Final Report – OTKA 75676

Structural and functional organization of thalamic regions involved in motor and executive functions.

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The original Research Plan consisted of four projects. I describe the results according to this plan.

Project 1) Cortical influence on basal ganglia recipient thalamic nucleus –comparison with other nuclei

Cortical influence over the thalamus has been studied using various methods in different nuclei. We utilized optogenetic methods using cortical light stimulation in vivo in a mouse line expressing channel rhodopsin in layer 5 pyramidal cells under ketamine xylazine anesthesia. In these experiments two nuclei were compared, the somatosensory n. posterior (Po) and the basal ganglia recipient ventromedial nucleus (VM). Po receives giant layer 5. cortical terminals, whereas the type of layer 5 terminals were not known in the VM. Light stimulation of S1 cortex resulted in faithful action potential generation in Po, demonstrating a powerful corticothalamic information transfer in this pathway. Repetitive stimulation resulted in strong attenuation of the response. In the VM first we characterized the layer 5 corticothalamic pathway by retrograde and anterograde tracing. Most layer 5 inputs to VM were found in the M2 cortex and neighboring frontal regions. Interestingly, contralateral layer 5 input has also been consistently found. In contrast to Po the layer 5 terminals were small in VM. 3D EM reconstructions showed that at the ultrastructural level VM terminals displayed a distinctive phenotype. Most layer 5 inputs targeted the head of spine-like structure which emanated from the proximal dendrites of VM cells via multiple, often branching asymmetrical synapses. The neck as well as the head of the spines showed highly variable dimensions, which indicates that the spines may display activity dependent morphological plasticity. While, the layer 5 inputs in VM were diminutive compared to their PO counterparts they were still significantly larger than the other major cortical terminal type in VM which originates in layer 6.

Light stimulation of M2 cortex with similar laser intensity as in S1 did not result in thalamic firing in VM. However, increased laser intensity was able to induce reliable short latency (5-6 ms) response in VM, indicating a monosynaptic response. In contrast to Po, repetitive stimulation (up to 50 Hz) did not result in short term depression of the response. Systematic variation of the location of laser stimulation resulted in “hot spots” of cortical sites, requiring the lowest intensity of laser light to

induce thalamic firing. Contralateral stimulation was also successful. We conclude that small layer 5 terminals are also sufficiently strong the activate thalamus but require the convergence of larger number of layer 5 cell to one VM neuron to be successful. The data has been presented as a conference abstract (FENS, Barcelona 2012) but has not been published since the first author of the project (Hajnalka Bokor) is on a maternity leave.

In case of Po we also studied the ascending sensory drivers and demonstrated that subcortical and cortical giant terminals can converge and interact on single thalamic cells. Ascending and descending information are known to be relayed through the thalamus via strong, "driver" pathways. According to our current knowledge, different driver pathways are organized in parallel streams and do not interact at the thalamic level. Using an electron microscopic approach combined with optogenetics and in vivo physiology, we examined in the Po whether driver inputs arising from different sources can interact at single thalamocortical cells. Both the anatomical and the physiological data demonstrated that ascending driver inputs from the brainstem and descending driver inputs from cortical layer 5 pyramidal neurons converge and interact on single thalamocortical neurons in POm. Both individual pathways displayed driver properties, but they interacted synergistically in a time-dependent manner and when co-activated, supralinearly increased the output of thalamus. As a consequence, thalamocortical neurons reported the relative timing between sensory events and ongoing cortical activity. We concluded that thalamocortical neurons can receive two powerful inputs of different origin, rather than only a single one as previously suggested. This allows thalamocortical neurons to integrate raw sensory information with powerful cortical signals and transfer the integrated activity back to cortical networks. These data challenge the text book view of thalamus and demonstrate that thalamus not always act as a faithful relay but can integrate powerful inputs arising from different sources. A paper on this topic has been published in Cerebral Cortex. (Groh, Bokor et al., 2013)

In further experiments we compared the cortical influence on somatosensory nuclei receiving different types of cortical afferents. To examine this question, we studied the phase of thalamic action potentials relative to cortical oscillations and established correlations among phase, the nuclear location of the thalamocortical neurons, and the frequency of cortical activity.

