J. Physiol. (1979), **289**, *pp.* 33–53
With 10 *text-figures*
Printed in Great Britain

INHIBITORY MECHANISMS INFLUENCING COMPLEX CELL ORIENTATION SELECTIVITY AND THEIR MODIFICATION AT HIGH RESTING DISCHARGE LEVELS

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(*Received 24 February 1978*)

SUMMARY

1. These experiments have investigated the contribution made by GABA-mediated inhibitory processes to the orientation tuning of complex cells in the cat's striate cortex. The GABA antagonist bicuculline has been ionophoretically applied to individual complex cells and the modifications produced in their orientation tuning documented.

2. In terms of the type of change produced in orientation tuning by the application of bicuculline, it seems that there are two categories of complex cells.

3. In one of these categories the orientation selectivity was eliminated during bicuculline application. The excitatory input to these cells would therefore appear to be non-orientation specific. Their orientation selectivity is presumably generated by a GABA-mediated inhibitory input.

4. In the other category of complex cells, although the orientation selectivity was decreased during bicuculline application, the cells retained a preference for a range of orientations that was generally centred around the original optimal orientation. It is suggested that for these cells the inhibitory input enhances the orientation tuning of an excitatory input that is already broadly orientation tuned.

5. Comparison of normal orientation tuning curves with those observed during the application of bicuculline provides a basis for estimating the orientation tuning of the GABA-mediated inhibitory input. In all cases, it is clear that at normal resting discharge levels, orientations either side of the optimal, and not those centred on the optimal, generate the most powerful inhibitory input.

6. These results would seem to be best explained by inhibitory interconnexions between cortical columns sensitive to different orientations. This type of lateral interaction between columns may serve to enhance the contrast in the orientation domain for the cortical representation of a specific stimulus orientation.

7. Increasing the resting discharge level of a complex cell, without blocking the action of GABA appeared to increase the gain of the inhibitory mechanisms acting on the cell. The normal excitatory responses to optimal or near optimal orientations were greatly reduced, or replaced by inhibitory responses, and non-optimal orientations produced only inhibitory responses. These inhibitory effects were blocked by the ionophoretic application of biculline.

8. These findings are discussed in the context of other observations in the literature. It is tentatively suggested that the interneurones providing the inhibitory drive to

0022-3751/79/2780-0310 $01.50

complex cells receive an input from recurrent collaterals of the recipient complex cells. Their other inputs would derive from neighbouring colums and from the afferent input to the parent column. The inputs from neighbouring columns would mediate the lateral inhibitory interactions in the orientation domain, and the recurrent collateral feed-back the decreased responsiveness at high resting discharge levels.

INTRODUCTION

Complex cells in the cat's striate cortex were first described by Hubel & Wiesel (1962). They suggested that the receptive field properties of complex cells reflected the nature of the excitatory input converging on them from simple cells in the same column. In these terms the orientation selectivity of complex cells was seen to be a consequence of the orientation selectivity of the input simple cells. However, it is now apparent that the excitatory input to complex cells cannot be assumed to originate only from simple cells. Many appear to receive a direct excitatory input from the lateral geniculate body (Hoffman & Stone, 1971; Singer, Tretter & Cynader, 1975; Toyama, Maekawa & Takeda, 1977). Moreover, there is a growing realization that intracortical inhibitory interactions exert a powerful influence over complex cell receptive field properties in general, and in particular, their orientation selectivity (Creutzfeldt & Ito, 1968; Benevento, Creutzfeld & Kuhnt, 1972; Blakemore & Tobin, 1972; Rose & Blakemore, 1974; Sillito, 1975*b*). Complex cell orientation tuning thus appears to be a product of the interaction of the excitatory and inhibitory inputs converging onto a given cell. The major unresolved question concerns the relative contribution of these two sets of inputs to the orientation tuning.

