

GABA_A-Receptor-Mediated Rebound Burst Firing and Burst Shunting in Thalamus

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Ulrich, Daniel and John R. Huguenard. GABA_A-receptor-mediated rebound burst firing and burst shunting in thalamus. *J. Neurophysiol.* 78: 1748–1751, 1997. The role of γ -aminobutyric acid-A (GABA_A)-receptor-mediated inhibitory postsynaptic potentials (IPSPs) in 1) generating rebound burst firing and 2) burst inhibition in thalamocortical (TC) relay cells and inhibitory neurons of nucleus reticularis thalami (nRt) was investigated. Experimental data from previous studies were used to generate artificial synaptic responses in neurons via a computer-driven dynamic clamp. On average, in nRt neurons trains of six or more 10-nS GABA_A IPSPs generated rebound bursts of action potentials with a mean delay of 605 ± 32 (SE) ms. In contrast, 10 IPSPs were required for rebound bursts in relay cells, and these occurred with a significantly shorter delay of 327 ± 35 ms. Ca²⁺-dependent burst responses could be shunted by single IPSPs. Half-maximal burst inhibition was obtained in nRt cells when IPSP conductance was 1.5 times the whole cell input conductance. Burst shunting in TC cells was less effective and required a synaptic- to input-conductance ratio of 3. The relative time window of IPSP burst shunting was broader in nRt (~20 ms) than TC cells (~10 ms). We conclude that in nRt cells GABA_A-dependent rebound burst responses would occur with a latency that is incompatible with pacemaking of fast (>3-Hz) thalamic rhythm generation such as spindles, yet burst inhibition is powerful. Therefore a likely role for reciprocal intra-nRt connectivity is to mediate lateral inhibition between nRt cells.

INTRODUCTION

Thalamic oscillations occur during stages of electroencephalographic synchronicity that are prevalent during certain stages of sleep and in absence epilepsy (for review see Steriade et al. 1993). Nucleus reticularis thalami (nRt) cells receive excitatory synaptic input from relay cells and in turn generate inhibitory postsynaptic potentials (IPSPs) in thalamocortical (TC) cells. These IPSPs have both γ -aminobutyric acid-A (GABA_A)- and GABA_B-receptor-mediated components and are capable of inducing rebound burst firing in relay cells (Steriade et al. 1993). nRt cells are mutually connected, but the role of nRt proper in synchronizing and maintaining thalamic oscillations is not completely understood. The surgically disconnected rostral pole of nRt sustains spindle activity, suggesting a pacemaker role for this nucleus (Steriade et al. 1987), and computer models have shown that assemblies of nRt cells can maintain oscillatory activity (Destexhe et al. 1994; Golomb et al. 1994). Disconnection of the perigeniculate nucleus (considered the visual equivalent of nRt) from the relay nuclei abolished spindle waves in slices of the ferret lateral geniculate nucleus, suggesting that spindles result from synergistic network interac-

tions between relay and perigeniculate nucleus cells (von Krosigk et al. 1993).

Another putative role of the intra-nRt connectivity is lateral inhibition, which has been inferred from double-shock inhibition experiments in vivo (Ahlsén and Lindström 1982) and a disinhibitory effect of focal blockade of GABA_A receptors in nRt (Huguenard and Prince 1994).

Here we use previously obtained experimental data on kinetics and reversal potentials of IPSPs in thalamic neurons to define an artificial hybrid computer synapse that allows the systematic variation of inhibitory postsynaptic current size over a wide range compatible with intact connectivity in vivo. We assessed the role of GABA_A IPSPs in the generation of rebound bursts and burst inhibition in nRt and TC cells.

METHODS

Experiments were performed as previously described (Ulrich and Huguenard 1996a,b). Briefly, rats of either sex (postnatal day 11–13) were anesthetized with pentobarbital sodium (50 mg/kg ip) and decapitated. The brain was removed and transferred into an ice-cold solution containing (in mM) 234 sucrose, 11 glucose, 24 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 10 MgSO₄, and 0.5 CaCl₂, equilibrated with 95% O₂-5% CO₂. Horizontal slices (200 μ m) were cut with a Vibratome (TPI, St. Louis, MO) and incubated at 32°C in physiological saline containing (in mM) 126 NaCl, 26 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 2 MgCl₂, 2 CaCl₂, and 10 glucose, equilibrated with 95% O₂-5% CO₂ for ≥ 1 h before recording.

