Relief of Synaptic Depression Produces Long-Term Enhancement in Thalamocortical Networks

Akio Hirata and Manuel A. Castro-Alamancos
Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, Pennsylvania

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Hirata, Akio and Manuel A. Castro-Alamancos. Relief of synaptic depression produces long-term enhancement in thalamocortical networks. J Neurophysiol 95: 2479–2491, 2006. First published December 28, 2005; doi:10.1152/jn.01145.2005. Thalamocortical synapses may be able to undergo activity-dependent long-term changes in efficacy, such as long-term potentiation. Indeed, studies conducted in vivo have found that theta-burst stimulation (TBS) of the thalamus induces a long-term enhancement (LTE) of field potential responses evoked in the neocortex of adult rodents. Because the thalamus and neocortex form a complex interconnected network that is highly active in vivo, it is possible that a change in thalamic excitability would be reflected in the neocortex. To test this possibility, we recorded from barrel neocortex and applied TBS to the thalamic radiation while the somatosensory thalamus was inactivated with muscimol. Thalamocortical LTE was absent when the thalamus was inactivated, suggesting that changes in thalamic excitability are involved. Single-unit recordings from thalamocortical cells revealed that TBS causes a significant reduction in the spontaneous firing rate of thalamocortical cells. Reducing the spontaneous firing of thalamocortical cells directly enhances the efficacy of the thalamocortical pathway because it relieves the tonic depression of the thalamocortical connection caused by thalamocortical activity. Because these changes in thalamic excitability are triggered by corticothalamic activity, this may be a useful top-down mechanism to regulate afferent sensory input to the neocortex during behavior as a function of experience.

Introduction

Sensory information, excluding smell, ascends toward the neocortex through the thalamus through thalamocortical pathways. In return, the neocortex communicates back to the thalamus through corticothalamic pathways (Sherman and Guillery 2001; Steriade et al. 1997). Thus thalamus and neocortex are intricately interconnected structures, so that activity in one of them may be reflected in the other. A particularly useful model system to study these pathways is the somatosensory cortex of rodents, and in particular, the representation of vibrissae (Bernardo and Woolsey 1987; Woolsey and Van der Loos 1970).

One important question refers to the ability of these pathways to change their efficacy as a function of activity. At the short-term level, thalamocortical and corticothalamic pathways show distinct short-term plasticity (for review, see Castro-Alamancos 2004); thalamocortical responses in the barrel cortex depress at frequencies >2 Hz, whereas corticothalamic responses in the ventrobasal thalamus (VB) facilitate at frequencies >5 Hz. At the long-term level, there is ample evidence that intracortical pathways in barrel cortex can undergo activity-dependent changes in synaptic efficacy such as long-term potentiation (LTP) and long-term depression (LTD) (Aroniadou-Anderjaska and Keller 1995; Castro-Alamancos et al. 1995; Egger et al. 1999; Glazewski et al. 1998; Malenka and Bear 2004; Takahashi et al. 2003) and that these mechanisms underlie sensory experience driven plasticity in adult and developing sensory cortex (Bear 2003; Foeller and Feldman 2004; Fox 2002). There is also evidence that both thalamocortical and corticothalamic pathways in sensory areas can undergo LTP and LTD (Castro-Alamancos and Calcagnotto 1999; Crair and Malenka 1995; Feldman et al. 1999; Heynen and Bear 2001; Isaac et al. 1997; Komatsu et al. 1988; Lee and Ebner 1992).

Interestingly, thalamocortical synapses can produce LTP in thalamocortical slices of somatosensory cortex, but only up to postnatal day 9 (Crair and Malenka 1995). This time seems to coincide with the critical period for developmental alterations in the barrel cortex caused by sensory perturbations and also with the time when silent synapses (i.e., synapses devoid of AMPA receptors, but containing NMDA receptors; Isaac et al. 1995; Liao et al. 1995) disappear from thalamocortical connections (Isaac et al. 1997). However, high-frequency thalamic stimulation in adult rodents in vivo causes long-term enhancement (LTE) of thalamocortical evoked responses in visual cortex (Dringenberg et al. 2004; Heynen and Bear 2001) and somatosensory cortex (Lee and Ebner 1992; see Fig. 12 in Castro-Alamancos and Connors 1997). Further work is needed to reconcile these in vitro and in vivo results. This is important because if thalamocortical connections are unvarying in the adult, it would impose restrictions on models of experience-dependent plasticity in adult sensory cortex.

In the barrel cortex of anesthetized rats, we studied thalamocortical LTE induced by theta-burst stimulation (TBS) applied to the thalamic radiation. Interestingly, thalamocortical LTE was abolished by inactivating the thalamus, indicating that the changes leading to thalamocortical enhancement after TBS are produced within the thalamus. Indeed, the results revealed that TBS leads to a long-lasting reduction in the firing rate of thalamocortical cells that can explain the enhancement of thalamocortical responses. Because this change in thalamocortical excitability seems to be triggered by corticothalamic activity, it is possible that corticothalamic activity serves as a regulator of thalamocortical activity by scaling the efficacy of the thalamocortical connection.

