Sensory information originating in individual whisker follicles ascends through focused projections to the brainstem, then to the ventral posteromedial nucleus (VPM) of the thalamus, and finally into barrels of the primary somatosensory cortex (S1). By contrast, the posterialmedial complex (PoM) of the thalamus receives more diffuse sensory projections from the brainstem and projects to the interbarrel septa of S1. Both VPM and PoM receive abundant corticothalamic projections from S1. Using a thalamocortical slice preparation, we characterized differences in intrinsic neuronal properties and in responses to corticothalamic feedback in neurons of VPM and PoM. Due to the plane of the slice, the majority of our observed responses came from activation of layer VI because most or all of the layer V axons terminating in PoM are cut.

We found that VPM neurons exhibit higher firing rates than PoM neurons when stimulated with injected current. Stimulation of corticothalamic fibers evoked monosynaptic excitation, disynaptic inhibition, or a combination of the two in both nuclei. A few differences in the feedback responses emerged: purely excitatory postsynaptic potentials (EPSPs) in VPM were smaller and facilitated more than those in PoM, and only the EPSPs in VPM had a strong NMDA component. For both nuclei, some of the feedback responses were purely disynaptic inhibitory postsynaptic potentials (IPSPs) from the thalamic reticular nucleus (TRN). This was due to EPSP failures within VPM and PoM combined with greater reliability of S1-originating synapses onto TRN. These findings suggest that despite the exclusively excitatory nature of corticothalamic fibers, activation of cortex can trigger excitation or inhibition in thalamic relay neurons.

**Keywords:** electrophysiology, excitation, higher order nucleus, inhibition, relay nucleus

**Introduction**

Sensory systems of the brain have hierarchical features, but there are also parallel pathways of sensory information. Within the parallel hierarchies of the forebrain lie enormous feedback connections. That is, each area of primary sensory cortex sends efferent fibers back to the relay area of thalamus from which it receives feedforward input. These corticothalamic feedback connections often greatly outnumber the thalamocortical feedforward connections, in some cases by 40 to 1 (Sherman and Koch 1990). Despite the dramatic scale of these anatomical findings, the functional significance of corticothalamic connections is poorly understood. Speculations about the function of corticothalamic connections include sharpening and/or amplifying receptive field responses, focusing attention, and modulating receptive field properties based on contextual information (Steriade et al. 1993; Sherman and Guillery 2002; Sillito and Jones 2002).

Corticothalamic synapses are glutamatergic and can activate D-aspartate (NMDA), and metabotropic glutamate postsynaptic receptors (Bromberg et al. 1981; De Biasi and Rustioni 1990; Deschénes and Hu 1990; Scharfman et al. 1990; McCormick and von Krosigk 1992; Long et al. 2004; Landisman and Connors 2005). Thus, the activation of corticothalamic fibers can cause a general increase in excitability of thalamic relay neurons. Though both feedforward and feedback fibers make glutamatergic synapses onto thalamic neurons, only the feedback fibers contact the metabotropic glutamate receptors (mGluRs; Martin et al. 1992; Godwin et al. 1996; Liu et al. 1998; Vidnyanszky et al. 1996). Activation of mGluRs can shift the state of thalamic neurons from burst firing to single spiking mode (McCormick and von Krosigk 1992). These 2 neuronal states are often associated with sleep and arousal, respectively (Steriade et al. 1993), although bursting may occur during wakefulness as well (Ramcharan et al. 2000; Swadlow and Gusev 2001).

In order to understand the functions of corticothalamic connections more completely, it is important to consider aspects of the anatomy as well as the physiological behavior of the local circuitry. In the rat whisker barrel system, the cortical barrel fields project onto several nuclei in somatosensory thalamus. Neurons in 2 of these nuclei exhibit very different receptive field sizes, and the projections they receive from primary somatosensory cortex (S1) differ in their source, number, and synapse characteristics (Hoogland et al. 1987; Chiaia et al. 1991; Diamond, Armstrong-James, Budway, and Ebner 1992; Diamond, Armstrong-James, Ebner 1992; Lu and Lin 1993, though see Alloway et al. 2003). The ventroposteromedial nucleus (VPM) and the medial portion of the posterior complex (PoM) are somatotopically organized in a mirror image fashion about their common border (Nothias et al. 1988). Under surgical levels of anesthesia, vibrisa neurons in the PoM have an average receptive field size of 5 whiskers (Chiaia et al. 1991; Diamond, Armstrong-James, Budway, and Ebner 1992). Neurons in VPM are clustered into discrete barreloids. Each barreloid contains neurons most sensitive to one particular whisker and receives input from (and sends output to) one particular S1 barrel (Senft and Woolsey 1991; Diamond, Armstrong-James, Ebner et al. 1992; Agmon et al. 1995; Land et al. 1995). By contrast, the anatomy and connectivity of PoM is more diffuse (Bourassa et al. 1995). Responses of PoM cells to whisker movement can be largely suppressed by inactivation of cortical inputs, whereas VPM neurons continue to respond briskly under similar conditions (Diamond, Armstrong-James, Budway, Ebner et al. 1992). The effects of silencing cortical input on PoM responses are large, yet the synaptic mechanisms of feedback onto PoM or VPM neurons are not known.

