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Effect of corticothalamic innervation on thalamic somatosensory nuclei

Sándor Borbély

Sleep Oscillation Research Group – RCNS

Thalamus acting as a relay station plays important role in the sensory information processing, each sensory pathway excluding the olfactory tract reaching the proper cortical region via specific thalamic nuclei. Nevertheless, thalamus is not only a relay station, it has bidirectional connections to the cortex. Most of the corticothalamic (CT) fibers arise from cortical layer 6 and layer 5, targeting primary and higher-order thalamic nuclei. Layer 6 (L6) corticothalamic cells give a very precise modulatory topographic innervation to the same primary thalamic nuclei, their thalamic input originates from. Since corticothalamic axons give collaterals to the inhibitory cells of thalamic reticular nucleus (NRT) their activation results in a very complex response. Relay cells (TC) of primary thalamic nuclei are directly excited and indirectly inhibited via a feed-forward inhibition with the same origin. The aim of my research was to reveal the exact role of layer6 modulatory corticothalamic innervation in the function of thalamocortical circuit.

Our research work was carried out on NTSR1-Cre or NTSR1-ChR2 transgenic animals. NTSR1 expressed specifically in L6 corticothalamic cells. Using in vivo electrophysiological methods, combined with optogenetics we wanted to get answers for the following questions:

1. What is the net effect of biphasic corticothalamic projection on thalamic cells?
2. How the properties of cortical feedback are dependent on sleep/wake cycle related state changes?
3. How the excitatory/inhibitory property of feedback can be overwritten experimentally?
4. What is the difference in layer 6 cortical feedback to primary and higher order thalamic nuclei?

Effect of corticothalamic innervation on thalamic cell activity

The modulatory effect of L6 corticothalamic pathway was investigated under acute and chronic conditions. In our acute experiments silicon-probe electrodes were inserted to the ventrobasal complex (VB) of thalamus and/or posteromedial thalamic nucleus, under urethane anesthesia. Chronic experiments were carried out on tungsten tetrode and optic fibers implanted animals in freely moving conditions. The common property of these experimental methods is the insertion of small size, high resistance electrodes into the brain, with tight proximity to each other. This approach makes the recording of a single cell's activity possible on

several electrodes and consequently the clusterization of neuronal activity in the brain. Based on firing pattern, auto-correlogram of firing and spike waveform we can distinguish more cell's activity from the same recording and make a classification of them. Using this method we can separate TC and NRT cells according to their electrophysiological characteristics.

In urethane anesthetized animals stimulation of L6 corticothalamic axon terminals resulted in a mixture of excitation and inhibition both in the case of TC and NRT cells, which effect was mostly independent of network state. When we repeated these experiments under freely moving conditions somewhat different response was detected. During sleep most of the TC cells showed a short excitation followed by inhibition, and approximately one fourth of these cells showed only inhibition. Occasionally excited cells or cells without any response were also found. If L6 CT terminals were activated in awake state, most of the TC cells were inhibited, while one fourth of them showed excitation/inhibition pattern. The response of NRT cells was also state-dependent, during sleep 90% of NRT cells showed excitation/inhibition, while rest of them were excited. In awake state equal proportion of cells were inhibited, excited/inhibited, only excited or showed no response.

The effect of optogenetic stimulation of L6 terminals was also investigated in higher order thalamic nuclei (mostly in posteromedial nucleus, PoM). TC cells of PoM showed a short direct excitation, followed by a continuous inhibition during pulse stimulation and an offset rebound excitation after the end of stimulation. Unfortunately, the number of detected PoM cells is low, and their response is very diverse, therefore we will not include these data to our final manuscript.

State dependence of L6 cortical feedback on the network level

The state dependence of cortical feedback was also investigated both in urethane anesthetized and in freely moving animals. Under urethane anesthesia activation of L6 CT terminals was able to induce sleep spindles in VB during spindling phase, but not in deep sleep-like and desynchronized phases. In freely moving animals sleep spindles could be elicited only with low reliability in each phase of sleep/wake cycle, both in thalamus and somatosensory cortex. The success rate of spindle induction is very low in freely moving animals, maybe slightly higher in spindling phase, however it is always an order of magnitude lower than in anesthetized animals.

Modulation of on-going network state via L6 efferents

In this part of the project, we implanted tungsten wires and optrodes to somatosensory cortical regions of both hemispheres, another wire to hippocampus and neck musculature. Our goal was to induce network state change using different stimulus paradigms. The resistance and size of tungsten electrodes made the recording of local field potential and multi-unit activity possible.