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METHODS

Surgery

Ninety-four adult male Sprague-Dawley rats (300–350 g) of between 2 and 4 mo of age were used in this study and cared for in accordance with National Institutes of Health guidelines for laboratory animal welfare. All experiments were approved by the Drexel University Institutional Animal Care and Use Committee. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and placed in a stereotaxic apparatus. The level of anesthesia was monitored with field potential recordings and limb-withdrawal reflexes. To maintain the anesthetic level, pentobarbital sodium was continuously supplied at a rate of 12.5 mg/kg/h (ip) through a cannula leading into the peritoneal cavity. Infusion was produced either manually every 15–30 min or using a constant flow pump. All skin incisions and frame contacts with the skin were injected with lidocaine (2%). A unilateral craniotomy extended over a large area of the parietal cortex. Small incisions were made in the dura as necessary, and the cortical surface was covered with artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 1.3 MgSO₄, 7 H₂O, 10 dextrose, 1 CaCl₂ 2 H₂O, and or saline. After the stimulating and recording electrodes were in place, the cortex was covered with a layer of 0.6% agarose. Body temperature was automatically maintained constant with a heating pad at 37°C.

Electrophysiology

Figure 1 shows a schematic depiction of the experimental setup and cresyl violet–stained coronal sections showing the positioning of electrodes. Two stimulating electrodes (bipolar concentric 200 μm in diameter; Frederick Haer, Bowdoinham, ME) and two recording electrodes were used. To record field potentials, tungsten insulated microelectrodes were used (100 μm diam; 0.5–1 MΩ). To record single units from the thalamus, glass pipettes were pulled to a fine tip (10–30 MΩ) and filled with ACSF or saline. These electrodes generally record only a well-discernible single unit of very large amplitude (>10 times the noise) that are usually stable for several hours. Every cell included in this study corresponds to a recording in which there was only one discernible large amplitude spike in the recording electrode. Extracellular recordings were obtained from the ventrobasal thalamus (VB) and from the primary somatosensory cortex (S1). The approximate coordinates used for VB from bregma were as follows (Paxinos and Watson 1982): posterior = 3.5, lateral = 3, depth = 5–6. The approximate coordinates used for S1 were as follows: posterior = 2–3, lateral = 5.5, depth = 1. The stimulating electrodes were placed in the thalamic radiation and in S1. The approximate coordinates used for the thalamic radiation stimulating electrode were as follows: posterior = 2.5, lateral = 4.5, depth = 3.5–5. The S1 stimulating electrode was placed between 0.5 and 1 mm anterior-lateral to the S1 recording electrode and was used as a control to test input specificity. In every case, the stimulating and recording electrodes were diligently aligned so that stimulation of the thalamic radiation resulted in short latency– and large amplitude–evoked responses in the thalamus and the neocortex. The cortical field potential response had an onset at ~1.5 ms and produced two major negative peaks; the first at ~3 ms post-stimulus followed by a second larger negative peak at ~4.5 ms post-stimulus (see Data analysis). The amplitude of the ~4.5-ms peak was between 5 and 10 mV for low-frequency stimuli of ~150-μA intensity. Although both of these peaks are not completely independent, because they overlap somewhat in time, we measured both of them. The first peak (3 ms) reflects a monosynaptic thalamocortical current sink in layer IV of barrel cortex, whereas the second peak (~5 ms) reflects this layer IV sink plus a propagating current sink into layer III (Castro-Alamancos and Connors 1996; Castro-Alamancos and Oldford 2002). Moreover, measuring both peaks also allows comparison with previous thalamocortical LTP studies, which have measured primarily the longer latency peak that produces the maximum response amplitude.

The simultaneously recorded thalamic single units fired at 3–6 ms post-stimulus with high reliability (>80%) in response to stimuli at 10–20 Hz (i.e., the response displayed strong facilitation at this frequency), but unreliably (<20%) in response to low-frequency stimuli (<1 Hz). These latencies and response properties are consistent with the simultaneous stimulation of thalamocortical and corticothalamic fibers producing monosynaptic responses in cortex and thalamus, respectively (Castro-Alamancos 2004). The thalamic cells included in this study did not show signs of being driven antidromically by the thalamic radiation stimulation. Testing for antidromic invasion was done by using a test train of five pulses at 50 Hz.

![Diagram](http://jn.physiology.org/)

**FIG. 1.** Schematic representation of the experimental setup (A) and cresyl violet–stained coronal sections (B and C) showing the location of electrodes in 1 animal. Frontal section (B) shows location of thalamic radiation stimulating electrode and barrel cortex recording electrode. The more posterior section (C) shows location of ventrobasal thalamus (VB) recording electrode and cortex stimulating electrode. Tips of stimulating electrodes are marked by lesions (small holes) produced by applying constant current. Tracts of recording electrodes can be followed in adjacent sections up to their destination. Note that in the schematic diagram (A), all electrodes are shown in the same coronal plane, but in fact, different electrodes are placed at different distances from bregma (as shown in B and C).
Antidromic cells produced a short-latency spike to each of the five test stimuli with little jitter (<0.1 ms) and were discarded. This was done to selectively study cells that were driven by corticothalamic inputs but not antidromically discharged to eliminate this variable (antidromic discharge) from the interpretation of the effects of stimulation on the recorded cells. Note that other cells are necessarily being driven antidromically as evidenced by the thalamic radiation-to-cortex response.

**Stimulation**

Electrical stimulation of the thalamic radiation or S1 consisted of 200-μs-duration pulses delivered at different frequencies. The intensity was adjusted to provide a robust response but was kept <200 μA in most experiments. In some experiments, we also tested intensities of 400 and 800 μA but found that the amount of LTE induced was not different from the lower intensities. Thus the range of intensities tested was 50–800 μA. Baselines were derived using single-pulses at 0.02 Hz (every 45 s). TBS was used to induce LTE. A TBS sequence consisted of 10 bursts (burst = 5 pulses at 100 Hz) delivered at 5 Hz. A total of five TBS sequences were delivered at 10-s intervals.