Dramatic differences in the anatomy of the corticofugal synapses onto PoM and VPM neurons suggest that the 2 pathways are functionally very different. The upper portion of layer VI of S1 sends projections to VPM, and the lower portion of layer VI...
sends the majority of its projections to PoM, though a small number of these neurons also send collaterals to VPM (Hoogland et al. 1987; Bourassa et al. 1995; Killackey and Sherman 2003). Furthermore, corticothalamic axons originating in layer V project exclusively to PoM. All of the axons originating from layer VI neurons are thin, and they terminate in small synaptic boutons on thalamic relay neurons. By contrast, the layer V terminals arise from large caliber axons and form large synaptic boutons. As a general rule, large synaptic terminals have more active zones, release transmitter more reliably, and are thus more efficacious (Pierce and Lewin 1994). It has been proposed that the layer VI inputs play a more modulatory role, whereas the layer V inputs serve as the primary “driver” of PoM cells (Sherman and Koch 1990; Feig and Harting 1998; Sherman and Guillery 1998; Reichova and Sherman 2004).

In this study, we first compare the intrinsic membrane properties of VPM and PoM neurons; differences in physiological properties could help to explain the response properties of these 2 neuronal groups in vivo. We then compare the consequences of activating corticothalamic feedback fibers on VPM and PoM neurons.

Materials and Methods

Slice Preparation
Sprague-Dawley rats aged P14–P21 were anesthetized using tiopental sodium (50 mg/kg). The rats were then decapitated, and the brains removed and quickly placed in ice-cold artificial cerebrospinal fluid (ACSF) bubbled with 95% CO2/5% O2. The composition of the ACSF was (in mM) 126 NaCl, 3 KCl, 2.0 MgSO4, 1.25 Na2HPO4, 26 NaHCO3, 2 CaCl2, and 10 dextrose. Thalamocortical slices were obtained as described previously (Agmon and Connors 1991) using a Vibroslice (Campden Instruments, Lafayette, IN). Three–400-μm sections (from each hemisphere) containing the somatosensory thalamus and cortex were prepared and placed either onto pieces of vinyl mesh in an interface holding chamber or into a submersion chamber. The chambers contained oxygenated ACSF, and slices were incubated for 1 h at 33 °C and subsequently at room temperature. All experiments were carried out in accordance with National Institutes of Health (NIH) guidelines.

Electrophysiological Recordings
After incubation in the holding chamber, an individual slice was transferred to either an interface recording chamber or a submersion chamber held at 32–33 °C. Whole-cell patch recordings were made using glass microelectrodes (5–7 MΩ) filled with internal solution (in mM: 138 K gluconate, 2 KCl, 2 NaCl, 10 HEPES, 1.25 Na2HPO4, 26 NaHCO3, 2 CaCl2, and 10 dextrose). Thalamocortical slices were obtained as described previously (Agmon and Connors 1991) using a Vibroslice (Campden Instruments, Lafayette, IN). Three–400-μm sections (from each hemisphere) containing the somatosensory thalamus and cortex were prepared and placed either onto pieces of vinyl mesh in an interface holding chamber or into a submersion chamber. The chambers contained oxygenated ACSF, and slices were incubated for 1 h at 33 °C and subsequently at room temperature. All experiments were carried out in accordance with National Institutes of Health (NIH) guidelines.

Anatomical tracer injections were made using dextran tetramethylrhodamine-bromide (10,000 MW), lysine fixable (Molecular Probes, now Invitrogen, Carlsbad, CA). Dry tracer crystals were placed on the end of a broken glass microelectrode, and the tip was lowered into the target region of the thalamocortical slice preparation, injections of the anterograde tracer rhodamine-dextran were made into layer VI and incubated for several hours (see Materials and Methods). Rhodamine labeling (Fig. 1A,B) confirmed that individual cells in layer VI sent axons to the thalamus via the internal capsule. In the example shown, fibers can be seen both in the TRN as well as the ventrobasal complex (VB) nuclei (Fig. 1A,B). Final injections were resectioned on a freezing microtome into 80-μm sections, mounted on gelatinized slides, and fixed using a cover slip with a solution made from 100 ml of 1 part 0.1 M phosphate-buffered saline, 3 parts glycerin, and 1 ml of saturated aqueous purpurogallin (a free radical scavenger; recipe courtesy of Dr. Stewart Hendry, Johns Hopkins University).

Results

Table 1
Intrinsic membrane properties of VPM and PoM neurons

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VPM</th>
<th>PoM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential (mV)</td>
<td>−58 ± 4 (n = 40)</td>
<td>−58 ± 5 (n = 44)</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>119 ± 35 (n = 40)</td>
<td>112 ± 45 (n = 44)</td>
</tr>
<tr>
<td>Action potential half width (ms)</td>
<td>1.06 ± 0.29 (n = 12)</td>
<td>1.19 ± 0.41 (n = 15)</td>
</tr>
<tr>
<td>Spikes per burst</td>
<td>5.3 ± 1.4 (n = 41)</td>
<td>4.4 ± 1.8 (n = 23)</td>
</tr>
<tr>
<td>Firing frequency during a burst (Hz)</td>
<td>348.5 ± 81.3 (n = 41)</td>
<td>231.7 ± 68.4 (n = 23)</td>
</tr>
<tr>
<td>Spike frequency adaptation ratio</td>
<td>11.9 ± 5.7 (n = 12)</td>
<td>18.8 ± 8.4 (n = 7)</td>
</tr>
<tr>
<td>From burst firing</td>
<td>2.0 ± 0.9 (n = 13)</td>
<td>2.8 ± 2.7 (n = 10)</td>
</tr>
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Note: All data are listed as mean ± SD. n = number of cells. All statistical values determined using unpaired, 2-tailed t-tests.

