Corticocortical Inhibition of Peripheral Inputs Within Primary Somatosensory Cortex: The Role of GABA\textsubscript{A} and GABA\textsubscript{B} Receptors

S. A. Chowdhury and D. D. Rasmusson

Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

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Chowdhury, S. A. and D. D. Rasmusson. Corticocortical inhibition of peripheral inputs within primary somatosensory cortex: the role of GABA\textsubscript{A} and GABA\textsubscript{B} receptors. J Neurophysiol 90: 851–856, 2003; 10.1152/jn.01059.2002. A conditioning-test pulse paradigm was used in combination with microiontophoresis to examine the corticocortical modulation of somatosensory processing. Single-cell recordings were made in the glabrous digit representation of primary somatosensory (S1) cortex in anesthetized raccoons. Test stimulation of the periphery (the on-focus digit) was preceded by conditioning stimulation of the cortical area that represents an adjacent digit at interstimulus intervals ranging from 5 to 200 ms. An early and prolonged inhibitory modulation was produced in most of the 61 neurons examined, and an early facilitation followed by inhibition was produced in about one-third of the cells. Microiontophoretic administration of a potent GABA\textsubscript{A} receptor antagonist, CGP 55845, blocked the inhibition and in many cases revealed a facilitation of the sensory response. Microiontophoretic administration of a GABA\textsubscript{B} receptor antagonist, gabazine, blocked inhibition at short interstimulus intervals and reduced the longer inhibition by half. These results indicate that connections between glabrous digit representations within S1 cortex produce predominantly inhibitory modulation of sensory input and that both GABA\textsubscript{A} and GABA\textsubscript{B} receptors contribute to this modulation. The relevance of these connections to the effects of peripheral nerve injury and subsequent reorganization is discussed.

INTRODUCTION

One of the interesting developments in the field of somatosensory function in the last 20 years is the discovery that mammals retain the capacity for functional plasticity well into adulthood (Jones 2000; Kaas 1991). This “reorganization” is seen clearly after digit amputation when the denervated portion of somatosensory (S1) cortex becomes responsive to stimulation of an adjacent digit (Kelahan and Doetsch 1984; Merzenich et al. 1984; Rasmusson 1982). The raccoon has been useful for studying this issue because of its highly developed somatosensory system (Welker and Seidenstein 1959), which allows precise delineation of the denervated areas. The site of the plastic changes has not been conclusively identified; there is clear evidence for subcortical reorganization (Rasmusson 1996; Rasmusson and Northgrave 1997), which would provide the denervated cortex with novel inputs as well as for physiological changes in corticocortical connections (Zarzecki et al. 1993). In the raccoon, the starting point of reorganization, i.e., the state of the system immediately after digit amputation, is paradoxically a strong inhibition when adjacent digits are stimulated (Rasmusson and Turnbull 1983; Rasmusson et al. 1992), but the response changes to excitation several months after denervation (Kelahan and Doetsch 1984; Rasmusson 1982; Rasmusson and Turnbull 1983; Rasmusson et al. 1992). To determine the mechanisms for denervation-induced plasticity, it is essential to clarify the interactions between peripheral and corticocortical inputs in the intact animal.

One traditional method for studying the interaction between two inputs is the conditioning-test pulse paradigm in which facilitatory or inhibitory effects can be seen in the response to the test stimulus as a result of a preceding conditioning pulse delivered to another pathway (Gardner and Costanzo 1980). The present study used this paradigm to determine if S1 cortical neurons that receive peripheral input from one digit (the “on-focus” digit) are influenced by an adjacent cortical area that receives input from an “off-focus” digit. Initial studies showed this to be primarily inhibitory. Consequently, additional neurons were tested after microiontophoretic administration of specific GABA\textsubscript{A} and GABA\textsubscript{B} receptor antagonists (gabazine and CGP 55845) to determine the contribution of these two receptor subtypes to this corticocortical inhibition.

METHODS

Recordings were obtained from nine adult raccoons (Procyon lotor) of either sex. The body weights of the raccoons were between 4.5 and 13.2 kg. The handling of animals and experimental procedures were carried out in accordance with the guidelines of the Canadian Council of Animal Care and were approved by the Dalhousie University Committee on Laboratory Animals.