The application of neuropharmacological techniques to the study of visual cortical cells has provided a means of examining the relative role of inhibitory and excitatory mechanisms in determining receptive field properties. Many of the stimulus specific features of the receptive fields of visual cortical cells appear to depend on a GABA-mediated inhibitory mechanism (Sillito, 1975*b*, 1977*a*). This mechanism can be blocked locally by the ionophoretic application of the GABA antagonist bicuculline (Sillito, 1975*a*). In the presence of a block of its inhibitory input a cell's receptive field properties must then reflect the nature of its effective excitatory input. Preliminary investigation of the orientation selectivity of a small population of complex cells with this technique indicated that for some of the cells the excitatory input was non-orientation specific (Sillito, 1975*b*). This implies that the inhibitory input has the major role in generating the orientation selectivity. The only other published work on this matter has suggested conversely, that the orientation selectivity is determined primarily by the nature of the excitatory input, although modified by an inhibitory input broadly orientation tuned to the same optimum (Blakemore & Tobin, 1972; Carpenter & Blakemore, 1973; Rose & Blakemore, 1974). Unfortunately there is very little experimental evidence to support either of these views. The present paper extends the original work with ionophoretically applied bicuculline and examines the influence of resting discharge level on complex responses. A preliminary report of the effect of resting discharge level has already been made (Sillito, 1976*b*).

METHODS

The experiments were carried out on cats paralysed with gallamine triethiodide and anaesthetized with a mixture of 70 % N_2O and 30 % O_2 supplemented with 0·1–0·4 % halothane as necessary. Further details of anaesthesia, operative procedure, the care of the animal throughout the experiment, optical and histological procedures are given elsewhere (Sillito, 1975*a*,*b*; 1977*a*,*b*).

The five barrel micropipettes for recording and application of drugs were prepared and filled according to the procedure described by Gent, Mayne, Sillito & West (1976). The centre recording barrel, which was also used to mark the recording site, was filled with a solution of 2 % pontamine blue in 0·5 M-Na acetate (Hellon, 1971). The outer drug barrels were filled with a selection of the following solutions: gamma-aminobutyric acid (GABA) (0·5 M, pH 3, HCl) DL-homocysteate (DLH) (0·2 or 0·5 M, pH 7·5, NaOH), L-glutamate (0·5 M, pH 7·5, NaOH), strychnine hydrochloride (10 mM in 165 mM-NaCl), bicuculline (5 mM in 165 mM-NaCl, pH 3, HCl), bicuculline methochloride or methiodide (5 mM in 165 mM-NaCl, pH 3, HCl). For most experiments the drug barrels contained respectively DLH, bicuculline and GABA with two barrels being used for bicuculline (for discussion of this latter point see Sillito, 1975*a*). In experiments where both strychnine and bicuculline were used, one of the bicuculline barrels was replaced with strychnine.

Experimental procedures

Stimuli were presented on a tangent screen 1 m in front of the animal. The standard stimulus situation involved a 34 cd/m^2 stimulus on a 17 cd/m^2 background. Stimulus intensity was varied in 0·1 log unit steps from this standard value. Receptive fields were studied using slits (0·25–1·5° wide, 4–12° long). The stimulus velocities used were in a range 2–20°/sec. The particular stimulus parameters initially chosen for a given cell were those producing the optimal excitatory response (see below for contrast, however). In addition, a careful check was made to ensure that the slit length selected was great enough to reveal the optimal orientation selectivity in the cell examined (Henry, Dreher & Bishop, 1974). All observations refer to monocular stimulus presentation using the dominant eye for the cell in question. The optimal orientation of the receptive field of the cells examined was initially determined qualitatively using hand moved stimuli. A quantitative evaluation of the responses was then made using the optical projection system described by Sillito (1976*a*). A series of peristimulus time histograms (p.s.t.h.s) were constructed for the cell's response to a range of orientations in 5° steps, extending 20° either side of the qualitatively determined optimal. If two of the orientations tested gave an equal response, an intermediate point was selected and tested. The whole procedure was repeated to ascertain that variations in excitability were not contributing to the response assessment, and where there was doubt over the optimal orientation, a careful check was made again using 2·5° steps in the vicinity of the optimal. Once the optimal had been established, a full tuning curve was constructed for orientations extending to 90° either side of the optimal in 10 or 20° steps.

