Nucleus specific inhibitory control of thalamocortical activity

OTKA K research project 10975 (2013-2018)

Final report

Project 1) GABA-A receptors:

GABA-A receptors (GABA-ARs) are typically expressed at synaptic or nonsynaptic sites mediating phasic and tonic inhibition, respectively. These two forms of inhibition conjointly control various network oscillations. To disentangle their roles in thalamocortical rhythms, we focally deleted synaptic, γ2 subunit-containing GABA-ARs in the ventrobasal complex of the thalamus using viral intervention in mice. After successful removal of γ2 subunit clusters, spontaneous and evoked GABAergic synaptic currents disappeared in thalamocortical cells when the presynaptic, reticular thalamic (nRT) neurons fired in tonic mode. This indicated complete absence of traditional synaptic inhibition. However, when nRT cells fired in burst mode, phasic GABA-AR-mediated events persisted, indicating a dynamic, burst-specific recruitment of nonsynaptic GABA-ARs. In vivo, removal of synaptic GABA-ARs reduced the firing of individual thalamocortical cells but did not abolish slow oscillations. Interestingly in the total absence of synaptic receptors faster sleep oscillation (sleep spindles) remained also intact. We conclude that nonsynaptic GABA-ARs are recruited in a phasic manner specifically during burst firing of nRT cells and provide sufficient GABA-AR activation to control major thalamocortical oscillations. This novel form of GABAergic synaptic transmission may be a critical mechanism in timing normal and pathological thalamocortical oscillations. The results have been published in an original research article with the PI as the last, corresponding author **(Rovó *et al.*, 2014)**.

Project 2) Pontothalamic (PnO-Thalamic) Pathway

We described of a novel, ascending, inhibitory pathway which link the rostral reticular formation in the brainstem and the thalamus. In, the thalamus this pathway selectively innervated the intralaminar nuclei (IL) with giant, multisynaptic terminals both in rodents and humans. With a French collaboration we proved that these inputs utilize both GABA and glycine as transmitter and can be labeled by glycine transporter 2 (GlyT2). Since IL are thought to play critical role in maintaining consciousness in humans and

the pathway was evolutionary highly conservative we tested the effect of selective activation of the inhibitory reticulo-thalamic pathway in freely moving mice.

GlyT2 fibers were optogenetically activated in freely moving animals through unilateral optic fibers, chronically implanted in the IL. Light activation of GlyT2 terminals in the IL using 30 Hz stimulation trains evoked a near-complete behavioral arrest for the duration of the stimulation. Within 1-2 s following stimulation onset all forms of movement were suspended (exploration, eating, grooming), while the posture of the animals was maintained. The animals either became motionless, displaying only small head movements, or performed hypo/bradykinetic movements, which were frequently asymmetric. After turning off the laser stimulation spontaneous activity reappeared. In order to identify the influence of the ponto-thalamic inhibitory projections on large scale forebrain loops, we recorded the local field potentials (LFP) from motor cortical areas which receive IL input and project to PRF in freely moving mice (n=4). Optogenetic activation of GlyT2 fibers in the IL of awake animals for a duration of 33 s resulted in a striking modification of the ongoing cortical electrical activity. The low-amplitude high-frequency fluctuations, which dominate awake states, were abruptly replaced by large amplitude slow oscillations (n=4 animals, 47 stimulations). A significant increase (Mann-Whitney U test, $p<0.01$) was evident in the frequency range of 2-6 Hz compared to pre-illumination periods. Slow wave activity disappeared following the termination of the stimulus, indicating causative role of the PnO-thalamic pathway. The results have been published in an original research article with the PI as the last, corresponding author **(Giber *et al.*, 2015).**

After disclosing the novel pontothalamic inhibitory pathway and finding significant motor disturbances following its selective activation we set out to figure out which top-down pathway may control its activity. Following retrograde tracing from the n pontine reticular formation (PRF) the layer 5 of higher order motor cortices were selectively labeled. We generated a transgenic CRE mouse line where layer 5 cells can be selectively labeled and at the same time the inhibitory brainstem cells are EGFP-positive (RBP4-Cre -GlyT2-EGFP line). Using this mouse line and conditional AAV vector based tracing and optogenetic activation we demonstrated that higher order layer 5 motor cortical cells innervate GlyT2 neurons and can be reliably activated via this input. Tight cortical control of inhibitory PRF cells were further demonstrated by pharmacological silencing of cortical activity while recording juxtecellularly recorded and labeled GlyT2 cells in the PnO, which led to the disruption of rhythmic PRF activity. These data demonstrate effective motor cortical control of the inhibitory brainstem neurons.

