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THE CONTRIBUTION OF INHIBITORY MECHANISMS TO THE RECEPTIVE FIELD PROPERTIES OF NEURONES IN THE STRIATE CORTEX OF THE CAT

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SUMMARY

1. The iontophoretic application of the GABA antagonist bicuculline to simple and complex cells in the striate cortex of the cat produced extensive modifications of receptive field properties. These modifications appear to relate to a block or reduction of GABA-mediated intracortical inhibitory influences acting on the cells examined.

2. For simple cells the effects of bicuculline on receptive field properties involved a loss of the subdivision of the receptive field into antagonistic ‘on’ and ‘off’ regions, a reduction in orientation specificity and a reduction or elimination of directional specificity.

3. The effect on the ‘on’ and ‘off’ subdivisions of the simple cell receptive field was such that all stationary flashing stimuli, whether covering the whole receptive field, or located within the receptive field over a previously determined ‘on’ or ‘off’ region, resulted in an ‘on and off’ response.

4. The orientation specificity of complex cells was reduced during the application of bicuculline such that in many cases the original specificity of the cell was virtually lost with the response to the orientation at 90° to the optimal being of similar magnitude to the optimal. The directional specificity of complex cells was generally less affected than that of simple cells. Often when large changes in orientation specificity were observed the directional specificity was relatively unaffected.

5. For some cells apparently showing to all visual stimuli only inhibitory responses, the application of bicuculline resulted in the appearance of excitatory responses.

6. In all cases receptive field properties reverted to the original state after termination of the bicuculline application. It was not generally possible to duplicate the effects of bicuculline by raising neuronal excitability with iontophoretically applied glutamate.
7. On the basis of these results it is suggested that the normal subdivision of the simple cell receptive field into separate 'on' and 'off' regions and its directional specificity are dependent on intracortical inhibitory processes that are blocked by bicuculline. The orientational tuning of simple cells conversely appears to be largely determined by the excitatory input but normally enhanced by lateral type inhibitory processes acting in the orientation domain.

8. It also appears that the excitatory input to some complex cells is not orientation specific. This suggests that for these cells it is extremely unlikely that they receive an orientation specific excitatory input from simple cells.

INTRODUCTION

Visual stimuli evoke both inhibitory as well as excitatory post-synaptic potentials in visual cortical neurones (Creutzfeldt & Ito, 1968). Consequently an understanding of the neural organization underlying the receptive field characteristics of visual neurones requires a detailed consideration of the properties of both the excitatory and inhibitory inputs. For example there is evidence indicating that the normal highly specific orientation tuning of cells in the visual cortex may be derived from the interaction of excitatory and inhibitory inputs that are each broadly tuned around the same optimal orientation, with respect to the range of orientations that will elicit an effect (Blakemore & Tobin, 1972). In other cases, it is possible to relate some properties of the receptive field directly to the nature of the excitatory input and others to the operation of an inhibitory input. This is exemplified by the characteristics of simple cell responses to an optimally oriented slit moving over their receptive field. In terms of the response to the leading or trailing edges of the slit (Bishop, Coombs & Henry, 1973) the simple cell reflects the response of lateral geniculate cells to a moving slit (Dreher & Sanderson, 1973) and hence that of the excitatory input. Conversely the specificity of some simple cells for one direction of the slit motion cannot be directly related to the response of geniculate neurones and must therefore involve an additional input. Benevento, Creutzfeldt & Kuhnt (1972) described inhibitory inputs to visual cortical neurones that were directionally specific with reference to the trigger stimulus. This type of inhibitory input would be effective with a non-directionally specific excitatory input in generating a directionally specific action potential discharge.

It is likely that all the inhibitory influences acting on visual cortical neurones are mediated via interneurones within the cortex rather than directly by afferents from the lateral geniculate body which would seem to exert only an excitatory effect (Watanabe, Konishi & Creutzfeldt, 1966;...
Armstrong, 1968). This view is supported on anatomical grounds by the work of Gary & Powell (1971), who found that all the terminals of lateral geniculate origin in the visual cortex seem to have the asymmetric membrane thickenings associated with excitation (Uchizono, 1965). There is evidence indicating that the inhibitory transmitter in the visual cortex may be GABA (Iversen, Mitchell & Srinivasan, 1971), and it has recently been demonstrated that in area 17 of the visual cortex, the GABA antagonist bicuculline will block both the inhibition produced by the iontophoretic application of GABA and visually evoked inhibition (Sillito, 1975).

The present work is an attempt to examine further the role of intracortical inhibitory processes in the genesis of the receptive field properties of visual cortical neurones. Iontophoretically applied bicuculline has been used to reduce or eliminate GABA-mediated inhibitory influence acting on neurones in area 17 of the cat's visual cortex and any consequent modifications occurring in their receptive field properties have been carefully assessed. Where bicuculline is effective in reducing influences acting on a neurone, the neurones receptive field properties should reflect the nature of the excitatory input alone. Thus a comparison with the normal response should provide evidence on the functional role of the inhibitory input and a basis for checking previous assumptions regarding the organization of inhibitory mechanisms in the visual cortex. Preliminary reports of these results have already been made (Sillito, 1974a, b).

**METHODS**

Details of anaesthesia, preparation and care of the cats throughout the experiment, electrodes, drugs and histological procedures have been described in the preceding paper (Sillito, 1975). An account is given here of the optical and visual stimulation techniques and the main experimental procedures.

