$GABA_B$-receptor-mediated inhibition reduces the orientation selectivity of the sustained response of striate cortical neurons in cats

JOHN D. ALLISON,[1] J.F. KABARA,[2] R.K. SNIDER,[2] V.A. CASAGRANDE,[1] AND A.B. BONDS[2]

[1]Department of Cell Biology, Vanderbilt University, Nashville
[2]Department of Electrical and Computer Engineering, Vanderbilt University, Nashville

(RECEIVED July 10, 1995; ACCEPTED November 7, 1995)

Abstract

Blocking $GABA_A$-receptor-mediated inhibition reduces the selectivity of striate cortical neurons for the orientation of a light bar primarily by reducing the selectivity of their onset transient (initial 200 ms) response. Blocking $GABA_B$-receptor-mediated inhibition with phaclofen, however, is not reported to reduce the orientation selectivity of these neurons when it is measured with a light bar. We hypothesized that blocking $GABA_B$-receptor-mediated inhibition would instead affect the orientation selectivity of cortical neurons by reducing the selectivity of their sustained response to a prolonged stimulus. To test this hypothesis, we stimulated 21 striate cortical neurons with drifting sine-wave gratings and measured their orientation selectivity before, during, and after iontophoretic injection of 2-hydroxy-saclofen (2-OH-S), a selective $GABA_B$-receptor antagonist. 2-OH-S reduced the orientation selectivity of six of eight simple cells by an average of 28.8 (± 13.2) % and reduced the orientation selectivity of eight of 13 complex cells by an average of 32.3 (± 27.4) %. As predicted, 2-OH-S reduced the orientation selectivity of the neurons' sustained response, but did not reduce the orientation selectivity of their onset transient response. 2-OH-S also increased the length of spike "bursts" (two or more spikes with interspike intervals ≤ 8 ms) and eliminated the orientation selectivity of these bursts for six cells. These results are the first demonstration of a functional role for $GABA_B$ receptors in visual cortex and support the hypothesis that two GABA-mediated inhibitory mechanisms, one fast and the other slow, operate within the striate cortex to shape the response properties of individual neurons.

Keywords: 2-hydroxy-saclofen, GABAergic inhibition, Orientation tuning, Visual cortex

Introduction

The firing rate of neurons in the primary visual (striate) cortex depends in part on the orientation of an elongated stimulus (Hubel & Wiesel, 1962). There are two general models for the substrate of this phenomenon. The first (Hubel & Wiesel, 1962) asserts that excitatory input from an organized, aligned array of lateral geniculate nucleus (LGN) cells produces the orientation selectivity of a cortical neuron. A stimulus with optimal orientation maximally stimulates the LGN cell array and generates a suprathreshold response from a cortical neuron. This model is supported by evidence that both the excitatory input from the LGN to cortex (Vidyasagar & Urbas, 1982; Leventhal, 1985; Soodak et al., 1987; Chapman et al., 1991) and the subsequent excitatory postsynaptic potentials (EPSPs) of cortical neurons (Ferster, 1986, 1994; Ferster & Jagadeesh, 1992) exhibit orientation selectivity. The insufficiency of this model is seen in evidence that intracortical inhibition sharpens the orientation selectivity of cortical neurons by suppressing their response to nonoptimal stimuli (Morrone et al., 1982; Bonds, 1989).

The second model proposes that intracortical inhibition mediated by γ-aminobutyric acid (GABA) is crucial for generating orientation selectivity. The strongest supporting evidence for this model is the marked reduction in orientation selectivity to a light bar when the $GABA_A$-receptor antagonist bicuculline is applied onto cortical neurons (Sillito, 1975, 1979; Pfleger & Bonds, 1995) or when GABA itself is applied remotely to promote disinhibition at the recording site (Eysel et al., 1990; Allison & Bonds, 1994). However, iontophoretic application of the $GABA_B$-receptor antagonist phaclofen at the recording site does not reduce orientation selectivity for light bars (Baumfalk & Albus, 1988). These disparate results might be a consequence of the stimulus dependence of the effect of GABA blockers. In complex cells, for example, bicuculline treatment produces a greater loss of selectivity for the orientation of a light bar than for a drifting sine-wave grating because it reduces the orienta-

Reprint requests to: A.B. Bonds, Department of Electrical and Computer Engineering, P.O. Box 1824 Station B, Vanderbilt University, Nashville, TN 37235, USA.

tion selectivity of their onset transient (initial 200 ms) response far more than the orientation selectivity of their sustained response (Pfleger & Bonds, 1995). This onset transient dominates the response to a single traverse of a light bar across the receptive field while stimulation with gratings accentuates the sustained response. Pfleger and Bonds (1995) suggest that the transient response is predominantly affected by bicuculline treatment because the $GABA_A$ receptor is a fast-acting ionotropic Cl^- channel (Connors et al., 1988; Connors, 1992; Mody et al., 1994). They hypothesize that although blocking the slow, G-protein coupled, metabotropic $GABA_B$ receptor (Connors et al., 1988; Connors, 1992; Mody et al., 1994) does not reduce cortical neurons' selectivity for the orientation of a light bar (Baumfalk & Albus, 1988), it might reduce their selectivity for the orientation of a prolonged stimulus (e.g. a drifting grating) by reducing the orientation selectivity of their sustained response.

We tested Pfleger and Bonds' (1995) hypothesis by stimulating neurons with drifting sine-wave gratings and measuring their orientation selectivity before, during, and after local iontophoretic injection of 2-hydroxy-saclofen (2-OH-S), a selective $GABA_B$-receptor antagonist (Curtis et al., 1988; Kerr et al., 1988*a*,*b*). 2-OH-S reduced the orientation selectivity of 14 of 21 neurons (67%). As predicted by Pfleger and Bonds (1995), 2-OH-S reduced the orientation selectivity of the neurons' sustained response, but did not reduce the orientation selectivity of their onset transient response. 2-OH-S did, however, increase the amplitude of both the transient and sustained responses. Finally, 2-OH-S increased the length of spike bursts (spike sequences with interspike intervals $\leq$8 ms) produced by cells and reduced the orientation selectivity of these bursts. These results indicate that $GABA_B$-receptor-mediated inhibition sharpens the orientation selectivity of some cortical neurons, offering the first demonstration of a functional role for $GABA_B$-receptor-mediated inhibition in the striate cortex.

