Inhibitory Contributions to Spatiotemporal Receptive-Field Structure and Direction Selectivity in Simple Cells of Cat Area 17

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Murthy, Aditya and Allen L. Humphrey. Inhibitory contributions to spatiotemporal receptive-field structure and direction selectivity in simple cells of cat area 17. J. Neurophysiol. 81: 1212–1224, 1999. Intracortical inhibition contributes to direction selectivity in primary visual cortex, but how it acts has been unclear. We investigated this problem in simple cells of cat area 17 by taking advantage of the link between spatiotemporal (S-T) receptive-field structure and direction selectivity. Most cells in layer 4 have S-T–oriented receptive fields in which gradients of response timing across the field confer a preferred direction of motion. Linear summation of responses across the receptive field, followed by a static nonlinear amplification, has been shown previously to account for directional tuning in layer 4. We tested the hypotheses that inhibition acts by altering S-T structure or the static nonlinearity or both. Drifting and counterphasing sine-wave gratings were used to measure direction selectivity and S-T structure, respectively, in 17 layer 4 simple cells before and during iontophoresis of bicuculline methiodide (BMI), a GABA_A antagonist. S-T orientation was quantified from fits to response temporal phase versus stimulus spatial phase data. Bicuculline reduced direction selectivity and S-T orientation in nearly all cells, and reductions in the two measures were well correlated (r = 0.81) and reversible. Using conventional linear predictions based on response phase and amplitude, we found that BMI-induced changes in S-T structure also accounted well for absolute changes in the amplitude and phase of responses to gratings drifting in the preferred and nonpreferred direction. For each cell we also calculated an exponent used to estimate the static nonlinearity. Bicuculline reduced the exponent in most cells, but the changes were not correlated with reductions in direction selectivity. We conclude that GABA_A-mediated inhibition influences directional tuning in layer 4 primarily by sculpting S-T receptive-field structure. The source of the inhibition is likely to be other simple cells with certain spatiotemporal relationships to their target. Despite reductions in the two measures, most receptive fields maintained some directional tuning and S-T orientation during BMI. This suggests that their excitatory inputs, arising from the lateral geniculate nucleus and within area 17, are sufficient to create some S-T orientation and that inhibition accentuates it. Finally, BMI also reduced direction selectivity in 8 of 10 simple cells tested in layer 6, but the reductions were not accompanied by systematic changes in S-T structure. This reflects the fact that S-T orientation, as revealed by our first-order measures of the receptive field, is weak there normally. Inhibition likely affects layer 6 cells via more complex, nonlinear interactions.

**INTRODUCTION**

The analysis of object motion in the visual world begins in primary visual cortex (area 17) through the action of direction-selective neurons (Hubel and Wiesel 1962). These cells respond well to motion in one direction across their receptive fields and weakly or not at all to motion in the opposite direction. The mechanisms underlying this selectivity remain unresolved. However, among simple cells, important insights have been gained through the study of spatiotemporal (S-T) receptive-field structure. Many direction-selective simple cells in cat area 17 have S-T–oriented receptive fields in which response timing changes gradually across the field (Albrecht and Geisler 1991; McLean and Palmer 1989; Movshon et al. 1978; Reid et al. 1991; Saul and Humphrey 1992a). This organization confers directional tuning: a stimulus moving in a direction that successively activates receptive-field positions with progressively shorter delays, or response phases, elicits a larger net excitatory response than a stimulus moving in the opposite direction. In contrast, nondirection-selective cells lack S-T–oriented receptive fields.

We recently showed (Humphrey and Saul 1998; Murthy et al. 1998) that S-T structure is well correlated with directional tuning in layer 4 of cat area 17. The degree to which cells are S-T oriented accounts for over half of their directional tuning on average. We also showed that a linear-nonlinear, or exponent, model (Albrecht and Geisler 1991; Heeger 1993) accounts well for directional tuning in most layer 4 cells. The model consists of two stages: a linear process in which S-T orientation confers a directional bias and a static nonlinear process that amplifies the bias to accentuate selectivity. The nonlinearity may be a threshold or, equivalently, an exponential amplification, either of which accentuates differences in response amplitude to optimal versus suboptimal stimuli. The exponent model, however, does not account for directional tuning in layer 6 because receptive fields there are weakly S-T oriented and unrealistically large static nonlinearities are required to account for their tuning (Murthy et al. 1998). Dynamic nonlinear interactions (Emerson and Citron 1992) likely predominate in layer 6.

Intracortical inhibition is important for direction selectivity, as evidenced by the fact that blocking GABA_A-mediated inhibition reduces selectivity in most simple cells (Sillito 1984). How inhibition acts is not clear, however. One hypothesis is that it creates or enhances S-T orientation. If so, then blocking inhibition should produce a reduction in S-T orientation that is correlated with a loss of directional tuning. An alternative, though not mutually exclusive, hypothesis is that inhibition is “flat,” merely suppressing weak responses (Sato et al. 1995). It might act by lowering membrane potentials relative to spike threshold. This iceberg effect should enhance an initial directional bias but not affect response timing. In terms of the exponent model, if this was the primary inhibition, then block-
ing it should reduce direction selectivity and change the value of the exponent but not alter S-T orientation.