**Optical and visual stimulation techniques**

The eyes were brought to focus on a tangent screen at 1·0 m with appropriately powered contact lenses selected on the basis of determining the refractive error by retinoscopy. The position of the optic disk and blood vessels defining the area centralis were determined by projecting an image of the retina on the tangent screen with a Keeler Pantoscope as in the method originally described by Fernald & Chase (1971). This procedure was repeated several times during the course of the experiments to check for eye movements. Focusing the image by selecting the correct pantoscope lens provided an additional estimate of the refractive error of the eye. Either 3·0 or 5·0 mm artificial pupils were carefully centred in front of the contact lenses.

The visual stimuli were projected on the tangent screen. A Leitz Diascriptor 4 was used for projecting hand controlled stimuli. A specially constructed optical system was used to produce precisely controlled moving and flashing stimuli. In this system the stimulus was generated by a variable slit aperture in a modified Leitz Prado Universal projector. The light beam from the projector was reflected from a front
surfaced mirror mounted on an optical scanner (Ealing Beck Ltd, Type 22-4774) and directed through a large aperture Dove prism (1 in., Spindler & Hoyer, Göttingen, Germany) to a final mirror mounted on a rotatable turret which reflected the beam on to the tangent screen. The orientation of slit stimuli was adjusted by rotating the Dove prism. The motion was produced by rotation of the scanner under the control of a linear ramp stimulus of variable rise time and amplitude. The direction of motion of a slit was always in a plane at 90° to its long axis. Flashing stimuli were generated by interrupting the light path from the projector with an Ealing electronic shutter (Ealing Beck type F22-8437). The neural response to these stimuli was examined by constructing post-stimulus histograms on line using a small purpose built computer (Lewin, 1974). For all the experiments the background illumination was in the range 17–22 cd/m² and the stimulus was 34-5 cd/m². These values were measured with an S.E.I photomotometer and the stimulus luminance refers to measurements made with the stimulus on background illumination.

**Experimental procedure**

For each cell an initial evaluation of receptive field properties was made with hand controlled stimuli. This was carried out for both eyes and the dominant eye determined. Subsequent tests were all carried out with monocular viewing using the dominant eye. The initial evaluation of receptive field characteristics was checked by constructing and comparing post-stimulus histograms for the neurones response to a range of moving and flashing stimuli selected to assess each of the proposed receptive field characteristics. Thus for example the optimal orientation was checked by constructing post-stimulus histograms for the response of the neurone to moving slits at orientations in a series of 5 or 10° steps either side of and including the proposed optimal. Likewise simple cell receptive fields (Hubel & Wiesel, 1962) were particularly carefully evaluated by comparing the response as judged from post-stimulus histograms to one or more stationary flashing stimuli of varying dimensions to check for spatial summation within a proposed ‘on’ or ‘off’ subdivision, to check the extent of the subdivision and to evaluate the antagonistic interaction between adjacent subdivisions when they were simultaneously stimulated.

Once the receptive field properties had been established in this manner a standard series of tests was routinely used for documenting them before, during and after the iontophoretic application of bicuculline. As it was difficult to apply steady bicuculline ejecting currents for long periods (see Methods, Sillito, 1975) and the time available during the bicuculline application was limited, these tests were not as comprehensive as those involved in the original examination. For orientation specificity eight standard testing orientations were used. These extended in a series of 22-5° steps starting from and including the previously determined optimal. In the results these testing orientations are represented as three 22-5° steps both clockwise (+) and anticlockwise (−) to the optimal, together with the optimal and 90° to the optimal. The full range is thus −67-5, −45-0, −22-5°, the optimal, +22-5, +45-0, +67-5, 90°. The orientation at 90° to the optimal can be represented at both ends of the list, a change from +90 to −90° implying a reversal of the direction of motion. However, for all the testing orientations the response to both directions of motion over the receptive field were recorded and hence the 90° test was generally carried out only once in each series of tests. There still remains a distinction between +90 and −90° in terms of which direction of motion represents the first pass over the receptive field in each stimulus cycle but with respect to the time available the possible error was not considered large enough to warrant recording the response to both directions of motion at both +90 and −90°. The slit dimensions and velocity of slit motion used for each series of tests were those judged to produce for the particular neurone
examine the largest excitatory response at the optimal orientation. For the response to stationary flashing stimuli simple cell fields were tested with stimuli located to each of the previously determined ‘on’ and ‘off’ regions and a stimulus covering the whole receptive field. All other cells were only routinely tested with a stationary flashing stimulus covering the whole receptive field. In certain cases additional specific tests were used to examine particular receptive field properties not covered by the standard series, as for example in the case of the length preference of hypercomplex receptive fields (Hubel & Wiesel, 1965).

In this way the receptive field properties of the neurones examined were documented as a series of histograms showing the response to a standard range of stimuli. All the tests involved at least twenty-five presentation of the stimulus and in many cases fifty. For moving stimuli one presentation of the stimulus is counted as a complete cycle of the stimulus motion forwards over the receptive field and then backwards over it to the starting point.

For most of the neurones examined the effect of bicuculline on receptive field properties was compared with the effect of raising neural spontaneous activity with iontophoretically applied glutamate. As prolonged application of glutamate tended to produce a depolarization block of the cells response this comparison was restricted to the particular receptive field characteristic most affected by the bicuculline, consequently avoiding the long application period required to cover the whole series of tests. In some cases the effect on receptive field properties of depressing neuronal excitability with iontophoretically applied GABA was also tested. The over-all time required to complete the investigation of a cell was generally 3–4 hr. Where it was possible to hold the cell for longer periods of time the application of bicuculline was repeated and more detailed tests of its effects on certain aspects of receptive properties were made.