In the next series of experiments, we tested to what extent extrathalamic inhibition from the PRF is able to modulate the activity of thalamic neurons in vivo. To this end we recorded and labeled intralaminar

thalamic neurons while optogenetically activating their PRF inputs in anesthetized animals. We found that the PRF input is able to strongly reduce the spontaneous or tail pinch induced firing of intralaminar thalamic neurons but only if the cell body was situated within or very close to PRF fibers. These and our earlier data together suggest that strong, movement related inhibition may reach thalamic neurons from the higher order cortical regions via the PRF. Data on the cortico-PRF pathway have been presented as a poster in the Thalamocortical Interaction Gordon Research Conference in 2018. The project will be completed by studying the behavioral effect of inhibiting the cortico-PRF pathway. The PRF project formed the basis of a PhD theses defended by Viktor Plattner in 2017, the PI being the supervisor.

Project 3) Activity and role of reticular thalamic nucleus in sleep oscillation

Photoactivation of nRT

One of the best characterized sleep oscillations are the sleep spindles (9-15 Hz frequency range) which characterize stage II NREM sleep. These transient oscillations very their duration depending on the age, mental and disease states as well as information acquired prior to sleep. After establishing the pattern of thalamic neuronal activity in freely sleeping rats we found that the duration of the spindles display tight correlations with the activity of the nRT cells. In order to test the direct, causal role of nRT cells in determining the duration of sleep spindles we induced sleep spindles optogenetically in parvalbumin-channelrhodospin (PV-ChR) (3 animals, 8 sessions) and vesicular-GABA transporter-channelrhodopsin (vGAT-ChR) mice (9 animals, 17 sessions). These strains express channelrhodopsin in both somata and axon terminals of nRT cells. Laser stimuli were delivered either to the nRT somata (n=10), or to nRT axon terminals in VB (n=15) with identical results. The experiments were performed under urethane anesthesia to gain large enough sample in a homogeneous state using the same multishank silicon probes as above. Under urethane anesthesia in mice, brain state showed cyclic fluctuations between patterns resembling slow wave sleep, light sleep with sleep spindles and desynchronized EEG states, mimicking natural sleep on a shorter timescale (10-30 min). Spindles were evoked by short stimuli of laser pulses with variable length and intensity (0.1-10 mW, 2-40 ms). Spindles could not be induced during desynchronized states or slow wave activity, but only in the intermediate states in which spindles also occurred spontaneously. During spindling epochs the length of both spontaneous and evoked spindles displayed large variability though there was a co-modulation between the two ($R=0.21$, $p<0.001$). Also, density of spindles showed a weak correlation with the length of both spontaneous ($R=0.09$,

p<0.001, 10s window) and evoked spindles (R=0.11, p<0.001, 10s window), indicating a slow background modulation.

We tested the effect of nRT population recruitment by varying either stimulus intensity (n=14) or duration (n=11) using stimulation parameters from subthreshold to maximal strength. The probability of evoking spindles increased both with stimulus intensity and duration ranging from 0% to 56%. This shows that the magnitude of nRT activation was highly variable under these experimental conditions using the stimulus intensity range we applied. Still in 20 out of 24 sessions there was no correlation between stimulus intensity or duration and spindle length (Figures 8C,D, bottom; p>0.05, Kruskal-Wallis test). The remaining 4 showed inconsistent and weak correlations in multiple directions. In four animals (6 sessions) we kept the stimulus parameters and recording locations constant and summed the data across animals. In this pooled data set also no significant difference was found between spindle length evoked by the 3 different stimulus intensities (0.14 mW, 4.4 mW, 10.5 mW, 1200 repetitions each; Kruskal Wallis test, p=0.11). These results indicate a constantly fluctuating network state controlling spindle duration probably via determining the size of recruitable nRT population. The results has been published as an original research article the PI being the corresponding author (Barthó *et al.*, 2014).

Heterogeneity of TRN cells

In contrast to the heterogeneity of cortical GABAergic cells the GABAergic cells of the nRT has been thought to form a homogeneous cell population. In collaboration with a research group in the Gladstone Institute of Neurological Disease, San Francisco we established that somatostatin containing nRT neurons form a distinct cell population in both mice and humans. Somatostatin neurons displayed different connectivity, cellular activity and had distinct roles in thalamocortical rhythmogenesis. We found that PV, but not SOM, cells are rhythmogenic, and that PV and SOM neurons are connected to and modulate distinct thalamocortical circuits. Notably, PV, but not SOM, neurons modulated somatosensory behavior and disrupt seizures. These results provide a conceptual framework for how nRT may gate incoming information to modulate brain-wide rhythms. The results has been published as an original research article the PI being a co-author (Clemente-Perez *et al.*, 2017).