To evaluate these two hypotheses, S-T structure and direction selectivity were assessed in simple cells by measuring responses to stationary counterphasing and drifting gratings, respectively. Bicuculline methiodide (BMI), a GABA_A antagonist, was applied iontophotically to reduce intracortical inhibition. We observed that BMI reduced direction selectivity in most cells. In layer 4, the effect was paralleled by a well-correlated reduction in S-T orientation. In layer 6, no systematic changes in S-T structure were seen. We also calculated for each layer 4 cell the value of an exponent that represents the static nonlinearity. Application of BMI reduced the exponent in most cells, but the reduction was not correlated with the changes in directional tuning. Thus inhibition affects direction selectivity in layer 4 primarily by enhancing S-T orientation and secondarily by accentuating the static nonlinearity. The measures used here do not allow us to discern how inhibition operates in layer 6.

**METHODS**

**Physiological preparation**

Details of surgical preparation are described elsewhere (Murthy et al. 1998; Saul and Humphrey 1990). Briefly, adult cats were anesthetized throughout the experiment using halothane in nitrous oxide and oxygen. A tracheostomy was performed, paralysis was induced using gallamine triethiodide and d-tubocurarine chloride, and the animal was ventilated artificially. Heart rate, mean arterial blood pressure, and the cortical electroencephalogram were monitored continuously to assess physiological state. The halothane level was adjusted to maintain the dominant frequencies of the electroencephalogram <4 Hz during all stages of the experiment. Lactated Ringer solution was infused intravenously to maintain hydration. The cornea were covered with contact lenses fitted with 3-mm artificial pupils.

**Recording, visual stimulation, and iontophoresis**

Extracellular recordings of single neurons were made using micropipettes filled with 0.6–2.0 M KCl (~35–20 MΩ). The recording electrode was glued to a three-barrel micropipette array, with its tip protruding from the array by ~20 μm (Hayve and Caspary 1980). The array tip was broken to 5–7 μm, yielding an inner diameter of ~1.5 μm per barrel. Each barrel contained one of the following solutions: bicuculline methiodide (BMI; 2.5 mM in 165 mM NaCl, pH = 3); gamma-amino butyric acid (GABA; 0.5 M, pH = 3); or sodium acetate (2.0 M) for current balancing, to which 4% Pontamine sky blue was added. Drug barrels were subject to constant retaining currents when not in use, using ~18 nA for GABA and ~10 to ~15 nA for BMI. Currents were controlled using a Neurophore iontophoresis unit.

Receptive fields initially were mapped manually on a tangent screen. All subsequent stimuli were presented monocularly at 57 cm on a Tektronix 608 monitor driven by a Picasso image synthesizer linked to an LSI-11/73 computer. Mean luminance was 15 cd/m² and Rayleigh-Michelson contrast was ~0.4. Simple cells were identified using standard criteria (Hubel and Wiesel 1962; Skottun et al. 1991).

Dristing sinewave gratings were used to determine each cell’s optimal stimulus orientation and spatial and temporal frequency (Humphrey and Saul 1998). A set of randomly interleaved counterphasing and drifting gratings then was presented before (control) and during iontophoresis of BMI and, when possible, after recovery from the drug (postcontrol). The counterphasong grating was presented at eight spatial phases over one-half cycle of the stimulus spatial fre-

**FIG. 1.** Example of a titration to assess the antagonistic effects of bicuculline methiodide (BMI) on exogenously applied GABA. Plot shows 1st harmonic amplitude in response to a grating drifting in the cell’s preferred direction. Horizontal bars mark the periods of drug application. Dashed line (left) marks the control response during the 1st 2 min. Iontophoresis of GABA suppressed the response within 5 min. Concurrent ejection of BMI antagonized the GABA effect and the control response returned. Cessation of BMI again led to GABA-induced suppression followed by a return to control-level responsiveness (recovery) when the GABA ejection current was turned off.

**Data analysis**

Action potentials were summed into peristimulus time histograms (PSTHs) to measure the average response per cycle of the periodic stimulus. First harmonic (±SE) amplitude and temporal phase were obtained for each PSTH, with response phase expressed in cycles relative to the stimulus (Saul and Humphrey 1990).

From responses to moving gratings we computed a directional index (DI) as $DI = (PD - NPD)/(PD + NPD)$, where PD and NPD are the response amplitudes in the preferred and nonpreferred directions of motion, respectively. Ratios of 0 and 1, respectively, reflect no and complete direction selectivity. An estimate of the standard error of DI was obtained from separate DI measurements on individual sets of trials. Only cells with DIs >0.33 were considered selective and subsequently tested with BMI.

Responses to counterphasing gratings were used to characterize S-T receptive-field structure. We relied primarily on a recently developed method described in detail by Murthy et al. (1998). Because the present results require understanding the method and its rationale, we summarize it here. In a strictly linear model of directional tuning, a stationary, counterphasing sinewave grating elicits predictable pat-
functions were shifted equally horizontally so that amplitude peaked in the preferred direction of motion. Second, the response amplitude and phase increase with increasing spatial phase, thereby normalizing for pre-existent direction of motion also were used to derive a predicted DI. From the four quadrature pairs, predicted amplitudes to each direction were expressed as a vector in polar coordinates. Temporal quadrature was simulated by translating the response phase of one grating in each directionally nonselective cell, response phase is constant (i.e., slope = 0) within each half of the grating cycle. However, amplitude varies sinusoidally with spatial phase and the AM ratio is 1. For a cell with intermediate directional tuning, amplitude also fluctuates sinusoidally and the modulation ratio lies between 0 and 1, and the response phase versus spatial phase data do not follow a straight line but are fit by an arctangent function (e.g., Fig. 4A). We used this fit to derive a spatiotemporal index (STI) for each cell that reflects the slope of the function at the spatial phase generating the maximum response. The STI is a metric that summarizes the S-T orientation of the receptive field. STI is 1 and 0, respectively, for receptive fields that are completely S-T oriented or unoriented. In a strictly linear system, S-T orientation determines directional tuning; thus STI and DI values are equal (see Murthy et al. 1998 for derivation of the relationship).

