The influence of input from the lower cortical layers on the orientation tuning of upper layer V1 cells in a primate

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Abstract

The receptive fields of cells in the primary visual cortex (area 17 or V1) show clear orientation selectivity, unlike those of the lateral geniculate nucleus (LGN) cells which provide their visual input. The intrinsic circuitry of V1 cells is believed to be partly responsible for this selectivity. We investigated the influence of ascending projections from neurons in the lower layers (5 and 6) of V1 on the orientation selectivity of single neurons in the upper layers (2,3, and 4) by reversibly inactivating ("blocking") lower layer neural activity with iontophoretic application of γ-aminobutyric acid (GABA) while recording from upper layer cells in the prosimian primate, *Galago crassicaudatus*. During lower layer blocking, the majority (20/28 = 71.4%) of upper layer neurons exhibited a change in the orientation of their preferred stimulus, a reduction in their orientation tuning, and/or an increase in their response amplitude. Twelve (42.9%) neurons exhibited shifts in their preferred orientation averaging 11 ($\pm$4) deg. These neurons were located, on average, 272 ($\pm$120) μm tangential from the vertical axis of the pipette center. Eleven neurons (39.2%) exhibited an average reduced orientation tuning of 52.5%. Their average location was 230 $\pm$ (115) μm away from the vertical axis of the pipette. Five (17.9%) neurons with average location 145 ($\pm$75) μm from the vertical axis exhibited both effects. Two (7.1%) neurons that exhibited significant increases in response amplitude to stimulus angles within 10 deg of the peak excitatory stimulus without changes in orientation selectivity or tuning were located less than 100 μm from the vertical axis. The effects on the orientation tuning of cells were restricted in all cases to within $\pm$30 deg of the preferred stimulus orientation. This suggests that layer blocking affects cells with preferred stimulus orientations similar to those of the recorded neurons. Only cells located within 500 μm tangential to the vertical axis of the injection site exhibited these effects. These results suggest that cells within layers 5 and 6 provide organized, orientation-tuned inhibition that sharpens the orientation tuning of cells in the upper cortical layers within the same, or closely neighboring, cell columns.

Keywords: Visual cortex, Orientation tuning, Layer blocking, Intracortical inhibition, GABA

Introduction

Hubel and Wiesel (1962) originally proposed that input from lateral geniculate nucleus (LGN) cells with appropriately aligned receptive fields produces the orientation selectivity exhibited by neurons in the primary visual cortex (area 17 or V1). Support for this hypothesis is provided by evidence that the excitatory postsynaptic potentials recorded from V1 neurons which receive monosynaptic excitatory input from LGN relay cells exhibit orientation tuning (Ferster & Lindström, 1983; Ferster, 1986, 1987, 1988). A large body of evidence, however, suggests that GABAergic inhibition which originates from within V1 can act to sharpen the orientation tuning of V1 neurons (Sillito, 1975; for review see Bonds & DeBruyn, 1985). Thus, a widely held alternative viewpoint is that the orientation biased input from the LGN is sharpened by intracortical inhibitory mechanisms that arise from a complex circuit of tangential and vertical interconnections between cortical layers and columns (Wörgötter & Koch, 1991; Casagrande & Kaas, 1994).

One possible source of intracortical inhibition responsible for shaping the orientation selectivity of cortical neurons is found in vertical interconnections (Creutzfeldt et al., 1974*a*,*b*). For example, neurons in the lower layers (5 and 6) contribute not only efferents to subcortical targets and extrastriate areas, but also heavily innervate neurons located within the layers above them. Layer 5 neurons in primates send substantial

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ascending projections to neurons in layer 3 as well as projections to layers 4 and 6 (Casagrande & Kaas, 1994). In macaque monkeys, a major intrinsic projection from layer 5 arises from GABAergic interneurons in 5A that likely can directly modify thalamic recipient neurons in layer 4 (Lund, 1988). Layer 6 neurons also provide light input to neurons in all cortical layers and heavy input to layer 4 (Blasdel et al., 1985). Inhibitory interneurons make a substantial contribution to these projections in monkeys (Fitzpatrick et al., 1985; Blasdel et al., 1985). Within V1 of the prosimian primate bush baby (*Galago crassicaudatus*), neurons in the upper layers are also heavily innervated by lower layer neurons, although the pattern of inputs varies between layers (Lachica et al., 1992, 1993). Layer 5 sends its heaviest input to layer 3B. Layer 6 sends a heavier projection to layer 4 with almost no input reaching layers 3A or 2 (Lachica et al., 1992, 1993; Casagrande & Kaas, 1994). These anatomical findings raise questions about the possible impact of inputs from infragranular neurons on the response properties of neurons within the upper layers.

Although the functional significance of these ascending projections from the lower layers in V1 of primates is unknown, several physiological studies have examined their impact in cats by reversibly inactivating (i.e. "blocking") neural activity in the lower layers while recording from neurons in the upper layers. Layer blocking produces a release of inhibition ("disinhibition") that leads to a reduction of length tuning in hypercomplex cells (Bolz & Gilbert, 1986) and a reduction in orientation tuning and/or a shift in orientation selectivity in simple and complex cells (Bolz & Gilbert, 1986; Allison & Bonds, 1994). There are, however, significant differences in the anatomy of V1 between cats and primates, including differences in the subdivisions of the lower layers, the intrinsic connections with other layers, and the organization of compartments (e.g. cytochrome oxidase (CO) blobs and interblobs). Furthermore, the physiological investigations in area 17 of cats (Bolz & Gilbert, 1986; Allison & Bonds, 1994) did not provide a detailed analysis of the topographic relationships between the blocked lower layer neurons and the subsequently affected upper layer neurons, which would help to interpret the results in the context of the columnar organization of orientation selectivity.

We therefore undertook to address the following questions in this report: (1) Does blocking neural activity in layers 5 and 6 of V1 in a primate affect the orientation tuning and responsiveness of upper layer neurons?; and (2) If lower layer blocking does affect the orientation tuning of upper layer neurons, what is the topographic relationship between the region of blocked lower layer tissue and the tangential and laminar location of upper layer cells affected by the inactivation? Bush babies were chosen for this study because detailed information is available on the laminar differences in the physiological properties of V1 cells (DeBruyn et al., 1993), and the laminar interconnections within V1 have been well described (Lachica et al., 1992, 1993; Casagrande & Kaas, 1994).

Materials and methods

Surgical preparation for recording

Four adult bush babies (*Galago crassicaudatus* of both sexes; 1.0–1.5 kg) were anesthetized, paralyzed, and prepared for recording, as described in detail elsewhere (DeBruyn et al., 1993). Paralysis was maintained with Pavulon (pancuronium bromide, 1.9 mg/kg-h) (Astra, Westboro, MA) in lactated Ringer's solution with 5% dextrose and the animals were artificially ventilated with a mixture of 75% nitrous oxide, 23.5% oxygen, and 1.5% carbon dioxide delivered at a rate of 40–50 strokes/min in a volume sufficient to maintain the peak expired CO_2 at 4–4.5%. Anesthesia depth was maintained with 1% Brevital (methohexital sodium) as needed by monitoring both the EEG and EKG.

Electrode placement

A three-barrel micropipette electrode was positioned approximately 1.5 mm lateral to the midline and inserted between 1400–1500 μm deep into the lower layers of V1. Two barrels of the pipette contained 0.5 M γ-aminobutyric acid (GABA) in saline (pH = 3.0). The third barrel contained a 2.5% solution of horseradish peroxidase (HRP) in 1 M NaCl to mark the pipette's position and to provide a balancing current during GABA iontophoresis. The final position of the pipette was marked with a deposit of HRP by passing a 500-nA current through the pipette for 15–30 min (an example is indicated by arrowheads in Fig. 1). A tungsten-in-glass microelectrode (Levick, 1972) was then placed roughly 750 μm medial to the pipette electrode and lowered into the upper cortical layers within the medial bank of V1. The medial bank of V1 in primates represents the peripheral visual field, so all cells recorded had receptive fields located 15 ($\pm$ 5) deg from *area centralis*.