Patch pipettes were filled with (in mM) 120 potassium gluconate, 11 KCl, 1 MgCl₂, 1 CaCl₂, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, and 11 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, pH adjusted to 7.3 with KOH, osmolarity 300 mosM. Whole cell recordings were performed at 30°C under visual control (Edwards et al. 1989). A dynamic clamp (Sharp et al. 1993; Ulrich and Huguenard 1996b) was applied at 10 kHz through a bridge amplifier (Axoclamp-2A, Axon Instruments, Foster City, CA). A liquid junction potential of -12 mV was subtracted on-line.

Low-threshold spike (LTS) amplitudes were measured at the most positive poststimulus membrane voltage (excluding Na⁺ spikes) compared with rest (Fig. 2, *A* and *B*). However, because the underlying passive response could not always be accurately assessed, values for small LTS amplitudes tend to be overestimates. Data are presented as means \pm SE; *n* designates the number of cells.

RESULTS

In the first part of the study we investigated GABA_A-IPSP-mediated rebound burst firing in thalamic cells to assess a

possible contribution of intra-nRt mechanisms to the synchronization of intrathalamic spindle oscillations. The numerical values used to simulate dynamic clamp IPSPs in each cell type are summarized in Table 1. Trains of IPSPs were generated at 200 Hz, a characteristic intraburst firing frequency of nRt cells (Mulle et al. 1986). Peak conductance of IPSPs was systematically varied between 1 and 10 nS and the number of IPSPs was varied between 1 and 20.

Characteristic examples of dynamic clamp experiments are shown in Fig. 1 for an nRt and a TC neuron. Transient hyperpolarization from trains of IPSPs induced an LTS in each cell type when the resting potential was positive to the GABA equilibrium potential (E_{GABA}) by at least ~ 10 mV. The current used to generate the IPSPs is shown in Fig. 1, *C* and *D*, and was based on the conductance waveform characteristic for GABA_A inhibitory postsynaptic currents (Fig. 1, *E* and *F*). In nRt cells, rebound LTSs could be generated by a train of 6 ± 1 (mean \pm SE) IPSPs with a peak conductance of 10 nS each ($n = 5$). In contrast, significantly longer trains of 10 ± 1 IPSPs were needed in TC cells to elicit an LTS ($n = 7$; $P < 0.05$). In addition, the relative burst onset in nRt cells (605 ± 32 ms) was considerably delayed compared with TC neurons (327 ± 35 ; $P < 0.0005$). No correlation was found between burst delay and resting membrane potential. Extrapolating the intervals to a physiological temperature of 38°C in rats with a Q_{10} of 2.1 (Otis and Mody 1992) results in an expected interburst frequency of 6 Hz for an interconnected TC-nRt network. This frequency is in the lower band characteristic for spindle waves. In contrast, the intra-nRt LTS would only generate oscillations at 3 Hz under the same conditions. We therefore conclude that the thalamic spindle network clock is mainly set by the recurrent excitatory-inhibitory loop. However, intra-nRt oscillations may still contribute to slower intrathalamic network activities (e.g., Warren et al. 1994).