**Microdialysis**

To inactivate the thalamus with muscimol (Sigma-Aldrich, St. Louis, MO), a microdialysis probe was placed in VB (see Fig. 1) at the following coordinates from bregma: posterior = 3 lateral = 2–2.5 depth = 4–6 as previously described (Castro-Alamancos 2002). ACSF was continuously infused through the probe at 4 μl/min. The microdialysis probe consists of a 2-mm dialyzing membrane with a 250-μm diameter. Muscimol was dissolved in the ACSF at 200 μM. In control experiments, we determined that muscimol application resulted in the abolishment of spontaneous neuronal discharges (multunit activity) within a 1-mm radius from the probe. This was determined by using an array of tungsten recording electrodes each placed at increasing distances from the probe at 1-mm intervals. Thus multunit activity at 1 mm was mostly abolished, at 2 mm was slightly impaired, and at 3 mm was unaffected. When AP5 (Sigma-Aldrich) was applied in the neocortex, the microdialysis probe was placed adjacent to the recording electrode (~500 μm lateral). The probe was inserted parallel to the cortical recording electrode and the dialyzing membrane extended the depth of neocortex (2 mm). d-AP5 or dl-AP5 was dissolved in the ACSF at 0.25–1 mM.

**Data analysis**

Field potential responses were measured by calculating the peak amplitude of the two major negative peaks of the evoked response produced between 2 and 3.5 ms post-stimulus (termed here the 3-ms peak) and between 3.5 and 5.5 ms post-stimulus (termed here the 5-ms peak; see Fig. 3A for an example). Note that the amplitude of the 3-ms peak is equivalent to a slope measurement of the onset response (2–3.5 ms post-stimulus), because both of these variables are covariant. For LTE experiments, every six consecutive responses evoked at 0.02 Hz were averaged (this corresponds to a 5-min period). Thus a 30-min baseline is composed of six data points, each corresponding to a 5-min period. Unless otherwise indicated, data are plotted as a percent of the baseline amplitude, which is the average of the six data points during the 30-min baseline period.

Spontaneous single-unit activity was measured by using rate meters with a 1- or 10-s bin. In addition, interevent time histograms (IETHs) were obtained by calculating the time interval between every spike with a 1-ms bin resolution. This provides information of the timing between spikes during spontaneous firing. Evoked single-unit responses were computed with peristimulus time histograms (PSTHs) using a 1-ms bin. A minimum of 20 trials was used to derive PSTHs.

Population data are presented as mean ± SD. Within-subject comparisons were conducted with paired t-tests.

**Histology**

The location of electrodes was marked by passing DC current for 4–6 s. At the end of the experiments, the animals were given an overdose of pentobarbital sodium and perfused through the heart with saline followed by paraformaldehyde (4%). The brains were sectioned in a vibratome (80–100 μM) and processed for Nissl staining. For the data included in the study, subsequent analysis confirmed the location of stimulating and recording electrodes in the intended targets. In fact, the electrophysiological responses were good predictors of the correct positioning of the stimulating and recording electrodes.
thalamic radiation. Note the simultaneous depression and facilitation of the thalamic radiation-to-cortex and thalamic radiation-to-VB pathways, respectively.

Figure 3B shows population data for each pathway. Data for the three pathways was obtained from the same rats (n = 10) under the same conditions (i.e., stimulation alternated between...
Plotted is the peak amplitude of the evoked response to the 10th stimulus in a train (steady state) measured between 2 and 8 ms post-stimulus for the thalamic radiation-to-VB and cortex-to-cortex pathways. For the thalamocortical pathway (thalamic radiation-to-cortex), we measured the amplitude of the first negative peak of the evoked field potential response between 2 and 3.5 ms (3-ms peak) and the amplitude of the second negative peak between 3.5 and 5.5 ms (5-ms peak), as shown in Fig. 3A. The thalamic radiation-to-cortex pathway shows robust frequency-dependent depression of both peaks measured, the thalamic radiation-to-VB pathway shows strong frequency-dependent facilitation, and the cortex-to-cortex pathway shows slight depression or no change (Fig. 3B).

Effect of TBS on thalamic radiation-to-cortex responses

We tested the ability of thalamic radiation TBS to change the efficacy of the thalamic radiation-to-cortex responses. First, we obtained a stable baseline by stimulating in the thalamic radiation and recording evoked responses in the cortex at 0.02 Hz for a minimum of 30 min. Every six consecutive responses were averaged, so that a 30-min baseline is composed of six data points, and each data point is the average of six consecutive responses. After obtaining a stable baseline, TBS (see METHODS) was applied to the thalamic radiation three times at 30-min intervals. In the experiments shown in Fig. 4, as a control, we also monitored responses in the cortex-to-cortex pathway. This allowed testing for input specificity. TBS applied to the thalamic radiation caused an increase in the amplitude of the thalamic radiation-to-cortex evoked response that was long lasting. For simplicity, we call this long-lasting change LTE. LTE produced by TBS in the thalamic radiation-to-cortex pathway is input specific because little change occurs in the cortex-to-cortex pathway after TBS is delivered to the thalamic radiation-to-cortex pathway. Figure 4B shows population data (n = 6) for experiments in which both thalamic radiation and S1 stimulation were used to monitor both pathways. Stimulation alternated between both pathways (each pathway was stimulated at 0.02 Hz). TBS applied to the thalamic radiation produced a significant 33.4 ± 12% long-

