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Neuronal mechanisms of selectivity for object features revealed by blocking inhibition in inferotemporal cortex

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The inferotemporal cortex (area TE) of monkeys, a higher station of the visual information stream for object recognition, contains neurons selective for particular object features. Little is known about how and where this selectivity is generated. We show that blockade of inhibition mediated by γ-aminobutyric acid (GABA) markedly altered the selectivity of TE neurons by augmenting their responses to some stimuli but not to others. The effects were observed for particular groups of stimuli related to the originally effective stimuli or those that did not originally excite the neurons but activated nearby neurons. Intrinsic neuronal interactions within area TE thus determine the final characteristic of their selectivity, and GABAergic inhibition contributes to this process.

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In the primate brain, visual information on object features is processed by cortical areas along the 'ventral pathway' from primary visual cortex (V1) in the occipital lobe through areas V2 and V4 to areas TEO and TE in the inferior temporal lobe[1]. Stimulus-selective response properties of neurons become gradually more complex along the pathway. V1 neurons respond to a simple spot or a line segment[2], whereas at the final stage of the pathway, TE neurons respond to complex object features such as a particular shape or a combination of shape with color or texture[3–5]. The stimulus-selective activity of TE neurons has been thought to underlie discrimination, recognition and memory of complex visual objects[3–5]. Elucidation of how and where this complex stimulus selectivity is generated constitutes a critical step toward understanding the neural mechanisms responsible for these cognitive processes.

The role of intracortical GABAergic inhibition in generating orientation selectivity of V1 neurons has been debated for over three decades[6–8]. Recent studies show that the intrinsic inhibition is involved in the generation of the selectivity of V1 neurons in macaques[9] and cats[10], as suggested by earlier work[11,12]. In macaque TE, neurons stained with antibodies against GABA or GABA-synthesizing enzymes are 25% of the total neuron population and are distributed across all cortical layers[13,14]. Most, if not all, TE neurons are likely to be under the influence of GABAergic inhibition, but the contribution of this inhibitory input to their feature selectivity is unknown.

To examine the effects of blocking GABAergic inhibition on visual responses of TE neurons, we applied bicuculline methiodide, a $GABA_A$ receptor antagonist, microiontophoretically to the neurons while extracellularly recording their action potentials with triple-barreled microelectrodes. TE neurons with similar preferences for object features are clustered in columns arranged in a mosaic pattern across the cortical surface[15,16], and connected to immediately adjacent columns by their local recurrent axons and to distant columns by long-range horizontal axons[17]. Neuronal interactions among these connected columns, as well as those within each column, may be involved in generating feature selectivity. We therefore first surveyed the stimuli effective for activating neurons in regions of 1–1.5 × 1–1.5 mm^2, each dimension spanning a few columns[15,16]. The critical stimulus features eliciting the maximal responses were determined for these neurons using a reduction procedure (Methods)[15,18,19]. We devised a set of 25–45 stimuli for each region by selecting effective stimuli and their modifications as well as ineffective stimuli. Then we examined the responses of neurons in these regions to the stimulus sets before, during and after bicuculline application.

Results

Bicuculline enhanced visual responses of most neurons tested (89%, 150 of 168) to some stimuli in the set (two-way ANOVA, drug and/or drug × stimulus interaction, $p < 0.05$; Table 1), whereas application of pH-matched vehicle (Methods) did not affect their responses. For neurons (89%, 133 of 150) in which the original responses were restored after drug application was stopped, responses were enhanced ($p < 0.05$, *t*-test) for 16% of the stimuli tested on average. The increase in the largest enhancement of visual responses was 18.1 ± 15.6 spikes per second (mean ± s.d., $n = 133$; Table 2). This was larger than that of the spontaneous firing (6.9 ± 6.9 spikes/s; paired *t*-test, $p < 0.0001$). The increase in visual responses thus cannot be explained by an increase in the spontaneous firing rate. These results indicate that GABAergic inhibition controls the magnitude of visual responses in TE neurons.

The role of GABAergic inhibition is not mere gain control, however, because the disinhibitory effects of bicuculline were highly stimulus specific. Bicuculline affected the stimulus selectivity of 74% of the neurons (99 of 133, drug × stimulus inter-

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Table 1. Effects of bicuculline on visual responses of TE neurons.

