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INHIBITORY PROCESSES UNDERLYING THE DIRECTIONAL SPECIFICITY OF SIMPLE, COMPLEX AND HYPERCOMPLEX CELLS IN THE CAT'S VISUAL CORTEX

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SUMMARY

1. The iontophoretic application of bicuculline, an antagonist of GABA, the putative inhibitory transmitter in the visual cortex, has been used to examine the contribution of post-synaptic inhibitory processes to the directional selectivity of simple, complex and hypercomplex cells in the cat's striate cortex.

2. The directional selectivity of simple cells was significantly reduced or eliminated during the iontophoretic application of bicuculline. This supports the view that the selectivity is derived from the action of a GABA-mediated post-synaptic inhibitory input modifying their response to a non-directionally specific excitatory input.

3. Complex cells were subdivided into three categories on the basis of the action of iontophoretically applied bicuculline on their directional selectivity, receptive field characteristics and distribution in terms of cortical layer. They are referred to as type '1', '2' and '3' complex cells.

4. The directional specificity of type '1' complex cells was eliminated during the iontophoretic application of bicuculline. It seems likely, therefore, that they receive a non-directionally specific excitatory input and that, as for simple cells, the directional specificity derives from the action of a GABA-mediated post-synaptic inhibitory input. No type '1' complex cells were recorded below layer IV.

5. The directional specificity of type '2' complex cells was unaffected by the iontophoretic application of bicuculline, despite increases in response magnitude, a block of the action of iontophoretically applied GABA and, in some cases, changes in other receptive field properties. It is suggested that these cells receive a directionally specific excitatory input. The type '2' complex cells were found both superficial and deep to layer IV with the majority in layer V.

6. Type '3' complex cells appear to have very similar receptive field

properties to those of the cells described by other workers as projecting to the superior colliculus. They were found predominantly in layer V. Their directional specificity was not eliminated by the iontophoretic application of bicuculline. However, they exhibited a powerful suppression of the resting discharge in response to stimulus motion in the non-preferred direction. Iontophoretic application of ammonium ions revealed a small excitatory response in place of the suppression. It appears from these observations that the directional specificity of the type '3' complex cells could be determined, at least in part, by an inhibitory process which is not GABA-mediated.

7. The directional specificity of hypercomplex cells found in layers II and III was unaffected by the iontophoretic application of bicuculline, and they showed no suppression of their background discharge level in response to stimulus motion in the non-preferred direction. This evidence is consistent with the view that they receive a directionally specific excitatory input.

INTRODUCTION

Many visual cortical cells exhibit a strong selectivity to one of the two possible directions of motion of an optimally oriented slit in a plane at 90° to its long axis. These cells are described as exhibiting directional selectivity. On the basis of data obtained from the use of intracellular recording techniques, it would appear that this derives from the action of a powerful directionally specific post-synaptic inhibitory input which modifies their response to a non-directionally specific excitatory input (Benevento, Creutzfeldt & Kuhnt, 1972; Innocenti & Fiore, 1974). Goodwin & Henry (1975) have recently argued however that, while this type of mechanism may operate for simple cells, it does not apply to complex cells which they suggest receive a directionally specific excitatory input. The main evidence for their conclusions was that a stimulus moving in the null direction over a complex cell receptive field exerted no effect on the resting discharge level, while for simple cells the spontaneous or driven background activity was suppressed. They suggested that the excitatory input to complex cells is derived from directionally specific simple cells in accord with the original model of Hubel & Wiesel (1962) concerning the hierarchical organization of the visual cortex. If the conclusions of Goodwin & Henry (1975) concerning the differing processes contributing to simple and complex cell directional specificity are correct, this would imply that the intracellular data of Benevento *et al.* (1972) and Innocenti & Fiore (1974) was only obtained from simple cells. Unfortunately, in neither of the intracellular studies are receptive fields defined as simple or complex, but with respect to evidence indicating that simple cells are stellate cells, and complex cells are pyra-

midal cells (Kelly & van Essen, 1974) it would seem likely that the sample would be biased towards the larger pyramidal cell type, and hence complex cells, rather than the other way round. It is also difficult to reconcile the conclusions of Goodwin & Henry (1975) with other evidence favouring a parallel, rather than a serial, processing of the visual input through simple and complex cells (Hoffman & Stone, 1971; Movshon, 1975; Hammond & McKay, 1975).

Neuropharmacological techniques have been used recently to evaluate the contribution of inhibitory mechanisms to the receptive field properties of neurones in the visual system (Sillito, 1975*a*,*b*, 1976). Iontophoretic application of bicuculline, the antagonist of GABA, the putative inhibitory transmitter in the visual cortex (Iversen, Mitchel & Srinivasan, 1971), to simple and complex cells has been reported to produce a large reduction in simple cell directional specificity but to have less effect on complex cell directional specificity (Sillito, 1975*b*). The implication is that GABA-mediated inhibitory processes are important to the generation of simple cell directional specificity but not that of complex cells. This is in accord with the conclusions of Goodwin & Henry (1975); however, the presence of some complex cells in which directional specificity was changed by bicuculline and the fact that the total sample of directionally specific cells studied was small leaves room for some doubt on the matter. The experiments described here constitute a more detailed investigation of this problem using neuropharmacological techniques and include a study of the directional specificity of hypercomplex cells. The results obtained confirm the original conclusions regarding simple cells but suggest a clear subdivision of the complex cell category with respect to directional specificity.

METHODS

The experiments were carried out on cats in the weight range 2·3–3·0 kg. Anaesthesia was induced with a mixture of 70% N_2O, 30% O_2 (v/v) and 2·0–3·5% (v/v) halothane. It was maintained subsequently throughout the course of the experiment by a 75% N_2O, 25% O_2 (v/v) mixture with the addition of 0·1–0·5% (v/v) halothane, as necessary, to sustain an adequate depth of anaesthesia. The animals were immobilized by an I.V. infusion of gallamine triethiodide at a rate of 10 mg/kg.hr. Further details of the preparation and care of the animals throughout the experiment and the general methods are given elsewhere (Sillito, 1975*a*,*b*).

