Supplementary Methods for:

Synaptic Basis for Intense Thalamocortical Activation of Feedforward Inhibitory Cells in Neocortex

Scott J. Cruikshank[1], Timothy J. Lewis[2] and Barry W. Connors[1]

[1]Department of Neuroscience
Division of Biology & Medicine
Brown University
Providence, RI 02912

[2]Department of Mathematics
One Shields Avenue
University of California, Davis
Davis, CA 95616

Address for proofs and correspondence:

Barry W. Connors
Department of Neuroscience
Box G-LN
Brown University
Providence, RI 02912

Tel: 401-863-2982
Barry_Connors@Brown.edu

Supplementary Methods I: Calculations of Conductances

For measurements of excitatory and inhibitory synaptic conductances, we applied the methods of Wehr & Zador, 2003[19]. The cells were held in voltage clamp mode. Thalamocortical currents were recorded at five different command potentials, beginning with the most depolarized (+25 to +35 mV), then sequentially stepping down to the most hyperpolarized (in 30 to 35 mV steps). Steps lasted ≥ 10 seconds, and thalamic stimuli were delivered 500 ms before the end of each step. A small biphasic voltage pulse (5 mV positive, –5 mV negative, 50 ms each) was inserted 500 ms before the thalamic stimulus, for the purpose of measuring input resistance. The sequence of 5 steps was repeated 5-20 times (usually 10) and the currents for each command potential were averaged. Generally, the two most depolarized steps were removed from analysis because of voltage escape during strong synaptic responses and because these responses introduced non-linearities into the synaptic I-V relationships (below). Series resistances were measured before and after the thalamocortical tests, using the membrane test feature in P-Clamp 9 (the peak current transients were measured in response to biphasic 10 mV trains, 10 Hz).

Series resistance compensation: Voltages were corrected for series resistance (R_{series}) using the following equation:

$$V_{corrected}(t) = V_{recorded}(t) - I_{recorded}(t) * R_{series,}$$

where $V_{recorded}(t)$ is the uncorrected holding potential recorded at time t, and $I_{recorded}(t)$ is the current recorded at time t.

Synaptic current: Thalamocortical synaptic current, $I_{synaptic}(t)$ was calculated with the following equation:

$$I_{synaptic}(t) = \Delta I_{recorded}(t) - \Delta V_{corrected}(t) / R_{in}$$

• $\Delta I_{recorded}(t) = I_{recorded}(t) - I_{recorded}(\text{baseline})$,

where "baseline" is the 10 ms period immediately preceding the thalamic stimulus.

• $\Delta V_{corrected}(t) = V_{corrected}(t) - V_{corrected}(\text{baseline})$.

• Input resistances (R_{in}) were calculated from current responses to small voltage pulses (described above), using Ohms law.

Thus, the calculated thalamocortical current equals the change in total recorded current relative to baseline, subtracting any nonsynaptic current involved in moving the somatic voltage away from its baseline value (i.e. subtracting $\Delta V_{corrected}(t) / R_{in}$)[19].

The off-line method of series resistance compensation applied here has the advantage of providing for relatively complete correction of voltage errors due to the series resistance. This contrasts with purely on-line correction methods, which generally leave 25 – 50% of the voltage errors uncompensated. However, it has the disadvantage of allowing for greater changes in membrane potential than on-line methods. Because of the filtering properties of neurons, the compensation current that is subsequently added may be temporally distorted. However, in the present case, the fraction of compensation current relative to total synaptic current was usually less than 20%, so the distortion should be fairly minimal.

Synaptic conductance and reversal potential: With $I_{synaptic}$ thus determined for each holding potential (usually 3 potentials were used for the analysis; see above), we constructed

synaptic I-V plots at each time point ($I_{synaptic}$ vs. $V_{corrected}$). We then fitted linear regression lines to those plots to calculate synaptic conductances [$G_{synaptic}(t)$] and reversal potentials [$E_{synaptic}(t)$], based on the slopes and voltage-intercepts, respectively (Fig. 2A, top). Because these calculations were made for every time point, continuous conductance and reversal potential waveforms could be constructed (Fig. 2A, bottom left).

