Corticothalamic Inhibition in the Thalamic Reticular Nucleus

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Zhang, Liming and Edward G. Jones. Corticothalamic inhibition in the thalamic reticular nucleus. J Neurophysiol 91: 759–766, 2004. First published October 29, 2003; 10.1152/jn.00624.2003. Mutual inhibition between the GABAergic cells of the thalamic reticular nucleus (RTN) is important in regulating oscillations in the thalamocortical network, promoting those in the spindle range of frequencies over those at lower frequencies. Excitatory inputs to the RTN from the cerebral cortex are numerically large and particularly powerful in inducing spindles. However, the extent to which corticothalamic influences can engage the inhibitory network of the RTN has not been fully explored. Focal electrical stimulation of layer VI in the barrel cortex of the mouse thalamocortical slice in vitro resulted in prominent di- or polysynaptic inhibitory postsynaptic currents (IPSCs) in RTN cells under the experimental conditions used. The majority of cortically induced responses consisted of mixed PSCs in which the inhibitory component predominated or of large IPSCs alone, implying inhibition of neighboring cells other, cortically excited RTN cells. Within the mixed PSCs, fixed and variable latency components could commonly be identified. IPSCs could be blocked by application of ionotropic glutamate receptor antagonists or of GABA_A receptor antagonists, also indicating their dependence on corticothalamic excitation triggering disynaptic or polysynaptic inhibition. Spontaneous GABA_A receptor-dependent IPSCs were routinely observed in the RTN and, taken together with the results of cortical stimulation, indicate the existence of a substantial network of intrareticular inhibitory connections that can be effectively recruited by the corticothalamic system. These results suggest activation of cortical excitatory inputs triggers the propagation of inhibitory currents within the RTN and support the view that activation of the RTN from the somatosensory cortex, although focused by the topography of the corticothalamic projection, is capable of disynaptically engaging the whole inhibitory network of the RTN, by local and probably by reentrant GABA_A receptor–based synapses, thus spreading the corticothalamic influence throughout the RTN.

INTRODUCTION

The thalamic reticular nucleus (RTN) is a sheet of GABAergic neurons that covers the external surface of the thalamus. In rodents and certain other species it forms the principal source of inhibition to the relay neurons of most dorsal thalamic nuclei (reviewed in Steriade et al. 1997). GABAergic synapses form ~30% of the synapses on rodent RTN cells (Liu and Jones 1999) and are mostly formed by the intranuclear collaterals of axons of RTN cells that project into the dorsal thalamus (Cox et al. 1996; Deschênes et al. 1985; Liu et al. 1995; Pinault et al. 1995a,b, 1997; Pinault and Deschênes 1998; Yen et al. 1985). In other species, there are also GABAergic dendrodendritic synapses between RTN cells in cats (Deschênes et al. 1985; Yen et al. 1985), but in monkeys they are described as common in some studies and rare in others (Asanuma 1994; Williamson et al. 1994). In rodents, their existence is debated (de Biasi et al. 1986; Pinault et al. 1997).

It is thought that the capacity of RTN neurons to inhibit one another is a key element in preventing the occurrence of hypersynchronized, low-frequency oscillations of the thalamocortical network that underlie certain forms of seizures (Huguenard and Prince 1994; Huntsman et al. 1999; McCormick and Bal 1997; Sohal et al. 2000; von Krosigk et al. 1993). The inhibitory interactions serve to dampen the recurrent excitation of RTN cells by bursting relay cells subjected to RTN-based inhibition that underlies oscillations in the spindle range of frequencies (Bal et al. 1995a,b; von Krosigk et al. 1993; Warren et al. 1994). Evidence for functional inhibitory synapses between RTN cells has been obtained in experiments in which the RTN was subjected to focal electrical stimulation in vitro (Huntsman et al. 1999; Huntsman and Huguenard 2000; Zhang et al. 1997) or in vivo (Bazhenov et al. 1999) or in which RTN or perigeniculate neurons were directly stimulated by local application of glutamate in vitro (Sanchez-Vives et al. 1997a; Shu and McCormick 2002). None of these studies, however, has addressed the issues of the capacity of extrinsic sources of input to induce inhibition in the RTN nor the extent of spread of this inhibition within the RTN.

