# Cortical layer 6 modulates network activity in thalamus

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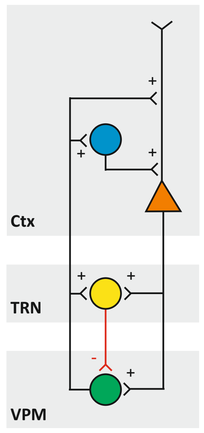
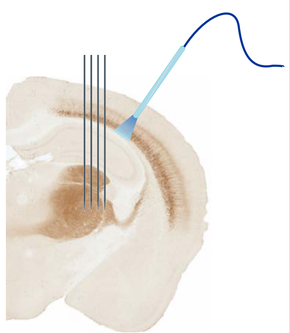
## Introduction

Layer 6 of the cortex provides a massive projection to the thalamus, the function of which is not utterly clear. This corticothalamic (L6) feedback has been implicated in sharpening of thalamic receptive fields, as well as spatial synchronization and termination of sleep spindles. Since L6 axons give collaterals to both thalamocortical (TC) and thalamic reticular (nRT) neurons, their activation is likely to produce a mixture of excitation and inhibition. The ratio and timing of these, however, may vary with the strength of L6 activation, as well as the state of the network. Here we investigate this system by optogenetically manipulation L6 cortico-thalamic neurons, while simultaneously recording thalamic and cortical activity.




## Methods

- animals: NTSR1-ChR mice, expressing channel-rhodopsin in L6 corticothalamic neurons exclusively
- recording: Silicon probe in VPM/VPL or Po thalamus and cortex. Multiple single units (TC cells and nRT axon terminals) in thalamus, in cortex also LFP.
- stimulation: 447 nm laser light on S1 cortex, or directly in thalamus
- anesthesia: urethane, convenient for fluctuating between synchronized and desynchronized states



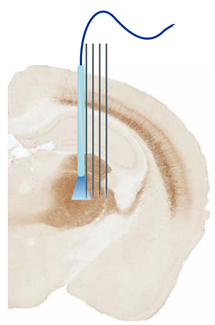
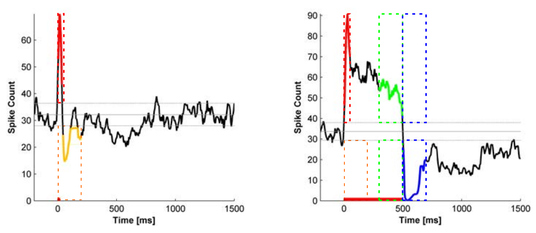
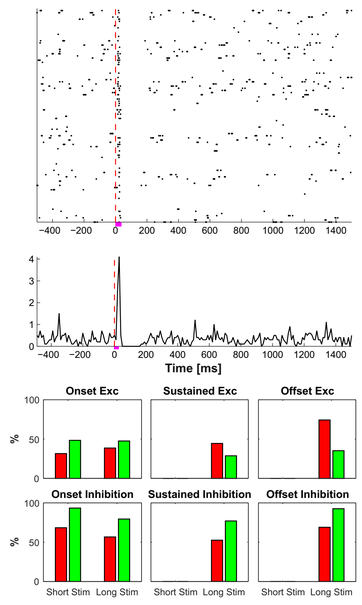