	Before and during bicuculline application		Before and after bicuculline application	
Two-way ANOVA	$p < 0.05$ (affected)	$p > 0.05$ (unaffected)	$p > 0.05$ (recovered)	$p < 0.05$ (unrecovered)
Drug factor (a)	38 (25%)		34 (26%)	4 (24%)
Drug × stimulus interaction (b)	27 (18%)		22 (16%)	5 (29%)
Both (a) and (b)	85 (57%)	18 (100%)	77 (58%)	8 (47%)
Total neurons[1]	150	18	133	17
Percentage	89%[2]	11%[2]	89%[3]	11%[3]

[1]All 168 neurons tested showed selective responses to the stimuli used (stimulus factor, $p < 0.05$); [2]of 168 neurons tested; [3]of 150 neurons whose responses were affected by bicuculline.

action, $p < 0.05$; Table 1). For example, a neuron (Fig. 1a) originally responded to five stimuli in the set (top row). Following bicuculline application, this neuron exhibited enhanced responses to the bottom view of an apple and to a pattern composed of a green star and yellow circle. However, responses to the most effective stimulus, a white circle, and the other two effective stimuli, a luminance gradation and a vertical grating, were not enhanced beyond the increase in spontaneous firing (Fig. 1a). The neuron also became responsive to six originally ineffective stimuli: a tomato and its simplified pattern, a padlock, monkey and human faces and a simplified face pattern (Fig. 1a). No such effect was observed on the other ineffective stimuli. The response selectivity of this neuron was thus greatly altered by the removal of GABAergic inhibition (Fig. 1b). Another neuron (Fig. 2a) responded only to the face of a doll monkey under control conditions among the stimuli tested. Disinhibition by bicuculline not only augmented its response to the preferred doll face, but also caused this neuron with highly specific initial selectivity to respond to six other originally ineffective face and non-face stimuli ($p < 0.01$), but not to many other ineffective stimuli. The disinhibitory effects by bicuculline thus occurred only in a proportion of originally effective and ineffective stimuli.

Bicuculline induced 84% of the neurons analyzed (112 of 133) to respond to at least one stimulus that did not activate them originally. On average ($n = 133$), each neuron responded anew to 3.2 of the 25.6 originally ineffective stimuli tested, whereas each neuron originally responded to 6.6 of 32.2 total stimuli. These results suggest that a substantial fraction of the excitatory inputs to TE neurons normally are not expressed because of GABAergic inhibition.

Forty-five percent of neurons tested (60 of 133) showed no change in their responses to the initially most effective stimulus following bicuculline application (for example, Fig. 1), whereas their responses to some other stimuli were enhanced significantly. The absence of enhancement of the responses to the most effective stimulus was not due to a ceiling effect (that is, the inability of neurons to fire above a certain firing rate), because the disinhibited responses of these neurons to originally suboptimal stimuli were often stronger than those to the most effective one (Fig. 1b). These results indicate that the effects of bicuculline on these neurons resulted from specific disinhibition of GABAergic synapses on the excitatory inputs of particular stimulus features, rather than removal of nonspecific inhibition.

The disinhibitory effects on the stimulus selectivity of TE neurons often seemed to occur for a particular feature dimension. One neuron (Fig. 3a), for example, responded to a black cross but not to a white one under control conditions. During bicuculline application, it responded to both crosses. Another neuron (Fig. 3b) recorded near the neuron in Fig. 3a also responded to the black solid cross. In the presence of bicuculline, it began to respond to an outline of a white cross, which originally was ineffective. Another neuron (Fig. 3c) originally responded to a concentric circle with a low spatial frequency, but after disinhibition, it responded to an originally ineffective concentric circle with a high spatial frequency even more strongly than to the initially effective stimulus. A radial grating activated another neuron (Fig. 3d), whereas a spiral grating did not; however, the ineffective grating elicited significant responses following bicuculline application. Of the neurons tested, 37% (40 of 109) showed changes in luminance contrast, spatial frequency or polar angle selectivity. As the population data (Fig. 4a) show, the change in selectivity for luminance contrast occurred only for a particular shape in each neuron (second column) and not for other shapes (fourth column). Responses of the neurons to a shape with an originally ineffective luminance contrast were enhanced from 2.4 ± 1.7 to 15.2 ± 9.5 spikes per second (mean ± s.d., $n = 24$; paired t-test, $p < 0.0001$; Fig. 4a). Similarly, responses to a grating of an ineffective type were augmented from 2.9 ± 2.1 to 15.8 ± 8.7 spikes per second ($n = 23$, $p < 0.0001$; Fig. 4b, second column), whereas no change occurred for gratings of different types (fourth

Table 2. Change in firing rates in TE neurons caused by bicuculline.