Experimental protocol

Receptive fields were first mapped for location and optimal orientation with a manually controlled light bar. Drifting sine-wave gratings were then presented with a microprocessor-based pattern generator and displayed on a CRT display (Tektronix 608; mean luminance 110 cd/m^2, P31 phosphor) with a 10-deg circular field. The display was compensated for linear modulation up to 60% contrast. The pattern generator allowed for independent control of the orientation, spatial frequency, temporal frequency, and contrast of the stimulus pattern. The primary data analysis tool was construction of 4 s, 128 bin/s poststimulus-time histograms. To reduce artifact from the inherent nonstationarity of visual cortex, the interleaved histogram technique of Henry et al. (1973) with randomization was used. A stimulus set consisted of each measurement condition as well as a null condition (uniform field at the mean luminance of the gratings) to assess the maintained discharge. Each element in the stimulus set was presented once, in random order with 1 s of mean luminance between each presentation, until the set was completed. Presentation of the set was then repeated in a different random order until each stimulus condition had been tested ten times. With 4-s presentation periods, results are based on 40 s of averaging for each condition. The number of impulses per presentation was tracked to permit calculation of response variability.

Each individual cell was initially characterized in terms of its orientation selectivity, spatial and temporal frequency tuning, and contrast response. Due to the time constraints involved in collecting the data, none of the cells were tested for end-stopping. Cells were classified as simple or complex according to their relative modulation (Skottun et al., 1991). Orientation selectivity was tested again with spatiotemporally optimized gratings at 40% contrast to provide a baseline for comparison with

subsequent measurements. After establishing the baseline orientation tuning and selectivity of the cell, GABA was iontophoresed into the lower layers using a total of 100 nA of current (50 nA per barrel). Five minutes after initiation of the GABA current, the orientation tuning of the cell was retested, in some cases as many as three times. Delivery of GABA was then discontinued, and, after a 5-min delay, the orientation tuning of the cell was retested three times or until the orientation tuning returned to pre-GABA control levels. The mean (± S.E.M.) response amplitudes generated by each cell before, during, and after GABA administration were plotted and a Student's *t*-test was used to compare each cell's response profile at each stimulus orientation between experimental conditions.

Histological verification

Each electrode penetration was marked by making pairs of electrolytic lesions (5 μA for 5 s) along electrode penetrations before retracting the electrode (see arrows, Fig. 1). At the conclusion of each recording session, the animal was given a lethal dose of sodium pentobarbital (Nembutal; Abbott; N. Chicago, IL) through the venous cannula until brain activity ceased. Each animal was then transcardially perfused with 0.9% saline followed by a solution of 4% paraformaldehyde, 0.2% picric acid, and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.4) and then by a solution of 0.5% paraformaldehyde–10% sucrose in phosphate buffer. The brain was removed, placed in 30% sucrose in phosphate buffer overnight, then frozen sectioned into 52-μm coronal sections. To locate the position of the GABA pipette, which was marked with a deposit of HRP, all sections were rinsed three times in phosphate buffered saline (PBS), incubated in 3,3-diaminobenzidene (50 mg/100 ml PBS) for 20 min, then 30 μl of 30% H_2O_2 was added as the chromogen. The reaction was ended after 10–20 min and the sections were then rinsed three times in PBS. The sections were then stained for cytochrome oxidase (CO) by the method of Wong-Riley (1979) to locate layer borders and CO blobs, mounted on gelatinized slides, dried, defatted in an ascending series of alcohols, cleared with xylene, then coverslipped with Permount.

The electrode tracks were reconstructed to determine the tangential and laminar position of each recorded neuron relative to the location of the pipette tip. Fig. 1 shows an example of

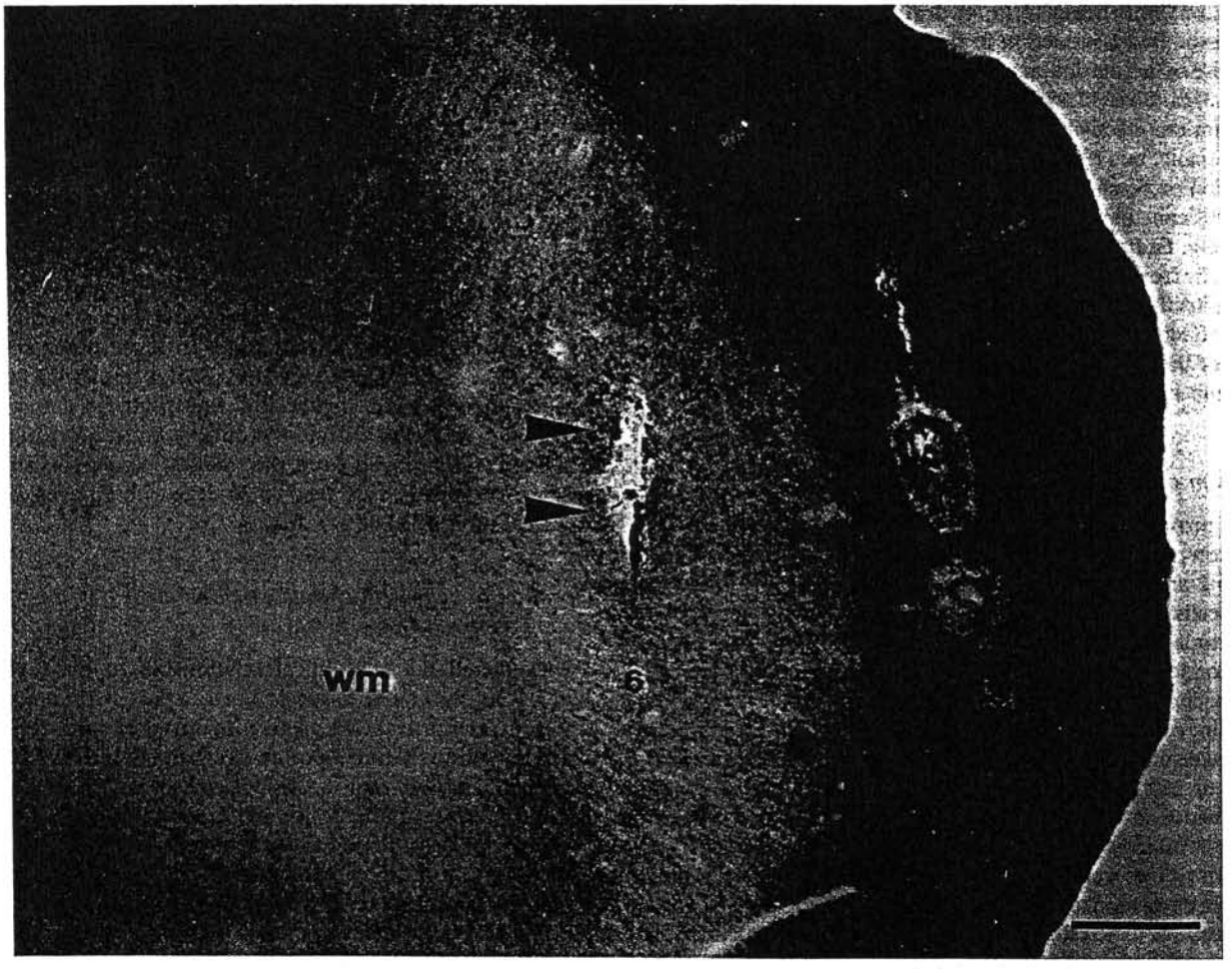

Fig. 1. A photomicrograph of a single 52-μm coronal section documents the proximity of the recording electrode track to the position of the tip of the pipette that contained GABA. Although there is no apparent HRP deposit in this particular case, the position of the pipette is easily seen in the slight damage caused by the pipette (indicated by arrowheads). Several lesions (marked by arrows) were made to aid in reconstruction of the recording electrode track. Cortical layers are numbered (1–6) and white matter (wm) is abbreviated. Scale bar = 250 μm. Medial is to the right, and dorsal is to the top.

the relative positions of the blocking pipette and recording electrode. The tip of the pipette which contained GABA is clearly within infragranular layer 6 (indicated by the arrowheads) while the recording electrode, indicated by a track and two lesions (arrows), passes through layer 3 and the top edge of layer 4. This example demonstrates the proximity of the blocking and recording electrodes. We found that such a close placement was necessary for layer blocking to have any effect on the responses of upper layer neurons.

