RESEARCH ARTICLE

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Role of cortical feedback in the receptive field structure and nonlinear response properties of somatosensory thalamic neurons

Abstract Previous studies have suggested that the descending pathway from the primary somatosensory (SI) cortex to the ventral posterior nucleus of the thalamus has only a mild facilitative influence over thalamic neurons. Given the large numbers of corticothalamic terminations within the rat somatosensory thalamus and their complex topography, we sought to examine the role of corticothalamic feedback in the genesis of spatiotemporal receptive fields and the integration of complex tactile stimuli in the thalamus. By combining focal cortical inactivation (produced by microinjection of the $GABA_A$ agonist muscimol), with chronic multielectrode recordings, we observed that feedback from the rat SI cortex has multiple influences on its primary thalamic relay, the ventral posterior medial (VPM) nucleus. Our data demonstrate that, when single-whisker stimuli were used, the elimination of cortical feedback caused significant changes in the spatiotemporal structure of the receptive fields of VPM neurons. Cortical feedback also accounted for the nonlinear summation of VPM neural responses to simultaneously stimulated whiskers, in effect "linearizing" the responses. These results argue that the integration and transmission of tactile information through VPM are strongly influenced by the state of SI cortex.

Keywords Corticothalamic · Corticofugal ·
Combination sensitivity · Dynamic receptive fields ·
Barrel cortex · Rat

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Introduction

The rat trigeminal somatosensory system exhibits many structural and functional characteristics which suggest that it may use feedback and parallel processing as a computational strategy to categorize tactile information. Structurally, neurons in the rat ventral posterior medial (VPM) nucleus of the thalamus receive feedforward parallel inputs from the principal and spinal trigeminal nuclei (Chiaia et al. 1991; Williams et al. 1994) and feedback inputs from the primary somatosensory (SI) cortex (Bourassa et al. 1995; Chmielowska et al. 1989; Wise and Jones 1977). Although both feedforward and feedback inputs are likely to contribute to the response properties of thalamic neurons, the role of SI cortical feedback has not been thoroughly explored. Two candidate thalamic response properties that may require cortical feedback are the spatiotemporal structure of receptive fields (RFs) and combination sensitivity (Ghazanfar and Nicolelis 2001).

The spatiotemporal (or dynamic) RF properties of neurons have been described in the visual (Ringach et al. 1997), auditory (deCharms et al. 1998), and somatosensory systems (Nicolelis and Chapin 1994; Ghazanfar and Nicolelis 1999). Neurons in these pathways have RFs whose centers (or feature selectivity) change as a function of poststimulus time (on the order of milliseconds). For example, in the rat somatosensory thalamus, VPM neurons exhibit RFs that respond best to one whisker at the earliest poststimulus time and then respond best to another whisker at a later time (Nicolelis and Chapin 1994). These spatiotemporal RFs of VPM neurons have been hypothesized to emerge through the asynchronous convergence of feedforward and feedback inputs onto these neurons (Nicolelis 1997). Several computational models have also suggested that feedback circuitry may underlie the time-varying properties of cortical and thalamic neurons in many sensory systems (Somers et al. 1995; Douglas et al. 1995; see Ghazanfar and Nicolelis 2001 for review), yet experimental data have not been forthcoming.

Complex, time-varying sensory signals are often integrated nonlinearly by neurons in higher forebrain areas – the neuronal responses do not equal the arithmetic sum of the responses to parts of the signal (Margoliash and Fortune 1992; Suga et al. 1983; Brosch et al. 1999). Recently, combination-sensitive neurons have been described in the rat SI cortex (Shimegi et al. 1999, 2000; Ghazanfar and Nicolelis 1997) and VPM (Ghazanfar and Nicolelis 1997), where neurons may respond either supralinearly or sublinearly to simultaneous multiple-whisker deflections. The circuitry which gives rise to these nonlinear somatosensory responses has not been investigated. Yet, the divergent pattern of SI corticothalamic terminations and their topographic arrangement in the rodent VPM and in the reticular nucleus (RTN) of the thalamus (Bourassa et al. 1995; Hoogland et al. 1987; Welker et al. 1988) suggest that feedback may play a role in the combination sensitivity of VPM neurons (Ghazanfar and Nicolelis 1997).

In the present study, we investigated the role of rat SI cortical feedback on the VPM by recording from the same populations of single VPM neurons, before and during inactivation of SI cortex with the GABA-agonist muscimol. We used this paradigm to measure how inactivation of SI cortex influenced the spatiotemporal RF structure of VPM neurons and their nonlinear responses to multiple-whisker stimuli. Our results indicate that SI cortical feedback has a significant influence on both the RF structure and nonlinear responses of VPM neurons, and that these effects are stimulus dependent.

Materials and methods

Animals and surgical procedures

Nine adult female Long-Evans rats (250–300 g) were used in these experiments. Details of surgical procedures have been described elsewhere (Nicolelis et al. 1997). Briefly, animals were anesthetized with intraperitoneal injections of sodium pentobarbital (Nembutal, 50 mg/kg) and transferred to a stereotaxic apparatus. When necessary, small supplementary injections of sodium pentobarbital (~0.1 cm³) were administered to maintain anesthesia during the surgery. Following retraction of the skin and soft tissue, a small, rectangular craniotomy was made over the VPM nucleus of the thalamus using stereotaxic coordinates. Two bundles of eight microwires, cut at two different lengths, were used. The distance between bundles was ~1 mm. The arrangement of microwires in each case maximized the number of barreloids sampled in each structure. Each microwire was Teflon-coated and had a 50-μm tip diameter. For all animals, the representation for the large, caudal whiskers was targeted. Upon proper placement, microwire implants were cemented to the animal's skull with dental acrylic. Following the implantation of the microwires, a second small craniotomy was made over the posteromedial barrel subfield. Here, a 27-gauge stainless steel, microinfusion guide cannula (23 mm in length) was stereotaxically lowered through a small hole made in the dura mater. The tip of the guide cannula was positioned 500 μm below the surface of SI cortex, and the cannula was then cemented in this position.

The location of microwire bundles was assessed by qualitative RF mapping during surgical implantation and quantitative analysis of response properties following experimental procedures (Nicolelis et al. 1993a, 1993b; Nicolelis and Chapin 1994; Ghazanfar and Nicolelis 1997; Krupa et al. 1999; Faggin et al.

1997). The location of both the microwire bundles and cannulae were confirmed by light-microscopic analysis of Nissl-stained sections through the SI cortex and VPM thalamus.

Data acquisition

Following a recovery period of 5–7 days, animals were anesthetized with sodium pentobarbital (50 mg/kg) and transferred to a recording chamber, where all experiments were carried out. A head stage was used to connect the chronically implanted microwires to a preamplifier, whose outputs were sent to a multineuronal acquisition processor (MNAP, Plexon, Dallas, Tex.) for online multichannel spike sorting and acquisition (sampling rate 40 kHz per channel). A maximum of four extracellular single units per microwire could be discriminated in real time using time-voltage windows and a principal component-based spike-sorting algorithm (Abeles and Goldstein 1977; Nicolelis and Chapin 1994). Previous studies have revealed that, under these experimental conditions, approximately 80% of the microwires yield stable single units and an average of 2.3 single units can be well-discriminated per microwire (Nicolelis et al. 1997). Further details regarding acquisition hardware and spike sorting can be found elsewhere (Nicolelis and Chapin 1994; Nicolelis et al. 1997).

