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# The Role of GABA-Mediated Inhibition in the Rat Ventral Posterior Medial Thalamus. II. Differential Effects of $GABA_A$ and $GABA_B$ Receptor Antagonists on Responses of VPM Neurons

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## SUMMARY AND CONCLUSIONS

*1.* Changes in the response properties of 106 ventral posterior medial (VPM) units were assessed after iontophoretic blockade of γ-aminobuturic acid-A or -B ($GABA_A$ or $GABA_B$) receptor-mediated inhibition using bicuculline methiodide (BIC) or 2-hydroxy-saclofen (2-OH-S), respectively.

*2.* The iontophoretic administration of either BIC or 2-OH-S did not alter significantly the average spontaneous firing rate of VPM neurons for current intensities between 40 and 80 nA. The presence of 10 mM 2-OH-S (60 nA) was effective in completely reversing the depressant effects of the selective $GABA_B$ receptor agonist, baclofen, on the spontaneous activity of VPM neurons.

*3.* The effect of BIC on whisker-evoked responses was a preferential enhancement in the responses elicited by the whisker giving rise to the highest probability response (center receptive field whisker or CRF). The effect of 2-OH-S (40–80 nA iontophoretic currents) was to increase the responsiveness of VPM neurons to the stimulation of whiskers in all parts of the receptive field (RF), although its influence was much more pronounced in the peripheral areas of the RF (surround receptive field whisker or SRF). This preferential enhancement of SRF-whisker responses after the blockade of $GABA_B$ receptor-mediated inhibition resulted in a 2.3-fold increase in the average RF size of VPM neurons; no statistically significant increases in the size of the RF were seen in the presence of BIC.

*4.* The primary influence of BIC and, to a lesser degree, 2-OH-S was to prolong the response duration of VPM neurons to CRF whisker stimulation. Under our recording conditions, ~25% of VPM neurons in normal animals responded with sustained discharges. In the presence of BIC and 2-OH-S, the percent of VPM units that could be classified as tonically responding increased to 82% and 67%, respectively.

*5.* The proportion of VPM neurons that was selective to the deflection of whiskers in a particular direction (87%) was not altered in the presence of BIC or 2-OH-S.

*6.* BIC was effective in antagonizing GABA-mediated inhibition within the first 40 ms of a stimulus; BIC was completely ineffective in reversing a late suppression seen between 80 and 140 ms. In contrast, no statistically significant changes in the initial GABA-mediated inhibition were seen in the presence of 2-OH-S, but 2-OH-S was partially effective in antagonizing the late suppression of responses in VPM neurons.

*7.* We conclude that $GABA_A$ receptor-mediated inhibition in the thalamic VPM nucleus of rats regulates the magnitude of the short-latency response to sensory stimulation. Our data suggest that $GABA_B$ receptor-mediated inhibition occurs at a time point significantly later than the fast, bicuculline-sensitive $GABA_A$ receptor-mediated inhibition. The late, 2-OH-S-sensitive suppression mediated by $GABA_B$ receptors is expressed at a time when the responses in VPM are dominated by the late-arriving, larger RF inputs, which were shown in a previous study to be mediated by the trigeminal subnucleus interpolaris.

## INTRODUCTION

In the companion article (Lee et al. 1994), changes in the receptive field (RF) size of ventral posterior medial (VPM) neurons were assessed after an excitotoxic destruction of the ipsilateral thalamic reticular nucleus (TRN). Because γ-aminobutyric acid (GABA)-mediated inhibition in rats arises almost exclusively from the ipsilateral TRN (Barbaresi 1986; Harris and Hendrickson 1987), our aim was to quantify the alterations in the RF properties of individual VPM neurons after an excitotoxic destruction of this nucleus. One of the striking features of the disinhibited responses after eliminating TRN was an immediate increase in the number of whiskers that could elicit responses in a given VPM neuron. This enlargement in the RF size was demonstrated to be an enhancement in the expression of the trigeminal subnucleus interpolaris input (Lee et al. 1994).

Results from other RF-mapping studies in the somatic thalamus of rats (Salt 1989) and cats (Curtis and Tebecis 1972; Duggan and McLennan 1971; Faingold et al. 1983; Hicks et al. 1986; Salt 1989) have failed to demonstrate significant changes in the RF size after iontophoretic administration of the $GABA_A$-receptor antagonist, bicuculline. In view of our results from the companion article (Lee et al. 1994) that the loss of inhibition from TRN leads to a threefold increase in the RF size of VPM neurons, we have reevaluated the effectiveness of bicuculline in altering the RF properties of VPM neurons under our recording conditions.

In addition, recent reports (Bowery et al. 1987; Chu et al. 1990) have indicated that the distribution of $GABA_B$-receptor binding sites in the ventrobasal thalamus is nearly as high as in other brain regions, such as the hippocampus, neocortex, and spinal cord, where the actions of $GABA_B$-receptor-mediated inhibition are well documented (Alger and Nicoll 1982; Connors et al. 1988; Curtis et al. 1988; Dutar and Nicoll 1988; Kaneko and Hicks 1988; Kerr et al. 1989). Therefore we have combined a rigorous quantitative analysis of the changes in the RF size and response dynamics of single units in VPM to controlled deflections of the vibrissae with an iontophoretic blockade of inhibition using a $GABA_A$-receptor antagonist, bicuculline meth-

iodide or a highly selective $GABA_B$-receptor antagonist, 2-hydroxy-saclofen (2-OH-S) (Curtis et al. 1988; Kerr et al. 1989).

A preliminary account of this work has appeared in abstract form (Lee et al. 1991).

## METHODS

The RF-mapping experiments were performed on 18 adult Long-Evans rats of either sex ranging in weight from 250 to 350 g (Charles River Laboratory). An additional six animals were used to stimulate electrically the trigeminal nucleus principalis (PrV) using a paired-pulse paradigm. The methods for maintaining animals during the recording session and single-unit RF mapping and analysis were identical to the companion article (Lee et al. 1994). Animals were maintained at the anesthetic stage III-3 as described in the companion article (Lee et al. 1994).

### *Iontophoretic techniques*

Single-unit recordings were obtained and the effects of the GABA antagonists measured using a five-barrel micropipette that contained an etched carbon fiber in one of the barrels (Armstrong-James and Fox 1983). The tip of the etched carbon fiber usually protruded beyond the other iontophoretic barrels by 15–20 $\mu$m. One of the barrels was filled routinely with 0.9% NaCl (pH 7.2) and used for current balancing. The remaining three barrels contained either bicuculline methiodide (BIC; 5 mM, pH 3.5; Sigma), L-glutamic acid (0.5 M, pH 7.5; Sigma), (±)-baclofen (BAC; 10 mM, pH 4.0; Research Biochemicals) or 2-OH-S (1 mM, pH 3.5; Research Biochemicals) depending on the specific experiment. A three-channel iontophoretic pump (Neurophore, Medical Systems) provided the ejection and retention currents as well as the automatic current balancing. Unless specified, the currents used for drug ejection varied from 40 to 120 nA and for retention, from 7 to 15 nA. The iontophoretic administration of bicuculline at these current intensities has been reported previously to be effective in antagonizing exogenously applied GABA in the VPM of rats (Salt 1989). The current intensity necessary for blocking $GABA_B$-receptor-mediated inhibition was estimated by antagonizing the effects of a selective $GABA_B$-receptor agonist, baclofen (Alger and Nicoll 1982), on the spontaneous firing of VPM neurons with 2-OH-S. In this study, no systematic comparisons were made between ejection currents and the changes seen in the response properties. Rather we have focused on quantifying the changes in the RF properties of individual neurons in the absence and presence of BIC and 2-OH-S at an intensity range where the effects of the drugs are more predictable.

### *Paired-pulse paradigm*

To selectively assess the time course of the feedback inhibition from TRN, a concentric bipolar stimulating electrode (diameter 100 $\mu$m; Rhoades) was placed in PrV using the coordinates derived from the Paxinos and Watson (1982) rat atlas (AP -9.5 mm, ML 2.7 mm, and D 7.5 mm from bregma). These stereotaxic coordinates were verified to be the appropriate location for vibrissa-evoked responses in PrV by crudely mapping each penetration using a tungsten microelectrode. The RF near the recording electrode was noted and the electrode was replaced with the stimulating electrode. From our previous study (Friedberg et al. 1989), PrV was known to lie directly underneath the transverse sinus.

Upon isolating a neuron in VPM whose RF was equivalent to the responses seen in PrV near the stimulating electrode, single shocks (100 $\mu$s in duration) were delivered to PrV that elicited short-latency (2–3 ms) responses in VPM. The intensity of the stimulus used was kept between 75 and 100 $\mu$A, as higher current intensities produced movement artifacts because of the current spread into the nearby motor trigeminal nucleus. If the isolated VPM unit could be activated orthodromically with a high response probability, i.e., every stimulus led to a response, a second shock of equal stimulus duration and intensity, was delivered at various interstimulus intervals. The typical interstimulus times tested were 10, 20, 40, 60, 80, 100, 120, 140, 160, 200, 500, and 1,000 ms. Each paired-pulse paradigm consisted of 15 trials at 0.33 Hz. After recording the normal time course of feedback inhibition from TRN, 40–83 nA of either BIC or 2-OH-S was administered iontophoretically for 3 min before reevaluating the feedback inhibition. Because the entire procedure for a single neuron lasted >30 min, the iontophoretic current was reduced by two-thirds after the 3-min application and left on for the duration of the paired-pulse measurements.

