Similarity of Direction Tuning Among Responses to Stimulation of Different Whiskers in Neurons of Rat Barrel Cortex

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INTRODUCTION

Rats obtain information on the surrounding space through simultaneous and/or sequential multiwhisker contact with objects (Carvell and Simons 1990). Individual whiskers are sensing probes of punctate receptive fields, and sequential deflections of whiskers are recognized as consecutive stimuli along a skin surface. Thus it is essential to integrate information arising from spatially and temporally patterned deflections of the mystacial vibrissae for the neural representation of a spatiotemporally continuous three-dimensional environment around animals (Shimegi et al. 2000; Shuler et al. 2001).

The information received through whisker stimulation is processed in the postero medial barrel subfield of the primary somatosensory cortex where functional modules called barrel columns are somatotopically arranged (Woolsey and Van der Loos 1970). Cells in a given barrel column respond to inputs from the somatotopically corresponding whisker [principal whisker (PW)] (Welker 1976). When the PW is deflected, barrel cortex cells respond differentially to a variety of stimulus parameters such as angular direction, velocity, frequency, and amplitude of deflections. It is also known that there is a convergence of excitatory inputs derived from stimulation of neighboring whiskers to cells in the barrel cortex (Moore and Nelson 1998; Zhu and Connors 1999) and that cells in all layers exhibit spike response to deflections of several adjacent whiskers (AWs) (Armstrong-James and Fox 1987; Armstrong-James et al. 1992; Chaplin 1986; Ito 1985). Thus barrel cortex cells integrate extensive spatial and temporal information derived from various combinations of whiskers. Cells in the barrel cortex exhibit nonlinear interactions of the response to multiwhisker stimulation. Simons and his colleague (Carvell and Simons 1988; Simons 1983, 1985) have found that suppressive interaction was predominant in the sequential whisker stimulation paradigm, in which the response to the second whisker stimulation was strongly suppressed by the first whisker–elicited inhibition. More recent studies (Ghazanfar and Nicolelis 1997; Shimegi et al. 1999) showed that the response facilitation was induced when multiwhiskers were stimulated simultaneously or sequentially at short interstimulus intervals [ISIs; optimal ISI = 2.0 ± 2.7 (SD) ms; n = 57] (Shimegi et al. 1999). The patterns and magnitudes of the response interaction are strongly dependent on various spatiotemporal parameters of stimulus such as ISI, sequence, angular direction of whisker deflection, and combination of whiskers (Fanselow and Nicolelis 1999; Mirabella et al. 2001; Shimegi et al. 2000; Simons 1985). The response suppression was also observed at ISIs ranging from 5 to >100 ms, suggesting the functional difference between facilitatory and suppressive interactions. Therefore it is reasonable to assume that the complex features of the three-dimensional environment of animals are represented as stimulus-specific facilitatory and inhibitory interactions of the responses to multiwhisker stimulation.

However, several fundamental questions regarding how multiwhisker stimuli are integrated and processed by the somatosensory system remain to be answered. For example, little is known about 1) the relationship between stimulus specificity of responses to single-whisker stimulation of PWs and AWs, 2) the neuronal mechanism underlying stimulus-specific response interaction, and 3) the functional role of facilitatory and suppressive interactions of responses in cortical representation of stimulus features. To address these issues, we focused on the direction selectivity of responses to single- and multiwhisker stimulations of cells in the barrel cortex.
METHODS

Single-unit recordings were performed on the barrel cortex of 47 Sprague-Dawley rats weighing between 180 and 450 g. All efforts were made to minimize the suffering of animals and the number of animals used. The surgical procedures used were all in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (1996) and the Guidelines of the Animal Care Committee of the Osaka University Medical School.

Preparation

The animals were anesthetized with urethane (1.25 g/kg, ip). Lidocaine, a local anesthetic, was applied at the pressure points and around the area of surgery. After the initial surgery, the animal was placed on a stereotactic headholder. The Ag-AgCl surface electrodes were placed on the shaven skin to monitor the electric cardiogram. The depth of anesthesia was monitored throughout the experiment by testing for reflexes and monitoring the changes in heart rate in response to tail pinching. When the heart rate changed in response to tail pinching, more urethane was administered. Regular respiration (80–100 breaths/min) and absence of spontaneous movements were ensured. The rectal temperature was maintained at 37–38°C by a thermostatically controlled heating pad. To prevent brain edema, the animals were administered dexamethasone (1 mg/kg, im) 24 h before the experiment, and experiments were finished within 12 h after starting them.

Device for whisker stimulation

Whiskers were stimulated mechanically using probes attached to a piezoelectric stimulator (a single bidirectional bimorph) (Simons 1983) or a galvanometer (Shimegi et al. 1999, 2000). Whiskers contralateral to the recorded barrel cortex were trimmed to a length of 15 mm and were securely held with a wedge at the tip of the probe. The tip of the stimulating probe was positioned at a distance of 10 mm from the facial skin. The stimulating probe was fixed using an angle-changeable joint to a stimulator, by which the probe could be set such that the natural position of the whiskers was not disturbed and the row of whiskers was aligned accurately. The motions of the probe tip itself and the stimulated whisker were measured by a CCD laser displacement sensor (LK-030, Keyence, Osaka, Japan) to ensure that there was no bias in whisker deflection to particular directions.

PIEZOELECTRIC STIMULATOR. A piezoelectric stimulator was used for stimulating single whiskers in eight (45° step) directions by either changing the polarity of voltage or rotating the orientation of the bimorph. The bimorph was fixed on a joint pedestal that can rotate on the axis collinearly along the bimorph for stimulating whiskers in eight directions. The piezoelectric stimulator generated a ramp-and-hold deflection in eight directions (45° step), in which 0.4-mm displacement during 4 ms (velocity, 100 mm/s) was followed by a 300-ms holding period.

GALVANOMETER. Galvanometer-driven stimulators were used for stimulating single or multiple whiskers in the rostrocaudal direction. The galvanometer generated a 1.1-mm excursion of the tip during 10 ms without a hold phase. The onset and offset velocity at the tip of the probe driven by the galvanometer was 110 mm/s in both directions observed using the CCD laser displacement sensor.