Selective innervation of rostral TRN by layer 5 neurons of the frontal cortex

As indicated in our interim report during the course of our studies we discovered a novel and highly selective innervation of the rostral TRN from the layer 5 (L5) pyramidal cells of the frontal cortex. Since thiese inputs are not present in other cortical regions and other TRN sectors the results indicated that

thalamocortical circuits are qualitatively different in frontal circuits than in the rest of the thalamocortical system. Due to the importance of frontal circuits in higher order cognitive processes with the approval of OTKA we launched a new project not originally indicated in our research plan to elucidate the role of the selective L5-TRN projections. In this study conditional viral tracing from the frontal but not parietal cortical areas in the L5-specific Rbp4-Cre mouse revealed dense, topographically-organized projection in the antero-ventral TRN. Compared to L6-TRN synapses the L5-TRN synapses showed distinct ultrastructural properties at the electron microscopic level. *In vitro* electrophysiological experiments demonstrated that in contrast to the L6-TRN pathway the L5-TRN pathway displays short term depression instead of short term facilitation and that L5-TRN EPSCs has higher NDMA/AMPA ratio. Optogenetic activation of L5 cells in anesthetized mice elicited action potentials in anterior TRN with short latency and high fidelity. During spontaneous activity single spike activity of L5-recipient TRN cells showed low correlation with the ongoing frontal cortical oscillations, while high frequency longer TRN burst were tightly coupled with fast high amplitude cortical events indicating efficient control in case of cortical synchrony. Confirming this, optogenetically recruiting progressively more L5 cells converging on the TRN neurons resulted in TRN bursts with higher spike number and intra-burst frequencies. L5 driven TRN sectors innervated thalamic nuclei with frontal cortical connections. The data together suggest powerful and temporally precise cortical control specifically in frontal cortico-thalamic circuits via strong coupling of TRN cells to synchronized L5 outputs. Data on the L5-TRN pathway have been presented as a poster in the Thalamocortical Interaction Gordon Research Conference in 2018 and formed essential contribution to a research plan awarded by the European Research Council with an Advanced Grant in 2017.

4) Review of our previous works

Due to our intensive focus on the GABAergic system of the thalamus, the topic of the present grant as well, together with professor Michael Halassa (New York University) we assembled a review on the diversity of thalamic inhibition. In this, we contrasted the structural and functional properties of inhibition arising the nRT with inhibitory afferents arising outside of the thalamus. The paper has been published in TINS (Halassa & Acsády, 2016).

5) Papers published in connection with this project:

Barthó, P., Slézia, A., Mátyás, F., Faradzs-Zade, L., Ulbert, I., Harris, K.D., & Acsády, L. (2014) Ongoing Network State Controls the Length of Sleep Spindles via Inhibitory Activity. *Neuron*, **82**, 1367–1379.

Clemente-Perez, A., Makinson, S.R., Higashikubo, B., Brovarney, S., Cho, F.S., Urry, A., Holden, S.S., Wimer, M., Dávid, C., Fenno, L.E., Acsády, L., Deisseroth, K., & Paz, J.T. (2017) Distinct Thalamic Reticular Cell Types Differentially Modulate Normal and Pathological Cortical Rhythms. *Cell Rep.*, **19**.

Giber, K., Diana, M.A., M Plattner, V., Dugué, G.P., Bokor, H., Rousseau, C. V, Maglóczky, Z., Havas, L., Hangya, B., Wildner, H., Zeilhofer, H.U., Dieudonné, S., & Acsády, L. (2015) A subcortical inhibitory signal for behavioral arrest in the thalamus. *Nat. Neurosci.*, **18**, 562–568.

Halassa, M.M. & Acsády, L. (2016) Thalamic Inhibition: Diverse Sources, Diverse Scales. *Trends Neurosci.*, **39**, 680–693.

Rovó, Z., Mátyás, F., Barthó, P., Slézia, A., Lecci, S., Pellegrini, C., Astori, S., Dávid, C., Hangya, B., Lüthi, A., & Acsády, L. (2014) Phasic, Nonsynaptic GABA-A Receptor-Mediated Inhibition Entrains Thalamocortical Oscillations. *J. Neurosci.*, **34**, 7137–7147.