*P < 0.0001.

indicated, drugs were bath applied via the ACSF that perfused the slice at 2 ml/min. Drug doses (in μM) were 50 bicuculline methiodide (BMI), 20 picrotoxin, 50 dl-2-amino-5-phosphonovaleric acid (APV), 20 6,7-dinitroquinoxaline-2,3-dione (DNQX), and 1 CGP-62349 (a γ-aminobutyric acid B [GABAB] blocker from Ciba-Geigy, now Novartis International AG, Basle, Switzerland).

Anatomy and Histology
Anatomical tracer injections were made using dextran tetramethylrhodamine-bromide (10,000 MW), lysine fixable (Molecular Probes, now Invitrogen, Carlsbad, CA). Dry tracer crystals were placed on the end of a broken glass microelectrode, and the tip was lowered into the target region of the slice (deep layer VI of S1 cortex). Slices were then left in the holding chamber at room temperature for 8–12 h. After tracer incubation, slices were placed in 4% paraformaldehyde with 20% sucrose overnight. Finally, slices were resectioned on a freezing microtome into 80-μm sections, mounted on gelatinized slides, and fixed using a cover slip with a solution made from 100 ml of 1 part 0.1 M phosphate-buffered saline, 3 parts glycerin, and 1 ml of saturated aqueous purpurogallin (a free radical scavenger; recipe courtesy of Dr. Stewart Hendry, Johns Hopkins University).

To confirm that the corticothalamic fibers were intact in the thalamocortical slice preparation, injections of the anterograde tracer rhodamine-dextran were made into layer VI and incubated for several hours (see Materials and Methods). Rhodamine labeling (Fig. 1A,B) confirmed that individual cells in layer VI sent axons to the thalamus via the internal capsule. In the example shown, fibers can be seen both in the TRN as well as the ventrobasal complex (VB) nuclei (Fig. 1B). However, transport to PoM was not seen, probably because we were unable to maintain the slice long enough to get full transport. This interpretation is supported by our subsequent observation of monosynaptic excitation of the thalamic nuclei from stimulation of corticothalamic fibers (see below).

To distinguish VPM from PoM cells, we first made sure we could accurately localize each nucleus in living slices cut in the thalamocortical plane (Agmon and Connors 1991). Under bright field illumination in the recording chamber, the PoM appears as a darker (in the interface chamber, lit from above) or lighter (in the submersion chamber, transilluminated; see Fig. 1C), triangle-shaped region medial to the arc-shaped band of VPM that, in turn, is medial to the ventral postero lateral nucleus (VPL) and the TRN. The clear distinction seen in the recording chamber assured proper targeting of each nucleus. Cresyl violet staining.
of resectioned slices confirmed the borders of VPM and PoM as previously defined from unstained visualization of live slices (data not shown).

**Intrinsic Membrane Properties of VPM and PoM Neurons**

We characterized the intrinsic membrane properties of individual cells by applying depolarizing and hyperpolarizing current steps. Both VPM cells and PoM cells exhibited intrinsic firing properties typical of most thalamic neurons (Llinas and Jahnsen 1982). They fired intrinsic bursts when activated from relatively hyperpolarized resting potentials ($V_{rest}$ more negative than $-58$ to $-60$ mV) and tonic spikes when activated from more depolarized potentials (more positive than $-58$ mV) (Fig. 2A,C). Both cell types fired only a single burst following a depolarizing step from hyperpolarized holding potentials (see above). If a long (several hundred milliseconds), strong depolarizing step was delivered from a holding potential equal to or more hyperpolarized than $-60$ mV, both cell types fired a fast burst of action potentials followed by tonic spikes (Fig. 2B,D, right).

When stimulated with the same intensity current pulses from $V_{rest}$, VPM cells fired at much higher frequencies than PoM cells during tonic firing. Figure 2B,D show examples of responses to current stimulation of a VPM neuron and a PoM neuron recorded from the same slice. Compared with PoM cells, cells from VPM had lower spike thresholds and much higher firing frequencies at every stimulus intensity tested (Fig. 2E). The differences in tonic firing frequency were not due to differences in $V_{rest}$ or input resistance, which were nearly identical in the 2 cell types (Table 1). The average widths of individual action potentials from the 2 nuclei were also indistinguishable, with means of 1.06 ms for VPM cells and 1.19 ms for PoM cells.

Both VPM and PoM cells showed spike frequency adaptation during repetitive spiking. We calculated the adaptation rates as $F_i/F_f$, where $F_i$ is the initial firing frequency and $F_f$ is the final firing frequency for the minimum depolarizing step that elicited a regular train of action potentials (Beierlein et al. 2003). $F_i$ was the inverse of the interval between the first 2 spikes in the train, and $F_f$ was the inverse of the interval between the last 2 spikes in the train during a 600-ms depolarizing step. Because both VPM and PoM cells adapt differently depending on whether they are in bursting or tonic firing modes, we calculated the firing frequencies and adaptation values from burst initiated as well as tonic firing. The mean adaptation ratios for VPM cells were 11.9 from holding potentials that evoked bursts and 2.0 during purely tonic firing (Table 1). Adaptation rates of PoM neurons were 16.8 from bursting trials and 2.8 from tonic firing trials. The small differences in adaptation rates between the 2 cell types were not statistically significant.