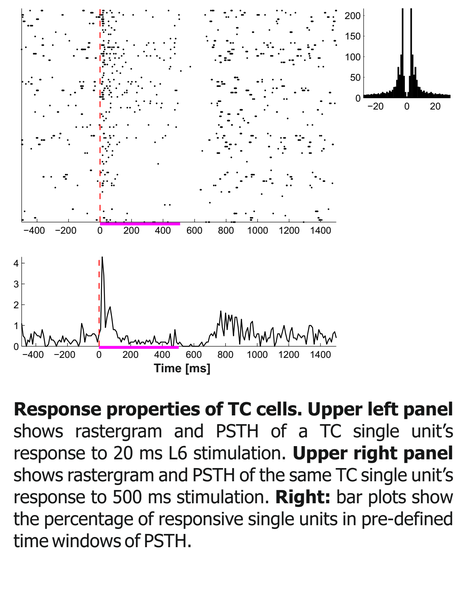
photo: www.gensat.org



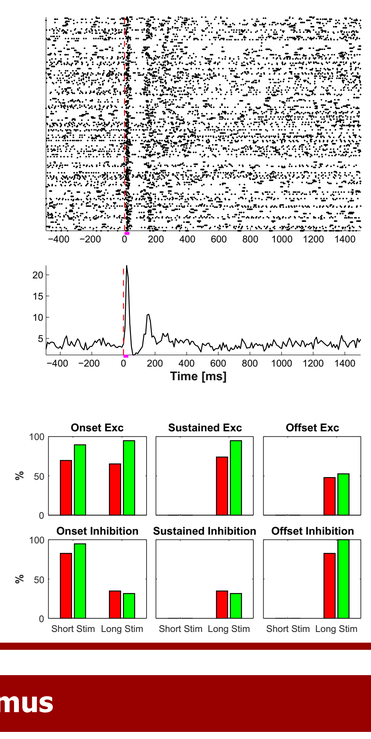

**Analysis windows of detailed PSTH analysis. Left panel: '**onset excitation' window (red) and 'onset inhibition' window (orange) were defined in the case of short stimulations. **Right panel: i**n the case of long stimulations two additional time segments were defined with detection of excitation and/or inhibition: 'sustained' windows (green) and 'offset' windows (blue).

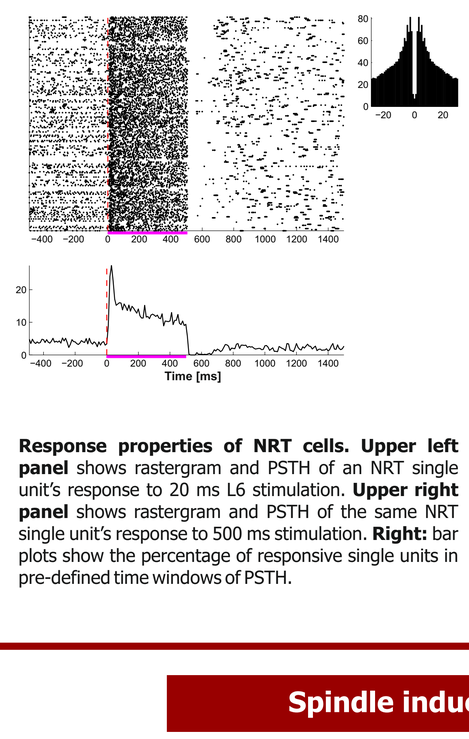
## Results



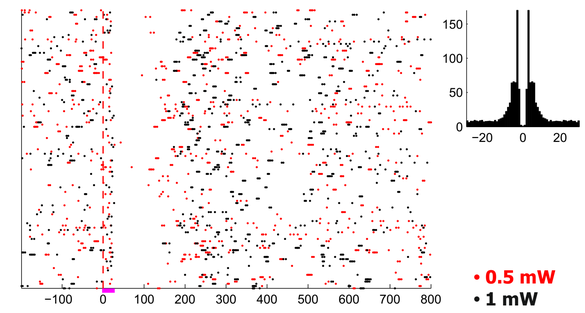

**Response properties of TC cells. Upper left panel** shows rastergram and PSTH of a TC single unit's response to 20 ms L6 stimulation. **Upper right panel** shows rastergram and PSTH of the same TC single unit's response to 500 ms stimulation. **Right:** bar plots show the percentage of responsive single units in pre-defined time windows of PSTH.



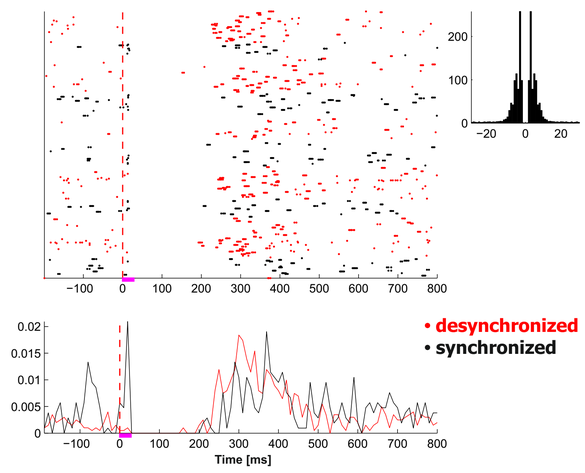

**Response properties of NRT cells. Upper left panel** shows rastergram and PSTH of an NRT single unit's response to 20 ms L6 stimulation. **Upper right panel** shows rastergram and PSTH of the same NRT single unit's response to 500 ms stimulation. **Right:** bar plots show the percentage of responsive single units in pre-defined time windows of PSTH.