In a linear system, S-T orientation and AM to counterphased gratings are related inversely. Hence either or both measures potentially predict direction selectivity. However, cells are subject to non-linearities that, in the context of direction selectivity, have been modeled as static nonlinearities (Albrecht and Geisler 1991; Heeger 1993). They accentuate differences in amplitude to optimal versus nonoptimal stimuli and hence increase AM ratios beyond those due to linear summation. Thus conventional linear predictions of direction selectivity that use response amplitude (e.g., Reid et al. 1991) underestimate the linear contribution because of nonlinear amplitude distortion. In contrast, response phase is not affected by static nonlinearities, so the phase-based measure, STI, provides a better estimate of the linear contribution. We used the STI to quantify changes in the temporal organization of the receptive field induced during blockade of GABA_A-mediated inhibition and to estimate their linear contribution to changes in direction selectivity. As described in the RESULTS, we also employed the STI to evaluate the contribution of static nonlinear processes to directional tuning.

In addition to the STI, we used phase and amplitude measures to make conventional linear predictions of directional tuning for comparison with our STI-based measures and to examine relationships between S-T structure and direction selectivity that require information about amplitude. We used a superposition method similar to that of Jagadeesh et al. (1997). It derives from the fact that the sum of two counterphasings gratings in spatial and temporal quadrature constitutes a drifting grating. Assuming linearity, the responses to the counterphasings gratings equal those to the drifting grating. We identified pairs of gratings in spatial quadrature from the eight spatial phases tested. First harmonic response amplitude and phase at each spatial phase were expressed as a vector in polar coordinates. Temporal quadrature was simulated by translating the response phase of one grating in each pair by a quarter cycle. The paired responses were summed vectorially to give predicted responses to a drifting gratings in each of two directions. A mean predicted amplitude and phase was calculated from the four quadrature pairs. Predicted amplitudes to each direction of motion also were used to derive a predicted DI.

For ease in viewing, the counterphase data from control trials were normalized in three ways. First, response phase was plotted so as to increase with increasing spatial phase, thereby normalizing for preferred direction of motion. Second, the response amplitude and phase functions were shifted equally horizontally so that amplitude peaked near 0.25 and 0.75 cycles. Third, the phase functions were shifted vertically to pass through the origin. The BMI and postcontrol data were shifted similarly to maintain spatial phase correspondence in the different conditions.

Reconstructing recording sites

Electrode penetrations were marked by extracellular deposits of Pontamine dye. Animals were administered a lethal dose of Nembutal and perfused with aldehydes. Brain sections were stained for Nissl substance, electrode tracks were reconstructed (Murthy et al. 1998), and cells’ recording locations were assigned using the laminar criteria of O’Leary (1941; Humphrey et al. 1985).

Statistics

All comparisons of means were made using a paired t-test (Miller and Freund 1985). Pearson product-moment (r) or Spearman rank (r_s) correlations were used for other comparisons.

RESULTS

Results are based on 27 simple cells from 17 cats; 17 cells were in layer 4 and 10 were in layer 6. We first describe changes in direction selectivity and S-T receptive-field structure produced by GABA_A blockade in three representative cells. We next summarize how the blockade affected the population and show that effects on S-T structure differed between layers 4 and 6. We then show that inhibition contributes to direction selectivity in layer 4 mainly by increasing S-T orientation.

Effect of BMI on direction selectivity and S-T structure in individual cells

Figure 2A shows average responses of a layer 4 cell to one cycle of a sinewave grating drifting at 2 Hz during control, BMI, and postcontrol conditions. During control trials, the cell was highly direction selective (DI = 0.92), discharging vigorously in the preferred direction of motion (Fig. 2A, bottom) and weakly in the nonpreferred direction (top). Within 4 min of iontophoresing BMI, selectivity was abolished (DI = 0). To facilitate comparison, control responses (* * *) are superimposed on the BMI data. Interestingly, the loss of direction selectivity in this cell reflected both an increase in response amplitude to the nonpreferred direction and a decrease in amplitude to the preferred direction. Additionally, there were shifts in response timing that were most visible in the preferred direction: the response during BMI was delayed by about a quarter cycle relative to the control response. The drug effect was reversible as evident in the postcontrol trials taken within 3 min of terminating BMI. We show later that BMI-induced changes in amplitude and timing to moving gratings reflect changes in the amplitude and timing structure of the receptive field.

The cell’s S-T structure during the three conditions is shown in Fig. 3. The PSTHs illustrate responses to a 2-Hz counterphasings gratings presented at different spatial phases in the receptive field. During control trials (Fig. 3A), the receptive field displayed clear S-T orientation, as evidenced by a gradual shift in response timing with increasing spatial phase. To further illustrate this, mean phase values are plotted against spatial phase in Fig. 4A. An arctangent function fit to the

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1 Response phases also were normalized to compensate for the difference between the spatial phases of the quadrature pairs. Taking spatial phases of 0 and 0.25 as the first or reference pair, we subtracted 0.0625 cycles from the second quadrature pair to simulate spatial alignment. Similarly, 0.125 and 0.1875 cycles were subtracted from the third and fourth pairs, respectively.
response phase data yielded an S-T orientation index (STI) of 0.62.