Methods

Preparation

We prepared 12 adult cats (2.0–4.0 kg) for single-unit electrophysiological recording following guidelines established by the American Physiological Society, the Society for Neuroscience, and Vanderbilt University's Animal Care and Use Committee. Each cat was injected (i.m.) with 0.5 ml acepromazine maleate (TechAmerica, Elwood, KS) and 0.5 ml atropine sulfate (Elkins-Sinn, Cherry Hill, NJ) then, approximately 45 min later, anesthetized with 5% halothane (Fluothane, Ayerst, Philadelphia, PA) in O_2. After cannulating a forelimb vein, anesthesia was maintained with i.v. injection of 2.5% sodium thiopental (Pentothal, Abbott, N. Chicago, IL), the trachea was cannulated, the head was mounted in a stereotaxic device, and a small craniotomy (2 × 5 mm) was performed over the representation of *area centralis* (H-C coordinates P4-L2). For recording, paralysis was induced with an i.v. injection of gallamine triethiodide (Flaxedil, American Cyanamid, Pearl River, NY; 10 mg/kg/h). Pentothal anesthesia was continued at 1 mg/kg/h, each cat was respirated at 30 breaths/min with a mixture of NO_2:O_2:CO_2 (70%:28.5%:1.5%), and pCO_2 was held at 3.9%. We monitored heart rate and brain activity *via* EKG and EEG, respectively, to ensure anesthetic stability and treated accelerated heart rate or lack of occasional sleep "spindles" with bolus i.v. injections of Pentothal. Rectal temperature was maintained at 37.5°C with a servo-controlled heat pad. The pupils were dilated with 1% atropine sulfate and the nictitating membranes were retracted with 10% phenylephrine hydrochloride. Contact lenses with 4-mm artificial pupils were applied and auxiliary lenses were added to ensure that the eyes were focused on the stimulus plane, usually a distance of 57 cm.

Recording, iontophoresis, and data acquisition

We glued a Levick (1972) electrode onto a three-barrel pipette (tip separation $\leq$50 μm) for recording and iontophoretic injection, respectively. The first barrel of the pipette was filled with 0.9% saline (pH 3.5) and was used for current compensation during injection from the other barrels. The second and third barrels of the pipette contained 25 mM 2-hydroxy-saclofen (2-OH-S) dissolved in 75 mM saline (pH 3.5) (Curtis et al., 1988; Kerr et al., 1988*a*,*b*). These dosages have been proven effective and nontoxic so we did not test directly their effectiveness for blocking iontophoretic administration of GABA. The combined electrode configuration was lowered into the striate cortex with an electronic microdrive (Stepper Mike, Oriel, Stratford, CT). Action potentials from the recording microelectrode were amplified (10,000×), filtered (300–10,000 Hz), reduced to standard pulses *via* a window discriminator, and stored by computer with a resolution of 1 ms. The computer controlled the global structure of experiments, sent stimulus parameters to the pattern generator, recorded the time of occurrence of every spike, and displayed and stored data as it was collected.

A manually controlled projection system produced a moving light bar which was used to locate a cell's receptive field and rapidly estimate the field parameters. After plotting the receptive field with the light bar, the cell was stimulated with sine-wave gratings of 4-s duration generated on a Tektronix 608 display (100 cd/m^2 mean luminance, 10-deg diameter field) driven by a microprocessor-controlled pattern generator. We measured each cell's optimal stimulus orientation, spatial frequency, and temporal frequency, then recorded the baseline orientation tuning curve by randomly interleaving (Henry et al., 1973) stimulus orientations in 10-deg increments across a 180-deg range centered on the optimum angle at 30% contrast. Each stimulus orientation was presented 10 times for a total averaging time of 40 s/orientation. After establishing the baseline orientation tuning, 2-hydroxy-saclofen (2-OH-S) was iontophoresed (100–150 nA per barrel) for 5 min and the cell's orientation selectivity was remeasured. Iontophoresis of the 2-OH-S was then discontinued and 10 min later the orientation selectivity of the cell was remeasured. We continued to measure the neuron's orientation selectivity until it returned to control values or until 1 h had expired.

To determine quantitatively whether 2-OH-S produced a significant change in the orientation tuning of any cell, we calculated the 99% confidence interval of the baseline average response amplitude, on the basis of sweep-by-sweep analysis, at each stimulus orientation. Orientation tuning functions were then drawn for the average response at each orientation and for the lower and upper confidence limits (i.e. three curves). The upper limit gave the maximum orientation tuning bandwidth of the cell. In some cases, the variability was less than a degree. Application of 2-OH-S was considered effective if iontophoresis of this substance caused the orientation tuning of the cell to exceed the 99% confidence interval.

Results

We stimulated 21 striate cortical neurons (eight simple cells and 13 complex cells) with drifting sine-wave gratings and measured their orientation selectivity before, during, and after blocking $GABA_B$-receptor-mediated inhibition with iontophoretic injection of 2-hydroxy-saclofen (2-OH-S) near the recorded neurons. The magnitude of the effect of 2-OH-S on orientation selectivity was modest compared to the effect of bicuculline (Sillito, 1979; Pfleger & Bonds, 1995). In further contrast to bicuculline, 2-OH-S did not change the maintained discharge of most (18 of 21) neurons, with increases seen in only two simple cells (circles) and one complex cell (triangles) (Fig. 1). The lack of effect on maintained discharge is consistent with studies of the actions of $GABA_B$-receptor antagonists in hippocampal and cortical slices (Mody et al., 1994).

