GABA-MEDIATED INHIBITION CORRELATES WITH ORIENTATION SELECTIVITY IN PRIMARY VISUAL CORTEX OF CAT

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Abstract—Orientation selectivity is an important emergent property of neurons in the primary visual cortex, and inhibition is thought to play an important role in establishing this selectivity. But the relationship between strength of inhibition and orientation selectivity is unclear. To investigate this relationship, we electrophoretically applied the inhibitory transmitter GABA and the GABA$_A$ antagonist bicuculline on the same individual area 17 neurons in anesthetized cats. Neurons were classified as weakly orientation-selective, moderately orientation-selective, or strongly orientation-selective, according to the values of an orientation bias index. Orientation bias, half-width of the tuning curve at half-height and an orientation-specificity index (orthogonal to optimal ratio) were compared with or without GABA and bicuculline administration. GABA improved orientation selectivity with the greatest effects on weakly orientation-selective cells, smaller effects on moderately orientation-selective cells, and minimal effects on strongly orientation-selective cells; bicuculline diminished orientation selectivity the most on moderately and strongly orientation-selective cells, with minimal effects on weakly orientation-selective cells. We also found that orientation selectivity correlated with the level of neurons' spontaneous activity. These results suggest that the degree of orientation selectivity of an area 17 neuron correlates with its endogenous inhibition strength, and that GABAergic inhibition can bi-directionally regulate orientation selectivity. This correlation indicates that GABA-mediated inhibition plays an important role in establishing sharp orientation selectivity of individual neurons. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: area 17, orientation bias, bandwidth, orthogonal to optimal ratio, GABA, bicuculline.

The great majority of neurons in the primary visual cortex are tuned for local attributes of the image, such as stimulus orientation and spatial frequency (Hubel and Wiesel, 1962; De Valois et al., 1982). The discovery of orientation selectivity of neurons in primary visual cortex is recognized as a key development in visual neuroscience (Martin, 1988). The relative contributions of excitation and inhibition in generating orientation selectivity serve as a model problem for how synaptic circuitry of the cortex performs a complex computation (Ferster and Miller, 2000).

The role of inhibition in establishing sharp orientation selectivity is still debated. Several studies suggested that inhibition has no effect on sharpening orientation selectivity: neurons retain their orientation selectivity in intracellular inhibition-blocked experiments (Nelson et al., 1994), cooling experiments (Ferster et al., 1996) and shock-inactivation experiments (Chung and Ferster, 1998); the tuning of synaptic excitation and inhibition, recorded intracellularly, appears to be similar to the tuning of their target neurons, and discounts any possibility of inhibitory contributions to their nonoptimal orientations (Ferster, 1986; Carandini and Ferster, 2000). However, iontophoretic application of the GABA$_A$ antagonist bicuculline close to a recorded cell (Sillito, 1975, 1979; Nelson, 1991; Sato et al., 1996), or iontophoretic application of GABA in the vicinity of a recorded cell (Eysel et al., 1990; Crook et al., 1991, 1997; Allison and Bonds, 1994; Allison et al., 1995), led to a broadening in tuning selectivity of the recorded cells. The role of inhibition in establishing sharp orientation selectivity was also supported by intracellular recording studies (Creutzfeldt et al., 1974; Sato et al., 1991; Volgushev et al., 1993; Pei et al., 1994) and experiments with multiple visual stimuli (Bonds, 1989; Hammond and Kim, 1996). Some models (Ben-Yishai et al., 1995; Somers et al., 1995; Ringach et al., 2002a) which were supported by recent results (Wilent and Contreras, 2005; Nowak et al., 2008; Sohya et al., 2007) indicated that suppression of neural responses to nonoptimal stimuli, induced by broad inhibition, plays an important role in the generation of sharp orientation selectivity.

However, previous work only focused on the presence of inhibition in the generation of sharp orientation selectivity. The relationship between strength of inhibition and orientation selectivity has not been examined. Moreover, bicuculline administration had little effect on orientation selectivity in some area 17 neurons (Sillito, 1975, 1979). Our recent result (Leventhal et al., 2003) also showed that monkey V1 neurons have weak orientation selectivity and are less sensitive to bicuculline treatment in aged animals, whereas administration of GABA and its receptor agonist resulted in improved orientation selectivity. Therefore, we hypothesize that cells with weak orientation selectivity receive weak inhibition, and that their selectivity could be sharpened by improving inhibition; cells with strong orientation selectivity should be associated with strong inhibi-

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tion. To test this hypothesis, neurons were classified as weakly orientation-selective (Weakly-OS), moderately OS (Moderately-OS), and strongly OS (Strongly-OS), according to the amount of bias in their responses to different orientations. We studied the effects of electrophoretic application of the inhibitory transmitter GABA and the GABA_A antagonist bicuculline on the orientation selectivity of individual area 17 neurons.