The corticothalamic projection forms one of the principal sources of extrinsic input to the RTN. Corticothalamic synapses form more than 60% of the synapses on rodent RTN neurons (Liu and Jones 1999) and are capable of powerfully exciting these neurons (Golshani et al. 2001; Liu et al. 2001). Corticothalamic stimulation is a potent initiator of spindle oscillations in vivo and in vitro (Steriade et al. 1972; Warren et al. 1994), serving to recruit large-scale ensembles of synaptically coupled RTN and relay cells into spindle activity. Yet the collaterals of corticothalamic axons that innervate the RTN in rodents terminate in highly focal domains that can influence directly only a limited number of RTN cells (e.g., Agmon et al. 1994; Bourassa et al. 1995). The present study attempts to determine the capacity of corticothalamic inputs to elicit inhibitory events in RTN neurons and the potential for this inhibitory influence to spread through the RTN.

METHODS

Preparation of in vitro slices

Postnatal day 2 (P2) to P17 ICR mice (Harlan Sprague–Dawley, Indianapolis, IN) were anesthetized by hypothermia (P2–P4) or with ether (P5–P17) and decapitated. The brain was quickly removed and put into chilled artificial cerebrospinal fluid (ACSF), in which 126 mM NaCl had been replaced by sucrose at equivalent osmolarity and...
containing (in mM) 252 sucrose, 3 KCl, 1.25 NaH₂PO₄, 1.3 MgSO₄, 7 H₂O, 2.5 CaCl₂, 26 NaHCO₃, and 10 dextrose, pH 7.4 when bubbled with 95% O₂–5% CO₂, osmolarity 300–315 mOsm. Slices (400 μm thick) containing the somatosensory cortex, RTN, and ventral posterior nucleus (VP) were cut at an angle that preserved corticothalamic and thalamocortical connectivity (Agmon and Connors 1991). The slices were cut on a vibratome while immersed in chilled ACSF and were then transferred to a submerged-type recording chamber containing (in mM) 130 potassium gluconate, 10 KCl, 2 MgCl₂, 0.1 CaCl₂, 1.1 EGTA, 10 HEPES, 2 K₂-ATP, and 0.5 GTP. The pH was adjusted to 7.3 with KOH, and final osmolarity was 290–310 mOsm/kg. Series resistance during recordings was regularly 5–25 MΩ. For voltage-clamp experiments, QX314 (5 mM) was routinely added to the internal solution to prevent voltage-sensitive Na⁺ channels from generating action potentials. The resting membrane potential (RMP) was measured just after rupturing the cell membrane and in most of the experiments the cells were held at −70 mV. Data were low-pass−filtered at 2–10 KHz and digitized at 1–10 KHz via a CED 1401 plus interface (Cambridge Electronic Design) with a Pentium-based computer (Gateway 2000) that stored the data and provided on-line display of responses and off-line data analysis. CED patch- and voltage-clamp software was used to acquire and analyze data. Spon-
taneous and evoked postsynaptic currents (PSCs) were also analyzed with MiniAnalysis 5.1.1 (Synaptosoft). The liquid junction potential error was not corrected. Plots were made using Microcal Origin 5.0 (Microcal Software) and Sigmaplot 2000 (SPSS).

Recording

The RTN was visualized with differential interference contrast and infrared optics (Fig. 1B). Whole cell patch-clamp recordings were performed using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Recording pipettes were pulled from borosilicate glass on a Narishige PP-83 two-stage puller. In the experiments, pipettes had resistances of 3–7 MΩ when filled with internal solutions containing (in mM) 130 potassium gluconate, 10 KCl, 0.1 CaCl₂, 1.1 EGTA, 10 HEPES, 2 K₂-ATP, and 0.5 GTP. The pH was adjusted to 7.3 with KOH, and final osmolarity was 290–310 mOsm/kg. Series resistance during recordings was regularly 5–25 MΩ. For

Synaptic stimulation

Corticothalamic synaptic responses were evoked in RTN neurons by electrical stimulation of layer VI of the somatosensory cortex, using 0.1 ms cathodal stimuli delivered at 0.07 Hz to a single site in layer VI via a monopolar tungsten microelectrode (Fig. 1A). Stimulus strength was set 10–15% above absolute threshold to elicit a stable response.

Morphology

Biocytin 0.5% was present in the internal solution of the recording pipette during all recordings. Slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, incubated in avidin—biotin−peroxidase complex (ABC kit PK-4000, Vector Laboratories) and reacted with 3,3′-diaminobenzidine—4HCl to visualize injected cells, which were then drawn with the aid of a camera lucida (Fig. 1C).