In the second part of the study we investigated burst inhibition by varying the amplitude and timing of individual dynamic clamp IPSPs. In this set of experiments neurons were hyperpolarized to about -80 mV through constant current injection and bursts of action potentials were elicited by brief threshold current pulses. Figure 2 shows the effectiveness of dynamic clamp IPSPs in reducing LTSs in an nRt and a TC neurons. In both cell types a relatively linear relationship was found between the amount of burst inhibition and the amplitude of the IPSP. The latter were normal-

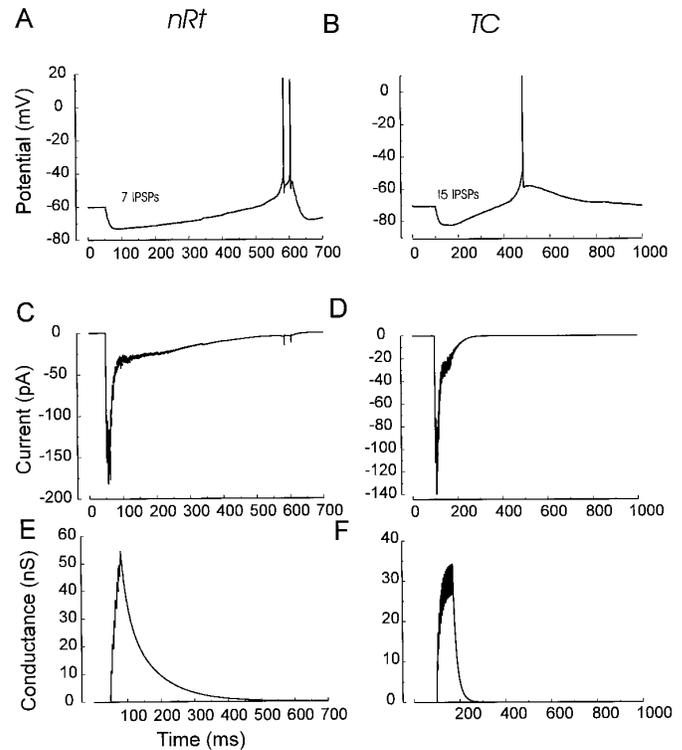


FIG. 1. Rebound burst firing in thalamic cells. Dynamic clamp recordings from a nucleus reticularis thalami (nRt) cell (*A*) and a thalamocortical (TC) neuron (*B*). *A*: time course of membrane potential of an nRt cell during a series of 7 inhibitory postsynaptic potentials (IPSPs) with an individual peak conductance of 10 nS. Note the rebound burst of action potentials following the repolarization of the membrane potential. *B*: analogous experiment in a TC cell. Train of 15 IPSPs at 10 nS each induced a rebound burst in this experiment. Note the considerably shorter burst onset in the TC cell compared with the nRt neuron. *C* and *D*: synaptic currents injected by the dynamic clamp based on a predefined, time-dependent conductance and a continuously updated driving force. *E* and *F*: summed conductances of the individual IPSPs. Dynamic clamp inhibitory postsynaptic current parameters are listed in Table 1.

ized to the input conductance to account for heterogeneity in cell size. IPSP-mediated LTS inhibition was more effective in nRt than TC cells. Half-maximal inhibition of LTSs in nRt cells is achieved with a conductance ratio of 1.5 (Fig. 2*C*; $n = 8$), compared with a ratio of ~ 3 in TC neurons (Fig. 2*D*; $n = 6$). The mean half-effective IPSP would be equivalent to ~ 5 nS in each cell type. As expected, the relative timing between LTS and IPSP onset affects the effectiveness of burst inhibition (Fig. 3). LTSs were generated in a time interval between 0 and 30 ms relative to a single 10-nS IPSP. Examples are shown for an nRt cell (Fig. 3*A*) and a TC neuron (Fig. 3*B*). Figure 3, *C* and *D*, shows pooled data from several experiments. In nRt cells, burst inhibition is mostly effective in a relative time window of 0 to +20 ms. In TC cells, a narrower time interval from 0 to 10 ms was found to be optimal (Fig. 3*D*). From these data we conclude that IPSPs are more effective in shunting burst firing in nRt than TC cells.