EXPERIMENTAL PROCEDURES

Preparation and recording

Experiments were performed on anesthetized and paralyzed adult cats (2.1–2.8 kg). All procedures were approved by the Institutional Animal Care and Use Committee of University of Science and Technology of China and conformed to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. The methods of preparation and single-cell recording are the same as previously described (Shou et al., 1996; Hua et al., 2006); thus, only a short summary follows. Cats were anesthetized prior to surgery with ketamine HCl (20 mg/kg, i.m.; Ben Venue Lab Inc., Bedford, OH, USA), and then i.v. and tracheal cannulae were inserted. After surgery the animal was placed in a stereotaxic apparatus. A long-acting anesthetic (1% lidocaine HCl; Abbott Labs, Chicago, IL, USA) was applied to all wound margins and pressure points. Nictitating membranes were retracted with Neosynephrine (0.5%; Bayer, Morristown, NJ, USA). Pupils were maximally dilated with atropine, and contact lenses were used to protect corneas. A mixture of urethane (20 mg/kg/h; SCR, Shanghai, China) and gallamine triethiodide (10 mg/kg/h; Sigma) was infused i.v. to maintain anesthesia and paralysis. Heart rate (about 180–220 pulses/min) and EEG were monitored to assess the level of anesthesia. End-expiratory CO_2 was maintained at approximately 4%, and body temperature at 38 °C. A craniotomy (8 mm) in midline was performed at 4 mm posterior to the ear bars, and the dura removed. After introduction of the electrode assembly, the hole was covered with a 4% solution of agar in saline. Action potentials of isolated cortical cells were recorded extracellularly with multibarrel glass microelectrodes having impedances of 2–5 MΩ (filled with 4 M NaCl).

Visual stimulation

Computer-controlled visual stimuli were generated on a CRT monitor (1024×768, 100 Hz, G220; Sony, Japan), placed 57 cm from animals' eyes. The mean luminance of the display was 45.2 cd/m² , and the environment luminance on the cornea was approximately 0.1 lux. The program to generate the stimulus was written in MATLAB (Mathworks, Natick, MA, USA), using the extensions provided by the high-level Psychophysics Toolbox (Brainard, 1997) and low-level Video Toolbox (Pelli, 1997). Luminance nonlinearities of the CRT were corrected through software. We used drifting sinusoidal gratings as stimuli (2 Hz), five cycles per trial (2.5 s). The Michaelson contrast of the stimuli was 99%. Each stimulus orientation was presented two trials for a total 10 cycles/orientation. Blanks (4 s) of the same mean luminance as the grating stimuli were interleaved with stimulus trials to determine the spontaneous firing rate and to avoid response adaptation. We selected optimal stimulus size and spatial frequency for each cell during stimulation to the dominant eye. Then a set of sinusoidal gratings with optimal stimulus parameters, moving in 24 different directions (0–345° in increments of 15°) was used to compile the orientation tuning curves. The direction of motion of each drifting stimulus was orthogonal to its orientation.

GABA and bicuculline application

The methods of GABA and bicuculline application are the same as previously described (Leventhal et al., 2003). GABA and bicuculline were delivered through multibarrel electrode arrays containing four pipette channels. Two barrels held GABA and bicuculline respectively, one barrel was filled with 4 M NaCl in order to record the action potentials of the cells and another was filled with vehicle solution (pH 4.5) for current balancing. The following solutions of drugs were prepared for each experiment: GABA (Sigma, 0.5 M, pH 3.5), and bicuculline methiodide (BMI; Sigma, 5 mM, pH3.5). All solutions for microiontophoresis were freshly prepared before the experiments, filtered, and kept frozen for subsequent use. Administration of one drug at a time was accomplished by passing (+)20 to (+)50 nA through the barrel. A retaining current of (−)20 nA was applied through the other barrel simultaneously in order to prevent leakage of the other drug.

Analysis of orientation selectivity

To study changes of orientation selectivity during administration of GABA and bicuculline, we used three different measures in our analysis. Our first measure was the orientation bias (OB) (Worgotter and Eysel, 1987; Thompson et al., 1994; Leventhal et al., 1995; Leventhal et al., 2003). OB is a global measure that is influenced by all of the data points on the tuning curve, and was calculated as follows:

$$OB = \left| \frac{\sum_k R_k e^{i2\theta_k}}{\sum_k R_k} \right|$$

where R_k is the mean spike rate in response to a drifting grating with orientation θ_k (in radians). The OB averages the responses for the two directions of motion at each orientation. OB is quite robust to noise in the data and provides a bounded range from 0 to 1, with 0 indicating complete insensitivity to orientation and one corresponding to response at only one orientation. Cells with an OB of less than 0.2 (including none orientation selective cells with an OB of less than 0.1) were classified as Weakly-OS, those with an OB in the intermediate range (0.2–0.3) were classified as Moderate-OS, and cells with an OB of greater than 0.3 were classified as Strongly-OS. Similar schemes for classification of orientation specificity were employed by others (Wolf et al., 1986; Girman et al., 1999).