**Corticothalamic Response Heterogeneity of VPM and PoM Cells**

Stimulation of the internal capsule evoked heterogeneous synaptic responses in both VPM and PoM cells. These included pure excitation (Fig. 3A), pure inhibition (Fig. 3B), and combinations of excitation and inhibition (Fig. 3C). As these examples also show, the strength and balance of EPSPs and IPSPs evoked...
from a single stimulus location could also vary widely as the stimulus intensity and number were varied. A comparison of the excitatory responses in Figure 3A to the inhibitory responses in Figure 3B shows that the amplitudes of both increased gradually with changes in stimulus intensity and both response types exhibited facilitation to 40 Hz train stimuli at low to moderate current intensities (up to 4 times threshold, or 4T for Fig. 3B and for all stimulus values for Fig. 3C). However, at higher stimulus intensities, the IPSPs in Figure 3B also exhibit evidence of depression.

Not surprisingly, responses that combined excitation and inhibition were more complex than the purely excitatory or inhibitory ones. As seen in Figure 3C, IPSPs could dominate and even completely obscure EPSPs. The source of these IPSPs is the thalamic reticular nucleus (TRN), whose GABAergic neurons directly contact both VPM and PoM neurons (see below). At low stimulus intensities, evidence of small monosynaptic EPSPs from corticothalamic sources can be seen in the responses of the PoM cell in Figure 3C, but these EPSPs were overwhelmed by concurrent IPSPs. In addition, the reliability of EPSPs in response to the first stimulus was very low, and thus they were not even evident following the first stimulus at moderate stimulus strengths. Only with very high stimulus intensities (>32T) or repetitive activation (>4T) could the EPSPs generate detectable responses.

However, in all 3 categories large increases in stimulus intensity make it harder to distinguish the synaptic origins of the responses because mixed fiber recruitment is likely. For this reason, characterizations of feedback responses throughout the paper are based on stimulus intensities between T and 2T.

Responses consisting of IPSPs alone or weak EPSPs together with strong, low-threshold IPSPs were common in both VPM and PoM cells in response to corticothalamic stimulation (Fig. 3D). In 60% of VPM cells (15 of 25) and 69% of PoM cells (9 of 13), the response profiles were strongly dominated by IPSPs: that is,
of excitation and inhibition (‘‘both’’). The spikes from VPM were not included in the graph (see Fig. 4).

Latencies and Failures of EPSPs and IPSPs

Previous studies have shown that corticothalamic axons originating from layer VI are distinguished by ‘‘supernormal’’ conduction properties (Swadlow and Waxman 1975; Kelly et al. 2001; Beierlein and Connors 2002): for a time interval following the propagation of a single action potential, and after its refractory period, the conduction velocity of a second spike is slightly increased compared with the first. Such a supernormal shift in axonal conduction can also be expressed as a decrease in the onset latency of an EPSP evoked during repetitive stimulation. By contrast, feedforward thalamocortical responses show no change in conduction velocity or slightly subnormal responses (Beierlein and Connors 2002). To test whether evoked EPSPs recorded in VPM and PoM exhibited supernormal latency shifts, we compared EPSP latencies of the first 2 responses during the 40-Hz stimulus trains. The supernormal shift for VPM neurons was, on average, 324 μs (7% of the total latency) and 350 μs (9%) for PoM cells, which were similar in magnitude to the supernormal shifts described by Beierlein and Connors (2002). Considering only responses showing supernormal shifts, the absolute latencies of EPSPs in VPM ranged from 1.7 to 8 ms (mean = 4.0 ± standard deviation [SD] 1.6 ms) and those of EPSPs in PoM ranged from 3.7 to 16 ms (mean = 5.6 ± 3.1 ms; Fig. 4A). Latencies of PoM responses were significantly longer than those of VPM (P < 0.02, unpaired, 2-tailed t-test).

The determination of sources of IPSPs was also evaluated, but supernormality could not be used because the responses were disynaptic. During stimulation of the internal capsule, TRN cells potentially can be activated either by input from collaterals of axons originating in layer VI of S1 or by collaterals of thalamocortical axons originating in VPM. But they cannot be activated antidromically because their axons do not leave the thalamus.