Recording session and whisker stimulation

After spike sorting, the simultaneous extracellular activity of all well-isolated single units was recorded throughout the duration of all stimulation experiments. A computer-controlled, multichannel whisker stimulator was used to deliver innocuous mechanical stimulation to single whiskers or multiple whiskers simultaneously on the mystacial pad contralateral to the microwire implant (Krupa et al. 2001). The stimulus paradigm included the deflections of 16 individual whiskers (B1–4, C1–4, D1–4, and E1–4) and eight combinations of multiple-whisker stimuli. Multiple-whisker stimuli always consisted of the simultaneous deflection of 3 of the 16 individual whiskers and were oriented either horizontally (whisker rows, B123, C123, D123, and E123) or vertically (whisker columns, CDE1, CDE2, CDE3, and CDE4). Two hundred trials were obtained per stimulated whisker(s), and whiskers were stimulated in random order at a rate of 2 Hz and with a step-pulse of 100 ms in duration. The output of the stimulator was calibrated to produce a ~0.5-mm upward deflection of each of the whiskers.

SI cortical inactivation

Immediately after completion of the stimulation paradigm, a 33-gauge infusion cannula was lowered through the chronically implanted guide cannula. The tip of the infusion cannula extended ~1 mm beyond the tip of the guide in order to target the infragranular layers of SI cortex (500 μm+~1,000 μm=~1,500 μm below the cortical surface). The γ-aminobutyric acid (GABA) agonist muscimol (500 ng in 500 nl of saline) was then infused into SI cortex using an electronic syringe pump to allow for a slow (~2 min) and controlled infusion of muscimol into SI cortex. The concentration and volume of muscimol was chosen to inactivate a large portion of SI barrel cortex (more than 2 mm²) for several hours (Martin 1991; Krupa et al. 1999). Following infusion of muscimol, 30 min was allowed for the drug to take effect and recordings were then resumed. The stimulation paradigm was repeated during cortical inactivation. For control animals, an identical volume of vehicle solution (500 nl of saline) was infused into SI cortex in lieu of muscimol. All these parameters were developed and reported by Krupa et al. (1999).

Sequence of events

Following online discrimination of single thalamic units, a recording session consisted of stimulating 16 single whiskers and eight

combinations of multiple whiskers (a total of 24 different stimuli, 200 trials each). This paradigm was completed in approximately 45 min and was followed by a microinfusion of saline or muscimol into SI cortex. Following a waiting period of 30 min, recording of neural activity was resumed and the stimulation paradigm was repeated (~45 min), for a total experimental time of approximately 2 h per animal.

Small supplemental doses of sodium pentobarbital (0.05 cm³) were administered as needed throughout the experiments to maintain the animal under a similar anesthetic state, as determined by brain activity, respiration rate, and eye-blink reflex.

Data analysis

Measurement of response magnitudes

The average evoked firing rate was quantified for each neuron using poststimulus time histograms (PSTHs) and cumulative frequency histograms (CFHs). CFHs were used to measure the statistical significance of sensory responses. These histograms depict the cumulative poststimulus deviations from prestimulus average firing seen in the PSTHs. In other words, the CFHs describe the probability that the cumulative frequency distribution in the histogram differs from a random distribution, as computed by a one-way Kolmogorov-Smirnov test. Neuronal responses were considered statistically significant if the corresponding CFH indicated a $P<0.01$ (Faggin et al. 1997; Nicolelis et al. 1993a). These analyses were carried out on commercially available software (Neural Explorer; Plexon, Dallas, Tex.).

Spatiotemporal RFs

As described previously (Ghazanfar and Nicolelis 1999; Nicolelis et al. 1993a; Nicolelis and Chapin 1994), most somatosensory cortical and VPM neurons exhibit time-dependent shifts of their RF centers. For this analysis, a single neuron's response to each whisker was divided into the following poststimulus time epochs: 0–5 ms, 5–10 ms, 10–15 ms, 15–20 ms, 20–25 ms, 25–30 ms, 30–35 ms, and 35–40 ms. In each epoch, the whisker that elicited the greatest response (as measured by spike counts or standard deviations above baseline activity) was defined as the "center." As the center whisker often varied as a function of the poststimulus time epoch, we were able to map the "spatiotemporal" RF for most neurons. This approach is nearly identical to the "response plane" techniques used to map spatiotemporal RFs in the visual system (Dinse et al. 1991; Stevens and Gerstein 1976). Spatiotemporal RFs were plotted as a series of 3D graphs in Matlab (Mathworks, Natick, Mass.), or as trajectories in whisker space (see Results).

#### Nonlinear responses to multiple-whisker stimuli

The responses to simultaneous, multiple-whisker stimuli were compared with the arithmetic sum of single-whisker responses. An arbitrary threshold of 10% was used to determine nonlinearity. If the response to multiple-whisker stimuli was 10% above or below the arithmetic sum of single whisker response of those same three whiskers, we judged it to be nonlinear. Similar or identical criteria for nonlinearity have been used in previous studies of the somatosensory system (Ghazanfar and Nicolelis 1997; Shimegi et al. 1999), the bat auditory system (Fitzpatrick et al. 1993), and for the birdsong system (Margoliash and Fortune 1992; Doupe 1997). Since there was some variability in multiple-whisker response across different experimental conditions, we compared saline-control conditions with muscimol conditions using a difference index. This index consisted of dividing the "after" response magnitude by the "before" response magnitude and then subtracting 1 from the dividend (see Results).

Results

Our findings are based on recordings from populations of single neurons located in the VPM nucleus of nine animals. Three of the animals served as the control group, which received microinfusions of saline, while the remaining six animals served as the experimental group, which received microinfusions of the GABA agonist muscimol in the SI cortex. Figure 1 shows representative examples of the microwire locations from five of the six animals in the experimental group. A total of 206 neurons were recorded, 52 in the control condition and 154 in the experimental group. The chronic nature of the electrode implants allowed the same set of thalamic neurons to be recorded before and during inactivation of primary SI cortex (Krupa et al. 1999; Nicolelis et al. 1997; Fig. 2).

A previous study from our laboratory has demonstrated the extent and duration of muscimol inactivation of

Fig. 1 Microwire locations. Photomicrographs of representative sections (80 μm thick, stained with cresyl violet) from five different rats (from the experimental group). Each section shows the tip of the recording electrode (*heavy arrow*) in that section. As can be seen, the tips of the electrodes were located within the ventral posterior medial (*VPM*) nucleus. Examination of all histological sections indicated similar placements within the VPM for other electrode tracts in these rats as well as the control rats. (*PoM* Posteromedial thalamic nucleus, *VPL* ventral posterolateral thalamic nucleus, *ZI* zona incerta, *ml* medial lemniscus). *Scale bar* 1 mm