### L6 activation strength changes excitation-inhibition pattern



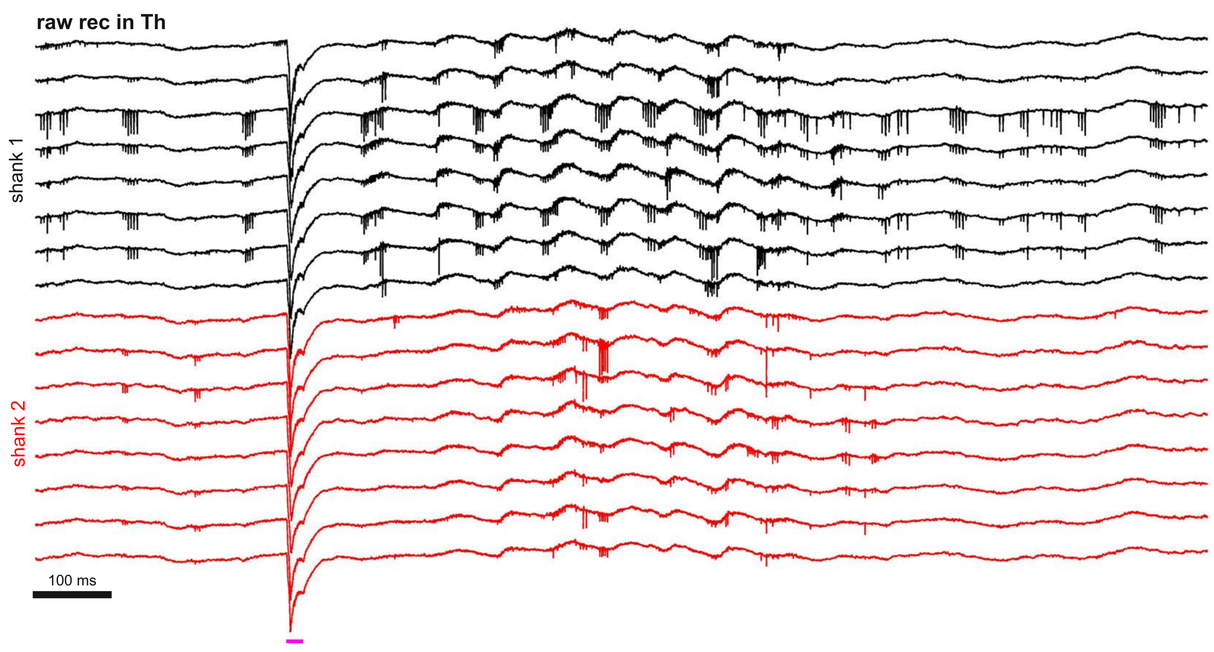

**Rastergram and PSTH of TC single unit's response to L6 stimulation of two different intensities (10 ms long pulses, 0.5 and 1 mW).** Lower stimulation intensity evokes excitation followed by inhibition, while at higher intensity only inhibition is present.