	Before bicuculline application	During bicuculline application	Increase
Spontaneous firing	6.1 ± 4.2	13.1 ± 9.2[+]	6.9 ± 6.9
Responses[1] evoked by the most effective stimulus before bicuculline application	18.7 ± 15.0	29.6 ± 25.5[+]	10.9 ± 15.7*
Responses[1] evoked by the most effective stimulus during bicuculline application	16.8 ± 14.5	32.5 ± 25.8 [+]	15.7 ± 16.1**
The largest increase[1] of responses evoked by a stimulus from before to during bicuculline application	11.4 ± 13.6	29.4 ± 25.1[+]	18.1 ± 15.6**

Firing rates (spikes/s, mean ± s.d., $n = 133$). [1]Net visual responses after the spontaneous firing rate was subtracted; [+]$p < 0.0001$, paired t-test compared with rate before bicuculline application; *$p < 0.0005$, **$p < 0.0001$, paired t-test compared with the increase of spontaneous firing.

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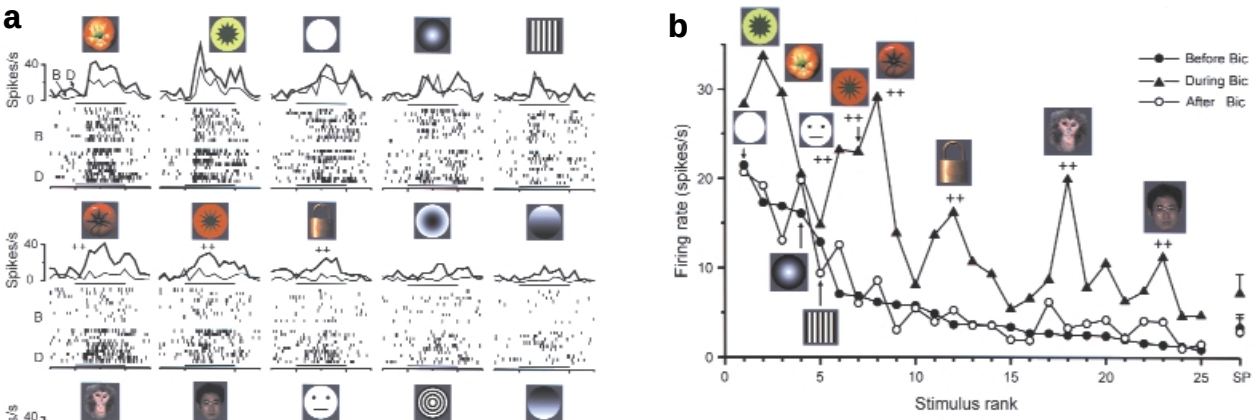

Fig. 1. Stimulus specific effects of blocking GABAergic inhibition on visual responses of a TE neuron. Stimulus selectivity of this neuron was markedly altered by bicuculline (ANOVA, both drug factor and drug × stimulus interaction, $p < 0.01$). Disinhibition of responses occurred only for a proportion of effective and ineffective stimuli. (**a**) Peristimulus time histograms (PSTHs) and rastergrams of responses to each stimulus (above PSTHs) are shown. Bin width of PSTHs is 100 ms. Underlines below PSTHs indicate the period of stimulus presentation. (**b**) Responses before, during and after bicuculline (Bic) application plotted against the stimulus rank determined by responses before bicuculline application. Before Bic, –30 nA retaining currents (RC); during Bic, 5 mM, +5 nA ejecting currents (EC); after Bic, –30 nA RC. Sp, spontaneous firing; ++statistically significant relative to spontaneous firing during bicuculline application (*t*-test, $p < 0.01$) but not before bicuculline application ($p > 0.05$). The maximal and minimal s.e.m. of the responses, respectively, are 3.2 and 0.4 spikes/s before bicuculline, 5.2 and 0.9 spikes/s during bicuculline, and 3.6 and 0.3 spikes/s after bicuculline. Error bars, s.e.m.

column). These results suggest that some of the inhibited and the originally effective stimulus features were related to each other in a specific feature dimension.

Furthermore, 26% of the neurons tested (23 of 88) seemed to receive inputs from subcomponents of an effective stimulus, which were masked under control conditions. The neuron in Fig. 3e, for example, was induced by bicuculline to respond to a T shape, which is a component of the originally effective cross. Another neuron (Fig. 3f) began to respond to an originally ineffective component, a star with a white outline, of the originally effective figure composed of the star and a circle drawn by a white line. Responses of this kind to ineffective components were, on average, increased from 3.1 ± 3.0 to 19.8 ± 10.5 spikes per second ($n = 23$, $p < 0.0001$, Fig. 4c). These results indicate that geometrically distinctive components are also integrated in some TE neurons.