Before infusion

After infusion
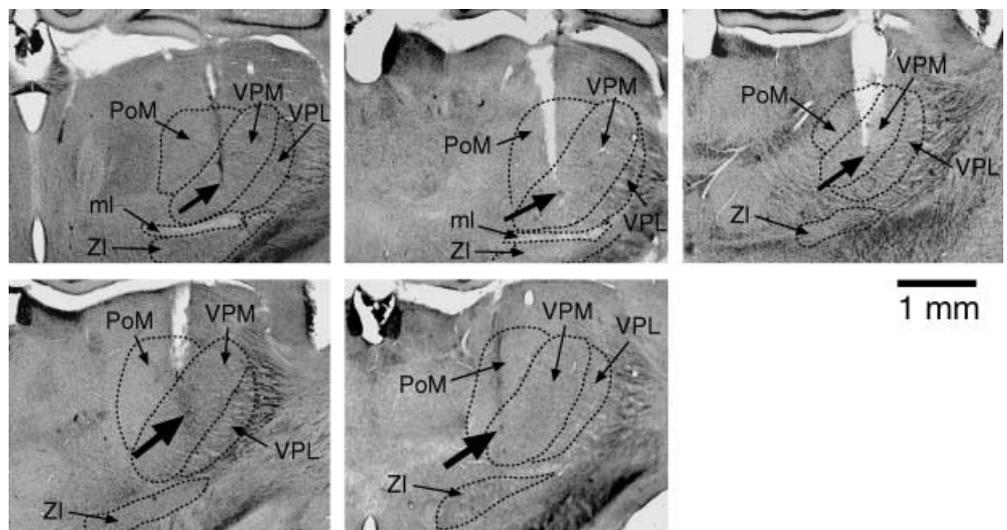
Fig. 2 Stability of thalamic single-unit recordings before and during cortical inactivation. Depicted here are the waveforms of four well-isolated single units recorded from a single microwire located in the VPM of a rat. The *left panel* shows the set of neurons before muscimol activation. The *right panel* shows that the same set of thalamic neurons can be recorded at least 2 h later during muscimol inactivation of SI cortex
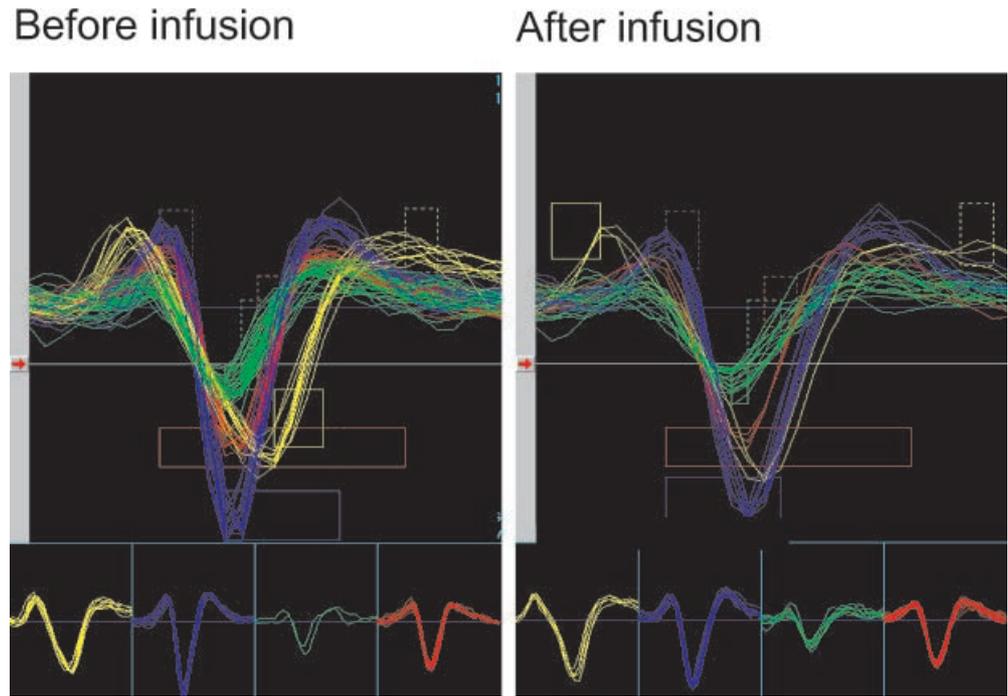
rat neocortex (Krupa et al. 1999). Control microinfusions of saline had little or no effect on somatosensory cortical responses, but muscimol inactivated the SI cortex within an hour, an effect that lasted for at least 6 h. Based on previous work, we estimated that the corticofugal projections, originating from a cortical area of at least 2 mm², were inactivated by our muscimol injections (Krupa et al. 1999; Martin 1991). The relatively long-lasting effect of muscimol allowed us to repeat the stimulation paradigm well within the duration of the SI cortical inactivation.

## General effects of eliminating SI cortical feedback on VPM thalamic responses to single whiskers

Under pentobarbital anesthesia, the majority of rat VPM neuronal responses to single-whisker stimuli consist of both short-latency (5 to 15 ms poststimulus) and long-latency (15–40 ms poststimulus) components (Nicolelis and Chapin 1994). Eliminating SI cortical feedback input to these neurons resulted in changes in both temporal domains. Figure 3 depicts PSTHs of four neurons to a single-whisker stimulus before and during SI cortical inactivation. Figure 3a shows a neuron whose long-latency response disappeared following SI cortical inactivation, but whose short-latency response remained unaffected. Figure 3b shows a similar effect, but in this example the short-latency response increased in magnitude following SI inactivation. Figure 3c shows a large reduction in a short-latency response, while Fig. 3d demonstrates an increase in the long-latency response following SI inactivation. Overall, measurement of 1,911 short-latency responses revealed that 54% of these components showed

a decrease and 35% percent showed an increase greater than 10% of their original response magnitude. Similarly, for 1,668 long-latency responses, 55% of responses decreased and 39% increased more than 10% of the original response. Thus, elimination of SI cortical feedback results both in the unmasking and suppression of short- and long-latency tactile responses in the rat VPM.

Influence of SI cortical feedback on VPM RF structure

VPM thalamic neurons have been characterized as having spatiotemporal RFs (Nicolelis and Chapin 1994). In at least 40% of these RFs, the whisker that elicits the strongest response (and defines the RF center) by a given neuron changes as a function of poststimulus time. Since the surrounds of these VPM RFs also shift as a function of poststimulus time, RFs are shaped by both the short- and long-latency responses to single-whisker stimuli. Given that SI cortical feedback can influence the two temporal components of the response of VPM neurons (Fig. 3), we examined how this might influence the spatiotemporal RF structure of VPM neurons. Figure 4 depicts the spatiotemporal RFs of three neurons before and during inactivation of SI cortex. Each block represents the matrix of facial whiskers and the color code represents the response magnitude (red indicates 6 SDs of baseline firing-measured, 100-ms prestimulus). Poststimulus time is represented by a series of these blocks from top (5–10 ms) to bottom (25–50 ms) in Fig. 4. Figure 4a demonstrates that eliminating SI cortical feedback can dramatically reduce both temporal components in the spatiotemporal RFs of VPM neurons. In neuron 1, the spatial extent of the RF was reduced from 5 to 15 ms,