Figure 3B illustrates the changes in S-T structure during iontophoresis of BMI. Control responses are shown superimposed on the corresponding BMI profiles. Each pair of PSTHs is normalized to equate maximum firing rates to better illustrate the relative changes in timing induced by the drug. Reduction of inhibition produced clear timing changes at all spatial phases; responses were elicited later than in control trials. These shifts are defined as phase lags. In Fig. 4C the lags are plotted as increases in response phase, and all were statistically significant \((P < 0.05)\).

Figures 3B and 4C also reveal that the BMI-associated response did not lag the control discharge uniformly across spatial phase. Phase lags were greatest at zero spatial phase and progressively less up to \(\approx 0.44\) cycles. Because of symmetry \((\text{Movshon et al. 1978; Reid et al. 1991})\), the same timings were duplicated in the second half cycle. The changes resulted in much more uniform timing within each half of the grating cycle, and the receptive field became essentially S-T unori-
ent (STI = 0.16). The loss of S-T orientation would be expected in a linear model of directional tuning.

In such a model, amplitude profiles vary systematically with direction selectivity (see methods) (Murthy et al. 1998). For a cell with a DI of 1.0, amplitude should be constant (i.e., unmodulated) as the position of a counterphased grating changes. As DI decreases, the degree of modulation should increase. Figure 4D shows that BMI altered the AM ratio, increasing it to 0.90 from a control value of 0.67. Overall, then, the changes in phase and amplitude during GABA_A blockade are consistent with a linear spatiotemporal model of direction selectivity. This was supported by the conventional linear predictions using the superposition method: BMI reduced predicted DI from 0.47 to 0.08.

After cessation of the GABA_A antagonist, the control pattern of response timings was reinstated (Figs. 3C and 4E). S-T orientation was again clearly discernable (STI = 0.70), and the AM ratio decreased to 0.75, its approximate control value. The conventional linear prediction of direction selectivity (0.40) also returned to a near-control value. The aforementioned cell was one of the most striking examples of the effects of reducing inhibition. Another simple cell in layer 4, the direction selectivity of which was reduced but not abolished by BMI, is shown in Fig. 2B. This result was the more typical one. The cell was highly direction selective (DI = 0.93) during control trials. Within 2 min of applying BMI, responses to both directions of motion increased by about the same amount. However, the relative increase in response to the nonpreferred direction was greater, resulting in a 53% reduction in DI. The effect of BMI was reversible, as seen in the postcontrol data. Unlike the previous cell, these changes were not accompanied by any significant shift in response phase to the drifting grating.

Figure 5, B and C, shows that the BMI-induced changes in the cell’s direction selectivity reflected changes in S-T orientation: STI decreased from 0.51 to 0.18. Unlike the previous cell, timing changes were associated with phase leads not lags, and they did not occur at all positions. Significant shifts occurred only between spatial phases of 0.25 and 0.38 cycles (and 0.75–0.88 cycles). However, as before, the phase shifts resulted in more uniform timing across the receptive field.

The decrease in this cell’s direction selectivity also was accompanied by increases in response amplitude to the counterphasing grating (Fig. 5D), although the AM ratio changed little, from 0.89 to 0.81. The superposition analysis predicted a reduction in DI from 0.31 to 0.19. After cessation of the GABA_A block, timings, S-T orientation and amplitudes returned to approximate control values (not illustrated).

Figure 2C illustrates the effect of reducing inhibition on a simple cell in layer 6. Like the previous example, the response to each direction of motion increased during BMI but the relative change in the nonpreferred direction was greater, reducing DI from 0.97 to 0.65. Strong directional tuning returned in the postcontrol trials. Figure 6 illustrates the cell’s S-T structure. Unlike the layer 4 cells, this receptive field lacked prominent S-T orientation during control trials (STI = 0.14; Fig. 6, A and C). BMI induced slight phase leads at some positions but the timing shifts did not significantly change S-T orientation (Fig. 6, B and C). This is not surprising given the initially low S-T orientation. The reduction in DI was accompanied by an increase in the AM ratio, from about 0.67 to 0.81 (Fig. 6D). The superposition analysis predicted a reduction in DI from 0.37 to 0.17. Thus for this and two other layer 6 cells (not illustrated), changes in S-T structure correctly predicted the reduction in direction selectivity. However, unlike layer 4 cells, the changes largely reflected alterations in the AM ratio rather than in S-T orientation. Further, in other layer 6 cells (see following text) the minor changes in S-T structure induced by GABA_A blockade predicted an increase in DI but a decrease was seen. Overall, changes in S-T structure accounted poorly for changes in directional tuning in this and other layer 6 cells.

Population results

EFFECT OF BMI ON DIRECTION SELECTIVITY. Figure 7 plots the DI under control versus BMI conditions for each cell. Most (89%) cells lie significantly below the line of unity slope, indicating that the drug reduced their direction selectivity. However, the strength of the effect varied widely, from slight

FIG. 4. A and B: response phase and amplitude plotted as a function of stimulus spatial phase for the control responses in Fig. 3A. Response phase increased monotonically with spatial phase. An arctangent fit to the phase data (— in A) yielded a spatio-temporal index (STI) of 0.62. C and D: responses during iontophoresis of BMI (•); control responses (●) are shown for comparison. Response phase at all positions was significantly delayed by BMI, compared with controls, and the STI was reduced to 0.16. Like response phase, amplitude changed systematically, and the amplitude ratio increased from 0.67 to 0.90. E and F: during the postcontrol run, S-T orientation and response amplitude and its modulation returned to approximate control values. All error bars in this and the following figures indicate ±1 SE.
reductions to complete loss. For 37% of the cells, DI was reduced to 0.33, our criterion for selectivity, but most of these cells maintained a directional bias. Interestingly, two cells reversed their preferred direction: one was patently selective and the other was biased for direction.