Results

We measured the orientation selectivity and tuning of 28 neurons located within layers 2–4 before, during, and after blocking neural activity in the lower layers. In two cases, the tip of the iontophoresis pipette was centered in the lower half of layer 5 beneath the recorded cells while in one case it was centered at the border of layers 5 and 6 and in another case it was centered in layer 6 (see Fig. 1). Iontophoretic administration of GABA into the lower layers produced several notable effects on the responses of some of the upper layer neurons studied. First, almost half of the cells (12/28 = 42.9%) showed a shift in their preferred stimulus orientation, the average (± standard deviation) shift being 11 (± 4) deg. Second, 11 (39.3%) neurons exhibited a reduction in their orientation tuning (i.e. an increase in orientation tuning bandwidth), which averaged 52.5 (±36.3)%. Five (17.9%) neurons exhibited both a shift in their orientation selectivity and a reduction in their orientation tuning. Finally, two (7.1%) neurons exhibited neither a shift in orientation preference nor a reduction of tuning, but did display a significant increase in their response activity during layer blocking. Examination of each cell's position relative to the vertical axis from the injection site showed that layer blocking was only effective for tangential distances of less than 500 μm. The main effects of layer blocking and the impact of the relative locations of the blocking electrodes and recorded cells are described in detail below.

Shifts in orientation preference

Fig. 2 provides two examples of cells that exhibited shifts in orientation preference. These particular cells were located within layers 2 and 3C. However, similar results were also obtained from cells located in layers 3A, 3B, and 4. The mean response amplitude (± S.E.M.) is plotted as a function of stimulus orientation for each case. In Fig. 2A, the baseline orientation tuning function recorded from the layer 2 neuron is shown by the solid line connecting filled circles. The amplitude of this response peaked around 23 spikes/s during stimulation with gratings oriented at 220 deg. The baseline orientation tuning bandwidth (half-width at half-height) of this neuron was 18 deg. The orientation tuning function measured during GABA iontophoresis with a pipette centered in the lower half of layer 5 is shown by open circles connected by a dashed line. The optimal response amplitude increased slightly to 26 spikes/s, but the peak response was found with stimulation by gratings oriented at 210 deg rather than the baseline excitatory peak of 220 deg. This shift in stimulus preference was the result of a significant increase in response amplitude during stimulation with an orientation of 210 deg ($t = 2.7$; $P < 0.05$) and a concurrent significant decrease in response amplitude to stimulation with an orientation of 220 deg ($t = 2.44$; $P < 0.05$). GABA administration appeared to reduce excitation at the preferred stimulus while increasing excitation 10 deg from the optimal orientation. The response amplitude at all other stimulus angles showed no significant change. The neuron's orientation preference returned to 220 deg and its response amplitude returned to control values approximately 20 min after GABA iontophoresis had been discontinued (filled triangles connected with a dotted line). Of course, these estimates of shift are limited by the resolution of the measurements, but the cell does show significant differences between points. The resolution was limited by the need to complete experiments in a timely fashion. Thus, time restriction required the use of large increments, usually 10 deg, to cover an adequate range of stimulus angles during the experiment. Notably, this simple cell exhibited no maintained discharge before, during, or after layer blocking.

Fig. 2B illustrates a larger and more dramatic shift in orientation preference exhibited by a layer 3C neuron during layer blocking. This neuron showed a baseline peak response amplitude of 27 spikes/s during stimulation with a grating oriented at 100 deg. During layer blocking (in which the pipette was centered at the border of layers 5 and 6 [see Fig. 5]), the peak response shifted to a stimulus orientation of 118 deg. This 18-deg shift occurred without any corresponding change in the response amplitude of the cell. The experimental data are quite reliable, representing the average of three separate tuning functions recorded from this neuron during delivery of GABA into the lower layers (i.e. about 45 min of data collection). In all three cases the neuron preferred a stimulus orientation of 118 deg, indicated by a significant increase in response amplitude at this stimulus orientation ($t = 3.1$; $P < 0.01$). Approximately 30 min after termination of GABA injection, the neuron again exhibited a preference for a stimulus angle of 100 deg. This neuron showed no significant changes in the rate of its maintained discharge (indicated by the small, horizontal lines near the vertical axis) or its response amplitude to most other stimulus orientations during the entire experiment. A total of 12 (42.9%) of the 28 neurons we tested exhibited qualitatively similar shifts in their orientation selectivity during layer blocking. The observed shifts ranged from the smallest changes in stimulus orientation used (i.e. 10 deg) to 25 deg, with an average shift in orientation of 11 (± 4) deg.

Location of cells with shifted orientation tuning relative to pipette tip

We examined the anatomical relationship to the region of blocked infragranular tissue of the 12 neurons that exhibited a shift in their orientation preference during lower layer blocking. Fig. 3 shows a tracing of an overlapping series of coronal sections taken from an individual case in which several cells exhibited a shift in orientation preference. The medial bank is to the right side of the figure and dorsal is to the top. The layers (1–6) and white matter (wm) are labeled. The final position of the tip of the pipette containing GABA is indicated by the hatched area in the medial portion of layer 5. Two penetrations (P1 and P2) of the recording electrode are represented by the solid lines. The tracks of the two recording electrode penetrations are located roughly 250 and 500 μm, respectively, medial to the pipette site and the location of recorded neurons are marked by ticks. The positions of four neurons which showed shifts are indicated by solid circles. The open square marks the

position of a neuron that exhibited only a reduction in orientation tuning and the open triangle shows the location of a cell that showed both effects. As shown in this example, the majority of neurons that displayed a shift in orientation preference were not located directly vertical to the pipette tip (i.e. toward the medial bank), but were found to lie somewhat tangential to the vertical column of cells in which the pipette tip was located.