Fig. 3a–d Examples of the responses of four different neurons to a single-whisker stimulus before and during SI cortical inactivation. **a** A neuron whose long-latency response disappeared following SI cortical inactivation, but whose short-latency response remained unaffected. **b** A neuron which shows a similar effect to that shown in **a**, but in this example the short-latency response increased in magnitude following SI inactivation. **c** This neuron shows a large reduction in a short-latency response. **d** Here, an increase in the long-latency response occurs following SI inactivation
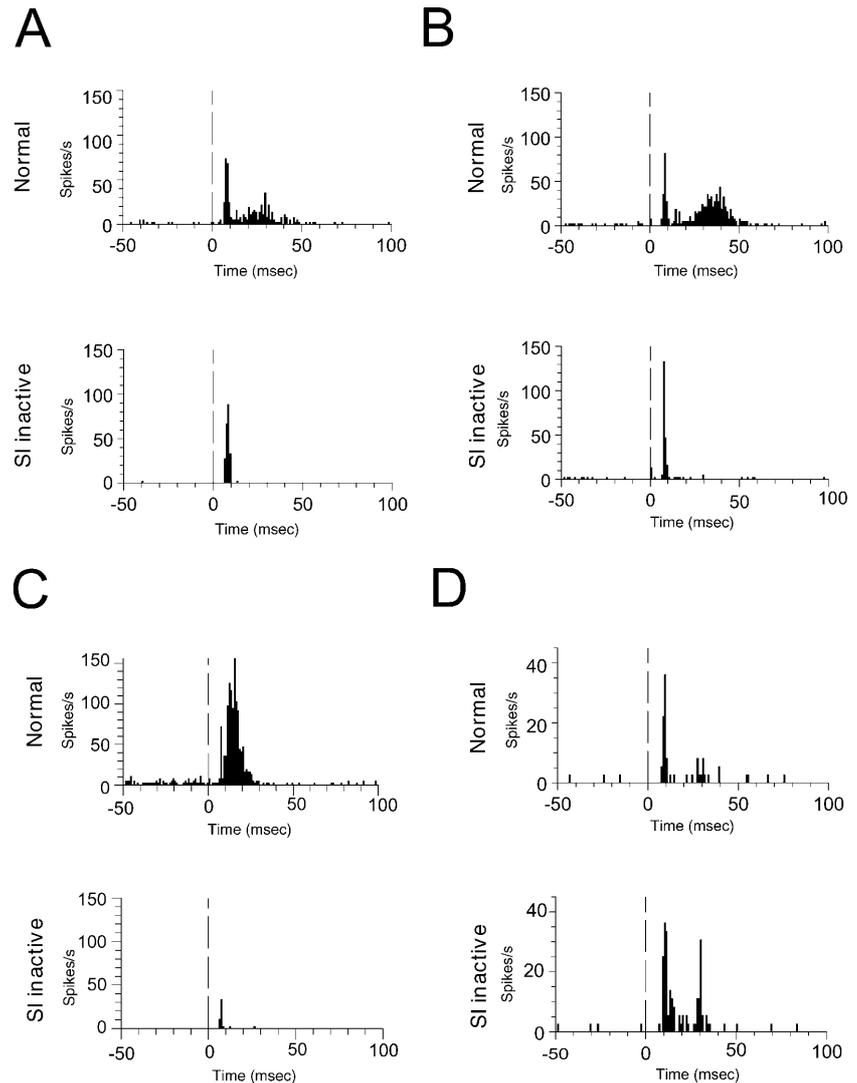
and the spatial structure of the RF between 15 and 25 ms was nearly eliminated following SI cortical inactivation. Similarly, for neuron 2 the spatial extent of the RF was reduced at 20–25 ms and completely eliminated between 25 and 30 ms. Figure 4b depicts the spatiotemporal RF of a neuron that was "unmasked" following SI cortical inactivation. With the thalamocortical pathway intact, this neuron had a small RF in the 25- to 35-ms time range; however, following SI inactivation, the RF expanded in both the temporal and the spatial dimensions.

Figure 5 shows more examples of spatiotemporal RFs plotted in the form of trajectory plots. Each panel represents the matrix of facial whiskers, and the vectors depict the path that the RF takes over eight 5-ms epochs of poststimulus time; vectors were drawn by connecting the whisker locations that elicited the maximum response in each epoch. Figure 5a shows three neurons' spatiotemporal RFs before and during SI cortical inactivation. We observed that eliminating SI cortical feedback can alter the trajectory by reducing, expanding, or even changing the overall shape of the RF. Figure 5b depicts the RFs of three neurons under the control condition. Microinfusion of saline had little or no

effect on the spatiotemporal RFs of VPM neurons and demonstrates that the structure of these RFs was otherwise stable over the long time-course of the experiments.

These observations indicated that feedback projections from SI cortex can profoundly influence the spatiotemporal structure of VPM neuronal RFs. Indeed, these results suggest that cortical feedback does not simply act as global or tonic modulator of the responses of VPM neurons. In further support of this, the mean spontaneous activity, measured as the number of spike counts in a 100-ms prestimulus epoch from two groups of animals (those that received saline infusions and those that received muscimol infusions), did not change significantly under any of the experimental conditions [normal versus saline, $t(2)=-2.82$, n.s.; normal versus muscimol, $t(2)=0.54$, n.s.].

Elimination of SI cortical feedback "linearizes" thalamic responses to multiple-whisker stimulation
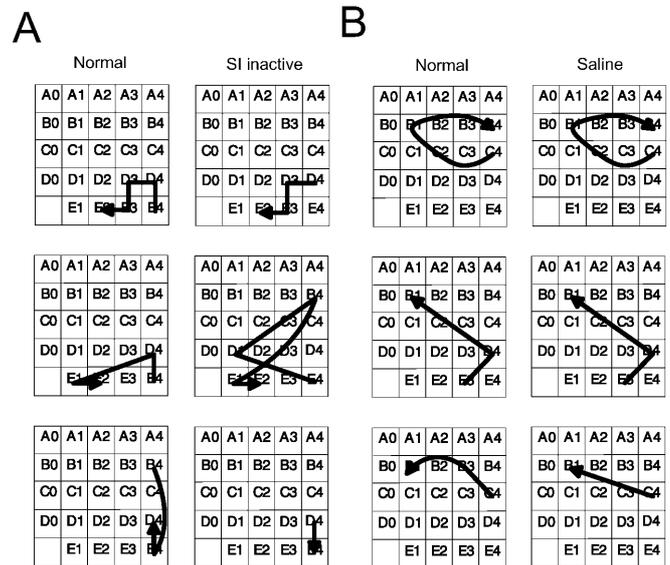
Particular combinations of multiple-whisker stimuli, where two or three whiskers were simultaneously de-
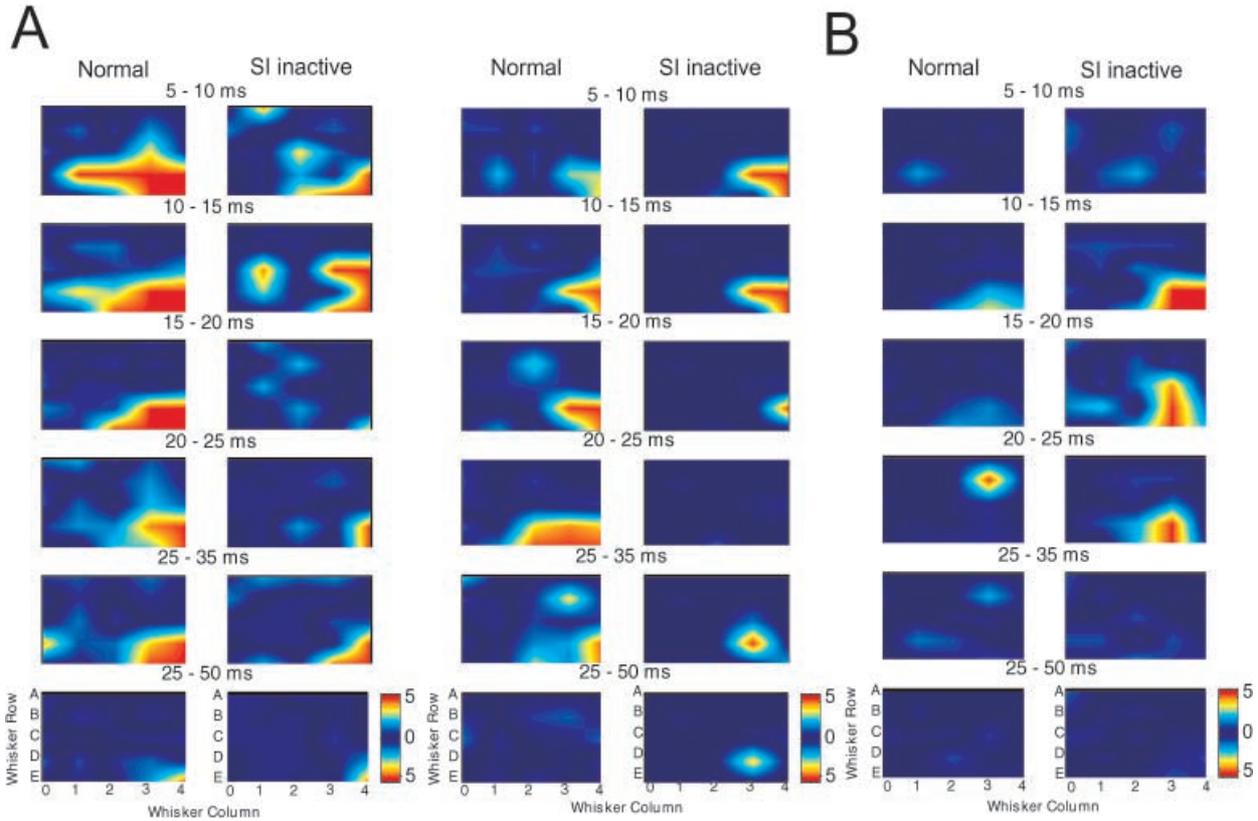
Fig. 4a, b Effect of SI cortical inactivation on the spatiotemporal receptive fields (RFs) of VPM neurons. Each block represents the matrix of facial whiskers, and the color code represents the response magnitude (*red* 6 SDs of baseline firing measured 100 ms prestimulus). Poststimulus time is represented by a series of these blocks from *top* (5–10 ms) *to bottom* (25–50 ms). **a** These two neurons demonstrate that eliminating SI cortical feedback can dramatically reduce both temporal components in the spatiotemporal RFs of VPM neurons. **b** The spatiotemporal RF of this neuron was "unmasked" following SI cortical inactivation. With the thalamocortical pathway intact, this neuron had a small RF in the 25–35 ms time range; however, following SI inactivation, the RF expanded in both the temporal and spatial dimensions