There was no laminar difference in the effect of BMI on direction selectivity. Mean control DI was 0.80 ± 0.05 both for layers 4 and 6. During BMI application the absolute value of each mean dropped to 0.41 ± 0.06 and 0.51 ± 0.06, respectively. Both values were significantly lower than normal (P < 0.05) but not different from each other (P > 0.1).

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**FIG. 5.** Effect of BMI on responses of the cell in Fig. 2B to a grating counterphasing at 4 Hz. Conventions are as in Figs. 3 and 4. A: receptive field was moderately S-T oriented during control trials. B: BMI produced a leftward shift (i.e., phase lead) in response timing at spatial phases 0.25–0.38 cycles (and 0.75–0.88 cycles). C: response phase vs. spatial phase for control and BMI conditions. BMI application reduced S-T orientation. D: response amplitude versus spatial phase for the 2 conditions shows that BMI increased amplitude.

**FIG. 6.** Effect of BMI on responses of the layer 6 cell in Fig. 2C to a grating counterphasing at 3 Hz. A: this direction-selective receptive field was weakly S-T oriented in control trials. B: BMI produced slight phase leads at most spatial phases, relative to control responses. C: response phase vs. spatial phase data reveal no significant change in S-T orientation induced by BMI. D: BMI increased response amplitude at most spatial phases. Conventions are as in Figs. 3 and 4.
EFFECT OF BMI ON SPATIOTEMPORAL STRUCTURE. Clear differences between layers 4 and 6 were observed in the action of BMI on S-T structure. Figure 8A plots the S-T orientation of each cell during control and BMI conditions. During control trials, layer 4 cells displayed a wide range of STI values, from 0.16 to 0.83 (mean $= 0.41 \pm 0.04$). During blockade of inhibition, STI was significantly reduced in 12 of the 16 cells (mean STI $= 0.17 \pm 0.03$; $P < 0.05$). Similar to the effect on DI, however, the change in STI varied among cells; a few became completely S-T unoriented but most continued to display an obvious spatiotemporal bias.

As noted above, direction-selective cells in layer 6 normally exhibit little or no S-T orientation. In the present sample, control STIs ranged from 0 to 0.22 (mean $= 0.09 \pm 0.02$). Reducing GABA_A-mediated inhibition did not systematically affect STI in this layer (mean STI $= 0.13 \pm 0.03$). It was unaltered in three cells, increased slightly in four cells, and reversed slightly in one cell.

Figure 8B summarizes the effect of BMI on predicted direction selectivity, obtained using the superposition method. Although inclusion of amplitude in these conventional predictions changed the distributions relative to Fig. 8A, similar trends were observed. In layer 4, BMI caused changes in S-T structure in most (81%) cells that predicted a reduction in DI, from $0.33 \pm 0.04$ to $0.21 \pm 0.04$ ($P < 0.05$), on average. In layer 6, predicted DI was not consistently affected (control mean $= 0.14 \pm 0.04$; BMI mean $= 0.18 \pm 0.04$). Because the action of BMI on S-T structure was clearest in layer 4, we focus on this layer for the rest of the RESULTS.

JOINT EFFECT OF BMI ON S-T STRUCTURE AND DIRECTION SELECTIVITY IN LAYER 4. Figure 9 shows that the changes in direction selectivity during blockade of GABA_A-mediated inhibition largely were accounted for by the alterations in S-T receptive-field structure. Figure 9A plots the percent change in STI versus percent change in DI induced by BMI. Most cells lie in the third quadrant, confirming that a reduced DI was almost always accompanied by a lowered STI. For these cells, reductions in the two measures were well correlated ($r = 0.81$).

A similar analysis comparing conventional linear predictions against actual direction selectivity is shown in Fig. 9B. The change in DI was correlated moderately with the change in predicted DI, although the relationship was more variable ($r = 0.67$) than between DI and STI.

We previously showed that the S-T structure of most layer 4 receptive fields accounts for a substantial fraction of their directional tuning (Murthy et al. 1998), although linear predictions nearly always underestimate actual tuning (Albrecht and Geisler 1991; DeAngelis et al. 1993b; Reid et al. 1991). Here we asked whether S-T structure accounted for a similar fraction of direction selectivity in control and BMI conditions despite the drug-induced changes. Figure 9C plots the proportion of DI attributable to STI for each cell in the two conditions. Values for 9 of the 16 cells fell on or near the line of unity slope, indicating that S-T orientation contributed to a similar proportion of directional tuning in both conditions. For four additional cells (with ordinate values of 0) STI accounted for a moderate to high proportion of DI in control trials. During BMI, their STIs dropped to near zero (mean $= 0.04$), as did most of their DIs (mean $= 0.16$), again revealing a strong dependence of directional
tuning on S-T orientation. For the three other cells, the fraction differed significantly in the two conditions. A similar analysis, done using conventional linear predictions, is shown in Fig. 9. Again, the fraction of selectivity accounted for by the predictions was roughly similar in the two conditions for about three-fourths of the cells.

Taken together, these results indicate that GABA_A-mediated inhibition affects directional tuning in layer 4 largely through changes in spatiotemporal receptive-field structure, particularly by increasing S-T orientation.

