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Role of cortical feedback in thalamocortical activity

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The project aimed to investigate the Layer 6. corticothalamic projection *in vivo* with multichannel electrophysiology combined with optogenetic methods. The main questions we addressed were the net excitatory / inhibitory effect of corticothalamic feedback on thalamic cells, its dependence on the arousal, and, in turn its ability to influence the thalamocortical network state.

Corticothalamic effect on thalamic cells

Layer 6. corticothalamic axons provide a direct excitation on thalamocortical (TC) cells, as well as an indirect inhibition via the thalamic reticular nucleus (nRT). Our first aim was to determine how the excitatory and inhibitory components form the net corticothalamic effect in the thalamus during sleep and wakefulness.

In the project we used optogenetic stimulation of corticothalamic axons or somata in a transgenic mouse line (NTSR1-cre), expressing cre-recombinase exclusively in thalamically projecting layer 6. neurons. Most animals were cross-bred with the Ai32 reporter line, yielding NTSR1-ChR2 double transgene mice, in other cases channelrhodopsin was introduced by adeno-associated viral vector containing double-floxed channelrhodopsin gene containing plasmid. We performed multiple single unit recordings with tetrodes in chronically implanted, or with silicon probes in head-restrained animals. Recording were made from the primary somatosensory thalamus (ventral posterior nucleus, VPL/VPM), or the higher order somatosensory posterior (Po) thalamic nucleus. After clustering, somato-dendritic signals of thalamocortical cells were

separated from axon terminal signals of locally projecting thalamocortical cells, thus yielding units of both cell types.

Thalamocortical units typically responded with a brief excitation followed by a period (~70 millisecond) of inhibition to brief (2-5 millisecond) corticothalamic stimulation, while thalamic reticular units almost exclusively responded with excitation. In thalamocortical cells, both the onset and the peak of excitation preceded that of the thalamic reticular units by 3-5 milliseconds, suggesting that the following decrease in firing is indeed active inhibition originating from the thalamic reticular nucleus. Interestingly, we also observed a silent period in thalamic reticular cells after the excitatory peak. Since the existence intra-nRT inhibition is to say the least debated, we tested the cells with longer (500 millisecond) long corticothalamic stimulation. The majority of thalamic reticular units were active throughout the stimulus, with a silent period after stimulus offset, suggesting a global or local down-state, rather than active inhibition.

State dependence of the corticothalamic effect

Thalamic cells operate in different (burst vs. tonic) mode during sleep and wakefulness, respectively. Sleep was staged by a machine learning algorithm, initially trained by visually labeled sets. We found a major difference in the corticothalamic response of thalamocortical cells between sleep and wake states. The initial excitatory component was decreased or absent on the same thalamocortical units compared to sleep, with a faster and stronger inhibitory component. The response of thalamic reticular units was excitatory in both states. The difference in excitation/inhibition profile may be explained by the slower onset of T-current in the thalamic reticular nucleus, providing a window without before feedforward inhibition on thalamocortical cells.

Comparison of first- and higher order nuclei

Both first-order VPM/VPL and higher-order Po recordings showed that TC units were responsive to corticothalamic stimulation, but while in VPM/VPL L6 stimulation often induced spindle oscillations, we saw no such effect in the Po. Recordings from axon terminals from the thalamic reticular nucleus in the Po suggest that this is due to a continuous tonic inhibition mediated by nRT cells projecting to higher-order, but not first order somatosensory thalamus. Trains of stimuli of different frequencies (5 Hz, 10Hz, 20 Hz, 40 Hz) showed a facilitation at 10 Hz in VPM/VPL, but not in Po, suggesting that first-order nuclei are more resonantly tuned to spindle generation than their higher order counterparts.

Activity of L6 corticothalamic cells during oscillations

Despite the abundance of anatomical and in vitro studies on the corticothalamic projection, their spontaneous firing has been poorly described, largely due to technical difficulties (their relatively sparse firing, and low separability from other cortical pyramidal cells in layer 6.). We identified corticothalamic cells by optical tagging in NTSR1-ChR animals, using juxtacellular recordings under urethane anesthesia. Corticothalamic cells fired phase locked to cortical slow oscillations, as well as cycles of sleep spindles. Surprisingly, the average firing rate during spindles was not significantly different from baseline, suggesting a lesser role of corticothalamic feedback in spindle termination than previously assumed.

Effect of corticothalamic feedback on sleep oscillations

According to Sherman and Guillery's hypothesis, the corticothalamic feedback behaves as a modulatory subsystem, similarly to the ascending modulatory pathways. Therefore we tested on chronically implanted, sleeping and behaving animals, whether prolonged corticothalamic activation can elicit a change in the ongoing network state. As previously, NTSR1-ChR mice were used, with stimulation sites either in the primary somatosensory cortex, or in the somatosensory thalamus. We found multiple state transitions, according to the pattern of activation.

First we investigated, whether corticothalamic activation has a propensity to trigger sleep spindles, as suggested by their preferential occurrence after K-complexes. Although under urethane anesthesia brief corticothalamic stimulation evoked spindles with high reliability, under natural sleep, only a mild increase was observed in sigma power. Prolonged low frequency pulse-like activation however, successfully entrained sleep spindles, though did not significantly increase the overall sigma power.

Next we tested, how tonic activity of corticothalamic cells affects thalamocortical state. 30 second long activation was used to elicit sustained tonic firing of L6CT cells at 13.58 ± 3.82 Hz, as verified by juxtacellular recordings in a separate set of experiments. Tonic corticothalamic activation showed an overall effect of suppressing sleep spindles. Data was analyzed separately for light, deep, REM sleep, as well as wake epochs, and was corrected for spontaneous state transitions due to infra-slow oscillation. Tonic activation during light (spindle-rich) sleep strongly decreased the occurrence of sleep spindles as well as sigma power, giving way to delta oscillations. Stimulation during deep (delta rich) epochs showed no significant change in the power spectrum. Correction for infra slow oscillation, however, revealed that it prevented the spontaneous shift to spindle-rich phase.

High intensity tonic stimulation, however, completely abolished both delta oscillations and sleep spindles, resulting in a desynchronized network, with elevated gamma rhythm. The effect was present across light, deep, REM sleep, and awake state.

Monitoring the animals' motion on video and electromyogram showed that none of the above state transitions coincided with a change in the sleep-wake state. This apparent contradiction was resolved by analyzing the effect on cortical sites 1 mm distant from the stimulation locus. Despite the robust spectral changes at the site of stimulation, we found minimal or no effect on the distal sites for either stimulation paradigm. In conclusion, corticothalamic feedback acts a local modulator of thalamocortical network state.