### *Histology*

After the termination of the experiments, the animals were killed and processed as described in the companion article (Lee et al. 1994). All thalamic recording sites were verified histologically to be within the dorsolateral aspect of VPM. Cases in which the paired-pulse paradigm were employed, horizontal sections were cut through the brain stem to verify the proper placement of the stimulating electrode.

### *Stimulus control and data acquisition*

Discrete, controlled movements of individual whiskers were carried out using a piezoelectric mechanical stimulator (Simons 1983). A metal probe attached to the piezoelectric stimulator deflected the whisker ~300 $\mu$m in one of four directions: up (U), down (D), backward (B), or forward (F). The whiskers were trimmed to a length of ~10 mm from the face, and the placement of the stimulator in relation to the whisker was kept constant at ~5 mm from the base of the whisker. The duration and frequency of the stimuli were maintained at 10 ms and 1 Hz, respectively, unless stated otherwise. The stimulator and data acquisition both were controlled by a computer interfaced through a series of high-speed clocks and memory modules (IBM-AT and Modular Instruments). The response to deflection of the whiskers was displayed "on-line" during the course of the experiment as a peristimulus time histogram (PSTH). A typical sampling time consisted of 300 ms of pre-stimulus activity and 200 ms sampling of poststimulus response time. The number of trials per stimulation protocol was kept constant throughout this study at 30 stimulus presentations. The 9 s of prestimulus activity (300 ms per trial × 30 trials) were used to calculate the spontaneous activity for each neuron.

All units isolated for this study were tested for sustained responsiveness. Responses in VPM units were classified as being tonically or phasically responding using the following criteria. After determining the RF map of a VPM neuron, the center receptive field (CRF) whisker was tested for tonic responsiveness using a 50-ms deflection of the whisker. If the first and second halves of the response period (the first 25 ms after stimulus onset) gave responses that were significantly higher than the prestimulus activity, the unit was classified as responding tonically. If either the first or second test periods failed to produce responses that were above background or if the second half of the response was <0.5 of the first half, the unit was classified as responding phasically. Typically, a tonically responding unit responded continually for the duration of the 50-ms deflection of a whisker.

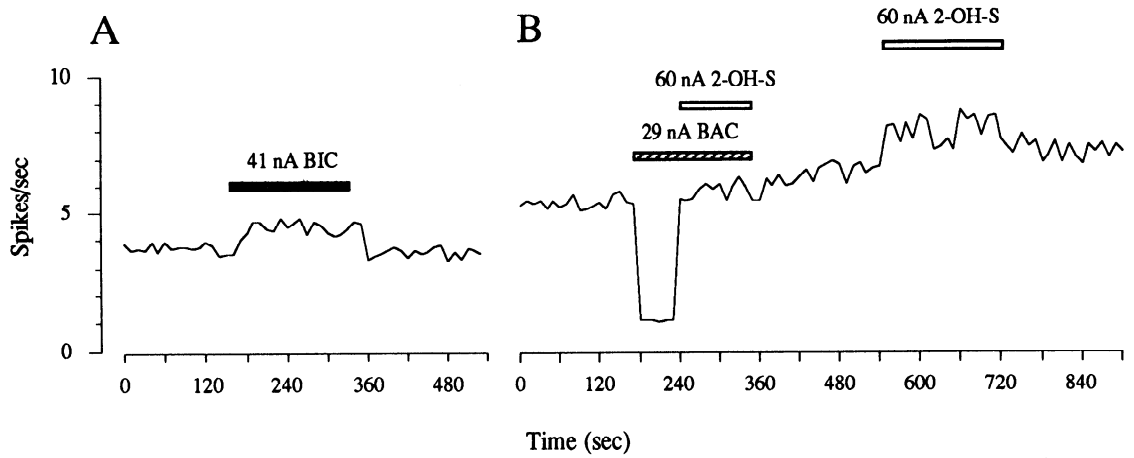


FIG. 1. Effect of bicuculline methiodide (BIC) and 2-hydroxy-saclofen (2-OH-S) on a spontaneously active neuron in ventral posterior medial (VPM). BIC and 2-OH-S had minimal effects on the average rate of firing for VPM neurons. *A*: rate of spontaneous firing for this representative VPM neuron increased from 3.7 to 4.5 spikes/s in the presence of 41-nA BIC. Most VPM neurons were unaltered in their spontaneous discharge in the presence of 40- to 83-nA BIC. *B*: spontaneous firing of VPM neurons was reduced by 80–100% with 29-nA baclofen (BAC). The selective γ-aminobutyric acid-B ($GABA_B$) receptor antagonist, 2-OH-S, was completely effective in reversing the depressant effect of BAC. 2-OH-S (60-nA) completely reversed the effects of BAC in this VPM neuron. The effects of 2-OH-S on the spontaneous firing of VPM neurons were variable. For this neuron, 60-nA 2-OH-S increased the spontaneous activity from 6.3 to 7.7 Hz. Bars indicate the duration of the drug injections. There were two reasons for using 29-nA for baclofen: it was the lowest injection current that reliably suppressed the spontaneous firing of VPM neurons and it was also the intensity used by Kaneko and Hicks (1988) to demonstrate the effect of BAC in SI cortex.

### *Data analysis*

For a cell discharge to qualify as a stimulus-evoked response during the response time period (2–50 ms after the onset of the stimulus), a distinct mode with ≥3 spikes had to accumulate in the PSTH at single latency after 30 stimulus presentations. The rationale for this criterion was based on the statistical improbability of three random events occurring at a given time bin. Each unit accepted as being responsive had a statistically significant increase in the rate of firing during the response time when compared with an equal duration of the prestimulus activity. These standards are similar to those reported previously in studies of the somatosensory cortex of rats (Armstrong-James and Fox 1987).

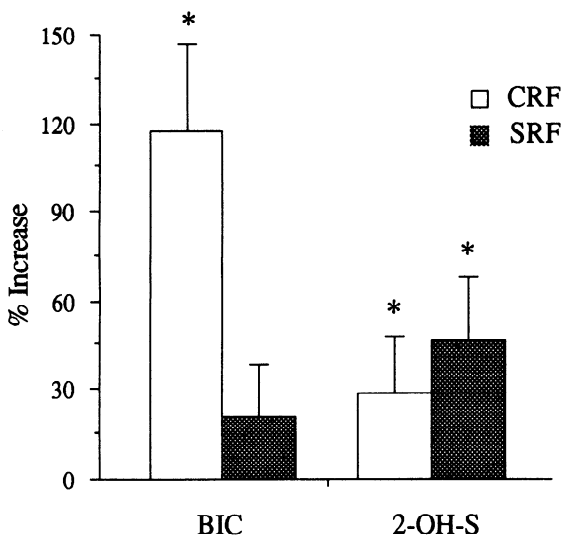


FIG. 2. Changes in the response magnitude of center receptive field (CRF) and surround receptive field (SRF) whisker evoked responses in the presence of BIC and 2-OH-S. BIC dramatically increased the number of spikes elicited by a 10-ms deflection of the CRF whisker, but was ineffective in altering the responses to the stimulation of SRF whiskers ($P > 0.20$, Student's $t$ test, $n = 63$). Iontophoretic application of 2-OH-S led to highly significant increases in the response magnitude (spikes per stimulus) for both the CRF and SRF whiskers ($n = 43$). The effect of 2-OH-S was proportionally greater on the SRF responses (*$P < 0.01$, Student's $t$ test). The number of spikes per stimulus for CRF and SRF whiskers before iontophoretic applications of BIC and 2-OH-S were 0.8 ± 0.12 and 4.5 ± 0.52 (means ± SE), respectively.

"Off-line" analysis of the data consisted of converting spike times to one of the following formats: response probability, response magnitude, response latency, and rate of spontaneous activity. *Response probability* was used as a measure of how well a unit followed a 1-Hz stimulation of a whisker in its RF. The maximum value of 1.0 indicated that the unit responded with at least one spike to each stimulus presentation. *Response magnitude* measured the average number of spikes per stimulus. This was computed as follows: Resp Mag = $[\Sigma(Spikes_R) - \Sigma(Spikes_{SA})] / NStim$ where $Spikes_R$ is total number of spikes during the response time, $Spikes_{SA}$ is total number of spikes during an equal length of the prestimulus sampling period, and *NStim* is the number of stimulus presentations. The *response latency* was determined as the mode of the latency histogram that plotted the frequency of the first spike during the response period. *Spontaneous activity* was computed as the average frequency during the prestimulus time in Hz.

For VPM units that were tested for direction selectivity in one of four directions, a vibrissa-responding unit was categorized as being "well tuned" if it responded vigorously to deflections of the CRF whisker in one direction, but displayed a null response to the opposite direction. Typically the difference in response probability between the best and worst directions for a well-tuned unit was ≥0.7. A neuron was classified as being "poorly tuned" if it responded to all four directions, but showed a statistically significant preference in one direction. All other units were classified as "not tuned."