Changes in orientation tuning

Fig. 2 shows the distribution of orientation tuning bandwidths recorded during administration of 2-OH-S as a function of baseline orientation tuning bandwidths. Although the effect of 2-OH-S on the orientation tuning bandwidth of most cells was not large, based on our criteria of exceeding the 99% confidence limit, the increase in orientation tuning was significant for 14 cells. 2-OH-S increased the orientation tuning bandwidth of six (75%) of the eight simple cells (circles) by an average of 28.8%. Eight (61.6%) of the 13 complex cells (triangles) displayed increases in orientation tuning bandwidth during treatment with 2-OH-S, with an average increase of 32.3%.

Fig. 3A illustrates the effect of 2-OH-S on the orientation selectivity of one simple cell. The normalized response amplitude of the cell is plotted as a function of stimulus orientation. Under control conditions (filled circles), the cell produced a peak response to a stimulus orientation of 285 deg and its baseline orientation tuning bandwidth was 11 deg (half-width, half-height). Iontophoresis of 2-OH-S (open squares) elevated markedly the cell's response to stimulus orientations of 275 deg and 295 deg. The orientation tuning bandwidth of the cell broadened to 15 deg. 2-OH-S increased the orientation tuning bandwidth of this simple cell by 36.4%, which was statistically significant.

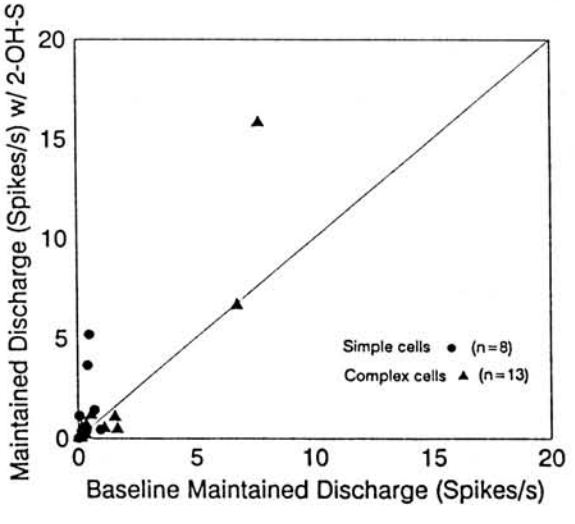

Fig. 1. Maintained discharge (spikes/s) during 2-OH-S treatment is plotted as a function of the baseline maintained discharge (spikes/s). Only two simple cells (circles) and one complex cell (triangles) exhibited increases in maintained discharge during treatment with 2-OH-S. The lack of effect on maintained discharge of 2-OH-S iontophoresis contrasts the marked increase seen with bicuculline treatment (Sillito, 1975; Pfleger & Bonds, 1995).

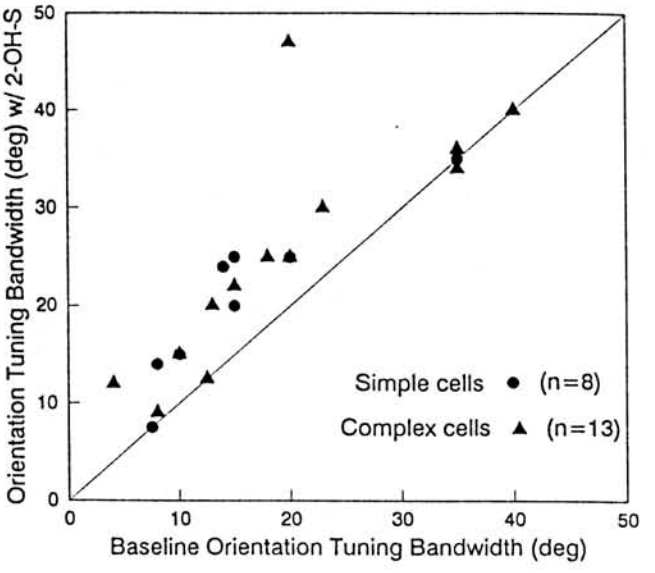

Fig. 2. Distribution of orientation tuning bandwidths during 2-OH-S treatment as a function of baseline orientation tuning. For six (75%) of eight simple cells and eight (61.6%) of 13 complex cells, the orientation tuning bandwidth during iontophoresis of 2-OH-S was greater than the upper limit of the 99% confidence interval of the cell's baseline orientation tuning bandwidth.

We analyzed the effect of 2-OH-S on the onset transient and sustained responses of the simple cells. For illustration, the normalized response that our example cell produced during its onset transient (i.e. during the initial 200 ms after stimulus onset; Pfleger & Bonds, 1995) is plotted as a function of stimulus orientation in Fig. 3C. Under control conditions (filled circles), the amplitude of the cell's onset transient peaked in response to a stimulus orientation of 285 deg. The baseline orientation tuning bandwidth of the onset transient response was 11 deg. With 2-OH-S (open squares), the cell's normalized transient response increased for a stimulus orientation of 275 deg and decreased for a stimulus orientation of 295 deg. Despite these response changes, 2-OH-S did not affect significantly the overall tuning bandwidth of the onset transient response, which decreased slightly to 10 deg. 2-OH-S did not affect the orientation selectivity of the onset transient response of any of the simple cells in a consistent manner.

The normalized sustained response of the cell is plotted as a function of stimulus orientation in Fig. 3E. Because of the longer duration, the cell produced many more spikes during its 3.8-s sustained response than during its 200-ms onset transient response. The orientation tuning function of the sustained response therefore more closely resembles the orientation tuning function of the cell's average firing rate (Fig. 3A). Under control conditions (filled circles), a stimulus orientation of 285 deg generated the cell's peak response and its baseline orienta-