Presentation of results

In the Results section where post-stimulus histograms are used to illustrate the response of a cell to moving stimuli they show the response of the cell to both the directions of motion of the slit in the plane at 90° to its long axis. The motion of the slit over the receptive field from its starting point at the beginning of the stimulus cycle is referred to as a ‘forwards’ motion. The return of the slit over the receptive field to the starting point is referred to as the ‘backwards’ motion. The movement of the slit either side of the field centre was always symmetrical. The vertical dashed line as shown in Fig. 2 and subsequent figures represents the middle portion of the stimulus cycle, consequently the portion of the histogram to the left of the dashed line corresponds to the forwards motion of the slit over the receptive field and the portion to the right the backwards motion. For some of the histograms the cycle time of the timing pulse, controlling the analysis cycle of the computer and initiating each stimulus cycle, was in fact slightly longer than the time taken for the cycle of stimulus motion. In these cases the dotted line indicating the mid point of the stimulus cycle is displaced to the left of the centre of the histogram.

In order to illustrate the over-all effect of bicuculline on the orientation specificity of a cell, tuning curves were constructed from the responses to the eight testing orientations. Because the testing orientations were in 22.5° steps these curves give only an approximate indication of the orientation tuning and its modification by bicuculline. Each point on the tuning curves represents the average spike count per trial for twenty-five passes of the slit at the particular orientation in one direction only over the receptive field. The direction chosen was based on the preferred direction at the optimal orientation and this was followed through the range of testing orientations except 90°. At 90° the responses to both directions were used, one for
the $+90^\circ$ point and the other for the $-90^\circ$ point. For each point the count was limited to a 500 msec period corresponding to the motion of the slit over the receptive field area. Spontaneous levels were assessed by running the test without a visual stimulus.

RESULTS

A detailed study was made of the effects of bicuculline on the receptive field properties of fifty-nine neurones in area 17 of the visual cortex. In each case the neurone was held in a satisfactory and stable recording situation for a period long enough (a minimum of 3 hr) to allow a careful comparison of the 'normal' receptive field properties and the receptive field properties during the iontophoretic application of bicuculline. Some of the neurones were examined for periods of up to 8 hr and the observations repeated several times. The receptive field properties of the cells considered here were stable throughout the period of examination, showing significant variations from normal only during the iontophoretic application of drugs. Significant 'spontaneous' changes in receptive field properties were only observed under conditions where the preparation was deteriorating or where there were fluctuations in the level of anaesthesia (as judged from the e.g.). The sample of fifty-nine neurones considered here excludes all neurones encountered in these conditions and also excludes all the data obtained from neurones where any instability in the recording situation made it difficult to be certain that the activity related to one particular neurone.

Details of the pharmacological effectiveness of bicuculline in terms of its ability to block the action of iontophoretically applied GABA and glycine are given in the preceding paper (Sillito, 1975). The present account refers only to the effect of iontophoretically applied bicuculline on receptive field properties. The classification of the neurones examined in terms of their receptive field properties and the specific effects of bicuculline on these properties are considered in the following sections.

1. Neurones with 'simple' type receptive field properties

Neurones were categorized as 'simple' type visual neurones if they exhibited a low spontaneous activity, had a small receptive field which could be subdivided into discrete antagonistic 'on' and 'off' regions when tested with stationary flashing stimuli, and if they showed spatial summation within the separate 'on' or 'off' regions (Hubel & Wiesel, 1962). Further to this they could generally be distinguished from complex cells on the basis of a slightly more sharply tuned orientation specificity and a smaller discharge field size to a slit at the optimum orientation moving across the region of the receptive field. For some of the cells examined
although it was not possible to divide the receptive field into discrete ‘on’ or ‘off’ regions all the other receptive field characteristics were reminiscent of those of simple cells and with respect to the comments of Bishop & Henry (1972) these were also considered to be simple.

On the basis of these criteria nineteen of the cells examined were considered to be simple cells. When tested with stationary flashing stimuli sixteen of these had receptive fields that could be subdivided into discrete ‘on’ and ‘off’ regions and three showed no clear subdivision of the receptive field but displayed all other characteristics associated with simple cells. In eighteen of the simple cells the iontophoretic application of bicuculline produced a block of the inhibitory action of iontophoretically applied GABA and at the same time a modification of receptive field properties. For one cell there was neither a block of the inhibitory action of iontophoretically applied GABA nor a change in receptive field properties.

Where the receptive fields could be subdivided into ‘on’ and ‘off’ regions the neurones exhibited prior to the application of bicuculline pure ‘on’ discharges in response to flashing slits over the ‘on’ regions, pure ‘off’ discharges from the ‘off’ regions and little or no response to a flashing stimulus consisting of a rectangle covering the whole receptive field. This is well illustrated by the responses of the simple cell shown in Fig. 1a–d. The receptive field in this example consisted of a central ‘on’ region flanked by ‘off’ regions. In fifteen of the simple cells bicuculline modified the normal responses to stationary flashing stimuli such that the cells gave mixed ‘on–off’ responses to slits located over previously determined ‘on’ or ‘off’ regions within the receptive field and also vigorous ‘on–off’ responses to stimuli covering the whole receptive field. This effect of bicuculline on simple cell receptive field properties can be seen in the responses of the simple cell shown in Fig. 1e–h. A slit located over either the upper (e) or the lower (g) ‘off’ region produced during the application of bicuculline both an ‘on’ and ‘off’ discharge, with the transient ‘on’ and ‘off’ components of similar magnitude. The magnitude of the ‘off’ component of this mixed response seen during the application of bicuculline was notably greater than the normal ‘off’ response elicited from this region. For a stimulus located over the ‘on’ area of the receptive field there was little increase in the magnitude of the evoked ‘on’ response but together with the ‘on’ response there was a transient ‘off’ component approximating in magnitude the transient phase of the ‘on’ response (f). A stimulus covering the whole field produced a vigorous transient ‘on–off’ response (h). The receptive field properties always reverted to the original state over a period of 2–30 min following cessation of the bicuculline application. The post-stimulus histograms in Fig. 1i–l clearly show the reappearance
of the ‘on’ and ‘off’ subdivisions of the receptive field and the loss of the response to flashing the whole receptive field. This recovery of the original receptive field properties would seem to exclude the possibility that the effects observed resulted from a spontaneous change in the receptive field of the neurone, or its excitability, or that the changes were secondary to eye movements. Further to this latter point, the receptive fields were