Five barrel micropipettes were used for the extracellular recording of neuronal activity and the iontophoretic application of drugs. The centre recording barrel contained a solution of 0·5 M-sodium acetate with 1% (w/v) pontamine blue, which enabled the recording site to be marked by the technique described by Hellon (1971). The other four barrels contained a selection of the following drugs made up in aqueous solution: DL-homocysteate (Fluka Chemicals Ltd) 0·2 or 0·5 M adjusted to pH 7·5 with NaOH; L-glutamate (Sigma Chemical Company), 0·2 M adjusted to pH 8 with NaOH; α-aminobutyric acid, GABA, (Sigma Chemical Company) 0·5 M adjusted to pH 3 with HCl; bicuculline (Fluka Chemicals Ltd) 5 mM in 165 mM-NaCl, solution

adjusted to pH 3 with HCl; strychnine (Sigma Chemical Company) 2 or 10 mM in 165 mM-NaCl; ammonium acetate or chloride (B.D.H.) 1·5 M solution. The retaining and ejecting currents used for controlling the release of the drugs were respectively: DL-homocysteate + 12 nA (retaining) and − 2–15 nA (ejecting); glutamate + 12 nA (retaining) and − 10–50 nA (ejecting); GABA − 18 nA (retaining) and + 1–50 nA (ejecting); bicuculline − 10 nA (retaining) and + 20–160 nA (ejecting); strychnine − 10 nA (retaining) and + 20–60 nA (ejecting); ammonium ions − 30 nA (retaining) and + 100–300 nA (ejecting). In many experiments, two barrels were filled with bicuculline. This procedure was adapted to overcome the difficulty in passing high currents for long periods through a micropipette containing bicuculline (Curtis *et al.* 1970). Generally, a current of between 20 and 80 nA was passed through each of the barrels simultaneously. Statements in the text referring to bicuculline-ejecting currents indicate total current passed with respect to both barrels.

Details of the optical stimulator and general optical procedures are given in preceding (Sillito, 1975*b*, 1976*b*). The maximum stimulus intensity was 34 cd/m^2 on a background of 17 cd/m^2. Where required, contrast was reduced in 0·1 log unit steps by interposing calibrated neutral density filters in the stimulator light path.

Histological procedures

The electrode recording sites were marked by passing a current of 2–8 μA for 3–10 min through the recording barrel with the electrode as the cathode; this deposited a small amount of pontamine blue in the tissue around the electrode tip. At the end of the experiment, the surface of the cortex underlying the small aperture in the skull (4 mm diameter), through which the penetrations has been made, was marked lightly with a black dye. The brain was then fixed in a solution of 10% (w/v) formal saline. A photographic record was made of the location of the mark on the surface of the cortex. Serial sections 50 μm thick were taken on a freezing microtome through the marked region of the cortex. The sections were stained with 1% (w/v) neutral red, mounted and the location of the blue spots marking the recording sites determined. Where statements are made about the distribution of cells in terms of cortical layer, these refer only to those cases where the recording position was marked clearly by pontamine blue dye. Layers were distinguished by criteria following the comments of Otsuka & Hassler (1962) and Garey (1971). The data obtained from the histological analysis is shown in Fig. 1.

Experimental procedure

In all cases, directional selectivity was assessed from the neuronal response to a slit at the optimal orientation moving over the receptive field in a regular cycle such that the dwell time during which the slit was stationary on either side of the field was equal for both directions of motion. Responses were assessed from either twenty-five or thirty-two complete cycles of stimulus motion (i.e. both directions) over the receptive field and the testing sequences were repeated several times to check for variability in the response pattern. Once the normal response had been documented, the response during the iontophoretic application of bicuculline was examined. The pharmacological effectiveness of bicuculline was routinely checked by ascertaining the degree of block produced in the inhibitory action of iontophoretically applied GABA. Cells tested have only been included in the sample if they showed a complete recovery from the effect of bicuculline within one hour after cessation of the drug application. For all cells where apparent reductions in directional specificity were observed, great care was taken to ensure that the increased excitability produced by the bicuculline had not resulted in a saturation of the excitatory response. Saturation of the excitatory response could limit the response to the preferred direction,

but still allow the response to the non-preferred direction to increase, thus producing an apparent reduction in directional specificity. This could lead to a false impression that a directionally specific inhibitory input had been blocked. The possibility that saturation had occurred was checked by ascertaining that further increases in magnitude of response could be produced after the time of making the observation on directional specificity. These subsequent increases were produced either by a further period of bicuculline application, or by increasing stimulus contrast or adjusting stimulus parameters in some other way. In many cases, it was possible to produce a doubling of the response magnitude and, in all cases, a significant increase was obtained. Where strychnine or ammonium ions were used to attempt to block the inhibitory input to a cell, the same testing procedures were used.

RESULTS

The data is derived from a total population of 102 neurones, all exhibiting directional selectivity and located in area 17. The interpretation of some of the observations is complicated by the problem of classifying those cells with receptive field characteristics intermediate to the categories of simple and complex. Often these could be assigned to one category or the other according to the particular classification scheme adopted (Hubel & Wiesel, 1962; Bishop & Henry, 1972; Sherman, Watkins & Wilson, 1976). Further to this, it is quite clear that the term complex cell, by any of the criteria adopted encompasses several distinct categories of cells. Even in the case of hypercomplex cells which are unequivocally defined in terms of length preference (Hubel & Wiesel, 1965) there is a problem in deciding whether certain directionally specific layer V cells exhibit a sufficient degree of length preference to be included in the category or not. For these reasons, the receptive field properties of each type of neurone discussed will be carefully defined.

Simple cells

Simple cells were categorized into two groups on the basis of whether or not they exhibited clearly defined 'on' and 'off' subdivisions in their receptive field. The first group comprises cells with such subdivisions. These cells correspond, in most ways, to the criteria applied by Hubel & Wiesel (1962) to simple cells. It was possible to demonstrate spatial summation within a subdivision, and for a given retinal eccentricity the receptive fields were smaller than complex cell receptive fields. The cells exhibited a higher degree of orientation selectivity than complex cells, and generally a lower level of spontaneous activity (0–3 impulses/sec), For all the cells in this group (fifteen), the iontophoretic application of bicuculline greatly reduced or eliminated directional selectivity as illustrated for two of the cells in Fig. 2(*A*, *B*). Each peristimulus histogram shows the response of the cell to a slit at the optimal orientation moving forwards over the receptive field (histogram to left of the dotted line) and then in

the opposite direction over the field to the original starting position (histogram to right side of the dotted line). In both cases, the response to the two directions of motion is almost equal during the application of bicuculline. The action of bicuculline on simple cell directional specificity developed rapidly and a maximal effect was often produced within 2–3 min of the on-set of the application with ejection currents in the range of 50–100 nA.