Statistical significance was determined using a Student's $t$ test at a confidence level of $P < 0.05$. Changes in direction selectivity were tested using an $\chi^2$ test.

## RESULTS

Alterations in the responses of VPM neurons to discrete movements of the contralateral vibrissae were quantified

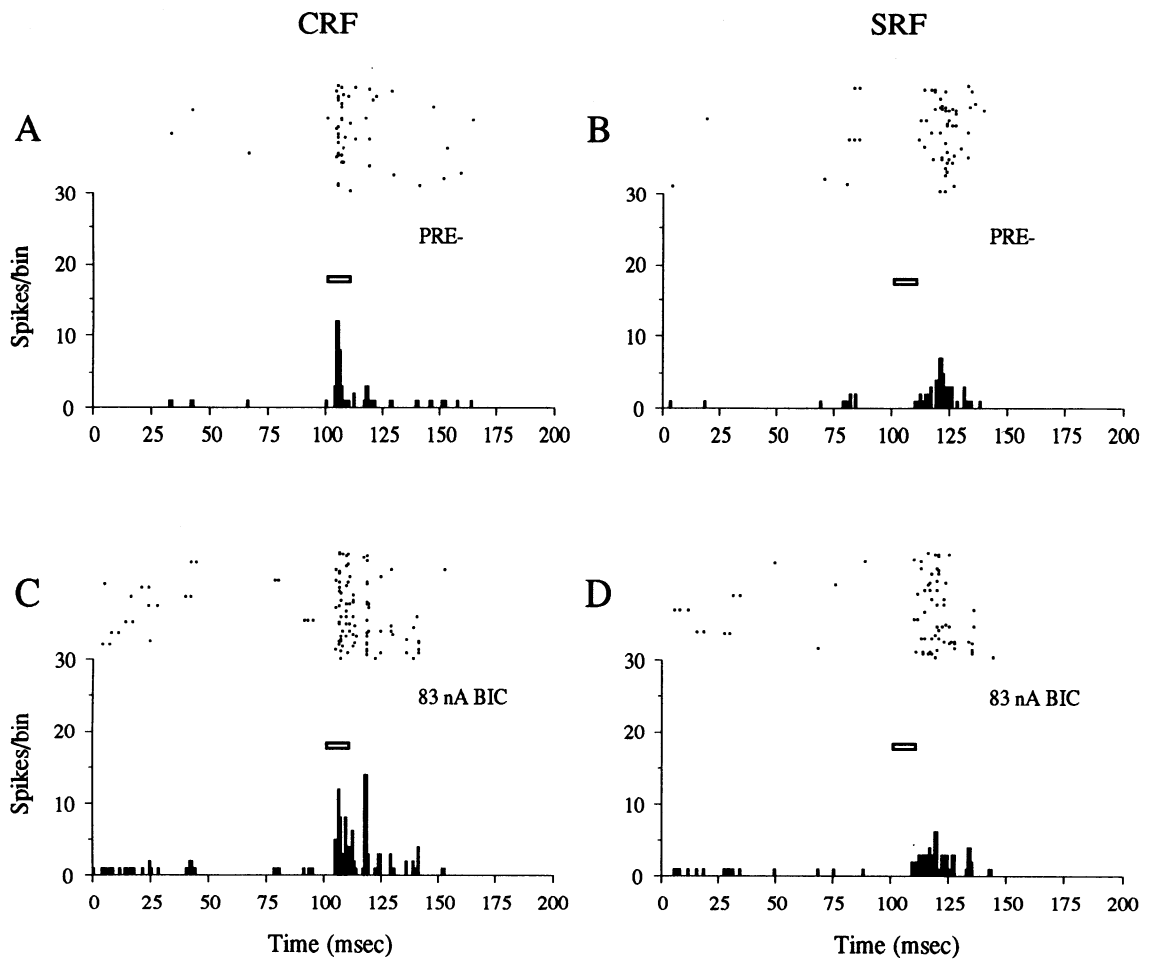


FIG. 3. Peri-stimulus time histograms (PSTH) of a representative VPM neuron to 30 stimulations of the CRF and SRF whiskers before (*A* and *B*) and after (*C* and *D*) 83-nA iontophoretic application of BIC. The duration of the whisker stimulus (10 ms) is indicated by the bars. The presence of BIC selectively increased the CRF-evoked responses, but failed in this atypical VPM neuron to convert the response characteristics from phasically responding to sustained discharges. The marked enhancement of the "off" response seen in this cell was not a consistent finding. Responses in VPM to whisker deflections were classified as being tonic or phasic by analyzing the response characteristics after increasing the stimulus duration of the CRF whisker to 50 ms (results not shown). The SRF-evoked responses (*B* and *D*) remained unaltered in the presence of BIC.

from 63 and 43 units before and after iontophoretic administration of BIC and 2-OH-S, respectively. The effect of the GABA antagonists on the time course and efficacy of the inhibitory feedback from TRN was assessed from 12 VPM units (6 with both BIC and 2-OH-S, 4 with 2-OH-S exclusively, and 2 with BIC alone).

### *Spontaneous activity*

The effect of BIC on thalamic activity in rats has been documented previously in detail (Curtis and Tebecis 1972; Duggan and McLennan 1971; Faingold et al. 1983; Salt 1989). In agreement with previous reports, the iontophoretic application of 40- to 80-nA BIC had minimal effects on the average spontaneous activity of VPM neurons. After a 1–2 min administration of 41 nA BIC, there was a greater tendency for individual neurons to fire spontaneous bursts of action potentials. Figure 1*A* shows a rare unit that increased its average spontaneous rate in the presence of BIC by 22%. The effect of 2-OH-S on spontaneous firing was similar to BIC. At iontophoretic current intensities shown to be effective in completely reversing the depressant effects of baclofen (Fig. 1*B*), the average firing rate of the VPM neuron depicted in Fig. 1 increased its rate only marginally in the presence of 2-OH-S.

The effectiveness of 2-OH-S in antagonizing the effect of a $GABA_B$ agonist, BAC, was less equivocal. Almost immediately after the onset of BAC iontophoresis in VPM, the spontaneous activity of units tested ($n = 12$) markedly decreased (Fig. 1*B*). The iontophoretic administration of 2-OH-S during the BAC application effectively reversed the depressant effects of BAC and returned the spontaneous firing to previous levels (Fig. 1*B*). The current intensities used to reverse the effect of 29-nA BAC ranged between 25 and 120 nA.

### *RF organization*

The effects of BIC and 2-OH-S on evoked responses in VPM were much more dramatic than on the spontaneous activity. The presence of BIC during the stimulation of the CRF whisker typically resulted in a dramatic increase in the number of spikes in response to a 10-ms deflection of the whisker. On average, the increase in the response of the

