Spatial Patterns of Excitation and Inhibition Evoked by Lateral Connectivity in Layer 2/3 of Rat Barrel Cortex

In the rat barrel cortex, neurons in layer 4 are topographically arranged in a precise columnar structure, and the excitatory feed-forward input from layer 4 to layer 2/3 projects almost exclusively within the home barrel column. Here we analyzed the lateral connectivity that links neighboring columns in layer 2/3, which is necessary for integrating information across whiskers. We examined the spatial distributions of three different functional types of lateral connections in layer 2/3 of the rat barrel cortex: glutamate receptor-mediated excitatory connections, GABA\textsubscript{A} receptor-mediated inhibitory connections and GABA\textsubscript{B} receptor-mediated inhibitory connections. Synaptic potentials of pyramidal neurons, which are measures of the strength of connections, were evoked by a horizontal array of stimulation electrodes. The synaptic potentials and their decrease with distance from the stimulation site were measured in two types of slices whose planes were parallel to or orthogonal to barrel rows. Excitatory and GABA\textsubscript{B} receptor-mediated inhibitory connections were stronger along barrel rows than across them, whereas GABA\textsubscript{A} receptor-mediated inhibitory connections did not show such a tendency. These results indicate that lateral connectivity in layer 2/3 varies on the basis of not only excitatory polarity but also receptor subtypes.

Keywords: apical dendrite morphology, barrel rows, biocytin-loaded cell, somatosensory, whole cell patch clamping

Introduction

Intercolumnar lateral connections are thought to play an important role in psychological and cognitive phenomena (Gilbert and Wiesel, 1990; Ramachandran and Gregory, 1991; Field et al., 1995; Buonomano and Merzenich, 1998b), but the mechanisms of these effects are uncertain because the physiological properties of the lateral connections remain poorly understood. Recently, the spatiotemporal patterns of excitation and inhibition in layer 2/3 have been observed using an optical recording technique with voltage-sensitive dye in tangential cortical slices (Tucker and Katz, 2003a,b). This result suggests that cortical inhibition driven by lateral connectivity sculpts the distribution of activity in the cortical network. However, the distribution of cortical excitation and inhibition evoked by lateral connectivity in layer 2/3 remains unclear.

It has been established that each cortical barrel represents a principal whisker on the contralateral side of the face in a one-to-one correspondence, and that neurons within a barrel respond most robustly to a deflection of the same principal whisker. Barrels receive inputs largely from their homologous barreloids, which are also in a one-to-one correspondence with whiskers (Woolsey and Van der Loos, 1970; Land and Simons, 1985). In the mature somatosensory cortex related to facial whiskers (barrel cortex), the excitatory feed-forward inputs from layer 4 to layer 2/3 are mediated by axons of spiny stellate and pyramidal cells in layer 4 that project to layer 2/3 almost exclusively within the home barrel column, forming a topographically precise vertical projection (Lubke et al., 2003; Foeller and Feldman, 2004). Considering that integration of information across whiskers by lateral connections that link neighboring columns in layer 2/3 is necessary, this anatomically robust structure of the barrel columns offers a good model for studying cortical lateral connections in layer 2/3 by an electrophysiological approach using brain slices. Since complete isolation of lateral connections in layer 2/3 from oblique connections from other layers is technically difficult, it should be noted that our measurements of inter-columnar connectivity in layer 2/3 probably include some afferent inputs from other layers. However, we feel that our recordings primarily reflect the horizontal intracortical connections of layer 2/3 because of series of findings suggesting that major connections are vertical and horizontal within the primary sensory cortex. The findings were made by a variety of approaches: anatomical traces (Staiger et al., 1999) pharmacological isolation by focal application of TTX (Ichinose and Murakoshi, 1996), and electrophysiological synaptic response measurement using brain slices (Chagnac-Amitai and Connors, 1989; Lubke et al., 2003).