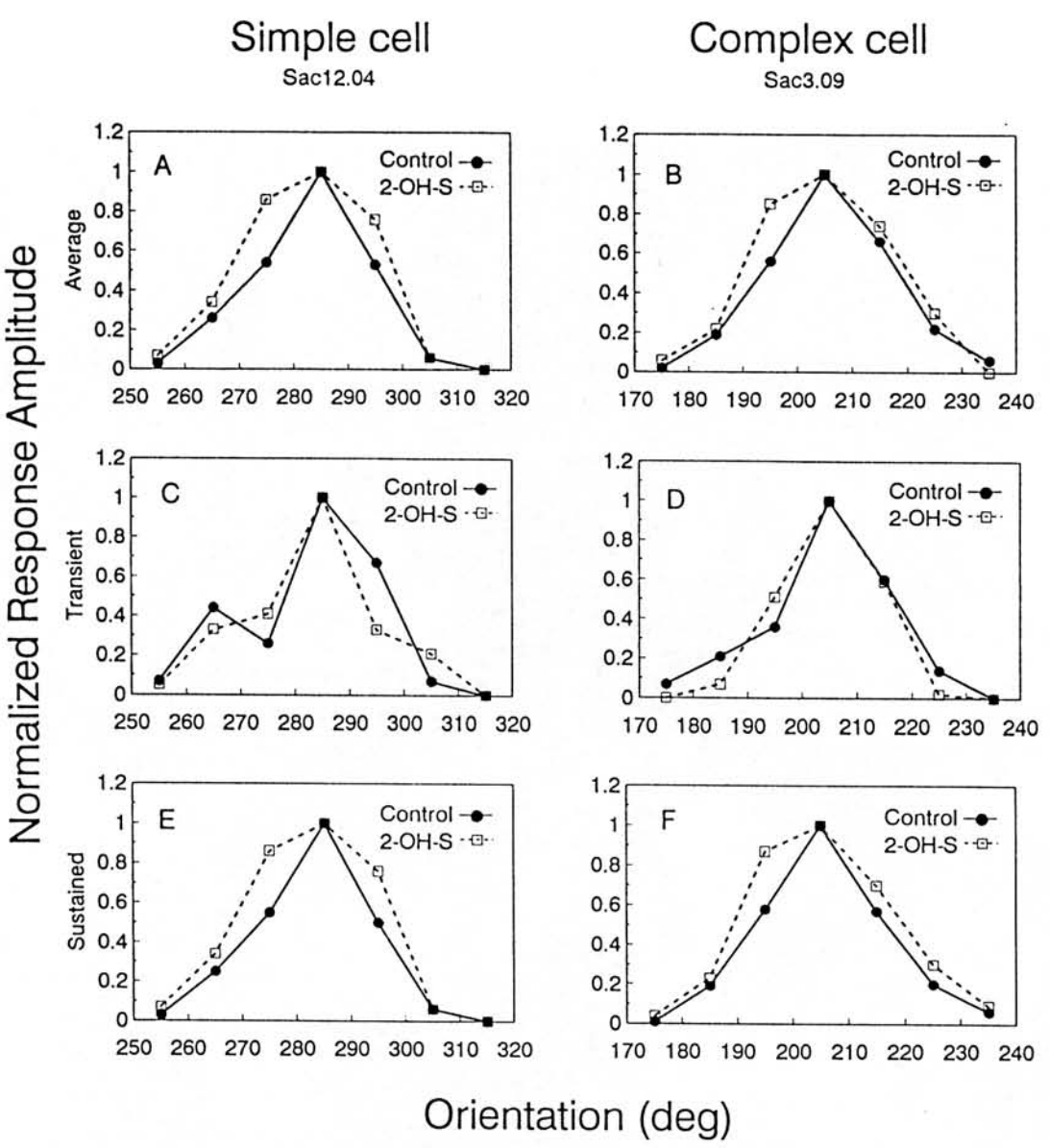

Fig. 3. Specific examples of impact of 2-OH-S on orientation selectivity. (A) The normalized response of a simple cell before and during treatment with 2-OH-S is plotted as a function of stimulus orientation. Iontophoresis of 2-OH-S increased the peak response amplitude of the cell from a baseline of 48 spikes/s to 52 spikes/s. 2-OH-S also broadened the orientation tuning bandwidth of this cell's average firing rate by 36.4%, from a baseline tuning bandwidth (half-width, half-height) of 11 deg (filled circles) to 15 deg during 2-OH-S treatment (open squares). (B) The normalized response of a complex cell before and after 2-OH-S treatment illustrates that 2-OH-S broadened the orientation tuning bandwidth of this cell's mean firing rate by 23.1%, from a baseline tuning bandwidth of 13 deg to 16 deg during 2-OH-S treatment. The cell's baseline peak response amplitude of 9 spikes/s increased to 13 spikes/s during iontophoresis of 2-OH-S. 2-OH-S did not affect significantly the orientation tuning bandwidth of the normalized onset transient responses of either the simple cell (C; total spike count = 28 spikes before and 40 spikes after 2-OH-S) or the complex cell (D; total spike count = 27 spikes before and 42 spikes after 2-OH-S). In contrast, 2-OH-S broadened the orientation tuning bandwidths of the normalized sustained response of the simple cell (E; total spike count = 1050 spikes before and 1150 spikes after 2-OH-S) by 36.4% and the sustained response of the complex cell by 23.1% (F; total spike count = 325 spikes before and 475 spikes after 2-OH-S).

tion tuning bandwidth was 11 deg. 2-OH-S (open squares) increased the amplitude of the sustained response to stimulus orientations of 275 deg and 295 deg. The orientation tuning bandwidth of the sustained response increased significantly to 15 deg. 2-OH-S increased the orientation tuning bandwidth of the cell's sustained response by 36.4%. The reduced orientation selectivity of the sustained response accounted entirely for the reduced orientation selectivity seen in the cell's average firing rate (Fig. 3A).

Fig. 3B shows the effect of 2-OH-S on the orientation selectivity of one complex cell. Under control conditions (filled circles), the cell produced a peak response to a stimulus orientation

of 205 deg and its baseline orientation tuning bandwidth was 13 deg. Iontophoresis of 2-OH-S (open squares) elevated its response to stimulus orientations of 195 deg and, to a lesser extent, 215 deg. The orientation tuning bandwidth broadened by 23.1% to 16 deg.