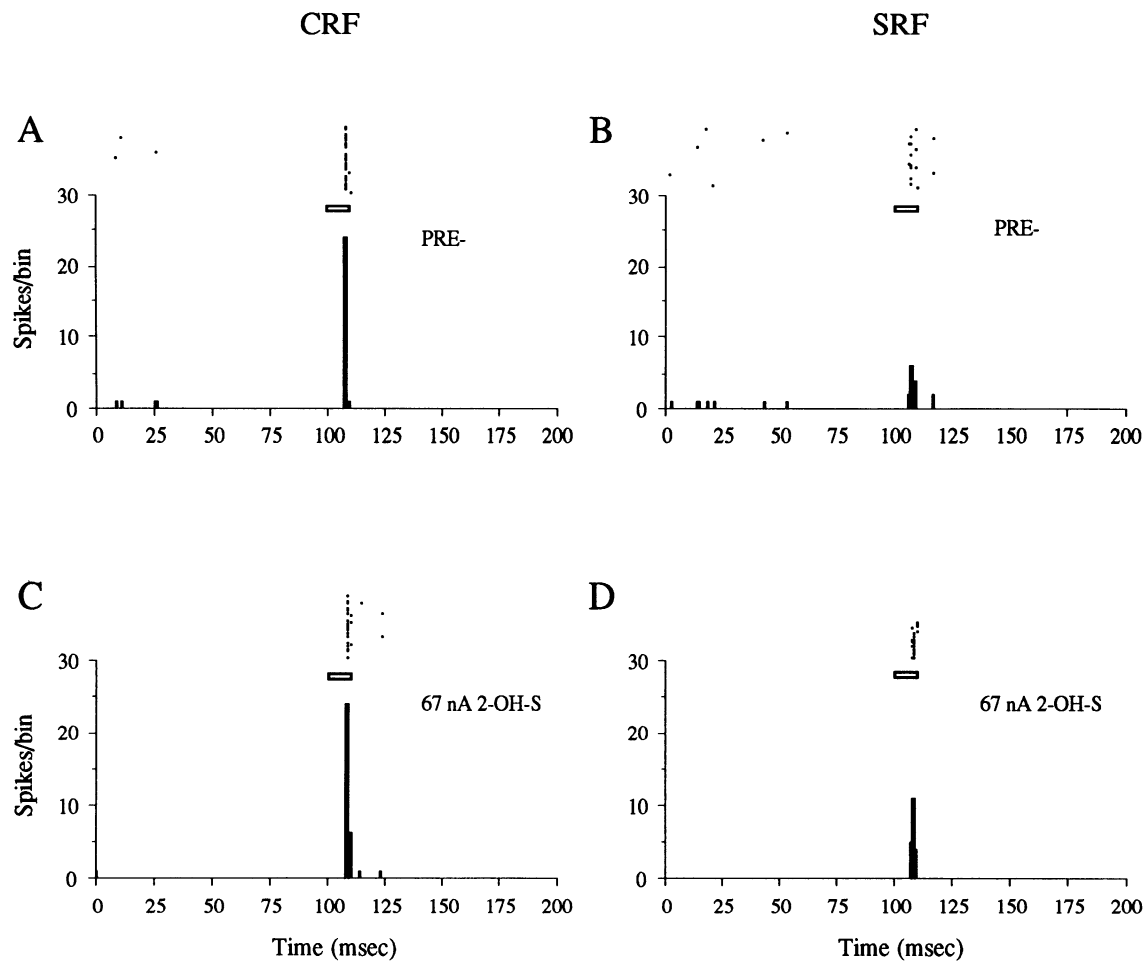


FIG. 4. PSTH of a representative neuron in VPM to 30 deflections of the CRF and SRF whiskers before (*A* and *B*) and after (*C* and *D*) 67-nA iontophoretic application of 2-OH-S. The effect of 2-OH-S for this VPM neuron was the opposite of the effects shown for BIC in Fig. 3; the CRF-evoked responses (*A* and *C*) remained unaffected by the 2-OH-S, whereas its presence nearly doubled the number of the spikes generated in the SRF-evoked response (*B* and *D*). The duration of the stimulus (10 ms) is indicated by the bars.

CRF whisker was 118% in the presence of 40- to 83-nA BIC. However, the surround receptive field (SRF) showed minimal enhancement in the responsiveness even after 120-nA BIC was ejected for 10 min. No statistically significant changes in the responsiveness of VPM neurons to the SRF whiskers were seen in the presence of BIC (Fig. 2). The effect of the $GABA_B$-receptor blockade during sensory stimulation was less dramatic than the enhanced responsiveness of the CRF whiskers seen in the presence of BIC. However, changes in the response elicited by 2-OH-S were almost identical for both the CRF and SRF whiskers (Fig. 2). The average increase in the responsiveness to CRF- and SRF-whisker stimulations was ~40% which was significant at the $P < 0.01$ level.

Examples of responses to the CRF and SRF whiskers before and after the iontophoretic administration of the two antagonists employed in this study can be seen in Figs. 3 and 4. The single-unit PSTH generated in response to the CRF whisker seen in Fig. 3*A* was typical of those seen in VPM under normal recording conditions. A VPM unit characteristically gave one or two spikes at a short latency (~6 ms) during the onset of the stimulus. Responses to the SRF whiskers, when present, occurred at variable latencies, generally 2–15 ms later than the CRF whisker (Fig. 3*B*). The iontophoretic administration of 40- to 83-nA BIC invariably increased the evoked response to the CRF whisker, as exemplified by the VPM neuron in Fig. 3 *C*. This unit increased its "off" response when the deflected whisker was released. This acute sensitivity to the change in the position of the whiskers has been reported previously to be a characteristic of VPM neurons (Simons and Carvell 1989; Sugitani 1979; Waite 1973). Our data suggests that blocking GABA-mediated inhibition did not alter drastically the nature of the response, but enhanced its expression. For the represented neuron in Fig. 3 and most units tested, BIC had a minimal effect on the SRF (Fig. 3 *D*).

The effects of 2-OH-S were much more subtle. Although in some cases, antagonizing $GABA_B$-receptor-mediated inhibition showed a similar effect to BIC, i.e., increased the number of spikes per stimulus to CRF-whisker stimuli, its effects on the SRF were greater than those of BIC. Figure 4 provides such an example. A 3-min iontophoresis of 67-nA 2-OH-S was ineffective in altering the response properties to CRF-whisker stimulation. In fact, the evoked response to the CRF whisker remained identical to preiontophoresis measurements (Fig. 4, *A* vs. *C*). However, the same drug application led to a 60% increase in the number of spikes generated in response to a SRF whisker (Fig. 4, *B* vs. *D*).

The RF size seen in VPM normally (preiontophoresis) and in the presence of BIC and 2-OH-S is summarized in

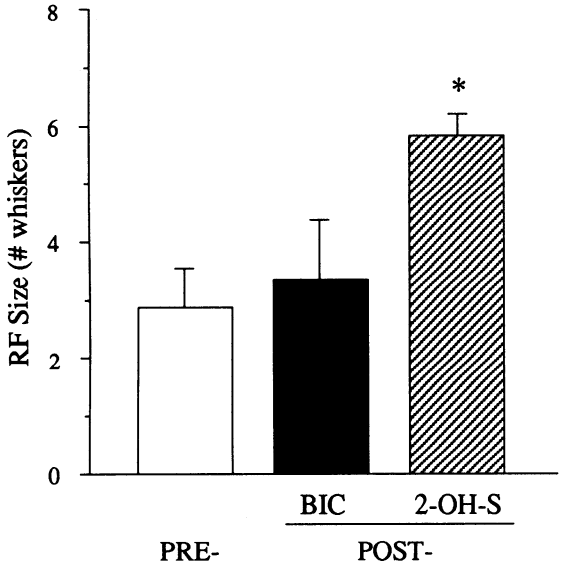


FIG. 5. Average receptive field size of 63 VPM units assessed after BIC and 43 units after 2-OH-S application. The average RF size in the presence of BIC was identical to preantagonist levels. The increase in the RF seen after iontophoretic application of 2-OH-S (40 – 80 nA) was mainly because of an enhanced responsiveness to SRF whisker stimulation. (*$P <$ 0.001, Student's $t$ test).

Fig. 5. The average number of whiskers that evoked responses in VPM neurons remained nearly identical to predrug measurements after BIC iontophoresis (2.87 ± 0.68 vs. 3.33 ± 1.04 whiskers, means ± SE). However, the average RF size in the presence of 2-OH-S enlarged to an average of 5.8 whiskers (± 0.37), which was highly significant ($P < 0.001$). Although this enlargement remains smaller than the changes seen after a complete loss of inhibition by lesioning TRN (Lee et al. 1994), these results suggest that $GABA_B$-receptor-mediated inhibition may play a crucial role in modulating the RF size in VPM.

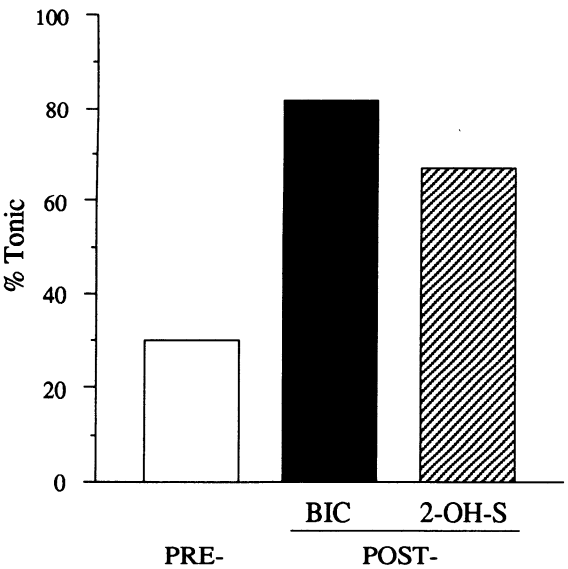


FIG. 6. Proportion of units that was classified as tonically responding before and after iontophoresis of BIC ($n$ = 63) and 2-OH-S ($n$ = 43). In contrast to the response profiles of VPM neurons before iontophoretic application of the antagonists, most neurons responded for the duration of a 50-ms deflection of the CRF whiskers in the presence of BIC and 2-OH-S.