Drug application

The following agents were applied through the superfusing ACSF: 6 cyan-7-nitroquinoxaline-2,3-dione (CNQX); (±)-2-amino-5-phos-

Statistics and measurement of synaptic responses

Amplitudes of whole cell PSCs were measured as the difference between the average of 0.5-ms regions spanning the baseline immedi-

RESULTS

During whole cell recording, biocytin 0.5% was present in the internal solution of the recording pipette. Injection of biocytin into neurons in the somatosensory sector of the RTN showed the typical dendritic morphology of RTN cells and the primary axon that entered and ramified focally in zones pre-
GABA A receptor

potentials held at –55 mV (mean, 42.8 ± 4.0), suggesting the presence of unitary events at the lower amplitudes and combined events at the higher amplitudes (Fig. 2B). All spontaneous responses were completely abolished by bath application of 10 µM BMI (Fig. 2A), indicating their dependence on GABA A receptor-based mechanisms.

Cortical stimulation evokes predominant IPSC/inhibitory postsynaptic potentials (IPSPs) in the RTN

Low-intensity electrical stimuli applied focally to the cells of origin of corticothalamic fibers in layer VI of the somatosensory cortex evoke excitatory PSCs (EPSCs) at two distinct latencies in RTN cells of the mouse thalamocortical slice (Golshani et al. 2001; Liu et al. 2001). EPSCs with latencies in the range of 2 to 4 ms are due to antidromic activation of thalamocortical fibers and orthodromic invasion of their collaterals in the RTN. EPSCs with latencies in the range of 6 to 9 ms are due to orthodromic activation of slower conducting corticothalamic fibers and their collaterals in the RTN. For the purposes of the present study, all responses with latencies < 6 ms were excluded.

Cortical stimulation will elicit N-methyl-d-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated excitatory postsynaptic potentials (EPSPs)/EPSCs in RTN cells of the mouse thalamocortical slice but these EPSPs/EPSCs are significantly attenuated in the presence of GABA A receptor-mediated inhibition (Warren and Jones 1997). In the present study, under the recording conditions used, pure EPSPs/EPSCs were rarely observed. In recordings from 51 RTN neurons showing responses to low-frequency electrical stimulation of layer VI, the typical evoked PSC consisted of either single or multiple peaked components with fixed or variable latencies (defined below) and with a distinctive slow decay (Fig. 3A). The majority of PSCs consisted of mixed IPSCs and EPSCs with a predominant IPSC component (34 neurons) and the remaining responses (17 neurons) consisted of pure IPSCs. Responses were not confined to a localized focus in the RTN and could be obtained at recording sites as distant as approximately 1 mm from one another (Fig. 1B).

All the evoked PSCs recorded in RTN neurons in response to cortical stimulation could be either fully or partially removed by 10 µM BMI (Figs. 3, A, C, and E and 4A). PSCs recorded in 17 of 51 neurons were completely blocked by 10 µM BMI, indicating that these PSCs were GABA A receptor-mediated IPSCs, and no EPSC component was found at several membrane potentials (IPSPs)/EPSCs in RTN cells of the mouse thalamocortical slice (Warren and Jones 1997). In the present study, all responses with latencies < 6 ms were excluded.