FIG. 3. Population data showing effect of frequency on amplitude of evoked responses in cortex-to-cortex pathway, thalamic radiation-to-cortex pathway, and thalamic radiation-to-VB pathway. A: example of a thalamic radiation-to-cortex field potential response showing measurement of amplitude of the 3- and 5-ms peaks. B: percent change of steady-state responses (10th stimulus) with respect to amplitude of response at 0.4 Hz. Thalamic radiation-to-cortex responses were measured as shown in A. Thalamic radiation-to-VB and cortex-to-cortex responses were measured as maximum amplitude of evoked negative field potential between 2 and 8 ms post-stimulus. Data are mean ± SD of n = 10 animals.

FIG. 4. Input-specific long-term enhancement (LTE) is induced in barrel cortex in response to theta-burst stimulation (TBS) of thalamic radiation. A: representative traces of cortical field potential responses from 1 experiment evoked before and 30 min after the 3rd TBS by cortical stimulation (cortex-to-cortex pathway) and thalamic radiation stimulation (thalamic radiation-to-cortex pathway). Note that TBS in thalamic radiation enhanced thalamic radiation-to-cortex–evoked response but had little effect on the cortex-to-cortex–evoked response. B: population data showing effect of TBS applied to thalamic radiation on cortex-to-cortex pathway and thalamic radiation-to-cortex pathway. Note that most of the enhancement occurred after the 1st TBS. Every data point is mean ± SD of 6 experiments. For each experiment, a data point was obtained by averaging the responses during a 5-min period (normally 6 responses were averaged). Numbers correspond to times for traces shown in A.
lasting enhancement of the 5-ms peak in the thalamic radiation-to-cortex pathway (paired t-test, \( n = 6 \); baseline vs. 30 min after the 3rd TBS; \( P < 0.01 \)) and a slightly larger increase of the 3-ms peak (37 ± 9%; paired t-test; \( P < 0.01 \)), but no significant change (2 ± 5%; paired t-test, \( n = 6 \), not significant) in the cortex-to-cortex pathway. In most experiments (80%; 12 of 15), the enhancement seemed to occur mostly after the first TBS, indicating that a single TBS saturated the enhancement.

We also monitored the effect of TBS on the short-term plasticity of the thalamic radiation-to-cortex response in 10 experiments. We tested short-term plasticity by measuring the amount of depression between the first and the third stimulus delivered at a 50-ms interstimulus interval before and after the induction of thalamocortical LTE for the 3- and 5-ms peak amplitudes. The results revealed that for the 3-ms peak amplitude, the amount of depression between the first and third stimulus before LTE was 64.2 ± 10% and increased to 72.4 ± 9% after LTE (measured 30 min after the last TBS). This enhancement in the amount of short-term depression caused by thalamocortical LTE was statistically significant (paired t-test, \( n = 10 \), \( P < 0.001 \)). Similar results were obtained for the 5-ms peak amplitude, which before LTE showed 78.5 ± 6% depression and after LTE showed 83.9 ± 5% depression. This enhancement of short-term depression was also significant (paired t-test, \( n = 10 \), \( P < 0.01 \)). Although it is complicated to interpret changes in facilitation and depression in intact networks, these results are consistent with thalamocortical LTE resulting from a presynaptic change, such as an increase in release probability. Moreover, an increase in release probability could result from reducing the spontaneous firing of thalamocortical cells in vivo because this would reduce tonic synaptic depression at the thalamocortical connection. If this is the case, inactivating the thalamus should abolish thalamocortical LTE.

**Inactivating the thalamus blocks LTE in the thalamic radiation-to-cortex pathway**

Stimulation of the thalamic radiation will lead to stimulation of both thalamocortical and corticothalamic fibers. This is obvious from Fig. 2D, where thalamic radiation stimulation leads to responses in both the thalamus and the neocortex. Because stimulation of the thalamic radiation will recruit corticothalamic fibers that can affect thalamocortical activity, we decided to inactivate the thalamus with muscimol. Inactivating the cell bodies of thalamocortical cells should have no effect on the ability of their axons to induce LTP because the mechanisms responsible for both presynaptic and postsynaptic forms of LTP are present at the synapse (Bear and Malenka 1994; Bliss and Collingridge 1993; Nicoll and Malenka 1995).

To apply muscimol into the thalamus, a microdialysis cannula was implanted into the thalamus, as previously described (Castro-Alamancos and Oldford 2002). Application of muscimol (200 \( \mu \)M) into the thalamus in most cases produced an enhancement of the thalamic radiation-to-cortex evoked response (Fig. 5A). This was expected because activity in thalamocortical neurons depress thalamocortical responses (Castro-Alamancos 2004; Chung et al. 2002; Oldford and Castro-Alamancos 2003; Swadlow and Gusev 2001). Thus by eliminating thalamocortical activity, the tonic depression of thalamocortical synapses is relieved. The amount of enhancement varied in different experiments but was on average 31.8 ± 12% for the 5-ms peak (\( n = 7 \); paired t-test, before vs. during muscimol; \( P < 0.01 \)) and 46.9 ± 13% for the 3-ms peak (\( n = 7 \); paired t-test; \( P < 0.01 \)). In no case did the thalamic radiation-to-cortex response depress during the application of muscimol into the thalamus. We interpret the different amounts of enhancement as a reflection of different basal amounts of spontaneous activity in the thalamocortical pathway. For larger spontaneous activity, there will be a larger enhancement of thalamocortical responses when this activity is abolished by muscimol.