As previously, we tried to induce single spindles or spindling state, but in this case by the stimulation of L6 CT cell bodies. In agreement with our earlier results spindles could be induced only with low probability, and induction of spindling phase – a longer period of time where occurrence of spontaneous spindles or sigma power increase was evoked but not maintained by the stimulation – was also impossible. At the beginning of this part of the project it seemed to us that some stimulus paradigms are efficient in spindle induction, but then it became clear that the reason of these false positive answers was the interaction of our stimulation protocols and the continuously pending spontaneous ultra slow oscillation between the stages of sleep/wake cycle. The cortical EEG of mice shows a continuous fluctuation of network states with a period of ~45-50 sec, most frequently between light and deep sleep stages, resulting in alternating power peaks in sigma (8-16Hz) and delta (<4Hz) range. We introduced a sham stimulation to our protocols, when real light stimulation did not occur, but all the analysis work was made similarly to real stimulations. We used sham stimulation every time in the rest of the project. Correction with spontaneous sleep/wake stage transitions revealed that, induction of spindling phase is not possible by the activation of L6 CT pyramids.

Due to the failure of inducing sleep spindles in naturally sleeping animals using short pulses, we decided to test long-lasting tonic stimulation paradigms to modulate the thalamocortical circuit. The 30s long mild (~3.9 mW laser output) ChR2 activation resulted in the suppression of spindles when the animal was in light sleep. This effect was highly reliable, even after correction with sham stimulation data, and also immediate. Spindles disappeared directly at the beginning of stimulation and were missing throughout the whole stimulation. While spindle suppression was most significant in light sleep, this type of stimulation reduced sigma power in deep sleep also, and slight decrease happened both in rem and awake phases. Our multisite LFP recordings also revealed that the spindle suppression effect remained local, it could not be observed in other parts of ipsi- or contralateral somatosensory cortical regions and did not affect the general arousal level of the animal. Our acute juxtacellular recordings also proved, that the applied stimulation protocol resulted in moderate steady-state firing rate increase in L6 CT cells.

We also wanted to know what happens if L6 CT get stronger external driving force. In the last phase of this topic we applied a 30s long strong stimulation (~6.0 mW laser output). This protocol evoked a high-reliability immediate desynchronization with increased power in gamma frequency range (>30Hz). Desynchronization could be induced in light and deep sleep stages, but sigma power decrease and gamma-power increase slightly appeared in rem and awake phases also. Desynchronization appeared immediately at the beginning of stimulation and persisted not only during the whole stimulation, but also shortly after the end of it, the recovery of spontaneous cortical LFP lasted for 3-5 sec. This effect similarly, to spindle suppression – was local, we could not detect it on other recording sites, and it had no effect on the arousal. Acute juxtacellular recordings revealed that the firing rate increase of L6 CT cells were more pronounced than in the case of spindle suppression protocol.

Summary

In agreement with my research plan we established our freely moving setup, introduced chronic experiments in our laboratory, and we carried out all the planned experimental work. These experiments demonstrated that stimulation of L6 corticothalamic pathways results in a mixture of excitation/inhibition of TC and NRT cells, which strongly affected and dependent of on-going network activity. We also demonstrated that in contrast to urethane anesthetized animal experiments, spindles could be induced only in light sleep at low probability, and spindling state could not be evoked by the activation of L6 CT cells. On the other hand, we were able to induce deep sleep-like stage reliably during light sleep state and evoke desynchronized (awake-like) state in light and deep sleep stages. The well localized nature of these state changes suggests that the most important role of this CT projection is a precise, topic modulation, and does not involved in general arousal induction mechanisms. We also could demonstrate that the observed network changes are independent of ultra-slow oscillations.

The above described results were published in several conference abstracts, the manuscript of this project is under preparation. Hopefully the paper will be published in 2020. During the 3 years of my scholarship several other papers and conference abstracts were published or are under preparation (for details see Publications).