The changes of OB induced by GABA and bicuculline administration were measured by the ΔOB:

$$\Delta OB = OB_{drug} - OB_{control}$$

The second measure of selectivity we used was the tuning curve half-bandwidth (Swindale, 1998), which is a more local measure dependent upon the shape of the curve around its peak and not the shape of the entire curve (Ringach et al., 2002b). To calculate this measure of selectivity, first the orientation tuning curves were fit with the von Mises distribution:

$$R = R_p e^{k[\cos2(\theta - \theta_p) - 1]}$$

where R represents the cell's response as a function of orientation θ, R_p is the value of the function at the preferred orientation θ_p, and k is a width parameter. Then half-width of the tuning curve at half-height (HWHH), which we used to describe the tuning width, was calculated as:

$$HWHH = 0.5 \arccos\left(\frac{\ln 0.5 + k}{k} \right)$$

In order to investigate effects of GABA and bicuculline on the ratio of optimal/orthogonal orientation, the orientation-specificity index (OSI, Wolf et al., 1986) was calculated:

$$OSI(\%)=\left(1-\frac{\text{orthogonal response}}{\text{optimal response}}\right)\times100$$

OSI ranges from 0% to 100%, with larger values indicating more difference between orthogonal and optimal responses.

We used a simple and objective method to classify cells as simple or complex (Skottun et al., 1991). The ratio of the first Fourier component (FFT1) to the mean component (FFT0) of response to a neuron's optimal drifting sinusoidal grating provides a measure of the relative response modulation. When the ratio is above 1, the neuron is classified as a simple cell. If the ratio is below 1, it is classified as a complex cell (Skottun et al., 1991; Bardy et al., 2006).

Whenever orientation-tuning curves are plotted in the range from 0 to 165°, we averaged the responses for the two directions of drift at each orientation.

RESULTS

For 86 neurons in the primary visual cortex, the recording conditions were stable and lasted long enough (at least 2 h) so that we could complete a full set of tests with GABA and bicuculline iontophoresis; the following analysis in-

cludes only those neurons for which all tests were completed. Consistent with previous reports (Nelson, 1991; Ringach et al., 2002a), in our present study no systematic differences were observed in the effects of GABA and bicuculline on cells classified as simple or complex, and therefore the results from both types of cells are considered together.

Effects of GABA and bicuculline on OB

We studied the effects of electrophoretic application of the inhibitory transmitter GABA and the $GABA_A$ antagonist bicuculline on the response properties of individual cells in different groups. Of the 86 neurons, 28 were classified as Weakly-OS, 28 as Moderately-OS, and 30 as Strongly-OS (see Experimental Procedures).

The effects of GABA and bicuculline on the responses of three typical cells are illustrated in Fig. 1. These cells exemplify what were seen in different groups upon GABA and bicuculline application. The upper plots show the results obtained prior to drug injection. The values of OB
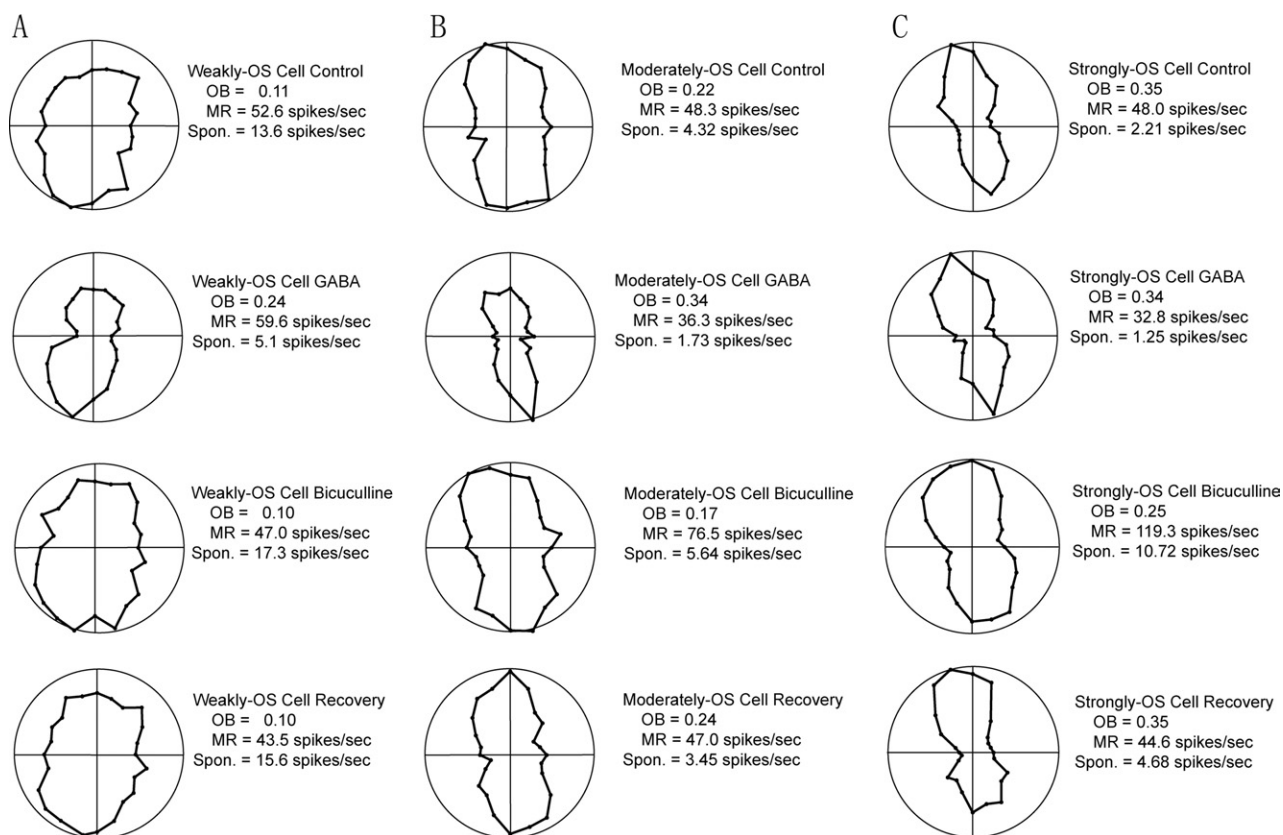