After responses stabilized during thalamic inactivation with muscimol, a new baseline was obtained, and the induction of LTE was attempted by applying TBS three times at 30-min intervals (muscimol application was continued throughout the experiment). Figure 5B shows population data measuring the 5-ms peak obtained from a total of 22 experiments, 15 of them with the thalamus intact and 7 with the thalamus inactivated.
with muscimol. In every case that TBS was applied with the thalamus inactivated, we failed to induce LTE (paired t-test, \( n = 7 \), baseline vs. 30 min after the 3rd TBS; not significant). In contrast, in the experiments where TBS was applied with an intact thalamus, a significant amount (25.5 ± 6%) of LTE was induced (paired t-test, \( n = 15 \), baseline vs. 30 min after the 3rd TBS; \( P < 0.01 \)). The results were similar when the 3-ms peak was measured (data not shown). In a few cases, we also addressed the possibility that after muscimol was applied to the thalamus, the induction of LTE required increasing or reducing the simulation intensity. Hence, in some experiments, we either reduced (\( n = 3 \)) or increased (\( n = 4 \)) the amplitude of the evoked response by lowering (down to 50 μA) or increasing (\( \leq 800 \) μA) the stimulation intensity, respectively, and attempted to induce LTE with TBS. In every case, we found that after reducing or increasing the response amplitude, TBS was still ineffective in producing any significant change (not shown but similar to Fig. 5B). Taken together, these results suggest that the changes leading to thalamic radiation-to-cortex response enhancement after TBS are occurring in the thalamus and that the LTE observed in thalamocortical pathways in vivo is different from traditional LTP. We next explored the changes that may be occurring in the thalamus to produce thalamocortical LTE.

What changes in the thalamus to produce LTE of thalamic radiation-to-cortex responses?

One possibility is that TBS applied to the thalamic radiation changes the excitability of thalamocortical neurons, such that their firing rate decreases significantly after TBS, and this change is long-lasting. Indeed, this would be similar to the effect of inactivating the thalamus with muscimol, which as shown in Fig. 5A, enhances thalamocortical responses. To test this possibility directly, single-unit recordings were performed from the VB thalamus, and a stimulating electrode was placed in the thalamic radiation to evoke corticothalamic responses. A hallmark of the corticothalamic pathway is that it produces strong frequency-dependent facilitation (see Figs. 2 and 3). To be sure that the recorded thalamocortical cell was being driven by corticothalamic inputs, we stimulated the thalamic radiation with four pulses at 10 Hz for a minimum of 20 trials. Thalamocortical cells that are being driven by corticothalamic fibers in response to stimulation of the thalamic radiation will display strong frequency-dependent facilitation. Indeed, Fig. 6A shows a PSTH derived by stimulating the thalamic radiation at 10 Hz for 20 trials. Note that the cell responds with low probability to the first stimulus but with very high probability to the fourth stimulus at 10 Hz. Figure 6B shows a PSTH derived from the cells included in this analysis (\( n = 10 \)). Note the strong facilitation. Notably, none of these cells were driven antidromically by the thalamic radiation stimulation at the intensities used. Obviously, other thalamocortical cells, whose axons are coursing closer to the stimulating electrode, are being antidromically discharged. This is not unexpected considering the different routes within the thalamic radiation taken by thalamocortical and corticothalamic fibers innervating the same area (Bernardo and Woolsey 1987). Figure 6C shows the average number of spikes produced by each stimulus in response to four pulses of thalamic radiation stimulation at 10 Hz, measured during a 3- to 15-ms time window after each stimulus. Again, the number of spikes produced by each stimulus showed strong facilitation at 10 Hz.

We next tested the effect of applying TBS to the thalamic radiation on the spontaneous activity of those cells that were driven by corticothalamic inputs. To obtain a baseline, we recorded the spontaneous firing during a 30-min period and calculated the number of spikes per 10-s bins. Also shown is the amplitude of the 3- and 5-ms peak measured from the simultaneously recorded thalamic radiation-to-cortex field potential response. After a stable baseline, TBS was applied to the thalamic radiation three times at 30-min intervals. Figure 7A shows an example from one typical cell. During the baseline period, the average firing rate of the cell was 2.9 ± 0.7 Hz. Application of TBS led to a strong and short-lasting suppression of the spontaneous firing (cells cease firing for tens of seconds after TBS) that was followed by a more modest but appreciable reduction in the spontaneous firing rate of the cell to 1.9 ± 0.8 Hz at 30 min after TBS. Moreover, the simultaneously recorded field potential in cortex revealed significant LTE concomitant with the thalamic firing suppression. Figure 7B shows population data (\( n = 10 \)) in response to application of TBS to the thalamic radiation three times at 30-min intervals. Note that TBS caused a significant reduction in the spontaneous firing rate of the cells. The spontaneous firing rate for the cells before TBS was 1.93 ± 0.6 Hz but was reduced to
1.2 ± 0.5 Hz 30 min after the third TBS (paired t-test, n = 10, before vs. 30 min after the 3rd TBS, P < 0.01). Thus after TBS, on average the spontaneous firing rate of thalamocortical cells is reduced significantly concomitant with thalamocortical LTE.