The two neurons in Figs. 1 and 2a were recorded from two nearby sites across the cortex in the upper layers. Although the effective stimuli differed between them under control conditions, most stimuli that became effective following bicuculline application were shared. The stimulus features effective in the first neuron under disinhibition (Fig. 1) could be grouped into faces, luminance gradations and patterns made up of a circle and a star, whereas those of the second neuron (Fig. 2a) consisted of faces, luminance gradations and gratings. Only these few particular groups of stimuli activated the two neurons under disinhibition, whereas other stimuli such as stars, radial gratings, crosses, T-junctions, squares, bars and fruits (Fig. 1a) did not activate them. Many of these newly effective stimuli activated other neurons along the same or surrounding vertical recording tracks under control conditions (without bicuculline). For example, the peaks in the response profile of the neuron in Fig. 2a under disinhibition corresponded to the peaks in the response profile without bicuculline of another neuron 200 μm away along the same penetration (Fig. 2b). Other neurons along this penetration responded under normal conditions to the face of a doll lemur (data not shown), to which the neuron in Fig. 2a became responsive under bicuculline application. These results suggest that the response properties of neurons under disinhibition are related to the normal response properties of nearby neurons as well as their own.

To test whether this holds true for TE neurons in general, we analyzed the response properties of 49 neurons using a fixed set of 30 stimuli that were predetermined for neurons in the recorded region (1×1 mm^2). We examined whether the number of neurons that became newly responsive to a stimulus in the set was correlated to the number of originally responsive neurons. Neurons ($n = 30$) recorded along nearby penetrations (< 0.7 mm) showed a positive correlation ($r = 0.47$, $p < 0.01$; Fig. 5a), whereas neurons ($n = 19$) from penetrations 1–1.4 mm apart within the recorded region did not (Fig. 5b; $r = –0.07$, $p = 0.69$). These results indicate that, at the population level, nearby neurons tend to show a greater correlation between changes in their selectivity induced by bicuculline and the normal selectivity of their neighbors. One may argue that there is a baseline difference in the number of neurons that responded to a feature between nearby neurons and distant ones (that is, the distributions along the *x* axes of Fig. 5a and b). This is expected because nearby neurons are more likely to respond to the same feature[15,16] (see also

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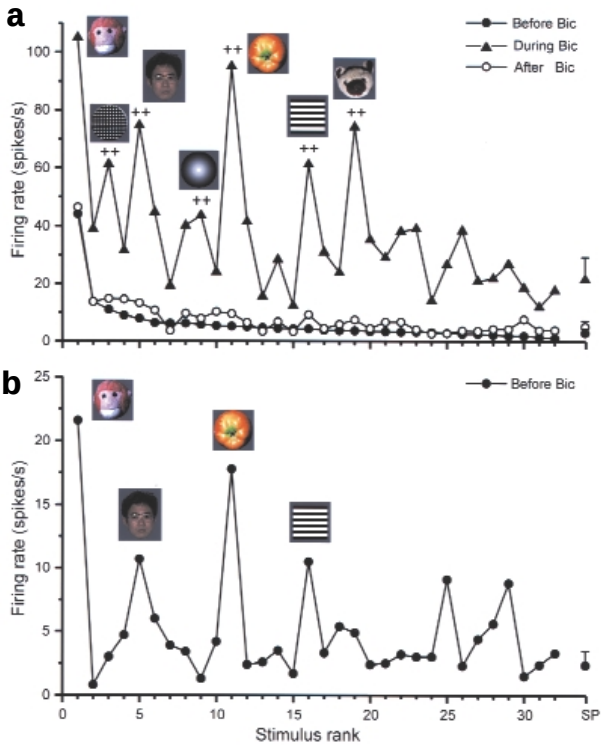

Fig. 2. Change in stimulus selectivity of a TE neuron following bicuculline application was correlated with normal selectivity of a nearby neuron. (**a**) A neuron with a highly specific preference for a doll face was induced by bicuculline to respond to several originally ineffective stimuli (ANOVA, both drug factor and drug × stimulus interaction, $p < 0.01$). Before Bic, –20 nA RC; during Bic, 5 mM, +2 nA EC; after Bic, –20 nA RC. (**b**) The response profile of another neuron without bicuculline. This neuron was located 200 μm away from the neuron shown in (**a**) along the same vertical penetration. Many of the effective stimuli for this neuron were effective stimuli during bicuculline application for the neuron shown in (**a**). The correlation coefficient of the difference in response of the neuron in (**a**) before versus during bicuculline application against the control responses of the neuron in (**b**) is 0.67 ($p < 0.01$). The maximal and minimal s.e.m. of the responses of the neuron in (**a**) are, respectively, 3.2 and 0.3 spikes/s before bicuculline, 12.2 and 2.3 spikes/s during bicuculline, and 3.9 and 0.5 spikes/s after bicuculline, and those of the neuron in (**b**) are 2.3 and 0.3 spikes/s before bicuculline. The other two small peaks of the response profile of the neuron in (**b**) are the responses to a polar grating and a pattern composed of a red circle and a green star (see **Fig. 1a**), which elicited responses from other neurons along the same track during bicuculline application.