The phase of thalamic action potentials depended on the exact frequency of the slow cortical oscillation both on long (minutes) and short (single wave) time scales. Faster waves were accompanied by phase advancement in both cases. Thalamocortical neurons located in different nuclei fired at significantly different phases of the slow waves but were active at a similar phase of spindle oscillations. Different thalamic nuclei also displayed distinct burst patterns. Bursts with a higher number of action potentials displayed progressive phase advancement in a nucleus-specific manner. Thalamic neurons located along nuclear borders were characterized by mixed burst and phase properties.

Our data demonstrate that the temporal relationship between cortical and thalamic activity is not fixed but displays dynamic changes during oscillatory activity. The timing depends on the precise location and exact activity of thalamocortical cells and the ongoing cortical network pattern. This variability of thalamic output and its coupling to cortical activity can enable thalamocortical neurons to actively participate in the coding and retrieval of cortical signals. The results has been published in Journal of Neuroscience (Slézia et al., 2011).

Project 2) Drivers of the thalamus in primates

We successfully characterized the entire primate thalamus regarding the origin and types of major excitatory inputs at the light and electron microscopic level. In contrast to the general assumption that the major role of the thalamus is to relay peripheral information to the cortex, our data showed that the larger part of the primate thalamus is devoid of giant vGLUT2-positive terminals. In addition, contrary to the stereotyped view of TC relay cells being driven by the activity of giant excitatory terminals, our data indicate variable composition of the major excitatory/inhibitory afferents.

As mentioned above the activity of thalamocortical neurons is largely determined by giant excitatory terminals, called drivers. These afferents may arise from neocortex or from subcortical centers; however their exact distribution, segregation or putative absence in given thalamic nuclei are unknown. To unravel the nucleus-specific composition of drivers, we mapped the entire macaque thalamus utilizing vesicular glutamate transporters 1 and 2 to label cortical and subcortical afferents, respectively.

Large thalamic territories were innervated exclusively either by giant vGLUT2- or vGLUT1-positive boutons. Co-distribution of drivers with different origin was present but not abundant. In several thalamic regions, no giant terminals of any type could be detected at light microscopic level. Electron microscopic observation of these territories revealed either the complete absence of large multisynaptic excitatory terminals (basal ganglia-recipient nuclei) or the presence of both vGLUT1- and vGLUT2-positive terminals, which were significantly smaller than their giant counterparts (intralaminar nuclei, medial pulvinar). In the basal ganglia-recipient thalamus, giant inhibitory terminals replaced the excitatory driver inputs. (The rodent and primate basal ganglia recipient nuclei displayed similar morphological organization.) The pulvinar and the mediodorsal nucleus displayed subnuclear heterogeneity in their driver assemblies.

These results show that in primates distinct thalamic territories can be under pure subcortical or cortical control; however there is significant variability in the composition of major excitatory inputs in several thalamic regions. Since thalamic information transfer depends on the origin and complexity of the excitatory inputs, this suggests that the computations performed by individual thalamic regions display considerable variability. The data can serve as a framework to explain primate behavioral

results and can be used to understand the morphological bases of human neurological diseases involving the thalamus (see below). The results have been published in the Journal of Neuroscience ((Rovó et al., 2012).

Project 3) The role of intralaminar and associated thalamic nuclei – morphological and behavioural approaches

A large forebrain circuit, including the thalamus, amygdala and prefrontal cortical regions, is responsible for the establishment and extinction of fear-related memories. Understanding interactions among these three regions is critical to decipher the basic mechanisms of fear. With the advancement of molecular- and optogenetics, the mouse has become the main species to study fear-related behaviors. However, the basic connectivity pattern of the forebrain circuits involved in processing fear has not been described in this species.

