Chronic Suppression of Activity in Barrel Field Cortex Downregulates Sensory Responses in Contralateral Barrel Field Cortex

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Li, Lu, V. Rema, and Ford F. Ebner. Chronic suppression of activity in barrel field cortex downregulates sensory responses in contralateral barrel field cortex. J Neurophysiol 94: 3342–3356, 2005. First published July 13, 2005; doi:10.1152/jn.00357.2005. Numerous lines of evidence indicate that neural information is exchanged between the cerebral hemispheres via the corpus callosum. Unilateral ablation lesions of barrel field cortex (BFC) in adult rats induce strong suppression of background and evoked activity in the contralateral barrel cortex and significantly delay the onset of experience-dependent plasticity. The present experiments were designed to clarify the basis for these interhemispheric effects. One possibility is that degenerative events, triggered by the lesion, degrade contralateral cortical function. Another hypothesis, alone or in combination with degeneration, is that the absence of interhemispheric activity after the lesion suppresses contralateral responsiveness. The latter hypothesis was tested by placing an Alzet minipump subcutaneously and connecting it via a delivery tube to a cannula implanted over BFC. The minipump released muscimol, a GABAA receptor agonist at a rate of 1 µg/h, onto one barrel field cortex for 7 days. Then with the pump still in place, single cells were recorded in the contralateral BFC under urethan anesthesia. The data show a ~50% reduction in principal whisker responses (D2) compared with controls, with similar reductions in responses to the D1 and D3 surround whiskers. Despite these reductions, spontaneous firing is unaffected. Fast spiking units are more sensitive to muscimol application than regular spiking units in both the response magnitude and the center/surround ratio. Effects of muscimol are also layer specific. Layer II/III and layer IV neurons decrease their responses significantly, unlike layer V neurons that fail to show significant deficits. The results indicate that reduced activity in one hemisphere alters cortical excitability in the other hemisphere in a complex manner. Surprisingly, a prominent response decrement occurs in the short-latency (3–10 ms) component of principal whisker responses, suggesting that suppression may spread to neurons dominated by thalamocortical inputs after interhemispheric connections are inactivated. Bilateral neurological impairments have been described after unilateral stroke lesions in the clinical literature.

INTRODUCTION

The integration of sensory information between the cerebral hemispheres is important for many perceptual tasks that require bilateral coordination. The function of the left and right somatic sensory cortex is closely linked through the corpus callosum (Ebner and Myers 1962; Glickstein and Sperry 1960; Krupa et al. 2001; Schnitzler et al. 1995). The integration of sensory information through the corpus callosum is still unexplained at a cellular level, but several recent papers have begun to explore cellular interhemispheric dynamics in the somatic sensory system (Calford and Tweedale 1990; Harris and Diamond 2000; Shuler et al. 2001; Swadlow 1988, 1989; Wiest et al. 2005). However, the extent to which real-time activity changes in one cortical area are reflected in that of its contralateral counterpart is still an open question.

The subcortical pathways from the whiskers on the right and left side of the face are kept quite separate from periphery to cortex (Chiaia et al. 1991; Peschanski 1984), leading to a functional lateralization in cortex. However, while barrel neurons respond best to stimulations of the contralateral whiskers, they are also influenced by the whiskers on the ipsilateral side of the face as well. Stimulating ipsilateral whiskers evokes both local field potential (LFP) (Pidoux and Verley 1979) and spikes in single layer V neurons in barrel field cortex (BFC) (Shuler et al. 2001). Responses of cortical neurons to the ipsilateral whiskers are mediated via callosal connections because blocking activity in one hemisphere eliminates all responses in the hemisphere ipsilateral to the whiskers stimulated (Pidoux and Verley 1979; Shuler et al. 2001). Behavioral studies (Harris and Diamond 2000) have shown that tactile learning occurring in one BFC leads to significant transfer to the homologous barrel on the other side. Indeed, rats exhibit the capability of integrating bilateral whisker information to determine whether movable walls on the left and right side of the face are close to the head, far from the head, or asymmetrically different distances from the head using only their whiskers (Krupa et al. 2001; Shuler et al. 2002). This capability of making a correct comparison with whiskers is a function of the corpus callosum (Shuler et al. 2002). These results indicate that integration of whisker information from both sides of the face occurs continuously during a rat’s normal behavior and cortex is the dominant location where this integration occurs.

Bilateral reorganization in cortex occurs after temporary and reversible inactivation of the periphery (Calford and Tweedale 1990; Shin et al. 1997). The peripheral sensory deafferentation induces immediate unmasking of new receptive fields at multiple levels along the sensory system (thalamus: Garraghty 1991; Nicoletis et al. 1993; spinal cord: Pettit and Schwark 1993), and these subcortical changes could also play a fundamental role in cortical plasticity. Studies with manipulations at the cortical level are needed to further specify the role of neocortex in bilateral interactions. One chronic study in our lab reported that a subpial aspiration lesion of one BFC significantly degraded both the background and evoked activity in the contralateral BFC (Rema and Ebner 2003). However, a cortical
lesion produces several types of change, including reactive events such as axon degeneration, decrease in growth factors, and neurotransmitter release from lesioned area as well as elimination of ongoing interhemispheric activity arising from the lesioned area. All of these events are possible modulators of firing characteristics of neurons on the contralateral side. In the present study, we tested the hypothesis that some or all of the interhemispheric effects of a cortical lesion are due to the decrease or elimination of interhemispheric activity. To test this idea, we silenced one cortex for 7 days with minipumped muscimol and measured the spontaneous and evoked activity levels of neurons in the contralateral D2 barrel column. The results show a significant reduction in principal whisker responses (D2) compared with controls with similar reductions in responses to the D1 and D3 surround whiskers. The results suggest that severe reductions in activity between the two hemispheres by itself may be a major contributor to the bilateral clinical deficits seen after unilateral cortical lesions.

METHODS

Adult male Long-Evans rats (250–350 g, 2–3 mo old, n = 24) were used in this study. All procedures were approved by the Institutional Animal Use Committee and followed guidelines set by the National Institutes of Health and the Society for Neuroscience. Figure 1 shows the design of the experiments. In each experimental case, one barrel field was silenced for 7 days using continuous muscimol infusion before responses were recorded in the other BFC to stimulation of its contralateral whiskers. Controls were carried out with saline minipumped onto BFC for the same period of time and with muscimol released onto visual cortex for the same 7-day period before recording cortical responses.