Fig. 5a, b Trajectory plots of spatiotemporal RFs for experimental and control neurons. Here, each matrix represents the whisker pad, and the *arrow* represents the movement of the RF center over poststimulus time. Movement was plotted by determining which new RF centers a neuron went through over eight 5-ms poststimulus time epochs. **a** Three examples of spatiotemporal RFs that were altered to varying degrees following SI cortical inactivation. Note that in some cases there were changes in the earlier part of the RF, while in another there could be massive changes in overall size and shape of the RF. **b** Three examples of spatiotemporal RFs in control animals before and after infusion of saline. Few, if any, changes occur in the RFs of neurons following saline infusions into SI cortex

flected, have been shown to elicit nonlinear responses in the rat VPM thalamus (Ghazanfar and Nicolelis 1997) and SI cortex (Ghazanfar and Nicolelis 1997; Shimegi et al. 1999). That is, the magnitude of the response is not equal to the arithmetic sum of the responses of the whiskers stimulated individually. Such stimuli, oriented along whisker rows or whisker columns (or *arcs*) can elicit either supralinear or sublinear responses. According to the pattern of corticothalamic terminations in VPM and the RTN (Bourassa et al. 1995; Welker et al. 1988), we hypothesized that cortical feedback could influence the supralinear responses to multiple-whisker stimuli oriented along whisker columns and the sublinear responses to whisker rows (Ghazanfar and Nicolelis 1997).

We tested this hypothesis by measuring the changes in nonlinearity of VPM responses to multiple-whisker stimuli before and after microinfusions of either saline or muscimol. Supralinear (more than 110% of the arithmetic sum of single-whisker responses) and sublinear (less than

90% of the arithmetic sum) responses were treated as separate groups. To control for the variability of neuronal responses over the long time-course of these experiments, the magnitude of the change in nonlinear neuronal responses following microinfusions of saline (or muscimol) was measured by dividing the "after" response by the "before" response and subtracting this ratio from 1. It should be noted that in the normal versus saline condition there was considerable variance in the responses to multiple whiskers (see Figs. 5, 6). However, the differences between the nonlinear responses under the normal versus saline conditions, while variable, were not significant for short- or long-latency responses to multiple-whisker stimuli in rows [short, $t(191)=1.9$, n.s.; long, $t(181)=-0.557$, n.s.] or columns [short, $t(191)=-0.447$, n.s; long, $t(188)=-0.466$, n.s.]. Thus, for the control group, this "difference index" provided an assessment of the natural variability of nonlinear responses which could then be used to assess whether changes following muscimol infusions in the experimental group exceeded this normal variability. The mean magnitude of this difference index for the control group (saline) was compared with the experimental group (muscimol) using unpaired t-tests. Short-latency and long-latency components of the nonlinear tactile responses of VPM neurons were considered separately.

Short-latency, supralinear responses

Figure 6a, b shows the effect of SI inactivation focuses on the short-latency responses of VPM neurons following multiple-whisker stimulation. Figure 7a shows the effect of SI inactivation on the supralinear responses to either whisker rows (left panel) or whisker columns (right panel). Inactivation of SI cortical feedback did not significantly influence the short-latency *supralinear* responses to *whisker row* stimuli [whisker rows, $t(117)=0.50$, n.s.], but did influence the short-latency supralinear responses to *whisker columns*, causing them to become even more supralinear [$t(88)=-2.33$, $P<0.05$].

Short-latency, sublinear responses

Figure 6 shows examples of an increase in the short-latency response to whisker row C123 or whisker column CDE4 stimulation following cortical inactivation. Figure 7b compares the short-latency sublinear responses for both whisker row and column stimuli. SI cortical inactivation linearized both types of sublinear responses [whisker rows, $t(452)=-2.18$, $P<0.03$; and whisker columns, $t(496)=-2.66$, $P<0.01$]. That is, less multiwhisker stimulus-driven inhibition was seen after SI inactivation. Thus, for short-latency responses, SI cortical feedback seemed to significantly influence the supralinear responses to whisker columns, making them more supralinear, and the sublinear responses to multiple-whisker stimuli oriented along both whisker rows and columns, causing them to become more linear.
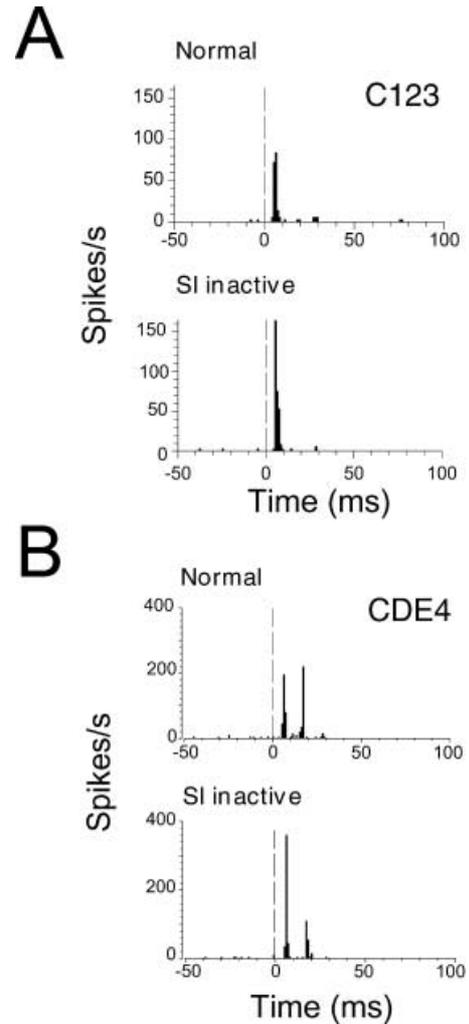

Fig. 6a, b Examples of the influence of cortical feedback on multiple-whisker responses. **a** PSTH depicts a neuron's response to the simultaneous deflection of whiskers C1, C2, and C3 (whisker row, *C123*). Notice that this short-latency response dramatically increases following SI cortical inactivation. **b** PSTH depicts another neuron's response to the simultaneous deflection of whiskers C4, D4, and E4 (whisker column, *CDE4*). Here both the short- and long-latency responses are affected by SI inactivation. The short-latency response increases, while the long-latency response decreases