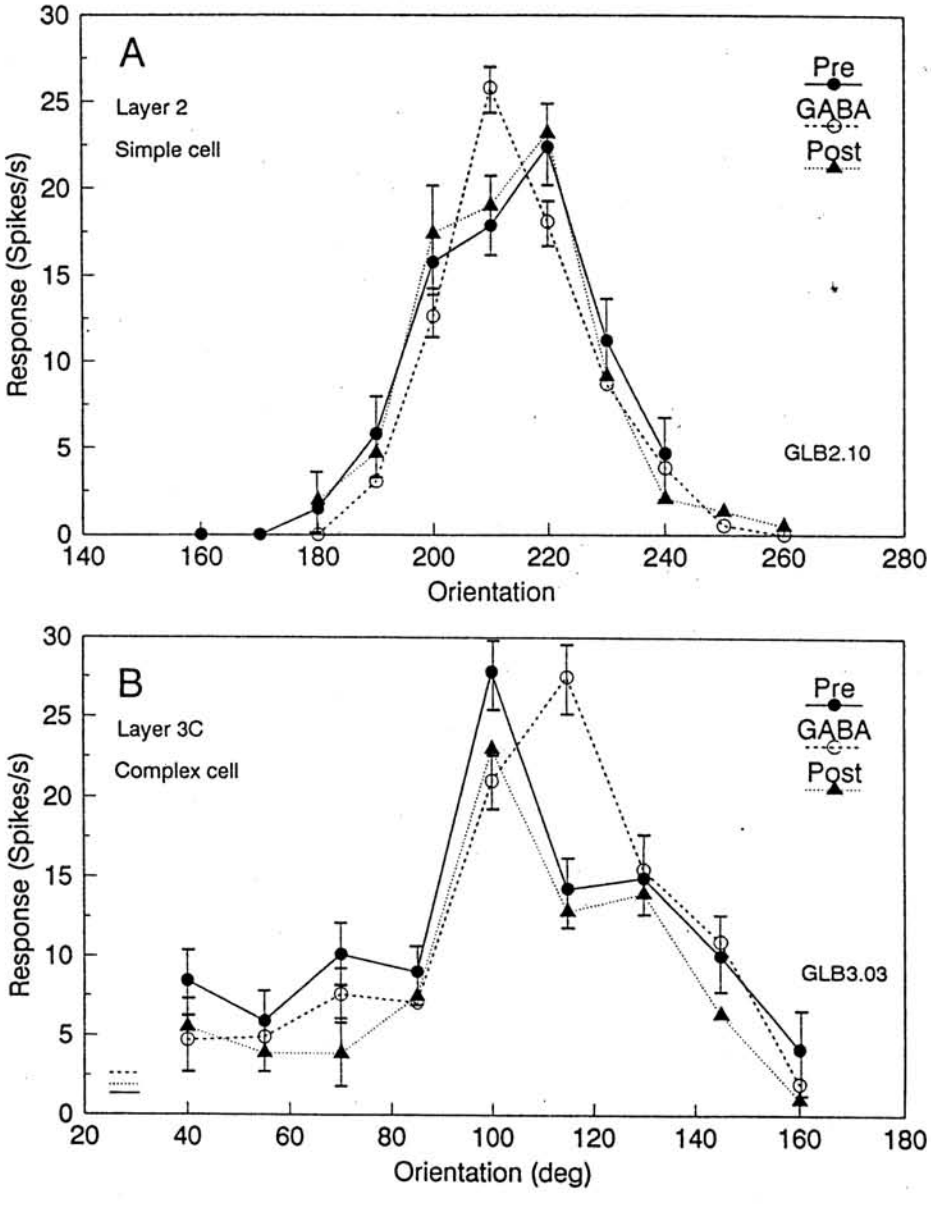

Fig. 2. The mean (±S.E.M.) number of spikes/s recorded from two neurons (A and B) is plotted as a function of stimulus orientation. In each case, the baseline activity recorded before layer blocking is illustrated by a solid line connecting filled circles. Open circles connected with a dashed line show the responses recorded during layer blocking and the filled triangles connected with a dotted line show the responses recorded 15–30 min after GABA administration had been discontinued. (A) This neuron exhibited a shift in orientation preference of 10 deg during layer blocking. This shift resulted from a significant increase in response amplitude to a stimulus orientation of 210 deg ($t = 2.7$; $P < 0.05$) and a concurrent significant decrease in response amplitude to a stimulus angle of 220 deg ($t = 2.44$; $P < 0.05$). This magnitude of shift in orientation preference was typical of most neurons that demonstrated this effect. This simple cell exhibited no maintained discharge before, during, or after the lower layers were blocked. The example neuron in (B) exhibited a much greater shift from a baseline preference for a stimulus angle of 100 deg to a preference of 118 deg during layer blocking. Moreover, this effect was maintained during three sets of recordings taken while GABA was administered. The data illustrated with open circles represents the mean (±S.E.M.) response amplitude averaged over three separate measurement periods (i.e. about 45 min of data collection). The neuron exhibited a highly significant response increase ($t = 3.1$; $P < 0.01$) during presentation of a stimulus at 118 deg. After recovery, the neuron again exhibited a preference for a stimulus orientation of 100 deg. This complex cell's baseline maintained discharge of 2 spikes/s (solid horizontal line near vertical axis) did not change significantly during layer blocking (horizontal dashed line near vertical axis). Animal (e.g. GLB2) and cell number (e.g. 10) are documented.

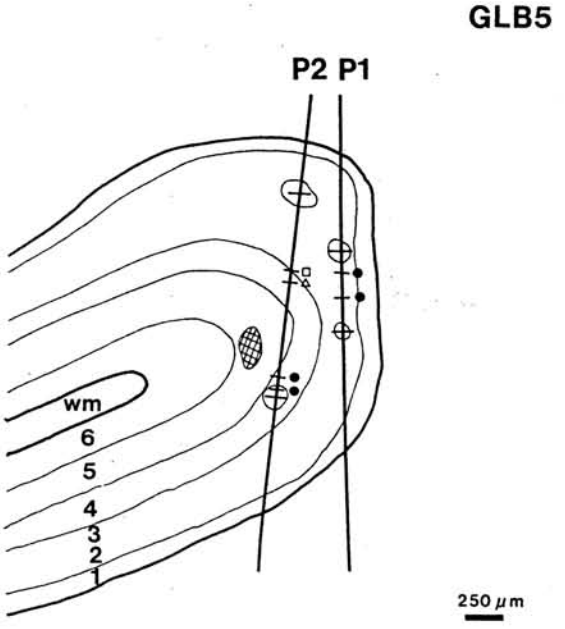

Fig. 3. An overlapping series of 52-μm sections from an individual case (GLB5) was traced to reconstruct two recording electrode penetrations (P1 and P2). The hatched area in layer 5 indicates the inactivated region of infragranular tissue. Ticks along the solid lines (i.e. recording electrode penetrations) represent the positions of recorded neurons. Solid circles show the location along the penetrations of neurons that exhibited *only* a shift in their orientation selectivity during layer blocking. The open square along P2 marks the location of a neuron that exhibited *only* a reduction in orientation tuning during layer blocking while the open triangle represents a neuron that exhibited *both* effects. Layers 1 through 6 and white matter (wm) are labeled. Scale bar = 250 μm.

Reduction in orientation tuning

The second effect observed during inactivation of the lower layers was a reduction in orientation tuning. This effect was seen in 11 (39.3%) of the 28 neurons. The orientation tuning bandwidth broadened either symmetrically around the original peak or asymmetrically with an elevation of responses to stimulus orientations to one side of the peak. In Fig. 4A, a layer 2 neuron exhibited a baseline peak response amplitude of 17 spikes/s to a stimulus orientation of 140 deg. The baseline orientation tuning bandwidth of this neuron was 15 deg. The orientation tuning function obtained during layer blocking with a pipette centered in layer 5 (dashed line connecting open circles) revealed an increase in orientation tuning bandwidth to 35 deg (a 133.3% reduction in orientation tuning) that resulted from a significant increase in response amplitude during stimulation with gratings oriented at 150 deg ($t = 2.6$; $P < 0.05$), 160 deg ($t = 2.8$; $P < 0.05$), and 170 deg ($t = 2.7$; $P < 0.05$). The layer blocking thus had an asymmetric effect on the orientation tuning function of this neuron which was restricted to within 30 deg of the peak excitatory stimulus. This neuron was unique, however, in that it also exhibited a slight reduction in response amplitude during layer blocking to the baseline excitatory peak orientation of 140 deg. Roughly 20 min after layer blocking was terminated, the orientation tuning function of this neuron (dotted line connecting filled triangles) returned to pre-blockade values. No significant changes in maintained discharge were observed during layer blocking.

Figs. 4B and 4C illustrate examples of neurons that were more typical in their response changes during layer blocking. The layer 4α neuron in Fig. 4B exhibited a peak baseline response to a stimulus orientation of 255 deg and had a baseline orientation tuning bandwidth of 8 deg. During layer blocking at the border of layers 5 and 6 (see Fig. 5), the preferred orientation was still 255 deg, but the response amplitude to a stimulus orientation of 245 deg was significantly elevated ($t = 3.2$; $P < 0.01$), resulting in an orientation tuning bandwidth of 13 deg (i.e. a 62.5% reduction in orientation tuning). A layer 3C neuron (Fig. 4C) exhibited a peak baseline response amplitude of 12 spikes/s to a stimulus angle of 250 deg and an orientation tuning bandwidth of 12 deg. During layer blocking, the response amplitude of this neuron significantly increased to stimulus orientations of 240 deg ($t = 3.15$; $P < 0.01$), 250 deg ($t = 3.2$; $P < 0.01$), 260 deg ($t = 3.4$; $P < 0.01$), and 270 deg ($t = 3.12$; $P < 0.01$). The increased response amplitude resulted in an increase in the orientation tuning bandwidth to 18 deg (a 50% reduction in orientation tuning). Moreover, this neuron also showed a shift of 10 deg in orientation preference. Thus, it is one of five neurons that exhibited both effects. The neuron showed no changes in its maintained discharge during layer blocking. The response of this neuron was lost during the recovery period so no post-GABA control run is shown.