Minipump implantation

Muscimol solution was prepared by dissolving muscimol (Sigma) in sterile saline (10 mM concentration) just prior to the experiment. Osmotic minipumps (Alzet, model 2001) were attached to right angle cannulae (Plastics One) with tip shortened to 0.5–1 mm before surgery so they would not extend past the thickness of the skull and then

FIG. 1. Experiment design. A: schematic diagram showing the location of minipump implantation over right SI (which delivers either muscimol or saline solution), the implantation on right visual cortex as control, and the recording site in the D2 barrel column of the contralateral (left) barrel field cortex (BFC). When the right SI was inactivated by muscimol for 7 days, the facilitatory influence of transhemispheric projections (dashed green arrow) would decrease. When this happens, the neurons in the left BFC could change their responsiveness to peripheral whisker stimulation. B: recording procedure in experimental and control groups.
polished by fine sand paper and sterilized. The whole assembly (Minipump chamber, connection tubing and cannula) were completely filled with muscimol solution, then immersed in sterile saline several hours prior to the implant procedure. The model 2001 minipump delivered the muscimol solution at the rate of 1 μl/h and had a capacity of 200 μl, which was more than enough capacity to provide the 168 μl muscimol needed for the 7-day pumping period. Rats were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip) then transferred to the stereotaxic headholder (Narashige). Supplements (10% of initial dose) were given as needed. Body core temperature was maintained at 37°C by a heating pad that was feedback controlled by a rectal thermometer (Harvard Labs). After a midline cut and soft tissue retraction, a small hole was carefully drilled on the right skull with a fine dental burr at the location of 2 mm posterior and 6 mm lateral to Bregma. The bone dust was frequently cleaned with saline during drilling. When the bone was thinned to a transparent layer, a fine forceps and 32-gauge syringe needle were used to strip off the bone film, expose the dura, and make only one or two openings in the dura to facilitate the penetration of the pumped solution. Care was taken to avoid any injury to blood vessels in the dura, and gel foam was applied to quench any oozing. After the surface of the parietal and temporal bone was dried as completely as possible, a thin layer of super glue was applied onto the dried bone surface while leaving the area around the bone opening uncontaminated. The minipump was embedded subcutaneously between the shoulder blades by blunt dissection of the connective tissues, and the pump with tube attached was inserted into the subcutaneous cavity. The cannula was then held in position just touching the dura with a micromanipulator, and the cannula and a short segment of the tube were securely cemented to the skull with dental cement. The skin edges were then sutured and antibiotic (Neuropracin) was applied to the incision. The rat was removed from the stereotax to recover from anesthesia. All animals were allowed to survive for 7 days from day of implantation surgery.

Two control groups were prepared: one with sterile saline minipumped onto the right BFC for 7 days (n = 5) and another by applying muscimol to the surface of visual cortex (P 6.0, L 2.0 mm from Bregma, n = 4) in the same way to determine the effect of visually applied muscimol on neurons in the contralateral BFC.

**Behavioral observations**

The animals were returned to their home cages after full recovery from anesthesia. The animals were observed every day for whisker movements, gait, response to whiskers, and skin touch when the animal was fully awake. Under each experimental and control condition, the animals were tested behaviorally in two ways. First we stimulated the ipsi- and contralateral whiskers and skin with a handheld probe to determine whether the rat reacted to the tactile stimulus (i.e., orientate head toward stimulated side, withdraw foot, etc.). We also checked the limb posture reflex. When the body of a normal rat is supported and the forelimb is placed in an unusual position, the animal will quickly return the limb to its anatomical position. However, if the forelimb area in SI is silenced or removed these reflexes are reduced or abolished. Because the cannula is placed at the medial edge of the BFC in SI cortex it is equally close to the forelimb representation, and the existence of the limb posture reflex can be used as a general indicator of muscimol release. Reflexes were tested on both forelimbs and both hindlimbs. The muscimol effect on cellular function was determined precisely by electrophysiology (see following text).

**Electrophysiological recording**

One week after the minipump implantation, the rat was anesthetized with urethan (1.5 g/kg, 30% aqueous solution, ip) and mounted in a stereotaxic apparatus (Narashige). With the muscimol cannula still in place and pumping, the skin was opened, and a craniotomy opening made from 4 to 7 mm lateral to the midline and from 0 to 5 mm posterior to Bregma to expose the left (untreated) BFC. Body temperature was maintained at 37°C. Supplementary injections (10% of the initial dose) were given as needed to maintain the anesthesia at stage III-3 (Friedberg et al. 1999; Guedel 1920). Both the spontaneous activity and evoked activity of single units were recorded using carbon-fiber microelectrodes with impedance from 0.2 to 1 MΩ (typically ~0.5 MΩ) at 1 kHz (Armstrong-James and Millar 1979; Armstrong-James et al. 1980). Single neurons were discriminated by their waveforms using a time-amplitude window discriminator (Bak Instruments). Spikes generated by each single unit were digitized in 1-ms bins by a CED 1401 Plus processor (Cambridge Electronic Design) controlled by a PC (Dell), preprocessed and displayed on-line using Spike 2 software (Cambridge Electronic Design) driven by in-house scripts and stored on the PC for further off-line analysis. All whiskers on the right face were trimmed to 10 mm. A piezoelectric stimulator was used to give standardized 3-ms duration, 300-μm ramp and hold forward deflections to one whisker at a time. The duration of each neuron’s response to the principal whisker spike was measured by displaying it on a storage oscilloscope. Microelectrodes were advanced perpendicularly to the cortex surface by a precision mechanical microdrive (Kopf Instruments). Contact of the electrode tip with the pia was identified both visually through an operation microscope and by noise reduction heard on the audiomonitor. Each unit was marked by its subpial depth read off of the microdrive control unit. All penetrations reported here were confirmed to be in the D2 barrel column. They were initially located by coordinates, then by short (7~10 ms)-latency layer IV cell responses to the principal whisker (Armstrong-James and Fox 1987; Armstrong-James et al. 1992, 1994). Electrodes were advanced in 70-μm intervals before searching for another cell to avoid repeated sampling from the same unit. Penetrations were continued down to layer VI. At each recording site, single units were isolated, tested, and recorded. For every unit, several measurements were made: spontaneous activity, responses to stimulation of the D2 (principal) whisker and responses to two immediately adjacent D-row surround whiskers (D1 and D3). Spike duration was measured, and neurons were separated into fast-spiking (<750 μs) and regular-spiking units (>750 μs). For each neuron studied, one block of 50 trials at 1 stimulus/s was presented to the D1–D3 whiskers in sequence. Spontaneous firing was recorded for 200 s after whisker stimulation, with the piezoelectric stimulator still close to the whisker but not moving it.

**Muscimol effective range detection in vivo**

**ACUTE.** We tested the effectiveness and reversibility of muscimol suppression acutely in one rat. After the left BFC was exposed as described in the preceding text, a small (6 mm diam) home-made plastic chamber was cemented watertight to the skull with dental cement. One 32-gauge syringe needle was used to make a small opening in the dura. A carbon-fiber electrode was advanced through the opening until the D2 barrel column was located. The chamber was filled with warm saline. Responses of D2 barrel column neurons were measured to stimulations of the principal and surround whiskers. Then saline within the chamber was removed and replaced by 10 mM muscimol. Spontaneous discharge and evoked response to whisker stimulations of the same neuron(s) were recorded. The period of muscimol effectiveness was estimated by measuring the response levels of neurons over time. Then the cortical surface was washed repeatedly with warm saline with the electrode still in place and test stimuli were delivered every 10 min to determine the duration of muscimol suppression.

**CHRONIC.** To assay the effective distance of muscimol suppression in vivo, two rats were implanted with muscimol minipumps identical to the BFC group. On the eighth postimplantation day, the rat was anesthetized with urethan (1.5 g/kg ip), and the skull bone within a 3-mm radius anterior and medial to the cannula was removed with the
pump still working. The same recording procedure was carried out as described in the preceding text. The brain area surrounding the cannula tip was examined using concentric movements of the electrode to see if neurons at various distances and depths from the cannula tip would respond to the whisker stimulation: first by using a hand-held probe stimulus and then the piezostimulator. Both spontaneous firing and evoked responses were recorded. The coordinate and depth of active neurons were charted for spontaneous firing or responses to whisker stimulation. The evoked response magnitudes were analyzed off-line.