![Diagram](image)

Fig. 1. Action of iontophoretically applied bicuculline on the response of a simple cell to stationary flashing stimuli. Each post-stimulus histogram represents twenty-five consecutive responses to the presentation of a 500 msec flash every 2.0 sec. The arrow on the diagram above each set of post-stimulus histograms indicates the region of the receptive field stimulated. The diagrams only show characteristics of receptive field organization, they do not represent relative dimensions of the ‘on’ or ‘off’ regions. Horizontal bars under each record mark the occurrence and duration of the flash. Vertical calibration bar shows range corresponding to 0–200 spikes/sec. Data accumulated in 20 msec bins. a–d, response of the cell prior to the application of bicuculline. e–h, response of cell during the iontophoretic application of bicuculline (+140 nA). Records taken after initial period of 20 min continuous application. i–l, response of cell 18 min after termination of bicuculline application.

carefully examined during the bicuculline application to ascertain whether there was any ‘new’ division of the receptive field into ‘on’ and ‘off’ regions with slightly different or more critically defined locations. In none of the fifteen cells was it possible to distinguish separate ‘on’ or ‘off’ regions in the receptive field during the iontophoretic application of bicuculline.

The orientation specificity and directional specificity of simple cells was also modified by the iontophoretic application of bicuculline. This is illustrated by the response of the simple cell shown in Fig. 2. In this example prior to the application of bicuculline the cell exhibited a signifi-
cant response only to the forwards motion of the slit at the optimal orientation (Fig. 2a–e). During the application of bicuculline there was a notable response to both directions of motion and a response to the testing orientations 22.5° either side of the optimal (Fig. 2f–j). In all the directionally specific simple cells examined (thirteen) bicuculline produced a large reduction or an elimination of the directional specificity.

Fig. 2. Modification of the response of a simple cell to a moving slit during the iontophoretic application of bicuculline. Post-stimulus histograms show responses of cell to a range of testing orientations comprising, −22.5°, the optimal, +22.5°, +45°, 90°. See text and Methods for further details. Each post-stimulus histogram constructed from forty cycles of stimulus motion over the receptive field. Vertical calibration indicates range corresponding to 0–125 spikes/sec. Bin size 20 msec. Horizontal calibration bar corresponds to 1.0 sec. Portion of post-stimulus histogram to left of dashed line shows response to forwards motion over field, portion to right corresponds to return of slit over field. Direction of motion and orientation indicated by diagrams above records. a–e, records taken prior to the application of bicuculline. f–j, effect of iontophoretic application of bicuculline (+60 nA), records taken after 20 min continuous application. (k) effect of raising neural spontaneous activity with iontophoretically applied glutamate (−40 nA) on response to optimal orientation. Record taken 30 min after termination of bicuculline application.

These changes were not reproduced by using iontophoretically applied glutamate to raise the excitability of the neurone. The record in Fig. 2k shows the effect of glutamate on the directional response of this cell. Although there is a small response to both directions of motion the magnitude of the evoked response to the normally effective direction of motion is considerably reduced both with respect to the record taken during the application of bicuculline and the normal record.

The application of bicuculline produced a similar reduction in the orientation specificity of all the eighteen simple cells showing a block of the inhibitory action of iontophoretically applied GABA. The over-all effect
of bicuculline in simple cell orientation specificity is best illustrated by the orientation tuning curves in Fig. 3 for two simple cells before and during the application of bicuculline. The curves show the response of the cells to the eight standard testing orientations (see Methods). The hori-

![Graph showing orientation tuning curves](image)

Fig. 3. (a) action of iontophoretically applied bicuculline on simple cell orientation tuning. Curves show response of cell to the range of testing orientations as indicated on the abscissa. Responses for one direction of motion over the receptive field, selected as the preferred direction at the optimal orientation and continued through other testing orientations (directionally specific cell). See section on presentation of results in Methods for further details. Continuous line with open circles shows response of cell prior to the application of bicuculline. Interrupted line with filled circles shows response of cell during the application of bicuculline (+150 nA). Results taken after initial period of 15 min continuous application. Horizontal lines to right of curves indicate levels of spontaneous activity. Cell illustrated shows no increase in magnitude of evoked response to optimal orientation during application of bicuculline. (b) tuning curves for cell showing large increase in magnitude of evoked response during the application of bicuculline. Bicuculline applied with +140 nA, results taken after initial period of 8 min application. All other details as for (a).