Each animal was initially sedated with ketamine hydrochloride (10–20 mg/kg im, MTC Pharmaceuticals, Cambridge, ON) and then given iso-flurane (5% in oxygen) so that an intravenous catheter could be inserted into a vein in the left forearm. The animal was subsequently maintained in an anesthetic, areflexic state with \textalpha-chloralose (intravenous, 5% in propylene glycol; Sigma, St. Louis, MO). Injections of a corticosteroid (0.5 ml 100 mg/ml, Solu-Delta Cortef, Upjohn; Orangeville, Ontario, Canada) were given before opening the skull and 2–3 h later (1 ml iv) to minimize cerebral edema. Body temperature was monitored and maintained near 37°C using a negative-feedback system comprised of a rectal probe and an electric heating pad (Harvard Apparatus, Saint Laurent, Quebec, Canada). End tidal CO\textsubscript{2} was monitored (CWE, Ardmore, PA) and lactated Ringer solution was delivered (intravenous) continuously throughout the experiment.

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The animal was mounted and positioned in a Kopf stereotaxic frame. The skull was exposed and a craniotomy was made over the left S1 cortex, exposing the cortical representations of the fourth digit, fifth digit, and palm. A well was created around the craniotomy using dental impression compound (Kerr, Romulus, MI) and filled with warmed Elliot’s solution (Abbott, Montreal, Quebec, Canada). The dura mater was cut and reflected to expose the brain.

Recording procedure

Recording and drug application were performed with five-barrel glass micropipettes (A-M Systems, Carlsborg, WA). The center barrel was filled with a fine (7 μm) carbon-fiber before being pulled on a vertical Narishige micropipette puller. The tapered end of the pipette was broken off, resulting in a total tip diameter of ~20–40 μm. The carbon fiber was then chemically etched (Armstrong-James and Millar 1979). The other end of this barrel was filled with 3 M NaCl for extracellular recording. The impedance of the carbon fiber-containing recording electrode was between 1 and 5 MΩ at 1 kHz. The output signal was amplified (10,000–50,000 times), band-pass filtered (0.2–5 kHz), and passed through a Humbug noise eliminator (Quest Scientific, North Vancouver, British Columbia, Canada). The resulting signal was monitored with an audio speaker and displayed on a storage oscilloscope.

The other barrels of the micropipette contained NaCl (0.9%) for current balancing, CGP 55845 (20 mM, pH 5.2), and gabazine (SR-95531, 20 mM, pH 3.3, Sigma). Both drugs were dissolved in 0.9% NaCl. Each barrel was filled immediately before recording using a syringe and Microfil needle (WPI, Sarasota, FL). Drug ejection and retaining currents were controlled by a Neurophore apparatus (Medical Systems, Greensville, NY). A holding current (~15 nA) was applied to each drug barrel to prevent leakage of drugs. The ejection currents were normally +40 nA for both CGP 55845 and gabazine and were applied for 5 min.

The micropipette was advanced in 10-μm steps using an Inchworm microdrive (Burleigh, Fishers, NY) until suitable neuronal activity was isolated. The location of each electrode penetration was marked on a photograph of the cortex. The neuron’s receptive field was determined by exploring the skin with a glass probe or von Frey hair monofilaments (Touch-Test Sensory Evaluator, North Coast Medical, San Jose, CA) and drawn on a photograph of the forepaw.

Testing procedure

The test stimulus was applied electrically to the digit containing the neuron’s receptive field through a pair of Teflon-coated silver wire electrodes inserted under the glabrous skin of the distal pad of the digit. The strength of this test pulse was set to produce a response from the cortical neuron on virtually every trial and ranged between 100 and 600 μA.

The adjacent cortical area (e.g., digit 4 representation when recording from a neuron in digit 5 cortex) was first identified using the recording electrode as receiving inputs from the adjacent digit. This is rapidly done in the raccoon using the sulcal patterns as landmarks (Rasmussen 1982; Welker and Seidenstein 1959). The conditioning stimulus was delivered to this adjacent area through a concentric bipolar electrode. The depth of the electrode was ~0.5 mm below the surface and strength of the stimulation electrode was adjusted to evoke a consistent response from the recorded neuron (30–100 μA). Both the conditioning and test pulses were delivered from the computer-controlled Master 8 stimulator (AMPI, Jerusalem, Israel) and were 0.2 ms in duration. The response of each cell was recorded before and after each drug administration using the same stimulation parameters.