We compared the latencies of all IPSPs recorded in VPM and PoM neurons (Fig. 4B). IPSPs in PoM had a distinctly bimodal distribution. A cluster of very short latencies (2–5 ms) is consistent with antidromic activation of rapidly conducting thalamocortical (VPM) axons (Gil and Amitai 1996; Beierlein and Connors 2002); IPSPs with much longer, more variable latencies are consistent with orthodromic activation of the slower corticothalamic axons (Golshani et al. 2001). The distribution of VPM latencies, however, was not obviously bimodal (Fig. 4C). Because VPM is physically closer to the cortex and TRN (see Fig. 1C), its disynaptic latencies should be shorter than those seen in PoM (see Kao and Coulter 1996; Gentet and Ulrich, 2003). This assumption is supported by the greater average EPSP latencies for PoM versus VPM neurons shown in Figure 4A. Thus, we used the largest value from the faster latency cluster in PoM (4.8 ms) to estimate the cutoff for corticothalamic-generated IPSPs in both cell types. Our analysis of pure IPSP responses and EPSP–IPSP combination responses in VPM and PoM are based on this latency threshold. The mean of the response of the neuron stayed negative to V_{rest} throughout trials of 40-Hz stimulus trains delivered at low stimulus intensities (< 2T of the first detectable response—either EPSP or IPSP). Only 31% of PoM responses (4 of 13) and 40% of VPM responses (10 of 25) showed EPSPs alone. Because EPSPs tended to be harder to detect than IPSPs, cells that appeared to have purely inhibitory responses were further tested by increasing stimulus intensity and by using train stimulation.
Corticothalamic Excitation of VPM and PoM Cells

To assess the strength of synaptic responses of thalamic neurons to the activation of single corticothalamic axons, we used minimal stimulation. Minimal EPSPs were measured by stimulating the internal capsule with near-threshold intensities, such that at least 50% of trials generated no response (Gil et al. 1999; Fig. 6A). The minimal stimulus intensities ranged from 5 to 60 μA. Even under these weak stimulus conditions, inhibitory events often obscured excitatory events, so inhibition was blocked pharmacologically (picrotoxin was used to block GABA<sub>A</sub> receptors and CGP-62349 to block GABA<sub>B</sub> receptors). The minimal EPSPs generated by internal capsule stimulation ranged in amplitude from 0.12 to 0.33 mV in VPM cells (mean = 0.24 ± SD 0.06 mV, n = 11) and 0.15 to 0.64 in PoM cells (mean = 0.39 ± SD 0.2 mV, n = 11). The PoM EPSPs were significantly larger than the EPSPs from VPM (P < 0.03, unpaired, 2-tailed t-test; Fig. 6B).

When stimulated with brief trains at 40–60 Hz, corticothalamic EPSPs onto both cell types showed facilitation that was independent of stimulus intensity. On average, facilitation was stronger in VPM cells than in PoM cells (Fig. 6C,D). Also, a greater number of VPM cells than PoM cells showed facilitation (92% in VPM versus 38% in PoM).

We tested whether slow NMDA receptor–mediated current contributed to synaptic responses and facilitation by adding the antagonist APV to the bath. APV strongly reduced facilitation of responses from all VPM cells tested (5 of 5) but had no effect on the facilitating short-term dynamics in PoM cells (3 of 3 cells) (Fig. 6E,F). There was no significant reduction of the first EPSP amplitude by APV for either cell type (control/APV = 0.82 ± 0.24i for VPM and 1.02 ± 0.12 for PoM). NMDA receptor blockade had a stronger effect on subsequent EPSPs in a train, particularly in VPM; the second to fourth EPSP amplitudes were reduced by 36–45% by APV in VPM cells (P = 0.026–0.007, 1-tailed, paired t-tests; significance increased with each successive pulse), compared with reductions in PoM of only 1.7–5.2% (not significantly different).

It should be noted that in both VPM and PoM, there were 4 cells (2 in VPM, 2 in PoM) that did not show supernormal latency shifts to train stimulation (Fig. 7A). Because it is possible that these responses are due to antidromic activation of feedback fibers, we have eliminated them from all population analyses. The 2 VPM cells had minimal EPSP amplitudes of 0.59 and 0.88 mV—well beyond the range of the cells with supernormal responses. However, the absolute latencies of these 2 VPM responses were within the distribution of those of the layer VI feedback responses (3.3 and 3.5 ms). One would expect significantly shorter latencies for antidromic activation. Thus, it is conceivable that these 2 responses are from unusually large layer VI fibers.

Likewise, PoM had 2 cells with subnormal responses (vs. supernormal). These responses were notable for their significantly corticothalamic-generated IPSP latencies were 8.1 ms for VPM cells and 11.9 ms for PoM cells.