Fig. 1. Polar plots obtained from three representative cells in the Weakly-OS (A), Moderately-OS (B) and Strongly-OS (C) groups, that received treatment with GABA and bicuculline. The OB, maximum (peak) responses (MR) and spontaneous activity (Spon.) are shown for each condition. The radius of each polar plot indicates the maximum response (MR). A typical Weakly-OS cell showing a lack of orientation sensitivity is illustrated in (A). Ten minutes after GABA application (50 nA), this cell exhibited improved orientation selectivity, as well as decreased spontaneous activity. GABA application was then discontinued, and bicuculline application (50 nA) was begun. Bicuculline reversed the effects of GABA. The responses of a Moderately-OS cell are shown in (B). After GABA administration (30 nA), this cell exhibited improved orientation selectivity, and decreased spontaneous activity. After bicuculline administration (50 nA), this cell exhibited reduced orientation selectivity, and increased maximum response and spontaneous activity. A polar plot typical of the majority of cells in the Strongly-OS group is shown in (C). Its orientation selectivity was not significantly affected by the administration of GABA (50 nA). On the other hand, application of bicuculline (50 nA) increased maximum response and reduced orientation selectivity. The spontaneous activities were not subtracted from the plotted responses.
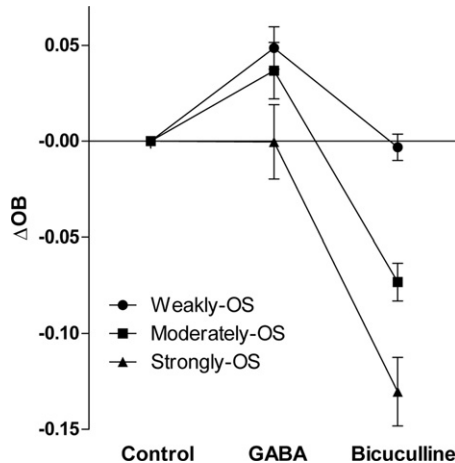
Fig. 2. Effects of GABA and bicuculline administration on orientation selectivity. For the Weakly-OS group (circles), GABA significantly improved OB ($P<0.001$, paired t-test); bicuculline showed no significant effect ($P=0.055$, paired t-test). For the Moderately-OS group (squares), GABA significantly improved OB ($P<0.05$, paired t-test) and bicuculline significantly diminished it ($P<0.001$, paired t-test). For the Strongly-OS group (triangles), GABA showed no significant effect on OB ($P=0.647$, paired t-test), but bicuculline greatly diminished it ($P<0.001$, paired t-test). Error bars denote S.E.

calculated from these tuning curves were 0.11, 0.22, and 0.35. The plots below show the results obtained after administration of GABA and bicuculline respectively. The fourth row shows the recovered responses obtained 10 min after the drug was discontinued. The weakly-OS cell (Fig. 1A) exhibited improved orientation selectivity on GABA application (OB from 0.11 to 0.24). Bicuculline administration had little, if any effect on stimulus selectivity (OB=0.10). The Moderately-OS cell in Fig. 1B represents an example of an increase in orientation selectivity on GABA application (OB from 0.22 to 0.34) in conjunction with a reduction on bicuculline application (OB from 0.22 to 0.17). Fig. 1C is an example of a Strongly-OS cell whose selectivity was diminished by administration of bicuculline (OB from 0.35 to 0.25), while the magnitude of response was greatly increased. However GABA administration did not affect the orientation selectivity of this cell (OB=0.34).