It is clear that before the application of bicuculline both the simple cells illustrated were sharply tuned towards the optimal orientation. In each case during the application of bicuculline there was a definite broadening of the orientation tuning curve. In Fig. 3a the magnitude of the evoked response to the optimal orientation was not increased after an initial 15 min period of bicuculline application but the testing orientation at $-22.5^\circ$ produced a response that was approximately
equal to the response to the optimal where previously it had little effect. Relative to the response to the optimal there was also some increase in the response to the testing orientations at +22.5° and +45°. This broadening of the orientation tuning curve did not appear to relate to a ‘saturation effect’ preventing further increases in the response magnitude to the optimal orientation, because a subsequent extended period of bicuculline application in an attempt to produce a further reduction in orientation specificity produced a considerable increase in response magnitude although no further reduction in orientation specificity. The simple cell in Fig. 3b although originally giving a similar magnitude evoked response to the optimal orientation as the cell in Fig. 3a showed an approximate doubling of the response amplitude during the initial period of bicuculline application as well as an apparent slight broadening of the tuning curve. Furthermore even when the increase in spontaneous activity is taken into account it appears that the cell actually responded to all the testing orientations including 90° to the optimal. Only four of the eighteen simple cells examined exhibited a response to all the testing orientations during the application of bicuculline. The effect of bicuculline on another of these four cells is illustrated by the post-stimulus histograms in Fig. 4. The response of the simple cell in Fig. 4 to the testing orientations at +45 and 90° to the optimal can be seen to include a transient peak as the slit moved forwards over the receptive field, in fact this was the only component of the response observed at 90° to the optimal and was moreover evoked by one direction of motion only. Where simple cells exhibited a response to the orientation at 90° to the optimal it always consisted of this type of phasic component evoked slightly before the slit crossed the field centre location as judged by the other responses of the cell. The records in Fig. 4 show the response of the cell 10 min after termination of the bicuculline application. There is no doubt that the original specificity of the cell was regained, in fact there was a reduction in the responsiveness of the cell. This type of subnormal responsiveness was often seen to occur for periods of up to 30 min after termination of the bicuculline application.

2. Neurones with ‘complex’ type receptive field properties

Complex cells were distinguished by their high spontaneous activity, larger receptive fields and slightly more broadly tuned orientation specificity in comparison with simple cells. None of the complex cells examined here had receptive fields that could be subdivided into ‘on’ or ‘off’ regions on the basis of their response to stationary flashing stimuli. Some, however, were activated by small stationary flashing stimuli located within their receptive field and gave ‘on–off’ responses. Large stationary flashing
stimuli covering the whole receptive field were in general relatively ineffective.

The effect of the iontophoretic application of bicuculline was examined in thirty-one complex neurones and found to produce a block of the inhibitory action of iontophoretically applied GABA and a modification of receptive field properties in twenty-four of these. In three of the remaining seven, bicuculline failed to produce a block of the inhibitory action of iontophoretically applied GABA and caused no detectable alteration in receptive field properties. For the other four neurones although receptive field properties were unaffected during the application of bicuculline the inhibitory action of iontophoretically applied GABA was blocked. Of these latter four neurones three were atypical in so far as they have an extremely high spontaneous activity and a rather low excitatory drive as judged by the amplitude of the evoked response to the optimal orientation.

Where bicuculline produced a modification of complex cell receptive field properties the most notable effect was a decrease in orientation specificity. For sixteen neurones within this group the decrease in orientation specificity involved a marked response to all the testing orientations including $90^\circ$ to the optimal. In contrast to the effects seen in simple cells the over-all orientation specificity of these complex cells was considerably reduced and often the response even at $90^\circ$ to the optimal closely approximated the response to the optimal orientation. This is illustrated by the

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*Fig. 4. Further example of the effect of bicuculline on simple cell orientation specificity. Range of testing orientations as for Fig. 3. Post-stimulus histograms constructed from response to twenty-five cycles of stimulus motion. Vertical calibration 0–266 spikes/sec. Bin size 15 msec. Horizontal bar calibration 1-0 sec. (a) normal response of cell. (b) response of cell during the iontophoretic application of bicuculline (+ 140 nA). Records taken after initial 18 min application period. (c) response of cell 20 min after termination of bicuculline application.*
post-stimulus histograms in Fig. 5 constructed for the response of a complex cell to moving slits at the optimal orientation and 90° to the optimal orientation. During the bicuculline application the magnitude of the evoked response to the optimal orientation was considerably increased and the slit at 90° to the optimal produced a large evoked response where previously it was ineffective. The response to the slit at 90° to the optimal appeared to be evoked from a rather larger discharge field than that for the slit at the optimal orientation. It can also be seen that the peak frequency of the evoked response to the orientation at 90° to the optimal was greater than that of the response to the backwards motion of the slit at the optimal orientation. The development of the response of this cell to the slit at 90° to the optimal is shown in Fig. 6. This consists of post-stimulus histograms constructed prior to the application of bicuculline and after 5, 10 and 15 min continuous bicuculline application. No additional changes were produced by the application of bicuculline beyond the 15 min period. Approximately 10 min after cessation of the bicuculline application the receptive field properties reverted to the original state as demonstrated by the records in Fig. 5c. There was a slight reduction in the neurone’s excitability at this period which is comparable to the similar effect observed in the simple cells. For most of the simple and complex cells examined the effect of bicuculline on receptive field properties developed over a period of 5–15 min with currents in the range 60–160 nA to reach a definite maximum beyond which no further changes were produced.