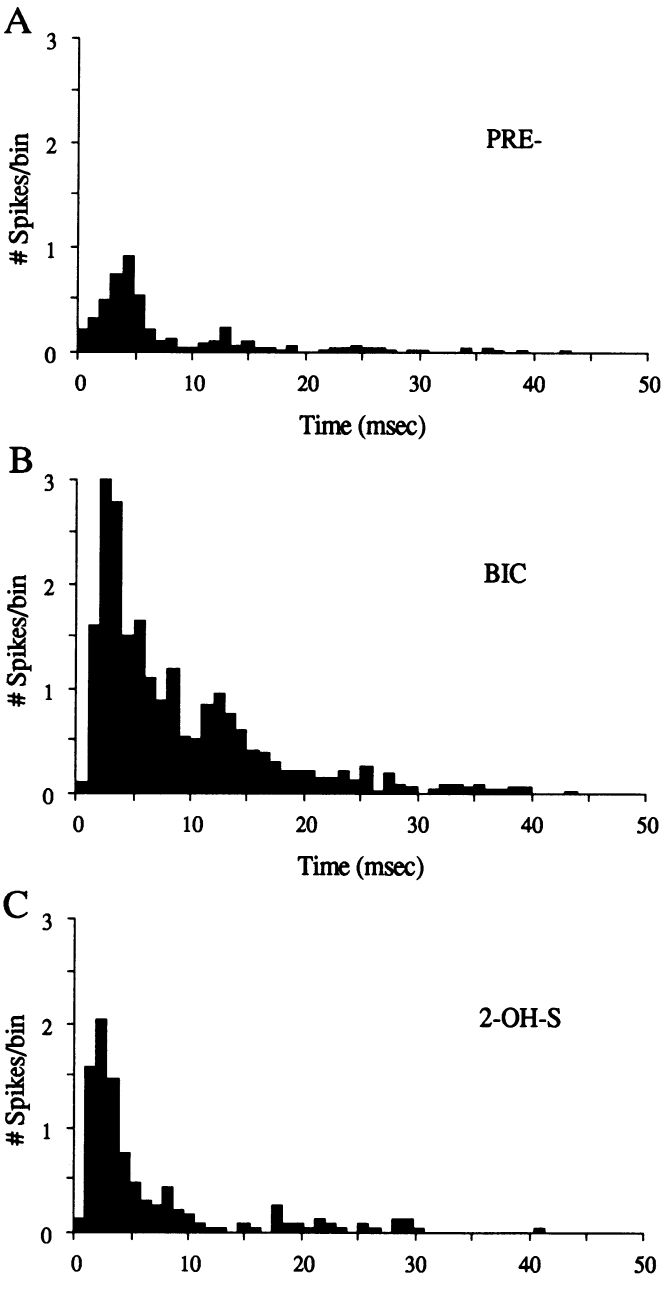


FIG. 7. Evoked interspike interval histograms (ISIH) for ($A$) VPM unit responses before antagonist applications ($n$ = 106), ($B$) responses in the presence BIC ($n$ = 63), and ($C$) under 2-OH-S ($n$ = 43). Each ISIH was averaged during the 2- to 50-ms response period after the onset of CRF whisker stimulations (stimulus duration, 10 ms). Each bin consists of 1 ms. Area under the histograms indicates the number of spikes elicited by the 30 whisker stimulations. Shortening of the mode for responses under BIC and 2-OH-S (3 vs. 5 ms mode for preiontophoresis responses, $P <$ 0.05, Student's $t$ test) indicates that whisker-evoked spikes were occurring closer together in the presence of BIC and 2-OH-S.

### *Response dynamics*

As demonstrated in the companion article (Lee et al. 1994), a prominent characteristic of VPM neurons as a result of the loss of GABAergic inhibition is the marked increase in the proportion of cells responding tonically to prolonged deflections of the whiskers. The proportion of units giving tonic responses before the application of antagonists was ~30%, which is nearly identical to previous find-

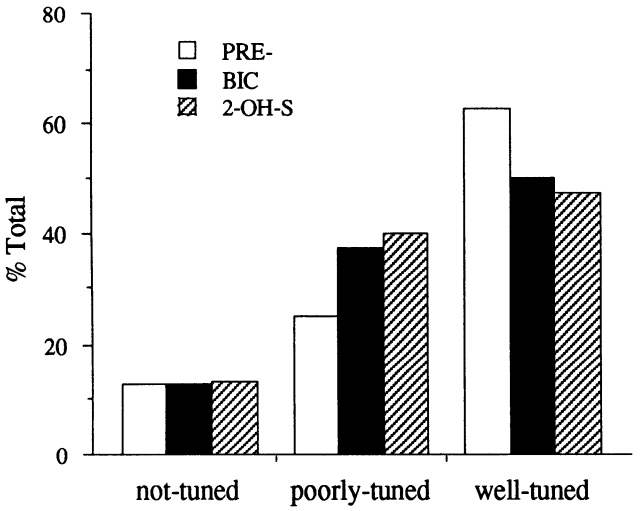


FIG. 8. Proportion of units untuned, poorly tuned, and well tuned to the deflection of CRF whiskers in one of four directions: up, down, backward, and forward for (*A*) control and in the presence of (*B*) BIC ($n = 63$), and (*C*) 2-OH-S ($n = 43$). Ratio of VPM units insensitive to direction remained unchanged in the presence of the GABA antagonists ($P > 0.10$, $\chi^2$ test).

ings (Ito 1988; Lee et al. 1994; Simons and Carvell 1989; Sugitani 1979; Waite 1973). For BIC treated units, nearly 84% of the population gave responses that were classified as being tonic. The ratio of units giving tonic responses after 2-OH-S was not quite as high (67%), but more than doubled the number seen in normal VPM (Fig. 6).

An interspike interval histogram (ISIH) graphically illustrates how the response dynamics changed in the presence of the GABA-receptor antagonists (Fig. 7). The mode of ISIH for the population of VPM neurons before drug applications centered around 5-ms interspike intervals (Fig. 7*A*). The changes in the magnitude of the response after disinhibition can be seen by comparing the areas under the histograms. The evoked response in VPM in the presence of BIC dramatically increased the number of spikes and drastically shortened the time between evoked action potentials (Fig. 7*B*; mode = 3 ms; $P < 0.05$, Student's $t$ test). The mode of the ISIH after 2-OH-S application was as similar to BIC treated cases, but the average number of evoked spikes were significantly less than with BIC (Fig. 7*C*).

Blocking GABAergic inhibition with either BIC or 2-OH-S had similar effects in terms of changing the response dynamics. There was an increase in the number of spikes generated in response to a brief, 10-ms stimulus, and more than two-thirds of units responded for the entire duration of a prolonged, 50-ms stimulus.

### *Direction sensitivity*

As in the companion article, the proportion of units not direction sensitive, poorly sensitive, and well tuned to the movement of whiskers in a particular direction was measured for predrug units and after iontophoretically blocking inhibition (Fig. 8). The ratio of VPM neurons selective to the movement of whiskers to a certain direction to units that were not selective remained unaltered even in the presence of GABA antagonists. There appeared to be some shift of well-tuned units to ones that were poorly tuned, but the changes were not statistically significant ($P > 0.20$, $\chi^2$ test)

### *Feedback inhibition*

The results of the paired-pulse paradigm for the 12 units tested prior to the application of BIC or 2-OH-S is plotted in Fig. 9. After the initiation of an action potential in a thalamocortical relay neuron in VPM, no subsequent spikes could be elicited in the same neuron for a period of ≤40 ms. The response to the second stimulus became indistinguishable from the first in terms of the response magnitude only when the interstimulus time was >200 ms (Fig. 9). Figure 10 illustrates PSTHs for a representative VPM neuron using the paired-pulse paradigm at various interstimulus times.

In the presence of BIC, all VPM units tested ($n = 8$) were able to generate a response to a second stimulus within the 40-ms, early inhibitory period. The response to the second stimulus varied from 47 to 110% (in response magnitude) of the first response (Fig. 11). In six out of eight neurons tested, there was a late BIC insensitive suppression in the response (Fig. 11). In such units, the iontophoretic application of 2-OH-S did not affect the early (<40 ms) suppression but greatly attenuated the late depression in the response (Fig. 12).

## DISCUSSION

The results from this study confirm a previous report that the blockade of $GABA_A$-mediated inhibition with BIC fails to unmask a larger excitatory RF in the VPM thalamic nucleus of rats (Salt 1989) or cats (Hicks et al. 1986). This finding was unexpected in view of the results from the preceding study (Lee et al. 1994) that the loss of inhibitory input produced by a lesion of TRN resulted in a three- to fivefold increase in the number of whiskers that normally evoked responses in a typical VPM neuron.

Several speculations have been offered for the failure of BIC to alter the cutaneous representation in somatic sensory thalamus: *1*) the concentration of BIC iontophoretically released may be insufficient to completely antagonize the GABA-mediated inhibition, *2*) the depth of anesthesia

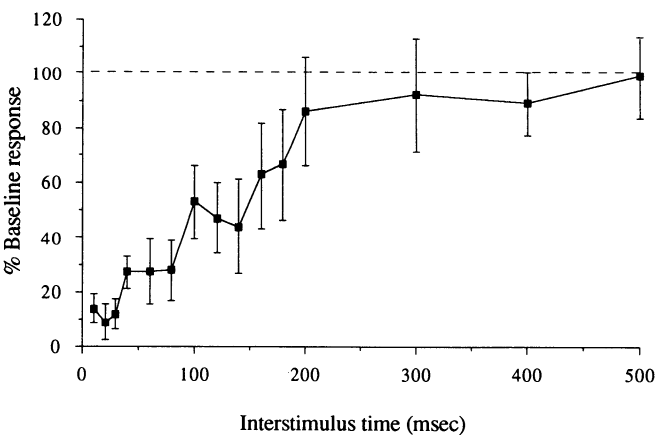


FIG. 9. Response of VPM neurons to the second of paired electrical stimuli to trigeminal nucleus principalis (PrV), expressed as a percentage of the response to the first pulse (baseline response). Baseline response refers to the response of VPM neurons to the first stimulus of a paired stimuli. Each data point represents the average response of 12 VPM units to 15 paired stimuli trials. The abscissa represents the delay between the 2 stimuli. The feedback inhibition from TRN suppressed nearly all responses in VPM for 40 ms after the initiation of an action potential (see text for description of the methods).