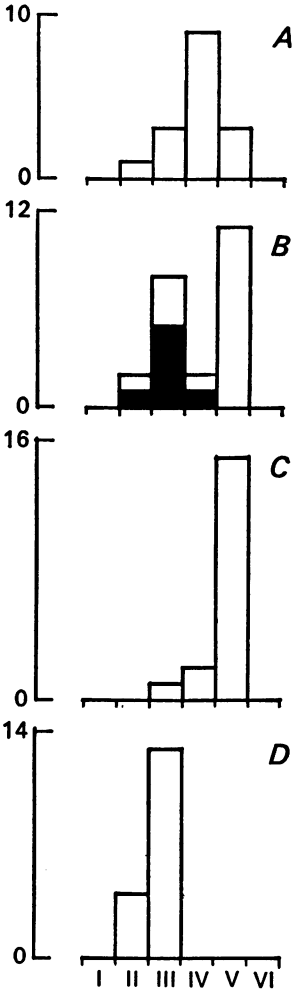

Fig. 1. Block histograms show distribution in terms of cortical layer of the various cell types discussed in this paper. In each case, vertical axis shows numbers of cells in a particular layer. *A*, simple cells, sixteen. *B*, type '1' and '2' complex cells, twenty-three. Shaded portion of histogram shows distribution of type '1' complex cells. *C*, type '3' complex cells, eighteen. *D*, hypercomplex cells, seventeen. Values following cell type indicate total number of cells marked.

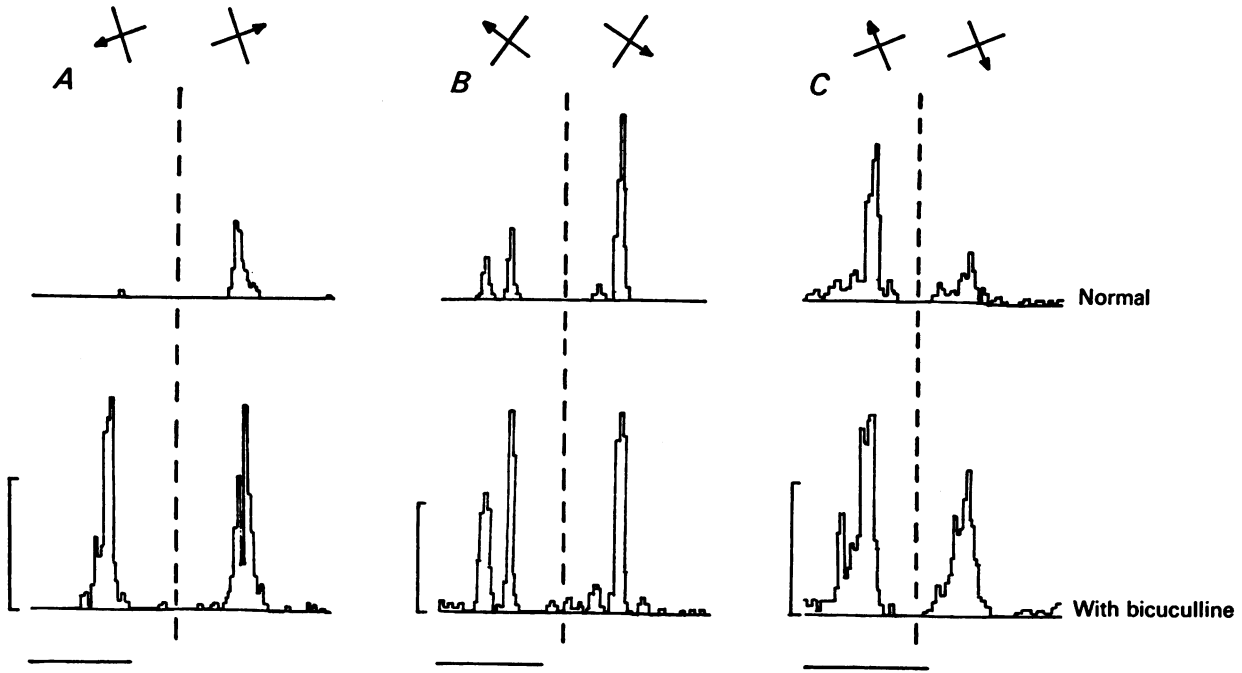

Fig. 2. Action of iontophoretically applied bicuculline on the directional specificity of simple cells. Post-stimulus histograms show the response of the cell to an optimally orientated slit of light moving forwards over the receptive field (histogram to left of the dotted line) and then returning across the field to original position (histogram to right of the dotted line). Upper records show normal responses of cells, lower records response during the iontophoretic application of bicuculline. *A*, responses of typical simple cell with clearly defined 'on' and 'off' subdivision to the receptive field. Vertical calibration bar indicates range corresponding to 0–100 counts/bin (0–200 impulses/sec). Horizontal calibration bar corresponds to 1·0 sec. Bin size 20 msec. No. of trials, twenty-five. Bicuculline applied with 70 nA current. *B*, further example of typical simple cell. Details as for *A* except that bicuculline applied with 100 nA current. *C*, responses of simple type cell lacking clearly defined 'on' and 'off' subdivisions. All details as for *A* except that records show responses for thirty-two trials and bicuculline applied with 90 nA current (vertical calibration 0–100 counts/bin, 0–156 impulses/sec). For further details see text.

The problem of classifying those cells which lack clearly defined 'on' and 'off' subdivisions, but otherwise exhibit simple cell receptive field characteristics, have been discussed elsewhere in the literature (Bishop & Henry, 1972; Sherman *et al.* 1976). It is still clearly open to debate as to whether they are or are not 'simple cells' in the terms originally envisaged by Hubel & Wiesel (1962). In the present work, the cells included in this second group either gave no response to a stationary flashing stimulus or gave weak and variable 'on' or 'off' responses, suggestive of the possibility of 'on' and 'off' subdivisions to the receptive field, but not consistent enough to test for spatial summation or map out the extent of the subdivisions. In all other respects, they appeared to be indistinguishable from typical simple cells. They showed low spontaneous activity (0–3 impulses/sec), a similar receptive field size for a given retinal disparity, good responses to slowly-moving stimuli ($< 5°$/sec), high orientation selectivity and were found mainly in the region of layer IV. The records in Fig. 2*C* show the effect of bicuculline on the directional selectivity of one of these cells. It produced a notable reduction but not an elimination of the original selectivity. Similar or smaller effects were produced in the directional selectivity of all the cells in this group (fourteen).

The distribution in terms of cortical layer of the cells classified here as simple, is shown in Fig. 1*A*. Although cells were found in layers II, III, IV and V, the majority of recordings were made in layer IV. This is consistent with the expected distribution of simple cells (Hubel & Wiesel, 1962).