Fig. 6a–c). Even if we removed the five rightmost points in Fig. 5a so as to match the distribution along the *x* axes between Figs. 5a and b, there was still a positive correlation for the nearby penetrations ($r = 0.46$, $p < 0.02$).

We further examined this correlation in a larger population of neurons ($n = 124$) by calculating the correlation coefficient (r) of the difference in responses of a neuron between bicuculline application and control conditions against the control responses of another to the same stimulus set. We first analyzed the response correlation under control conditions between a pair of neurons along the same penetration (first group), different penetrations within a distance of 0.7 mm (second group) and with a distance of over 1 mm (third group). The distribution of r for the first group of neuron pairs (0.35 ± 0.29, mean ± s.d., $n = 254$ pairs; Fig. 6a) was markedly shifted toward more positive values than that for the second group (0.02 ± 0.28, $n = 724$; Fig. 6b; $p < 0.0001$) or for the third group (–0.04 ± 0.21, $n = 528$, Fig. 6c; $p < 0.0001$). The correlation between pairs of neurons thus retained a high value in the vertical direction, but decreased rapidly in the horizontal direction. The r of the difference in responses of a neuron between before and during bicuculline application against control responses of another showed a similar change with respect to the horizontal distance. The r for the first group of neuron pairs (0.25 ± 0.28, $n = 254 \times 2$ neurons; Fig. 6d) was larger than those for the second group (0.03 ± 0.25, $n = 724 \times 2$, Fig. 6e; $p < 0.0001$) or the third group (–0.02 ± 0.22, $n = 528 \times 2$; Fig. 6f; $p < 0.0001$). The r for the second group was significantly larger than that for the third group ($p < 0.0001$; Fig. 6e and f). These results further demonstrate that excitatory inputs to a neuron masked by local GABAergic inhibition are correlated with normal responses of their neighbors. The rapid decline of the correlation with the horizontal distance suggests that GABAergic inhibition acts mainly within a columnar cortical region or among immediate neighbors.

Discussion

The selectivity of neurons for object features such as shape, color, texture and their combination has been suggested to be generated in areas V4 and TEO, and conveyed to area TE[4,19]. Projections

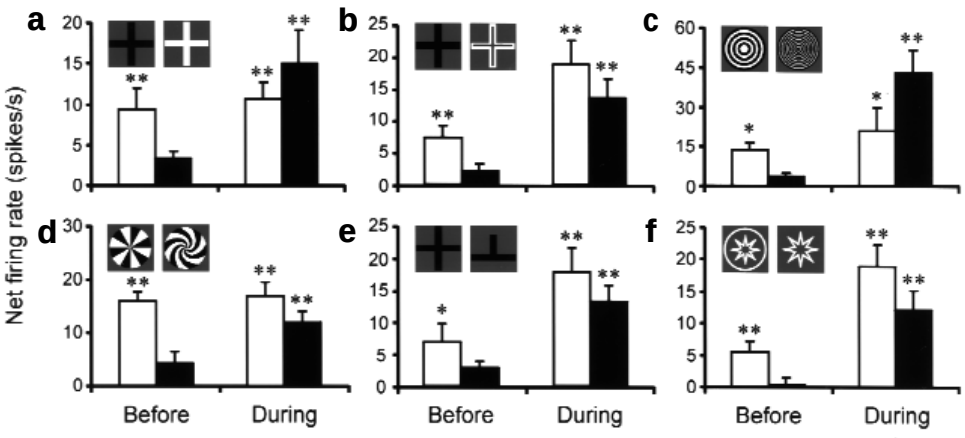

Fig. 3. Stimulus features disinhibited by bicuculline were related to the originally effective stimulus features in specific parameter domains. The neuron in (**a**), for example, responded to a black cross, but not to a white cross without bicuculline. It responded to both crosses during bicuculline application. Examples of disinhibited features include the same shape with a different luminance contrast (**a**, **b**), the same type of grating with a different spatial frequency (**c**), the same radial grating with a different grating angle (**d**) and a component of the effective stimuli (**e**, **f**). Mean net responses to the stimuli before and during bicuculline application. Asterisks, statistically significant responses relative to spontaneous firing rate (*t*-test, $**p < 0.01$, $*p < 0.05$). Error bars, s.e.m.