In vivo optical recording of voltage-sensitive dye signals has revealed that the neuronal activity in the barrel cortex spreads laterally in an oval pattern, preferentially along the rows of the barrel field (Derdikman et al., 2003; Petersen et al., 2003). In a previous in vivo intrinsic signal optical imaging experiment, we found that timing sensitivity of two-whisker stimulation occurs only between whiskers in the same barrel row (Ajima and Tanaka, 2002). We have also reported that intrinsic signals evoked during the application of GABA\textsubscript{A} receptor antagonists extended preferentially along barrel rows in the tangential cortical plane (Ajima et al., 1999; Ajima and Tanaka, 2002). Because intrinsic signals originated mainly from layer 2/3 of the cortex in in vivo imaging, lateral connections in layer 2/3 of the rat barrel cortex are suggested to have a spatially biased distribution.

To reveal the spatial patterns of excitatory and inhibitory lateral connectivity, we used slice preparations whose planes were parallel to barrel rows (1R slice) or orthogonal to barrel rows (5R slice), and we carried out whole-cell patch recordings from pyramidal neurons in layer 2/3. We found that excitatory lateral connectivity and GABA\textsubscript{A} receptor-mediated inhibitory lateral connectivity were stronger in the 1R slice than in the 5R slice, but we found no such differences in GABA\textsubscript{B} receptor-mediated inhibitory connectivity between the two
types of slices. That is, excitation and GABA<sub>a</sub> receptor-mediated inhibition were stronger along barrel rows than across them, and these spatial patterns depended on mediated receptors.

**Materials and Methods**

**Slice Preparation**
The experiments were performed using young Long Evans rats (19–35 postnatal days old). The animals were deeply anesthetized with ether and decapitated. The brain was quickly removed, submerged in an ice-cold artificial cerebrospinal fluid (ACSF) solution, placed on a flat tray and sliced at a 45° angle. The slice was 350 µm thick. The slices were kept in a holding chamber for at least 1 h at room temperature before recording. The normal ACSF solution used was composed of the following (in mM): 124 NaCl, 3.0 KCl, 1.0 NaH<sub>2</sub>PO<sub>4</sub>, 1.2 MgCl<sub>2</sub>, 2.4 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub> and 10 glucose; it was saturated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The membrane potentials of cells in the barrel cortex were recorded in the whole-cell mode at 34°C and observed under a microscope using a ×40 water immersion objective and infra-red differential interference contrast optics.

**Electrophysiological Recording**
Whole-cell patch recordings were performed at 34°C in a submersion-type chamber. A visualized whole-cell patch recording was obtained from cells in the upper layers. Patch electrodes were pulled from thin-wall borosilicate glass; they had a resistance of ~5 MΩ. A linear tungsten stimulating array of six electrodes arranged at 150–300 µm intervals was placed on neighboring upper layers, which were visually identified. The interval between stimulations was 5 sec, the duration was 100 ms and the intensity was varied from 10 to 500 µA. Data were acquired using PCLAMP (Axon Instruments, Union City, CA) and analyzed using ORIGIN (Origin Lab., Northampton, MA). The electrode solution used in the current-clamp recording consisted of the following (in mM): 140 potassium gluconate, 10 HEPES, 0.2 EGTA, 4.0 Mg-ATP, 0.3 Na-GTP, 2.0 NaCl and 4.0 KCl, or 122.5 potassium gluconate, 10 HEPES, 0.2 EGTA, 4.0 Mg-ATP, 0.3 Na-GTP, 8.0 NaCl, 17.5 KCl and 10 biocytin. The pH of the solution was adjusted to 7.3 with KOH, and the osmolarity of the solution was 295–300 mOsm.

GABA<sub>a</sub> receptor-mediated IPSP was measured at depolarized membrane potentials between −55 and −60 mV by passing a positive current, while NMDA and non-NMDA glutamate receptors were blocked by their antagonists, N-2-amino-5-phosphonovaleric acid (D-APV) (50 µM) and 6-cyano-7-nitroquinazoline-2,3-dione (CNQX) (20–40 µM), respectively. The IPSP completely disappeared following the application of a GABA<sub>a</sub> receptor antagonist, (+)-bicuculline methiodide (BMI). GABA<sub>a</sub> receptor-mediated IPSP was also measured at depolarized membrane potentials between −55 and −60 mV under the application of D-APV, CNQX and BMI, and a difference in this IPSP after application of CGP55845, an antagonist of selective GABA<sub>a</sub> receptor (Cunningham and Enna, 1996; Deisz, 1999; Chen and Pan, 2004), was obtained.