**Histology**

At the termination of recording in one penetration, cortical recording sites were marked in vivo by passing a DC current (electrode tip positive) of 2 μA for 10 s. This current produced a spherical microlesion, which was clearly visible in histological sections stained for cytochrome oxidase (CO) staining. On completion of recording, animals were given a lethal dose of urethan and perfused transcardially with PBS followed by phosphate-buffered 4% paraformaldehyde. Brains were postfixed overnight, saturated in 10, 20, and 30% sucrose, and the cortex was flattened, sectioned tangentially, and stained for CO activity (Wong-Riley and Welt 1980) to localize barrels and microlesion sites. A penetration was considered to be within the D2 barrel column if the recording sites were localized within, above, or below the horizontal boundaries of the D2 barrel as defined by the appropriate patch of high CO activity in layer IV. Only penetrations located within D2 barrel territories were included in the results.

**Identification of the depth of layer IV**

The location of layer IV was identified in three separate cases by comparing the location of lesions made at micrometer measured depths (450 and 800 μm, respectively) with that of the “barrels” revealed by CO staining. The rat was prepared as described in the preceding text. The carbon-fiber microelectrode was oriented perpendicular to the surface of the barrel field cortex (~40–45° lateral for the D row barrels), then advanced slowly (~10 s between 20-μm steps) into the cortex. Contact of the electrode tip with the surface of the cortex, which is the zero point, was carefully identified by two persons independently and by the transient amplifier noise created by the pulsing of the surface vessels. Whiskers were deflected by a hand-held probe as the electrode advanced into the cortex to ensure the electrode was in the barrel column and poststimulus time histograms (PSTHs) were built as needed (see METHODS for detailed description). Once the electrode was confirmed within the barrel column, the electrode was advanced into layer V (~1,000 μm underneath cortical surface, from the read-out of the microdrive) and held in place for ~5 min to compensate for the possible compression of the tissue during the penetration. Then the electrode was retracted at the same rate to 800 μm. And the recording site was marked by passing a DC current (electrode tip positive) of 2 μA for 5 s. Another electrolytic lesion was made at 450 μm using the same method. The loss of local spike activity always signaled a successful lesion. The depth data from the read-out of the microdrive were calibrated as we withdrew the electrode out of the cortex after lesion making. Then the electrode was moved ~300 μm lateral to aim at another barrel column. And an additional pair of lesions were made parallel to the electrode penetration for CO reaction.

**Data analysis**

In this study, units sampled were grouped by their depth read from the microdrive and corroborated by histology. Neurons collected from depths of ~450–800 μm in vivo were usually found within a CO-dense patch corresponding to the D2 barrel. Neurons <450 μm were categorized as supragranular (layer II–III) cells. Neurons collected >800 μm were considered to be in the infragranular layers, mainly layer V. The criteria used here are consistent with previous reports from several labs (Armstrong-James and Fox 1987; Brecht and Sakmann 2002).

Responses evoked by whisker stimulation were assessed by averaging PSTHs. PSTHs were constructed using 1-ms bins, the bin 0–1 was registered as the first bin after stimulus. Response magnitude was constructed by subtracting the averaged number of spontaneous events per 1-ms bin occurring 100 ms before each stimulus. Both the onset response latency (1st bin in a latency histogram) and the modal response latency (bin with the greatest number of spikes) were calculated. Poststimulus responses were further grouped into several intervals, namely, 5–10, 10–20, 20–50, and 50–100 ms for PSTH “epoch” analysis. For a first approximation, the first 10 ms are dominated by thalamic (VPM) inputs, 10–20 ms by adjacent barrel columns, 20–50 ms by other cortical areas (Armstrong-James and Fox 1987; Armstrong-James et al. 1991). Spikes within 3 ms poststimulus were rejected as being too early to be evoked responses to whisker stimulation. For each unit, the center/surround response ratio was also calculated. The center/surround ratio (C/S ratio) was defined as:

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\text{C/S ratio} = \frac{\text{response to D2 whisker}}{\text{[(response to D1 + response to D3 whisker)/2]}}.
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Thus the ratio indicates changes of the selectivity (contrast) of neurons in D2 barrel column. The 200-s spontaneous firing was analyzed separately. Student’s t-test was performed on the data to estimate significant effects.

**RESULTS**

The first result of these studies was to determine the depth of layer IV in the barrel field cortex of adult Long Evans rats. Lesions were localized after CO staining and photographed to demonstrate the relationship between the micrometer readings and the histological borders of layer IV. The histological results from one animal are illustrated in Fig. 2, which is representative of all of the cases studied. Figure 2 shows that the centers of lesions at 450 and 800 μm, made in vivo, are co-extensive with the top and bottom boundary of CO dense barrels that define layer IV in this cortex. This was also supported by the direct measurement from the section. Using section measurements, the layer III/IV boundary is ~370 μm from the surface, and the thickness of layer IV is ~300 μm. After correction for the shrinkage of the tissue in histological procedure, the predicted depth of layer IV in vivo should be ~480 μm and the thickness of layer IV should be ~400 μm. The histological data are close enough to our micrometer readings of layer IV to make reasonable estimates of layer position from the micrometer lesions alone. We realized that a ±30 μm or less error existed between the depth read-out of the microdrive and the real depth of the lesion sites when we made the lesion in the cortex. However, this error could only constitute a minor error in the depth classification. We repeated our experiment in three rats; results from six penetrations in different barrel columns in different animals were highly consistent. Our data clearly demonstrate that our classification of layer IV is reliable in the current experiment paradigm. In the animals used for recording, the brains must be cut tangentially to the cortical surface to accurately identify the barrel and...
septal position of each penetration, and in these cases, the histological estimate of depth is more difficult to reconstruct than in coronal sections.

Histology confirmed 122 neurons were within the D2 barrel column of the left SI in nine rats after 7-days of muscimol inactivation of the right BFC. Thirteen units were in supra-granular layer II/III of the D2 barrel column, 92 were in layer IV, and 17 were in infragranular layers (mostly layer V). Within the total population of cells, the waveform duration of 111 units was measured, and 27 units were categorized as fast-spiking units, 84 were regular-spiking units, and the remaining 11 units could not be classified for various reasons. Seventy neurons were identified within the D2 barrel column in five animals with sterile saline pumped onto the right BFC for 7 days. Twenty units were localized in superficial layer II/III, 42 units were in layer IV, and 8 units were in layer V. The waveform duration of these 70 neurons was known in most cases. Twenty units were fast spiking units, 49 units were

FIG. 2. Histology showing the depth of layer IV barrels in vivo. Image was taken from 1 coronal section of animal 03 showing the location of the lesions after CO staining. Two penetrations were made in this animal with 2 lesions in each penetration at 450 and 800 μm (indicated from the read-out of the microdrive) respectively as described in METHODS. Centers of the lesions were determined by reconstructing the lesions from several adjacent sections and marked by stars. Black stars mark penetration 1 which was localized by physiology as in the septum close to D1. Blue stars mark penetration 2 in D2 barrel. Layer IV barrels were roughly delimited by dashed line. Scale bar: 350 μm (contrast was adjusted in Photoshop). Note that the lesions are located near the upper and lower border of layer IV.
regular spiking units, and only 1 unit was unclassified. Forty-four neurons were also identified within the D2 barrel column in four control animals with muscimol solution pumped onto the right visual cortex for 7 days. These units were also analyzed here. See Table 1 for details.