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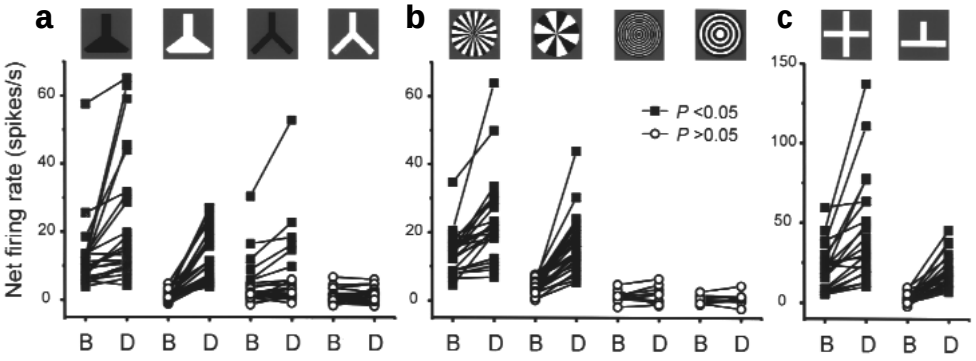

Fig. 4. Population data for changes in stimulus selectivity for specific feature dimensions or for components of a stimulus. Squares and/or circles joined by a line indicate responses of each of the neurons tested before (B) and during (D) bicuculline application. Pictures above the plots represent examples of stimuli, and not necessarily the exact stimulus presented for each neuron. (**a**, **b**) Bicuculline changed luminance polarity preferences of neurons (n = 24) for a particular shape (second column) but not for another shape (fourth column) (**a**), or selectivity of neurons (n = 23) for a particular type of grating, but not for other types of gratings (**b**). In the third and fourth columns in (**b**), data for different types of gratings are available only for 10 of the 23 neurons. (**c**) Other neurons (n = 23) were induced by bicuculline to respond to an originally ineffective components of an originally effective shape. $p < 0.05$, $p > 0.05$, t-test, relative to spontaneous firing rate.

from neurons having the same stimulus selectivity but with different receptive field positions in these former areas are hypothesized to converge onto TE neurons to render them translation-invariant selective for a particular feature. The present study showed that blockade of GABAergic inhibition within area TE itself, which affected single TE neurons or small groups of neurons and/or interaction between area TE and its afferent areas, altered the stimulus selectivity of TE neurons. This indicates that generation of selectivity is in progress in area TE, and that intrinsic inhibition is involved in the process. Information on object images is likely to be gradually integrated into complex forms in the successive areas leading to TE along the ventral visual pathway. Together with previous studies from V1 neurons of macaques[9,20,21] and cats[10,11,12], these results suggest that intracortical inhibition is important in generating the response properties of visual cortical neurons.

Anatomical studies show that excitatory horizontal axons are extensively connected to nearby and distant columns, and axons of GABAergic interneurons are distributed within a short distance (< 1 mm) in area TE[14,17]. Cross-correlation analyses have also indicated that nearby TE neurons share common inputs or are mutually connected with each other[22,23]. Our results show that a certain portion of the excitatory inputs to TE neurons are inhibited by local GABAergic interneurons. The stimulus features conveyed by these suppressed inputs are correlated to those originally effective for the neurons and other nearby neurons, and some of them are component features of an effective stimulus. These results indicate that TE neurons receive excitatory inputs with related or distinctive stimulus features, that their stimulus selectivity is formed by integrating these multiple inputs, and that GABAergic inhibition contributes to the process. The action of GABAergic inhibition is stimulus specific in many neurons, although it might involve a general, nonspecific effect in some neurons. The stronger correlation of the masked stimulus of a neuron with the effective stimulus of another in the same vertical penetration than in different penetrations indicates that excitatory and inhibitory interactions across the cortical layers are important in this process.

Under blockade of inhibition, many neurons seemed to change their stimulus selectivity along a particular feature parameter (Figs. 3 and 4). The results suggest that groups of neurons with different specificity in a particular feature domain (for example, luminance) interact with one another to tune to the best parameter in that domain (such as a particular luminance