The previous analysis provides information about the total number of spikes regardless of the interval between spikes. Although an absolute reduction in the total number of spikes is sufficient to relieve the tonic depression of the thalamocortical pathway, this relief would be stronger if, after TBS, the cells fired fewer spikes at frequencies that depress the thalamocortical pathway (i.e., >2 Hz). Thus to characterize the timing of the spontaneous firing of the thalamocortical cells before and after TBS, we derived IETHs for each cell during the last 15 min of the baseline period before TBS and for the same period corresponding to 1–15 min after TBS and 16–30 min after TBS. We choose the first TBS because normally little subsequent effect is produced by additional TBS bouts. Figure 8A shows IETHs corresponding to 10 cells before and after TBS.

**FIG. 7.** TBS of the thalamic radiation produces a long-lasting depression of spontaneous activity of thalamocortical cells. A: example of effect of TBS delivered to thalamic radiation on spontaneous firing of a VB cell. Middle panel: rate meter of the spontaneous firing derived using a 10-s bin. Immediately after TBS, activity of the cell is sharply suppressed, and it slowly recovers until reaching a new lower-frequency firing rate. Bottom plot: means ± SE of the 10-s bins during a 5-min period shown as the number of spikes per second. Top plot: amplitude of the 3- and 5-ms peaks of simultaneously recorded thalamic radiation-to-cortex response. Each data point was obtained by averaging the responses during a 5-min period (normally 6 responses were averaged). B: population data derived from 10 cells (same cells as shown in Fig. 6, B and C) on effect of TBS on spontaneous firing rate of thalamocortical cells. Specifics are as in A. Note that TBS produces a drop in the spontaneous firing rate of thalamocortical cells and concomitant thalamocortical LTE.

**FIG. 8.** Interevent time histogram (IETH) reveals that TBS suppresses firing rate of cells at frequencies that depress thalamocortical connections. A: population data showing an IETH of the cells shown in Figs. 6B, 6C, and 7B during a 15-min period before TBS, the 1st 15-min period after the 1st TBS (1–15 min), and the 2nd 15-min period after the 1st TBS (16–30 min). Left plot: close-up of spikes occurring at intervals below 10 ms (100 Hz). Right plot: spikes occurring at intervals between 10 and 500 ms (2–100 Hz). B: percent suppression by TBS of spikes occurring at frequencies above (>2 Hz) and below 2 Hz (0.2–2 Hz) during the 1st (black columns) and 2nd (gray columns) 15-min periods after TBS. Also shown is percent suppression by TBS of spikes occurring at frequencies 2–100 Hz and above 100 Hz (i.e., data shown in A). Paired t-test: *P < 0.01, **P < 0.05.
Figure 8B quantifies the percent suppression of spikes at different intervals. Spikes occurring at frequencies between 0.2 and 2 Hz showed on average 27 ± 15 and 19.9 ± 14% suppression for the first and second 15-min periods after TBS, but this was not significant (paired t-test, n = 10, not significant) because, although some cells suppressed their activity, others did not change at frequencies between 0.2 and 2 Hz. However, spikes occurring at frequencies >2 Hz, which is the frequency above which thalamocortical synapses depress, were reduced significantly (44.4 ± 6 and 37.3 ± 5% suppression for the 1st and 2nd 15-min periods after TBS; paired t-test, n = 10, P < 0.01). Specifically, while spikes occurring at frequencies between 2 and 100 Hz were also reduced significantly after TBS (13.6 ± 9 and 12.6 ± 8% suppression for the 1st and 2nd 15-min periods after TBS; paired t-test, P < 0.05), spikes occurring at frequencies above 100 Hz were more strongly reduced after TBS (52.4 ± 6 and 48.9 ± 7% suppression for the 1st and 2nd 15-min periods after TBS; paired t-test, P < 0.01). Because spikes occurring at >100 Hz form bursts (in well-isolated single-unit thalamocortical recordings) (Sherman and Guillery 1996; Steriade et al. 1997), this suggests that there is a particularly strong reduction in the number of bursts that occur after TBS. Thus both burst and single spikes that occur at frequencies >2 Hz are significantly reduced after TBS. Consequently, after TBS, thalamocortical synapses should be significantly relieved from the tonic depression caused by spontaneous thalamocortical firing.

Thalamocortical response efficacy depends on the spontaneous firing rate of thalamocortical cells

The previous results indicate that after TBS, the spontaneous firing rate of thalamocortical cells shows a long-lasting reduction, and this directly results in thalamocortical LTE. If this is the case, spontaneous changes in the firing rate of thalamocortical cells should be reflected in the amplitude of the thalamocortical-evoked response. To test this possibility, we monitored the spontaneous firing of thalamocortical cells and the amplitude of simultaneously recorded thalamic radiation-to-cortex field potential responses. In these experiments, to allow for a...
larger variability in the firing of thalamocortical cells, the level of anesthesia was allowed to fluctuate by delaying the supplementation of anesthesia. Figure 9, A–C, shows an example from one of these cases. Note that as the firing rate of the thalamocortical cell changes spontaneously from a low rate to a high rate, the amplitude of both the 3- and 5-ms peaks of the thalamic radiation-to-cortex evoked response is suppressed. Conversely, as the firing rate of the thalamocortical cell decreases, the thalamocortical response increases. Figure 9C also shows IETHs for two periods of 5-min duration each taken from the example shown in Fig. 9B (periods 1 and 2), which reveal strong suppression of spikes occurring at frequencies >2 Hz during period 2, when the firing rate of the thalamocortical cell was low and the thalamocortical response was enhanced. Population data obtained from seven cases is presented in Fig. 9D. This figure shows the relation between spontaneous firing of thalamocortical cells and the amplitude of the 3-ms peak of the thalamic radiation-to-cortex responses from seven experiments that showed spontaneous changes in cell firing. In every case, there was a very strong negative correlation between these variables; the average correlation was $-0.92 \pm 0.7$ for the 3-ms peak and $-0.91 \pm 0.8$ for the 5-ms peak ($n = 7$). Therefore these results show that the spontaneous firing rate of thalamocortical cells sets the efficacy of the thalamocortical pathway.