Examination of the transient (Fig. 3D) and sustained (Fig. 3F) responses of the complex cells showed that, as for simple cells, the reduced orientation selectivity resulted from an increase in the orientation tuning bandwidth of their sustained response, but not their transient response. Under control conditions (filled circles), the amplitude of the example cell's onset transient response peaked during presentation of a stimulus orientation of 205 deg and the orientation tuning bandwidth of the onset transient response was 13 deg. 2-OH-S increased the amplitude of the transient response to a stimulus orientation of 195 deg and the orientation tuning bandwidth of the transient response broadened slightly, but not significantly. Under control conditions (filled circles), the amplitude of the cell's sustained response peaked at a stimulus orientation of 205 deg and the baseline orientation tuning bandwidth of its sustained response was 13 deg. 2-OH-S increased significantly the amplitude of the sustained response to stimulus orientations of 195 deg and 215 deg, and the orientation tuning bandwidth of the sustained response broadened from 13 deg to 16 deg. 2-OH-S therefore increased the orientation tuning bandwidth of this complex cell's sustained response by 23.1%.

The distribution of changes during 2-OH-S iontophoresis in the orientation tuning bandwidths of the eight simple cells and 13 complex cells is illustrated in Fig. 4. All of the simple and complex cells that produced broadened orientation tuning bandwidths during iontophoresis of 2-OH-S exhibited similar patterns of an increase the orientation tuning bandwidth of the sustained, but not the onset transient, response. In fact, comparison of the distribution of changes in the orientation tuning of the sustained responses with the distribution of changes in the average response amplitude (Fig. 2) show the plots to be nearly identical. This is different from the situation resulting from the use of n-methyl-bicuculline to block $GABA_A$ receptors, in which changes in orientation tuning are dominated by broadening of the tuning of the onset transient response (Pfleger & Bonds, 1995).

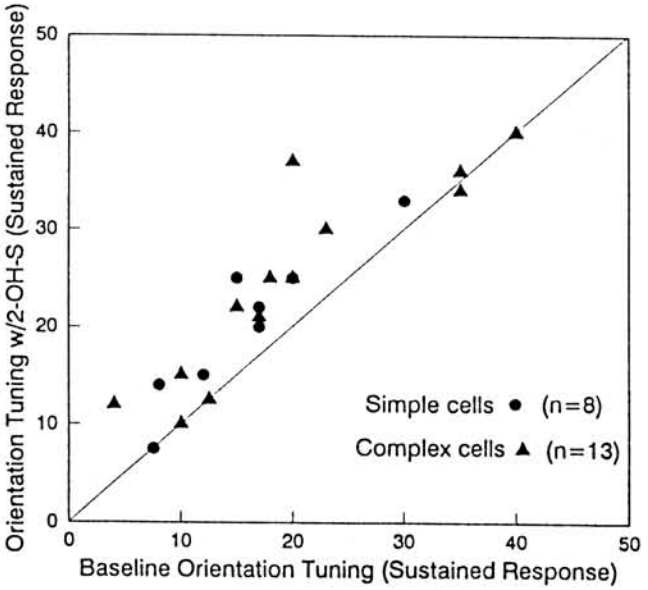

Fig. 4. The distribution of orientation tuning bandwidths of the sustained responses of eight simple cells and 13 complex cells before and after treatment with 2-OH-S. The six simple cells and eight complex cells which displayed increased in the orientation tuning bandwidths of their average response (Fig. 2) displayed changes in the orientation tuning bandwidths of their sustained responses.

Effects on burst length and percentage of spikes within burst epochs

During the experiments, we observed that for many neurons 2-OH-S shortened the intervals between the spikes being produced. Many cortical neurons generate spike sequences in which two or more spikes are separated by 8 ms or less (Cattaneo et al., 1981; DeBusk, 1992; Mandl, 1993). The length of these spike "bursts" (i.e. the number of spikes/burst) depends in part on stimulus orientation (Cattaneo et al., 1981; DeBusk, 1992; Bonds, 1994), with longer bursts (at a constant overall firing rate) occurring at optimal orientations. 2-OH-S reduced the orientation selectivity of bursts and increased their average length. For example, one complex cell normally produced an average burst length of about three spikes in response to stimulus orientations between 125 deg and 145 deg (Fig. 5A, filled circles). Although this cell did respond to stimulus orientations outside this range (data not shown), those orientations did not generate any incidences of two or more spikes separated by 8 ms or less (i.e. bursts). During iontophoresis of 2-OH-S (open squares), the length of the average burst produced by this complex cell increased by 15% to about 3.5 spikes in response to stimulus orientations between 125 deg and 145 deg. More importantly, 2-OH-S caused the cell to produce bursts about three spikes long in response to stimulus orientations which under control conditions generated no bursts. Thus, the dependence of burst length on stimulus orientation was eliminated. A total of six cells exhibited a complete loss of the orientation tuning of their bursts during 2-OH-S administration.

2-OH-S increased the firing rate of the 14 neurons that showed reduced orientation selectivity and also increased the length of bursts produced by some of these cells in response to optimal stimulus orientation. Fig. 5B shows the percentage change in burst length during 2-OH-S treatment plotted as a function of the percentage of spikes normally produced within bursts. Cells that normally produced 20% or less of their spikes within bursts did not generate bursts during 2-OH-S treatment. The length of bursts increased by about 10% for cells which normally produced 40–60% of their spikes within bursts. The length of bursts for cells which normally generated 75% or more of their spikes within bursts increased by 15–20%. The correlation (r^2) between the percentage change in burst length during 2-OH-S treatment and the percentage of spikes produced within bursts under normal conditions was 0.6. These results suggest a tendency for cells which normally produce a large percentage (>50%) of their spikes within bursts to show increased burst length and less selectivity for burst generation when the $GABA_B$ receptor is blocked. Although the change in burst length was correlated with the percentage of spikes produced within bursts, no such correlation was found between the per-