Recordings and the timing of stimuli were controlled using DataWave interface and software (Thornton, CO). The sampling rate was 20 kHz. Individual spikes were recorded and saved after discriminatio

n on the basis of amplitude and duration. Data were collected in 350-ms trials in which the conditioning pulse (cortex) was delivered at the beginning of the trial and the test pulse (digit) was delivered after an interval of 5, 25, 50, 100, or 200 ms. Control trials were also collected with stimulation of the digit alone. The interval between trials was 2 s. The sequence of interstimulus intervals (ISIs) was randomized initially and then remained the same for all animals. Thirty trials were obtained at each interval for each neuron; therefore the time necessary to collect a complete data set was ~7 min. The response for each ISI was counted as the number of spikes from 10 to 50 ms after the digit stimulation and expressed as a percent of the response to digit stimulation alone. In those cases where the conditioning stimulus produced a response after 10 ms, this response was measured in the 50-, 100-, and 200-ms trials and subtracted from the response at 5- and 25-ms trials where it might have interfered with the test response (Gardner and Costanzo 1980). Changes in background activity (i.e., in the absence of peripheral stimulation) were also monitored and recorded over several minutes before, during, and after drug administration. In those neurons that had a spontaneous rate >2 s⁻¹, the responses to skin stimulation were corrected for the spontaneous activity.

Comparisons were made between groups and before and after drug treatment using repeated-measures ANOVA and t-test (StatView, SAS Institute, Cary, NC). All error values in the figures and text are SE.

Results

The effect of corticocortical activation on peripheral input was determined in 61 neurons. These neurons were located between 123 and 1,200 μm below the cortical surface at a mean depth of 570 μm. Eighty percent of the neurons were above 800 μm and were therefore considered to be in the supragranular layers. The distance between cortical stimulation and recording sites measured on the cortical surface ranged from 2 to 5 mm, with a mean value of 3.5 ± 0.1 mm and a median of 3 mm. Most of the neurons (n = 51) had little or no spontaneous activity (~2 s⁻¹). The average spontaneous activity was 1.63 ± 0.37 s⁻¹ with a median of 0.58 and a range of 0–13.2.

The effects of conditioning stimulus on peripheral inputs (Fig. 1) was primarily inhibitory at longer intervals (50, 100, and 200 ms), whereas at 5 and 25 ms, about half of the neurons showed an increase in responsiveness. The mean responses

![Fig. 1](http://jn.physiology.org/) The effect of conditioning stimulus on the responses of all 61 primary somatosensory (S1) cortical neurons before drug administration. Histograms show the distribution of cell responses at each interval. The number of neurons with a response <100% at each interval is: 5 ms, 32; 25 ms, 37; 50 ms, 55; 100 ms, 47; 200 ms, 53.
(Fig. 2A) revealed an overall facilitation of the response at 5 ms and an inhibition at each of the later intervals. While the maximum inhibition occurred at 50 ms, it was still quite strong at 200 ms. The difference between intervals was statistically significant [repeated-measures ANOVA, $F(4,240) = 7.74, P < 0.001$]. The four longer intervals were significantly less than test alone values ($t = 2.16, P = 0.03–6.26, P < 0.001$), indicating a significant inhibitory effect, but the increase at 5 ms was not statistically significant ($t = 1.58, P = 0.12$) because of the bimodal distribution at this interval.

**Effect of GABA\textsubscript{B} receptor blockade**

A total of 57 neurons were studied before and after administration of the GABA\textsubscript{B} receptor antagonist, CGP 55845 did not have an effect on either the spontaneous activity (1.61 and 1.69 s\(^{-1}\) before and after the drug; paired $t = 0.23, P = 0.82$) or the response to the peripheral (test) stimulation alone (mean total spikes = 62.1 and 64.8, before and after the drug; paired $t = 0.49, P = 0.63$).

The effect of blocking GABA\textsubscript{B} receptors on the response following conditioning-test pairing is shown for a representative neuron in Fig. 3. This cell was inhibited at the four longer intervals and this inhibition was largely reversed by CGP 55845. The overall effect of CGP 55845 on all 57 neurons tested is shown in Fig. 2B. The asterisks in Fig. 2B indicate statistical differences between pre and post drug responses (repeated-measures ANOVA and paired t-test, $P < 0.001$) and were significant at all intervals except 5 ms. In addition, the means at the 3 short intervals were statistically $>100\%$ ($t = 3.6, 2.3, \text{and} 2.2, P < 0.05$), indicating that CGP 55845 revealed a significant facilitation at these intervals.