Drugs were applied by superfusing the slice with a solution containing the desired concentration of the drug. D-APV (50 µM), CNQX (20–40 µM) and CGP55845 (1.0 µM) were obtained from Tocris (Bristol, UK), and BMI (20–50 µM) was obtained from Sigma-RBI (St Louis, MO).

**Histological Procedures for Postfixed Slices**
Tissue slices containing biocytin-loaded cells were fixed by immersion in 4% paraformaldehyde in 0.1 M sodium phosphate buffer. Each slice was incubated overnight at 4°C with HRP-avidin D (Vector Laboratories, Burlingame, CA) in 0.05 M Tris-HCl-buffered saline (TBS) containing 0.5% Triton X-100 (TX). After washing in TBS, the slices were reacted with 3,3′-diaminobenzidine tetrahydrochloride (DAB) (0.05%) and H<sub>2</sub>O<sub>2</sub> (0.005%) in Tris-HCl buffer.

**Results**
To clarify the spatial patterns of cortical lateral connectivity, we used angled slice preparations that enabled us to visually identify barrels in the same row (1R slice) or in five different rows (5R slice) (Ajima et al., 1995; Finnerty et al., 1999) in a living slice (Fig. 1). Visualized whole-cell patch recordings were made from pyramidal neurons in layer 2/3, and synaptic responses were evoked by the electrical stimulation in the same layer using a horizontal array of six extracellular stimulation electrodes (S1–S6 in Fig. 1) arranged at 150–300 µm intervals. Stimulus intensity was scaled to the current threshold to produce excitatory postsynaptic potentials (EPSPs) with the stimulus electrode placed closest to the recording electrode (Fig. 1, S1) (S1 in the 1R slice: 31.6 ± 11.5 µA, n = 48; and in the 5R slice: 340.2 ± 20.2 µA, n = 34); this intensity was then used to scale stimuli for all six electrodes (S1–S6). Since the stimulus intensity for the current threshold to produce EPSPs is used as an indicator of brain slice freshness, we applied current at this intensity for all postsynaptic potential measurements. Stimulation was sequentially applied using six electrodes; the amplitude and initial slope of evoked EPSPs, and the amplitude of inhibitory postsynaptic potentials (IPSPs) were plotted as functions of the distance between the recording and stimulation electrodes. We performed exactly the same experiments in 1R slices and 5R slices to compare the amplitude and initial slopes of synaptic potentials evoked by S1. We also compared 1R and 5R slices regarding attenuation of synaptic potential with increasing distance between the recording and stimulating positions. For morphological analysis, biocytin was loaded into recorded neurons through the microelectrode used during recording, and the slices were fixed and stained with DAB after the experiments.

**Excitatory Connectivity**
We selected 75 pyramidal neurons (n = 45 in the 1R slice, n = 30 in the 5R slice) in layer 2/3 based on their morphology viewed under a microscope prior to recording, and they were all regular-spiking (RS) neurons when stimulated with current pulses (Conners and Gutnick, 1990). Some of the recorded neurons were biocytin-loaded and their pyramidal cell morphology was confirmed. The stimulus intensity was always set to be twice the threshold intensity for the induction of EPSPs with the closest electrode, S1, and the same current was used for S1 to S6 (Fig. 1). The stimulus intensities were almost the same for both 1R and 5R slices (2T = 16.7 ± 0.5 µA in the 1R slice, and 2T = 17.7 ± 0.53 µA in the 5R slice).

Figure 2A shows typical traces of EPSPs obtained from the 1R and 5R slices, respectively. In the stimulation, the electrode closest to the recording site generally produced the highest EPSP amplitude among the six stimulation electrodes. In Figure 2C, we plotted EPSP amplitude of EPSP at the same cells as in Figure 2A, B against the distance between the recording and stimulation electrodes. As the distance increased, the EPSP amplitude decreased in both the 1R and 5R slices. The attenuation of EPSP with the increase in the distance between the recording and stimulation sites obtained from the 1R slice was smaller than that from the 5R slice (Fig. 2C). To focus our attention on the attenuation of EPSP, all EPSP amplitudes were normalized by those at the closest stimulation electrode (S1). Normalized EPSP amplitudes were averaged for each stimulation electrode, and are shown in Figure 2D. The attenuation of the average EPSP amplitude obtained from the 1R slice was significantly smaller than that obtained from the 5R slice. The average values of EPSP amplitudes obtained for the closest stimulation electrode (S1) were also compared between the 1R and 5R slices (Table 1).
The attenuations of EPSP amplitude and slope were identical for sites lateral and medial to the recorded neuron in both 1R and 5R slices, suggesting that the density of excitatory lateral connections does not have a mediolateral directional bias (data not shown).