Complex cells

On the basis of the action of bicuculline on their directional specificity, certain aspects of their response pattern, their distribution in terms of cortical layer and their over-all receptive field properties, complex cells were subdivided into three categories. For convenience the cells in the three categories will be referred to as type 1, 2 and 3 complex cells with the proviso that the type 3 category encompasses cells which could be regarded as hypercomplex. This is discussed in detail below.

(*a*) *Type '1' complex cells.* The type '1' category refers to the complex cells in which the application of bicuculline produced large reductions or an elimination of the directional specificity (eight). On initial examination, these cells appeared to be rather like simple cells, except that they gave a vigorous 'on–off' response throughout their receptive to a small stationary stimulus. The spontaneous activity was low (0–3 impulses/sec) and the receptive field widths were 1° or less for locations with 15° of the area centralis. They were sharply orientation-tuned, showing a marked reduction in response to small deviations (5°) from the optimal, and no

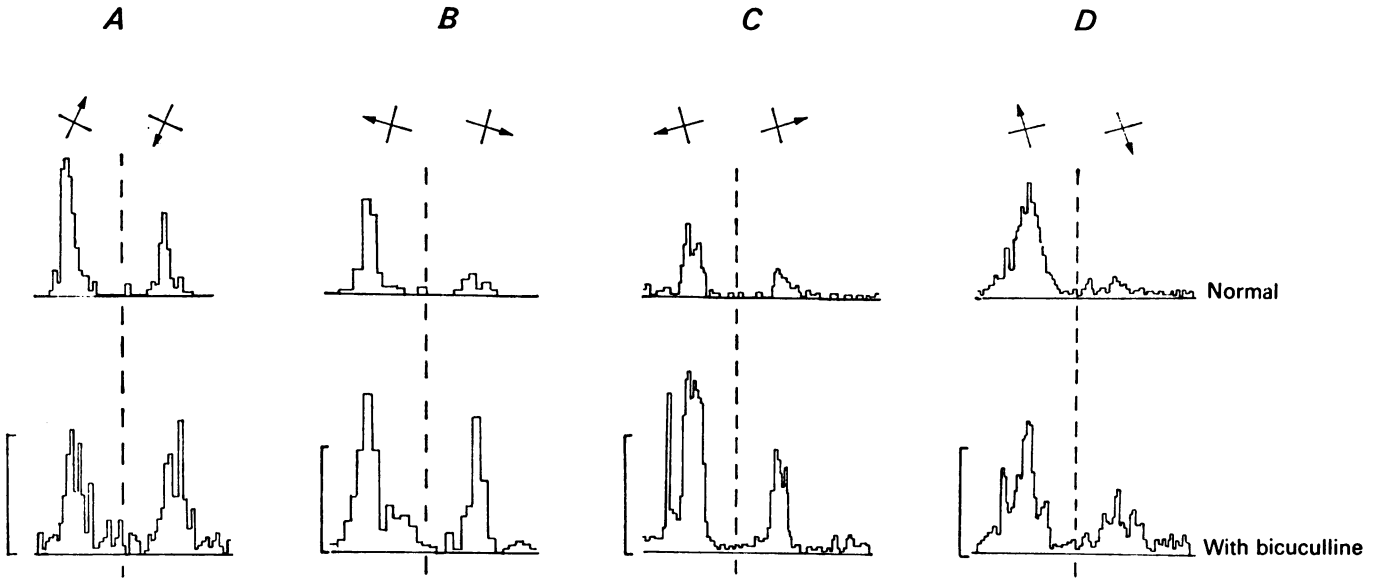

Fig. 3. Action of iontophoretically applied bicuculline on complex cell directional specificity. General details of records as in Fig. 1. *A*, type '1' complex cell. Vertical calibration bar indicates range corresponding to 0–100 counts/50 msec bin (0–80 impulses/sec) for the upper record (normal) and 0–200 (0–160 impulses/sec) for the lower record (during bicuculline application. No. of trials, twenty-five. Horizontal bar corresponds to 1·0 sec. Bicuculline applied with 40 nA ejecting current. *B*, further example of type '1' complex cell. Vertical bar indicates range corresponding to 0–100 counts/50 msec bin (0–80 impulses/sec); calibration same for upper and lower record. Horizontal bar indicates 1·0 sec. No. of trials, twenty-five. Bicuculline applied with 100 nA ejecting current. *C*, type '2' complex cell. All other details as for *B* except that bin size is 20 msec (vertical calibration 0–100 counts/bin, 0–200 impulses/sec) and bicuculline ejecting current 140 nA. *D*, type '2' complex cell. All details as for *B* except that bin size is 20 msec (vertical calibration 0–100 counts/bin, 0–156 impulses/sec). No. of trials, thirty-two and bicuculline ejecting current 90 nA. See text for details of type '1' and '2' complex cells.

significant response to testing orientations 30° either side of the optimal. They were found mainly in layer III, in contrast to simple cells, which were found mainly in layer IV (Fig. 1).

The effect of iontophoretically applied bicuculline on the directional selectivity of type 1 complex cells is illustrated in Fig. 3*A*,*B*. The application of bicuculline increased the magnitude of the evoked response and produced a considerable reduction in directional selectivity which, in the case of the cell in Fig. 3*A*, amounted to its virtual elimination. It is to be noted that the vertical amplification of the record in Fig. 3*A* is halved with respect to that of the upper record. The distinction between type 1 complex cells and simple cells is emphasized when their response during the application of bicuculline (Fig. 3*A*,*B*, lower records) is compared with that of typical simple cells (Fig. 2*A*,*B*, lower records). Quite different levels of spontaneous activity are involved, and the shape of the response profile in the post-stimulus histogram is also different. This was substantiated by the subjective impression derived from the sound of the response over the audio monitor, and the much higher level of driving that could be achieved in the case of the complex cells.

(*b*) *Type '2' complex cells.* The type '2' category refers to complex cells in which the application of bicuculline produced little or no reduction in directional selectivity. The cells in this group (nineteen) had larger receptive fields than type '1' complex cells, and generally a higher level of spontaneous activity (2–20 impulses/sec). The orientation selectivity was less than that of type '1' complex cells insofar as 5° deviations in the stimulus orientation from the optimal produced only small reductions in response magnitude, and generally they gave some excitatory response to orientations up to 45°, or even 60°, from the optimal. They gave either no response or an 'on–off' response to a stationary flashing stimulus. They were found in both the superficial layers of the cortex and layer V with the majority in layer V (Fig. 1*B*).