DISCUSSION

Repetitive IPSPs originating from nRt cells can lead to rebound burst firing in thalamic relay cells and are essential

TABLE 1. Parameters used to simulate IPSPs in a dynamic clamp in thalamic neurons

Parameter	nRt Cell	TC Cell
τ_{rise}	0.5 ms	0.5 ms
$\tau_{\text{decay, fast}}$	20.0 ms (0.54)	8.7 ms (0.58)
$\tau_{\text{decay, slow}}$	95.0 ms (0.46)	20.0 ms (0.42)
E_{Cl}	-72 mV	-81 mV

The time constants of decay of spontaneous inhibitory postsynaptic currents (IPSCs) were taken from (Zhang et al. 1997) and were temperature corrected with a Q_{10} of 2.1 (Otis and Mody 1992). The reversal potentials of IPSPs in nucleus reticularis thalami (nRt) and thalamocortical (TC) cells were obtained from perforated patch-clamp experiments in thalamic slices (Ulrich and Huguenard 1997). Values in parentheses are relative weights. E_{Cl} , chloride reversal potential.

in maintaining intrathalamic oscillations (Steriade et al. 1985). Retrograde HRP labeling of nRt cell axon collaterals from LGN indicates the presence of reciprocal intra-nRt connections (Ohara and Lieberman 1985) located mainly on the soma and proximal dendrites in rats (De Biasi et al. 1986), and repetitive IPSPs occur in nRt cells, indicating that the collaterals make functional connections (Bal et al. 1995b). However, the contribution of such IPSPs to synchronizing nRt cell firing is less clear. Here we show that repetitive IPSPs of 10 nS can lead to rebound burst firing in nRt cells. Single-fiber IPSPs in TC cells can be up to 13 nS in amplitude (Cox et al. 1997) but are likely to be smaller in nRt cells (Zhang et al. 1997). The comparison between TC and nRt cells in this study shows that the onset of the rebound burst is considerably delayed in the latter. Because the interburst interval of thalamic oscillations is largely occupied by the IPSP (Bal et al. 1995b), we can conclude that different oscillatory frequencies result from the TC-nRt and intra-nRt connections.

The decay of GABA responses in nRt cells tends to become slower with progressive maturation (Gibbs et al. 1996) in parallel with the appearance of dendrodendritic contacts within nRt (Pinault et al. 1996). It is conceivable that these ontogenetic events will influence intra-nRt rhythm generation. However, the frequency of spindle waves has been shown to remain constant during maturation of ferrets (McCormick et al. 1996). In a previous study, we found slower GABA_B-IPSP-mediated rebound bursts in nRt compared with the values in relay cells (Ulrich and Huguenard 1996a). These results suggest that

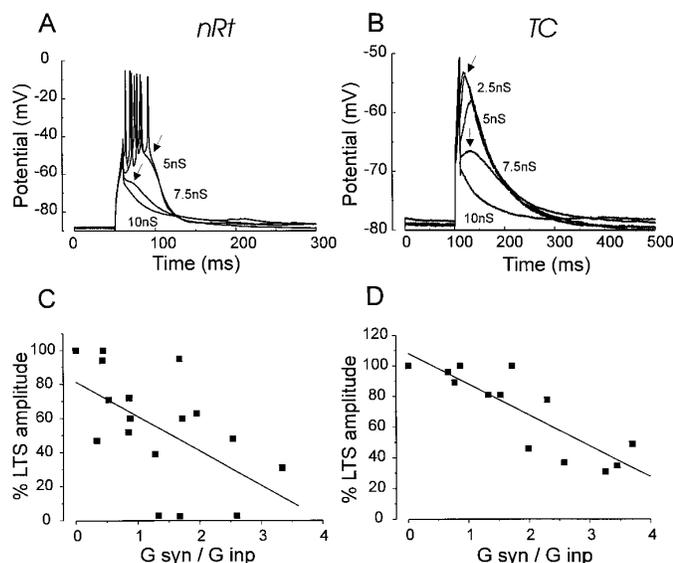


FIG. 2. IPSP-mediated burst shunting. Bursts were elicited by threshold intracellular current injections. Single IPSP was elicited by the dynamic clamp at the onset of the current pulse. *A*: nRt. Peak conductances of the IPSPs in this experiment were 0, 5, 7.5, and 10 nS. Note the significant shunt of the burst for $G > 5$ nS. *B*: TC. Low-threshold spike (LTS) was elicited by current injection and individual dynamic IPSPs of various peak conductance (0, 2.5, 5, 7.5, and 10 nS) were applied. Note the incrementing shunt of the burst for $G > 5$ nS. LTS amplitude was measured at time points indicated by arrows (*A* and *B*). *C* and *D*: scatter plots of the relative size of LTS amplitude (in % of control) vs. the peak conductance of the IPSP normalized to the input conductance. Straight lines were fitted by linear regression. *C*: $R = -0.6$, $P < 0.03$. *D*: $R = -0.9$, $P < 0.0002$.