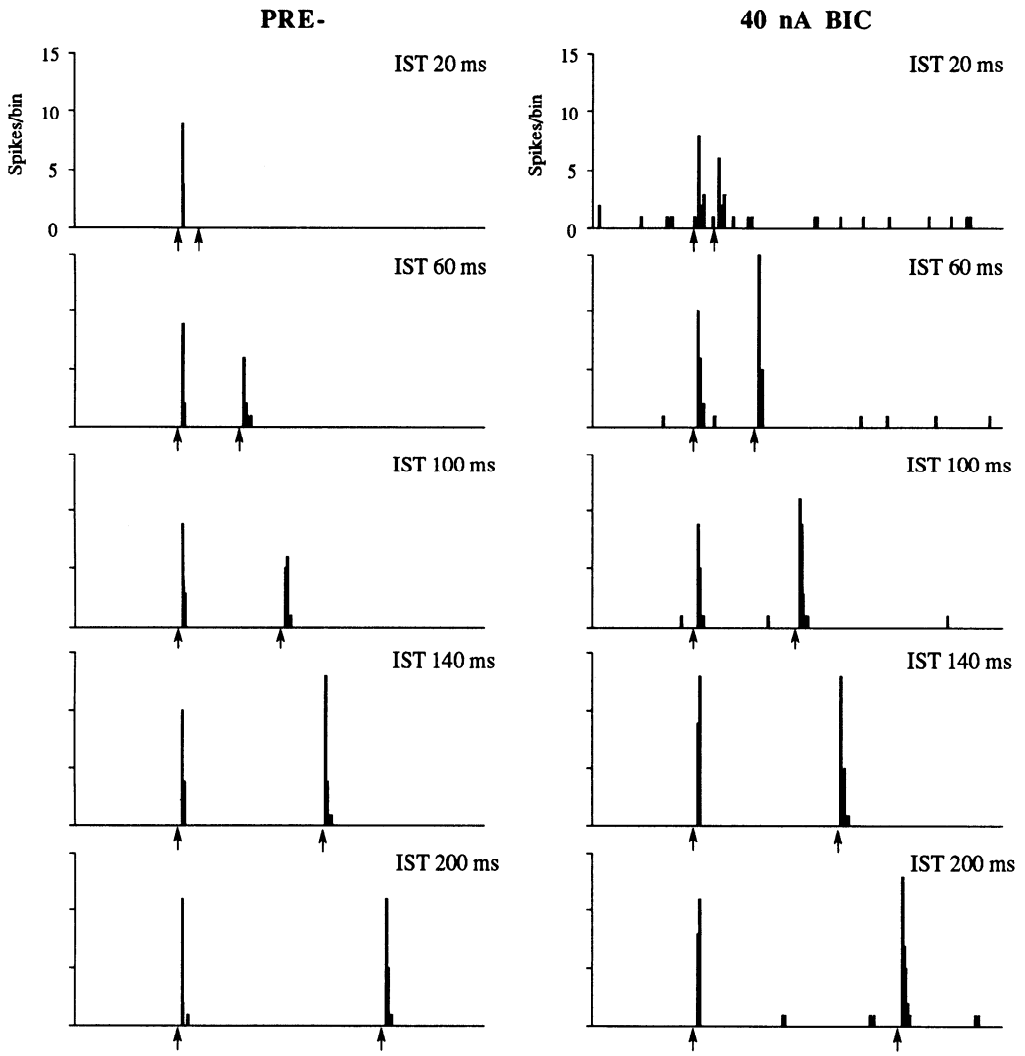


FIG. 10. Effect of BIC on a representative VPM neuron using the paired-pulse procedure as in Fig. 9. VPM units, as exemplified by this neuron, were generally unable to respond to the second of 2 electrical stimuli at interstimulus times between 0 and 40 ms. The response to the second stimulus became indistinguishable from the first only at interstimulus intervals >100–120 ms. However, significant responses were evoked by the second stimulus at interstimulus times as short as 10 ms in the presence of BIC. Arrows indicate the occurrence of the first and second stimuli.

may affect the expression of RFs in the thalamus, and *3*) $GABA_B$-mediated inhibition may play a crucial role in modulating the RF size in the thalamus. Although the feedback inhibition from TRN could not be abolished completely for all neurons tested in this study using the paired-pulse paradigm, nearly 90% of the feedback inhibition was overcome in the presence of 47-nA BIC (for example, compare percent suppression with and without the presence of BIC, Fig. 11, *A* and *B*). Further, no changes in the RF size were seen even when the 80-nA BIC was applied for nearly 30 min. Thus it appears unlikely that amount of BIC released was insufficient to show some of the changes seen in the preceding study after an excitotoxic lesion of TRN.

The level of anesthesia in this study was monitored at all times using the dominant electrocorticogram frequency and various other physiological signs to estimate the depth of the anesthesia at the time each unit was analyzed. Previous results from this laboratory (Friedberg et al. 1991) have indicated that the RF size of relay neurons in the thalamic VPM nucleus of rats decreases in proportion to the depth of anesthesia. As discussed in detail in the companion article (Lee et al. 1994), the level of anesthesia we have adopted as our standard recording conditions produces an average RF size in VPM neurons of 2.7 whiskers. At this anesthetic depth, the spontaneous activity in VPM neurons, which ranged from 0.5 to 10 Hz, was present in ~87% of the neurons isolated. Thus the failure of BIC to unmask additional excitatory connections cannot simply be attributed to the depressive effects of anesthesia seen at deeper anesthetic depths.

The interpretation that we favor and have focused on in this study is the unique role that $GABA_B$-mediated inhibition may play in the somatic thalamus of rats. Nearly all VPM neurons included in this study showed a remarkable sensitivity to the $GABA_B$ agonist, BAC and further, the depressant effect of the BAC application on neuronal activity could be completely reversed by the $GABA_B$ antagonist, 2-OH-S. In addition, the majority (75%) of neurons tested for the effectiveness of 2-OH-S in altering evoked activity showed a late (80 – 150 ms) suppression in the response, which was insensitive to BIC but partially reversed in the presence of the $GABA_B$ antagonist.

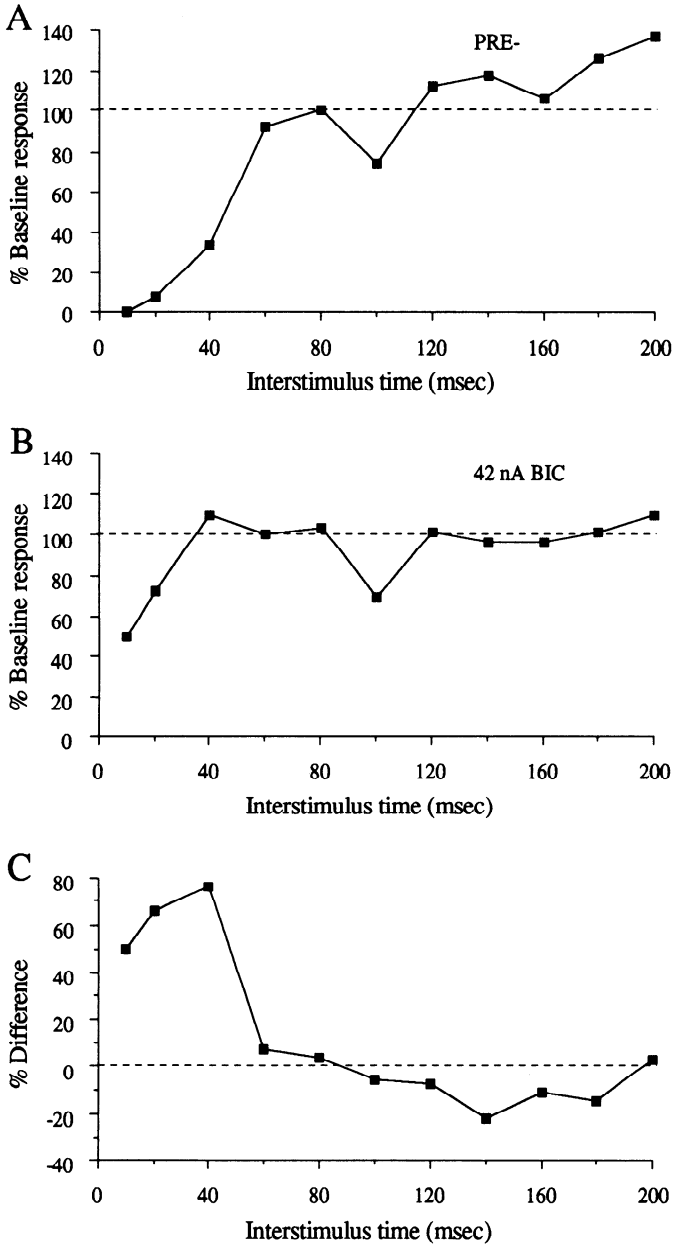


FIG. 11. Change in the efficacy of feedback inhibition before (*A*) and after (*B*) iontophoretic application of BIC during the paired-pulse procedure for a representative VPM neuron (15 paired stimuli). Baseline response refers to the response of VPM neurons to the first stimulus of a paired stimuli. *C*: difference between the 2 plots. The presence of BIC during the paired-pulse procedure returned nearly 50–80% of the response during the initial 40 ms after the first stimulus. Note the longer-latency suppression, which was insensitive to BIC. This late suppression was seen in six out of eight VPM neurons tested using the paired-pulse paradigm in the presence of GABA antagonists.