**GABAA Receptor-mediated Connectivity**

For IPSPs, the stimulus intensity was set to be five times larger than that of the threshold for the EPSP induction with the S1 electrode. IPSP amplitude decreased with increasing distance from the recording site to stimulation site (Fig. 3A,B). Since NMDA and non-NMDA glutamate receptors were pharmacologically blocked, the IPSPs we measured were from purely monosynaptic connections. No significant difference was observed in the amplitude-distance plot between the 1R and 5R slices (Fig. 3C). IPSP amplitudes normalized to the IPSP evoked by the closest stimulation electrode were averaged for each stimulation electrode, and are shown in Figure 3D. The average IPSP amplitudes from the 1R and 5R slices for S1 stimulation are shown in Table 1. Neither IPSP attenuation with increased distance nor average IPSP amplitude for S1 stimulation showed any significant differences between the 1R and 5R slices. Even when stimulus intensity was increased to twice that used in most of the recordings, the IPSP amplitudes and attenuations obtained from neurons in the 1R and 5R slices did not show statistical differences (data not shown), but the suppressed areas were enlarged by stronger stimulation in both slices. These results indicate that the spatial distribution of GABA<sub>A</sub> receptor-mediated inhibition is isotropic in the tangential cortical plane.

**GABA<sub>B</sub> Receptor-mediated Connectivity**

GABA<sub>B</sub> receptor-mediated IPSPs were obtained by calculating the difference between responses with and without the GABA<sub>B</sub> receptor antagonist CGP55845 (1 μM), because we found some positive and early phase components which are clearly different from GABA<sub>B</sub> receptor-mediated IPSPs under the existence of CGP55845, with glutamate and GABA<sub>B</sub> receptor antagonists. These IPSP responses were elicited by blocking EPSPs and fast IPSPs with APV, CNQX and BMI, while the membrane potential was maintained between -55 and -60 mV. The reversal of these IPSPs (~-85 mV) was close to the equilibrium potential of potassium ions, which was also consistent with GABA<sub>B</sub> receptors being permeable to potassium ions. The stimulus intensities in these measurements were again determined by testing at S1 and were set to be five or 10 times the threshold for EPSP. Figure 4A,B shows typical traces of GABA<sub>B</sub> receptor-mediated IPSP, which also decreased with the distance between the recording and stimulating sites. Figure 4C,D shows the distance-dependence of the IPSP amplitude evoked by weak or strong stimulation from the same neurons as in Figure 4A,B. For a weak stimulation (five times the threshold) there were no differences in the distance dependence of IPSP amplitude between the 1R and 5R slices. However, the IPSP amplitude decreased more sharply in the 5R slice than in the 1R slice for a strong stimulation (10 times the threshold). The amplitudes of GABA<sub>B</sub> receptor-mediated IPSP normalized to that obtained by S1 stimulation were averaged at each stimulation electrode. The attenuation of IPSP evoked by strong stimulation in the 1R slice showed a significant difference from that in the 5R slice.
whereas there was no significant difference for weak stimulation (Fig. 4E,F). The average IPSP amplitude for strong stimulation in the 1R slice was 40% higher than that for the 5R slice (Table 1), but no clear difference was observed for weak stimulation.

The similar difference in the attenuation of normalized synaptic potential amplitude was observed in the EPSP measurements. However, in the case of GABA<sub>B</sub> receptor-mediated IPSP, it is clear that the average IPSP amplitude evoked by S1 stimulation was higher in the 1R slice than in the 5R slice. This difference was only evident when stronger stimuli were applied.

We also tried to fit the IPSP decay to exponential functions, in order to examine whether the GABA<sub>B</sub> receptor subunits were differently distributed along versus across barrel rows. Since there was no significant difference in IPSP decay time constant in the 1R and 5R slices (145.1 with the 1R slice and 146.0 with the 5R slice), we conclude that there are not distinct populations of GABA<sub>B</sub> receptor subunits along versus across barrel rows.