**EFFECT OF BMI ON RESPONSE AMPLITUDE TO DRIFTING GRATINGS.** Because the BMI effects on direction selectivity in layer 4 largely could be accounted for by changes in S-T structure, we wondered whether alterations in response amplitude to drifting gratings could be predicted similarly from the counterphase data. Using the superposition method, we computed a predicted response amplitude for each direction of motion in the control and BMI conditions. Subtraction of the BMI predicted amplitudes from control predicted amplitudes yielded the predicted amplitude change for each direction due to the reduced inhibition. Likewise, the measured amplitudes to drifting gratings in the two conditions were subtracted to yield the observed amplitude change due to BMI.

Figure 10, A and B, plots the predicted versus observed amplitude change for the preferred and nonpreferred directions of motion, respectively. For most cells, BMI increased response amplitudes to drifting gratings for each direction. For both directions, most points in the sample fell on or near the line of unity slope, indicating that the amplitude changes were well accounted for by the predictions. Interestingly, BMI caused a decrease in amplitude in three cells (Figs. 2A and 10A), which were predicted by the changes in S-T structure. In general, BMI had a similar effect on response amplitudes in individual cells to drifting and counterphasing gratings, increasing amplitudes to both stimuli in most cells, and decreasing it to both in the three cells. The stronger responses during BMI were expected given its action in reducing inhibition. The
weaker responses in a few cells were surprising, and may reflect the action of BMI on complex neural networks (e.g., disinhibition of inhibitory neurons feeding back on the cell being studied).

**EFFECT OF BMI ON TIMING OF RESPONSE TO DRIFTING GRATINGS.** The reduction in direction selectivity often was accompanied by a shift in the phase of response to the drifting grating (e.g., Fig. 2A). To assess whether these timing shifts also could be explained by changes in S-T structure, we performed the superposition analysis as above but focused on response phase. Figure 10, C and D, plots the change in predicted versus observed phase for the preferred and nonpreferred direction, respectively. For most layer 4 cells, BMI induced a response phase lag to drifting gratings; other cells underwent a slight phase lead. Importantly, most of these shifts were well predicted by the concomitant changes in S-T structure.

Although the BMI-induced changes in timings and amplitudes across the receptive field underlie the shifts in timing to moving stimuli, the causal relationships are not obvious from simple inspection of the static plots. Clearly, the response to a drifting grating reflects the convolution of the stimulus profile with the amplitude and temporal structure of the receptive field. For layer 4 simple cells, the superposition method captures essential aspects of these S-T interactions, revealing causal relationships between changes in S-T structure and changes to moving gratings.

**DOES BMI ALSO AFFECT DIRECTION SELECTIVITY IN LAYER 4 VIA CHANGES IN THE STATIC NONLINEARITY?** Although S-T structure correlated with DI in control and BMI conditions, linear predictions underestimated DI in most cells, indicating that nonlinear processes also operate in both conditions. In layer 4 these processes can be modeled as a static nonlinearity—an exponent—that follows linear summation (Albrecht and Geisler 1991; Heeger 1993; Murthy et al. 1998). Here we asked whether reduction of inhibition altered the exponent and, if so, whether the change contributed systematically to decreased directional tuning.

The nonlinearity was evaluated using a procedure developed by Murthy et al. (1998). To summarize, the exponent can be estimated using the amplitude and STI data. One compares the modulation in amplitude to a counterphased grating with the modulation expected from the STI value. As described in METHODS, the STI fully predicts the AM ratio and the two measures are inversely related if summation is strictly linear. If, however, a static nonlinearity follows the linear stage, then the modulation predicted by the STI will underestimate the actual modulation. This is because the nonlinearity accentuates differences in amplitude to the null and optimal spatial phases of the grating, increasing modulation. The nonlinearity can be estimated by calculating an exponent, $n_{CG}$, which is required to raise the STI-predicted AM ratio to that observed. We previously showed (Murthy et al. 1998) that $n_{CG}$ approximates another exponent, $n_{DG}$, that is required to precisely match linearly predicted and actual direction selectivity to drifting gratings. Thus $n_{CG}$ is a reasonable estimate of the static nonlinearity. Here we compared $n_{CG}$ in control and BMI conditions for each layer 4 cell.

Figure 11A shows that BMI application reduced the value of $n_{CG}$ in most cells; geometric means were 1.7 and 1.1 in control and BMI conditions, respectively. The reduction reflected the fact that although BMI increased the AM ratio in most cells, the decrease in STI was disproportionately greater so that the STI-predicted modulation more closely approximated that observed during BMI. Figure 11B plots the percent change in $n_{CG}$ versus DI for these cells. Although both measures were reduced by BMI in most cells, the changes were not correlated. We note that expressing change in $n_{CG}$ in terms of percentage is not ideal, given its exponential nature. As alternatives, we analyzed change in terms of absolute reduction in $n_{CG}$ and DI and by normalizing for concomitant changes in STI. Neither procedure improved the correlation. We conclude that although reducing GABA receptors reduced inhibition altered both S-T structure and the static nonlinearity, the structural change was the more sensitive predictor of the reduction in direction selectivity.