Examination of device-related artifacts

To exclude the possibility that the results were contaminated by device-related artifacts, we examined three points: 1) whether two types of device evoke equivalent magnitudes of neuronal responses, 2) whether whiskers were stimulated equally in any direction, particularly in the rostrocaudal direction mainly used in this study, and 3) whether the stimulation of a whisker did not cause any movement of AWs caused by a facial conduction. Concerning the first point, we compared the magnitudes and latencies of the responses of regular-spiking units (RSUs) to whisker stimulations with two types of stimulator. The comparison of response magnitudes was performed by Welch’s t-test because the variances of responses to stimulations with two types of stimulator were statistically different from each other (test for equal variance, \( P < 0.05 \)). Significant differences were not observed between the stimulators in either magnitude (piezoelectric stimulator, \( n = 12 \) RSUs, 40.0 ± 33.6 spikes/25 stimuli; galvanometer, \( n = 80 \) RSUs, 24.9 ± 20.3 spikes/25 stimuli; Welch’s t-test, \( P = 0.16 \)) or latency (piezoelectric stimulator, \( n = 11 \) RSUs, 13.4 ± 3.7 ms; galvanometer, \( n = 78 \) cells, 14.8 ± 3.5 ms, Student’s t-test, \( P = 0.21 \)). It is consistent with the finding that a stimulation velocity of 100–110 mm/s was sufficient to elicit supramaximal responses in the barrel cortex cells, as previously reported (Ito 1985; Ito et al. 1979). Thus both types of stimulator had a comparable performance in evoking responses in cells. Concerning the second point, the motion velocities were 101.3 ± 1.8 mm/s in the rostral direction and 97.5 ± 3.6 mm/s in the caudal direction for the piezoelectric stimulator and 114.0 ± 2.2 mm/s in the rostral direction and 111.0 ± 2.9 mm/s in the caudal direction for the galvanometer. There were no significant differences in these velocities between the two directions for both stimulators (piezoelectric stimulator, Student’s t-test, \( P = 0.31 \); galvanometer, Student’s t-test, \( P = 0.15 \)). There might be a drift of piezoelectric stimulator to a particular direction, which is supposed to occur within a short (days to weeks) time (Temereanca and Simons 2003). If there is, it may enhance a bias of response toward a particular direction. To examine this possibility, we measured the distance and velocity of displacement of the stimulator in two directions. This measurement was repeated once a week over 3 wk. However, there were no significant differences in the distance and velocity with respect to the direction factor (2-factor ANOVA, \( P = 0.91 \)), the week factor (\( P = 0.77 \)), or the interaction (\( P = 0.56 \)).

To examine the third point, we measured the movement of whiskers adjacent to the stimulated whisker. Adjacent whiskers hardly moved during the whisker stimulation; for both stimulators, the displacement of adjacent whiskers was ≤25 μm at a distance of 10 mm from the facial skin, which could not elicit spike responses in recorded cells. Therefore it seems unlikely that there was a significant facial conduction of the stimulatory effect to adjacent whiskers.

Whisker stimulation

To examine directional preference of the response to single- and multiwhisker stimulations, whiskers were usually deflected rostrally or caudally from its natural position (Shimegi et al. 1999, 2000). The piezoelectric stimulator was used to obtain direction tuning curves of responses to single-whisker deflections in eight directions. The PW was determined as the whisker that evoked the greatest magnitude of response, and the shortest latency of the response was also used as another criterion when multiple whiskers showed responses of equivalent magnitudes. However, when the histologically identified PW did not agree with that identified electrophysiologically, the histological identification was adopted (\( n = 2 \)). For the multiwhisker stimulation, a pair of a PW and an AW in the same row was deflected either simultaneously or successively at varying ISIs. For the sequential stimulation, the PW was stimulated before or after the AW at varying ISIs. Multiwhisker stimulation caused both response facilitation and suppression, and the facilitation was evoked mainly at ISIs ≤5 ms and the suppression at ISIs >10 ms (Shimegi et al. 1999, 2000). Therefore a set of short ISIs of 0, 1, 2, 3, 4, 6, 8, and 10 ms and long ISIs ranging from 10 to 400 ms were tested to examine the temporal characteristics of response facilitation and suppression, respectively. In multiwhisker stimulation trials at short ISIs, two whiskers were deflected in the same direction either rostrally or caudally to examine the direction specificity of the facilitatory interaction of the response. To examine
the direction selectivity of the suppressive effect of the preceding AW stimulation on the response to PW stimulation, the direction of AW deflection was either the same or opposite to that of PW deflection.

**Electrophysiological recordings**

A rectangular opening (3 x 4 mm) of the cranium and a slit of the dura (<1 mm in length) were carefully made above the left barrel cortex (0–4 mm posterior to the bregma and 4–7 mm lateral to the midline) to allow the penetration of the recording electrodes. Single-pipette glass microelectrodes that were filled with 0.5 M sodium acetate containing 4% Pontamine Sky Blue (Tokyo Kasei, Tokyo, Japan) were used in this study to achieve the best isolation of a single-unit activity, as well as to obtain well-localized dye marks of the recording sites. The resistance of the electrodes ranged from 17 to 30 MΩ, as measured in situ. In most recordings, we could obtain well-isolated single cells that exhibited unitary spikes with the same waveform, amplitude, and time course.

On the basis of electrophysiological properties such as the firing pattern and time-course of action potentials, cells were classified into two types: RSUs and fast-spiking units (FSUs). Cells that fulfilled the following criteria were classified as FSUs: 1) the duration of the entire action potential was <0.8 ms, 2) exhibiting a high spontaneous firing rate (mostly 5–10 Hz), and 3) exhibiting an evoked response with burst-like multiple spikes. The remaining cells were classified into RSUs.

Once a single-unit activity was isolated, a PW was assessed qualitatively by manually deflecting the whiskers. The single-whisker stimulation of the PW and AWs and the following multiwhisker stimulation were performed. Peristimulus time histograms (PSTHs) were constructed on-line during 25 successive stimulations at a frequency of 0.5 Hz for each stimulus condition. This stimulus frequency seemed to be appropriate to avoid response adaptation, because we did not find any significant difference in the number of evoked spikes between the first and last 10 stimulations for rostral (first 10 stimulations, 5.2 ± 3.7 spikes/25 stimuli; last 10 stimulations, 5.9 ± 3.7 spikes/25 stimuli, paired t-test, P = 0.42) or caudal stimulation (first 10 stimulations, 5.3 ± 3.1 spikes/25 stimuli; last 10 stimulations, 5.1 ± 3.0 spikes/25 stimuli, paired t-test, P = 0.19) in 35 cells whose spike data of individual stimulations were available. The test order of direction of whisker deflection was randomized to avoid artifact related to routine methods. In this study, only cells that recorded reliably >2 h were analyzed. Therefore PW stimulation was periodically inserted as a control trial, by which the stability of single-unit activity, as well as to obtain well-localized dye marks of the recording sites. The resistance of the electrodes ranged from 17 to 30 MΩ, as measured in situ. In most recordings, we could obtain well-isolated single cells that exhibited unitary spikes with the same waveform, amplitude, and time course.