As expected, corticothalamic EPSPs arrived faster than the disynaptic IPSPs in all but one case (Fig. 5). But inhibition can also precede and dominate excitation if the reliability of transmitter release onto TRN cells and from its inhibitory synapses exceeds that of the excitatory synapses onto relay cells. In Figure 5A,B, the cells indicated by filled dots are cases where the EPSPs consistently failed in response to single stimuli or to the first stimulus in a train, effectively allowing IPSPs to arrive first. Cases of this type were observed in both VPM (4 of 12 cells) and PoM (4 of 14 cells), but only the 4 VPM responses to the right of the dotted line are consistent with feedback excitation of both pathways (because all responses to the left are probably IPSPs of thalamocortical origin, as shown in Fig. 4). By comparison, EPSPs evoked in TRN cells by single stimuli to the internal capsule showed no failures (0 of 11 cells; data not shown). Thus, the disynaptic pathway by which the corticothalamic circuit inhibits relay cells, via TRN, appears to be more reliable than the monosynaptic pathway by which it excites relay cells. More detailed discussion of cortically generated EPSP responses in TRN cells is provided below.
As shown previously for VPM neurons, stimulation of the corticothalamic pathway evokes both slow and fast inhibitory responses (Kao and Coulter 1997). Similar responses can also be seen in PoM neurons (Fig. 8). The timing of the 2 inhibitory components as well as their different reversal potentials suggests that these are GABA<sub>A</sub> and GABA<sub>B</sub> receptor-mediated IPSPs (Crunelli and Leresche 1991; Ulrich and Huguenard, 1996). The first IPSP (Fig. 8A) had a faster rise time and reversed near ~71 mV, consistent with the predicted chloride reversal potential for these cells. The second IPSP (Fig. 8A) had a slower rise time and reversed near ~89 mV, consistent with the more negative reversal potential of potassium. Blockade with a GABA<sub>B</sub> receptor-specific antagonist completely eliminated the second IPSP (Fig. 8B) and left the faster GABA<sub>A</sub> component intact. We used IPSP latency and the rate of rise as criteria to distinguish GABA<sub>A</sub> from GABA<sub>B</sub> responses in VPM and PoM cells and/or blockade of either component with the antagonists BMI (for GABA<sub>A</sub>) or CGP-62349 (for GABA<sub>B</sub>). All PoM cells (20/20) and all but one VPM cell (16/17) showing inhibition had a GABA<sub>A</sub>-like component. The average GABA<sub>A</sub>-IPSP amplitudes from Vrest were ~3.1 mV for PoM cells and ~2.4 mV for VPM cells. VPM cells were much less likely than PoM cells to have a GABA<sub>B</sub> receptor-like component as part of their inhibitory responses: 7 of 17 VPM cells and 20 of 22 PoM cells had IPSPs with a GABA<sub>B</sub> component. The average amplitudes of the GABA<sub>B</sub>-IPSP components for PoM and VPM cells were similar (~1.3 mV vs. ~1.4 mV, respectively).

**Corticothalamic Activation of the TRN**

Our measurements of evoked IPSPs in VPM and PoM neurons suggested that corticothalamic axons induce strong, reliable excitation of TRN neurons, as described above. We recorded directly from TRN cells to test this possibility. Figure 9A illustrates an example of the response of a TRN neuron to minimal corticothalamic stimulation. The test for supernormality was used to determine the origin of activation (see above), and all data shown in Figure 9 were consistent with activation by corticothalamic axons. The corticothalamic IPSPs in TRN cells were relatively strong, and half of the cells studied showed clear facilitation in response to 40 Hz train stimulation; IPSPs in the other half were stable (n = 10 cells; Fig. 9A, B).

The minimal EPSPs from cortical feedback onto TRN cells were of much larger amplitude than those onto VPM and PoM cells (Fig. 9C). The average minimal EPSP for TRN cells was 1.41 mV compared with 0.24 and 0.39 mV for VPM and PoM, respectively. There was almost no overlap among the EPSP amplitudes of TRN responses and those of the other 2 thalamic nuclei. These data suggest that corticothalamic feedback excites TRN neurons much more strongly than it does VPM or PoM neurons.

**Discussion**

The VPM and PoM nuclei provide distinctly different spatial and temporal features of somatosensory information to the neocortex (Sosnik et al. 2001; Trageser and Keller 2004; Golomb et al. 2006). Our measurements showed that the neurons in the 2 nuclei differ in their intrinsic membrane properties and excitability and in the nature of the synaptic feedback they receive from corticothalamic connections.

We initially characterized the axonal connectivity of our slice preparation, which had previously been used primarily for studies of thalamocortical pathways but only rarely for examining corticothalamic pathways. Jones et al. (1979) found that focal injection of a cocktail of retrograde and anterograde tracer...
into sensorimotor cortex in vivo yielded superimposed labeled cells in cortex and thalamus (i.e., feedforward and feedback neurons). Our labeling study in slices showed that at least some of the layer VI projections from primary somatosensory cortex (S1) to thalamus are intact in the slice. Anatomical confirmation of this pathway in the slice makes it very likely that the responses we and others (Kao and Coulter 1997; Reichova and Sherman 2004) recorded indeed had a corticothalamic origin.

Distinguishing the VPM and PoM nuclei in both the submersion and interface chambers was clear and unambiguous due to differences in their optical properties, as seen in Figure 1C, and by comparison with nuclear positions in standard atlases (Paxinos and Watson 1998).

**Figure 6.** Characterization of EPSPs. (A) Response of a VPM cell to single minimal shocks to the internal capsule (10 μA; 20 sweeps). (B) Plot of the distribution of minimal EPSPs for the population of VPM and PoM neurons tested. Bin size = 0.1 mV, range = 0.1-0.7 mV. N = 11 VPM cells and 11 PoM cells. Comparison of VPM (C) and PoM (D) cell EPSP response amplitudes to train stimulation (40 Hz). The x axes plot the stimulus number, and y axes plot the EPSP response amplitude normalized to the first EPSP, plotted on a log scale. Each line represents the response of one cell. For pulse numbers 2-4, values >1 indicate facilitation and values <1 indicate depression. Solid gray lines at y value 1 indicates the location of unchanging EPSP amplitudes. Dotted lines in each graph show the population average for the given cell type. (E) and (F): Examples of a VPM cell (E) and a PoM cell (F) EPSP responses before and after application of the NMDA blocker APV. Black responses are before APV application, and gray superimposed responses are during APV application. All EPSPs for this were recorded in ACSF with full inhibitory blockade using BMI and CGP-62349 (see Materials and Methods).