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FIG. 3. A: a common type of response evoked by cortical stimulation (arrowhead). Eight control traces recorded with membrane potential held at 0 (top) and 20 mV (middle), and an average (bottom) taken from recordings at 0 and 20 mV in the presence of BMI (10μM). The amplitudes of evoked currents differ with different membrane potentials (around 0.9 nA at 0 mV and above 1.8 nA at 20 mV). The currents last hundreds of milliseconds and have fluctuation peaks. At least 2 PSCs were evoked. A small PSC with a fixed latency (left arrow, mean onset 7.6 ms) and a second large PSC with a variable latency (right arrow, mean onset 45 ms) can be observed in the traces recorded in the absence of BMI. (See text for a definition of fixed and variable latencies). Addition of BMI completely blocked the evoked PSCs, indicating the PSCs are mediated by GABA_A receptors. B: another type of current evoked by cortical stimulation and recorded at a holding potential of ~20 mV. Fourteen raw traces show PSCs with 2 fixed latencies at 16 and 24 ms from stimulus artifacts (arrowheads). Boxed area, magnified at upper right, shows the two PSC components. C: single PSC with fixed latency recorded in a P11 cell in response to cortical stimulation. The PSC was completely abolished in the presence of BMI (10μM) and after recovery (not shown) it could be reabolved by 6 cyano-7-nitroquinoxaline-2,3-dione (CNQX: 20μM) (+)-2-amino-5-phosphonopentanoic acid (APV; 50μM), indicating that the PSC depends on monosynaptic excitation by glutamatergic corticothalamic fibers and di- or polysynaptic inhibition by GABAergic intrareticular connections. The PSC was recorded in 6 different membrane potentials (from 0 to ~100 mV in 20-mV increments). Each trace is an average of 5 original sweeps at each holding potential. D: current–voltage (I–V) relationship of the cell in C recorded in the control state and in the presence of BMI or CNQX + APV. The reversal potential for this cell was ~ −70 mV. Dotted line in C indicates position where current was measured and I-V relationship drawn. E: PSC recorded at holding potentials from −100 to 20 mV that could be partially attenuated by BMI (10μM), leaving a residual excitatory PSC displaying slower kinetics, and incompletely blocked by CNQX, suggesting involvement of N-methyl-D-aspartate receptors in the uncovered excitatory response. F and G: I–V relationships of the cell in E recorded in the absence (F) or presence (G) of BMI or of BMI and CNQX, and at two different times poststimulus [peak in 28 or 17 ms (Early) and 70 ms (Late)]. Dotted lines in E indicate the positions where currents were measured and I-V relationships drawn. The reversal potential for this cell was ~ −60 mV before BMI was added, and during BMI it was 0 mV.

FIG. 4. A: PSPs recorded in an RTN cell in response to stimulation of layer VI. The control response is multipeaked with 2 major peaks occurring at variable latencies centered around 20 and 12 ms. The 20 ms and longer latency responses disappeared after BMI (10μM) was added to the bath, leaving the response centered around 12 ms intact but with its potential reversed, suggesting the uncovering of a monosynaptic excitatory postsynaptic potential (EPSP; of the four traces, the lowest trace was taken 2 min after BMI application and the top three after 7 min). CNQX (20μM) + APV (50μM) removed the uncovered EPSP in this cell. Ten minutes after washout of the antagonists, the shorter and longer latency responses reappeared. Membrane potential for this cell was −62 mV. Two dotted lines indicate the time from stimulus artifact (arrowhead) to onset of the 2 principal response peaks. B: relationship between onset latency and the amplitude of postsynaptic potentials recorded in the presence or absence of BMI. Filled circles indicate onset latencies that shorten in the presence of BMI; open circles indicate amplitudes of short latency PSPs that increase in the presence of BMI. C: IPSCs with variable latencies recorded in a RTN cell in response to stimulation of layer VI (top). In the presence of CNQX + APV all currents disappear. The two arrows indicate responses recorded before (left arrow) and during (right arrow) the application of CNQX + APV. Bottom: currents starting to return after washout of CNQX + APV for over 1 h. Right arrow indicates responses before and left arrow indicates responses after washout of CNQX + APV. Shorter latency components returned first. D: time course and relationship between onset latencies and amplitudes of PSCs recorded in the presence or absence of APV + CNQX. Filled circles indicate the latencies of responses recorded before, during, and after washout of APV + CNQX. Open circles indicate the latencies of the PSCs mediated component with a slow decay time and were substantially reduced by APV (50μM, data not shown).

Cortically evoked PSCs were also sensitive to application of the glutamate receptor antagonists CNQX and APV and the response could be completely abolished by bath application of 20μM CNQX and 50μM APV (Figs. 3C and 4A and C), as readily as with 10μM BMI. Seventy percent of cells showing pure IPSC/IPSPs (5/7) were also equally sensitive to BMI or CNQX/APV, indicating the dependence of the responses on monosynaptic corticothalamic excitation of other RTN cells followed by di- or polysynaptic inhibition. IPSCs removed by CNQX and APV were characterized by a rapid, “all-or-none” disappearance in about 90 s, and recovered with a gradually shortened latency in a very slow manner after washing (Fig. 4, C and D), suggesting that the key factor in initiating an inhibitory chain reaction in the RTN is the monosynaptic, excitatory corticothalamic synapse. This result clearly indicates that RTN cells excited by glutamatergic corticothalamic collateral synapses are closely coupled to their neighbors by GABA_A receptor-containing inhibitory synapses.
Corticothalamic stimulation elicits postsynaptic responses with fixed and variable latencies in RTN neurons