When the injection currents were discontinued, the responses of the cells recovered and were nearly identical to the pre-application state.

A comparison of the changes in OB of the three groups that occurred during the administration of GABA and bicuculline is shown in Fig. 2. For Weakly-OS cells (circles), GABA could significantly improve orientation selectivity, but it was not significantly affected by the administration of bicuculline. However for Strongly-OS cells (triangles), GABA failed to improve OB, while bicuculline greatly diminished the orientation selectivity. For Moderately-OS cells (squares), both GABA and bicuculline affected OB: GABA improved orientation selectivity and bicuculline diminished it. We also analyzed effects of GABA and bicuculline on the F1/F0 ratio. GABA significantly improved the F1/F0 ratio ($P<0.05$, paired t-test) and bicuculline significantly decreased it ($P<0.05$, paired t-test). This result is consistent with previous results that improving the strength of suppression could improve the F1/F0 ratio of some cells (Bardy et al., 2006). At present we do not observe any obvious correlation between F1/F0 ratio and the effects of drugs on OB, but a larger sample would be required for a careful investigation of this correlation.

Effects of GABA and bicuculline on orientation bandwidth and OSI

In our data, the orientation tuning curve of area 17 cells usually exhibits strong responses within a central "core" tuning band of orientations, and a wide plateau of weaker responses across all orientations. OB depends not only on the width of the core tuning but also on the relative height of the plateau response compared with the response at the preferred orientation. The two factors, bandwidth and orthogonal/optimal response, in principle can affect OB independently (Ringach et al., 2002b). Thus OB analysis cannot tell us whether the changes of orientation selectivity should be attributed to changes of bandwidth or orthogonal/optimal responses or both. In order to address this question, we studied the effects of GABA and bicuculline on both orientation bandwidth (measured as HWHH) and orthogonal/optimal ratio.

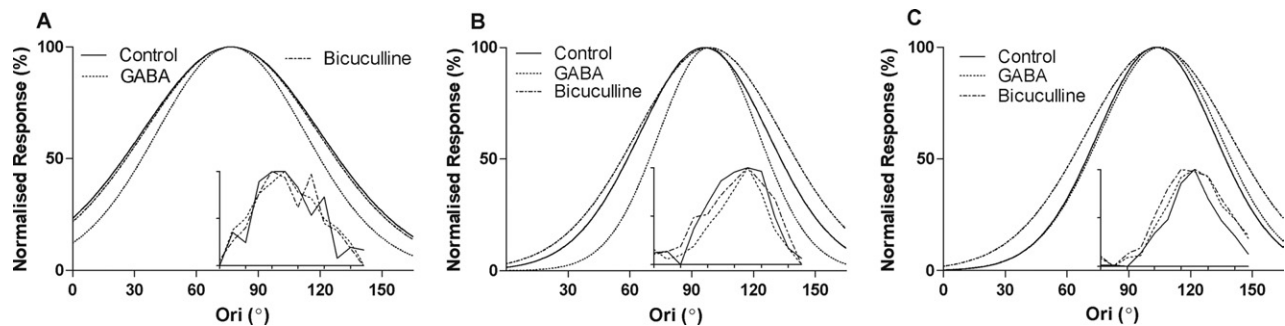In Fig. 3, responses during the control, GABA and