**Intrinsic Membrane Properties**

The membrane properties of a neuron largely determine how it transforms its synaptic inputs into output patterns of action potentials. Relay neurons of the dorsal thalamus tend to have certain electrophysiological characteristics in common, regardless of their nucleus of origin. Most notably, they display 2 modes of spiking: when activated from a relatively negative resting potential, they generate bursts of spikes driven by low-threshold calcium currents and when activated from more depolarized resting potentials, they generate tonic trains of spikes (Llinas and Jahnsen 1982; Jahnsen and Llinas 1984).

We compared the intrinsic firing properties of VPM and PoM neurons. Although their passive membrane properties were
similar, when presented with similar suprathreshold stimuli the VPM neurons had lower thresholds, were able to fire tonically at higher frequencies, and generated higher frequency spike bursts than PoM neurons. The relatively lower excitability of PoM neurons may contribute to their sensory responsiveness in vivo.

In anesthetized rats, PoM neurons are generally more difficult to excite with somatic sensory stimulation than are VPM neurons (Hoogland et al. 1987; Chiaia et al. 1991; Diamond, Armstrong-James, Ebner et al. 1992, b; Lu and Lin 1993; Sosnik et al. 2001). In response to whisker inputs, PoM cells fire fewer spikes at longer and more variable latencies than VPM cells.

Figure 7. Distinction between layer V and layer VI inputs to PoM. (A) Responses to paired-pulse stimuli (50 Hz). Thin axons from layer VI can be identified by their tendency to evoke shorter latencies from the second pulse compared with the first (supernormality). Larger diameter fibers exhibit either no change in latency or slightly longer latencies to the second of 2 pulses (normality or subnormality). In the top example ("layer V"), the second EPSP arrived 0.1 ms later than the first. In the bottom example ("layer VI"), the second EPSP arrived 0.6 ms earlier than the first. (B) Corticothalamic responses of PoM cells sorted by change in latency and minimal amplitude (N = 13 cells). (C) PoM responses of the same cells shown in panel B but plotted by absolute latency versus minimum EPSP amplitude. Filled circles represent responses from layer VI activation; open circles are from possible layer V activation.

Response Heterogeneity
The main goal of this paper was to characterize feedback effects of S1 cortex onto the 2 thalamic nuclei, VPM and PoM. We observed 3 different types of responses after stimulating the corticothalamic pathway: excitation alone, inhibition alone, or a combination of excitation and inhibition. Despite the purely excitatory nature of the feedback fibers themselves, the strength and prevalence of inhibitory responses in both cell types was significant (Fig. 3). Several previous studies have described excitation and inhibition in multiple thalamic areas as a result of activating cortical inputs (Pare et al. 1991; Warren et al. 1994, 1997; Kao and Coulter 1997; Bartlett and Smith 1999; Zhang and Jones 2004).

Latencies of both excitatory and inhibitory events originating from cortex had a much broader distribution than latencies originating from thalamocortical activation (Figs. 4 and 5). These findings are consistent with data from other studies that show tight clustering of thalamocortical latencies (Gil and Amitai).
be much larger than those from layer VI. The distinction between these 2 pathways is consistent with the idea that layer V inputs serve as "drivers" to the PoM, and those from layer VI provide a modulatory effect on activity both in VPM and PoM (Sherman and Koch 1990; Feig and Harting 1998; Sherman and Guillery 1998; Reichova and Sherman 2004). We think the differences we have observed in EPSP magnitude in VPM versus PoM largely arise from axons originating in layer VI, despite the existence of the large layer V terminations in PoM. First, we saw no bimodal distribution of PoM response amplitudes that would indicate activation from layer V versus VI. This is not surprising because the majority of layer V fibers traverse an anatomical plane that is not preserved in the thalamocortical slice (Veinante et al. 2000). Second, there is a large overlap in EPSP response amplitudes (Fig. 6B), which is not consistent with anatomical findings showing large synaptic boutons from layer V (up to 5 μm in diameter) and very small ones from layer VI (~1.5 μm; Bourassa et al. 1995; also see Veinante et al. 2000). This difference in terminal size is more consistent with previous studies of responses in higher order thalamic nuclei originating from layer V versus VI (Li et al. 2003; Reichova and Sherman 2004). In both of those studies, the EPSPs from layer V axons were reported to be 4–10 times the amplitude of those from layer VI.

We did observe 2 PoM responses that may have had layer V as their origin (Fig. 7). These 2 neurons had noticeably larger minimal EPSP amplitudes and showed no evidence of latency supernormality. However, their amplitudes were only modestly larger than those observed by Li et al. (2003) and Reichova and Sherman (2004). Another difference between our study and that of Reichova and Sherman is that they observed no significant difference in the amplitude of layer VI EPSPs in VPM versus PoM. This could be due to different stimulation methods because we used minimal stimulation and they reported amplitudes based on 1.5T stimulus intensities. This would also explain why their observed EPSPs (presumably from layer VI) were significantly larger than ours (~1.5 mV vs. ~0.2 mV).

Short-term facilitation of excitatory responses was present in both cell types, but much stronger and more common for VPM cells (Fig. 6C,D). In fact, the average PoM response showed no facilitation because there were virtually equal numbers (and equal amplitudes) of facilitating and depressing responses from the 40-Hz train stimulation. The combination of larger EPSPs and less facilitation suggests that layer VI terminations onto PoM may be more reliable and thus have a stronger influence, than those onto VPM cells.