The PSCs recorded in response to stimulation of layer VI commonly showed complex, multipeaked waveforms which were dominated by early, relatively fixed and later, variable latency components that could be identified (Figs. 3A–C and 4, A and C). We shall define a fixed latency response as a response in which, on a trial-by-trial basis, the variance between individual responses was < 1 ms. On this basis, 36 of 51 neurons showed a fixed latency response with some of them followed by a second response of variable latency. In Fig. 3A, the evoked response consisted of a small PSC with a fixed latency of 7.6 ms, followed by a second large PSC with a variable latency (range 35.6–48.6 ms; mean 42.3 ± 1.7 ms, n = 8 traces). The remaining 15 cells showed only variable response latencies ranging from two more or less consistent responses to responses with widely differing latencies (Fig. 4A). To determine the range and accuracy of the timed responses, we examined the mean and SD of the PSCs with variable latencies. The latencies of the 15 cells shown in Fig. 5B ranged from 9 to 92 ms with a mean of 32 ± 5.9 ms. There was a correlation between the mean latency and SD (r = 0.82, P = 0.0001; Fig. 5B), indicating that the accuracy of the timed response decreases as the interval increases.

In cells with fixed response latencies, as defined above, the mean latency of this response was 9.4 (±2.1 ms; n = 36; Fig. 5A). The mean latency was significantly shorter in these neurons than in those with variable latencies of response (Fig. 5C; independent t-test, P < 0.0001). The PSCs with the shorter fixed latencies usually presented a single peak (Fig. 3, C and E), suggesting that PSCs with shorter latencies are largely mediated by disynaptic transmission following monosynaptic corticotectal excitation of neighboring RTN cells. Responses with fixed latencies did not show changes in latency in the presence of BMI. For 21 cells studied in detail, the latency in control conditions was 9.57 ± 0.4 ms, and in the presence of BMI it was 9.45 ± 0.4 ms. The slight difference was not statistically significant.

In 4 of 17 cells whose responses consisted of pure IPSCs (see above), the IPSC was of fixed latency. Pure IPSCs were observed in the majority (87%) of the neurons (13/15) that exhibited variable latencies of response. In cells with mixed IPSCs/IPSPs and EPSCs/EPSPs, the mean latency became significantly shorter during the presence of BMI in the bath solution (Figs. 4A and 5C); 19.47 ± 2.93 in control; 10.89 ± 1.26 in BMI (Student’s t-test, P = 0.01, n = 5). After washout of BMI from bath solution, the latencies recovered to their original length (Fig. 4, A and C). This result suggests that, although dominant, the IPSC/IPSPs have longer latencies than the EPSC/IPSPs and are thus likely to be di- or polysynaptic; it further suggests that inhibitory activity driven by excitatory corticoreticular inputs propagates in the RTN.

Reversal potentials of the cortically evoked PSCs were examined in 23 neurons (Fig. 5, B and D). The mean reversal potential for the responses showing variable latencies was −50.1 ± 3.3 mV (range −34 to −69.8 mV; n = 10), whereas the mean reversal potential for fixed latency responses was −37.0 ± 6.1 mV (range 5 to −65 mV; n = 13). There was a significantly negative correlation between age and reversal potential in the responses with variable latencies (r = −0.73, P = 0.01, n = 10; Fig. 5D). The average reversal potential for PSCs with variable latencies (−50 mV) was closer to the calculated chloride equilibrium potential of −57 mV under these recording conditions, but there were 4 cells with reversal potentials less than −50 mV that were recorded in younger animals (less than P5). The average reversal potential for PSCs with fixed latencies (−37 mV) was 10 mV more positive than the PSCs with variable latencies, implying that the fixed latency PSC, although mixed, contained a substantial AMPA/NMDA receptor–mediated component (which alone would have a reversal potential of approximately 0 mV). These results indicate that reversal potential correlates negatively with developmental age for Cl−-based GABA A receptor–mediated inhibitory components. However, in the younger animals, reversal potentials might not match the chloride equilibrium potential. A more positive reversal potential might be attributable to a chloride uptake mechanism in the younger cells or, alternatively, to an increased permeability of the synaptic channel in the younger cells (Agmon et al. 1996; Ben-Ari et al. 1989; Zhang et al. 1991).