The action of bicuculline on complex cell receptive field properties is further illustrated in Fig. 7. In this case the response of the cell is represented as a post-stimulus histogram using a dot display where the height of the dots above the base line indicates the number of counts in a given bin rather than the solid bars used in the other records. Although in contrast to the cell shown in Fig. 5 the application of bicuculline had little effect on the response to the optimal orientation (Fig. 7b) it still revealed a clear response to the testing orientation at 90° to the optimal. The recovery of the cell from the effects of bicuculline was relatively slow, and the cell which tended to have a high spontaneous activity, exhibited a mixed excitatory and inhibitory response to the slit at 90° to the optimal when tested 10 min after terminating the bicuculline application. Recovery was essentially complete after 20 min although the spontaneous activity was reduced with respect to normal (Fig. 7d). Raising the neural spontaneous activity with iontophoretically applied glutamate did not result in any response to the slit at 90° to the optimal (Fig. 7e) but a repeated application of bicuculline once more revealed a clear response (Fig. 7f).
The over-all effect of bicuculline on complex cell orientation specificity was such that even when it produced large responses to the testing orientations at 90° to the optimal as shown in Figs. 6 and 7 it did not produce an equal increase in the response to all the testing orientations. This is illustrated in Fig. 8a by the orientation tuning curves for a complex cell before and during the iontophoretic application of bicuculline. During the application of bicuculline, although the response to the testing orientations at −67.5°, −45° and 90° actually exceeded the response to the optimal orientation, the responses to −22.5°, +22.5° and +45° were not increased in proportion. The variation in the orientation response did not have any obvious relation to the sequence in which the data were taken, with for example the least responsive points being neither the first nor the last data taken during the application. It was however for this cell and all the

![Fig. 5. Effect of the application of bicuculline on the response of a complex cell to a moving slit at the optimal orientation and at 90° to the optimal. Post-stimulus histograms constructed from twenty-five cycles of stimulus motion. Vertical calibration 0–200 spikes/sec. Bin size 20 msec. Horizontal calibration bar indicates 1.0 sec. (a) normal response of cell. (b) response of cell during application of bicuculline (+140 nA). Records taken after initial period of 15 min continuous application. (c) response of cell 25 min after termination of bicuculline application.](image-url)
cells examined very difficult to maintain a steady bicuculline current throughout the whole course of the tests and small adjustments to the applied voltage were nearly always necessary. Hence the effectiveness of the block might be expected to vary. It is also notable in Fig. 8a that prior to the application of bicuculline several of the testing orientations reduced the spike count well below the spontaneous level, presumably reflecting

![Diagram](image)

**Fig. 6.** Post-stimulus histograms showing the development of the response of the complex cell in Fig. 5 to the slit at 90° to the optimal. (a) response prior to the application of bicuculline. (b) response after 5 min application. (c) response after 10 min. (d) response after 15 min. All other details as for Fig. 5.

the presence of an inhibitory input evoked by these stimuli. During the application of bicuculline none of the testing orientations reduced the spike count below the spontaneous level.

The orientation specificity of the remaining eight of the twenty-four neurones which exhibited modifications of receptive field properties during the iontophoretic application of bicuculline was affected to a much smaller
Fig. 7. Further example of the effect of bicuculline on the response of a complex cell to a moving slit at the optimal orientation and 90° to the optimal orientation. Post-stimulus histograms constructed from response to thirty-two cycles of stimulus motion. Vertical calibration 0–156 spikes/sec. Bin size 20 msec. Horizontal calibration 1·0 sec. (a) response of cell prior to the application of bicuculline. (b) effect of iontophoretic application of bicuculline (+120 nA) on the response of the cell. Records taken after an initial period of 15 min continuous application. (c) response of cell 10 min after termination of bicuculline application. (d) response of cell 20 min after termination of bicuculline application. (e) response to slit at 90° to optimal whilst raising spontaneous activity with iontophoretically applied glutamate (−40 nA). (f) effect of repeated application of bicuculline (+120 nA) on response of cell to slit at 90° to optimal. Record taken after 12 min continuous application.
extent. The cells exhibited increases in the evoked response and in some cases a response to all the testing orientations but not a reduction in the over-all orientation specificity. The tuning curves in Fig. 8b show the effect of bicuculline on one of these cells. During the iontophoresis of bicuculline there was a response to all the testing orientations but the magnitude of the evoked responses to the previously effective orientations were increased approximately equally. The orientation specificity was slightly reduced with the testing orientations at +22.5 and +45° to the optimal producing relatively to the optimal larger effects than before. It is not possible to ascertain on the basis of the present results whether or not the effects of bicuculline on the eight cells in this group of complex cells reflects a functional difference with respect to the other sixteen cells showing larger changes in the orientation specificity. It is feasible that the
difference merely reflects variations in the effective diffusion of bicuculline to the requisite synapses.

The directional specificity of complex cells tended to be rather less affected by the application of bicuculline than that of simple cells. In only two of the complex cells examined was it essentially eliminated and in most cases it was slightly reduced or unaffected. Thus even where the iontophoresis of bicuculline resulted in large changes in orientation specificity the directional specificity was often unaffected. For example in the case of the neurone illustrated in Fig. 5, it is interesting to note that the application of bicuculline although increasing the evoked response to the optimal orientation, did not markedly affect the directional specificity, because the relationship between the evoked responses to the forwards and backwards motion of the slit remained very similar. Conversely the response to the slit at 90° to the optimal as shown in Fig. 5 during the bicuculline application was not directionally specific. However if the development of the response to the slit at 90° to the optimal as shown in Fig. 6 is considered, it is clear that the response after 5 and 10 min of the bicuculline application is directionally specific but not after 15 min. It is possible that the mechanisms contributing to directional specificity are not as effective for slit orientations at 90° to the optimal and hence the difference. The iontophoretic application of bicuculline also resulted in complex cells giving large ‘on–off’ responses to stationary flashing stimuli covering their whole receptive field. Complex cells were normally found to be relatively unresponsive to these stimuli. A detailed study of the effects of bicuculline on the response of complex cells to stationary flashing stimuli was however not made in the experiments reported here.