Behavioral observations

Observations of animal behavior began within 24 h after minipump-implantation surgery. Each animal was tested for forelimb placing and posture reflex every day at random times. Nine rats with muscimol minipumped onto BFC all showed a clearcut absence of forelimb posture reflex during the 7-day recovery period. The earliest onset of hyporeflexia was the next day after surgery. On the other hand, they responded quite well to the posture, pinch, and placing with the forelimb ipsilateral to the muscimol and with both hindlimbs. These nine rats also failed to orientate or move their heads when the whiskers contralateral to the implantation side were stimulated by handheld probe, whereas in contrast they did turn their head when whiskers ipsilateral to the implantation side were stimulated. Behavioral observations were consistent across the nine animals. These animals didn’t show any apparent dyskinesia. Behavioral asymmetry has been reported after unilateral vibrissae removal (Steiner et al. 1986). In our experiment, rats also tended to maintain contact with the wall of the cage most of the time although they behaved normally in walking or forelimb usage in their cages. Thus from the behavior we conclude that the release of muscimol onto cortex significantly suppressed neuronal activity in cortex and that chronic muscimol application by minipump covered a brain area larger than the BFC in SI. However, the effect of pumped muscimol was restricted to the extent that it didn’t appear to spread to visual cortex. The same behavioral tests showed no deficits in either the saline pumped or the VI muscimol infused rats (no visual tests were carried out).

Estimating the area of muscimol suppression

It has been shown that suppression of neuronal activity in cortex by muscimol is reversible using an experimental paradigm similar to that of the current study (Reiter and Stryker 1988). Our recording in BFC with muscimol acutely applied confirmed that suppression of cortical activity by 1 μl of 10 mM muscimol solution per hour is completely reversible in rat BFC. At 20 min after muscimol application, layer IV neurons in the D2 barrel reduced their spontaneous activity and evoked responses to whisker test stimuli by >50%. However, it is still important to define the size of the area that was blocked by muscimol 7 days after implanting the minipump, and results from chronic muscimol application in vivo with electrophysiology in two rats were very consistent. The effective range of muscimol is near circular in shape with a point release site, which centered around the tip of the cannula. Neuronal activity, both the spontaneous firing and evoked activity to whisker stimulation, was completely suppressed in any radius within 1.5 mm away from the tip of the cannula. No action potentials (occasionally injury discharges) were recorded from the pial surface to the white matter within this zone. When the whiskers were deflected with moderately intense manual stimulation, axonal background noise could be heard on the audiomonitor. The deep layer cells around the 1.5 mm distance showed some activity but still far below normal. The brain area that was from 1.5 to 2.5 mm away from the cannula tip was partially suppressed by muscimol. Neurons in this region exhibited response decrements, but they had more spontaneous activity and more responses to test stimuli compared with the fully blocked region. Neurons would often fire in clusters at low frequency (<1/3 s), especially infragranular neurons when whiskers were stimulated. These results are consistent with previous studies which reported blockade of muscimol was still detectable 2 mm anterior to the cannula after 2 days of 10 mM muscimol application (Reiter and Stryker 1988). During this type of recording, muscimol was still being pumped onto the brain surface, but we also applied warm saline to the cortical surface when it appeared dry. This dilution may shrink the size of the area delineated by our recording. We conclude that 10 mM muscimol from the minipump can diffuse horizontally over a large enough area to suppress much of the BFC and some of the forelimb area if applied near the medial border of the BFC.

Evoked response magnitude in muscimol BFC group

The responses evoked in neurons from layer II to layer V of D2 barrel column in left BFC to test stimuli applied to the principal whisker D2 and surround whiskers D1 and D3 were reduced significantly after 7 days of muscimol application to the right BFC. Applying muscimol to the right visual cortex for 7 days had no effect on the evoked response of neurons in the left BFC. Figure 3A plots the average number of action potentials generated per 50 stimuli by the neurons from layer II to layer V from three groups in response to D1–D3 whisker stimulations. The effect of muscimol diffusion can be measured by data from the visual cortex group because we chose to implant minipumps over the visual cortex at a location that would ensure the distance from the cannula tip (either over right SI or right visual cortex) to the area studied (left BFC) is the same between muscimol BFC group and muscimol visual group. Our data indicate 7 days of muscimol application to right visual cortex didn’t alter the response magnitude of neurons in D2 barrel column of left BFC to principal whisker D2 stimulation (42.9 spikes/50 stimuli vs. 43.8 spikes/50 stimuli, muscimol visual vs. saline, P < 0.43, 1-tail t-test, same in the following text unless specified). Responses to surround whiskers D1 and D3 were decreased but not statistically significantly (D1: 19.3 spikes/50 stimuli vs. 24.9 spikes/50 stimuli, muscimol visual vs. saline, P < 0.07; D3: 16.9 spikes/50 stimuli vs. 21.4 spikes/50 stimuli, muscimol visual vs. saline, P < 0.08). Hence neurons in the left BFC were not significantly suppressed by diffused muscimol. However, applying muscimol to the right SI for 7 days significantly de-
graded the responsiveness of D2 barrel column neurons in the left BFC to both the principal whisker and the surround whiskers. When compared with the saline group, muscimol decreased responses to the principal whisker D2 by 42% (23.1 spikes/50 stimuli vs. 43.8 spikes/50 stimuli, muscimol BFC vs. saline, $P < 8.7 	imes 10^{-10}$). Similarly, responses to surround whiskers D1 and D3 decreased by 47% (13.2 vs. 24.9, $P < 4.4 	imes 10^{-6}$) and 55% (9.6 vs. 21.4, $P < 3.3 	imes 10^{-8}$). Responses in muscimol BFC group were also significantly smaller than those from the visual group. Compared with the visual group data, responses to the principal whisker D2 reduced 46% in BFC group (23.1 spikes/50 stimuli vs. 42.9 spikes/50 stimuli, muscimol BFC vs. muscimol visual, $P < 1.7 	imes 10^{-10}$). Response to D1 and D3 whiskers also decreased 31 and 43%, respectively (D1: 13.2 vs. 19.3, $P < 0.005$; D3: 9.6 vs. 16.9, $P < 3 	imes 10^{-4}$). Figure 3A shows the comparison of evoked responses of neurons from these three groups.

**Effects on spontaneous activity**

Spontaneous activity was assessed in 117 neurons of 122 units from nine muscimol BFC rats, 65 of 70 units from five saline animals, and 42 of 44 units from four muscimol visual rats. After 7 days of pumping sterile saline onto the right BFC, the average spontaneous activity level of 65 neurons from layer II to layer V of the D2 barrel column in the contralateral BFC was 0.95 spikes/s. This number is not significantly different from the spontaneous firing rate (typically ~1 spike/s) in adult Long Evans rats under urethane anesthesia (Armstrong-James and Fox 1987). Therefore we concluded that the implantation and surgery didn’t alter the excitability of BFC neurons and the saline group served as a good control for this parameter. After 7 days of muscimol application to right visual cortex, the spontaneous firing of neurons didn’t change from layer II to layer V in D2 barrel column of left BFC. The average spontaneous firing rate of 42 neurons from muscimol visual group is 1 spike/s, which is statistically insignificant when compared with saline group (1 vs. 0.95 spike/s, $P < 0.69$, 2-tail t-test). Unlike the evoked response, applying muscimol to the right BFC for 7 days slightly decreases the spontaneous activity level of neurons in the D2 barrel column of the left BFC to 0.75 spikes/s (averaged from 117 units), which is not statistically significant (muscimol BFC vs. saline, $P < 0.10$, 1-tail t-test; muscimol BFC vs. muscimol visual, $P < 0.06$, 1-tail t-test). Figure 3B compares the mean ± SE value for spontaneous activity when all units in each of three groups are pooled together.