Long-latency, supralinear, and sublinear responses

Figure 6b shows an example of a decrease in the long-latency response to whisker column stimuli as well as a large increase in the short-latency response (whiskers CDE4). The effects of SI cortical feedback on the nonlinear summation of the long-latency component of VPM responses are summarized in Fig. 8. While SI cortical inactivation had no effect on the supralinear responses to whisker row stimuli [$t(84)=-0.23$, n.s.], it had a very significant impact on the supralinear responses to whisker column stimuli, resulting in a linearization of the responses [$t(132)=5.83$, $P<0.000001$; Fig. 8a]. In contrast, for long-latency sublinear responses to multiple-whisker stimuli, SI inactivation had no significant

Fig. 7a, b Effect of SI inactivation on the supra- and sublinear short-latency VPM responses. The effect of SI inactivation on the nonlinear responses to multiple-whisker stimuli was assessed by measuring the magnitude of the difference in the nonlinear responses in control animals following saline infusions and comparing it to the magnitude of the difference that occurred in experimental animals following infusion of muscimol into SI cortex. The *y*-axis represents the magnitude of the change in nonlinearity, where ''ϕ'' means that no change occurred, greater than ϕ indicates that responses became more robust, and less than ϕ indicates that response became more suppressed. *Error bars* indicate 1 SEM. **a** Supralinear response to whisker rows (*left panel*) and columns (*right panel*). Inactivation of SI cortex had no effect on the supralinear responses to whisker row stimuli. Responses to whisker column stimuli, however, became significantly more supralinear (*P*<0.05). **b** Sublinear response to whisker rows (*left panel*) and columns (*right panel*). Inactivation of SI cortex caused the sublinear responses to both whisker row and whisker column stimuli to become significantly more linear (*P*<0.03 and *P*<0.01, respectively)
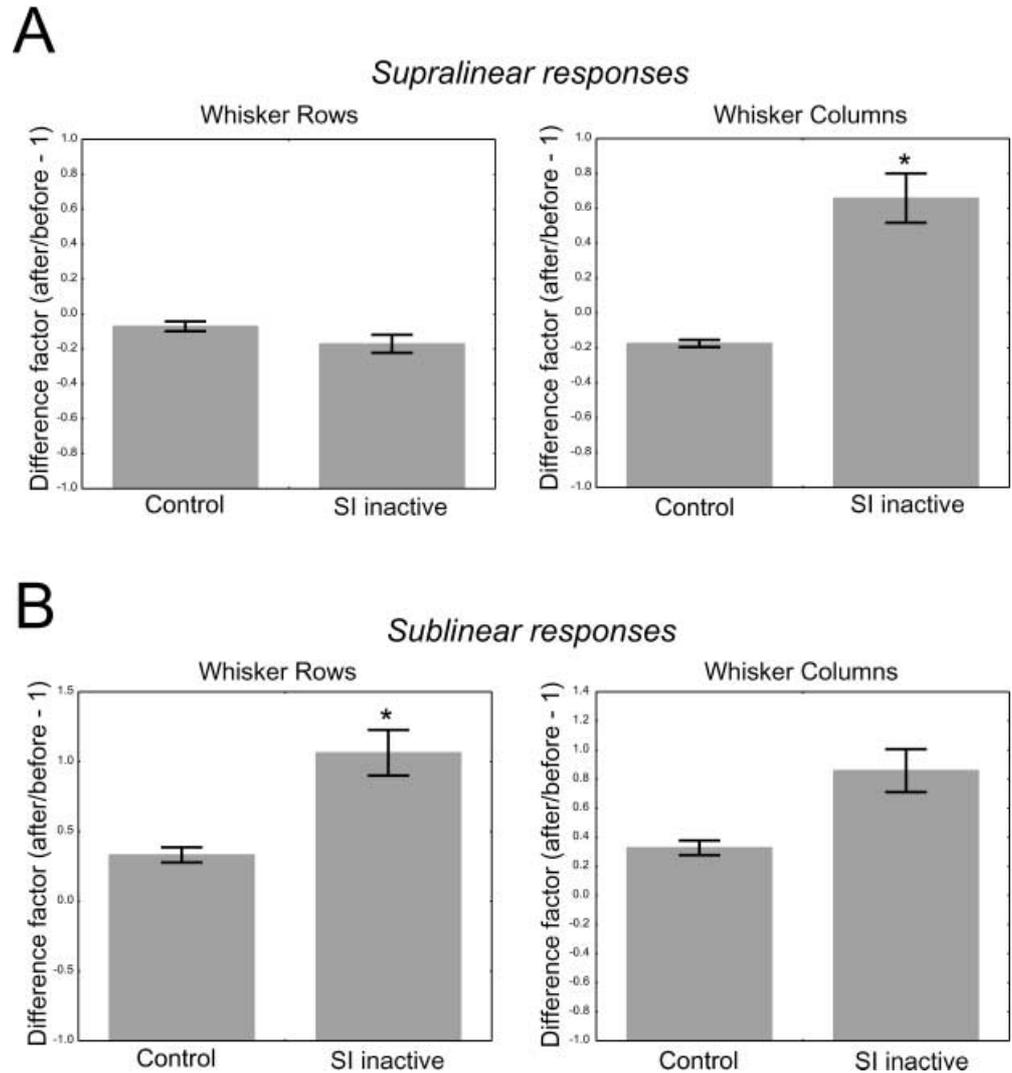
effect on responses to either row or column stimuli [whisker rows: $t(577)=-0.33$, n.s.; whisker columns: $t(558)=0.35$, n.s.; Fig. 8b]. Thus, in contrast to the short-latency responses to multiple-whisker stimuli, inactivation of SI cortical feedback seems to remove an excitatory influence that contributed to the supralinear responses to whisker column stimuli.

In summary, the hypothesis that SI cortical feedback influences the nonlinearity of thalamic responses to multiple-whisker stimuli was supported. Inactivation of SI cortex caused short-latency supralinear responses to whisker column stimulations to become more supralinear and sublinear responses to whisker row stimuli to become more linear. In contrast, long-latency supralinear responses to whisker column stimuli tended to become more linear. In other words, removal of SI cortical feedback removed stimulus-driven inhibition in the short-latency period, but removed stimulus-driven excitation in the long-latency period.

Discussion

We examined the potential influence of SI cortical feedback on the dynamic RFs and combination sensitivity of VPM neurons in the rat. By combining chronic electrode recordings, we were able to quantitatively map the RF properties and the responses to single- and multiple-whisker stimuli before and during cortical inactivation. We found that corticothalamic feedback has a robust influence on the spatiotemporal RF structure and the nonlinear response properties of VPM thalamic neurons.