Although the contribution of $GABA_B$-mediated inhibition in rat sensory thalamus has not yet been reported, Kaneko and Hicks (1988) have provided evidence that the primary effect of BAC was to suppress more selectively the responses of cat SI neurons that were evoked from the peripheral areas of the receptive field rather than the central regions. Our results showing that $GABA_B$-receptor blockade preferentially enhanced the surround receptive field responses in the rat thalamus, are consistent with their findings in neocortex.

A recent study by Connors et al. (1988) using an in vitro slice preparation of rat SI cortex has indicated that pyramidal neurons in layers II and III can generate two distinct inhibitory postsynaptic potentials (IPSPs) to electrical stimuli or exogenous applications of GABA. A short-latency, BIC-sensitive fast inhibition was accompanied by a large membrane conductance change and a longer-latency, BIC-insensitive inhibition that resulted in a proportionally smaller conductance change. In direct relevance to this study, they have reported a unique contribution of the "late" $GABA_B$-receptor-mediated IPSP (1-IPSP) in modulating the frequency of repetitive action potentials induced

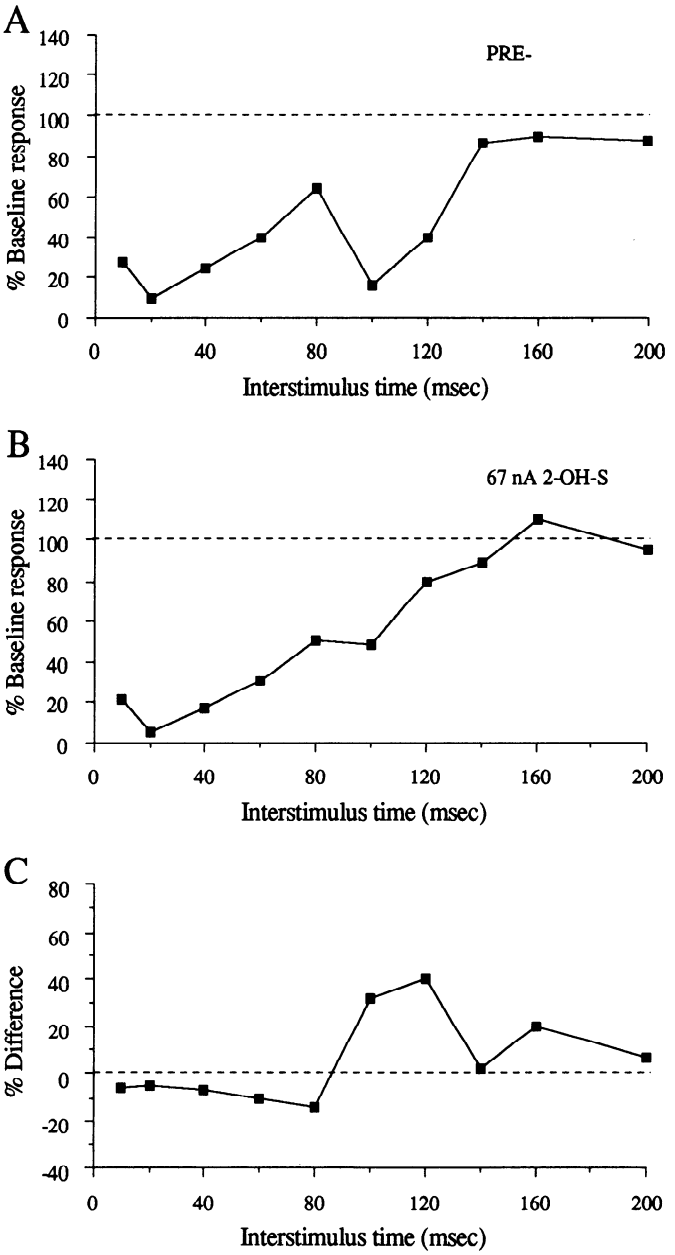


FIG. 12. Change in the efficacy of feedback inhibition before (*A*) and after (*B*) iontophoretic application of 2-OH-S during the paired-pulse procedure for a representative VPM neuron (15 stimuli). Baseline response refers to the response of VPM neurons to the first stimulus of a paired stimuli. The presence of 2-OH-S was ineffective in reversing the early feedback inhibition. However, the longer-latency suppression seen in six out of eight neurons assessed, was blocked by the iontophoretic application of 2-OH-S during the paired-pulse procedure (*C*).

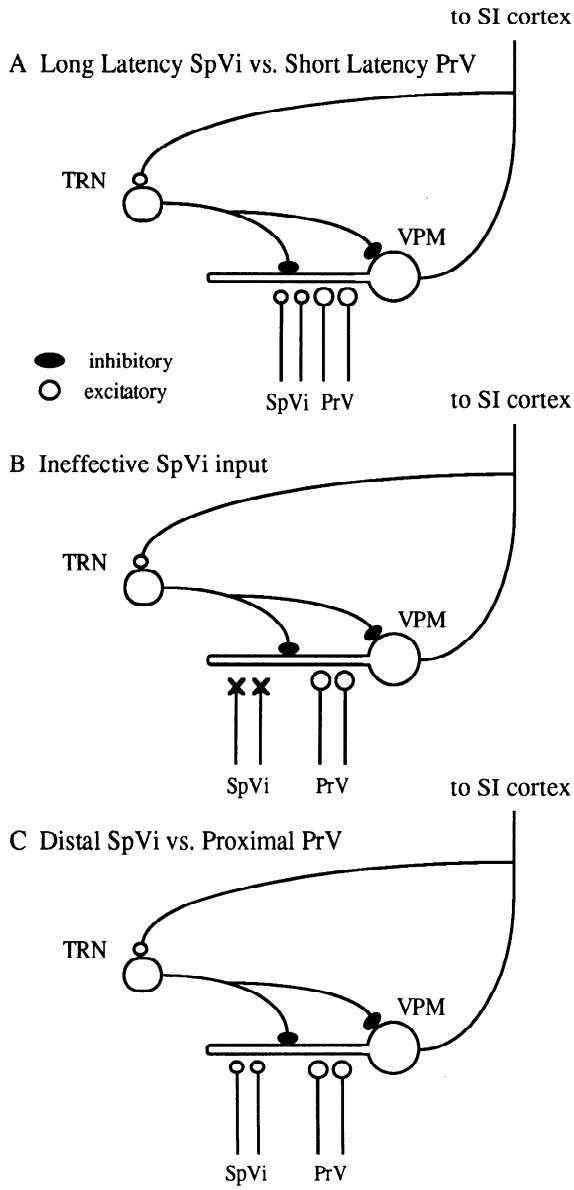


FIG. 13. Schematic diagrams of the proposed influence of TRN-mediated inhibition on the 2 trigeminal inputs related to whisker sensation. The precise, feedback inhibition from TRN, which primarily synapses on the cell body and proximal dendrites (Peschanski et al. 1983), is the only form of inhibition in the rat thalamic VPM nucleus. The loss of this GABA-mediated inhibition, e.g., lesioning of TRN (Lee et al. 1994) or iontophoretic blockade of $GABA_B$ receptors, results in an enhanced expression of trigeminal subnucleus interpolaris (SpVi) inputs as manifest by the marked increase in the receptive field size. The GABA-mediated suppression of the SpVi inputs may arise in 1 of 3 ways as illustrated in the figure. In hypothesis *A*, a dominant, short-latency input from PrV invokes $GABA_A$- and $GABA_B$-receptor-mediated inhibition, which minimize the influence of SpVi whose input arrives in VPM at a significantly longer latency. In hypothesis *B*, the SpVi input may be ineffective or "weak" synapses as proposed by Rhoades et al. (1987). These synapses are unable to evoke action potentials in VPM neurons. In hypothesis *C*, the influence of SpVi remains minimal because of a "shunting" inhibition that is proximal to the SpVi input (see text for further discussion).

by a direct depolarizing current injection. At low current injection intensities, the 1-IPSP effectively suppressed the repetitive firing, but at higher current levels, the initial frequency of firing was slightly enhanced. These results lead to the notion that $GABA_B$-receptor-mediated inhibition, demonstrated in this study to regulate the extent of functional convergence onto a single VPM neuron, may have a unique role in modulating the firing pattern of VPM neurons to prolonged deflections of multiple whiskers in their receptive field.