**Influence of muscimol on FSU and RSU**

BFC neurons can be categorized by their spike duration into fast- and regular-spiking unit (FSU and RSU) cells (Mountcastle et al. 1969; Simons and Carvell 1989). Because FSUs are mostly GABAergic inhibitory neurons (McCormick et al. 1985), it is of particular interest to see if inactivating one BFC with muscimol for 7 days changes the excitatory-inhibitory balance in the contralateral homotopic area. Twenty FSUs and 49 RSUs were identified in the saline group. Most of the FSUs were in layer III and IV while RSUs were evenly distributed from layer II to layer V. Generally the FSUs were more responsive than RSUs in that they generated more spikes on average to test stimuli. The frequency of encountering FSUs in the muscimol suppressed cortex is reduced compared with controls. Of the population of cells that were found, contralateral BFC suppression affected FSUs and RSUs quite differently. After muscimol application, responses of contralateral FSUs were significantly reduced to all three whiskers. On average, responses of FSUs to the D2 principal whisker stimulations are only 47% of the control value ($P < 0.0005$). The FSU reductions are 38% ($P < 0.0002$) and 26% ($P < 0.0004$), respectively, to surround D1 and D3 whisker stimulations.

RSUs are more resistant to muscimol application with responses to the principal whisker stimulation being more reduced than responses to surround whiskers. After 7 days of muscimol application, the response of RSUs to the D2 whisker stimulation being more reduced than responses to surround whiskers.
stimulations decreased significantly (63% of the control value, \( P < 0.0003 \)) while changes of response to D1 and D3 whiskers stimulations were less prominent. Figure 4 shows the effects of muscimol on FSUs and RSUs. RSUs still generated average responses that were 80% (\( P < 0.12 \)) and 70% (\( P < 0.03 \)) of the control value to surround whiskers D1 and D3, respectively. Figure 4 shows the details.

Laminar analysis

To see whether inactivation by muscimol depressed neurons in contralateral BFC in a layer specific manner, units were grouped by their subpial depth and layer after histological correction. Muscimol application considerably reduced the frequency of isolating supragranular neurons in each electrode penetration. The percentage of neurons recorded in superficial layers in muscimol animals is 11% (13/122), while in control (saline) animals sampled in the same way the supragranular subset constitutes 29% (20/70) of the sample. Supragranular neurons in muscimol animals typically produce small spike amplitudes, and while they show robust spontaneous activity, they are less responsive to whisker stimulation. Figure 5 compares the average response of neurons in the same laminar category between different animal groups. The data indicate that reduction of responsiveness mainly affects neurons in the layers II/III and IV of the D2 barrel column. The infragranular neurons also decrease their average response magnitude, but the decrease does not achieve significant levels. The lack of significance in infragranular neurons could be due to the limited size of the sample. Responses to surround whiskers D1 and D3 were affected as well as principal whisker D2. In brief, layer II/III neurons in the D2 barrel column in muscimol animals only gave an average response at 45% to D1 (12 spikes/50 stimuli vs. 26.5 spikes/50 stimuli, \( P < 0.03 \)), 47% to D2 (25.2 vs. 53.9, \( P < 0.006 \)), and 23% to D3 (5 vs. 21.1, \( P < 0.0005 \)) of the control value respectively. Layer IV neurons (\( n = 92 \)) in muscimol BFC group were also compared with 42 granular layer neurons from saline animals. Their relative response magnitude is 68% (15.1 spikes/50 stimuli vs. 24.9
spikes/50 stimuli, $P < 0.03$) to D1, 62% (24.8 vs. 42.3, $P < 0.0002$) to D2, and 61% (11.7 vs. 21.6, $P < 0.01$) to D3 stimulations compared with controls, respectively. We also recorded 17 infragranular neurons (mostly layer V neurons) in muscimol animals and 8 neurons from saline rats. Although these neurons exhibited response decrease, statistical test failed to prove significance: 67% (11.8 vs. 21, $P < 0.12$) to D1, 72% (16.9 vs. 26.1, $P < 0.11$) to D2, and 58% (11.4 vs. 20.6, $P < 0.05$) to D3.

Barrel neurons in layer IV were examined separately because it was not expected that these neurons would show a response decrement after muscimol application. Eleven layer IV FSUs and 31 RSUs were identified from 42 barrel cells in saline control, and 22 FSUs and 61 RSUs were localized within layer IV in muscimol BFC rats. Figure 6A compares the average response to peripheral whisker stimulation in 22 layer IV FSUs (BFC muscimol group) and 11 barrel FSUs in saline controls. Responses of FSUs in the barrel to both the principal whisker and surround whiskers were heavily suppressed. The response reduction was 50% ($P < 0.02$) to D2 and 65% ($P < 0.02$) and 76% ($P < 0.02$) to D1 and D3, respectively. However, as shown in Fig. 6B, layer IV RSUs decreased their response to the principal D2 whisker ($P < 0.01$) after muscimol application, whereas the responses to the surround whiskers D1 and D3 remained unchanged ($P < 0.75$, $P < 0.37$, 2-tail $t$-test).

![A Layer IV FSU](image1)

![B Layer IV RSU](image2)

**FIG. 6.** Influences of chronic unilateral inactivation of SI by muscimol for 7 days on the layer IV FSUs and RSUs in contralateral D2 barrel column. A: bar graph comparing the average response for 22 FSUs in layer IV from muscimol BFC group and 11 FSUs from saline control. Muscimol application significantly reduced the mean response magnitude of FSUs to stimulations of both principal whisker D2 and row surround whiskers D1 and D3. B: bar graph represents the average response for 61 RSUs from muscimol BFC group and 31 RSUs from saline control group. Only the response to the principal whisker D2 reduced significantly.

![A Response to D2](image3)

![B Response to D2](image4)

**FIG. 7.** Effect of muscimol on response latency epochs of neurons in contralateral D2 barrel column. A: comparison of the average response magnitude within 4 latency epochs to principal whisker D2 stimuli. Muscimol application reduced both the short (3–10 ms)- and longer (10–50 ms)-latency components. B and C: comparison of the average response in 4 latency epochs to surround whisker D1 and D3 stimulations, respectively. Muscimol application only decreased the long (10–100 ms) latency components in surround whiskers.