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sion between rostral and caudal deflections, was calculated using the equation

\[
\text{DI}(\text{SI}) = (\text{SI}_c - \text{SI}_r)/(\text{SI}_c + \text{SI}_r)
\]

where \( \text{SI}_c \) and \( \text{SI}_r \) are the SIs calculated from the responses to PW stimulation when an AW was antecently deflected in the caudal and rostral directions.

To assess the significance of direction bias of responses to rostrocaudal stimulations, we performed Student's \( t \)-test for responses to rostral and caudal stimulations in each cell. Cells with significant differences (\( P < 0.05 \)) were defined as direction-biased cells and the remainder as non–direction-biased cells, although rostrocaudal directions were not always the best direction for individual cells.

**Histological analysis**

At the end of each penetration, the electrode was pulled back to the depths where units were recorded, and dye marks were produced by passing tip-negative currents (intensity, 5 \( \mu \)A; duration, 1 s at 0.5 Hz; 200 pulses) through the electrode. After the recording experiment, the animals were deeply anesthetized with an overdose of anesthetics and perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in phosphate buffer (PB). The recorded hemispheres of the cortex were flattened and postfixed in 4% paraformaldehyde/30% sucrose in PB for 4–12 h. Sixty-micrometer-thick frozen tangential sections of the barrel cortex were cut on a microtome and immersed in PBS. Serial sections were histochemically stained for cytochrome oxidase (CO) (Wong-Riley 1979). The laminar locations of the recording sites and barrels in layer IV were identified under a microscope. Finally, the locations of cells in relation to the barrel columns were reconstructed by drawing, using a camera lucida. In our sample, the majority of cells were recorded from the CO-poor region. This seems to be because we estimated the CO-poor region to be much larger than the CO-rich region. The CO-rich column contained the barrel territory showing a strong CO staining in layer IV and regions above and below it; the rest of the cortex was classified as the CO-poor column. Because the area of the barrel in layer IV that was delineated by CO staining is smaller than that of the barrel demarcated by the Nissl-stained cell assembly, the CO-poor column can include not only the septal column but also the margin of the barrel column.

**RESULTS**

The well-isolated single-unit activity of 107 cells (RSU, 92 cells; FSU, 15 cells) was recorded from the barrel cortex. Among them, the laminar locations of 78 cells were identified histologically (RSU, layers II/III, \( n = 21 \), IV, \( n = 19 \), V/VI, \( n = 24 \), unknown, \( n = 28 \); FSU, layers II/III, \( n = 7 \), IV, \( n = 5 \), V/VI, \( n = 2 \), unknown, \( n = 1 \)).

**Direction selectivity of response to single-whisker stimulation**

The direction selectivities of responses to the deflections of single PWs and AWs were assessed.

**RSUs**

Twelve RSU-type cells were tested using an eight-directional (45° step) protocol with the piezoelectric stimulator. Among them, only five cells were histologically identified to be layers V/VI cells. Six of the remaining seven cells seemed to be also layers V/VI cells estimated from their cortical depth (1,062–1,449 \( \mu \)m from the cortical surface). The remaining one cell could be a layer IV cell (635 \( \mu \)m from the cortical surface). A typical example of an RSU-type cell representing clear direction bias for the responses to single-whisker stimulation is shown in Fig. 1. The cell responded almost equally to the deflection of whiskers E2 (left column) and E3 (right column). The E2 was defined as PW on the basis of the shortest response latency (E2 = 9 ms, E3 = 11 ms), which was ascertained histologically. When the E2 whisker was stimulated, the cell responded selectively to stimulations in the caudal directions (90–270°; Fig. 1, left column), and the maximal response was evoked by caudal (180°) whisker deflection (29.3 spikes/25 stimuli). The preferred direction \( [D_{\text{pref(E2)}}] \), the direction selectivity index \( [\text{DI}_c(E2)] \), and the orientation selectivity index \( [\text{OI}_c(E2)] \) were 165°, 0.46, and 0.06, respectively. The same direction preference was observed in responses to AW stimulation (Fig. 1, right column), and the shape of the direction tuning diagram was very similar to that of responses to E2 stimulation \( [D_{\text{pref(E3)}}] = 158°, \text{DI}_c(E3) = 0.64, \text{OI}_c(E3) = 0.04] \).

These results suggest a possibility that, in each cell, the preferred direction and magnitude of the direction bias are common between the responses to single-whisker stimulation of PW and AW. To analyze this point quantitatively, the \( D_{\text{pref}} \) and \( \text{DI}_c \) of the response to stimulation of AW in the same row as PW \( [D_{\text{pref}}(\text{PW}) \text{and DI}_c(\text{PW})] \) were plotted against those of the response to PW stimulation \( [D_{\text{pref}}(\text{PW}) \text{and DI}_c(\text{PW})] \) in Fig. 2 for 12 RSUs tested. A strong and positive correlation was observed between \( D_{\text{pref}}(\text{PW}) \) and \( D_{\text{pref}}(\text{AW}) \) (correlation coefficient, \( r = 0.84, P < 0.01 \), Fig. 2A). The slope of the regression line (1.04, solid line) was almost 1, which supported the above notion. Moreover, there was a moderate but statistically significant positive correlation between \( \text{DI}_c(\text{PW}) \) and \( \text{DI}_c(\text{AW}) \) (\( r = 0.64, P < 0.05 \), Fig. 2B). Although all 12 RSUs did not show a clear orientation selectivity \( [\text{OI}_c(\text{PW}) = 0.06 \pm 0.05; \text{OI}_c(\text{AW}) = 0.07 \pm 0.05] \), the degree of DI varied largely from cell to cell [Fig. 2B; \( \text{DI}_c(\text{PW}) = 0.18 \pm 0.15, \text{DI}_c(\text{AW}) = 0.22 \pm 0.21 \)]. These results, that is, small \( \text{OI}_c \) and varying value of \( \text{DI}_c \), imply that whisker responses of cells in the barrel cortex do not exhibit orientation selectivity; however, they exhibit wide range of direction tunings. Particularly, in Fig. 2A, the responses of 8 of 12 RSUs (66.7%) were tuned to the caudal direction (90–270°), even though the sample size was too small to test a statistical significance of the bias. As mentioned above, most of the cells analyzed were recorded from below layer IV. Therefore the common directional tuning property was preserved among the responses to the single-whisker stimulation of PW and AWs, at least in deep layers as suggested qualitatively by Simons (1985).

