INHIBITION REGULATES BURST INITIATION IN RETICULAR NEURONS DURING THALAMIC OSCILLATIONS

Vikaas S. Sohal and John R. Huguenard
Room M016, Department of Neurology and Neurological Sciences
Stanford University School of Medicine
Stanford, CA 94305-5122

Oscillations are ubiquitous in neural systems, and inhibitory synapses often regulate their properties. For example, modulating $GABA_A$ receptors switches thalamic oscillations between different modes. In thalamic slices, stimulating internal capsule activates thalamic reticular (RE) neurons, initiating oscillations via the following mechanism: RE neurons fire bursts mediated by low-threshold, T-type Ca^{2+} currents. RE neuron bursts produce IPSPs in thalamocortical (TC) relay neurons, which deinactivate another T-type Ca^{2+} current. As a result, following the decay of IPSPs, some TC neurons rebound burst and re-excite RE neurons (von Krosigk et al., 1993). In control conditions, these oscillations resemble spindles, typical of slow wave sleep. However, the $GABA_A$ receptor antagonist picrotoxin (PTX) transforms these spindle-like oscillations into hypersynchronized, epileptiform rhythms resembling spike-wave discharges characteristic of generalized absence epilepsy (Jacobsen et al., 2001). This transformation is thought to result from the blockade of inhibitory synapses between RE neurons, since genetic disruption of these synapses also generates epileptiform activity *in vitro* (Huntsman et al., 1999) and spike-wave discharge *in vivo* (DeLorey et al., 1998). Note that inhibitory synapses between RE neurons are mediated almost entirely by $GABA_A$ receptors whereas those from RE neurons to TC neurons also contain $GABA_B$ components (Ulrich and Huguenard, 1996). In contrast to the effects of blocking intra-RE inhibition with PTX, selectively strengthening intra-RE inhibition using clonazepam (CZP), a benzodiazepine that is clinically effective against absence epilepsy, suppresses synchronous thalamic oscillations *in vitro* (Sohal et al., 2003). Here we first show experimental evidence that both PTX and CZP alter burst initiation in RE neurons during thalamic oscillations *in vitro*. Then we use simulations and mathematical analysis to show how, by regulating burst initiation in RE neurons, intra-RE inhibition may control thalamic oscillations.

Recording intracellularly from RE cells during spindle-like oscillations in thalamic slices, we have found that after blocking intra-RE inhibition with PTX, RE cells burst more times per oscillation. The same result is obtained whether PTX is applied to the entire thalamic slice (4.0 ± 1.1 additional bursts per oscillation; $p < 0.01$; $n = 14$), or locally, just to RE neurons (2.8 ± 0.6 additional bursts per oscillation; $p < 0.01$; $n = 7$). Conversely, strengthening intra-RE inhibition with CZP reduces the number of times per oscillation that each RE cell bursts (1.5 ± 0.5 fewer bursts per oscillation; $p < 0.05$; $n = 8$). Through all of these manipulations, the number of spikes per burst remains approximately constant, changing by at most one spike per burst.

These changes in the number of RE cell bursts per oscillation result, at least in part, from an altered propensity for RE cells to burst when intra-RE inhibition is modulated. To quantify this propensity, we analyzed intracellular recordings from RE cells which burst many times per oscillation in both control conditions and after drug application. In these recordings, we detected the maximum voltage attained during both EPSPs which failed to elicit bursts, and during EPSPs immediately preceding bursts (within 25 msec). Thus, for each recording, we calculated the

probability that an EPSP of given peak voltage elicited a burst, in various conditions. In every case PTX (n = 3) significantly increased the probability that an EPSP peaking 0-10 mV above V_{rest} elicited a burst ($p < 0.05$, χ^2 test), while CZP (n = 3) significantly decreased this probability ($p < 0.05$, χ^2 test).