Extent of inactivation

Whether it is drug-induced or by cooling, a problem in any inactivation study is ascertaining the extent and duration of the inactivation. In the current study, we used muscimol, a GABA agonist, as our inactivation agent. Using identical surgical and infusion procedures as described here, Krupa et al. (1999) have found that the ef-

Fig. 8a, b Effect of SI inactivation on the supra- and sublinear long-latency VPM responses. Same analysis as in Fig. 7 except that here it is the long-latency responses that are examined. *Error bars* indicate 1 SEM. **a** Supralinear response to whisker rows (*left panel*) and columns (*right panel*). Cortical inactivation did not affect the long-latency responses to whisker row stimuli, but significantly linearized the supralinear responses to whisker column stimuli ($P<0.000001$). **b** Sublinear response to whisker rows (*left panel*) and columns (*right panel*). Inactivation of SI cortex had no effect on the long-latency sublinear responses to either whisker row or column stimuli
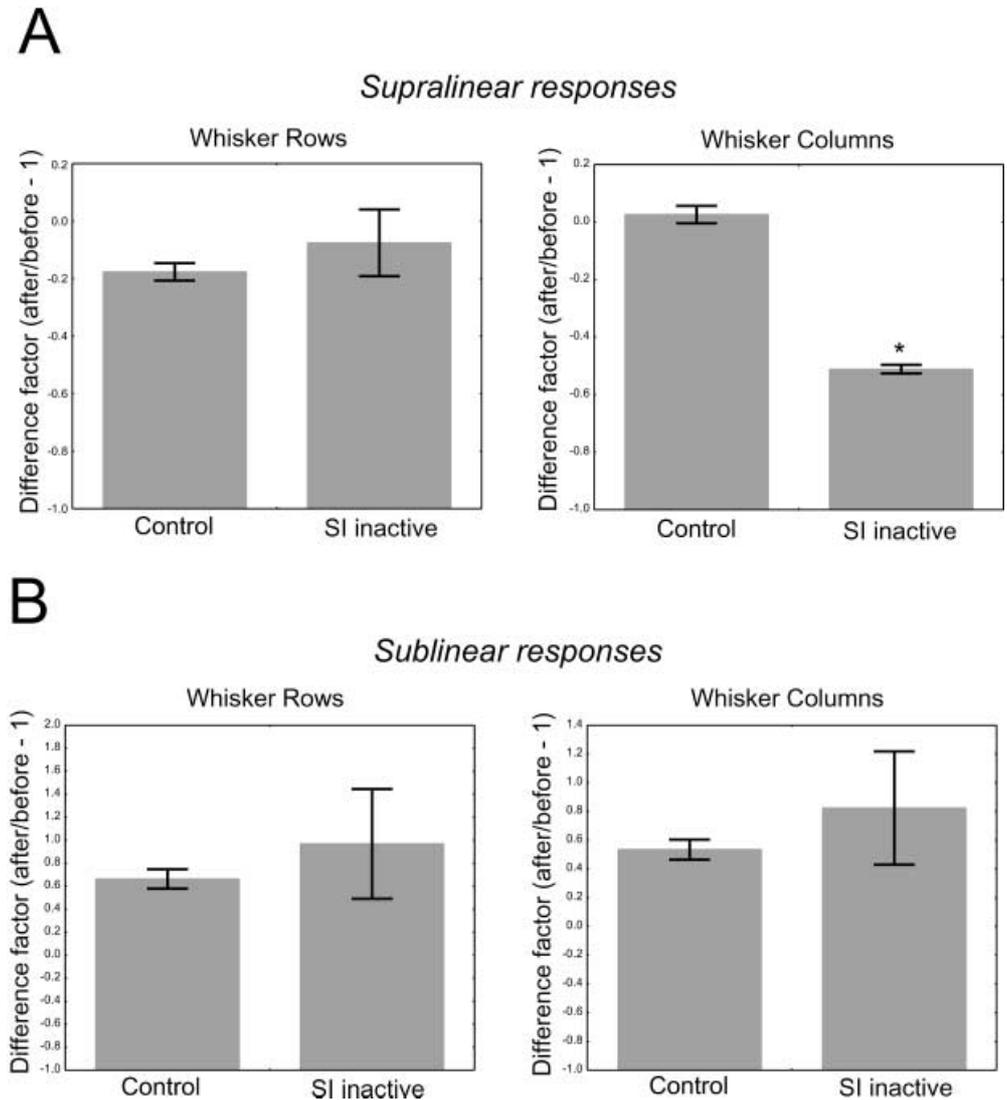
fective spread of cortical inactivation following a 150-ng muscimol infusion is approximately 2.2 mm (see Fig. 3C by Krupa et al., 1999). This is supported by an independent study of muscimol inactivation by Martin (1991). In our study, we used a 500-ng muscimol infusion. Thus, it is likely that we completely abolished activity in most of the whisker area of SI cortex. The duration of muscimol inactivation of cortex is approximately 9 h (see Fig. 3A by Krupa et al., 1999; Martin 1991). This is well within the range of time needed for us to complete our stimulus protocol (~2 h).

Reduction and enhancement of thalamic responses following cortical inactivation

We found that a large number of VPM neurons showed a reduction in response magnitude in both the short- and long-latency components following SI inactivation. However, we also found a large percentage of VPM neurons that showed an increase in response magnitude fol-

lowing SI inactivation. Previous studies of the rat somatosensory corticothalamic pathway support our finding of response suppression following cortical inactivation. For example, in both the awake and anesthetized rat, Yuan et al. (1985, 1986) have found that lidocaine-induced cortical inactivation reduces the responses of ventral posterior lateral (VPL) neurons following stimulation of their center RFs. Using magnesium or cooling to suppress SI cortex, Diamond et al. (1992) have found that, for the eight VPM neurons they examined, the cortex seems to exert only a slight facilitation effect on the VPM thalamus. Interestingly, they found that the principal whisker responses of nine neurons in the rostral sector of the posterior complex (the paralemniscal thalamic pathway) are profoundly suppressed following cortical inactivation. Neither of these studies report any significant response enhancement following the SI cortical inactivation that we observed in this study. This is very likely due to the fact that neither of the previous studies quantified responses to tactile stimulation outside the classic RF.

A heterogeneous range of corticothalamic influences has been reported by Shin and Chapin (1990). They recorded from neurons in the rat VPL while microstimulating the overlying SI cortex. Similar to our findings, they have found that following microstimulation, thalamic neurons exhibit both response suppression and facilitation. Similarly findings have been observed in other sensory systems as well. In the visual system of cats and macaques, inactivation of corticothalamic projections does not lead to simple facilitation or suppression of all responses in the lateral geniculate nucleus (LGN) – both types of response patterns are seen (Kalil and Chase 1970; Marrocco et al. 1996). In the bat auditory system, focal inactivation of a part of auditory cortex tuned to a particular frequency range causes a reduction in the responses of thalamic neurons tuned to the same frequency range, but an increase in the responses of thalamic neurons tuned to different frequencies (Zhang et al. 1997).

Thus, in light of the complex topography of corticothalamic terminations within the somatosensory thalamus (Welker et al. 1988; Deschenes et al. 1998), these data, together with ours, argue against the existence of a single, direct, and homogeneous influence of SI cortex upon the VPM. Instead, our data suggest that the SI cortex exerts a dual effect on VPM neurons.

SI cortical feedback influences the spatiotemporal structure of thalamic RFs

VPM neurons have complex spatiotemporal RFs – the whisker that triggers the strongest response of a neuron (the RF center) changes as a function of poststimulus time (Nicolelis et al. 1993a; Nicolelis and Chapin 1994). We have hypothesized that these spatiotemporal RFs are the outcome of the asynchronous convergence of multiple afferents, both feedforward and feedback, onto VPM neurons (Nicolelis and Chapin 1994; Fig. 9). According to this hypothesis, altering the ascending or descending flow of sensory information should affect the spatiotemporal structure of RFs.

Previously, this hypothesis has been tested in the VPM by disrupting the flow of ascending cutaneous information from the whisker pad by injection of a local anesthetic (Nicolelis et al. 1993b). It was found that blockade of *ascending* information immediately altered the spatiotemporal RF structure of VPM thalamic neurons by affecting both the short- and long-latency components of single-whisker responses. Short-latency components are frequently unmasked or lost, while long-latency components are enhanced or remain the same. In the present study, we further tested the hypothesis by disrupting the flow of *descending* information to VPM by inactivating SI cortex. Our results demonstrate that the spatiotemporal RFs of thalamic neurons are dramatically altered by cortical inactivation.