Do all AWs eliciting discharges exhibit the same direction selectivity as that of PW? Is the directional bias of responses to single-whisker stimulation consistent with that of responses to multiwhisker stimulation? To address these points, it is necessary to test as many whiskers and conditions as possible. Therefore we adopted a previously reported method to stimulate PW and AWs, in which direction selectivity was examined only in the rostrocaudal stimulations (Shimog et al. 1999, 2000) in 93 cells (80 RSUs, 13 FSUs).

First, we examined whether the similar directional preference of responses to single-whisker stimulation of PW and AW found in the eight-directional stimulation protocol could be confirmed in responses to rostrocaudal stimulations. Figure 3 shows typical examples of three RSU-type cells showing...
direction bias (cells 1 and 2) or nondirection bias (cell 3) in the responses to single-whisker stimulations in rostrocaudal directions. Cells 1 and 2 responded more vigorously to either the rostral or caudal deflection of their PWs than to the other directions (Fig. 3A). The same directional preferences in the rostrocaudal axis were observed in the responses to AW stimulation in each cell (Fig. 3, B and C). To quantify the directional bias of responses, direction selectivity index (DI) was calculated for individual whiskers; the value itself indicates the magnitude of bias, and the positive and negative signs indicate the preference to caudal and rostral directions, respectively. DI(AW) were very close to DI(PW) in each cell [Fig. 3, cell 1, DI(E1) = 0.51, DI(δ) = 0.60, DI(E2) = 0.76; cell 2, DI(E2) = −0.57, DI(E3) = −0.77]. The similarity of direction selectivity between the responses to single-whisker stimulation of PW and AW was also observed in non–direction-biased RSUs, which did not exhibit direction preference to any whiskers tested [Fig. 3, cell 3, DI(E1) = −0.01, DI(δ) = −0.01, DI(E2) = 0.10]. It should be noted that the magnitude and latency of response to single-whisker stimulation varied from whisker to whisker in a given cell, but the direction preference was consistent among whiskers.

To quantitatively analyze the similarity of direction selectivity between the responses to single-whisker stimulation of PW and AW in the rostrocaudal axis, the DIs of response to AW stimulation [DI(AW)] were plotted against those of response to PW stimulation [DI(PW)] in Fig. 4A for 60 RSUs. Twenty RSUs were excluded from this analysis, because their responses to AW stimulation in both directions were not significantly different from spontaneous discharges (Student’s t-test). When DIs were calculated for more than one AW for a given cell, the mean DI(AW) was plotted for the cell in the graph. The distributions of DI(PW) and DI(AW) are shown as histograms at the top [DI(PW)] and on the right [DI(AW)] of the graph, respectively. Also, the distribution of the distance of each dot from the diagonal line is indicated on the top right of Fig. 4A. The DI(PW)s of RSUs were distributed in a wide range from −0.85 to 1, which means that the direction preference of RSUs varies among cells, although the distribution of DI of RSUs was biased toward the caudal direction in both
responses to single-whisker stimulation of PW and AW (1-sample t-test, \( P < 0.05 \)). The bias toward the caudal direction is consistent with the results observed for the eight-directional stimulation protocol in 12 RSUs (Fig. 2A). However, we confirmed that this asymmetry of direction preference was not attributed to technical artifacts, for example, an anisotropic motion of the stimulator (see METHODS). Data were distributed close to the diagonal line (dotted line), indicating that each cell has similar direction preferences between PW and AWs. RSUs exhibited a statistically significant positive correlation between DI(PW) and DI(AW) \(( r = 0.88, P < 0.01, \text{Fig. 4A})\). The slope of the regression line \((0.86, \text{solid line})\) was close to 1.

To verify that such a similarity of directionality is not attributable to the nature of the stimulator, the same analysis was applied to 12 RSUs tested using the eight-directional stimulation protocol. DI8(D2) and DI8(AW) exhibited a statistically significant positive correlation between them (piezoelectric stimulator, 0.09 \(\pm\) 0.39; galvanometer, 0.15 \(\pm\) 0.40, Student’s \( t\)-test, \( P = 0.62 \)). Thus consistency in direction selectivity between responses to single-whisker stimulation of PW and AW in rostrocaudal directions was comparable with that observed for the eight-directional stimulation protocol. These results suggest that there is a functional network connecting neuronal populations that share a common specificity to the direction of deflection among whiskers.

**FSU**

An FSU has a larger receptive field than an RSU and exhibits a poor direction selectivity in responses to PW stimulation (Simons 1978). Therefore we examined whether direction selectivity in responses to AW stimulation is similar to that in responses to PW stimulation in FSU-type cells. Among 15 FSU-type cells analyzed in this study, 2 cells in layers II/III were tested using the eight-directional stimulation protocol and the remaining 13 cells using the two-directional stimulation (rostrocaudal) protocol. A typical example of an FSU tested using the eight-directional stimulation protocol is shown in Fig. 5. The cell responded to the stimulation of at least three whiskers (D1, D2, and D3). The PW was D2. In accordance with previous studies (Simons 1978; Swadlow and Gusev 2002), the direction selectivity of responses to PW stimulation was weak, and the direction tuning diagram was circular \([\text{DI}_8(D2) = 0.20; \text{Fig. 5}]\). The tuning diagrams of responses to AW stimulation were also circular, and their \(\text{DI}_8\)s were small \([\text{DI}_8(D1) = 0.19, \text{DI}_8(D3) = 0.07]\), which were consistent with the poor direction selectivity in responses to PW stimulation.

In the population analysis of direction selectivity for 13 FSU-type cells (Fig. 4B) tested using the rostrocaudal stimulation protocol, most of cells did not exhibit a strong direction preference, and their DI8s were smaller than that in FSUs (test for equal variance, \( P < 0.01 \)). Thus FSUs tended to have a weak direction selectivity for any whiskers that evoked spike responses.