**DISCUSSION**

Numerous studies have shown that iontophoretic application of bicuculline reduces direction selectivity (Sato et al. 1995; Sillito 1975, 1977; Sillito et al. 1980; Tsumoto et al. 1979; Wollman and Palmer 1993), thus demonstrating an important role for intracortical inhibition in generating this perceptually important (Pasternak et al. 1985) response property. However, translating these results into an understanding of how the inhibition operates has proved to be more difficult. Our study...
reveals that, at least for layer 4 simple cells, inhibition sculpts the spatiotemporal structure of the receptive field, accentuating S-T orientation so as to produce greater directional tuning. In terms of the linear-static nonlinear exponent model, inhibition appears to operate primarily at the linear stage of spatiotemporal summation.

Here we discuss the results in light of the spatiotemporal model. We then consider the heterogeneity in the effect of BMI on direction selectivity and S-T structure, and its implications for excitatory mechanisms. Finally, we illustrate a connectional scheme that accounts for our observations. We focus on layer 4 because BMI effects there can be interpreted in the context of the exponent model, although we briefly consider the layer 6 results.

Understanding BMI effects in the context of the LN model

Two aspects of our results clearly show that inhibition contributes to direction selectivity by altering S-T structure. First, BMI-induced reductions in selectivity nearly always were accompanied by reductions in S-T orientation, and changes in the two parameters were well correlated. Second, conventional linear predictions, based on response timing and amplitude, accounted well for absolute changes in the amplitude and phase (Fig. 10) of responses to drifting gratings. The success of these predictions confirms other evidence (Albrecht and Geisler 1991; DeAngelis et al. 1993a,b; Humphrey and Saul 1998; Jagadeesh et al. 1997; McLean et al. 1994; Reid et al. 1991) for linear spatiotemporal summation as a critical mechanism of directional tuning in single cells. Also, it extends those population studies by showing how S-T structure and direction selectivity covary in single cells.

Although changes in structure account for much of the reduction in DI, the STI and conventional linear predictions underestimate directional tuning in control and BMI conditions (Fig. 9, C and D). This is expected because, in both conditions, nonlinearities exist that amplify directional biases produced by linear spatiotemporal summation. Although this helps to explain discrepancies between predicted and observed DI, we still must ask why the absolute changes in amplitude to drifting gratings were well predicted by the linear model (Fig. 10, A and B) given the static nonlinearity. The answer may lie in the fact that, for most cells, BMI reduced $n_{CG}$, our measure of the nonlinearity. Thus any change in amplitude to drifting gratings should have been well predicted by the linear model. In contrast to amplitude, the excellent predictions of response phase changes to drifting gratings are expected because response phase is not influenced by static nonlinearities.

Interpreting the weaker static nonlinearity ($n_{CG}$) during BMI application requires knowing what the nonlinearity reflects biologically. We have modeled it as an exponent but it may reflect a spike threshold or threshold plus amplification. In practice, exponents and thresholds produce similar effects: the accentuation of differences in cell discharge rates to optimal versus nonoptimal stimuli (Albrecht and Geisler 1991; Tolhurst and Heeger 1997). Carandini and Ferster (1998) recently provided support for threshold and amplification as processes underlying the static nonlinearity in simple cells. They measured the modulation of membrane potentials and discharge rates to drifting gratings. Directional tuning of the spikes was always greater than that of the potentials (Jagadeesh et al. 1997). The firing rates could be accounted for by applying a simple threshold to the membrane potentials followed by a linear gain.

These results suggest that the reduction in $n_{CG}$ may be linked to changes in membrane potential relative to spike threshold. Given that inhibition acts to keep a cell’s membrane potential below threshold (Berman et al. 1992; Ferster and Jagadeesh 1992), reducing inhibition by BMI should increase the proportion of time the potential rises above threshold. This will lead to increased firing rates in the preferred and nonpreferred directions, a change that we observed in most cells (Fig. 10). However, because the increase in the nonpreferred direction is proportionately greater, due to its smaller control response, direction selectivity will decrease. The BMI-induced reduction in $n_{CG}$ thus may reflect an increase in membrane potential relative to spike threshold.

Whereas the reductions in $n_{CG}$ help to account for heightened amplitudes and decreased direction selectivity, the reductions were not correlated with changes in DI, unlike the changes in S-T orientation. This indicates that inhibition affects direction selectivity primarily by accentuating S-T orientation. Inhibition secondarily affects the static nonlinearity, probably by lowering membrane potentials relative to spike threshold so as to suppress responses in the nonpreferred direction (Movshon et al. 1978). An additional possibility, which our data cannot address, is that inhibition also alters the gain of feedforward and recurrent excitation (Suarez et al. 1995).

The ability to dissociate the influence of BMI on linear versus static nonlinear mechanisms rests on the assumption that the nonlinearity does not affect response phase and hence does not alter S-T orientation. This assumption is reasonable given our measure of timing: fundamental response phase. For example, membrane potential fluctuations in response to sine-wave gratings are not always sinusoidal (cf. Figs. 7 and 8 in Jagadeesh et al. 1997). For a waveform that deviates from a sinusoid, simple DC shifts in the waveform relative to spike threshold might alter the resulting discharge profile and the timing of some spikes. However, the phase of the fundamental response would be affected little, as would be the S-T orientation. Only temporal shifts of the whole profile would significantly alter fundamental phase. Additionally, if a static nonlinearity did affect response phase and S-T orientation, then BMI-induced reductions in STI should correlate with the reductions in $n_{CG}$, but they did not ($r_s = -0.12$). Thus it is unlikely that changes in the static nonlinearity contributed to the reductions in S-T orientation. Those reductions likely reflect changes in spatiotemporal interactions among cells’ inputs.