The trigeminothalamic circuitry depicted in Fig. 13 for rat VPM is somewhat simpler than the ones found in cats and monkeys primarily because of the near absence of interneurons in the rat VPM (Barbaresi et al. 1986; Harris 1986; Harris and Hendrickson 1987). The feedback inhibition from TRN has been shown in this study and others (Salt 1989; Simons and Carvell 1989) to be effective in suppressing evoked responses in VPM for ~30 - 40 ms after a VPM neuron fires an action potential. Given the result from the companion paper (Lee et al. 1994) that the loss of inhibitory drive from TRN results in an enhanced expression of trigeminal subnucleus interpolaris input, we propose that the level of inhibition from TRN determines the relative strength of the two trigeminal inputs, PrV and interpolaris (SpVi) (Fig. 13, *A–C*). Our data from this and the companion paper demonstrate that the receptive fields in VPM can be enlarged almost immediately by blocking $GABA_B$-receptor-mediated inhibition or by destroying the TRN. The enlargement seen was dependent on the presence of SpVi as its destruction in TRN-lesioned cases led to a dramatic shrinkage in the receptive field size of VPM neurons (Lee et al. 1994). These results indicate that the SpVi input to VPM is not an ineffective or "weak" pathway as suggested by Rhoades et al. (1987) (hypothesis *B* in Fig. 13), but rather appears to be regulated by the inhibition from TRN (hypothesis *A* and/or *C* in Fig. 13).

Previous results from our laboratory (Friedberg et al. 1989, 1991; Lee et al. 1990) have shown that the PrV and SpVi inputs related to whisker sensation relay two fundamentally different types of information. The PrV pathway is dominated by a short-latency, sensory-mediated activity related to a single whisker. The SpVi pathway, on the other hand, relays information from a number of different whiskers at a significantly longer latency than the PrV input. The response latencies in VPM to whisker stimulation range from 4 to 11 ms when the SpVi pathway is removed; in contrast, the range of latencies increases to 40 ms when the PrV pathway is selectively destroyed (Friedberg et al. 1991). In view of these findings, the late IPSP mediated by the $GABA_B$ receptors appears to play a crucial role in determining the relative strength of the long-latency input from SpVi.

Thus we propose two unique roles for the GABAergic inhibition in rat VPM arising primarily from the TRN. The fast $GABA_A$-receptor-mediated inhibition can modulate the strength of sensory activation without altering the receptive field characteristics of the sensory input, e.g., size and directional information. To a lesser extent, the late $GABA_B$-receptor-mediated inhibition regulates the efficacy of sensory input, but perhaps more importantly, can integrate the inputs from a larger sensory area.

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## REFERENCES


ALGER, B. E. AND NICOLL, R. A. Pharmacological evidence for two kinds of GABA receptors on rat hippocampal pyramidal cells studies *in vitro*. *J. Physiol. Lond.* 328: 125–141, 1982.

ARMSTRONG-JAMES, M. AND FOX, K. Effect of iontophoresed noradrenaline on the spontaneous activity of neurons in rat primary somatosensory cortex. *J. Physiol. Lond.* 355: 427–447, 1983.

ARMSTRONG-JAMES, M. AND FOX, K. Spatiotemporal convergence and divergence in the rat SI "barrel" cortex. *J. Comp. Neurol.* 263: 265–281, 1987.

BARBARESI, P., SPREAFICO, R., FRASSONI, C., AND RUSTIONI, A. GABAergic neurons are present in the dorsal column nuclei but not in the ventroposterior complex of rats. *Brain Res.* 382: 305–326, 1986.

BOWERY, N. G., HUDSON, A. L., AND PRICE, G. W. $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. *Neuroscience* 20: 365–383, 1987.

CHU, D. C. M., ALBIN, R. L., YOUNG, A. B., AND PENNEY, J. B. Distribution and kinetics of $GABA_B$-binding sites in rat central nervous system: a quantitative autoradiographic study. *Neuroscience* 34: 341–357, 1990.

CONNORS, B. W., MALENKA, R. C., AND SILVA, L. R. Two inhibitory postsynaptic potentials and $GABA_A$- and $GABA_B$-receptor-mediated responses in neocortex of rat and cat. *J. Physiol. Lond.* 406: 443–468, 1988.

CURTIS, D. R., GYNTHER, B. D., BEATTIE, D. T., KERR, D. I. B., AND PRAGER, R. H. Baclofen antagonism by 2-hydroxy-saclofen in the cat spinal cord. *Neurosci. Lett.* 92: 97–101, 1988.

CURTIS, D. R. AND TEBECIS, A. K. Bicuculline and thalamic inhibition. *Exp. Brain Res.* 16: 210–218, 1972.

DUGGAN, A. W. AND MCLENNAN, H. Bicuculline and inhibition in the thalamus. *Brain Res.* 25: 188–191, 1971.

DUTAR, P. AND NICOLL, R. A. A physiological role for $GABA_B$ receptors in the central nervous system. *Nature Lond.* 332: 156–158, 1988.

FAINGOLD, C. L., HOFFMANN, W. E., AND CASPARY, D. M. Bicuculline-induced enhancement of sensory responses and cross-correlations between reticular formation and cortical neurons. *Electroencephalogr. Clin. Neurophysiol.* 55: 301–313, 1983.

FRIEDBERG, M. H., LEE, S. M., AND EBNER, F. F. Anesthetic stage as a determinant of VPM receptive field properties. *Soc. Neurosci. Abst.* 17: 324, 1991.

FRIEDBERG, M. H., LEE, S. M., WEISSKOPF, M. G., AND EBNER, F. F. Plasticity in the rat trigeminal somatosensory pathway: return of cortically dependent behavior following lesion of main V nucleus. *Soc. Neurosci. Abst.* 15: 167, 1989.

HARRIS, R. M. Morphology of physiologically identified thalamocortical relay neurons in the rat ventrobasal thalamus. *J. Comp. Neurol.* 251: 491–505, 1986.

HARRIS, R. M. AND HENDRICKSON, A. E. Local circuit neurons in the rat ventrobasal thalamus—a GABA immunocytochemical study. *Neuroscience* 21: 229–236, 1987.

HICKS, T. P., METHERATE, R., LANDRY, P., AND DYKES, R. W. Bicuculline-induced alterations of response properties in functionally identified ventroposterior thalamic neurones. *Exp. Brain Res.* 63: 248–264, 1986.

HOUSER, C. R., VAUGHN, J. E., BARBER, R. P., AND ROBERTS, E. GABA neurons are the major cell types of the nucleus reticularis thalami. *Brain Res.* 200: 341–354, 1980.

ITO, M. Response and topography of vibrissae sensitive VPM neurons in the rat. *J. Neurophysiol.* 60: 1181–1197, 1988.

KANEKO, T. AND HICKS, T. P. Baclofen and γ-aminobutyric acid differentially suppress the cutaneous responsiveness of primary somatosensory cortical neurones. *Brain Res.* 443: 360–366, 1988.

KERR, D. I. B., ONG, J., JOHNSTON, G. A. R., ABBENATE, J., AND PRAGER, R. H. 2-Hydroxy-saclofen: an improved antagonist at central and peripheral $GABA_B$ receptor. *Neurosci. Lett.* 91: 92–96, 1989.

LEE, S. M., FRIEDBERG, M. H., AND EBNER, F. F. The role of GABA-mediated inhibition in the rat ventral posterior medial (VPM) thalamus. I. Quantitative assessment of receptive field changes following excitotoxic lesion of the thalamic reticular nucleus. *J. Neurophysiol.* 71: 1702–1715, 1994.

LEE, S. M., FRIEDBERG, M. H., AND EBNER, F. F. Differential effects of $GABA_A$ and $GABA_B$ receptor antagonists to somatic sensory stimuli. *Soc. Neurosci. Abst.* 17: 324, 1991.

LEE, S. M., FRIEDBERG, M. H., AND EBNER, F. F. Long-term loss of GABAergic inhibition leads to plasticity in the rat thalamic VPM nucleus. *Soc. Neurosci. Abst.* 16: 45, 1990.

PAXINOS, G. AND WATSON, C. *The Rat Brain in Stereotaxic Coordinates.* Sydney: Academic, 1982.

PESCHANSKI, M., RALSTON, H. J., III, AND ROUDIER, F. Reticularis thalamic afferents to the ventrobasal complex of the rat thalamus: an electron microscope study. *Brain Res.* 270: 325–329, 1983.

RHOADES, R. W., BELFORD, G. R., AND KILLACKEY, H. P. Receptive-field properties of rat ventral posterior medial neurons before and after selective kainic acid lesions of the brain stem trigeminal complex. *J. Neurophysiol.* 57: 1577–1600, 1987.

SALT, T. E. Gamma-aminobutyric acid and afferent inhibition in the cat and rat ventrobasal thalamus. *Neuroscience* 28: 17–26, 1989.

SIMONS, D. J. Multi-whisker stimulation and its effect on vibrissa units in rat SmI barrel cortex. *Brain Res.* 276: 178–182, 1983.

SIMONS, D. J. AND CARVELL, G. E. Thalamocortical response transformation in the rat vibrissae/barrel system. *J. Neurophysiol.* 61: 311–330, 1989.

SUGITANI, M. Electrophysiological and sensory properties of the thalamic reticular neurones related to somatic sensation in rats. *J. Physiol. Lond.* 290: 79–95, 1979.

WAITE, P. M. E. The responses of cells in the rat thalamus to mechanical movements of the whiskers. *J. Physiol. Lond.* 228: 541–561, 1973.