**Relationship between direction selectivity and cell type**

There were studies showing that RSUs tuned better to a particular stimulus direction than FSUs (Kyriazi et al. 1996; Simons 1978). To confirm this point quantitatively, we performed two population analyses. The absolute DI, which is the degree of direction bias, of RSUs was significantly larger than that of FSUs (RSUs, 0.30 \(\pm\) 0.31, \(n = 80\) cells; FSUs, 0.11 \(\pm\) 0.14, \(n = 13\) cells; Welch’s \( t\)-test, \( P < 0.05 \)). The percentage of direction-biased cell tended to be greater in RSUs (37.5\%, 30/80) than in FSUs (23.1\%, 3/13), although a statistical comparison between them \((\chi^2\text{ test})\) could not be performed because of the small number of FSU samples.

There is a possibility that a direction bias is related to a spiking threshold of individual cells. That is, even when two given cells receive excitatory inputs with the same direction bias, the cell with a higher threshold should exhibit a greater direction bias in spike responses than the cell with a lower threshold. It is possible that a difference in threshold level is...
reflected in the spontaneous and the evoked firing level of cells. To examine this possibility, the spontaneous activity level and maximal spike response of direction-biased RSUs were compared with those of non–direction-biased RSUs. Spontaneous activity was defined as the spikes occurring during 25 stimulations without the actual whisker deflection. However, significant differences were not observed between two RSU groups either in spontaneous activity (Student’s $t$-test, $P > 0.05$, direction-biased cells, 0.17 ± 0.44 spikes/25 stimuli ($n = 30$ cells); non–direction-biased cells, 0.21 ± 0.39 spikes/25 stimuli ($n = 50$ cells)) or in maximal evoked response (Student’s $t$-test, $P = 0.20$, direction-biased cells, 21.1 ± 11.7 spikes/25 stimuli; non–direction-biased cells, 27.2 ± 23.9 spikes/25 stimuli). Those results suggest that the difference in the direction bias of spike responses reflected the difference in the direction bias of excitatory inputs rather than that in firing threshold.

Analysis of shortest latency of response to single-whisker deflection

It is possible that the difference in the direction of whisker movement is represented not only by the number of spikes evoked but also by the temporal aspects of response such as the response latency. To address this issue, minimal spike latency was compared between two directions of whisker deflection (Student’s $t$-test, $P = 0.93$, $P < 0.01$, for PW and AWs ($r = 0.90$, $P < 0.01$). In a population of direction-biased cells ($n = 21$; 18 RSUs, 3 FSUs) that exhibited significant magnitudes of responses to stimulation both in the preferred and nonpreferred directions, the latency of response to a preferred direction was not significantly shorter than that to a nonpreferred direction (preferred, 13.7 ± 3.9 ms; nonpreferred, 14.2 ± 4.1 ms). In this analysis, 12 cells among the 33 direction-biased cells were excluded because they did not respond at all or responded with a very small number of spikes ($\leq 2$ spikes/25 stimulations) or responded with spikes that were markedly scattered, making it difficult to discriminate the exact latency of response to stimulation in the nonpreferred direction. Moreover, the difference in the latency of response to PW stimulation between two directions was not associated with absolute DIs (Fig. 6E), suggesting that, with response latencies, cells code a difference in stimulated whiskers, but not a difference in stimulation direction.

In our result of latency analysis, the difference in the latency of responses to PW stimulation between two directions was not associated with absolute DIs (Fig. 6E). Regarding this point, Temereanca and Simons (2003) reported that, in the thalamic ventral posteromedial nucleus (VPm), the early component (i.e., initial 1.2–7.5 ms) of whisker-deflection-driven local field potential (LFP) is more direction-specific than the late component. If these early and late components of thalamic response
James and Callahan (1991), barrel cells that are driven by monosynaptic inputs from VPm discharge after 2 ms of firing of VPm cells. That is, the response latencies of the 68 cells analyzed in this study fell in the range corresponding to the late component of LFP of VPm. Therefore the direction selectivity and the time-dependent change of LFP in VPm are not directly represented in those of spike response of cells in the barrel cortex, presumably because LFP represents the integral of postsynaptic potentials evoked by lemniscal, reticular, and corticothalamic inputs in a population of thalamic neurons. Thus this point needs further consideration. However, there was a noticeable difference in response latency between PW and AW for both directions (Fig. 6, C and D). That is, the mean differences were 2.8 (Student’s t-test, P < 0.01) and 2.6 ms (P < 0.01) for caudal and rostral deflections, respectively. Thus the difference in response latency correlated well with the difference in whisker rather than in the direction of whisker movement.

Laminar analysis of direction selectivity of response to PW stimulation

We analyzed the laminar distribution of DIs for each cell response to PW stimulation to assess the laminar difference in the strength of direction selectivity (Fig. 7). Histograms were constructed with the absolute DIs of the response of each cell to PW stimulation only in the rostrocaudal axis (n = 71). There was no significant difference in either the mean DI or the median DI of layers (mean DI: layers II/III, 0.19; IV, 0.26; V/VI, 0.28; median DI: layers II/III, 0.08; IV, 0.06; V/VI, 0.10; Kruskal-Wallis test, P = 0.50). Among 59 RSUs that were tested using the rostrocaudal stimulation protocol and histologically identified, there was a tendency that direction-biased responses to PW stimulation were more frequently observed in layers IV (42.1%, 8/19) and V/VI (42.1%, 8/19) than in layers II/III (19.0%, 4/21), although the difference among layers was not significant (χ² test, P = 0.20). As for FSUs, this analysis was not performed because of the small number of samples (n = 12).

Direction selectivity and cell location in barrel columns

To examine the representation of direction selectivity in terms of barrel column, cells were divided into two groups on the basis of their location in barrel columns, that is, in the CO-rich (n = 9 cells) and CO-poor (n = 50 cells) columns. Mean absolute DI(PW) was compared between the groups, but no significant difference was found (CO-rich, 0.21 ± 0.26; CO-poor, 0.26 ± 0.29; Student’s t-test, P = 0.66). Moreover, the percentages of direction-selective cells were 44.4% (4/9 cells) in the CO-rich region and 32.0% (16/50 cells) in the CO-poor region, which were not significantly different (χ² test, P = 0.46). These results suggest that direction selectivity is not differentially represented between CO staining–defined subdivisions of barrel cortex.