The records in Fig. 3*C*,*D* show the responses of type '2' complex cells. The cell in Fig. 3*C* exhibited a similar increase in response magnitude to the cell in Fig. 3*A*, but the peak height of the response to the null direction remained approximately 50% of the preferred direction response. In fact, that absolute difference between the response magnitudes even increased. The cell in Fig. 3*D* showed little change in response magnitude and a slight decrease in directional specificity. The ineffectiveness of the iontophoretic application of bicuculline on the directional specificity of these cells cannot be attributed to a lack of pharmacological effectiveness because, in all cases, it blocked the inhibitory action of iontophoretically applied GABA and, in many cases, produced changes in the magnitude of the evoked response and orientation selectivity.

(*c*) *Type '3' complex cells.* These complex cells all exhibited a high resting discharge (8–30 impulses/sec) which was suppressed by stimuli moving in the null direction. This contrasts with the complex cells in groups 1 and 2 which generally gave a small excitatory response to stimuli moving in the null direction. Reducing the length of the testing slit caused a large reduction in the orientation selectivity but did not affect the directional

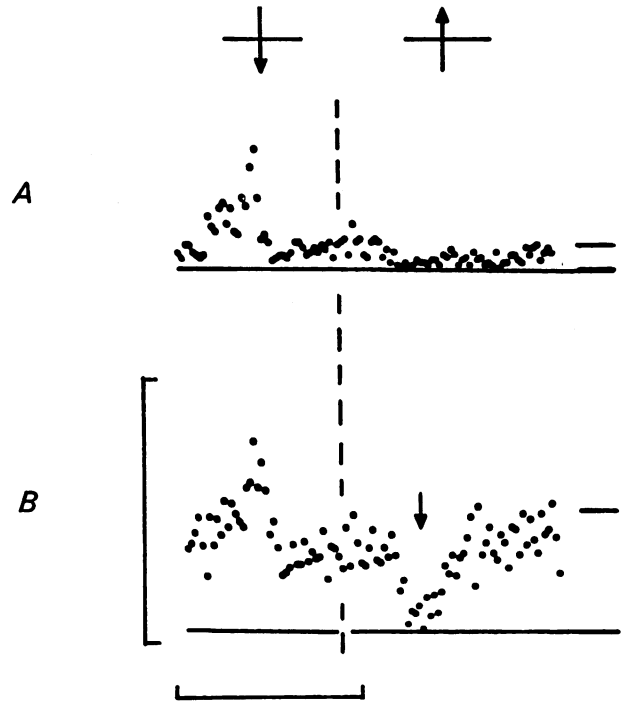

Fig. 4. Response of type '3' complex cell to optimally oriented slit moving over the receptive field. See text for details of type '3' complex cells. *A*, dot display post-stimulus histogram showing normal response of cell. *B*, response of cell when nesting discharge level increased by iontophoretic application of DL-homocysteate with 10 nA ejecting current. Vertical arrow above record indicates position at which slit moving in the non-preferred direction crosses the receptive field. Horizontal bars to right of each record indicate level for zero counts/bin (lower bar) and level corresponding to average count/bin for the background discharge (upper bar). Vertical calibration to left of record indicates range for 0–100 counts/20 msec bin. Horizontal time calibration is 1·0 sec. No. of trials, twenty-five.

selectivity. While the length of the testing slit affects the orientation tuning of all visual cortical cells to some extent (Henry, Bishop & Dreher, 1974), the effect of these cells was much more marked such that, with a 2°-long slit, it was very difficult in some cases to determine a clear optimal

within a range of $\pm 30°$. Most of these cells (nineteen out of twenty-one) also exhibited a varying degree of length preference, giving a larger response to an optimally oriented slit of similar dimensions to the excitatory discharge region of the receptive field than to a slit extended beyond this. This length preference was very marked in some of the cells, but much less so in others. However, while both the length preference and orientation selectivity of these cells were somewhat variable properties that appeared to be affected by changes in the level of excitability, the directional preference was not. Most of these cells were recorded in layer V (Fig. 1*C*). They appear to conform to the description made by Palmer & Rosenquist (1974) of the layer V cells projecting to the superior colliculus with the proviso that only a few of the cells described by them exhibited length preference in contrast to the majority of the cells in the present sample.

The length preference exhibited by these cells raises the question of whether they should be considered as hypercomplex rather than complex cells. Certainly for the cells in this group that did not exhibit length preference (two out of twenty-one), there is no alternative in the present terminology but to classify them as complex. For the others, approximately half could be considered hypercomplex while the remainder would be better described as complex cells exhibiting some length preference, as discussed briefly by Rose (1974).

The suppression of the resting discharge of these cells elicited by stimuli moving in the non-preferred direction was often marked, and would even block an artificially high resting discharge driven by the iontophoretic application of DL-homocysteate. This is illustrated in Fig. 4 where the upper records hows the normal response of the cell and the lower record shows the response during the iontophoretic application of DL-homocysteate. The stimulus motion in the non-preferred direction reduced the high resting level produced by the application of DL-homocysteate to almost zero. This effect was very notable over the audio monitor where a distinct pause in the firing could be heard as the stimulus passed over the field in the non-preferred direction. This type of observation suggests the possibility that these cells receive a powerful inhibitory input elicited by stimulus motion in the non-preferred direction. In this context, the effect of the application of bicuculline was surprising. In no case did it eliminate the directional selectivity of these cells and, where the suppression of the resting discharge by stimuli moving in the non-preferred direction was strong, it failed to block this despite large increases in the resting discharge levels and clear antagonism of the action of iontophoretically applied GABA. This is illustrated in Fig. 5. A small arrow above the right-hand side of each record marks the point at which the stimulus moving in the non-preferred direction crosses the receptive field. The suppression of

the normal resting discharge is clear (Fig. 5*A*), but it is emphasized during the application of bicuculline because, although there is a massive increase in spontaneous activity, the non-preferred direction of stimulus motion still almost completely silences the cells activity. The records show the response after 6, 9 and 15 min bicuculline application with an ejection