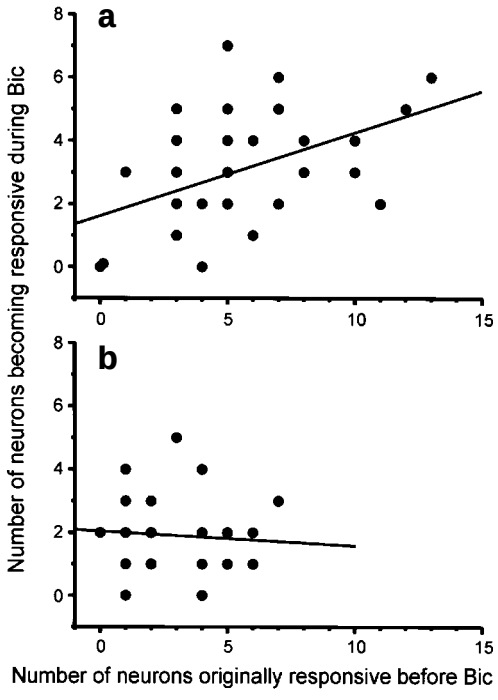

Fig. 5. Correlation between the change in stimulus selectivity induced by blocking GABAergic inhibition and the selectivity of nearby TE neurons under normal conditions. Each dot represents one of 30 stimuli in the set. Some dots, such as the dot shown at (0,0) in (**a**), represent more than one stimulus. Forty-nine neurons recorded from a cortical region 1×1 mm^2 were analyzed. For each stimulus, the number of neurons that responded to the stimulus before bicuculline application (abscissa, $p < 0.05$, t-test) are plotted against the number of neurons that became responsive during bicuculline application ($p < 0.05$, relative to spontaneous firing rate) but did not respond originally to that stimulus (ordinate, $p > 0.05$). (**a**) Neurons (n = 30) recorded along nearby penetrations (< 0.7 mm). (**b**) Neurons (n = 19) recorded along penetrations at a distance of 1–1.4 mm.

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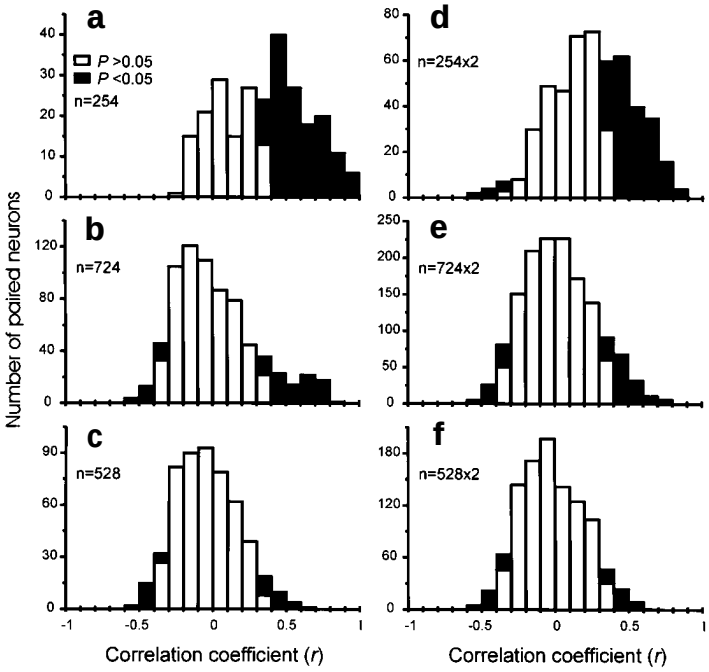

Fig. 6. Response correlation between neurons. Histograms (**a**, **b**, **c**) of correlation coefficient (*r*) between the control responses of a pair of neurons and those (**d**, **e**, **f**) between the response change of a neuron induced by bicuculline and the control responses of the other. A total of 124 neurons from three monkeys were analyzed. (**a**, **d**) Neuron pairs along the same penetration. (**b**, **e**) Neuron pairs from different penetrations within 0.7 mm distance. (**c**, **f**) Neuron pairs from different penetrations > 1 mm apart. Filled bars indicate neuron pairs with a significant correlation ($p < 0.05$); open bars indicate neuron pairs without a correlation.

contrast). The neurons then become selective to the best combination of features (a particular shape with a particular luminance contrast). This result should be interpreted with care, however, because we do not know what parametric domains might be potentially important for the responses of the studied neurons, and because we could not test responses systematically over all possible feature domains. Although the data in Figs. 1, 2, 5 and 6 clearly demonstrate that neurons responding to different 'stimuli' inhibit one another, enhancing the selectivity for complex object images, it remains to be established whether the change caused by bicuculline can be explained by a change in selectivity for a small number of 'features'.

The correlation between responses under normal conditions for a pair of neurons recorded from the same recording tracks was stronger than that from different tracks (Fig. 6a–c), supporting the notion that TE neurons in a vertical column share similar stimulus selectivity[15]. The mean correlation coefficient for pairs of neurons in a vertical track, however, was not very high (0.35, Fig. 6a). Although the absolute value of the correlation coefficient depends on the stimulus set used and is difficult to interpret, nearby neurons often responded to different stimuli in addition to shared stimuli (for example, Fig. 2a and b), and many individual neurons responded to apparently distinct stimuli (Figs. 1 and 2b). Response properties of neurons within a column are thus correlated, but are more heterogeneous than previously thought[15]. A columnar region in area TE thus receives excitatory inputs of multiple, heterogeneous stimuli. GABAergic inhibition suppresses some of these inputs, and thereby contributes not only to the stimulus selectivity of individual neurons, but also to the formation of functional columnar organization in area TE.