Direction selectivity of response to multiwhisker stimulation

EXCITATORY INTERACTION OF RESPONSE TO MULTIWHISKER STIMULATION. The combined stimulation of PW and AW at short (<5 ms) ISIs often induces response facilitation depending on
the direction of whisker deflection, which is called direction-selective facilitation (Shimegi et al. 2000). An induction of stimulus-specific response facilitation is associated with the difference in the relative magnitude of excitatory responses elicited by single-whisker stimulation under different stimulus conditions (Shimegi et al. 2000). Therefore it is possible that direction-selective facilitation reflects the direction preference in responses of each cell to single-whisker stimulation. To examine this possibility, the direction selectivity of the response to multiwhisker stimulation was compared with that of single-whisker stimulation in the rostrocaudal axis.

Figure 8 shows a cell representing a typical direction-selective response facilitation. This cell responded to the stimulation of δ and E1 whiskers (Fig. 8, A and B). The PW was δ. Responses to both δ and E1 whisker stimulation were strongly biased toward the caudal direction [Fig. 8, A and B; DI(δ) = 0.83, DI(E1) = 0.77]. When the whiskers were deflected caudally from the resting position, the E1 whisker stimulation, antecedent to δ whisker stimulation by 2–3 ms, evoked a strong response facilitation (FI = 1.3–2.0; Fig. 8, C and D, left). In contrast, no facilitatory interaction was observed when both whiskers were deflected in opposite directions (Fig. 8, D, right). Among the 16 cells tested, 13 cells exhibited significant response facilitation ($P < 0.05$; mean FI = 2.40 ± 1.05) to either one or two directions of whisker deflection. Six of 13 cells were recorded from layers II/III, 2 from layer IV, and 2 from layers V/VI. The laminar location of the remaining three cells was not identified histologically. Five (38.5%) of 13 cells showing significant response facilitation exhibited direction-selective facilitation [layers II/III, 1/6 cell (16.7%); layer IV, 1/2 cell (50%); layers V/VI, 2/2 cells (100%); unknown, 1/3 cell (33.3%)]. In all five cells that exhibited direction-selective response facilitation, the stimulus direction able to induce response facilitation was consistent with the cell’s preferred direction, which was determined by single-whisker stimulation. As we reported previously (Shimegi et al. 1999), the optimal ISI for including response facilitation nearly corresponded to the difference in the latency of responses between PW and AW stimulations.

Figure 9 shows an example representing non-direction-selective response facilitation, that is, bidirectional facilitation.
The PW was E2, and no direction bias was observed in the response to PW stimulation [Fig. 9A; DI(E2) = 0.10]. The stimulation of AW, E1, evoked only a negligible number of spikes (Fig. 9B). In the combined stimulation of two whiskers, however, the facilitation was observed in responses to both directions of whisker deflection when the stimulation of E1 whisker preceded that of E2 by 3 ms (Fig. 9, C and D, left, caudal deflection FI = 1.55, and right, rostral deflection FI = 1.45). This implies that AW stimulation evoked the subthreshold excitatory response with no direction bias that contributed to an induction of bidirectional facilitation in this cell.

**FIG. 6.** Latency analysis. A and B: relationship of shortest response latencies between 2 (caudal and rostral) stimulus directions. C and D: relationship of shortest response latencies between 2 (PW and AW) whiskers stimulated. E: relationship between [DI(PW)] and difference in response latency between 2 stimulus directions. F: relationship between [DI(PW)] and the shortest latency of response to the preferred direction. Single or multiple AWs were tested for each cell, and obtained data were all plotted in the graph. A: response to PW stimulation. n = 68 for 68 cells. Regression line (solid line): f(x) = 0.98x + 0.62. Correlation coefficient: r = 0.93 (P < 0.01). Dotted line indicates the diagonal. B: response to AW stimulation. n = 57 for 42 cells. f(x) = 0.88 + 2.34. r = 0.90. C: response to caudal stimulation. n = 69 for 53 cells. f(x) = 0.91x + 4.36. r = 0.81. D: response to rostral stimulation. n = 67 for 47 cells. f(x) = 0.88x + 5.56. r = 0.77. E: difference in latency of response to PW stimulation in 2 directions was plotted against absolute value of cell DI. n = 68 for 68 cells. Regression line (solid line); f(x) = 0.88x + 3.23, correlation coefficient: r = 0.93 (P < 0.01). F: latency of response to PW stimulation in the preferred direction was plotted against absolute DI. n = 68 for 68 cells. Regression line (solid line): f(x) = 0.001x + 14.1. Correlation coefficient: r = 0.009 (P = 0.40).

The direction preference of response to multiwhisker stimulation was consistent with that to the single-whisker stimulation of the cells shown in Figs. 8 and 9. To examine this point quantitatively, the DIs of the response to the multiwhisker stimulation [DI(multi)] were plotted against the DI of the response to PW stimulation in Fig. 10. Data for 16 cells were plotted in the graph. There was a positive and statistically significant correlation between DI(PW) and DI(multi) (r = 0.92, P < 0.01), and the slope value of the regression line (solid line) was 0.84. The same analysis was applied to DI(AW) of 13 cells exhibiting responses to AW stimulation sufficient to calculate DI of the 16 cells. A significant correlation was also observed between DI(AW) and DI(multi) (r = 0.59, P < 0.05). Thus, in a population, the direction preference of responses to multiwhisker stimulation is well consistent with that of responses to single-whisker stimulation.

In all five cells showing direction-selective response facilitation, the facilitation was induced when whiskers were stimulated in the cell’s preferred direction, assessed with single-whisker responses. These results suggest that the direction selective of response facilitation is based on the direction-selective excitation of responses to single-whisker stimulations.

### Suppressing interaction of response to multiwhisker stimulation

Multiwhisker stimulation causes not only facilitatory response interaction but also a suppressive one (Shimegi et al. 1999, 2000; Simons 1983, 1985). The induction and magnitude of the suppressive interaction was consistent with that of the facilitation interaction in the single-whisker stimulation of the cell’s preferred direction.
of response suppression are also dependent on the direction of whisker deflection (Carvell and Simons 1988; Simons 1983, 1985). Thus we examined the relationship between direction selectivities of facilitatory and suppressive response interactions.