Excitatory and inhibitory synaptic conductance: The total thalamocortical synaptic conductance ($G_{synaptic}$) was finally decomposed into excitatory and inhibitory parts: $G_e(t)$ and $G_i(t)$[19]. The following 4 assumptions were made:

• Excitatory synaptic reversal potential (E_e) = 0 mV

• Inhibitory synaptic reversal potential (E_i) = –69 mV

E_i derived from recordings between connected cell pairs, using Cs-gluconate internal:

(FS to RS pairs, n = 9, mean E_i = –69 mV; FS to FS pairs, n = 11, mean Ei = –70 mV)

• $G_{synaptic}(t) = G_e(t) + G_i(t)$

• $E_{synaptic}(t) = [G_e(t)*E_e + G_i(t)*E_i] / [G_e(t) + G_i(t)]$

Then G_e and G_i were solved for:

$$G_e(t) = G_{synaptic}(t) * \left(\frac{E_{synaptic}(t) - E_i}{E_e - E_i} \right)$$

$$G_i(t) = G_{synaptic}(t) * \left(\frac{E_{synaptic}(t) - E_e}{E_i - E_e} \right)$$

As with the total synaptic conductance (above), G_e and G_i values were also plotted as continuous waveforms (Fig. 2A, bottom right; Fig. 2B, bottom).

Supplementary Methods II: Computational Modeling

Basic cell model: The passive responses of FS and RS cells were described by single-compartment neuron models with passive membrane properties and conductance-based synaptic input:

$$C_m \frac{dV}{dt} = -G_m(V - E_{rest}) - G_e(t)(V - E_e) - G_i(t)(V - E_i),$$

where V is the membrane potential in mV, t is time in msec, C_m is the membrane capacitance in pF, $G_m=1/R_{in}$ is the resting membrane conductance in nS, E_{rest} is the resting membrane potential in mV, $G_e(t)$ and $G_i(t)$ are respectively the excitatory and inhibitory synaptic conductances in nS, and E_e and E_i are respectively the excitatory and inhibitory synaptic reversal potentials in mV. Intrinsic properties and synaptic reversal potentials were the mean values found experimentally, using potassium based internal recording solution (see main text and Supplementary Fig. 2). The E_i values observed with potassium based internal solution were more negative than those with cesium solution (–91 mV vs. –69 mV), possibly because of the suppressive effect of cesium on the potassium chloride cotransporter KCC2[43]. The default synaptic conductances $G_e(t)$ and $G_i(t)$ were set to the averages of the experimentally determined waveforms (as described above; Supp. 2). Simulations with the models were performed using a forward Euler method programmed in MatLab. A time step of 0.05ms was used; this corresponded to the experimental sampling rate. Note that the instantaneous membrane time constants were always more than 2ms, even at the time of the peak synaptic input.

Cortical FS cells are extensively coupled to each other via gap junctions[44]and this could affect responses to complex patterns of thalamocortical inputs[45]. However, results from a model FS cell network suggest that electrical coupling effects are not significant for responses to the

simple stimuli considered here (not shown). Therefore, no gap junctional conductances were included in the simulations.

Swapping synaptic kinetics or amplitudes within the computational models: The amplitude of each synaptic conductance was taken to be the total synaptic conductance integrated over time, i.e. from the onset of the conductance to 50 msec later, at which time the synaptic conductances were effectively zero. When swapping synaptic kinetics or amplitudes, synaptic conductances were normalized (divided) by their amplitudes and then multiplied by the appropriate synaptic amplitude. For example, when swapping excitatory synaptic conductance amplitude (keeping kinetics at respective default values), the excitatory conductance delivered to the FS cell consisted of the normalized FS excitatory conductance waveform multiplied by the amplitude of the RS excitatory conductance.

References listed exclusively in the supplementary materials:

43. Payne, J.A., Rivera, C., Voipio, J. & Kaila, K. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci* **26**, 199-206 (2003).
44. Amitai, Y.*, et al.* The spatial dimensions of electrically coupled networks of interneurons in the neocortex. *J Neurosci* **22**, 4142-4152 (2002).
45. Schneider, A.R., Lewis, T.J. & Rinzel, J. Effects of correlated input and electrical coupling on synchrony in fast-spiking cell networks. *Neurocomputing* **69**, 1125-1129 (2006).
46. Rudy, B. & McBain, C.J. Kv3 channels: voltage-gated K+ channels designed for high-frequency repetitive firing. *Trends Neurosci* **24**, 517-526 (2001).