3. Neurones with ‘hypercomplex’ type receptive field properties

Only two hypercomplex cells were examined in the present work. They were both typical ‘end stopped’ cells (Hubel & Wiesel, 1965) and in neither case did the iontophoretic application of bicuculline produce any modification of their receptive field properties. For one of these cells however the bicuculline application produced only a partial block of the action of iontophoretically applied GABA although an effective block was produced in the other. Considering the fact that a number of both simple and complex cells in the present sample were also unaffected as regards receptive field properties despite a block of the action of iontophoretically applied GABA, these results cannot in isolation be taken as indicating that hypercomplex cells are, or are not, unique from other types of visual neurone, with respect to the action of bicuculline.
4. Neurones with low orientation specificity

Three cells with a very low orientation specificity were encountered. In most ways they were typical of complex cells with high spontaneous activity and large discharge fields. However their orientation specificity, although biased towards an optimal range was much lower than normally seen in complex cells. None of the three responded to large stationary flashing stimuli although two were well activated by small rectangular stimuli flashing within the receptive field. The application of bicuculline resulted in a response to large flashing stimuli but had little other effect.

![Diagram](https://example.com/diagram.png)

**Fig. 9.** Action of bicuculline on cell that appeared to be unaffected by visual stimuli. Post-stimulus histograms constructed from response of cell to twenty-five cycles stimulus motion. Vertical calibration 0–120 spikes/sec. 50 msec bins. Horizontal bar calibration 1.0 sec. (a) before application of bicuculline. (b) during application of bicuculline (+140 nA). Record taken after 12 min period of continuous application.

5. Neurones apparently unaffected by visual stimuli

During the course of the present experiments the effect of bicuculline was examined on four neurones that as judged in terms of an excitatory response were apparently unaffected by the usual range of visual stimuli. In each case the response of the cell was tested during the application of bicuculline by a slit moving over the receptive field at an orientation that had previously been found to drive the background activity recorded in the vicinity of the electrode. In three of these cells the iontophoretic application of bicuculline produced a block of the inhibitory action of iontophoretically applied GABA and revealed a response to the testing slit. This is illustrated for one of these cells in Fig. 9. The other two of these three cells were found to show an inhibition of their resting discharge in response to the testing slit prior to the application of bicuculline. The effect of bicuculline on one of these is shown in Fig. 3 in the preceding paper (Sillito, 1975). The application of bicuculline produced a block of the
action of iontophoretically applied GABA in the remaining one of these four cells tested but did not reveal a response to visual stimuli. The possibility cannot be excluded that these cells were in fact hypercomplex cells for which the appropriate stimulus had not been determined. Although recently there have been other reports of cells in the visual cortex showing only inhibitory responses (Innocenti & Fiore, 1974).

**DISCUSSION**

In the present work the iontophoretic application of bicuculline has been found to produce modifications of the receptive field properties of simple and complex cells in the striate cortex of the cat. For simple cells these modifications involved an apparent loss of the antagonistic 'on' and 'off' subdivisions of the receptive field, a small reduction in orientation specificity and a large reduction or elimination of directional specificity. The main effect of bicuculline on complex cell receptive field properties was to produce a change in orientation specificity resulting in many cases in what appeared to be a virtual loss of the original specificity. The assumption underlying these experiments has been that during the application of bicuculline, because of a block of GABA-mediated intracortical inhibitory processes (Iversen et al. 1971; Curtis & Felix, 1971; Sillito, 1975), the responses of the cells will reflect the nature of their excitatory input. Whilst some of the receptive field characteristics observed during the application of bicuculline do in fact reflect the predicted nature of the excitatory input others are not easily equated with the previously accepted views on this matter (Hubel & Wiesel, 1962).

With respect to the elimination of simple cell directional specificity, the present results are consistent with data obtained from intracellular investigations (Benevento et al. 1972; Innocenti & Fiore, 1974) which indicate that the directional specificity is generated by the specific action of a post-synaptic inhibitory process modifying the response to a non-directionally specific excitatory input. The action of bicuculline on directional specificity is thus what would be predicted if this post-synaptic inhibitory process were blocked. Likewise the reduction in simple cell orientation specificity produced by bicuculline is compatible with the loss of a lateral type inhibitory process acting in the orientation domain (Benevento et al. 1972; Blakemore & Tobin, 1972) and serving to enhance the orientation tuning of an already orientation specific excitatory input (Hubel & Wiesel, 1962).

In contradistinction to its action on the orientation and directional specificity of simple cells the effects of bicuculline on the 'on' and 'off' subdivisions of the simple cell receptive field are not easily explained. It
INHIBITORY MECHANISMS IN THE STRIATE CORTEX 325

has generally been accepted that the elongated and parallel 'on' and 'off' subdivisions of the simple cell receptive field relate to the convergent excitatory input from one or more groups of respectively all 'on' or all 'off' centre geniculate cells with their receptive fields extending in a 'row' through visual space (Hubel & Wiesel, 1962). However the loss of the antagonistic 'on' and 'off' subdivisions during the application of bicuculline implies the presence of an excitatory input that is not spatially organized in terms of forming discrete 'on' and 'off' response areas. This can be interpreted in two ways, either the 'on' and 'off' subdivisions of the simple cell receptive field result from the action of a highly specific intracortical inhibitory process modifying an excitatory input from a randomly mixed group of 'on' and 'off' centre geniculate cells, or there are two types of excitatory input converging on simple cells. In the latter case one excitatory input would be the organized convergence of specific groups on 'on' and 'off' centre geniculate fibres (Hubel & Wiesel, 1962) and the other would involve an excitatory input without this organization and normally suppressed by an intracortical inhibitory mechanism. Whilst neither of these explanations are entirely satisfactory it appears clear that the 'normal' appearance of the 'on' and 'off' subdivisions of the simple cell receptive field is dependent in some way on the action of an intracortical inhibitory process.