Figure 11 depicts an example of a cell showing unidirectional response to PW stimulation. The PW was E2, and its stimulation only in the rostral direction evoked responses (Fig. 11, A and C; DI(E2)/H11005/H11002 1.00). The stimulation of E1 whisker evoked only negligible number of spikes (Fig. 11, B and C). When the stimulation of the E1 whisker preceded that of the E2 whisker by 30–60 ms, the response to E2 stimulation was significantly suppressed (Fig. 11, D and E; Student’s t-test, P < 0.01). To quantify the response suppression, suppression index (SI; see METHODS, Eq. 2) was calculated. The responses to E2 stimulation were maximally suppressed at an ISI of 30 ms to the same extent in both caudal and rostral deflections of the E1 whisker, and the SI was 0.89. The profiles of the ISI tuning of responses showed that the suppressive effect became weak but more direction-selective at longer ISIs (Fig. 11E). The stimulation of the E1 whisker in the caudal direction, which by itself did not evoke spikes, suppressed the response to E2

FIG. 8. An example of the direction-selective response facilitation. A cell in layers V/VI. A–C: PSTHs of responses. Responses to single deflection of δ (PW) (A) and E1 (AW) (B). C: responses to combined stimulation of δ and E1, in which E1 stimulation preceded that of δ by 3 ms. Whiskers were deflected caudally (left) and rostrally (right) from the resting position. D: relationship between response magnitude expressed as the number of spikes per 25 stimuli (left ordinate) or facilitation index (right ordinate) and ISI (abscissa). Two open circles indicate the responses to individual stimulations of δ and E1 whiskers. Left graph: results for caudal deflection of whiskers. Right graph: results for rostral one. Filled circles indicate responses to combined stimulation. Note that facilitatory interaction was observed only when whiskers were deflected in the preferred direction of response to single-whisker stimulation, that is, caudal direction.

FIG. 9. An example of non-direction-selective facilitation. A cell in layers II/III. A–C: PSTHs of responses. Responses to single deflection of E2 (PW) (A) and E1 (AW) (B). C: responses to combined stimulation of E1 and E2, in which E1 stimulation preceded E2 by 3 ms. Whiskers were deflected caudally (left) and rostrally (right) from the resting position. D: interstimulus interval (ISI) tuning curve. Other notations as in Fig. 8. Note that direction bias was not observed in responses to single- and multiwhisker stimulations.
stimulation more strongly than that in the rostral direction at ISIs of 45 and 60 ms. Thus in this cell, the direction bias of response suppression was more pronounced at ISIs longer than that caused the maximal suppression, and the more suppressive direction was opposite to the preferred direction of the excitatory response to PW stimulation.

Among the 23 cells tested for suppressive interaction of multiwhisker stimulation, all cells exhibited significant suppression (Student’s t-test, \( P < 0.05 \)) at ISIs that induced the maximal suppression, which were mostly 20–30 ms.

To examine the ISI dependency of the strength and directional difference in the response suppression for the 13 RSUs tested, the relative magnitudes of the responses to stimulations in both directions during the suppression and the percentage of the directional difference of the suppression were plotted against ISI in Fig. 12, A and B, respectively. In Fig. 12A, the magnitudes of response suppression were compared between two directions of stimulation. In this analysis, the stimulation direction that caused a stronger or longer response suppression than the other direction in each cell was determined as the “strongly suppressive direction” and the other as the “weakly suppressive direction.” At ISI of 30 ms, the maximal suppression was observed without directional difference [\( \text{DI(SI)} = 0.08 \)]. As ISI increased, the effect of response suppression decreased, but its directional difference increased (Fig. 12B). A statistically significant difference in the magnitude of response was observed at ISIs of 60 and 100 ms (paired t-test, \( P < 0.05 \)). The directional difference in response to two directions was largest at ISI of 100 ms, and the DI(SI) calculated for this ISI was 0.34. Thus the strength and directional differences of response suppression were strongly dependent on ISI, and directional difference was reflected not in the maximal suppression but in the time-course of recovery from the suppressive effect.

To study the relationship between the direction selectivity of the response to PW stimulation and that of the response suppression by preceding AW stimulations, the direction selectivity of the suppression index [\( \text{DI(SI)} \)] obtained from multiwhisker stimulation was plotted against the DI(SI) of the response to PW stimulation [\( \text{DI(PW)} \)] in Fig. 13. The DI(SI)s were calculated in two ways: either from the maximal values of SI for each stimulus direction for 23 cells [Fig. 13A; \( \text{DI(SI}_{\text{max}} \)] or from SI at the ISI showing the maximal value of directional difference for 13 cells tested for long ISIs [Fig. 13B; \( \text{DI(SI}_{\text{dif}} \)]. The distributions of DI(SI) and DI(PW) are indicated as histograms at the right and top of Fig. 13, A and B, respectively. Except for cells that responded to PW stimulation only in one direction (\( \text{DI} = \pm 1.0 \)), we tested suppressive effect of AW stimulation on the responses to PW stimulation in both directions; thus the number of DI(PW) was shown as “sample” in the histogram. As shown in Fig. 13A (total number of samples = 52), most DI(SI_{max}) were within \( \pm 0.20 (0.06 \pm 0.10 \text{ SD}) \), suggesting that directional difference of the maximal effect of response suppression was very small, whereas the

![FIG. 10. Relationship between DI(SI) for responses to PW stimulation and the selectivity (DI(SI)) for responses to multiwhisker stimulation, in which cases (Box 16). Dots line, the diagonal line.](http://jn.physiology.org/)

![FIG. 11. Example of direction-selective inhibitory interaction of response of cell in layer IV, PSTHs of responses to single deflections of E2 (PW) (A) and E1 (AW) (B) and those of responses to combined stimulation of E1 and E2 (D) in which E1 stimulation preceded E2 by 30 ms. Whiskers were deflected caudally (top) and rostrally (bottom) from the resting position. C: magnitudes of response (mean \pm SD) to single-whisker stimulation. E: ISI tuning curves. Other notations as in Fig. 8. Note that direction bias in response suppression induced by preceding AW stimulation was observed at ISIs >30 ms, for which the Aw-induced stronger suppression of response to PW stimulation than the rostral (preferred direction) deflection.](http://jn.physiology.org/)
maximal value of the directional difference of the suppression was distributed over a wide range (Fig. 13B; total number of samples = 22). However, in both cases, there was no correlation between the directionality of excitatory response to PW stimulation and that of response suppression (Fig. 13A; $r = 0.18$, $P = 0.09$; Fig. 13B; $r = -0.17$, $P = 0.77$). Thus the relationship of directionality of suppressive effect of antecedent AW stimulation to that of excitatory response to single PW stimulation varies among cells and cannot be predicted from the direction preference of the response to PW stimulation.

