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Characterization of interactions in thalamic microcircuitry by multichannel physiological methods

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Analysis of reciprocally connected excitatory and inhibitory populations in the thalamus during sleep oscillations.

a) *Characterization of excitatory and inhibitory units in thalamic recordings*

The main challenge in analyzing thalamic circuitry is, that the excitatory thalamocortical (TC), and the inhibitory thalamic reticular (nRT) cells are located in different nuclei, therefore it is difficult to obtain recordings from topographically coupled excitatory-inhibitory populations. We employed multi-channel silicon probe recordings from the primary somatosensory thalamus (VPL/VPM) of anaesthetized and chronically implanted, naturally sleeping animals. This method allows the separation of several (up to 50 in our recording setup) single units, the activity of which can be simultaneously recorded. In our recordings we were able to distinguish action potentials of both the thalamocortical (TC) cells, and axon terminals of topographically projecting thalamic reticular (nRT) neurons. The latter appeared as spikes with narrow waveforms, but showed the characteristic long bursts of nRT cells, and phase-locked to local spindle oscillation at a different phase as TC cells. Later, with juxtacellularly recording and labelling a neuron in nRT while recording its axon terminal activity in VPM as a narrow waveform unit, we provided direct evidence that we are indeed simultaneously recording the excitatory and inhibitory components of the same, topographic thalamic circuitry.

b) *Excitatory – inhibitory dynamics during sleep spindles*

Sleep spindles arise as an interaction between TC and nRT neurons, with a ping-pong mechanism of TC cells exciting nRT cells to produce a burst, which in turn inhibits TC cells for tens of milliseconds, after which they produce a rebound burst, exciting nRT neurons again. Since spindles are not continuous oscillations, but discrete packets with a waxing and waning phase, during which the dynamics of this two coupled populations is still to be determined. First, we tested the hypothesis that the timing of the TC and nRT cells from cycle to cycle may change during a spindle. We found that the delay between TC and nRT firing was constant from the beginning to the end of the spindle. Next, we examined whether the

compactness of firing around the trough of spindle cycles changed during a spindle event, but found no change till the very last cycle.

Examining the firing rate, however, revealed striking changes in the dynamics of both cell types. TC cells showed a continuous recruitment during from cycle to cycle, while nRT cells got recruited in the first half (waxing phase) of the spindle, but dropped sharply few cycles before spindle termination. The number of spikes / burst of nRT cells monotonically decreased throughout the spindle. In conclusion, the dynamics of sleep spindles can be explained by the recruitment of thalamocortical units in the waxing phase, and the breakdown of inhibition in the waning phase. The results were published in Neuron (Barthó et. al., 2014).

Manipulation of thalamic circuitry by selective lesion of synaptic GABA-A receptors

In this subproject we examined the effect of synaptic GABA-A receptors on thalamocortical oscillations. Our working hypothesis was that deletion of synaptic GABA-A inhibition would eliminate certain oscillations, such as sleep spindles, but leave others, like delta wave, intact. We selectively deleted synaptic GABA-A receptors in the thalamus by injecting AAV-CMV-Cre viral construct in GABA-AR-γ2-flox mice. In vitro experiments conducted in Anita Luthi's lab showed that spontaneous IPSP-s almost completely disappeared from thalamocortical neurons, and histological staining confirmed the absence of γ2-subunits form GABA synapses.

Next we tested how deletion of synaptic inhibition in the thalamus influenced sleep oscillations, such as delta waves and sleep spindles. To our surprise we found no major difference in the properties of sleep rhythms between control and deleted animals (with an exception of a minor, 1 Hz decrease in mean sleep spindle frequency). This contradiction was resolved by in vitro results showing that under burst firing conditions, such as during sleep and anesthesia, the GABA-A currents are different from tonic mode. In tonic mode, nRT cells fire single spikes, and most GABA-A currents work through synaptic GABA-A receptors, therefore disappear after γ2-deletion. In burst mode, on the other hand, in addition to synaptic currents, an extrasynaptic GABA-A component appears, which provides sufficient hyperpolarization in TC cells for the generation of sleep oscillations. The results have been published in Journal of Neuroscience (Rovó et. al. 2014)

Manipulation of thalamic circuitry by optogenetic methods

Our data obtained in the previous subprojects strongly suggests a causal relationship between the pool of active nRT neurons at the beginning of the spindle oscillation, and the duration of the following spindle. In this project we tested this hypothesis by optogenetically inducing spindles in transgenic mice. The strains used were parvalbumin-cre and vesicular-GABA-transporter-cre lines, crossed with floxed channelrhodopsin reporter lines, resulting in hybrids, in which cells of the nRT expressed channelrhodopsin. Stimulation of nRT cells by blue (447nm) laser light resulted in distinct responses in thalamus, depending on the arousal level of the animal. Under urethane anesthesia, the level of network synchrony shows a

periodic fluctuation between deep synchronization (analogous to stage III-IV sleep, characterized by up- and down-states), though mild synchronization (resembling stage II. sleep, exhibiting sleep spindles), to desynchronized epochs. During desynchronized states, nRT stimulation elicited a brief inhibition in the ongoing activity, while under deeply synchronized conditions, it evoked a pause followed by an up-state. Only during lightly synchronized epochs, analogous to stage II. of natural sleep, were spindles evoked by nRT stimulation.

Within spindling epochs, properties of spindles such as frequency or duration also showed a slow modulation, both for spontaneous and evoked cases. On the other hand, varying the stimulation parameters had significant effect on the properties of evoked spindles. We therefore concluded that spindle oscillations are all or none phenomena, dependent on the actual network state, but not on the evoking stimulus. The results were published in Neuron (Barthó et. al., 2014).

Modelling of spindle-generating thalamic networks based on in vivo data

This subproject is currently underway in collaboration with Szabolcs Kali (MTA KOKI). We have adapted the thalamocortical and thalamic reticular cell models of Desthexhe et al, 1996, and generated artificial input patterns based on our silicon probe recordings in naturally sleeping and urethane anaesthetised animals. Results show that simulated groups of thalamocortical cells are able to produce output statistics similar to in vivo scenarios in response to realistic nRT input patterns. Currently we are incorporating the extrasynaptic GABA-A conductances, to provide a better fit to real-life spindle dynamics. This project is to be published in the coming year.

Publications

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