**DISCUSSION**

Corticocortical connections are known to contribute to the normal response properties of neurons in somatosensory cortex and to be modifiable. For example, plasticity has been demonstrated between nearby sites in rat cortical slices (Lee et al.)

![Fig. 2](image-url) Mean response before and after CGP 55845. A: mean ± SE before drug administration relative to the response to test alone (n = 61). Statistical significance for difference from 100%: *$p < 0.05$; ***$p < 0.001$. B: the effect of the GABA\textsubscript{B} receptor antagonist, CGP 55845 (n = 59). ***, difference between CGP 55845 and predrug, $P < 0.001$.

![Fig. 3](image-url) The effect of CGP 55845 on a representative neuron. Poststimulus time histograms (PSTHs) show the 1st 40 ms after test stimulation without (control) or with conditioning stimulation at the time indicated preceding the test stimulus (left: before drug administration; middle: after CGP 55845 administration). Bins, 1 ms, sum of 30 trials. Right: total number of spikes at each interval.
between gabazine and predrug, 854 S. A. CHOWDHURY AND D. D. RASMUSSON

Mean ± SE before (●) and after (○) administration of gabazine. *, difference between gabazine and predrug, P < 0.05.

FIG. 4. The effect of GABA$_A$ receptor blockade by gabazine (n = 19). Mean ± SE before (●) and after (○) administration of gabazine. *, difference between gabazine and predrug, P < 0.05.

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The results show that inhibitory modulation of adjacent digit areas of S1 cortex is much more prevalent than excitatory modulation (Fig. 1). Only about half of the neurons showed an increase in response to peripheral stimulation at any interval tested, whereas almost all showed a decrease at one or more intervals. A pattern consisting of inhibition alone at one or more intervals was the most common, whereas about one-third of the neurons showed an excitatory-inhibitory pattern. Facilitation was most frequently seen at the shortest interval tested, whereas almost all showed a decrease at intervals >25 ms. In most of the neurons that were facilitated, the facilitation was followed by inhibition. This could be a feedback inhibition following the firing of the recorded neuron or feedforward activation of inhibitory neurons in parallel with the excitatory connections.

These results indicate that some neurons in a digit representational area of S1 cortex can be excited via an adjacent cortical area, but this facilitation is relatively short lasting. The ISIs used here do not take into account the different conduction times for the two pathways. The time for corticocortical transmission over 2–5 mm would be on the order of 5–10 ms, whereas the latency following peripheral stimulation is 15–20 ms (Smits et al. 1991). Therefore it would be expected that if both cortex and skin were stimulated at the same time, the corticocortical input would arrive ~10 ms earlier than the peripheral input. Anatomical evidence indicates that there are no monosynaptic connections between digit areas in raccoon S1 cortex (Doetsch et al. 1988; Herron and Johnson 1987), although there are extensive projections to and from the sulcal regions separating the digit areas that could provide a pathway for polysynaptic connections. The short latency of the strong facilitation seen here suggests that the number of intervening synapses is small. Nearly all neurons, however, can be inhibited from an adjacent digit area either with or without a preceding facilitation, and this inhibitory influence often lasted ≥200 ms. This likely results from activation of inhibitory interneurons near the site of recording.

The only physiological study on corticocortical connections within raccoon S1 cortex (Smits et al. 1991) examined possible projections from an area just anterior to the digit representation called the “heterogeneous” zone by Johnson et al. (1982). This region contains neurons with multidigit receptive fields as well as joint, skin, and possibly muscle inputs, and Smits et al. found evidence of many excitatory projections back into the glabrous skin representation. In contrast, the regions examined in the present study are both glabrous skin representations in which the neurons have small receptive fields restricted to a single digit. The dominance of inhibitory connections between adjacent glabrous digit representations seen here may contribute to tactile localization and acuity.