**Latency analysis**

Responses of a barrel neuron to whisker stimulation consist of the temporal interactions of excitation/inhibition from different circuits. Thus investigating the temporal structure of the response offers an opportunity to differentiate the role of various brain areas in cortical function. Latency epochs are dominated by different inputs to barrel column neurons. It is of interest to see how inactivation of BFC influences the temporal structure of BFC neuron responses on the contralateral side. Figure 7 compares the mean values of four latency epochs from cells in all layers. Effects of muscimol on latency epochs substantially differ between principal whisker D2 and row surround whiskers D1 and D3. Response decrease was observed in the longer (10–100 ms)-latency components in response to D1 and D3 whisker stimulation where the short latency component is normally small. Response to the principal whisker D2 decreased prominently in both the robust short-latency (3–10 ms) and the more variable long-latency (10–50 ms) components. On average, spike number to D2 stimuli in 3-
to 10-ms component decreased 49% \((P < 9.6 \times 10^{-6})\), 10- to 20-ms epoch component reduced \(-47\% \(P < 1.3 \times 10^{-7}\), 20–50 ms reduced 54% \((P < 3.4 \times 10^{-5}\), but responses in the small 50- to 100-ms component remained unchanged \((P < 0.22)\). The response to the surround whisker D1, the spike number within 3–10 ms decreased by 62% \((P < 0.05)\), by 45% \((P < 0.0004)\) in 10- to 20-ms epoch, by 54% \((P < 1.8 \times 10^{-6}\) in 20–50 ms, and by 36% \((P < 0.03)\) in 50–100 ms, respectively. Similarly, response to D3 decreased by 59% \((P < 3.8 \times 10^{-5}\) in 10- to 20-ms epoch, 66% \((P < 1.2 \times 10^{-6}\) in 20–50 ms, 43% \((P < 0.008)\) in 50–100 ms, whereas the 3- to 10-ms component was unchanged \((P < 0.41)\). Latency epochs of FSUs and RSUs within layer IV were also analyzed, and the results were shown in Fig. 8. Comparison of the data between 22 layer IV FSUs in muscimol BFC group and 11 FSUs from the saline control in response to the principal whisker D2 found that only the neural response within 50–100 ms in barrel FSUs was significantly suppressed by muscimol application \((P < 0.03)\). Responses in other latency epochs, although also lower than controls, failed to reach significance \((3–10 \text{ ms}: P < 0.08; 10–20 \text{ ms}: P < 0.06; 20–50 \text{ ms}: P < 0.06)\). This can be largely attributed to the small sample size and the huge variance we have for the layer IV FSUs. However, the influences of muscimol infusion were more prominent on the latency epochs of layer IV RSUs. After comparing 64 barrel RSUs in muscimol animals with 31 layer IV RSUs in control, significant response reduction was found in 3- to 10-ms \((P < 0.02)\) and 10- to 20-ms \((P < 0.0003)\) latency epochs, whereas the responses within 20–50 ms and 50–100 ms were less unaffected \((P < 0.05, P < 0.07\), respectively). On the other hand, responses of these layer IV FSUs to the surround whiskers D1 and D3 shifted significantly in temporal structure, whereas no change of latency epochs was found in barrel RSUs after muscimol application. Figure BB shows the influences of muscimol infusion on the latency epochs of layer IV FSUs and RSUs in response to D1 whisker stimuli. Responses within 10–100 ms were affected \((3–10 \text{ ms}: P < 0.18, 10–20 \text{ ms}: P < 0.05, 20–50 \text{ ms}: P < 0.02, 50–100 \text{ ms}: P < 0.0007)\). But all four latency epochs of layer IV RSUs in response to D1 and D3 stimulations failed to show significant changes (D3 data not shown).

**Effects of muscimol on C/S ratio**

When all units were pooled together, the data lead to the conclusion that muscimol didn’t have an effect on the C/S ratio. The mean value of the C/S ratio of 120 units from layer II to layer V in nine muscimol BFC rats is 4.20, which is not significantly different \((P < 0.13, 2\text{-tail } t\text{-test}, \text{ same in the following text unless specified})\) from the number of 3.03 from 70 units in five saline control animals. In saline control rats, the average C/S ratio of FSUs in all layers \((n = 20)\), which is 3.39, is not different from that of RSUs \((n = 49, P < 0.62)\), which is 2.92. Muscimol treatment didn’t alter the C/S contrast between FSUs and RSUs. The average C/S ratio for FSU in all layers \((n = 27)\) is 4.73 in muscimol BFC rats. When compared with the mean value of 4.25 from RSUs \((n = 82)\), statistical test failed to prove the significance \((P < 0.70)\). Furthermore neither FSU nor RSU showed a significant change in C/S ratio after muscimol \((FSU: P < 0.31, \text{ muscimol BFC vs. saline control}; RSU: P < 0.19)\), although the numbers did tend to increase after muscimol application. However, when layer IV

**FIG. 8.** Effect of muscimol application on latency epochs of layer IV FSUs and RSUs in the contralateral D2 barrel column. A: comparison of latency epochs of layer IV neurons in response to D2 whisker stimuli between muscimol BFC and saline group. Left: bar graph comparing the latency epochs of 22 layer IV FSUs from muscimol BFC group with that of 11 barrel FSUs from saline control group. Right: bar graph comparing the latency epochs of 61 layer IV RSUs from muscimol BFC group with that of 31 barrel RSUs from saline control group. B: comparison of latency epochs of layer IV neurons in response to D1 whisker stimuli between muscimol BFC and saline group. Left: bar graph comparing the latency epochs of 22 layer IV FSUs from muscimol BFC group with that of 11 barrel FSUs from saline control group. Right: bar graph comparing the latency epochs of 61 layer IV RSUs from muscimol BFC group with that of 31 barrel RSUs from saline control group.
units were examined separately, it was found that muscimol application did have a significant influence on the C/S ratio of barrel neurons. For 42 layer IV neurons in normal rats, the average C/S ratio was 2.70. Muscimol significantly increased the value to 4.59 ($P < 0.03$, 1-tail $t$-test) when the data from 83 barrel neurons in muscimol BFC group were analyzed. The increase was mainly due to changes in FSUs. The C/S ratio of FSUs significantly increased from 1.95 ($n = 11$) to 5.29 ($n = 22$) after muscimol treatment ($P < 0.003$, 1-tail $t$-test). But RSUs didn’t change their ratio: the mean value of 4.33 from 61 layer IV RSUs after muscimol application didn’t differ from the 2.96 ratio in 31 barrel RSUs in control ($P < 0.27$). Muscimol also seemed to alter the selectivity profile between FSUs and RSUs. In saline control animals, layer IV RSUs were more selective than layer IV FSUs (C/S ratio $2.96 vs. 1.95$, $P < 0.02$, 1-tail $t$-test). However, in muscimol animals, the difference between RSUs and FSUs disappeared ($4.33 vs. 5.29$, $P < 0.54$).