**DISCUSSION**

This study reveals that electrical stimulation of layer VI of the somatosensory cortex, after excluding responses due to antidromic activation of thalamocortical axons, elicits PSCs in RTN cells that are dominated by inhibition. The currents elicited in some RTN cells had fixed latencies and were made
up of an EPSC accompanied by a predominant IPSC. Currents elicited in many other RTN cells had variable latencies and were composed of IPSCs only. The results suggest that local excitation of RTN cells by focused corticothalamic terminations are capable of engaging the network of inhibitory connections throughout a large part of the RTN.

Most IPSCs evoked in RTN cells by cortical stimulation were multicomponent, with variable and often long latencies, temporal persistence, and fluctuations. These characteristics suggest that they were the result of polysynaptic propagation of activity through the circuitry of the RTN and in this they resembled the effects recorded in a number of other defined circuits excited polysynaptically (Agmon et al. 1995; Buonomano 2003; Kaneko et al. 2000; Metherate and Cruikshank 1999). Ando et al. (1995) had earlier shown latency fluctuation and the presence of temporal facilitation in the responses of RTN cells to cortical stimulation. Shortening of mean latency of PSCs/PSPs, as we found after IPSCs/IPSPs were abolished, is also a likely indication of polysynaptic transmission in the RTN. That is to say, polysynaptic spread of inhibition will normally last longer than an initial excitatory effect. Altered latencies of evoked responses or shortening of the duration of IPSP fluctuations in the presence of GABA A receptor antagonists have also been reported in the cerebral cortex (Agmon et al. 1996; Buonomano 2003; Metherate and Cruikshank 1999).

Low-intensity electrical stimuli applied focally to the cells of origin of corticothalamic fibers in layer VI of the somatosensory cortex evokes EPSCs at two distinct latencies in RTN cells of the mouse thalamocortical slice (Golshani et al. 2001; Liu et al. 2001). EPSCs with latencies in the range of 2 to 4 ms are due to antidromic activation of thalamocortical fibers and orthodromic invasion of their collaterals in the RTN. EPSCs with latencies in the range of 6.5 to 9.5 ms are due to orthodromic activation of slower conducting corticothalamic fibers and their collaterals in the RTN. In the present study, only the longer latency, orthodromic responses were examined, although we cannot exclude that antidromic activation of other RTN cells could have contributed to longer latency inhibitory responses. The distance between the stimulating and recording sites was 1000 to 2000 μm, and assuming an axonal conduction velocity of approximately 0.2 mm/ms (Buonomano 2003; Tanifuji et al. 1994), the orthodromic latency of corticothalamic EPSCs in the RTN should be 5–10 ms, which is in the range derived by Golshani et al. (2001) and Liu et al. (2001). The mean latency of evoked PSCs with variable latencies was distinctly longer than that of evoked PSCs with fixed latencies. The longer latencies of the variable responses imply, again, that these longer latency IPSCs are the result of polysynaptic activation of the intrareticular network.

The presence of a small number of fixed latency IPSCs in the responses to cortical stimulation could indicate close coupling of the cell recorded from to a focus of corticothalamic fiber terminations that do not synapse directly on the cell, although it is conceivable that an excitatory input to the cell could have been severed by the slicing procedure.

Morphological data show that the axonal collaterals of corticothalamic fibers in the RTN are relatively short and that the projection of a single-barrel-associated part of layer VI terminates in a very constrained focus approximately 50 μm in extent in the mouse RTN (Agmon et al. 1995). Hence, local stimulation in layer VI is likely to activate only a limited number of RTN cells and spread of activity within the RTN will be by inhibitory connections between RTN cells. There is also the possibility that GABA A receptor-mediated currents are actually depolarizing under conditions in vivo, and spread activity directly through the RTN, eliciting burst discharges in the neurons and predisposing the network to spindle oscillations (Bazhenov et al. 1999).