Comparable difficulties relate to the interpretation of the changes in complex cell orientation specificity produced by bicuculline. The large loss of the original specificity observed in many of the cells implies that for these cells the excitatory input was relatively unspecific as regards orientation, and that the orientation specificity derived from the action of a highly specific intracortical inhibitory process. This is a somewhat controversial conclusion because it has generally been accepted that the excitatory input to complex cells is derived from a group of simple cells of similar orientation specificity (Hubel & Wiesel, 1962). This type of excitatory input would theoretically confer a basic orientation specificity on the cell which would not be eliminated by the application of bicuculline. The change in orientation specificity cannot be explained in terms of bicuculline diffusing to the simple cells providing the input because when applied directly to simple cells bicuculline produced only small changes in their orientation specificity. This point is particularly notable for example when comparing the responses of simple and complex cells to a slit at 90° to the optimal during the application of bicuculline. There is however other evidence indicating that the excitatory input to complex cells may not be derived from simple cells (Hoffman & Stone, 1971; Movshon, 1974). In so far as the present results indicate that the excitatory input to all complex cells may not be orientation specific they support this view.
A further possibility to be considered in the interpretation of these results is that changes observed in orientation specificity appeared larger than they in fact were because the excitatory responses to the optimal orientation were 'saturating'. This would exert a levelling effect with respect to the response to other orientations and give for example, in the case of complex cells, a false impression that the excitatory input was not orientation tuned. This possibility cannot be completely excluded for all the cells examined, for some of both the simple and complex cells described, further increases in the amplitude of the excitatory response were produced by an extended period of bicuculline application after the data for the tuning curves had been taken. Moreover for many of the cells decreases in orientation specificity were produced by bicuculline at a stage that the evoked response to the optimal orientation was relatively unaffected, and in some cases responses to non-optimal orientations exceeded the response to the optimal. It seems unlikely therefore that response 'saturation' represents the main factor in the reductions observed in either complex or simple cell orientation specificity. However further investigation of this problem is at present in progress.

The fact that iontophoretic application of bicuculline did not produce changes of equal magnitude in the orientation specificity of all the complex cells examined may not necessarily reflect differences between complex cells but rather the varying position of the micro-electrode with respect to the neurone and the effective distribution of the drug. Considering complex cells to be pyramidal cells (Kelly & van Essen, 1974) and regarding the extent of the dendritic arborisation of a pyramidal cell in the visual cortex (Szentagothai, 1973) it seems extremely unlikely that it would be possible to position the micro-electrode so that there was an effective recording of the neuronal response and uniform distribution of the drug over the dendritic field. Hence it is likely that only some inhibitory synapses would be affected in any particular recording situation and that this would vary from neurone to neurone according to the relative electrode position. Much less variation was seen in the effects of bicuculline on simple cell receptive field properties than complex cells. If simple cells are stellate cells (Kelly & van Essen, 1974) this may reflect a rather more uniformly spreading dendritic field in closer proximity to the cell body, such that there would be a more uniform distribution of the drug over the dendritic field from an electrode effectively positioned for recording the neuronal discharge.

In two recent investigations intravenously injected bicuculline and bicuculline topically applied to the cortical surface have been used to attempt to block the action of GABA at inhibitory synapses in the visual cortex (Daniels & Pettigrew, 1973; Rose & Blakemore, 1974). In both
cases modifications in receptive field properties were reported, these included a small reduction in complex cell orientation specificity but not the large changes observed for many complex cells in the present work. Daniels & Pettigrew found that intravenously injected bicuculline produced only a depression of simple cell responses and no decrease in receptive field specificity, whilst with topically applied bicuculline Rose & Blakemore found a reduction in orientation tuning and a three- to fivefold increase in receptive field size. The present results certainly do not support Daniels & Pettigrew's observations concerning the effect of bicuculline on simple cells, but they are essentially similar to those of Rose & Blakemore with respect to simple cell orientation tuning although not with respect to a three- to fivefold increase in receptive field size. As judged from the response to moving stimuli in the present work, the iontophoretic application of bicuculline produced little increase in the discharge field size of simple cells. There are certain problems in interpreting the effects of intravenously injected or topically applied bicuculline which make the results difficult to interpret. Intravenously injected bicuculline produces changes in the excitability of many parts of the central nervous system including possible actions on the retina and lateral geniculate body (Straschill & Perwein, 1969; Curtis & Tebecis, 1972) and the changes in receptive field properties could reflect these rather than a specific block of inhibition within the visual cortex. In applying bicuculline directly to the cortex Rose & Blakemore avoided the latter criticism. However bicuculline applied in this manner would still affect inhibitory processes throughout the visual cortex, producing changes in the over-all excitability and hence possible modifications of receptive field properties via changes in intracortically mediated excitatory influences acting on the neurones examined. Furthermore in these conditions the properties of the inhibitory neurones and hence their action on the cells examined may also be altered. Finally it is highly likely that large changes in the over-all excitability of the visual cortex would result in considerable modifications of the corticofugal input to the lateral geniculate body, with resultant changes in the input to the cortex.

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The contribution of inhibitory mechanisms to the receptive field properties of neurones in the striate cortex of the cat.

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