In many cases, it was virtually impossible to elicit an EPSP after one shock in both VPM and PoM cells (see Fig. 5A, filled circles), suggesting a low probability of release from corticothalamic synapses onto both cell types. This idea is supported by the findings of Golshani et al. (2001) who have recorded pairs of layer VI and VPM neurons. They reported a 68% failure rate of VPM EPSPs in response to the first spike in a train from a cell in layer VI. Our observations of such failures were slightly more common in VPM than PoM neurons (33% vs. 20%, respectively), further supporting our suggestion that layer VI terminations onto PoM are more reliable than those onto VPM. We attribute these differences to the fact that PoM cells receive input only from lower layer VI, whereas VPM cells receive most of their input from upper layer VI along with sparse input from lower VI (Bourassa et al. 1995; Killackey and Sherman 2003). But it is also possible that a single axon can have different effects on

**Excitatory Synaptic Responses**

The direct effect of corticothalamic feedback on the VPM and PoM is excitation. The amplitudes of minimal EPSPs from cortex were significantly larger in PoM cells than in VPM cells. One of the most important aspects of characterizing these EPSPs is distinguishing their points of origin: whether the larger EPSPs seen in PoM come from layer V or from layer VI inputs. Anatomically, the axons and terminals from layer V appear to

Figure 9. Cortical feedback responses of TRN neurons to internal capsule stimulation. (A) Example response of a single TRN neuron to 1.5× threshold stimulus train (40 Hz), showing short latency. (B) Summary of the population of TRN responses to 40-Hz train stimulation. All EPSP amplitudes are normalized to the response to the first pulse. The y axis is plotted on a log scale. Each line represents the responses of a single neuron. Solid gray line indicates unchanging EPSP amplitude. Lines below solid gray show response depression, and lines above solid gray show response facilitation. Dotted gray line indicates population average (N = 10 cells). (C) Distribution of minimal EPSP amplitudes for the 3 thalamic cells types: VPM, PoM, and TRN. Bin size = 0.5 mV, range = 0.5–4 mV. All EPSPs smaller than 0.5 mV are pooled in the first bin. (N = 11 VPM, 11 PoM, and 10 TRN cells).
separate postsynaptic targets, as seen in neocortex (Markram et al. 1998).

**Inhibitory Synaptic Responses**

GABA released onto VPM and PoM neurons activated both GABA_a and GABA_b responses, as determined by their time courses (early and late), reversal potentials, and effects of receptor-specific antagonists. Almost all cells of both nuclear types had a GABA_a component in their inhibitory responses. However, many more PoM cells than VPM cells showed GABA_b responses (91% vs. 37%, respectively). As with the intrinsic firing properties, the increased prevalence of GABA_b-IPSPs may contribute to the in vivo sluggishness of PoM neurons compared with VPM neurons. That is, the GABA_b responses may keep the cells more hyperpolarized for longer periods of time during a similar level of TRN activation (Roy et al. 1984; Bloomfield and Sherman 1988; Salt and Eaton 1990; Crunelli and Leresche 1991; Pare et al. 1991; Huguenard and Prince 1994; Kao and Coulter 1997; Kim et al. 1997; Warren et al. 1997; Kim and McCormick 1998; Bartlett and Smith 1999; Ziburkus et al. 2003). In fact, strong inhibitory input from zona incerta (ZI) has been demonstrated to robustly reduce PoM responses to whisker stimulation elicited responses consistent with both feedforward and feedback activation, based on latency analysis. However, the majority of responses (10 of 11) had properties consistent with an anatomical and physiological organisation with feedback axons that activate only the disynaptic inhibitory pathway and do not activate direct excitation onto certain VPM and PoM cells (Deschénes et al. 1998; Pinault and Deschénes 1998a, 1998b). Our data strongly support the first idea, but at least one case (Fig. 5B) also supports the second possibility.

More specifically, we propose a model of how the feedback circuit achieves these varied responses originating from a purely excitatory set of corticothalamic synapses (Fig. 10). The third possibility has been demonstrated anatomically and is also consistent with our electrophysiological findings (see Fig. 3BD). By providing different strengths of activation to VPM, TRN, and PoM, cortex can differentially modulate these areas over space and time and thus change the balance of activity in each thalamic nucleus. In addition to varying the strength of activation, indicated by EPSP amplitudes, cortical inputs to thalamus can also vary in their reliability and speed. The disynaptic inhibitory pathway from cortex, to TRN, to VPM or PoM may "win out" over direct monosynaptic excitation by gaining speed and reliability at the second synapse.

Finally, differences in the balance of excitation and inhibition to VPM and PoM suggest a mechanism for maintaining a relatively quiet baseline in PoM compared with VPM in vivo. The
fact that a feedback inhibitory circuit from cortex may be involved in quieting PoM suggests the possibility that feedforward modulation by PoM is triggered very selectively and possibly very little during periods of high activity in cortex.

Thus, feedback from cortex can actually trigger net inhibitory effects in both VPM and PoM cells of the thalamus—effectively changing the sign of the strictly excitatory fibers from primary cortex.

Notes
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