Publications

Full Publications

Horváth Á.Cs., **Borbély S.,** Boros Ö.C., Komáromi L., Koppa P., Barthó P., Fekete Z. (2019) Infrared neural stimulation and inhibition using an implantable silicon photonic microdevice *(under peer review)*

Bencsik N., Pusztai Sz., **Borbély S.,** Fekete A., Dülk M., Kis V., Pesti Sz., Vas V, Szűcs A., Buday L., Schlett K. (2019) Caskin scaffold protein regulates dendritic spine morphology, learning and memory. *Scientific Reports (accepted)*

Szádeczky-Kardoss K., Varró P., Szűcs A., **Borbély S.,** Világi I. (2019) Kainate receptors have different modulatory effect in seizure-like events and slow rhythmic activity in entorhinal cortex ex vivo. *Brain Research Bulletin 153 (2019) 279-288*

Csernai M., **Borbély S.,** Kocsis K., Burka D., Fekete Z., Balogh V., Káli Sz., Emri Zs., Barthó P. (2019) Dynamics of sleep oscillations is coupled to brain temperature on multiple scales. *Journal of Physiology 597.15 (2019) pp 4069–4086*

Balogh V., Szádeczky-Kardoss K., Varró P., Világi, I., **Borbély, S.** (2019) Analysis of propagation of slow rhythmic activity induced in ex vivo rat brain slices. *Brain Connectivity doi: 10.1089/brain.2018.0650.*

Benkő Zs., Moldován K., Szádeczky-Kardoss K., Zalányi L., **Borbély S.,** Világi I., Somogyvári Z. (2019) Causal relationship between local field potential and intrinsic optical signal in epileptiform activity in vitro. *Scientific Reports. 2019 Mar 26;9(1):5171.*

Borbély, S., Világi, I., Haraszti, Z., Szalontai, Ö., Hajnik, T., Tóth, A., Détári, L., (2018) Sleep deprivation decreases neuronal excitability and responsiveness in rats both in vivo and ex vivo. *Brain. Res. Bull., 137:166-177*

Borbély S., Jocsák G., Moldován K., Sedlák E., Preininger E., Boldizsár I., Tóth A., Atlason PT., Molnár E., Világi I. (2016) Arctigenin reduces neuronal responses in the somatosensory cortex via the inhibition of non-NMDA glutamate receptors. *Neurochemistry International 97: pp. 83-90.*

Conference abstracts

Borbély S., Zalatnai A., Balogh V., Csernai M., Borján D., Gulyás É., Barthó P. (2019) Cortical layer 6 modulates on-going network state, MITT 2019, P57

Borbély S., Balogh V., Csernai M., Burka D., Barthó P. (2018) Cortical layer 6 regulates network state. FENS 2018, E009.

Csernai M., Kocsis K., Burka D., **Borbely S.,** Fekete Z., Balogh V., Kali S., Emri Z., Bartho P. (2018) Sleep spindle frequency is modulated by temperature in vivo and in silico. FENS 2018, E010.

Balogh V., **Borbély S.**, Csernai M., Barthó P. (2017) Corticothalamic effect on thalamic neurons. *SfN 2017, 240.05.*

Borbély S., Balogh V., Csernai M., Burka D., Barthó P. (2017) Cortical layer 6 regulates network state. *SfN 2017, 240.06.*

Schlett K., Bencsik N, Pusztai S., Fekete A., **Borbély S.,** Kis V., Szucs A., Buday L. (2017) Caskin scaffold protein regulates dendritic spine morphology, learning and memory. *SfN 2017, 123.07.*

Csernai M., Kocsis K., Burka D., **Borbely S.,** Fekete Z., Balogh V., Kali S., Emri Z., Bartho P. (2017) Temperature modulates sleep spindle frequency in vivo and in silico. *SfN 2017, 239.13.*

Gáspár A., Major K., Moldován K., Molnár E., **Borbély S.,** Varró P., Világi I. (2017) Repeated seizures modify behaviour and memory processes in rats. *FENS Regional Meeting 2017, P1-018*

Balogh V., **Borbély S.,** Csernai M., Barthó P. (2017) Shaping of thalamic network activity by layer 6 corticothalamic feedback. *FENS Regional Meeting 2017, P2-156*

Benkő Zs., Moldován K., Major K., Zalányi L., **Borbély S.,** Világi I., Somogyvári Z. (2017) Causal relationship between local field potential and intrinsic optical signal in epileptiform activity in vitro. *FENS Regional Meeting 2017, P1-219*

Bencsik N., Pusztai Sz., Fekete A., **Borbély S.,** Kis V., Pesti Sz., Szűcs A., Buday L., Schlett K. (2017) The caskin scaffold protein regulates dendritic spine morphology, learning and memory. *FENS Regional Meeting 2017, P1-339*