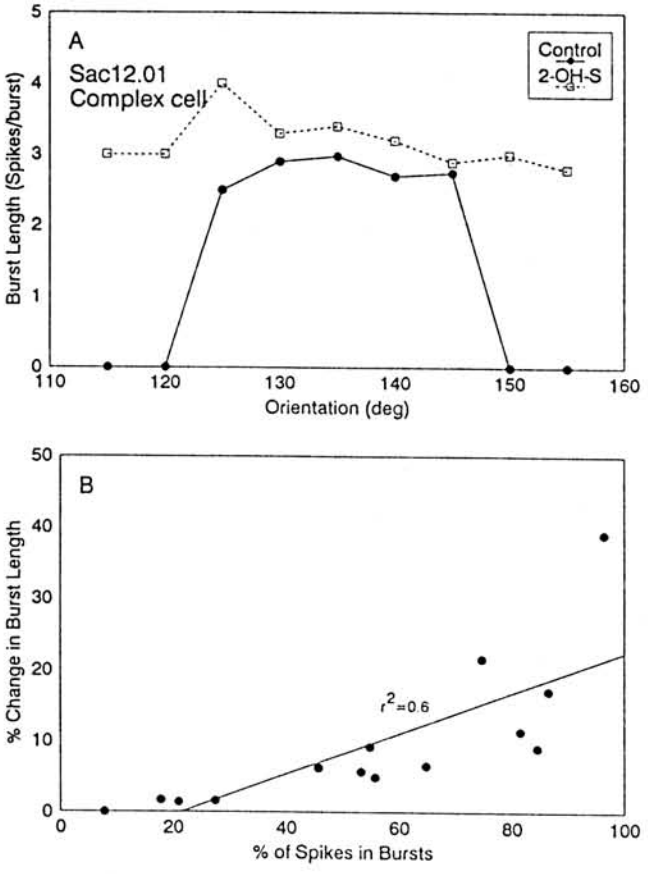

Fig. 5. Influence of 2-OH-S on burst activity. (A) Data from an example neuron shows that under control conditions (filled circles), the length (i.e. number of spikes) of the bursts is broadly dependent on stimulus orientation. Although the cell did not exhibit burst behavior in response to stimulus orientations outside the range of 125 deg to 145 deg, it did respond to stimuli outside this range (data not shown). 2-OH-S increased this cell's average burst length by 15%, from 3 spikes/burst to 3.5 spikes/burst (open squares). Most importantly, 2-OH-S caused the cell to burst in response to stimulus orientations which normally did not cause bursts, eliminating the orientation selectivity of the cell's burst behavior. (B) The percentage change in spike burst length during iontophoresis of 2-OH-S is plotted as a function of the normal percentage of spikes produced within bursts for 14 cells that increased their firing rate during 2-OH-S treatment. Cells which normally produced fewer than 20% of their spikes in bursts were unaffected by 2-OH-S. The average burst length of cells which normally produced 40–60% of their spikes in bursts was elevated by 5–10% during 2-OH-S treatment. The average burst length of cells which normally produced 80% or more of their spikes in bursts increased by 15% or more during 2-OH-S. The correlation (r^2) between the percentage of spikes normally produced within bursts and the percentage change in burst length during 2-OH-S treatment was 0.6. Thus, cells which normally produced a large percentage of their spikes in bursts were more greatly affected by 2-OH-S.

centage spikes within bursts and an increase in the orientation tuning bandwidth of a cell (Fig. 6).

Discussion

The most important result of this study is that blocking $GABA_B$-receptor-mediated inhibition by iontophoretic injection of 2-OH-S reduces the orientation selectivity of some neurons in the striate cortex of cats. As predicted by Pfleger and Bonds (1995), this reduced selectivity is found in the sustained response, but not the onset transient response, of these neurons. The orientation selectivity of these neurons' sustained response must therefore be influenced, at least in part, by $GABA_B$-receptor-mediated inhibition. However, the data also suggest that $GABA_B$-receptor-mediated inhibition is not the only source of the orientation selectivity of the sustained response. 2-OH-S reduced the orientation selectivity of only 14 of 21 neurons and, although the orientation selectivity of the sustained response was reduced, it was never completely eliminated. Other mechanisms must therefore be involved in shaping the orientation selectivity of the sustained response. The nature of these other mechanisms is unclear, but the contribution of $GABA_A$-receptor-mediated inhibition has been reported as minimal (Pfleger & Bonds, 1995).

At first glance, the current results seem to contradict the finding that blocking $GABA_B$-receptor-mediated inhibition with phaclofen does not reduce the orientation selectivity of striate cortical neurons (Baumfalk & Albus, 1988). There are two possible explanations for the disagreement between the previous and current results. First, 2-OH-S is a more selective and potent antagonist of the $GABA_B$ receptor than phaclofen (Curtis et al., 1988; Kerr et al., 1988*a*,*b*) and therefore may have a more pronounced affect on cortical $GABA_B$-receptor function. A second, more likely explanation is that Baumfalk and Albus (1988) did not observe any reduced orientation selectivity in their sample of neurons because they stimulated those neurons with sweeps of a light bar. The onset transient response dominates the cortical cell's response to a light bar (Pfleger & Bonds, 1995) and, although we stimulated cells with a 4-s drifting sine-wave grating, we also did not observe a reduction in the orientation selectivity of the onset transient response when $GABA_B$-receptor inhibition was blocked. Rather, blocking $GABA_B$-receptor-mediated inhibition with 2-OH-S reduced only the orientation selectivity of cells' sustained response to the grating. This effect of 2-OH-S would probably be minimal in the case of stimulation with a light bar. The differences between the results of the current study and those of Baumfalk and Albus (1988) are therefore most likely due to the different methods used for stimulating cells.

The finding that blocking $GABA_B$-receptor-mediated inhibition with 2-OH-S is limited to the long latency (i.e. >200 ms) sustained response of cortical cells is consistent with the notion that the $GABA_B$ receptor operates through a "slow," G-protein coupled second-messenger system that increases membrane K^+ conductance (Mody et al., 1994). Electrical stimulation of $GABA_B$ receptors produces a "long latency" inhibitory postsynaptic potential (IPSP) which reaches its peak in 100–200 ms and lasts between 250–1000 ms (Connors et al., 1988; Connors, 1992). An important point to note is that although the 2-OH-S did not reduce the orientation selectivity of the onset transient response, it often increased the amplitude of this response component. This result suggests that some component of $GABA_B$-receptor function occurs before the 200-ms latency we used to define the onset of the sustained response.