**Contribution of GABA$_A$ and GABA$_B$ receptors to corticocortical inhibition**

Microiontophoretic administration of GABA$_A$ and GABA$_B$ receptor antagonists was used to determine the contribution of these two receptor subtypes to the inhibitory modulation. The selectivity of gabazine and CGP 55845 for GABA$_A$ and GABA$_B$ receptors, respectively, and their reversibility have been demonstrated repeatedly over the last 20 years (e.g., Bittiger et al. 1993; Wermuth and Biziare 1986). However, direct comparison of the effectiveness of the two drugs in reversing corticocortical inhibition is problematic using the microiontophoretic technique since the exact concentration of the drug present at any synapse depends on many variables in addition to amount present in the pipette. Consequently, we used a prolonged administration of the drug (5 min) that should saturate the relevant receptors within the area of diffusion. We have found that the same duration and concentration of CGP 55845 can produce changes in receptive-field size in this preparation (Chowdhury and Rasmusson 2002a,b). With this technique, we found that GABA$_B$ receptor blockade produced a reversal of inhibition at all intervals and in fact revealed greater facilitation at short intervals. The GABA$_A$ antagonist, on the other hand, produced complete reversal at the shortest interval and ~50% reversal at longer intervals.

CGP 55845 is a highly specific GABA$_B$ antagonist (Bittiger et al. 1993) and has been shown to produce receptive-field enlargement in raccoon cortex (Chowdhury and Rasmusson 2002a). The almost complete elimination of inhibitory modulation produced by CGP 55845 in the present study indicates that GABA$_B$ receptors play a major role in the corticocortical inhibition. GABA$_B$ receptors are present presynaptically, where they act to suppress GABA and glutamate release (Dutar and Nicoll 1988; Waldmeier et al. 1994; Zilberter et al. 1999). In addition, presynaptic GABA$_B$ receptors contribute to paired-pulse suppression (Chowdhury and Matsunami 2002; Chowdhury et al. 1996; Zilberter et al. 1999). Blockade of the GABA$_B$ autoreceptors on GABAergic terminals in the present experiment should increase GABA release and consequently lead to greater inhibition via GABA$_A$ receptors, but this was not observed. Blockade of GABA$_B$ heteroreceptors on glutamate terminals, on the other hand, should facilitate the release of glutamate by thalamocortical and intrinsic excitatory
neurons and could account for the switch from inhibition to facilitation that was seen in many cells after CGP 55845.

GABA<sub>B</sub> receptors are also present postsynaptically. These metabotropic receptors produce a postsynaptic hyperpolarization with slower onset but much longer duration than GABA<sub>A</sub> receptor activation (Connors et al. 1988). The block of inhibitory modulation at long intervals in the present study is consistent with the time course of a postsynaptic GABA<sub>B</sub>-mediated hyperpolarization.

Gabazine was used to block GABA<sub>A</sub> receptors because it has higher affinity for the receptor than bicuculline and is less likely to induce seizure activity (Heaulme et al. 1986). In addition, bicuculline methiodide has been described as having likely to induce seizure activity (Heaulme et al. 1986). In

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tion with slower onset but much longer duration than GABA<sub>A</sub>

receptor blockade (Chowdhury and Rasmusson 2002a; Tremere et al. 2001). It might be expected that this would also yield an increase in spontaneous activity and in the evoked response to the peripheral stimulus alone. While this was seen in some neurons, overall the effect was not significant for either CGP 55845 or gabazine. This may be due to the fact that receptive-field expansion is largely a reflection of changes in threshold at the edges of the receptive field, whereas the present study used a constant stimulus at one site.

The overall results of the present study are consistent with several possible pathways. The finding that one or both GABA antagonists reversed inhibition in most of the neurons indicates that the GABAergic neurons are acting directly on the neuron that was being recorded or presynaptically on its inputs. Differences between neurons in the amount of reversal of inhibition could be due to the location of the GABAergic receptors on the neuron relative to the micropipette. In addition, the inhibition that remained in some neurons could be occurring earlier in the polynuclear circuit (Bindman et al. 1988) at a site that is too far from the site of microiontophoresis to be affected by these drugs.

Possible relevance to injury-induced reorganization

Immediately after digit amputation, neurons in S1 cortex of the raccoon show predominantly inhibition from stimulation of adjacent digits (Rasmussen and Turnbull 1983). This inhibition is consistent with the largely inhibitory connections from the adjacent digit representational area of S1 revealed in the present study. The relative predominance of inhibitory corticocortical connections in the raccoon may account for some of the differences that have been observed between it and other species (Byrne and Calford 1991; Calford and Tweedale 1988; Li et al. 1994). Evidence in many models shows that the cortical GABAergic circuits are gradually downregulated after the removal of sensory input (Jones 2000); this would allow any weak excitatory inputs greater opportunity to be strengthened. The existence of some short-latency excitatory corticocortical connections provides one potential pathway that could be gradually strengthened during the process of long-term reorganization.

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DISCLOSURES

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