**Thalamocortical LTE is sensitive to thalamic, but not cortical, blockade of NMDA receptors**

Thalamocortical LTE in adult rats in vivo is NMDA receptor dependent because systemic (intraperitoneal) application of the competitive NMDA receptor antagonist $\pm$-3-(2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid (CPP) abolishes the ability of TBS to induce thalamocortical LTE (Heynen and Bear 2001). Systemic CPP may well abolish thalamocortical LTE by acting in the thalamus, cortex, or elsewhere. Thus we tested the effects of applying an NMDA receptor antagonist directly into either the thalamus or the neocortex, through microdialysis, on the ability of TBS to induce thalamocortical LTE. The NMDA receptor antagonist $\alpha$-AP5 (0.25–1 mM) was dissolved in the ACSF and applied through a cannula in the thalamus or through a cannula located in the neocortex placed adjacent to the cortical recording electrode. The antagonist was applied for the duration of the recording (i.e., during the baseline and after TBS). Figure 10 shows population data from several experiments that tested the ability of TBS applied to the thalamic radiation to induce LTE. When AP5 was applied in the thalamus ($n = 5$), TBS was completely ineffective in producing any significant enhancement. However, when AP5 was applied in the neocortex, we found that, in every experiment, there was a significant enhancement of the evoked responses. During cortical AP5, there was a $23 \pm 6\%$ enhancement of the 5-ms peak above the baseline measured 30 min after the last LTE ($t$-test; $P < 0.01$), whereas during thalamic AP5, there was a $2.8 \pm 4\%$ decrease in thalamic-radiation-to-cortex response amplitude of the 5-ms peak measured at the same time (Fig. 10). These results show that cortical NMDA receptors are not involved in producing thalamocortical LTE.

The results suggest that NMDA receptors within the thalamus, but not within the neocortex, mediate the changes that may be taking place to suppress the spontaneous firing of thalamocortical cells. It is also plausible that blocking NMDA receptors in the thalamus directly reduces the firing rate of thalamocortical cells, and therefore application of TBS has no added effect because thalamocortical synapses are already relieved from synaptic depression during thalamic AP5. To address this possibility, we recorded from thalamocortical cells and infused AP5 into the thalamus to measure the effect of AP5 on their firing rate. Figure 11A shows population data about the effect of AP5 on the spontaneous firing of thalamocortical cells (middle and bottom) and on the thalamic radiation-to-cortex evoked field potential response (top). Application of AP5 produced a significant reduction in the spontaneous firing rate of thalamocortical cells ($n = 6$; paired $t$-test; $P < 0.001$) and simultaneously enhanced the amplitude of the thalamic radiation-to-cortex response at the 3- ($P < 0.001$) and 5-ms peak ($P < 0.001$). This result indicates that the reason AP5 blocks LTE is because it suppresses the activity of thalamocortical cells and consequently thalamocortical responses are relieved from synaptic depression and enhance. We also tested if application of TBS during AP5 in the thalamus would produce additional suppression of thalamocortical activity (Fig. 11B). We found that during thalamic AP5, TBS had no additional enhancing effect on the thalamic radiation-to-cortex response and did not produce any significant suppression of spontaneous thalamocortical firing ($n = 6$; paired $t$-test; not significant). These results show that thalamocortical LTE is produced by relieving synaptic depression at thalamocortical synapses as a consequence of suppressing thalamocortical activity and that AP5 blocks thalamocortical LTE because it directly suppresses spontaneous thalamocortical activity, hence occluding the effect of TBS.

**Discussion**

This study found that TBS applied to the thalamic radiation leads to an input specific and long-lasting enhancement of the thalamocortical response evoked in the cortex, which we call LTE. Interestingly, we found that thalamocortical LTE was
completely abolished by inactivating the thalamus with muscimol. This result led to the hypothesis that changes in thalamic excitability may be responsible for thalamocortical LTE. To test this, we measured the spontaneous firing rate of thalamocortical cells before and after application of TBS to the thalamic radiation. In accordance with the hypothesis, we found that the firing rate of thalamocortical cells significantly decreases after TBS is applied to the thalamic radiation and that abolishing thalamocortical firing enhances thalamocortical responses in neocortex. In conclusion, a reduction in the firing rate of thalamocortical cells after TBS relieves the tonic depression of thalamocortical synaptic connections in the neocortex and leads to an enhancement of thalamocortical synaptic efficacy (Fig. 12).