In the first part of our study we mapped the connectivity pattern between the basolateral nucleus of amygdala (BLA), the mediodorsal nucleus of the thalamus (MD) and the medial prefrontal cortex, which were shown to have closed triangular connectivity in rats. However, in mice we found no evidence for direct connectivity between BLA and MD and little overlap between medial prefrontal regions having reciprocal connection with both BLA and MD in mice. We attribute the difference between our results and earlier rat studies to methodological problems rather than to a genuine species difference. The common nodes in the cortex, which displayed mutual reciprocal connection to both nuclei, proved to be the agranular insular cortex and the border zone of cingulate and M2 cortex. In addition BLA had unidirectional projection to orbital cortex which is in connection with MD, providing an indirect pathway between the two regions. Our data demonstrate cortical crosstalk between BLA and MD via cortical sectors whose roles in fear-related behaviour have not been extensively studied. The results have been published in the European Journal of Neuroscience (Mátyás et al., 2014)

Next we examined the hypotheses that hemispheric difference may exist in a rodent model of a complex emotional behavior and whether it can be connected to asymmetries in medial thalamic nuclei. The mechanism of this lateralization is entirely unclear due to the inaccessibility of human brain to experimental manipulations. Together with a Korean group using unilateral inactivation as well as intracranial stimulation of the anterior cingulate cortex (ACC), we demonstrated that observational fear learning is controlled by the right but not the left ACC. Since no overt anatomical difference was found in the thalamocortical connectivity of the two ACC hemispheres, the question arose if hemispheric difference exists already at the level of thalamus. Thus, we lesioned the intralaminar nuclei and the anteromedial nuclei together with anterograde and retrograde tracing from the ACC. Interestingly, in contrast to the cortex, inactivation of both the left and the right thalamic nuclei, which are in reciprocal connection to ACC, induced similar impairment of this behavior. The data suggest that lateralization of negative emotions is an evolutionary conserved trait (since it is

present both in rodents and primate) and mainly involve cortico-cortical operations. Lateralization of a complex emotional behavior in a rodent model will allow detailed analysis of cortical asymmetry in cognitive functions. The results of the project has been published in PNAS (Kim et al., 2012).

Project4) Structural and functional properties of a novel, ascending, glycinergic input in the forebrain

Project 4 has now four subprojects all of which has been successfully finished. *1) Anatomy of the pontothalamic (PnO-thalamic) pathway*. Tract tracing together with 3D EM reconstruction of glycinergic terminals from serial electron microscopic sections demonstrated that PnO fibers establish multisynaptic contacts on the proximal dendrites of intralaminar thalamic relay cells with dual GABA-ergic glycinergic phenotype. High resolution confocal light microscopy demonstrated the colocalization of GABAerg and glycinergic receptors postsynaptic to glycinergic terminals. Interestingly, the exact ratio of GABAergic and glycinergic receptors was highly variable from terminal to terminal.

2) In vitro-physiology of the PnO-thalamic pathway. Photostimulation experiments in vitro revealed large amplitude fast rising IPSCs, which maintained their charge transfer even at high frequency laser stimulation (40 Hz). Consistent with the anatomical findings the IPSCs had dual GABAergic and glycinergic component. Confirming the anatomical results, we found large variability in the ratio of GABAergic/glycinergic component from cell to cell ranging from pure GABAergic (17%) to pure glyicinergic (18%) contacts.

3) In vivo activity of the PnO-thalamic pathway. In vivo recording of GlyT2 cells in the PnO (some of which has been antidromically activated from the thalamus) revealed rhythmic clusters of action potentials coupled to the slow cortical oscillation. Interestingly, we found large heterogeneity in the phase of firing. PnO cells firing in the early and late phases of the UP-states were found together with neurons preferring the down state. As a population PnO cells covered the entire Down-UP transition of cortical activity.

4) The cortico-PnO influence. Anterograde and retrograde tracing revealed that layer 5 cells of the M2 cortex project to PnO. Systematic activation and inactivation of this cortical region in vivo resulted in the alteration of PnO firing. More specifically, focal application of TTX or procaine in the cortex to transiently block cortical activity resulted in the cessation of rhythmic actvitity in PnO. Electrical stimulation of M2 with different frequency resulted in short latency (8-10 ms) firing of PnO cells inidicating monosynaptic response. PnO neurons were able to follow faithfully high frequency cortical stimulation.

In conclusion we described and characterized a novel ascending inhibitory system from the brainstem reticular core which opposed to the well-known reticular activating system (RAS) express

strong inhibition via the intralaminar nuclei. The activity of this novel brainstem pathway is under strong cortical control. The first manuscript of these data is under re-revision in Nature Neuroscience (one minor experiments were asked in the third round by one reviewer, the other two reviewers have already accepted the paper as it is) (Giber et al., 2014).

PUBLICATIONS

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