**DISCUSSION**

This study documents a significant decrement of evoked responses in rat SI cortical neurons to peripheral whisker stimulation, especially in the supragranular layers, after 7 days of activity suppression in the contralateral BFC. These data support the hypothesis that the cerebral hemispheres maintain an active bilateral balance of sensory processing activity. The crosstalk between the two hemispheres is achieved by modulating the responsiveness of neurons to sensory inputs at a single-unit level independent of the general level of background excitability. The present results provide insights into the way that callosal activity modulates cells in primary sensory cortex. Because the responses of BFC neurons after 7 days of contralateral muscimol treatment are reduced by roughly the same magnitude as in animals with BFC lesions, we conclude that most the neural response decrement 8 days after BFC neurons is induced by the effect on callosal activity. Comparison of muscimol and lesion data revealed a high degree of consistency in the evoked response profile (Fig. 10). After brain lesions, the response magnitude of neurons to test stimuli in the contralateral D2 barrel column was equally severely depressed in rats (Rema and Ebner 2003); this is in agreement with the present results. However, lesions of one BFC also decreased the background activity of neurons in contralateral BFC by nearly 80%, which was not true after reversible pharmacological silencing. We conclude that lesions produce a more global deficit than simple silencing as produced in the present study. Taken together, both the previous and current studies speak to the specific cellular changes that underlie interhemispheric “diaschisis-like” effects in sensory cortex and supports the hypothesis that primary sensory cortex in each cerebral hemisphere actively interacts in such a way that changes in activity on one side are registered continuously by neurons in connected areas. An unexpected finding was that the short-latency component of the evoked response that is usually associated with thalamocortical inputs were affected in both lesion and muscimol studies. We speculate that the contralateral VPM thalamic nuclei may be indirectly affected by the chronic unilateral cortical suppression by muscimol, and we are currently testing this possibility.

**Methodological concerns**

**SURGICAL AND RECORDING PROCEDURE.** The present study was designed to distinguish between impairments induced by reduced callosal activity resulting from cortical lesions that trigger degenerative events as well as activity decrements. Whether the cortex was effectively silenced without cell death emerges as a crucial issue. In the present studies, certain precautions were taken to prevent damage to cortex. Special care was taken during stabilizing and cementing to ensure the muscimol was delivered onto the surface of cortex without any direct damage. Furthermore, the brain was examined by histology after recording. Data from one rat with muscimol on SI were excluded after histology showed a small zone of cell loss directly under the cannula that must have occurred during implantation. We feel confident that direct brain damage had been minimized and the role of activity in interhemispheric interaction could be isolated. Spontaneous firing rate remained stable and equivalent between the experimental and control
animals. Thus we concluded that changes in responsiveness in BFC after contralateral BFC inactivation is not due to "exogenous" factors such as changes in our recording conditions.

We concluded that muscimol application decreased the occurrence of recordable neurons using our set of selection criteria, especially in the superficial layers. Neurons responsive to whisker stimulation in muscimol animals typically tend to have rather smaller amplitude spikes as displayed individually compared with units isolated in normal rats, although we have not quantified this difference. Neurons with large waveforms tend to be spontaneously active, but less drivable. Although we realize that the ratio of the more responsive but less discriminable units to the higher amplitude but less responsive neurons should be similar between experimental and control animals, this difference is also difficult to quantify. These changes may become important when comparing how neurons were selected in data from different studies.

### Physiological and pharmacological effects of muscimol in vivo

Muscimol is a powerful and selective GABA<sub>A</sub> receptor agonist (DeFeudis 1980a,b; Krogsgaard-Larsen et al. 1979; Naik et al. 1976). Because GABA is the primary inhibitory neurotransmitter in all layers of cerebral cortex, pharmacological activation of GABA<sub>A</sub> receptors successfully suppresses activity in almost all neurons (Hendrickson et al. 1981; Hess and Murata 1974). The inhibitory action of GABA is generally associated with a direct postsynaptic effect (Newberry and Nicoll 1985). Muscimol had been widely used to reversibly inactivate a circumscribed neuronal population, but it is still necessary to characterize its physiological and pharmacological effects in each application. First we needed to establish whether the pharmacological effect of muscimol introduced any bias to our observations and, second, whether the muscimol solution at the concentration used (10 mM) effectively suppress neuronal activity over the target area.

The acute muscimol experiments demonstrated that 10 mM muscimol solution, applied directly to the surface of barrel area, produces a reversible suppression of cortical activity. The suppression developed over time and by 20 min postmuscimol application, spontaneous firing and responses of layer IV neurons in D2 barrel column to peripheral stimuli were reduced by >50%. Although there is little question that muscimol suppresses neural activity in cortex, it is also the case in the acute experiments that muscimol could enter cortex along the electrode tract and alter the onset time. We mapped the effect area of muscimol in vivo by electrophysiology, and the results are highly consistent with what had been reported using muscimol solution with the same concentration (Edeline et al. 2002; Reiter and Stryker 1988). Our data show that 10 mM muscimol can diffuse horizontally over a large area to affect the majority of BFC. An autoradiographical study (Martin 1991) in rat spinal cord showed that when 5 mM (1 mg/ml) radioactive muscimol was injected into the spinal cord, a sphere of suppression with radius of 1.5–1.7 mm was formed. Given the concentration of muscimol solution (5 mM) is one half of ours (10 mM), and assuming that muscimol diffuses with similar speed in the brain and spinal cord, it is reasonable to conclude that the 10 mM muscimol solution we applied to the surface of cortex could suppress a large enough area to affect the majority of the BFC.