Fig. 3. Normalized orientation tuning curves of the same cells shown in Fig. 1. Each tuning curve is normalized by the peak response, to facilitate comparison of the tuning width, and then fit with the von Mises distribution. Inset plots show raw data. For a Weakly-OS cell (A), after GABA application, this cell exhibited narrowed tuning width. Bicuculline showed no significant effect on this cell. The responses of a cell in the Moderately-OS group are shown in (B). After GABA administration, this cell exhibited narrowed tuning width; after bicuculline administration, the cell showed broadened tuning width. For a Strongly-OS cell (C), GABA administration had almost no effect on tuning width, but application of bicuculline broadened it.
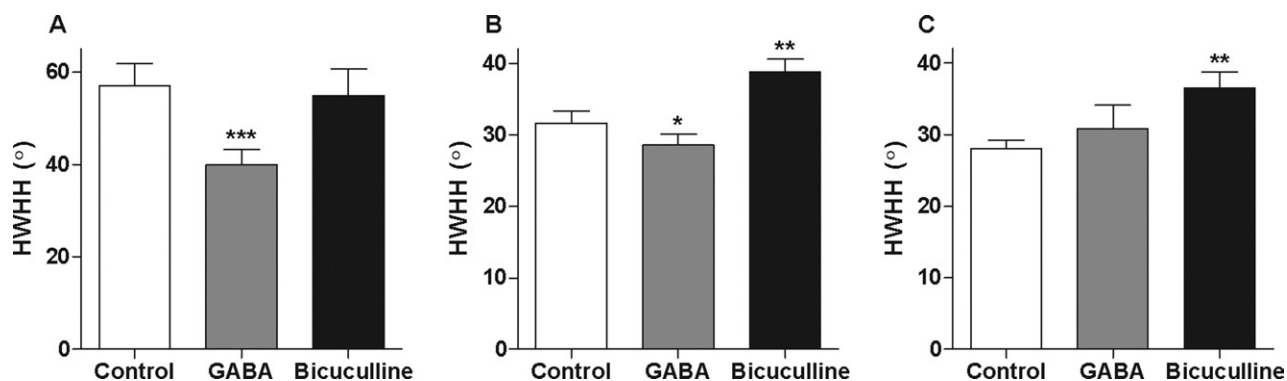
Fig. 4. Effects of GABA and bicuculline administration on tuning bandwidth, as measured by HWHH. For the Weakly-OS group (A), GABA significantly narrowed tuning width ($P<0.001$, paired t-test); bicuculline showed no significant effect on tuning width ($P=0.61$, paired t-test). For the Moderately-OS group (B), GABA significantly narrowed tuning width ($P<0.05$, paired t-test) and bicuculline significantly widened it ($P<0.01$, paired t-test). For the Strongly-OS group (C), GABA showed no significant effect on tuning width ($P=0.27$, paired t-test), but bicuculline significantly widened it ($P<0.01$, paired t-test). Error bars denote S.E.

bicuculline trials of individual cells (same neurons as in Fig. 1) were normalized and averaged, and then fit with the von Mises distribution.

The orientation tuning exhibited similar sharpening effects during the GABA administration (dotted line) in the cells from the Weakly-OS and Moderately-OS groups (Fig. 3A and 3B, respectively), but not from the Strongly-OS groups (Fig. 3C). The effects of bicuculline (dash–dotted line) on the bandwidth of the orientation tuning curves were evident in the cells from the Moderately-OS and Strongly-OS groups, but absent in the Weakly-OS group. These effects of GABA and bicuculline administration on bandwidth are summarized in Fig. 4. The results indicate that change of bandwidth is one of the reasons for change of orientation selectivity during GABA and bicuculline administration.

Additional analysis of the population data reveals that other factors, besides those that govern the tuning curve bandwidth, determine orientation selectivity. The difference between optimal and orthogonal responses, measured by the OSI, is illustrated in Fig. 5.

Responses to the orthogonal orientation were reduced more than to the optimal orientation during GABA admin-

istration in the Weakly-OS and Moderately-OS groups, but not in the Strongly-OS cells. This differential effect on optimal and orthogonal orientations suggests that the effect of GABA is not simply to non-selectively decrease the responses of cells to all visual stimuli. The facilitatory effect on responses to the orthogonal orientation during bicuculline administration was more evident in the Moderately-OS and Strongly-OS cells than in the Weakly-OS group.

The foregoing results suggest that the changes of orientation selectivity during GABA and bicuculline administration were due to changes of both bandwidth and orthogonal/optimal responses.

Relationship between orientation selectivity and spontaneous activity

Orientation selectivity could be related to spontaneous activity if the threshold level and the excitatory/inhibitory balance, which both influence spontaneous activity, also have a large influence on orientation tuning (Ringach et al., 2002b).
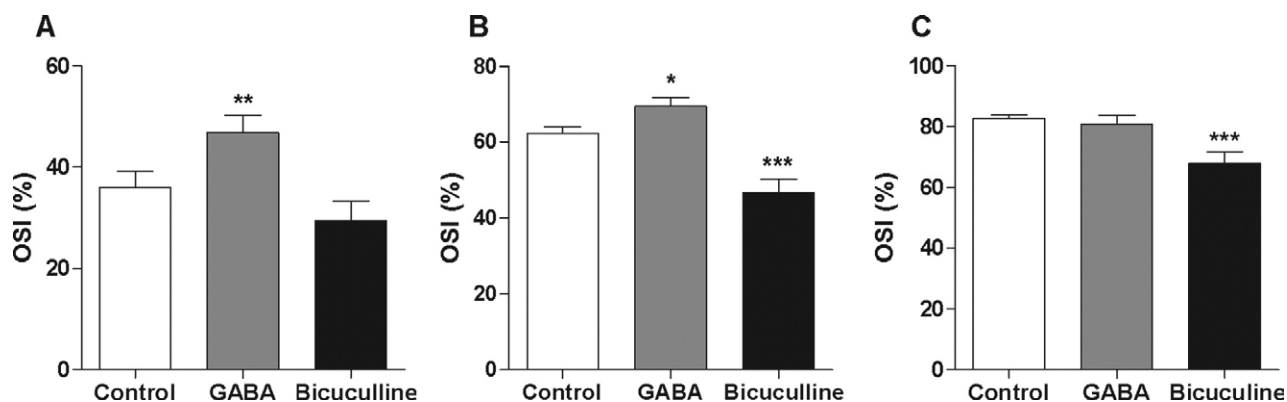