Location of cells with reduced orientation tuning relative to pipette tip

Fig. 5 shows a tracing of an overlapping series of coronal sections drawn from an individual case (GLB3). As in Fig. 3, the hatched area located along the border of layers 5 and 6 represents the location of the pipette tip and the center of the area believed to be affected by iontophoresis of GABA. The solid lines show two recording electrode penetrations (P1 and P2) and the ticks indicate the locations of recorded neurons. The open circle illustrates the position of a neuron that exhibited *only* a shift in orientation tuning. The solid squares represent locations of neurons that showed *only* a reduction in orientation tuning and the open triangle shows the position of a neuron (see Fig. 4C) that displayed *both* effects. In this example, the four neurons that exhibited reduced orientation tuning during layer blocking were in closer vertical register (i.e. directly above the blocking pipette) relative to the inactivated infragranular tissue than was the neuron that exhibited no reduced orientation tuning. This pattern was also observed in the other cases.

Increases in response amplitude with no receptive-field changes

An example of a symmetrically affected neuron that exhibited significant increases in response amplitude to stimulus orientations near the peak excitatory stimulus with no corresponding changes in orientation tuning or selectivity is shown in Fig. 4D. This layer 3B complex cell exhibited a peak baseline response of 40 spikes/s to a stimulus orientation of 270 deg and a broad orientation tuning bandwidth of 25 deg. The orientation tuning function recorded from this neuron during layer blocking exhibited no shift in orientation preference nor was the orientation tuning reduced. However, the response amplitude to stimulus orientations of 260 deg ($t = 2.9$; $P < 0.05$), 270 deg ($t = 2.75$; $P < 0.05$) and 280 deg ($t = 2.4$; $P < 0.05$) was significantly elevated during layer blocking.

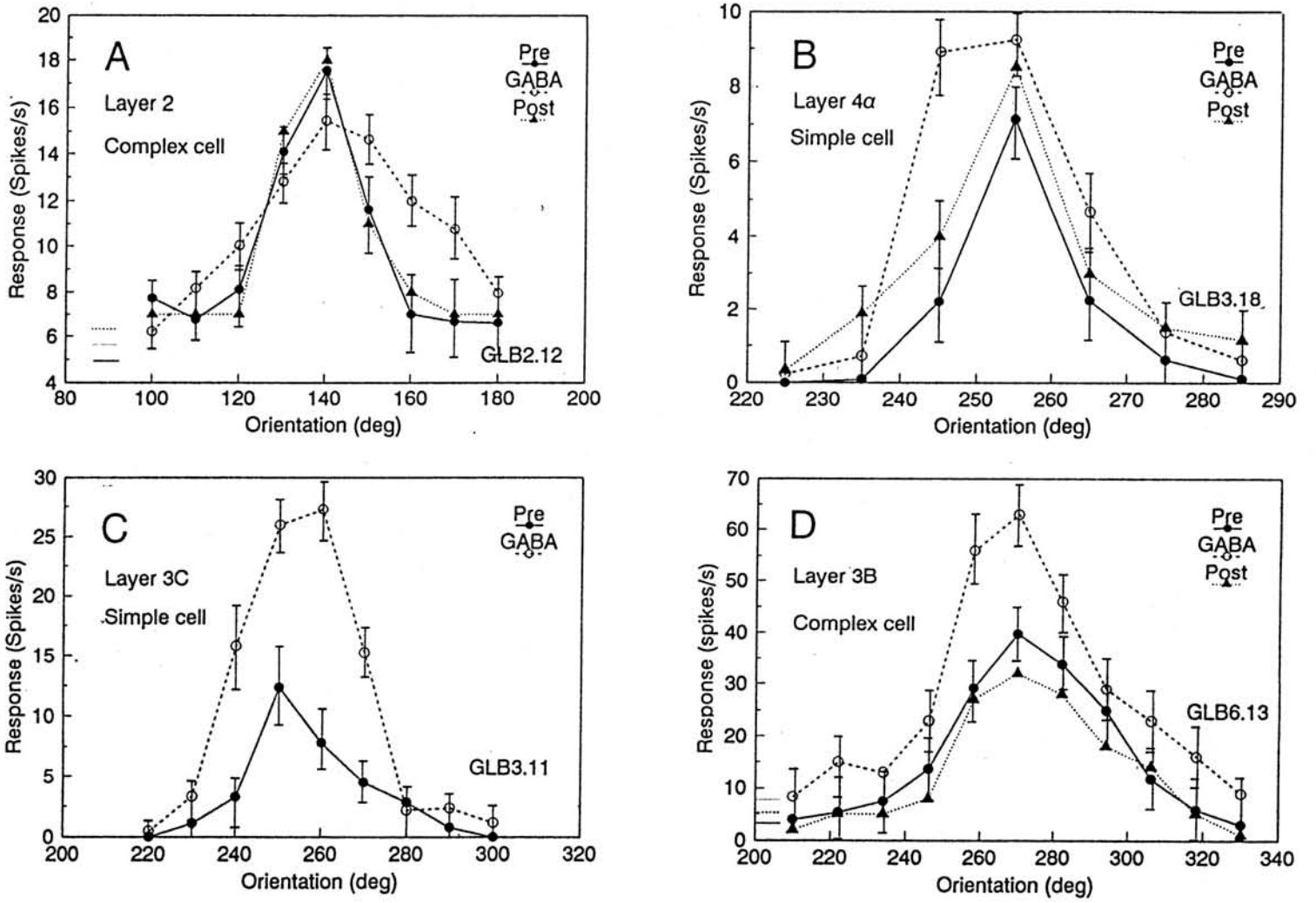

Fig. 4. A: Layer blocking significantly elevated the response amplitude of this neuron during stimulation with grating orientations of 150 deg ($t = 2.6$; $P < 0.05$), 160 deg ($t = 2.8$; $P < 0.05$), and 170 deg ($t = 2.7$; $P < 0.05$). This resulted in a decrease in orientation tuning of 133.3%. This neuron was unique in that its response amplitude to the optimal excitatory stimulus (i.e. 140 deg) decreased slightly. No significant changes in maintained discharge were observed during layer blocking (horizontal lines near the vertical axis). B: This layer 4α neuron illustrates the more typical, asymmetric change in the orientation tuning function recorded during layer blocking. The response amplitude increased at the peak excitatory stimulus of 255 deg, but was significantly elevated at 245 deg ($t = 3.2$; $P < 0.01$). The orientation tuning bandwidth increase from 8 deg to 13 deg (a 62.5% reduction in tuning). C: This neuron demonstrated both a shift in its preferred stimulus angle and a broadened orientation tuning function during layer blocking. The response amplitude increased significantly to stimulus angles of 240 deg ($t = 3.15$; $P < 0.01$), 250 deg ($t = 3.2$; $P < 0.01$), 260 deg ($t = 3.4$; $P < 0.01$), and 270 deg ($t = 3.12$; $P < 0.01$). The orientation tuning decreased by 50% from 12 deg to 18 deg. D: This neuron exhibited neither a shift of its preferred stimulus orientation nor a reduction in orientation tuning during layer blocking. The response amplitude of this neuron, however, was significantly elevated during stimulation with gratings orientated within 10 deg of the optimal stimulus. Increases in response amplitude were significant at 260 deg ($t = 2.9$; $P < 0.05$), 270 deg ($t = 2.75$; $P < 0.05$), and 280 deg ($t = 2.4$; $P < 0.05$). No such increases were observed in the maintained discharge (horizontal lines near the vertical axis).