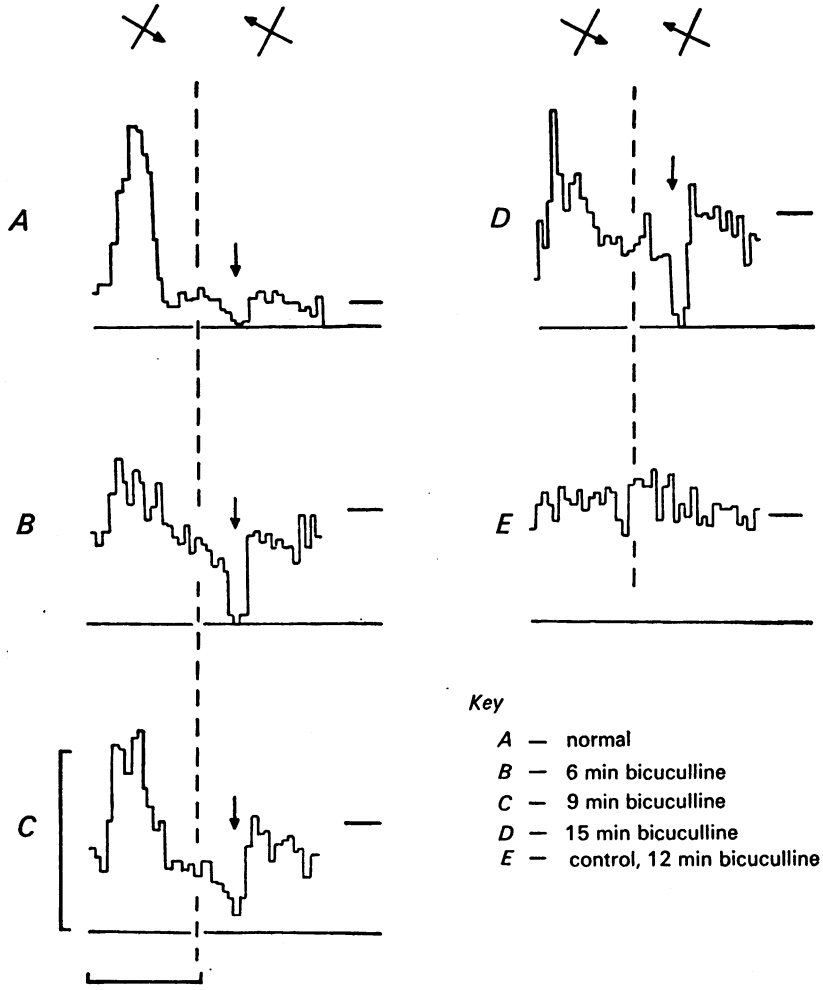

Fig. 5. Action of bicuculline on the directional specificity of type '3' complex. General details and calibration as for Fig. 4 except that bin size is 50 msec. Vertical arrows over right-hand portion of the records indicate position at which slit moving in non-preferred direction crosses the receptive field. *A*, normal response of cell. *B*, response after 6 min bicuculline application. *C*, after 9 min application. *D*, after 15 min application. Ejection current 140 nA (for *B*, *C*, *D*). *E*, Control. Spontaneous activity after 12 min bicuculline application.

current of 140 nA. In the case of this cell, the effect of iontophoretically applied GABA appeared to be completely blocked. The resting discharge level during the application of bicuculline in the absence of visual stimulation is shown by the control record in Fig. 5*E*. In one case, where suppression of the resting discharge by stimulus motion in the null direction was weak, the application of bicuculline revealed the presence of a small excitatory peak.

The general lack of effect of bicuculline on the inhibitory response of these cells to stimuli moving in the non-preferred direction introduced the possibility that an alternative inhibitory transmitter to GABA could be involved. The iontophoretic application of strychnine had no effect on the directional selectivity which would suggest that glycine was not involved. However, the interpretation of the action of strychnine is complicated by the fact that, in the visual cortex, it tends to depress cell excitability and resting discharge levels when applied with ejecting currents of more than 30 nA. In an attempt to confirm that the suppression of the resting discharge in the non-preferred direction reflects the action of a post-synaptic inhibitory input to the cell, rather than a loss of facilitation due to the withdrawal of a tonic excitatory input, the effect of the iontophoretic application of ammonium ions was examined. Ammonium ions have been reported to produce a block of the ionic mechanism underlying the i.p.s.p. (inhibitory post-synaptic potential) hyperpolarization, although not blocking the decrease in membrane resistance *per se* (Lux, Loracher & Neher, 1970; Raabe & Gunnit, 1975; Llinas, Baker & Precht, 1974). This action has been argued to reflect the fact that, ammonium ions block the active pumping of chloride ions which normally act as the generator for the e.m.f. of the i.p.s.p. In the cat motor cortex, for example, Raabe & Gunnit (1975) found that ammonium ions would block the thalamically induced inhibition of the antidromic invasion of pyramidal cells. While the action of ammonium ions on processes underlying the i.p.s.p. cannot be regarded as resolved, their application to central nervous system does provide a possibility of reducing the effectiveness of inhibitory processes acting on cells in a situation where the transmitter is not known.

In the present work, either 1·5 M-ammonium acetate or chloride solution was used in one or two barrels of the micropipettes and its action on the directional specificity of these layer V cells examined. This is illustrated by the cell in Fig. 6. The upper record shows the normal response with a clear inhibition of the resting discharge in the non-preferred direction and the lower records the response at 2, 5 and 8 min of iontophoretic application of ammonium ions with a 200 nA ejecting current. After 8 min application, there is a clear excitatory response in the non-preferred direction. This was the largest type of effect that could be produced and

200 nA was found to be the minimum effective current. This type of result suggests that a post-synaptic inhibitory input does underly the suppression of the resting discharge evoked by stimulus motion in the non-preferred direction. The magnitude of the excitatory responses produced, however,