Fig. 5. Effects of GABA and bicuculline administration on OSI. For the Weakly-OS group (A), GABA significantly improved OSI ($P<0.01$, paired t-test), while bicuculline showed little effect ($P=0.051$, paired t-test). For the Moderately-OS group (B), GABA significantly improved OSI ($P<0.05$, paired t-test) and bicuculline significantly decreased it ($P<0.001$, paired t-test). For the Strongly-OS group (C), GABA showed no significant effect on OSI ($P=0.43$, paired t-test), but bicuculline significantly decreased it ($P<0.001$, paired t-test). Error bars denote S.E.
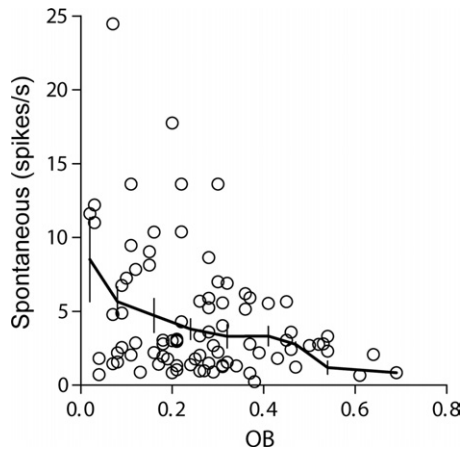
Fig. 6. Relationship between OB and spontaneous firing rate. The middle (thick) curve represents an average spontaneous activity, with a bin width of 0.08 OB. Error bars denote S.E.

Fig. 6 illustrates the relationship between orientation selectivity and spontaneous activity in our population. Cells with higher OB values had lower spontaneous rates, while cells with lower OB values tended to have relatively higher spontaneous rates. These results are consistent with previous measurements of spontaneous activity and orientation selectivity in monkey V1 (Ringach et al., 2002b).

The change of spontaneous rates of area 17 neurons in different groups after GABA and bicuculline administration is shown in Table 1. GABA results in a larger percentage decrease in spontaneous activity in Weakly-OS than in Strongly-OS cells (paired t-test, $P<0.01$ in all cases). Bicuculline, on the other hand, resulted in larger percentage increases in spontaneous activity in Strongly-OS than in Weakly-OS cells (paired t-test, $P<0.01$ in all cases).

Taken together, these results suggest that cells with high orientation selectivity receive more inhibition; cells with weak orientation selectivity do not receive sufficient inhibition.

DISCUSSION

In this study we employed microiontophoretic application of GABA and bicuculline to bi-directionally modulate the input inhibition strength in area 17 neurons, and compared the effects on their orientation selectivity and spontaneous responses. The main finding is that GABA-mediated inhibition was greater in Strongly-OS cells than in Weakly-OS cells. GABA was more effective in Weakly-OS cells than in Strongly-OS cells, and conversely, the GABA antagonist bicuculline was more effective in Strongly-OS cells than in Weakly-OS cells. We also found that the changes of orientation selectivity during GABA and bicuculline administration were due to changes of both bandwidth and orthogonal/optimal responses.

Strength of inhibition and orientation selectivity

The effects of GABA and bicuculline correlate with the strength of orientation selectivity in the population of cat area 17 neurons, which is evident in OB, bandwidth and OSI data. This result is consistent with the hypothesis that cells with high orientation selectivity receive strong inhibition, and that such strong inhibition could be a mechanism for improving orientation selectivity (Troyer et al., 1998; McLaughlin et al., 2000). Sharp selectivity for orientation has previously been associated with signs of suppressive interactions (Ringach et al., 1997, 2002a). That hypothesis is also supported by the overall lower spontaneous firing rate of the high OB cells (Fig. 6), which could arise from strong cortico-cortical inhibition (Ringach et al., 2002b).

Our finding that GABA exerts a weaker effect on strongly selective cells than in weakly selective ones supports the idea that, once the inhibition is sufficiently strong, further increasing inhibition will not improve the orientation selectivity significantly. This result is consistent with our previous finding that GABA administration did not affect the selectivity of most of the already strongly selective cells in young monkeys (Leventhal et al., 2003). Our results partly confirm some models' predictions (Somers et al., 1995; Carandini and Ringach, 1997; Troyer et al., 1998) that high orientation selectivity might reflect the balance of excitation and inhibition. Increasing the strength of inhibition, when the strength was relatively low, could decrease the amplitude of the responses and sharpen their orientation tuning; when strength of inhibition was high, however, increasing the strength of inhibition would further decrease responses while maintaining the strength of orientation selectivity.