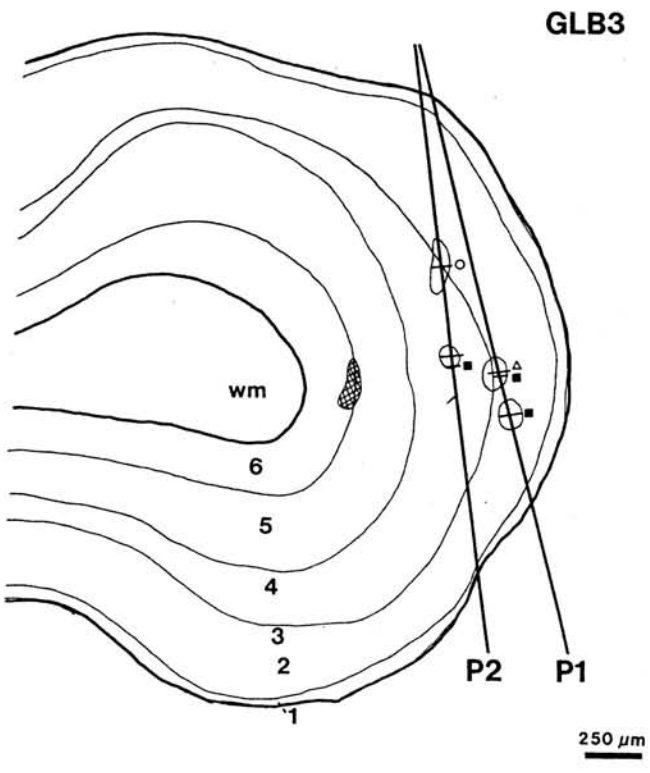

Fig. 5. A tracing from an overlapping series of sections from case number GLB3 illustrates the position of several neurons that exhibited *only* a reduction in orientation tuning (solid squares) or *only* a shift in orientation selectivity (open circle) or both (open triangle). The neurons that exhibited changes in orientation tuning are located closer to the vertical column of infragranular neurons (i.e. directly toward the medial bank) than is the neuron that exhibited only a shift in orientation selectivity. Abbreviations are as in Fig. 3.

Fig. 6 plots the tangential location from the pipette tip of all 28 recorded neurons. This figure schematically represents the planar projection of a cylinder of cortex viewed from looking directly at the medial bank. The location of the pipette tip within the lower layers is represented by the intersection of the lines, and radial distances from this point are labelled. The average tangential distance from the pipette tip of the seven neurons (solid circles) that exhibited *only* a shift in their preferred stimulus orientation during layer blocking was 272 (± 120) μm. The six neurons (solid squares) that exhibited *only* reduced orientation tuning during layer blocking averaged 230 (± 115) μm tangential from the pipette tip. The five neurons (solid triangles) that exhibited a shift in preferred stimulus orientation *and* a reduced orientation tuning during layer blocking were located an average of 145 (± 75) μm tangential from the pipette tip. The two neurons ("X"s) which exhibited increased response amplitudes with no changes in orientation tuning or selectivity were located an average of 50 (± 15) μm from the pipette tip. Thus, the tangential distances from the pipette tip of the neurons that exhibited shifts in preferred stimulus orientation were not significantly greater than the distances of the neurons which did not show tuning shifts. However, the more important point to note is that *all* of the upper layer neurons affected by lower layer blocking were tangentially located within 500 μm of the inactivated tissue. Conversely, only two of the eight upper layer neurons unaffected during layer blocking (*asterisks*) were within 500 μm of the blocked tissue. In fact, the remaining six (*asterisks*) were located well outside of 500 μm (their distances are numerically indicated). This finding suggests that the orientation-dependent response characteristics of most supragranular neurons are affected by inputs from infragranular neurons located within a limited tangential distance.

Discussion

Blocking the lower layers of V1 in bush babies clearly alters the responses to stimulus orientation of many (71.4%) upper layer neurons. These upper layer cells exhibit either a change in their preferred stimulus orientation, a reduction in their orientation tuning, and/or a generalized increase in their response amplitude to stimuli near the peak excitatory orientation. Only cells located within 500 μm tangential to the vertical axis of the blocking pipette center exhibit any response changes dependent on stimulus orientation. This suggests that the orientation tuning of the blocked lower layer neurons could relate in specific ways to the recorded upper layer neurons. In the following discussion, we consider our results first within the context of the cortical circuitry of bush baby V1. Emphasis is placed on the patterns of innervation of the upper layers by lower layer cells. Next, we compare our findings with previous studies in cat area 17. The similarities between the neural mechanisms generating orientation tuning in area 17 of both bush babies and cats suggest a common role for ascending projections from lower layer neurons in sharpening the orientation tuning of upper layer neurons in mammalian visual cortex. Finally, we develop a functional model based on lateral inhibition that could account for the results observed during this investigation.

Cortical circuitry: anatomical support for physiological results

The current results demonstrate that lower layer cells influence the orientation tuning of upper layer cells within both the same and, more often, closely neighboring columns. This influence could be produced by either direct or indirect projections of lower layer neurons to the upper layers. We first consider how the effect on upper layer neurons depends on the specific blocking site (layer 5 or 6). Changes in response properties were found following blockade from pipettes located within the center of layer 5 (two cases), the border of layers 5 and 6 (one case), and within the center of layer 6 (one case). While it is difficult to specify the exact laminar location of the blocked infragranular neurons, the results from these different cases show a pattern that is consistent with known vertical projections in bush baby V1.

Fig. 7 schematically illustrates how the potential for direct influence on upper layer cells differs between neurons in layers 5 and 6 of bush baby V1 (Lachica et al., 1993; Fitzpatrick et al., 1985; Blasdel et al., 1985). For example, layer 6 neurons project most heavily to layer 4, but layer 5 neurons project most heavily to layer 3. In fact, few projections from layer 6 of bush baby V1 extend higher than layer 3C (Lachica et al., 1993). In contrast, layer 5 sends a significant input to layers 2 and 3A as well as to other subdivisions of layer 3 (Lachica et al., 1993). The current physiological results fit this anatomical innervation pattern. All cells affected during layer blocking with the pipette centered in layer 6 were located within layer 3C [4B of Brod-