It is important to recognize that this long-lasting enhancement in thalamocortical responses after TBS we call LTE, does not qualify as LTP in its classical definition (Bear and Malenka 1994; Bliss and Collingridge 1993; Nicoll and Malenka 1995). Classical LTP consists of a change in the efficacy of synaptic communication generally induced by a rise in postsynaptic calcium and independent of the spontaneous firing rate of the presynaptic cell. In fact, the spontaneous firing rate in hippocampus or neocortex slices, where LTP is most intensely studied, is generally nil. The situation in vivo is obviously different because neural circuits are generally constantly bombarded by spontaneous activity. This study revealed that
changes in the firing rate of thalamocortical cells affects the tonic efficacy of its synapses in neocortex by taking advantage of their short-term plasticity (i.e., activity-dependent depression). This could well serve as a way to regulate the efficacy of the thalamocortical connection in vivo during behavior.

NMDA receptors are critically involved in the generation of LTP in many structures, including the neocortex (Malenka and Bear 2004). We found that thalamocortical LTE is abolished when NMDA receptors are blocked in the thalamus but not when they are blocked in the neocortex. These results argue that thalamic mechanisms are indeed responsible for the generation of thalamocortical LTE. One possibility is that the reduction in the spontaneous firing of TC cells is mediated through an NMDA receptor–dependent mechanism in the thalamus. Alternatively, the application of the NMDA receptor antagonist into the thalamus may have caused the suppression of the spontaneous firing of thalamocortical cells, and thus TBS would have no additional suppressing effect. Our results indicate that this is indeed the case. Application of an NMDA receptor antagonist into the thalamus directly enhances thalamocortical responses because it suppresses the spontaneous firing of thalamocortical cells. This suppression relieves synaptic depression at thalamocortical synapses and LTE ensues. Hence, subsequent application of TBS when NMDA receptors are blocked in the thalamus causes no additional effects because the effects TBS would normally produce have already been produced by the NMDA antagonist.

The results show that TBS leads to a reduction in thalamocortical firing rate at frequencies that depress the efficacy of the thalamocortical pathway, resulting in a concomitant enhancement of the efficacy of the thalamocortical pathway (see Fig. 12). Interestingly, the reduction in the firing rate of thalamocortical cells contrasts with the sharp increase in firing rate when afferents from the brain stem reticular formation are stimulated with similar high-frequency trains (Castro-Alamancos 2002; Castro-Alamancos and Oldford 2002).

What may cause such a reduction in firing rate as a consequence of thalamic radiation stimulation?

Stimulation of the thalamic radiation results in two main consequences in the thalamus (for a discussion, see Castro-Alamancos 2004): the antidromic activation of thalamocortical cells and the orthodromic activation of corticothalamic synapses. Both of these effects will lead to the synaptic activation of inhibitory cells in the reticular nucleus (nRt) by collaterals of thalamocortical fibers and by collaterals of corticothalamic fibers, respectively. In addition to this recurrent inhibition, corticothalamic synapses directly excite thalamocortical cells. Hence, the main effect in the thalamus of the antidromic discharge of thalamocortical cells (i.e., recurrent synaptic stimulation of nRt cells) is also produced by corticothalamic synapses. Consequently, it seems reasonable to suggest that, at a functional level, the change in thalamocortical excitability observed after TBS is triggered by corticothalamic inputs acting on nRt cells, on thalamocortical cells, or both. However, we emphasize that a role of thalamocortical fiber collaterals on nRt cells in triggering the changes in thalamocortical excitability cannot be discarded.

It is also noteworthy that these results rule out the participation of the intracortical collaterals of corticothalamic cells in producing thalamocortical LTE because, when the thalamus is inactivated, which should have no effect on the intracortical collaterals, LTE is abolished. This indicates that the intracortical collaterals of corticothalamic cells have no role in triggering or expressing LTE. If these intracortical collaterals did have a role, LTE in cortex should be present after thalamic inactivation.

It is interesting that despite the fact that the thalamocortical cells recorded were being strongly excited by corticothalamic fibers in response to high-frequency thalamic radiation stimulation, the net lasting effect on these cells after TBS was a reduction (inhibition) of their spontaneous firing rate. This is interesting because, although the functional role of corticothalamic activity is poorly understood, one of the main hypothesized roles for this pathway is that it could serve to regulate the excitability of thalamic cells (McCormick and von Krosigk 1992; Sherman and Guillery 1996). There is also evidence that the spatial and temporal properties of thalamocortical receptive fields are affected by corticothalamic activity (e.g., Diamond et al. 1992; Ergenzinger et al. 1998; Krupa et al. 1999; Murphy and Sillito 1987; Murphy et al. 1999; Sillito et al. 1994; Singer 1977; Temereanca and Simons 2004; Yuan et al. 1985).

One possibility is that corticothalamic activity produced by TBS affects the tonic inhibition that the nRt exerts over thalamocortical cells. Indeed, corticothalamic fibers innervate both VB and the nRt. In fact, corticothalamic EPSPs produced on nRt cells are stronger than those produced on thalamocortical cells (Gentet and Ulrich 2004; Golshani et al. 2001). At a functional level, this strong corticothalamic input to nRt neurons may underlie the strong capacity for corticothalamic activity to drive feedforward inhibition in the thalamus (Zhang and Jones 2004). Thus it is possible that, after TBS, thalamocortical cells are subjected to a much stronger tonic inhibition from the nRt that could result from enhanced efficacy of inhibitory synaptic potentials and/or from an increased tonic firing of nRt cells. Further work will have to tease apart these other possibilities.

Because corticothalamic activity seems to be the main trigger of the change in spontaneous thalamocortical firing, corticothalamic activity may well serve as a top-down regulator of the efficacy of the thalamocortical connection by setting the level of thalamocortical firing. This mechanism may be functionally useful to scale thalamocortical efficacy according to experience or behavioral state.

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