Methods

Experimental procedures. Experiments were done in the dorsal part of area TE of the inferior temporal lobe (A5–18) of three adult monkeys (*Macaca fuscata*). In one monkey, electrode penetrations were guided by magnetic resonance imaging. The general methods used for animal preparation and recording were similar to those described previously[18,19]. All surgical and animal care procedures conformed to the guidelines of the NIH (1996), and were approved by the animal experiment committee of Osaka University Medical School. After initial anesthesia with ketamine (5 mg/kg, i.m.) and endotracheal cannulation, monkeys were ventilated artificially with air and anesthetized with 1–2% isoflurane, and a small hole was drilled in the skull over area TE for electrode penetrations. During recording sessions, monkeys were immobilized with pancuronium bromide (0.08 mg/kg/h, i.v.), and anesthesia was maintained with a mixture of N_2O:O_2 (7:3) and 0.5–1.0% isoflurane[9]. Electrocardiogram, body temperature and expired CO_2 concentrations were monitored throughout the experiment. Action potentials were recorded extracellularly from single TE neurons driven by stimulation of the contralateral eye and amplified, and the timing of the potentials was recorded by a computer.

Before bicuculline experiments, stimulus selectivity of neurons within a cortical region of 1–1.5 × 1–1.5 mm was tested by making vertical penetrations one after the other with single metal microelectrodes (2–3.5 MΩ at 1 kHz) at the center and the four corners of the region. Visual responses of the recorded neurons were tested with more than 90 hand-held real objects and many two-dimensional paper cutouts to search for effective stimuli. The image of the most effective stimulus was taken with a video camera and modified on a computer. The critical stimulus feature essential for neuronal activation was determined by stepwise simplification of the most effective stimulus ('reduction process'[15,18,19]). We recorded more than 200 neurons in these experiments, for most of which their feature selectivity was surveyed using the reduction process. Stimuli (about 4° × 4°) were presented for 1 s with a small-amplitude motion (up to 1.2°) around the center of the predetermined receptive field on a gray background of a CRT monitor at 1.5- or 2-s intervals. All stimuli were presented pseudorandomly with 10 repetitions of each stimulus.

Microiontophoresis. Triple-barreled microelectrodes were constructed using a method improved by us. The triple-capillary glass-coated tungsten assembly was constructed based on a reported method[24]. Our preparation allowed a small amount of melted glass to coat the tip of tungsten wire in the central capillary, then 5–15 µm of the tip was exposed by techniques developed for a single glass-coated tungsten microelectrode[25]. Tips 2–4 µm in diameter on the other two glass barrels for iontophoresis were simultaneously formed, and were closely attached to the tip of tungsten for recording. One of the barrels was filled with 2.5 or 5 mM bicuculline methiodide (Sigma, St. Louis, Missouri; pH 3.5, adjusted with HCl) and the other with 0.9% NaCl (vehicle, pH 3.5) for control and balancing current. A low-intensity current (+1–5 nA) was used to eject a minimal amount of bicuculline to the immediate vicinity of neurons to avoid causing bursts of firing. When bicuculline was not administered, retaining currents of –15 to –30 nA were employed to prevent leakage of bicuculline. Under all conditions, a balancing current with the same intensity and opposite polarity was applied to the vehicle barrel to offset possible current effects on the recorded neurons. The effects of bicuculline on visual responses were tested after bicuculline had been applied for 5 min, and the recovery data were obtained at least 5–10 min after bicuculline was removed. Occasionally, bicuculline application caused epileptiform activity of

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the recorded neurons. In such cases, we decreased the ejecting current until the activity disappeared; otherwise recording was abandoned at these sites. For a full technical discussion of bicuculline microiontophoresis, see ref. 26.

Data analysis. Firing rates during 1 s of visual stimulation were averaged over 10 presentations. Net visual responses were then obtained by subtracting the spontaneous firing rate measured 0.5 s before presentation of each stimulus. Two-way analysis of variance (ANOVA, visual stimulus condition versus drug condition) was applied to the net responses evoked by one stimulus set before and during bicuculline application to detect the effects of bicuculline, or to those before and after bicuculline application to assess the recovery from the effects. A *t*-test was done to examine whether a stimulus evoked significant responses in a neuron relative to spontaneous firing, and whether bicuculline application enhanced responses to the stimulus, by comparing the net visual responses during bicuculline application with those before. The significance level in all tests was $p < 0.05$.

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