### Dendritic Morphology of Pyramidal Neurons

To find a structural basis of the oriented functional connectivity, we also analyzed the morphology of the basal and apical dendrites of recorded pyramidal neurons. The Sholl (1953) method was applied to hand-traced biocytin-loaded neurons. As in the typical Sholl method, the number of dendritic intersections was counted using an overlay of concentric rings centered on the soma. We applied the method to all the dendrites (Fig. 5A) and to the apical dendrites alone (Fig. 5B). For all the dendrites, there was no significant difference in density of dendrites between neurons in the 1R and 5R slices, though the estimated total length of dendrites (4177 ± 104 µm for the 1R slice and 3877 ± 172 µm for the 5R slice) showed slight differences. On the other hand, in the analysis of only apical dendrites, the number of dendritic crossings of neurons in the 1R slice was significantly greater than that in the 5R slice ~100 µm away from the soma.

In order to examine the two-dimensional patterns of basal or apical dendrites, we modified the Sholl method with a grid at 50 µm intervals (Fig. 6A). The origin was placed at the position of a soma and the number of dendrites in each square of the grid was counted. The apical dendritic density we measured tended to be similar to that found in the conventional Sholl method, namely, the number of dendrites in the 1R slice was greater than that in the 5R slice ~50–100 µm away from the soma (Fig. 6C,D).

### Table 1

<table>
<thead>
<tr>
<th>Amplitudes of EPSPs and IPSPs at the closest stimulation electrode (S1) obtained from both 1R and 5R slices</th>
<th>1R</th>
<th>5R</th>
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<tr>
<td>EPSP amplitude</td>
<td>30.54 ± 0.95 mV (n = 45)</td>
<td>26.65 ± 1.12 mV (n = 30)</td>
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<tr>
<td>IPSP amplitude (GABA&lt;sub&gt;A&lt;/sub&gt;)</td>
<td>0.64 ± 0.15 mV (n = 11)</td>
<td>0.91 ± 0.21 mV (n = 9)</td>
</tr>
<tr>
<td>IPSP amplitude (GABA&lt;sub&gt;B&lt;/sub&gt;) under weak stimulation</td>
<td>2.85 ± 0.35 mV (n = 29)</td>
<td>2.57 ± 0.07 mV (n = 17)</td>
</tr>
<tr>
<td>IPSP amplitude (GABA&lt;sub&gt;B&lt;/sub&gt;) under strong stimulation</td>
<td>5.73 ± 0.43 mV (n = 42)</td>
<td>4.11 ± 0.58 mV (n = 23)*</td>
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Data are means ± SEM. *P < 0.05 between 1R and 5R slices using a paired t-test.

Figure 2. Spatial patterns of excitatory lateral connections in rat barrel cortex. Representative recordings of EPSPs evoked by the array of six stimulation electrodes (S1–S6) from layer 2/3 pyramidal neurons (with 1R slice in A and with 5R slice in B). Arrows in (A) and (B) indicate stimulation time. The EPSP amplitudes (C) obtained in the same neurons in (A) and (B) are plotted with respect to the distance between recording and stimulating electrodes. EPSP amplitude normalized to those evoked by S1 was averaged for each stimulation electrode in (D). Blue circles/bars and red circles/bars show data from the 1R and 5R slices, respectively. *P < 0.05, **P < 0.001 and ***P < 0.005; paired t-tests.
We did not find any such tendency in the case of basal dendrites (Fig. 6B). To characterize the directional difference of the apical dendrites between the 1R and 5R slices, the number of dendrites in each square of the grid was summed up along the vertical or horizontal axes. In this analysis, apical dendrites in the 1R slices were significantly more abundant than those in the 5R slices at a distance of 50--100 µm around the soma. These results again indicate that the proximal apical dendrites are denser along barrel rows than across them.