GABA A receptor–based inhibition has been demonstrated between RTN neurons, and it has now been shown that these synapses can be engaged by the corticothalamic projection. Inhibitory synapses between GABAergic cells have been demonstrated morphologically in several species (Deschênes et al. 1985; Liu and Jones 1999; Ohara and Lieberman 1985; Williamson et al. 1994; Yen et al. 1985) and physiologically in vitro in ferrets and rodents (Huntsman et al. 1999; Sanchez-Vives et al. 1997a,b; Shu and McCormick 2002; von Krosigk et al. 1993; Zhang et al. 1997) and in vivo in cats (Bazhenov et al. 1999). The consensus of most morphological studies is that most of the GABAergic synapses in the RTN are formed by the intranuclear collaterals of axons of the RTN cells. There is uncertainty about the extent to which dendrodendritic synapses between RTN cells are a feature of the RTN, since these have been described as extensive in some species such as cats (Deschênes et al. 1985; Yen et al. 1985), either common (Asanuma 1994) or rare (Williamson et al. 1994) in monkeys, and absent in rodents (Ohara and Lieberman 1985). Physiological studies based on whole cell or single-unit recordings in association with local electrical (Huntsman et al. 1999; Huntsman and Huguenard 2000; Zhang et al. 1997) or glutamate (Sanchez-Vives et al. 1997a,b; Shu and McCormick 2002) stimulation of RTN or perigeniculate GABAergic neurons, or made during network operations of the connected RTN and dorsal thalamus (Kim and McCormick 1998; Warren et al. 1994), have shown that GABA A receptor–based inhibitory events can be readily identified as spontaneous events or after local stimulation of the RTN. GABA B receptor–based effects in the RTN are relatively uncommon or difficult to elicit (Sanchez-Vives et al. 1997a; Ulrich and Huguenard 1996), a feature that probably reflects the low expression of GABA B receptor gene expression in the RTN (Liang et al. 2000; Muñoz et al. 1998).
tating the spread of inhibitory activity in the RTN. The spread of hyperpolarizing inhibitory currents through the network of RTN neurons could also be potentiated by the presence of low-resistance gap junctions between the RTN cells (Landisman et al. 2002).

Previous studies that relied on local electrical stimulation of the RTN, although demonstrating the presence of GABA<sub>A</sub> receptor–based inhibition between RTN or perigeniculate neurons, could have stimulated diverse inputs to the RTN in addition to exciting the RTN cells directly. Studies involving local activation of RTN cells by glutamate did not reveal the extent of these inhibitory effects through the RTN. The present study stresses the efficacy of the corticothalamic projection in di- and polysynaptically recruiting intrareticular synapses and the potential for network operations in the thalamus to spread corticothalamically induced inhibition at long range through the RTN.

The corticothalamic projection is a powerful stimulus to the initiation of spindle oscillations that are typical of the thalamocortical network during slow wave sleep and drowsy inattentiveness, when RTN cells and relay cells are relatively hyperpolarized (Steriade et al. 1997). The effectiveness of the corticothalamic projection in promoting spindle oscillations appears to depend on its capacity to generate higher amplitude EPSCs in RTN cells than in relay cells, an effect that is likely to depend, in turn, on the enrichment of GluR<sub>4</sub> receptor subunits at corticothalamic synapses on RTN cells (Golshani et al. 2001). The more powerful activation of RTN cells ensures that the monosynaptic EPSP in relay cells is quickly overcome by the disynaptic RTN-based IPSP. As relay cells recover from the resulting hyperpolarization, the low-threshold calcium conductance is released and the relay cells fire a burst of action potentials that reexcite the RTN cells, so promoting continuation of the oscillation. The spread of inhibition through the RTN as more and more RTN cells are excited by the collaterals of thalamocortical fibers may be responsible for the shortening of the burst of discharges of RTN cells that ultimately leads to the fading of spindles as RTN cells and relay cells get out of synchrony (Bal and McCormick 1993; Kim and McCormick 1998). Synchronization of the cells is promoted by the blockade or absence of GABA<sub>A</sub> receptor–mediated inhibition in the RTN, leading to prolonged bursts and hypersynchronized, low-frequency oscillations at 3–4 Hz, resembling absence seizures (Blumenfeld and McCormick 2000; Destexhe 1998; Huguenard and Prince 1994; Sohal et al. 2000; von Krosigk et al. 1993). Shortening of RTN bursts or shunting of excitatory inputs by intrareticular inhibition could be a powerful influence in preventing seizures and corticothalamic activity could reinforce this effect. On the other hand, as pointed out by Sohal et al. (2000), intrareticular inhibition, perhaps enhanced by corticothalamic influences, could also permit focal activity in the thalamocortical network, serving to promote particular spatio-temporal patterns.

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