The results of our analysis of the burst behavior of cells suggests that $GABA_B$-receptor-mediated inhibition is involved in regulating the length of bursts produced by cortical neurons. Many cortical neurons produce bursts that are broadly selective for stimulus orientation (Cattaneo et al., 1981; DeBusk, 1992; Bonds, 1994) and, for cells that produced orientation

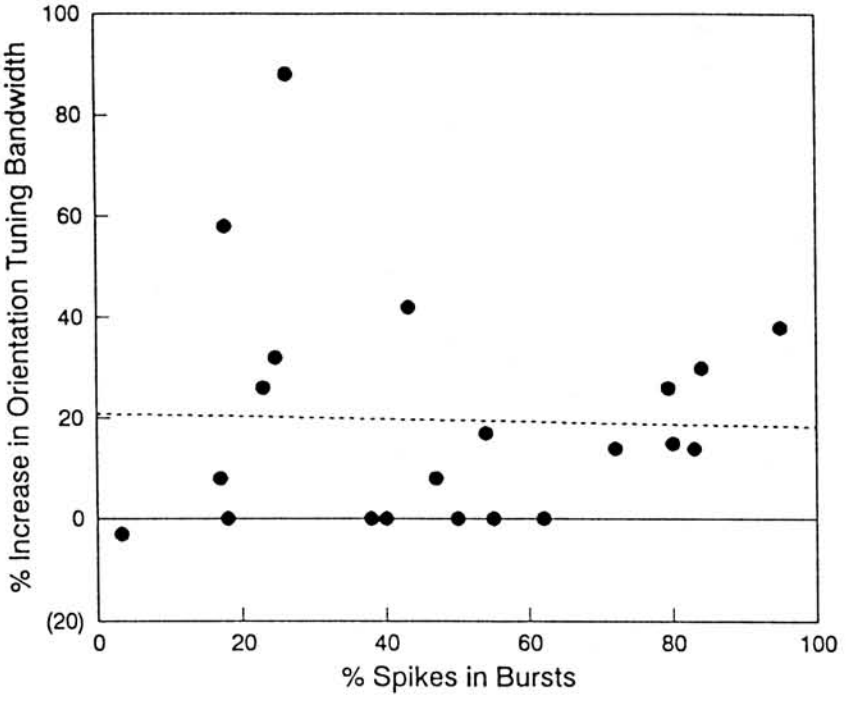

Fig. 6. Increase in orientation tuning bandwidth as a function of bursts. No correlation was found between the percentage increase in a cell's orientation tuning bandwidth during 2-OH-S and the percentage of spikes the cell produces within a burst.

selective bursts, 2-OH-S eliminated the orientation selectivity of the bursts and increased the number of spikes produced within bursts. The neural mechanisms that generate bursts are unknown, but two general models have been proposed (DeBusk, 1992). In the first model, cortical circuits form an excitatory (positive) feedback path to produce burst activity. Inhibition is required to break the excitatory loop and prevent the circuit from firing indefinitely. In the second model, strong synchronized input to a cell generates a high firing rate limited only by the refractory period of the cell and high threshold excitatory currents cause the cell to remain in an excited state. Inhibitory neurons stimulated by the cell provide feedback that reduces the high discharge rate after a brief delay. In both models, bursts occur due to the temporal lag required for the inhibitory circuit to be activated and, in turn, suppress the excitatory cell's discharges. While both of these models require inhibition to halt ongoing high-frequency activity to yield discrete bursts, the neurotransmitter involved is unknown. GABA is a likely candidate because it is distributed throughout visual cortex (Gehlert et al., 1985; Bowery et al., 1987). In fact, the average length of bursts (i.e. number of spikes/burst) is reduced by GABA and, conversely, increased by the $GABA_A$-receptor antagonist bicuculline (Dykes et al., 1984; Bonds et al., 1992). The finding that 2-OH-S increases the length of spike bursts supports the hypothesis that spikes bursts are controlled by GABAergic, inhibitory feedback (Bonds, 1994).

This report demonstrates that $GABA_B$-receptor-mediated inhibition plays a role in shaping the orientation selectivity of the sustained response of cortical neurons, shapes the orientation selectivity of bursts produced by cortical neurons, and increases the length of these bursts. In our limited sample, blocking $GABA_B$-receptor-mediated inhibition was less effective for complex cells than for simple cells. Furthermore, the average decrease in orientation selectivity during iontophoresis of 2-OH-S was much smaller than analogous treatment with bicuculline (Sillito, 1975, 1979; Pfleger & Bonds, 1995). However, although the data reported here are modest, these results are important because they are the first demonstration that $GABA_B$-receptor-mediated inhibition plays a functional role in visual processing within the striate cortex.

Acknowledgments

We thank Jason Dinger and Peter Melzer for their help collecting the data. This work was supported by NEI Grants EY06410 to J.D. Allison, EY01778 to V.A. Casagrande, EY03778 to A.B. Bonds, and a Vision core grant (EY08126) to Vanderbilt University.

References

Allison, J.D. & Bonds, A.B. (1994). Inactivation of the infragranular striate cortex broadens orientation tuning of supragranular visual neurons in the cat. *Experimental Brain Research* **101**, 415–426.

Baumfalk, U. & Albus, K. (1988). Phaclofen antagonizes baclofen-induced suppression of visually evoked responses in the cat's striate cortex. *Brain Research* **463**, 398–402.

Bonds, A.B. (1989). Role of inhibition in the specification of orientation selectivity of cells in the cat striate cortex. *Visual Neuroscience* **2**, 41–55.

Bonds, A.B. (1994). Dual inhibitory mechanisms for definition of receptive field characteristics in cat striate cortex. *Advances in Neural Information Processing Systems* **6**, 75–82.