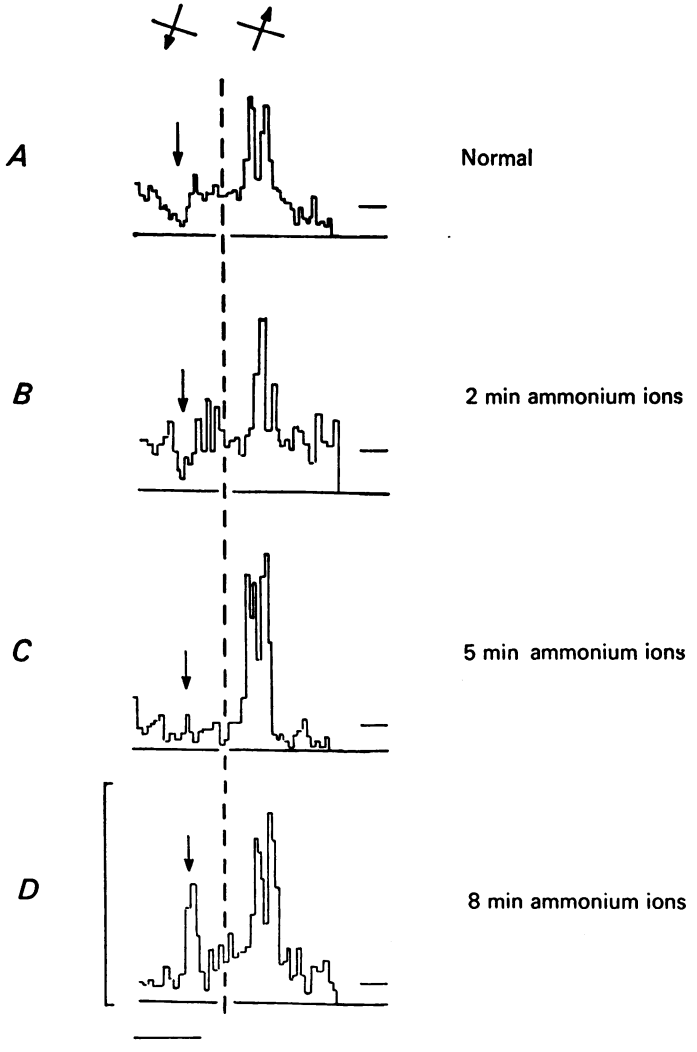

Fig. 6. Action of iontophoretically applied ammonium ions on the directional specificity of a type '3' complex cell. All general details and calibration as for Fig. 4. *A*, normal response. *B*, *C* and *D*, response after 2, 5 and 8 min period of iontophoretic application of ammonium ions with 200 nA ejecting current. Note response to non-preferred direction is to left of records in this Fig.

were small and in no way could be regarded as constituting an elimination of the directional selectivity.

Cells with hypercomplex receptive field properties

The cells discussed here were all located in layers II and III of the cortex as illustrated in Fig. 1*D*. They all (twenty-nine) exhibited a very high

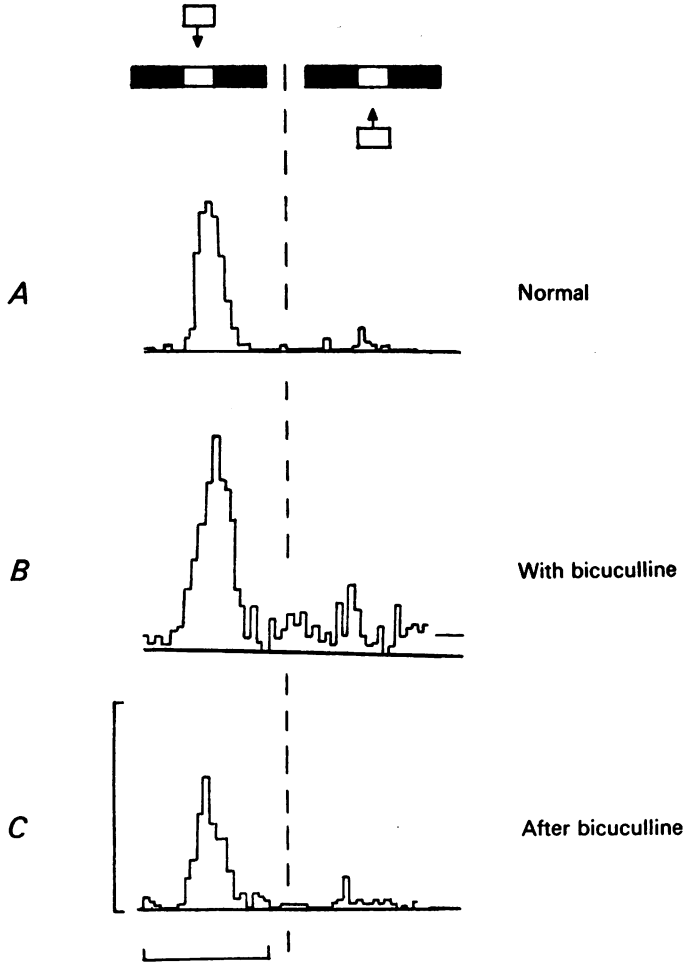

Fig. 7. Action of iontophoretically applied bicuculline on hypercomplex cell directional specificity. Each record shows the response to an appropriately oriented slit of optimal length passing over the receptive field. Diagram above records indicates direction of stimulus motion over field. Shaded portion of field represents apparent inhibitory sidebands. Calibrations as for Fig. 5. Bin size 50 msec. Twenty-five trials. *A*, normal response. *B*, response during iontophoretic application of bicuculline with 140 nA ejecting current. *C*, response after cessation of bicuculline application.

degree of length preference, giving no response or a residual response to a slit extended byond the boundaries of a central excitatory region. The length preference was an invariant receptive field property in contrast to that of the type 3 complex cells, discussed above. They were also quite sharply orientation-tuned when tested with a slit of similar dimensions to the excitatory region of the receptive fields. A study of the inhibitory processes contributing to the length preference and orientation selectivity of these cells is presented elsewhere (Sillito & Versiani, 1976, 1977). The present account refers only to the action of bicuculline on their directional selectivity. In no case was the directional selectivity eliminated during the ionotophoretic application of bicuculline and, in most cells, it was unaffected. The records in Fig. 7 show a typical example. The response was assessed with a slit of optimal length at the optimal orientation. Despite an increase in the magnitude of the evoked response, there was no significant response to stimulus motion in the non-preferred direction. In only one cell was a significant response to stimulus motion in the non-preferred direction produced, and this resulted in a response of approximately 50 % of that in the preferred direction.

DISCUSSION

To summarize the evidence presented here, it appears that iontophoretically applied bicuculline will cause an elimination or signicant reduction of the directional specificity of simple cells as defined by any of the current criteria. It also will eliminate the directional specificity of a distinct population of complex cells (referred to here as type 1 complex cells), but it does not affect the directional specificity of other complex cells (referred to as type 2 and 3 complex cells) nor does it, in general, affect the directional specificity of hypercomplex cells. One of the significant features of these results is that the effect of bicuculline on directional specificity appears to vary with cell type. This is particularly important in the case of complex cells, where the modification of the directional specificity of some cells, but not others, would seem to be a rather anomalous observation, were it not for the correlation of the effect with an apparently distinct subgroup of the complex category. In part, these results also act as a control concerning the possibility of bicuculline not only diffusing to the cell under observation but also diffusing to the cells providing an input to that cell. Thus, for example, in the context of the view that simple cells provide the excitatory input to complex cells in the same column (Hubel & Wiesel, 1962) it could be argued that the action of bicuculline on complex cell directional specificity reflects its diffusion to the input simple cells and a modification of their directional specificity. However, if this were the case it would be

extremely surprising that it always diffused to the simple cells providing an input to type '1' complex cells, but never to those providing an input to type '2' complex cells. The type '1' complex cells were found in layers II, III and IV and the type '2' cells in layers II, III and V, and hence both groups have a similar proximity to the population of directionally specific simple cells (see Fig. 1).