Heterogeneity in the effect of BMI on direction selectivity

The action of BMI on direction selectivity varied widely among cells, from small reductions to complete loss. These results conflict with those of Sillito et al. (1980), who reported that BMI eliminated direction selectivity in all simple cells studied. However, our results are in general agreement with those of Tsumoto et al. (1979), Wollman and Palmer (1993), and Sato et al. (1995), who also reported a wide range of BMI effects on direction selectivity. Numerous observations indicate that the heterogeneity here was not due to methodological...
activity in many simple cells varies with temporal frequency in a
fashion similar to those in the LGN (Saul and Humphrey 1992a). Also,
direction selective cells in layer 4 are observed readily in simple-cell
receptive fields. The unique timing signatures of the two af-
fected groups are observed in the same cell relatively independent of
the effect on direction selectivity, indicating that it effectively
reduced some level of inhibition. Fourth, the strengths of BMI
ejection currents were often less (~30 vs. >50 nA) for cells showing
the direction selectivity of which was abolished than for cells
produced by inputs from other simple cells having certain
spatiotemporal relationships. Simulated responses to a count-
erphasing grating are shown for an excitatory (A) and inhibi-
tory (B) simple cell and their target (C). The tangent fit to the respon-
s phase versus spatial phase data is
0.64) and hence stronger directional tuning. An arc-
tangent fit to the response phase versus spatial phase data is
shown in Fig. 12A (●). Removing the inhibitory input to cell C
would expose the excitatory structure, resulting in systematic
shifts in the cell’s response phase and a reduction in S-T
orientation (○), similar to that observed experimentally (e.g.,
Fig. 5C). Note that receptive fields receiving direct LGN input
may be more or less S-T oriented than shown here, depending
on the range of response timings among the inputs.

The geniculate inputs likely contribute to S-T structure
and directional tuning in layer 4 both by their direct
connections to simple cells (Bullier and Henry 1979; Ferster
and Lindstrom 1983; Martin and Whitteridge 1984) and by
indirect connections via other cortical cells, some of which
are inhibitory. In this regard our results and those of others
(Sillito 1984) appear to conflict with the conclusion of
Ferster et al. (1996) that, at least at the membrane potential
level, inhibition is not necessary to produce directional
tuning. However, the discrepancy may be less than it ap-
ppears. The average DI in our layer 4 cells during BMI
ejection was ~0.4. This is on the high end of the DI values
measured from membrane potential fluctuations (Jagadeesh
et al. 1997). Perhaps the residual selectivity in our cells
reflects geniculate-based directional biases of the membrane
potentials. We would expect our DIs to be higher than those
observed intracellularly because spike thresholds still affect
the BMI data, accentuating directional biases. Nevertheless,
we also found that inhibition accentuates S-T orientation
and simple changes in spike threshold do not account for
this. Therefore we predict that the removal of inhibition by
cortical cooling should produce changes in S-T orientation
that are observable at the membrane potential level. Unfor-
nately, no data on S-T structure during cooling exist to test
this prediction. It remains to be determined whether the BMI
and cooling results are compatible with a common interpre-
tation.

Figure 12 provides a simple illustration, compatible with the
present and previous (Saul and Humphrey 1990, 1992a) find-
ings, of how an S-T well-oriented receptive field may be
produced by inputs from other simple cells having certain
spatiotemporal relationships. Simulated responses to a coun-
terphasing grating are shown for an excitatory (A) and inhibi-
tory (B) simple cell and their target (C). Only the first half of
the grating spatial cycle is illustrated. Although not shown,
responses in Fig. 12A are produced by rectified inputs from two
LGN-like units—lagged and nonlagged—having relative spa-
tial and temporal offsets of 0.1 and 0.15 cycles, respectively.
Profiles in Fig. 12B reflect two other LGN inputs with similar
relative offsets. The cortical receptive fields that result are each
slightly S-T oriented (STIs = 0.23) and share the same pre-
ferred direction of motion. Linear summation of these two
units (C) produces a receptive field with greater S-T orientation
(STI = 0.64) and hence stronger directional tuning. An arc-
tangent fit to the response phase versus spatial phase data is
shown in Fig. 12D (○). Removing the inhibitory input to cell C
would expose the excitatory structure, resulting in systematic
shifts in the cell’s response phase and a reduction in S-T
orientation (○), similar to that observed experimentally (e.g.,
Fig. 5C). Note that receptive fields receiving direct LGN input
may be more or less S-T oriented than shown here, depending
on the range of response timings among the inputs.
of the timings that produce S-T orientation. Clearly, in the cat, the necessary range of timing delays is present in the LGN relay cells (Saul and Humphrey 1990). Their existence precludes the need to create timing delays in cortex using polysynaptic circuits, N-methyl-D-aspartate receptors (Maex and Orban 1996) and/or GABA B receptors (Suarez et al. 1995).

### Directional mechanisms in layer 6

Unlike layer 4, direction-selective cells in layer 6 display weak first-order S-T orientation, and even the addition of static nonlinearities does not account for their directional tuning (Murthy et al. 1998). Similarly, BMI had no consistent effect on the cells’ first-order S-T structure despite reducing direction selectivity. These results indicate that dynamic nonlinear interactions predominate in layer 6. Such interactions are detectable using two bars flashed sequentially across the receptive field (Emerson and Citron 1992). The second-order S-T oriented structures revealed by this indicate that directional tuning reflects nonlinear facilitatory and suppressive interactions in the preferred and nonpreferred directions. An obvious prediction is that BMI should lessen the suppression and reduce second-order S-T orientation.

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