### Network state also shapes excitation-inhibition pattern



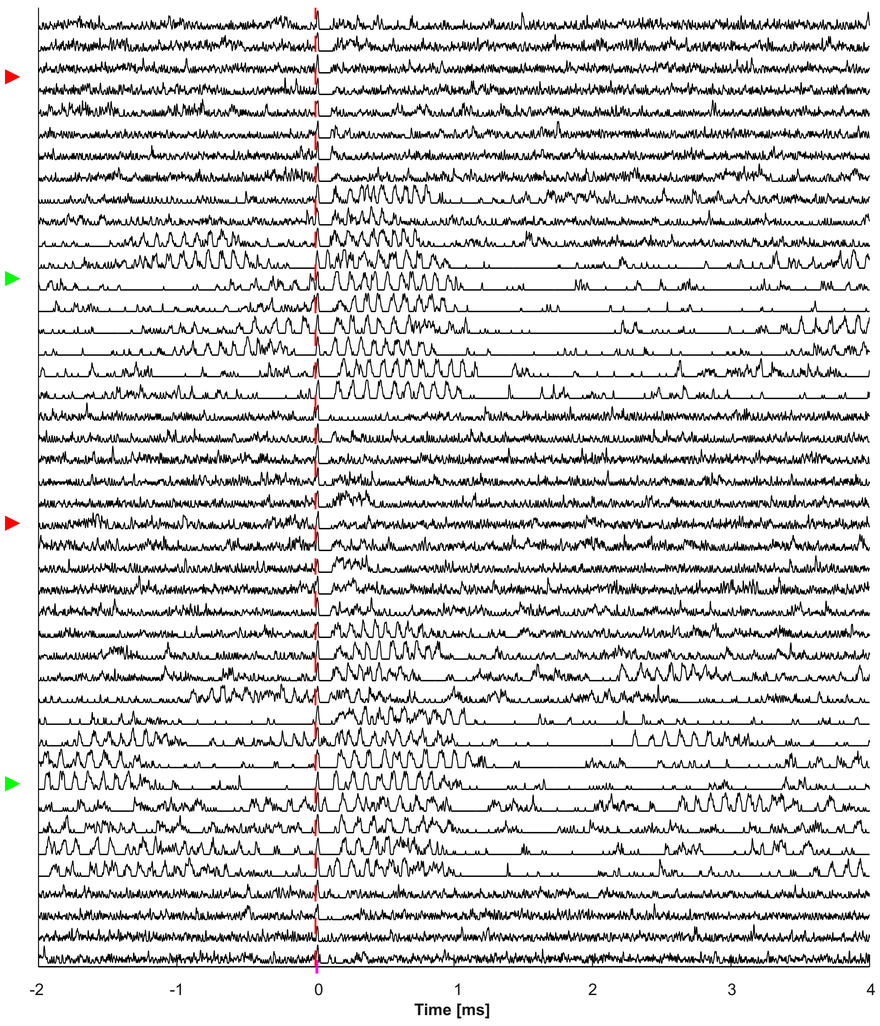

**Rastergram and PSTH of a TC single unit's response to stimulation (10 ms long pulses, 1 mW) during synchronized and desynchronized epochs.** Initial excitation is present only during synchronized, but not desynchronized periods.

### L6 stimulation can evoke spindles in thalamus



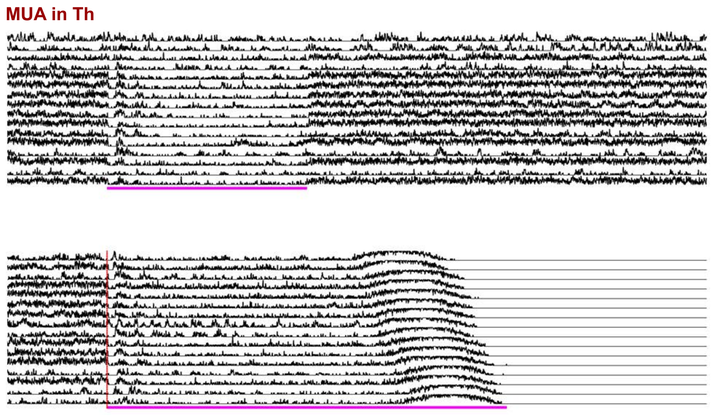

**Silicon probe recording (2 shanks of 4) from VPM/VPL, in response to 20 ms local optogenetic stimulation (magenta line) of L6 terminals.** Note the induced rhythmic firing in the multiunit (arrows) on one of the shanks.

### Spindle induction is dependent on network state

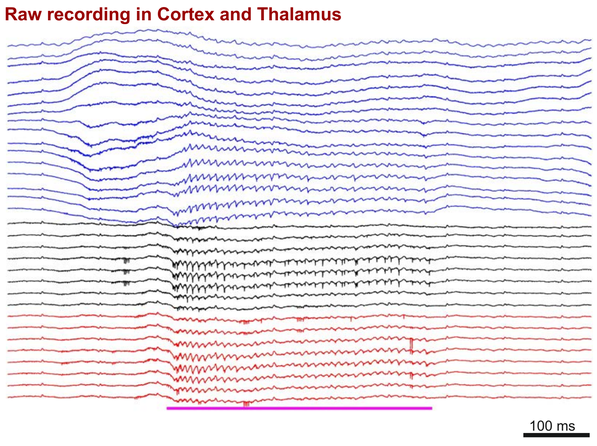



**Smoothed multiunit activity (MUA) for several trials of L6 stimulation (20 ms long laser pulse).** Note that the pulse evokes inhibition in MUA in all trials, but only evokes spindles during lightly synchronized (green arrows), but not in desynchronized epochs (red arrows)

### Tonic stimulation of L6 induces no state change




**Smoothed multiunit activity from VPM/VPL during tonic optogenetic stimulation of L6 terminals.** L6 activation inhibits the MUA activity, but induces no state change (upper figure), and eventually leads to depolarization block (lower figure), lasting over a second.




**Direct optogenetic stimulation of layer 6.** stimulation of the S1 cortex induces high-frequency gamma oscillations both locally (blue trace), as well as in the coupled thalamic regions (black and red traces).

## Conclusions

- L6 has a excitatory-inhibitory effect in TC cells, while mainly exciting nRT
- the pattern of excitation/inhibition depends on network state and strength of L6 activation
- corticothalamic stimulation can induce sleep spindles depending on the network state
- no evidence for a classical modulatory role of L6 was found

*This work was supported by Hungarian Scientific Research Fund OTKA NF101773, and Hungarian Brain Research Program KTIA_NAP_13-2014-0016*