GABA-mediated divisive gain control signals and orientation selectivity

We found the changes of orientation selectivity during GABA and bicuculline administration were due to changes of both bandwidth and orthogonal/preferred responses. This result suggested that inhibition of neural responses to nonoptimal stimuli may play an important role in the development of neural selectivity. In our results, the selectivity for stimulus orientation is higher in neurons whose responses exhibit GABA-mediated inhibition for nonoptimal stimuli than in cells with no such inhibition. This result is consistent with previous reports on orientation-tuning dynamics (Sillito, 1975, 1979; Sato et al., 1996; Eysel et al., 1990; Crook et al., 1991, 1997; Allison and Bonds, 1994;

Table 1. Effects of GABA and bicuculline application on spontaneous firing rate of area 17 cells

Group	Spontaneous rate		
	Control	GABA	Bicuculline
Weakly-OS	100% (6.42)	−76.1% (1.53)	+87.0% (12.01)
Moderately-OS	100% (4.72)	−70.3% (1.40)	+132.8% (10.99)
Strongly-OS	100% (3.61)	−36.8% (2.28)	+174.0% (9.89)

Changes in spontaneous firing rate induced by GABA and bicuculline in the Weakly-OS, Moderately-OS and Strongly-OS groups. GABA resulted in larger percentage decreases in spontaneous firing rate in the Weakly-OS and Moderately-OS groups than in Strongly-OS cells. Bicuculline resulted in the largest percentage increases in spontaneous firing rate in the Strongly-OS group, and the least increase in the Weakly-OS cells.

Ringach et al., 1997, 2002a) in which sharp selectivity for orientation was associated with suppression of neural responses to nonoptimal stimuli.

Our GABA administration presumably elicited inhibition which was not tuned, or only broadly tuned, for orientation, yet it could enhance orientation selectivity in weakly-selective area 17 neurons. Broadly tuned inhibition was also observed in highly OS cells by others (DeAngelis et al., 1992; Hammond and Kim, 1996; Wilent and Contreras, 2005; Nowak et al., 2008; Sohya et al., 2007). Our results might be explained by a linear-nonlinear model (Movshon et al., 1978) with a divisive gain control signal, not tuned for orientation (Carandini et al., 1997; Ringach et al., 2002a). When GABA was injected locally, $GABA_A$ receptors mediated inhibition via an increase in membrane conductance (shunting) and/or membrane potential hyperpolarization (Ulrich, 2003). Such shunting inhibition was proposed to act divisively on the gain of neural output (Blomfield, 1974; Rose, 1977). Therefore, this model does not specifically require inhibition from cells that are tuned to particular orientations (Heeger, 1992; Carandini and Heeger, 1994).

Source of inhibition

A remaining question for this model is the source of non-oriented inhibitory signals. Strong direct input from inhibitory geniculate relay cells (Carandini and Heeger, 1994) could suppress the response at orientations far from the optimal, and account for cells with both narrow bandwidth and high values of OSI. However no physiological evidence for direct thalamo-cortical inhibition has been found (Ferster and Lindstrom, 1983; Martin and Whitteridge, 1984). Non-oriented cortico-cortical inhibition could contribute to narrowing the bandwidth and inhibiting responses of orthogonal orientation in highly selective neurons. One possibility is that the non-oriented inhibitory neurons are strongly dominated by the thalamic input signals (Nowak et al., 2008). In the visual system, LGN neurons are weakly orientation selective (Vidyasagar and Urbas, 1982; Shou et al., 1986; Shou and Leventhal, 1989; Thompson et al., 1994). Another possibility is that the divisive inhibition may arise from a large pool of cells, which respond to essentially every orientation (Heeger, 1992). However, we cannot distinguish in this study whether the suppression signal is generated in a feed-forward fashion or in a feedback fashion, since the feed-forward inhibition models can be made to generate results that resemble those of the feedback models (Ringach et al., 2002a). Other inhibitory mechanisms may also be involved in shaping the orientation selectivity of primary visual cortex neurons. Allison et al. (1996) indicated that the orientation selectivity of striate cortical neurons' sustained response must be influenced, at least in part, by $GABA_B$-receptor-mediated inhibition. We cannot rule out the effects of $GABA_B$ mediated inhibition on orientation selectivity because, in our present study, we did not find a significant impact of bicuculline administration on the onset transient activity as observed by Pfleger and Bonds (1995). One explanation for this discrepancy could be the different electrode configurations and/or injection currents.

The actual organization of the inhibitory circuitry remains a question for further research.

The body of evidence that is presented here significantly advances our knowledge of the relationship between inhibition strength and orientation selectivity. Cells with weak orientation selectivity may be accounted for by insufficient inhibition, and those with strong selectivity tend to receive strong inhibition. It supports the model that GABA-mediated inhibition is an essential contributor to establishing sharp orientation selectivity.

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(Accepted 17 June 2008)
(Available online 19 June 2008)