### Physical connections underlying the functional downregulation

Early studies reported an unmasking and expansion of receptive fields of homologous areas in neocortex after a peripheral denervation on the ipsilateral side (Calford and Tweedale 1990; Clarey et al. 1996). The extensive homotopic/nonhomotopic commissural projections presumably serve as the underlying circuit for the interhemispherically transferable plasticity (Krubitzer et al. 1998). In rats, the anatomy of commissural connections correlates well with our finding that suppressing one BFC with muscimol for 7 days significantly downregulates...
the evoked responses of neurons in the contralateral BFC in most layers (infragranular neuron changes failed to show significance). Extensive connections exist between SI in the two hemispheres of rats as well as other mammals including cats, raccoons, gray squirrels, and monkeys (Gould and Kaas 1981; Jones and Wise 1977; Jones et al. 1975; Koralek et al. 1988, 1990; Krubitzer et al. 1986; Olavarria and Van Sluyters 1995; Olavarria et al. 1984; Wise and Jones 1976; for review, see Innocenti 1986). Commissural projections were reported to be mostly reciprocal between homotopic and nonhomotopic SI areas in rats, and the density of these connections varies as a function of the body part representation examined (Akers and Killackey 1978; Koralek et al. 1990). It was once believed that commissural cells and terminals mainly distributed in the agranular zone medial to the BFC with a columnar structure, while the callosal projections in BFC were absent (Akers and Killackey 1978; Wise and Jones 1976). However, more recent studies using axonal transport tracing techniques have revised this picture, so the vibrissae area in SI is not devoid of direct callosal projections. By injecting HRP into one BFC, callosal cell bodies and terminations were visualized in both supra- and infragranular layers of BFC on the other side (Olavarria et al. 1984). Those projections also terminated within the “septa” region in layer IV and preferred whisker rows to arcs, whereas only sparse terminals were observed in layer IV barrels (Olavarria et al. 1984). Similar results have also been reported in the upper and lower jaw representation areas of the granular zone in rat SI (Hayama and Ogawa 1997). These authors also reported moderately dense labeled callosal cell bodies and terminals in the septa of the postero medial barrel subfield (whisker input cortex) after injections in the contralateral barrel area compared with sparse to absent labeling inside of the layer IV barrels per se (Hayama and Ogawa 1997). Anatomical studies provide a substrate for physiological observations of interhemispheric interactions. Bilateral receptive fields have been described in several components of the somatosensory system in rats (Armstrong-James and George 1988; Shuler et al. 2001) and non-human primates (see Iwamura 2000; Iwamura et al. 2001 for review). Although it is possible that the ipsilateral body part close to the midline is represented in cortex via subcortical structures (Armstrong-James and George 1988), distal body parts such as digits (Iwamura et al. 1994) and vibrissae (Shuler et al. 2001) are bilateralized through the corpus callosum. On the other hand, plasticity induced in one hemisphere transfers to the other hemisphere after either peripheral deafferentation (Calford and Tweedale 1990; Clarey et al. 1996; Shin et al. 1997) or a central lesion (Reinecke et al. 2001; Rema and Ebner 2003) in mammals, and these interhemispheric changes are also mediated by the corpus callosum. Previous studies showed that the exchange of sensory information between two cerebral hemispheres is dependent on the intactness of the contralateral cortex and corpus callosum either by evoked field potential (Pidoux and Verley 1979), single unit recording (Shuler et al. 2001) or behavior tasks (Krupa et al. 2001; Shuler et al. 2002). These studies unequivocally demonstrated that functional interlink between two BFCs is mediated via callosal connections in an activity dependent way. More details were revealed by examining the laminar distribution of callosal projections in BFC. Callosal axons originate almost entirely from pyramidal neurons in layer II/III and layer V/VI in rat (Hayama and Ogawa 1997; Wise 1975; Wise and Jones 1976). They terminate with excitatory asymmetric synapses on spines of the apical or basal dendrites of pyramidal neurons in the superficial layers of the target regions of the contralateral cortex (Cipollioni and Peters 1983; Wise and Jones 1976; but see Vaughan 1983 for the callosal terminals in infragranular layers). Thus it is expected to see that BFC neurons in the superficial layers would be affected most after the contralateral BFC was silenced. This is indeed what we found in the present experiments. Both the current study and our previous lesion study (Rema and Ebner 2003) converge at this point. This result further indicates an integrative role of supragranular layers in the barrel cortex: it is the place where the integration of the thalamic and callosal information collides. Because layer IV barrels are more or less devoid of direct callosal inputs, it is rather surprising to find a significant decrease of responses in layer IV barrels. Two possible explanations may account for the layer IV effects. One is that changes in nearby septa and layers above and/or below layer IV barrels after inactivation of contralateral BFC alter the response properties of barrel neurons. It has been reported that whisker pairing plasticity was first observed in supra and infragranular layers before they occur in layer IV (Diamond et al. 1994). Similarly, because neurons in septa and superficial layers receive more callosal inputs relative to barrel neurons, layer IV neurons may be influenced indirectly. The difficulty with this explanation is that available evidence suggests that callosal inputs are segregated from thalamic inputs in the primary sensory areas in mammals (Gould and Kaas 1981; Jones and Wise 1977; Jones et al. 1975; Koralek et al. 1988, 1990; Krubitzer et al. 1986; Olavarria and Van Sluyters 1995; Olavarria et al. 1984; Wise and Jones 1976), but little is known about the details of the recipients of callosal projections in septa in the sense of cell types (e.g., FSU versus RSU). Thus no consistent theory emerges to explain the response decrease observed in layer IV neurons. Another explanation is that changes in the layer IV barrels are due to plasticity in dorsal thalamus. Callosal fibers also terminate in infragranular layers (see preceding text), and stimulating the corpus callosum activates only two classes of cells: suspected inhibitory neurons (SINs) and corticothalamic neurons in layer VI (Swadlow 1988, 1989). Because corticothalamic projections are the driving input to POM neurons and an important modulator to VPM neurons (Diamond et al. 1992a,b; Krupa et al. 1999; Sherman and Guillery 1996), it is possible that response decrease in layer IV is actually a reflection of the indirect effects of chronic silencing of contralateral BFC on thalamic relay neurons. We found that muscimol suppression had profound effects on several latency components of a response. When neurons from different layers were averaged, the longer-latency components (>10ms) exhibited significant decreases after both principal and surround whisker stimulation (Fig. 6). These latency components are thought to be responses generated by ipsilateral intracortical and interhemispheric connections. However, even layer IV neurons are influenced by muscimol application (Figs. 5 and 7). Because barrel neurons are driven predominantly by VPM, it is unexpected to find decrements in response to sensory stimuli in layer IV neurons. More surprisingly, the shortest-latency component (3–10 ms) decreased significantly. Responses of BFC neurons generated within 10 ms after stimulus onset are strongly driven by direct connections from
VPM (Armstrong-James et al. 1993). These findings also indicate that thalamus may be affected indirectly by muscimol suppression as well. Traditionally thalamic sensory relay nuclei were viewed as an autonomous relay conveying information to cortex with little modification. The classical view can hardly explain the current results. As a matter of fact, cortex projects extensively back to thalamus, which can have an impact on thalamic cell responses (Diamond et al. 1992b; Li and Ebner 2003).

The ascending lemniscal thalamocortical projections ending in layer IV determine the center of the classic receptive field of barrel neurons, whereas the surround of receptive field is thought to be largely generated via the intracortical connections between layers/barrel columns (Armstrong-James and Fox 1987; Armstrong-James et al. 1991; Simons and Carvell 1989). Hence the C/S ratio of barrel neurons indicates the interactions of neural circuits that the cortex employs in whisker information processing. As discussed in the preceding text, the thalamocortical and callosal projections form a complementary and layer-specific pattern in BFC, suggesting that in adult rat BFC the thalamic inputs may have complex interactions with the function of callosal inputs. Thus by examining the C/S ratio of barrel neurons before and after removal of the callosal projections we can detect changes in the balance of center and surround circuits. Our data show that FSUs are more sensitive to the removal of commissural inputs than RSUs. Callosal projections seem to converge more on FSUs because stimulating the corpus callosum activated SINs but not RSUs (Swadlow 1988, 1989), thus removal of excitatory callosal inputs to SINs can degrade their response, but the predicted outcome would be disinhibition. Commissural fibers also contact pyramidal neurons in layer II/III of the contralateral cortex (Cipolloni and Peters 1983; Wise and Jones 1976), the excitatory nature of commissural fibers and their spatial segregation indicates that in normal rats, the interhemispheric inputs reach a general equilibrium with the thalamic inputs so that the responsiveness of neurons in SI is maintained. Hence after chronic inactivation of one BFC, a mixed effect should be observed. When interhemispheric plasticity was acutely induced in cortex, suppression was recorded after an initial increase of responsiveness of neurons in contralateral homotopic area (Clarey et al. 1996; Reinecke et al. 2003). Removal of callosal inputs has a complex impact on the neural circuits on the contralateral side. First disinhibition occurs when some FSUs lose their excitatory input from the corpus callosum; however, because pyramidal neurons in superficial layers as well as in septa receive commissural influences, effects on RSUs diminish the disinhibition from FSUs and the responses to both principal and surround whiskers decrease significantly after chronic inactivation of the contralateral BFC. Thus the effect reported would be strongly biased by the duration of the silencing. It is important to emphasize that the decrease in FSU responses also can be influenced by changes in the level of thalamocortical inputs. Corticothalamic projections to both VPM and POm could affect transmission through thelemniscal and/or paralemniscal pathways back to the BFC. Because infragranular neurons are generally less responsive to whisker stimuli, paradigms other than comparing spike numbers to whisker stimuli may be more sensitive to these changes.

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