The cortical influence on thalamic RFs has recently been reported in other systems as well. In the primate somatosensory system, Ergenzinger et al. (1998) have
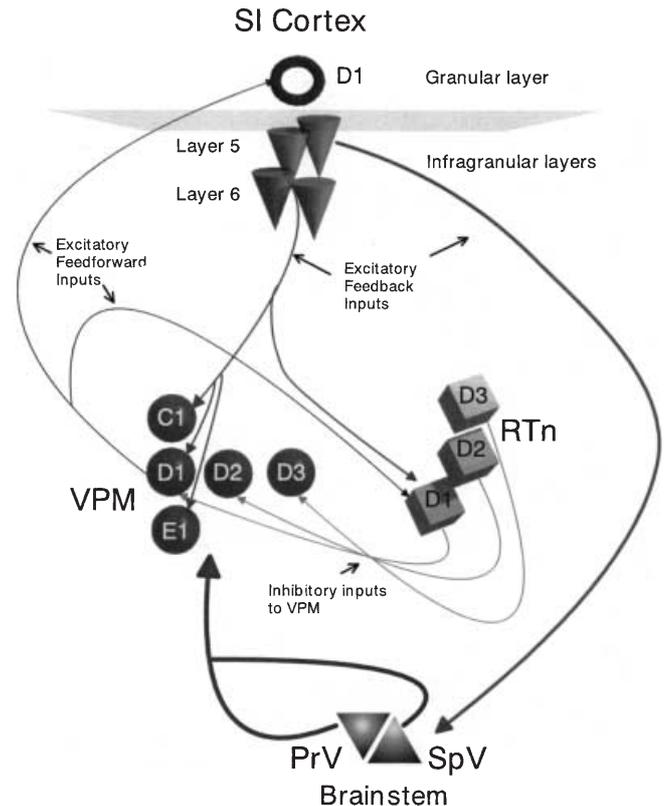
Fig. 9 Model of the effects of SI corticofugal feedback. Tactile information ascends from the trigeminal ganglion through the spinal (*SpV*) and principal (*PrV*) trigeminal brainstem nuclei. These two structures both send projections to the ventral posterior medial (*VPM*) nucleus of the thalamus. The VPM receives all its GABA-mediated inhibition from the reticular nucleus (*RTn*) of the thalamus, in a one-to-one topographic pattern. Projections from the VPM terminate in the granular and infragranular layers of the primary somatosensory (*SI*) cortex; collaterals from these axons also terminate in RTn. SI cortex sends descending fibers from neurons in the infragranular layers. These projections terminate in a restricted fashion in both the VPM and the RTN. In VPM, these fibers terminate on barreloids that together represent a column of whiskers. Corticofugal projections from layer V also terminate in the trigeminal brainstem nuclei

found that chronic suppression of SI cortex over a period of months results in a profound enlargement of thalamic RFs. Similarly, the frequency map in the bat inferior colliculus can be modified by pairing acoustic stimulation with electrical stimulation of the auditory cortex (Yan and Suga 1998). Such paired stimuli result in a shift in the preferred frequency ranges of the spectrotemporal RFs of inferior collicular neurons toward that of the stimulated cortical neurons.

Nonlinear processing of multiple-whisker stimuli: the role of cortical feedback

We found previously that rat VPM neurons respond nonlinearly to the simultaneous deflection of multiple whiskers, a form of "combination-sensitivity" to complex

stimuli (Ghazanfar and Nicolelis 1997), and here we tested whether such nonlinearities were influenced by SI cortical feedback. We found that short-latency supralinear and sublinear responses to whisker row and column stimuli and long-latency supralinear responses to whisker column stimuli were significantly affected by cortical inactivation. Thus, our results support the hypothesis that the nonlinear thalamic responses to such complex tactile stimuli are influenced at least in part by corticofugal projections.

The influence of corticothalamic feedback on the nonlinear responses of thalamic neurons is also supported by findings in the auditory and visual systems. In the bat auditory system, subcortical neurons respond supralinearly to the presentation of two tones separated by a particular time interval (echo delay tuning). By electrically stimulating cortical regions containing neurons of a particular delay tuning while recording from the inferior colliculus, Yan and Suga have found that collicular neurons tuned to the same echo delay increased their responses, while neurons tuned to different echo delays decreased their responsiveness (Yan and Suga 1996). In the visual system, there are neurons in the cat LGN which exhibit sensitivity to the length of a moving contour. Murphy and Sillito (1987) have found that, by cooling the visual cortex and recording in the LGN, corticofugal feedback has a strong influence on the length tuning of LGN neurons.

A model for the corticofugal influences on VPM

Based on our data and the anatomy of the rat somatosensory corticothalamic pathway, we propose the following model to explain our results. Layer VI neurons of SI cortex send projections directly to the dorsal thalamus. One branch of layer VI axons terminates in the VPM, while a second branch terminates in the reticular nucleus. VPM neurons send a collateral branch to the RTN from their ascending afferents to SI cortex. This pathway is summarized in Fig. 9. We hypothesize that the short-latency sublinear responses (for both row and column multiple-whisker stimuli) are generated by the interplay of ascending trigeminothalamic inputs and by the following disynaptic pathway: the glutamatergic synapse from SI cortex to the RTN and the GABAergic synapse from the RTN to VPM. According to this model, inactivation of SI cortex will reduce the ability of RTN neurons to inhibit VPM neurons, increasing the short-latency responsiveness to multiple-whisker stimuli. In fact, that is exactly what we observed (Figs. 6, 7).

We propose that the long-latency, supralinear responses to whisker column stimuli are facilitated by the direct glutamatergic projection from SI cortex to VPM. Anatomically, corticothalamic fibers from a single barrel cortical column terminate not only the isomorphic whisker representation in the VPM, but also onto regions that represent a column of whiskers (Bourassa et al. 1995; Welker et al. 1988). For example, the corticothalamic fibers from the D1 barrel cortical column would terminate on the D1, C1, and E1 whisker representations in the VPM. Functionally, this SI cortex→VPM projection is slower than the SI cortex→RTN→VPM pathway for two reasons: (1) its terminations are on the distal dendrites of VPM neurons; (2) part of its excitatory action is likely to occur via NMDA and metabotropic glutamate receptors (Salt and Eaton 1996; Turner and Salt 1999). In contrast, the RTN→VPM synaptic interaction is mediated through fast-acting GABA$_A$ receptors followed by the relatively slow activating GABA$_B$ receptors. Thus, following SI cortical inactivation, the long-latency supralinear responses to whisker column stimuli (but not whisker row stimuli) inactivation of SI cortex should linearize these responses as the relatively delayed excitation to VPM neurons is removed (Figs. 6b, 8).

Corticofugal projections from layer V to the spinal trigeminal (SpV) nucleus of the brainstem could also influence VPM neuronal responses (Fig. 9). Elimination of this corticofugal projection causes SpV neurons to increase their responsiveness to whisker stimuli (Jacquin et al. 1990; Woolston et al. 1983). This increase in excitability is transmitted to the VPM, where it may increase responsiveness to multiple-whisker stimuli.

Conclusions: drivers and modulators

The influence of one neuron on another has been dichotomized as either a "driving" influence or a "modulatory" influence (Crick and Koch 1998; Sherman and Guillery 1998). In this scheme, "drivers" are the neurons that influence the RF properties and "modulators" are neurons that influence the probability of responses (Sherman and Guillery 1998). Sherman and Guillery (1996, 1998) argue that the anatomical and physiological evidence suggests that cortical feedback onto primary thalamic relays has only a modulatory influence on their response properties. Our results show that the role of cortical feedback onto a primary somatosensory thalamic relay cannot be clearly dichotomized. It appears to act as both a driver and a modulator, depending on what response features are being examined. Specifically, we demonstrated that feedback "drives" the spatiotemporal structure of RFs and "modulates" the nonlinearity of responses to multiple-whisker deflections. Such a dual influence is revealed only when the dimension of time is included in the analysis, and when different stimulus configurations are employed.

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