Figure 1 shows how PTX and CZP affect burst initiation for one particular RE cell. Figure 1, left, shows the distribution of maximum voltages attained by EPSPs immediately preceding bursts, in control conditions, PTX, and CZP. PTX increases the number of bursts, particularly those following weak EPSPs, while CZP has the opposite effect. Figure 1, right, shows that these changes reflect, at least in part, the fact that in PTX, this neuron has a greater probability of bursting in response to weak EPSPs, whereas in CZP, this probability decreases.

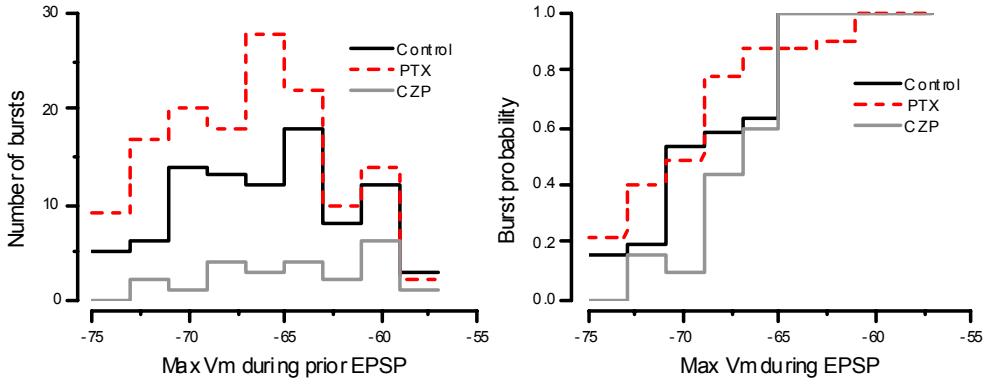

Figure 1

These experimental results show that the strength of intra-RE inhibition determines RE cells' responsiveness to excitation. In an earlier study, we found that a simulated thalamic network generated hypersynchronized, epileptiform activity when intra-RE inhibition was absent, and desynchronized, spindle-like oscillations in the presence of sufficiently strong intra-RE inhibition (Sohal and Huguenard, 2002). In these simulations, intra-RE inhibition shunted excitatory input to RE cells, occasionally preventing individual RE cells from bursting, and this was sufficient to desynchronize both RE cell activity and network oscillations. Here we describe mathematical analysis which elucidates how, by shunting excitatory input to RE cells, intra-RE inhibition desynchronizes RE cell bursts.

Consider two identical, simplified RE neurons receiving a rhythmic excitatory train. Each RE neuron contains a leak current, a T-type low-threshold calcium current (I_{Ts}), a calcium-dependent potassium current (I_{KCa}) responsible for the burst afterhyperpolarization, and a $GABA_A$ receptor-mediated synapse from the other neuron. For simplicity, assume that the time constants of the passive membrane, I_{KCa}, I_{Ts} activation, and the decay of $GABA_A$ receptor-mediated currents, are all fast with respect to the period with which excitatory input arrives. Then the only slow variable is T-current inactivation. Finally assume that only one neuron bursts in response to each cycle of excitatory input. Let $h_{i,j}$ denote the T-current inactivation of neuron i when the jth cycle

of excitatory input arrives. Then the mapping from ($h_{1,j}$, $h_{2,j}$) to ($h_{1,j+2}$, $h_{2,j+2}$) is a contraction, so that there exists a stable solution in which the two RE neurons burst on alternate cycles. The assumption that only one RE cell bursts in response to each cycle of excitatory input is equivalent to requiring that intra-RE inhibition be sufficiently strong, and arrive sufficiently quickly. Thus, we are able to derive, to leading order, a threshold value for the strength of intra-RE inhibition, above which this simple 2-RE cell network generates a stable desynchronized oscillation.

Taken together, these experimental observations, simulations, and mathematical analysis strongly suggest that intra-RE inhibition shunts excitatory input to RE neurons, preventing RE cells from bursting in response to some EPSPs, and that this effect is sufficient to desynchronize both RE cell bursting, and ultimately, thalamic network oscillations.

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