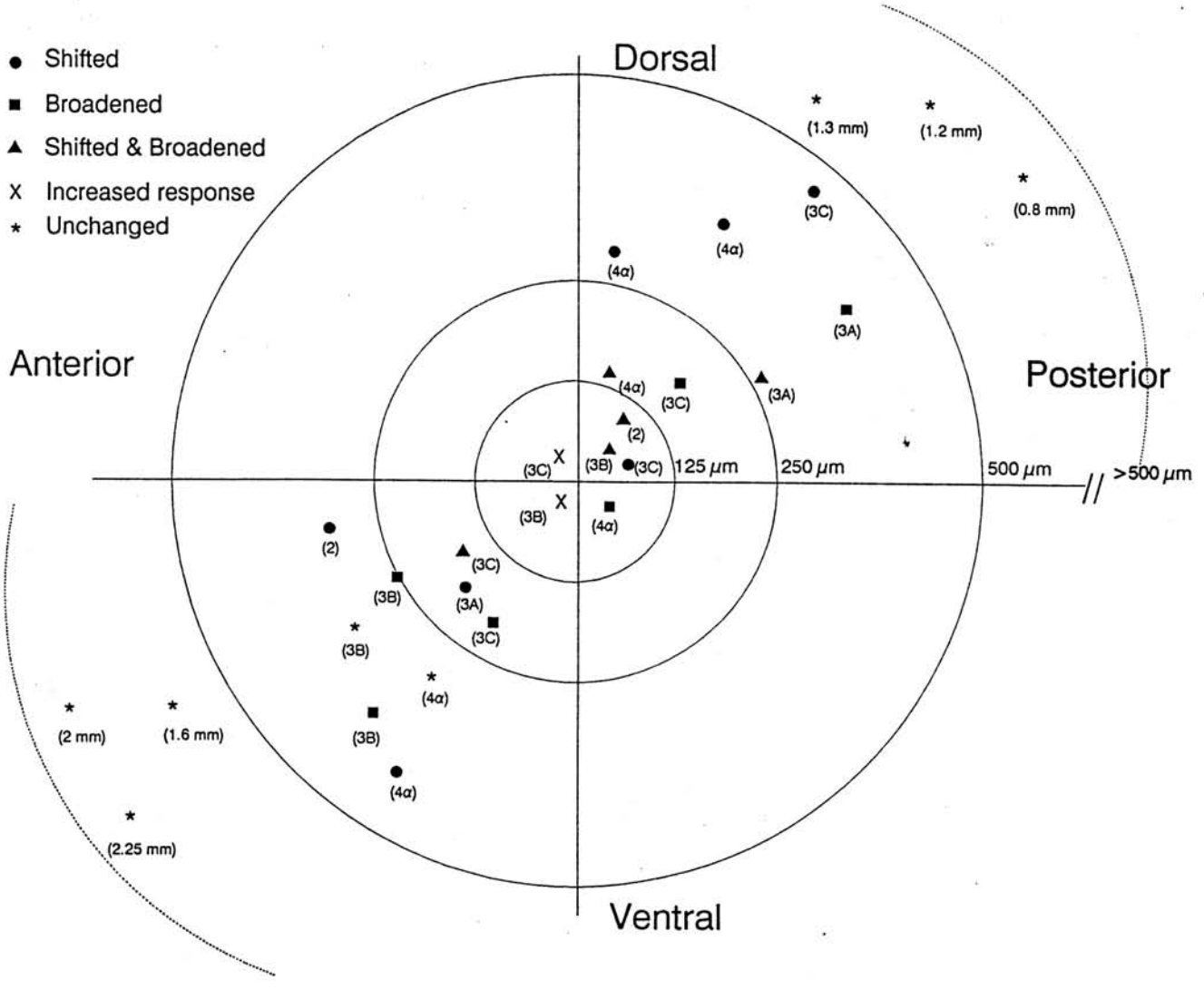

Fig. 6. This schematic illustration, which summarizes the anatomical data, represents a cylinder of area 17 viewed from the medial bank. The pipette is centered within the lower layers at the intersection of the vertical and horizontal lines. Dorsal, ventral, medial, and lateral directions are indicated and the rings illustrate radial distances of 125 μm, 250 μm, and 500 μm tangential to the vertical axis of the cell column in which the pipette is centered. The tangential locations of all neurons that exhibited *only* a shift in orientation tuning are indicated by solid circles ($n = 7$). Cells demonstrating *only* a reduction of orientation tuning (solid squares, $n = 6$) during layer blocking and those showing *both* effects (solid triangles, $n = 5$) are also included. The layers in which each cell was found are noted. The two neurons that exhibited an increase in response amplitude with no changes in orientation tuning are shown by "X"s. Asterisks indicate the positions (and tangential distances) of upper layer neurons unaffected by lower layer blocking. The clustering of data points within the upper right and lower left quadrants is an artifact which is due to the angle of penetrations by the recording electrode. Notably, all of the supragranular neurons affected by layer blocking were located within 500 μm of the pipette while the majority (75%) of unaffected cells were located outside this radius.

mann (1909)] or 4α. No cells that exhibited changes in response profiles following blockade with a pipette centered in layer 6 were located higher than layer 3C. One layer 3B cell was affected by layer blocking when the blocking pipette was located at the border of layers 5 and 6. The influence on this neuron could result from inactivation of neurons in either layer 5 or 6 since layer 3B does receive some input from both infragranular layers (Lachica et al., 1993), although it more likely results from blocking the lower portion of layer 5. In the two cases when the pipette was clearly centered in layer 5, a number of cells within layers 2, 3A, 3B, and 4 showed response changes during administration of GABA. Changes in response of cells within these layers is consistent with the innervation patterns described by Lachica et al. (1993).

Layers 5 and 6 of primate V1 contain a mixture of excitatory and GABAergic inhibitory neurons and both types (based upon morphology) project to the upper layers (Lund, 1988). The activity of both cell classes is likely to be reduced by GABA, but in our experiments the dominant effect of blocking the lower layers on the activity of upper layer neurons was an increase, as opposed to a decrease, in response amplitude. This suggests that lower layer blocking produces a disinhibitory effect on

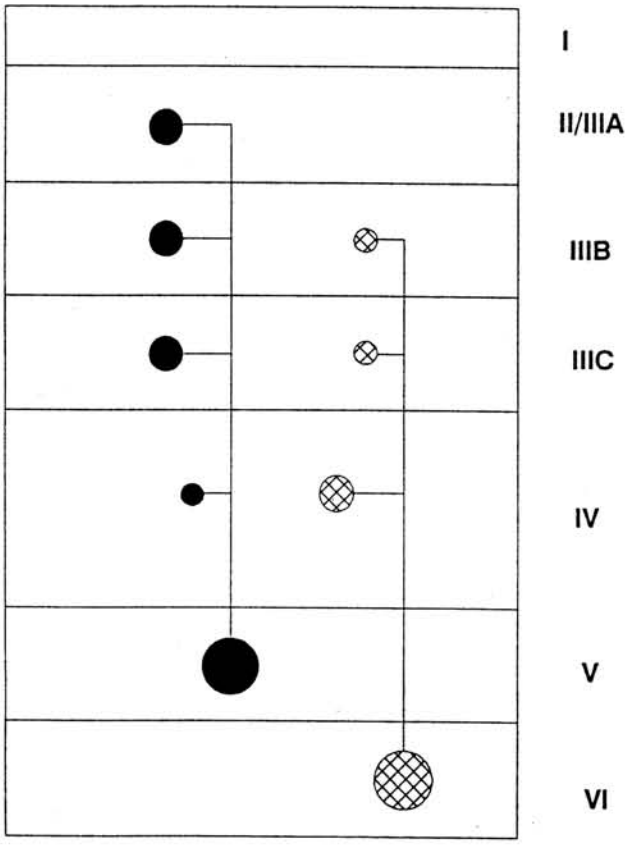

Fig. 7. A highly schematized diagram illustrates the relevant intralaminar projections from layers 5 and 6 to cortical layers 2, 3, and 4 in *Galago*. Layer 5 neurons (large solid circle) heavily innervate each subdivision of layer 3 (medium solid circle) and lightly innervate layer 4 (small solid circle). In contrast, layer 6 neurons (large hatched circle) project heavily to layer 4 (medium hatched circle), but provide a much smaller input to layers 3B and 3C (small hatched circle). Analysis of the laminar locations of recorded neurons confirmed that blocking layer 5 affected cells in all layers while blocking layer 6 affected cells in layers 3C and 4. Roman numerals indicate cortical layers according to Hässler's (1967) nomenclature. Hässler's layers IIIC and IV and the same as Brodmann's (1909) layers IVB and IVC.

upper layer cells *via* the inactivation of the lower layer inhibitory neurons. The marked absence of response reduction from the majority of recorded cells implies that either (1) excitatory influences from the lower layers are minimal or (2) the excitatory projections from the lower layers pass through local inhibitory interneurons within the upper layers. Although the major observed effect was disinhibitory, there was some minimal evidence of disfacilitatory effects. For example, the response amplitude of the cell illustrated in Fig. 2A exhibited a decrease at the baseline peak excitatory stimulus during layer blocking. Thus, there may be some direct excitatory influence from the lower layers on the responses of this cell. Furthermore, the changes in response amplitude noted in Fig. 2A suggest that the excitatory and inhibitory inputs may be spatially organized, since they are each restricted to a portion of the tuning curve.

Our results further suggest that the influence of lower layer inhibitory interneurons is restricted to upper layer neurons that are either directly above them or within a 0.5-mm radius from the center of the pipette. Projections to the upper layers from layer 6 are generally vertical although some can extend laterally up to 250 μm (Lachica et al., 1993; Blasdel et al., 1985). Projections from layer 5 diverge more broadly, although again the main projections are vertical (Lachica et al., 1993; Blasdel et al., 1985). In macaque monkeys, extensive intralaminar lateral projections that extend for more than 1.0 mm have been identified in both layers 5 and 6 (Blasdel et al., 1985). These extensive horizontal projections could account for the disinhibition observed from a few upper layer neurons located more than 250 μm away from the cell column in which the pipette was centered (see Fig. 6). We cannot rule out, however, that the distance over which the effects occurred is not due to the horizontal spread of GABA during iontophoresis.