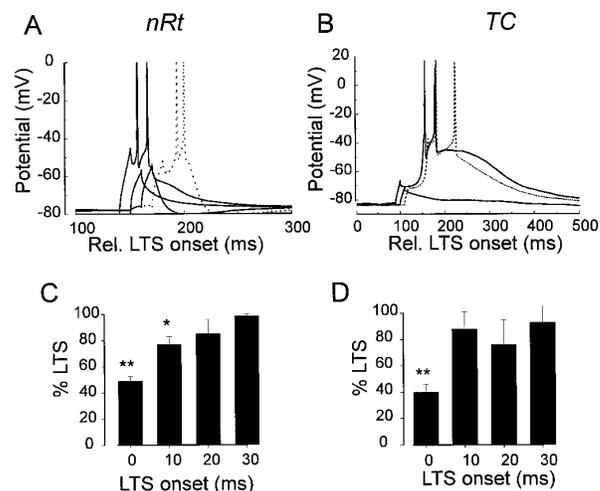


FIG. 3. Effective timing of burst shunting inhibition. LTSs were elicited in (*A*) an nRt cell (250 pA, 10 ms) and (*B*) a TC neuron (300 pA, 10 ms) by threshold intracellular current injection from a hyperpolarized resting state. *A*: LTSs were generated at -10 , 0 , $+10$, and $+20$ ms relative to the onset of a dynamic clamp IPSP with a 10-nS peak conductance. The response at -10 ms was indistinguishable from control and was used for reference. Note the drastic reduction of the LTS at $\Delta t = 0$ and $+10$ ms and the recovery at $+20$ ms (\cdots). *B*: similar experiment was performed in a TC neuron. Dynamic clamp IPSP of 10 nS was effective in reducing the burst at $\Delta t = 0$ ms but not at $\Delta t = 10$ ms (\cdots). *C* and *D*: histograms of normalized LTS size (in % of control) vs. LTS onset (relative to the IPSP) binned in 10-ms steps. In nRt, LTS is significantly reduced at 0 ms and $+10$ ms (*C*) but in TC, LTS is only significant reduced at 0 ms (*D*); $**P < 0.0001$; $*P < 0.005$.

in an intact thalamic circuitry, oscillations mediated in the delta and spindle mode are mainly mediated by the interplay between TC and nRt/perigeniculate nucleus cells, as shown in the *in vitro* slice model (von Krosigk et al. 1993). Although burst delay did not correlate with the membrane resting potential in our data set, it remains to be elucidated to what degree (modifiable) intrinsic membrane conductances shape the decay of the IPSPs.

IPSPs of various amplitudes up to 10 nS proportionally reduced and eventually abolished evoked LTS in thalamic cells most effectively in a relative time window of ~ 10 – 20 ms. Similarly, evoked IPSPs are capable of entirely blocking action potentials or Ca²⁺-dependent dendritic spikes in pyramidal cells of rat somatosensory cortex (Kim et al. 1995). The relative onset of the IPSPs is an important factor that determines the efficacy in counterbalancing excitation (Koch et al. 1983). In agreement with the present results, IPSPs can inhibit calcium transients in Purkinje cells and hippocampal pyramidal cells when coactivated in a narrow time window of ~ 10 ms (Callaway et al. 1995; Midgaard 1992). We therefore conclude that lateral inhibition within nRt can lead to burst shunting in individual nRt cells. This may explain why nRt cells tend to fire more vigorously during slow oscillations after blockade of GABA_A receptors (Bal et al. 1995a).

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