Bonds, A.B., DeBruyn, E.G. & DeBusk, B.C. (1992). Burst length modulation by GABA and bicuculline in cat cortical cells: A substrate for spatial selectivity. *Investigative Ophthalmology and Visual Science* (Suppl.) **33**, 1255.

Bowery, N.G., Hudson, A.L. & Price, G.W. (1987). $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. *Neuroscience* **20**, 365–383.

Cattaneo, A., Maffei, L. & Morrone, C. (1981). Two firing patterns in the discharge of complex cells encoding different attributes of the visual stimulus. *Experimental Brain Research* **43**, 115–118.

Chapman, B., Zahs, K.R. & Stryker, M.P. (1991). Relation of cortical cell orientation selectivity to alignment of receptive fields of the

geniculocortical afferents that arborize within a single orientation column in ferret visual cortex. *Journal of Neuroscience* **11**, 1347–1358.

CONNORS, B.W. (1992). $GABA_A$- and $GABA_B$-mediated processes in visual cortex. In *Progress in Brain Research, Vol. 90*, ed. MIZE, R.R., MARC, R.E. & SILLITO, A.M., pp. 335–348. Amsterdam: Elsevier.

CONNORS, B.W., MALENKA, R.C. & SILVA, L.R. (1988). Two inhibitory post-synaptic potentials and $GABA_A$ and $GABA_B$ receptor-mediated responses in neocortex of rat and cat. *Journal of Physiology* **406**, 443–468.

CURTIS, D.R., GYNTHER, B.D., BEATTIE, D.T., KERR, D.I.B. & PRAGER R.H. (1988). Baclofen antagonism by 2-hydroxy-saclofen in the cat spinal cord. *Neuroscience Letters* **92**, 97.

DEBUSK, B.C. (1992). Neural encoding within and beyond receptive fields in cat visual cortex. Doctoral Thesis, Vanderbilt University, Nashville, TN.

DYKES, R.W., LANDRY, P., METHERATE, R. & HICKS, T.P. (1984). Functional role of GABA in cat primary somatosensory cortex: Shaping receptive fields of cortical neurons. *Journal of Neurophysiology* **2**, 1066–1093.

EYSEL, U.T., CROOK, J.M. & MACHEMER, H.F. (1990). GABA-induced remote inactivation reveals cross-orientation inhibition in the cat striate cortex. *Experimental Brain Research* **80**, 626–630.

FERSTER, D. (1986). Orientation selectivity of synaptic potentials in neurones of cat visual cortex. *Journal of Neuroscience* **6**, 1284–1301.

FERSTER, D. (1994). Assembly of simple cell receptive fields in cat visual cortex. *Investigative Ophthalmology and Visual Science* (Suppl.) **35**, 1649.

FERSTER, D. & JAGADEESH, B. (1992). EPSP-IPSP interactions in cat visual cortex studied with in-vivo whole cell patch recording. *Journal of Neuroscience* **12**, 1262–1274.

GEHLERT, D.R., YAMAMURA, H.I. & WAMSLEY, J.K. (1985). γ-aminobutyric $acid_B$ receptors in the rat brain: Quantitative autoradiographic localization using [3H](-)-baclofen. *Neuroscience Letters* **56**, 183–188.

HENRY, G.H., BISHOP, P.O., TAPPER, R.M. & DREHER, B. (1973). Orientation specificity of cells in cat striate cortex. *Vision Research* **13**, 1771–1779.

HUBEL, D.H. & WIESEL, T.N. (1962). Receptive fields, binocular interactions and functional architecture in the cat's striate cortex. *Journal of Physiology* **160**, 106–154.

KERR, D.I.B., ONG, J., JOHNSTON, G.A.R. & PRAGER, R.H. (1988*a*). 2-hydroxy-saclofen: An improved antagonist at central and peripheral GABA-B receptors. *Neuroscience Letters* **92**, 92.

KERR, D.I.B., ONG, J., JOHNSTON, G.A.R. & PRAGER, R.H. (1988*b*). 2-hydroxy-saclofen: A new $GABA_B$-receptor antagonist. *Neuroscience Letters* (Suppl.) **30**, S85.

LEVENTHAL, A.G. (1985). Retinal design of the striate cortex. In *Models of the Visual Cortex*, ed. ROSE, D. & DOBSON, V.G., pp. 380–389. Chichester, England: John Wiley and Sons.

LEVICK, W.R. (1972). Another tungsten microelectrode. *Medical and Biological Engineering* **10**, 510–515.

MANDL, G. (1993). Coding for stimulus velocity by temporal patterning of spike discharges in visual cells of cat superior colliculus. *Vision Research* **33**, 1451–1475.

MODY, I., DE KONINCK, Y., OTIS, T.S. & SOLTESZ, I. (1994). Bridging the cleft at GABA synapses in the brain. *Trends in Neurosciences* **17**, 517–525.

MORRONE, C.C., BURR, D.C. & MAFEI, L. (1982). Functional implications of cross-orientation inhibition of cortical visual cells. I. Neurophysiological evidence. *Proceedings of the Royal Society* (London) **216**, 335–354.

PFLEGER, B. & BONDS, A.B. (1995). Dynamic differentiation of $GABA_A$-sensitive influences on orientation selectivity of complex cells in the cat striate cortex. *Experimental Brain Research* **104**, 81–88.

SILLITO, A.M. (1975). The contribution of inhibitory mechanisms to the receptive-field properties of neurones in the striate cortex of the cat. *Journal of Physiology* **250**, 305–329.

SILLITO, A.M. (1979). Inhibitory mechanisms influencing complex cell orientation selectivity and their modification at high resting discharge. *Journal of Physiology* **289**, 33–53.

SOODAK, R.E., SHAPLEY, R.M. & KAPLAN, E. (1987). Linear mechanisms of orientation tuning in the retina and lateral geniculate nucleus of the cat. *Journal of Neurophysiology* **58**, 267–275.

VIDYASAGAR, T.R. & URBAS, J.V. (1982). Orientation sensitivity of cat LGN neurones with and without inputs from visual cortical areas 17 and 18. *Experimental Brain Research* **46**, 157–169.