Comparisons to studies in cats

Our results are consistent with previous studies in cats which reported that lower layer inactivation alters the orientation selectivity and tuning of upper layer neurons (Bolz & Gilbert, 1986; Allison & Bonds 1994). The effects reported here are similar in several ways to the effects found in cat area 17. First, in both cat and bush baby the primary effect observed during layer blocking was an increase in response amplitude to stimuli oriented near the baseline excitatory peak (within $\pm$30 deg for bush baby and $\pm$20 deg for cat; Allison & Bonds, 1994). Responses to stimuli oriented outside of 30 deg were unaffected in both species. However, blocking horizontal inputs in the supragranular layer of area 17 in cats does produce an increase in response amplitude to stimulus orientations greater than 30 deg (Eysel, 1992). One possible explanation for why the effect is seen to spread 10 deg further away from the excitatory peak in bush babies than in cats is that, due to the restrictions of recording from the medial bank in bush babies, these receptive fields were located in the peripheral visual field, rather than on or near *area centralis* as was the case in cats (Allison & Bonds, 1994). Cells with receptive fields in the periphery tend to be more broadly tuned and have larger receptive fields.

Second, a shift in orientation selectivity was also observed by Allison and Bonds (1994) during infragranular layer blocking in cat area 17. The shift is slightly larger, on average, in bush babies (11 deg) than found in cats (6 deg), again possibly reflecting differences due to eccentricity. Shifts in orientation tuning can also result from the use of compound stimuli. Gilbert and Wiesel (1990) found similar shifts when stimulating the receptive-field surround of cat cortical neurons with bars oriented 20–30 deg away from the peak excitatory stimulus. Similarly, Kabara et al. (1994) found that a second sine-wave grating superimposed on the receptive field center oriented 30 deg to 60 deg away from the excitatory peak shifts the orientation tuning curve away from the second grating by an average of about 10 deg. While a precise mechanism linking these stimulus-induced shifts with inactivation-induced shifts is not yet apparent, the nature and degree of the shifts suggests a causal relationship.

Model of excitation, intracortical inhibition, and orientation tuning

The existence of an intracortical inhibitory mechanism that sharpens the orientation selectivity of V1 neurons remains controversial. Arguments abound over the influences of geniculate excitation, intracortical excitation, and intracortical inhibition

on the orientation tuning of V1 neurons (e.g. Wörgötter & Koch, 1991). The recordings of postsynaptic potentials in V1 cells by Ferster (1986, 1987, 1988) and his colleagues (Ferster & Lindström, 1983; Ferster & Koch, 1987) support the original proposal of Hubel and Wiesel (1962) that much of the orientation selectivity displayed by cortical neurons can be explained by a purely excitatory model resulting from an aligned array of inputs from the LGN. More recent whole-cell recordings (Volgushev et al., 1993) and computer simulation models (Wörgötter & Koch, 1991), however, suggest that a variety of mechanisms can be involved, including excitation alone, inhibition alone, or a combination of both. A likely combination is an orientation-biased excitatory input from the LGN coupled with an orientation-tuned intracortical inhibitory mechanism.

Our results support the arguments that intracortical inhibition that originates from within the infragranular layers is a factor in sharpening the orientation tuning of neurons in the upper layers. The results are also consistent with the presence of a broadly tuned intracortical inhibitory mechanism centered on or near the excitatory tuning of a neuron. However, a possible alternative interpretation is that layer blocking merely inactivates a portion of a nonselective inhibitory mechanism (Jones & Palmer, 1987) that does not enhance a neuron's orientation tuning under normal conditions, but instead might serve to normalize the responses of the recorded neuron (Albrecht & Geisler, 1991; Heeger, 1992). While the present results cannot directly address this issue, they do show that however the inhibition is organized, it comes through discrete channels and some of these channels can be inactivated to change the shape of a recorded neuron's orientation tuning curve. If the inhibitory mechanism was completely nonselective, one would expect to get shifts in tuning beyond 30 deg from the excitatory peak, but we never observed this during our experiments. Since the inhibition is processed through discrete channels, it is unlikely that the inhibition is completely untuned and it cannot solely serve to normalize responses since its strength will be different for different orientations. Finally, while GABAergic inhibition is clearly involved in orientation-selective tuning (Sillito, 1975), it is not involved in gain control (DeBruyn & Bonds, 1986).

The present results are summarized by the conceptual diagram in Fig. 8. An upper layer neuron (center circle) tuned to a vertical stimulus (indicated by the line within the circle) receives excitatory input from the LGN that is broadly tuned to a vertical stimulus. Inputs from three lower layer neurons, one in the same orientation column with identical orientation tuning and two in closely neighboring columns which are tuned slightly off the vertical axis, provide inhibitory inputs onto the upper layer neuron. The envelope of the three inhibitory tuning curves (indicated by the dotted line) represents the broadly tuned inhibitory mechanism which is a product of the individual inhibitory tuning curves. This inhibitory envelope combines with the orientation-biased LGN input to produce the orientation tuning of the upper layer neuron. Inhibition from the lower layers does not necessarily have to be summed laterally to provide a broad inhibitory envelope, since in both cats (Berman et al., 1982, 1987) and bush babies (DeBruyn et al., 1993), cells in the lower layers exhibit broader orientation tuning bandwidths, on average, than do upper layer neurons. The present results as well as those of Gilbert and Wiesel (1990), Allison and Bonds (1994), and Kabara et al. (1994), however, support the existence of inhibitory contributions from nearby orientation columns. This model also produces an overall reduction in response amplitude,

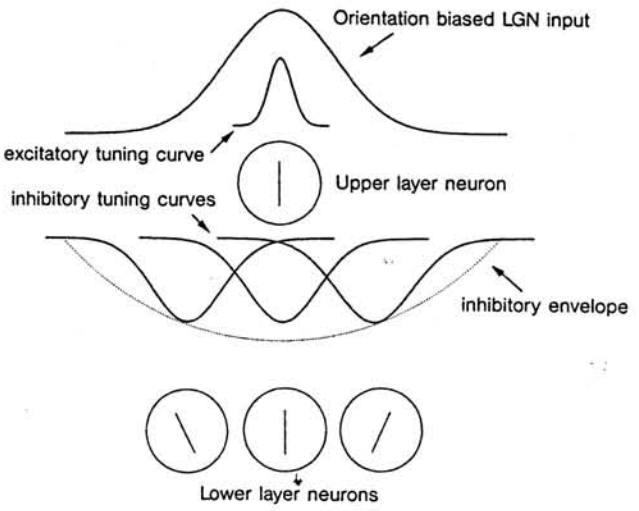

Fig. 8. A conceptual model, based on the current data, illustrates how inhibition originating from within the lower cortical layers might sharpen the orientation tuning of an upper layer neuron. An upper layer cell receives input from the LGN that is "biased" for a vertical stimulus (represented by the vertical line within the circle). Three lower layer neurons, one lying within the same orientation column and two within adjacent columns, provide inhibitory input to the upper layer neuron. The inhibitory envelope produced by the overlap in their orientation tuning curves impinges upon the orientation biased LGN input to generally suppress the responsiveness of the upper layer neuron as well as sharpen its orientation tuning. This lateral inhibition is analogous to the "center/surround" mechanism found within the retina, but is vertically organized.

including the responses to the optimal excitatory stimulus. This is consistent with the results obtained from the majority of neurons in this study which exhibited, during layer blocking, an increase in response amplitude to the optimal excitatory stimulus and stimuli oriented with ±30 deg of the peak stimulus. We suggest that lower layer neurons sharpen the orientation tuning of upper layer neurons by generating lateral inhibition in the orientation domain. This can be considered analogous to the enhancement of spatial selectivity in retinal receptive-field centers by interaction between the receptive-field center and surround. The mechanism of lateral inhibition that originates from within the vertical connections of cortex has now been seen in both cats and primates, which suggests that it is a general principle of mammalian visual cortical processing.

Acknowledgments

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