a behaviour task disrupts the proper execution of goal directed actions

We are going to publish a research paper about our results. This paper is in preparation.