Discussion

In this study, we investigated the spatial patterns of excitation and inhibition evoked by lateral connectivity in layer 2/3 in the rat barrel cortex. We demonstrated that excitatory and GABA_B receptor-mediated inhibitory lateral connections tend to be stronger along barrel rows than across them, whereas GABA_A receptor-mediated inhibitory connections did not show any directional bias in the tangential cortical plane (Fig. 7). According to our estimation based on the barrel sizes and the amplitude--distance relationships, the excitatory connection at 2.2--3.8 barrels away along the barrel row and 1.7--2.5 barrels away across the barrel rows was 10% of that evoked by S1 stimulation. In the case of GABA_A receptor-mediated inhibitory connectivity, 10% of IPSPs at S1 remained at 1.4--2.5 barrels away along the row and 1.1--1.6 barrels away across the rows.

Directional Bias of Cortical Connections

Connectional asymmetries in barrel cortex have been suggested by anatomical studies (Chmielowska et al., 1989; Bernardo et al., 1990; McCasland et al., 1991; Aroniadou-Andrjaska and Keller, 1996). Most of these studies focused on the directional bias of the connections, namely, that more neurons connect along barrel rows than across them. Recently, optical imaging studies have demonstrated that directionally biased cortical activities are caused by whisker stimulation (Petersen et al., 2003; Derdikman et al., 2003). Because optical signals originate mainly from layer 2/3 of the cortex, as seen by in vivo imaging, these studies suggest that functional excitatory connections in layer 2/3 are also stronger along the rows. Our findings about the directional bias of excitatory lateral connectivity are in agreement with these previous studies.

Brumberg et al. (1996) reported that ventrocaudal whiskers evoked a strong inhibition of neighboring barrel neurons. Our results showed no spatial bias in GABA_A receptor-mediated inhibition, but we did find that GABA_B receptor-mediated inhibition has a spatial bias. However, there was no mediolateral bias in 1R or 5R slices. Brumberg and colleagues observed unit activities of neurons in layer 4, whereas we monitored synaptic responses in layer 2/3; therefore, the different findings might be attributable to different recording techniques or different recording layers, or both.

In our present study, spatial patterns of inhibitory lateral connections were different depending on the subtype of GABA receptor involved. These spatial patterns agree fairly well with our previous findings based on intrinsic optical imaging, in which
we used whisker stimulation to evoke cortical activity during the iontophoretic application of GABAA or GABAB receptor antagonists (Ajima et al., 1999). In that study, we found that intrinsic signals appeared isotropically in the presence of a GABAA receptor antagonist; however, they appeared in oval domains elongated along barrel rows in the presence of a GABAB receptor antagonist. The difference in the spatial distribution between GABAA and GABAB receptor-mediated responses suggests that there is a functional difference between these two receptors. It appears that GABAA receptors inhibit surrounding neuronal activity to enhance contrast of excitation by afferent inputs, and that GABAB receptors modulate afferent excitation in the same barrel rows.

Slow inhibitory connections mediated by GABAB receptors were stronger along barrel rows than across them, but only under strong stimulation. Under weak stimulation, there was no significant difference in the amplitude of slow IPSP between the 1R and 5R slices (Table 1). Because the 1R and 5R slices are unlikely to have different electrical properties, the 1R slice probably has more lateral connections that have a higher current threshold for IPSPs than the 5R slice, or more extrasynaptic GABAB receptors along barrel rows that are activated by strong stimulation. In fact, in various brain areas, the GABAB receptors are known to be distributed at both synaptic and extrasynaptic sites (Scanziani, 2000; Gonchar et al., 2001; Kulik et al., 2003). The extrasynaptic receptors may be involved during strong or frequent stimulation, when the spillover of GABA release from synaptic clefts to the extrasynaptic space occurs.

**Direction Bias of Neuronal Morphology**

There are two possible origins of the directionally biased connectivity found in this study: presynaptic axons that make synapses on the recording neuron and/or postsynaptic dendrites that have a row-directed bias. Preferred axonal arborizations...
along the row are observed in layer 2/3 pyramidal neurons (Petersen et al., 2003), and this axonal spatial bias probably contributes to the directional bias of excitatory lateral connections found in this study. In other words, more neurons and axons are stimulated in 1R slices than in 5R slices. To our knowledge, there are no reports to date about the spatial preferences of axonal arborizations from the inhibitory neurons.