**DISCUSSION**

**Summary of main findings**

We examined the direction selectivity of neuronal responses in the barrel cortex to single- and multiwhisker stimulations. The results are summarized as follows. 1) RSUs, i.e., putative excitatory cells, exhibited variability in direction bias of responses to single-whisker stimulation ($-1 \leq \text{DI(PW)} \leq 1$), and the direction specificity of the response to PW stimulation closely correlated with that of the response to AW stimulation in each cell (Fig. 2B, $r = 0.84$ for $D_{\text{rect}}$ of 8-directional stimulations; Fig. 4A, $r = 0.88$ for DLs of rostrocaudal stimulations) supporting the previous results by Simons (1985). 2) The direction selectivity of FSUs, i.e., putative inhibitory cells, was usually weak ($-0.38 \leq \text{DI(PW)} \leq 0.50$) for not only PW but also AW. 3) Multiwhisker stimulation in the rostrocaudal direction caused direction-selective response facilitation at short ISIs ($\leq 5$ ms) in which stimulus direction to evoke the facilitation was consistent with the preferred direction of the response to PW stimulation. 4) Stimulation of AW preceding that of PW by $>6$ ms induced the suppression of the response to PW stimulation, whose maximal effect was observed at ISI of 20–30 ms without direction bias.

**Direction-selective network**

We assessed the similarity in direction selectivity between responses to PW and AW stimulation in each RSU and found a strong correlation between them. This suggests the presence of a neuronal network connecting excitatory cells with different whisker preferences but with a preference for common stimulus direction. Multiwhisker stimulation causes response
facilitation in a direction-selective manner mainly in layers II/III (Shimegi et al. 2000). Therefore we hypothesized that direction-specific response facilitation would be generated through a summative interaction of multiwhisker inputs biased in a particular direction within the direction-specific intercolumnar network. We examined this point and found that the directionality of the response to multiwhisker stimulation strongly correlated with that of the response to PW stimulation \( (r = 0.92) \). This supports the above hypothesis and suggests that direction-specific network contributes to generate direction selectivity for not only the response to single-whisker stimulation but also the response to multiwhisker stimulation. As we previously suggested (Shimegi et al. 1999, 2000), the spatiotemporal properties of tactile stimuli are expressed as the stimulus-specific response facilitation of cells in the barrel cortex. Information of direction and velocity of the deflection and location of whiskers are all indispensable for the detection of object motion or relative movement of animals and objects. Excitatory networks seem to be highly specific in terms of stimulus properties for pigeonholing various features of single- and multiwhisker information.

At any levels of hierarchical processing of whisker information from the trigeminal complex to the barrel cortex, cells exhibit direction selectivity in response to PW stimulation, and the direction tuning of which tends to broaden as the hierarchical level rises (Bruno and Simons 2002; Lichtenstein et al. 1990; Minnery and Simons 2003; Simons and Carvell 1989). The cortical direction selectivity of the response to PW stimulation seems to mainly reflect the converging direction-selective inputs from the thalamus with the same whisker preference.

However, inputs from the surrounding whiskers seem to arise from the corresponding neighboring columns through intercolumnar connections. Recent studies showed that either an inactivation (Fox et al. 2003) or an ablation (Goldreich et al. 1999) of the neighboring barrel reduced the responses to stimulation of the corresponding neighbor whiskers. These suggest that the surrounding receptive field is produced mainly through the intracortical mechanism for layers II/III cells (Armstrong-James and Callahan 1991; Armstrong-James et al. 1991) and through the intercolumnar and/or subcortical interaction for layer IV cells (Minnery and Simons 2003; Simons and Carvell 1989). In this study, we found that RSUs exhibited direction selectivity consistent among responses to stimulation of different single whiskers throughout the depth of the cortex. This suggests that both the subcortical and intracortical networks processing multiwhisker information operate in a direction-selective manner.

The directional preference of RSUs was more biased toward the caudal direction than the rostral direction (Fig. 4A). This seems to be meaningful with respect to rodent’s behavior in the sense that whiskers are more often deflected toward the caudal direction when rodents go forward along a wall or palpate objects. Therefore the direction-biased cell population with a preference toward the caudal direction might be formed in an experience-dependent manner. Even though we confirmed that there was no directional bias in devices for whisker stimulation, this point should be further assessed with a larger population of cells.

**Nature of inhibition**

Compared with RSUs, FSUs, i.e., putative inhibitory cells, had a larger receptive field and did not show direction selectivity in accordance with previous studies (Kyriazi et al. 1996; Simons and Carvell 1989; Swadlow 1989; Zhu and Connors 1999). Moreover, combined stimulation of AW preceding that of PW at long ISIs \( (\geq 6 \text{ ms}) \) inhibited the response to PW stimulation, which lasted for a long period, and the magnitude of the inhibition did not correlate with the cell’s direction selectivity estimated from the response to single-whisker stimulation. These results suggest that the cortical inhibition is less specific to the direction of whisker deflection.

Recent studies have shown that the large receptive field and poor direction selectivity of FSUs in layer IV are generated from an unselective pooling of convergent inputs from thalamic cells with different preferences for whisker and stimulus direction (Swadlow and Gusev 2002). In this study, the FSUs of all layers exhibited poor direction selectivity, suggesting that FSUs are possibly driven by a converging inputs with varying preference from intracortical (Roerig et al. 2003) and thalamocortical (Bruno and Simons 2002; Swadlow and Gusev 2002) sources. Thus FSU seems to operate in the cortical network regardless of direction preference.

From our results, it is assumed that the deflection of a whisker would activate a particular neuronal network sharing a common direction preference followed by non-direction-specific inhibition of cells in its own and the neighboring barrel columns. Moreover, only when two neighboring whiskers are stimulated at very short intervals in the same direction, is the direction-specific network activated more than that in the case of single-whisker stimulation. Therefore the inhibitory interaction is considered to enhance not only the spatiotemporal contrast between successive whisker deflections, but also the difference between stimulated and nonstimulated directions in the responses to single- and multiwhisker stimulations.

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