The action of bicuculline on simple cell directional specificity is consistent with the view of Goodwin, Henry & Bishop (1975) that simple cell directional specificity depends on an intracortical 'blanking inhibition', modifying the properties of the excitatory geniculate input to them. This confirms the conclusions drawn in a previous paper (Sillito, 1975*b*) concerning the contribution of GABA-mediated intracortical inhibition to simple cell directional specificity. However, the tendency to produce a rather smaller effect on the directional specificity of the simple cells lacking distinctive 'on' and 'off' subdivisions to their receptive field emphasizes the possibility that these may not be 'simple cells' in the terms originally conceived by Hubel & Wiesel (1962). The elimination of the directional specificity of the type '1' complex cells by the iontophoretic application of bicuculline is inconsistent with the view of Goodwin & Henry (1975) that complex cell directional specificity is a property conferred by the excitatory input. The first question arising from this observation is that of whether these 'complex cells' are really simple cells which, for some reason, have been incorrectly classified. Purely in terms of their normal receptive field properties the main argument against this is that they gave a clear 'on–off' response to stationary flashing stimuli throughout their receptive field. Moreover, their response profile during the iontophoretic application of bicuculline is distinct from that of typical simple cells and, on these grounds also, they differ, therefore, from the simple cell category. With their clear exclusion from the simple cell category they must be regarded as complex cells in the present terminology (Hubel & Wiesel, 1962). With respect to the elimination of their directional specificity by the application of bicuculline, the assumption made here is that this reflects a block of the action of a GABA-mediated post-synaptic inhibitory process serving to generate a directionally specific response in a cell which receives a non-directionally specific excitatory input. Some of the reasons for excluding the possibility of diffusion of the bicuculline to putative input simple cells have already been discussed. Further, to this it has always been found to be necessary to achieve a good resolution of the cell spike, and thus presumably to be in close proximity to the cell before either a block of the action of iontophoretically applied GABA or a change in receptive field properties could be achieved. Diffusion of the bicuculline to the input cells would imply an effective action of bicuculline on cells at a distance beyond the capability

of the electrode to resolve the action potential discharge, which with reference to the present experimental situation seems unlikely. A non-directionally specific excitatory input to these cells could derive either from a direct input from the lateral geniculate body (e.g. Hoffman & Stone, 1971) or an input from non-directionally specific simple cells.

The lack of effectiveness of bicuculline on the directional specificity of the type '2' complex cells, despite its apparent pharmacological effectiveness and, in many cases, its modification of other receptive field properties, is consistent with the view that their excitatory input is already directionally specific. This observation fits in with the conclusions of Goodwin & Henry (1975) and encompasses a group of cells that, by all criteria must be regarded as typically complex (Hubel & Wiesel, 1962; Bishop & Henry, 1972). The same considerations do not apply to the type '3' complex cells. Although bicuculline was ineffective in eliminating their directional specificity, they exhibited a powerful suppression of the resting discharge in response to stimuli moving in the non-preferred direction. This indicates the possibility of a directionally specific inhibitory input acting on them, and it was the presence or absence of this very type of effect that led Goodwin & Henry (1975) to conclude that simple cells, but not complex, receive a directionally specific inhibitory input. The possibility, however, that the suppression reflects a disfacilitation of a tonic excitatory input, rather than a post-synaptic inhibitory input, has to be considered. The over-all lack of effectiveness of bicuculline would be consistent with this view, but the fact that movement in the non-preferred direction reduced to virtually zero even very high background discharge levels maintained by the application of DL-homocysteate militates against it, as does the replacement of the suppression with an excitatory response during the iontophoretic application of ammonium ions. The excitatory response to motion in the non-preferred direction revealed by the application of ammonium ions was nonetheless small in comparison with the response to the preferred direction of motion. However, if the interpretation of the action of ammonium ions put forward by Raabe & Gunnit (1975) and Llinas *et al.* (1974) is correct, then they are only likely to cause a partial reduction of the effectiveness of the inhibitory mechanism because they would block the hyperpolarization generated by a post-synaptic inhibitory input, but not the conductance change. Clearly, any conclusion regarding the action of ammonium ions in this situation must be highly controversial, and all that can be said at present is that the results support the possibility that a post-synaptic inhibitory input contributes to the directional specificity of these cells. If this is correct, then apparent lack of effectiveness of bicuculline may reflect the fact that an alternative inhibitory transmitter to GABA is involved, or that the location of the inhibitory synapses on the cell is such

that there is not an adequate diffusion of bicuculline to them from an electrode effectively positioned for recording the action potential discharge of the cells. Further work on the mechanisms contributing to the directional specificity of these cells is necessary. However, the present data is clearly not consistent with the view that the directional specificity of these cells simply reflects a directionally specific excitatory input.

The iontophoretic application of bicuculline to the hypercomplex cells in layers II and III was also generally ineffective in changing their directional specificity but, while most of these cells gave no response to stimulus motion in the non-preferred direction, they also showed no signs of a suppression of the background discharge. The most simple interpretation of this data is that they receive a directionally specific excitatory input. It is interesting that the type '1' complex cells, for which the application of bicuculline eliminated directional specificity, were found mainly in layer III. Thus, the contrast between the effect of bicuculline on type '1' complex cells and hypercomplex cells located in layer III emphasizes the possibility of a differing synaptic organization underlying the directional specificity of the two types of cell.

It is not intended that the subdivision made here of the complex cell category into three groups should constitute yet another classification scheme for cortical cells. The subdivision has been used purely for convenience and simplicity in describing the present results. The results do emphasize, however, that the complex cell category cannot be regarded as representing a single functional cell type. Hence, attempts to group a sample of cortical cells as complex, on an arbitrary basis, by exclusion from the category of simple and hypercomplex cells must, at best, be regarded as misleading unless careful reference is made to their laminar distribution and receptive field characteristics. There is some question concerning the possibility of including some of the type '3' complex cells in the hypercomplex category. They can, however, be distinguished clearly from the cells classified here as hypercomplex by laminar distribution, orientation selectivity to a short slit, high resting discharge levels and suppression of the resting discharge by stimulus motion in the non-preferred direction. The type '3' complex cells appear to represent the cells described by Palmer & Rosenquist (1974) as projecting to the superior colliculus. On these grounds, to classify them as only either complex or hypercomplex is irrelevant, because it does not give any useful information concerning the distinctive features of the cell group.

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