Our morphological analysis showed that apical dendrites had a row-directed bias, whereas basal dendrites did not. Basal dendrites are known to accept synapses from layer 4 inputs (Feldmeyer et al., 2002), while apical dendrites, because of their morphology, are thought to be important for integration of cortical inputs. It was suggested that the branching pattern of apical dendrites is important features for the integration of the inputs in layer 5 pyramidal neuron (Scaefer et al., 2003).

We found a spatial bias of dendritic arbors only at the proximal apical dendrites and it was restricted to a distance of 50–100 μm from the recorded soma. Since the stronger connections mean more synaptic contacts and/or closer contacts to the soma on the recording pyramidal neurons, these proximal apical dendrites may serve the synaptic sites for the excitatory and slow inhibitory axons. Actually it has been reported that GABA_A receptors are located all over the dendrites, while GABA_B receptors are located relatively closer to the soma (Eder et al., 2001).

We also need to consider the maturation of cortical circuits. In this study we used young developing rats to prepare living slices, because of technical difficulties in the usage of rats older than 6 weeks old. Older animals may have much denser lateral connections in the 1R slice than in the 5R slice, because the formation of lateral connections is thought to be experience-dependent (Fukuda et al., 1993; Rioult-Pedotti et al., 1998; Trachtenberg and Stryker, 2001).

**Functional Role of Direction-biased Lateral Connections in Layer 2/3 of Rat Barrel Cortex**

Lateral connections in layer 2/3 of the barrel cortex are quite important for the earliest intercolumnal integration, because excitatory inputs from the whiskers are individually conveyed to the pyramidal neurons in particular barrels of layer 4. The discovery of directionally biased lateral connections in such an important cortical layer (layer 2/3) led us to speculate that inputs from the whiskers to the barrels tend to be firstly integrated within rows as if there was one set of sensors. Since a rat moves its whiskers in the row direction while exploring its environment, whiskers in the same row will be displaced sequentially as they brush along an object. The temporal
sequence of inputs for the whiskers may be an important piece of information that is firstly integrated within rows. We have already found that timing of inputs to neighboring whiskers in the same row affects cortical activity under anesthesia (AJima et al., 1999). Of course, during exploration of its environment, a rat repeatedly moves its whiskers, with a frequency of ~8 Hz. Repeated activation of the cortex, such as oscillatory activity, may be caused by whisker stimulation. Cortical oscillatory activities (7–12 Hz) were also observed in barrel cortex during active discrimination by whiskers (Wiest and Nicolelis, 2003), and oscillation of a different frequency (16 Hz) was observed in barrel cortex under anesthesia (Derdikman

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**Figure 6.** Spatial patterns of dendritic morphology of pyramidal neurons in layer 2/3 of rat barrel cortex. Fifty-micrometer interval grids were placed on the traces (A) and the number of dendrites in each square of the grid was determined for the basal (B) and apical dendrites (1R slice, C, 5R slice, D). Soma located at the origin. (E) and (F) represent the summation of dendrite number along the vertical and horizontal sections, respectively. Blue plots denote neurons in the 1R slice and red ones denote neurons in the 5R slice. Data from neurons in the 1R slice and those from neurons in the 5R slice were statistically analyzed by paired t-test: *P < 0.1, **P < 0.05 and ***P < 0.005.

**Figure 7.** The EPSP, fast IPSP (GABA<sub>A</sub>-mediated IPSP) and slow IPSP (GABA<sub>B</sub>-mediated IPSP) amplitude-distance relationships were summarized. Blue and red lines represent data from the 1R and 5R slices, respectively. Distance means the distance between the recording and stimulating electrodes.
et al., 2003). These kinds of oscillatory activity are widely suggested to have an important role in the encoding and integration of cortical information. Since GABA_AB receptor-mediated inhibition is known to modulate the frequencies of oscillatory activity in thalamic neurons (McCormick, 2002), cortical GABA_AB receptor-mediated inhibitory connections may also modulate these oscillation frequencies in a direction-biased manner.

We conclude that excitatory and GABA_AB receptor-mediated inhibitory lateral connections are stronger among barrel rows than across them. This is, at least in part, due to the spatially biased apical dendrites of pyramidal neurons. The data presented here strongly suggest that the directional bias of both lateral connections and apical dendrites are linked with particular functions in rat barrel cortex. These findings provide new insight into how cortical networks manage neuronal activities in order to carry out their function.

Notes

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References