The effect of ionophoretically applied bicuculline on orientation selectivity was assessed by constructing a further tuning curve using 10 or 20° steps. In some cases the tendency of the bicuculline barrels to block during a period of sustained ejection made this impossible, because of the time required to plot the curve. For these cells a quick assessment of the over-all changes was made using 45° steps. The response to the optimal orientation was regularly rechecked during the bicuculline application and was always checked at the end of the test sequence. This procedure ensured that for a given level of effectiveness of bicuculline, there was a valid comparison between the orientation tested and the optimal. Subsequent assessments and comparison of the data were made with respect to the largest response seen at the optimal in the test sequence. Thus the data illustrated represent the least favourable situation for demonstrating a decrease in a cell's selectivity towards its optimal orientation.

Saturation of the complex cell excitatory mechanism can give rise to an apparent decrease in orientation selectivity. The reason for this is that if the response at the optimal is at the maximal possible for the cell, further increases in excitability will bring up the responses to orientations either side of the optimal, but not to the optimal orientation. This possibility was eliminated in the present experiments by ensuring that the cell was capable of giving a larger excitatory response than that recorded in any of the test sequences. Two procedures were used for this. Tuning curves were generally plotted at contrast levels producing a submaximal response and it was ascertained at the end of the testing sequence that increasing contrast produced a larger

response. Alternatively, the bicuculline-ejecting current was briefly increased following the testing sequence, or continued for a further period of time to produce a higher level of neuronal excitability and larger response. Saturation of the excitatory mechanism was never found to be a significant factor in the changes in orientation tuning produced by the ionophoretic application of bicuculline.

The pharmacological effectiveness of bicuculline was checked by ascertaining its ability to block the inhibitory action of ionophoretically applied GABA. As a routine procedure the GABA-ejecting current necessary to suppress completely the cell's response to an optimal stimulus moving over its receptive field was determined. The application of bicuculline was then judged to be functionally significant when it blocked the suppressive effect of GABA at this ejecting current level. This was the minimal level of GABA block found to be consistent with a significant change in receptive field properties. For most cells the GABA-ejecting current could be doubled or trebled beyond this value during bicuculline application without attenuating the driven response. Data from cells in which the application of bicuculline failed to achieve the minimal degree of GABA-block are not included.

One of the two methods was used to increase the resting discharge levels of complex cells without blocking the action of GABA. Either a small visual 'conditioning' stimulus was oscillated in the excitatory discharge zone of the receptive field, or an excitatory amino acid (L-glutamate or DL-homocysteate) was ionophoretically applied to the cell. The stimulus cycle of the visual conditioning stimulus was independent of that of the testing stimulus and always at a higher frequency. The conditioning stimulus luminance was always within 0·2 log units of the testing stimulus luminance.

Representation of stimulus orientation

For simplicity of discussion, on all records the optimal orientation of the cell's receptive field is arbitrarily given the value of zero and testing orientations are described as being at the optimal orientation, or '+' so many degrees (clockwise from the optimal), or '−' so many degrees (anticlockwise from the optimal).

RESULTS

The results are based on a study of 106 complex cells recorded in layers II, III and V of area 17. These cells were distinguished from simple cells because they lacked spatially separate, and antagonistic 'on' and 'off' subdivisions in their receptive field. They gave either an 'on–off' response or no response to a stationary flashing stimulus. Slits producing an optimal response when flashed on and off were usually narrower than the receptive field, and the location of the slit within the receptive field did not seem to effect the response. Cells with a receptive field comprising a single 'on' or 'off' excitatory region flanked by inhibitory zones, were classified as simple, and excluded from the present sample. In most ways the population of cells studied here falls within the 'standard complex' group as described recently by Gilbert (1977) following the original work of Hubel & Wiesel (1962). The 'standard complex' group also includes, however, cells showing end inhibition. In the present study cells showing any significant degree of end inhibition were excluded. The effect of bicuculline on the orientation selectivity of cells showing a high level of end stopping is discussed elsewhere (Sillito & Versiani, 1977). The corticotectal type cells (Palmer & Rosenquist, 1974) described by Gilbert (1977) as 'special complex' cells are not included in the present sample.

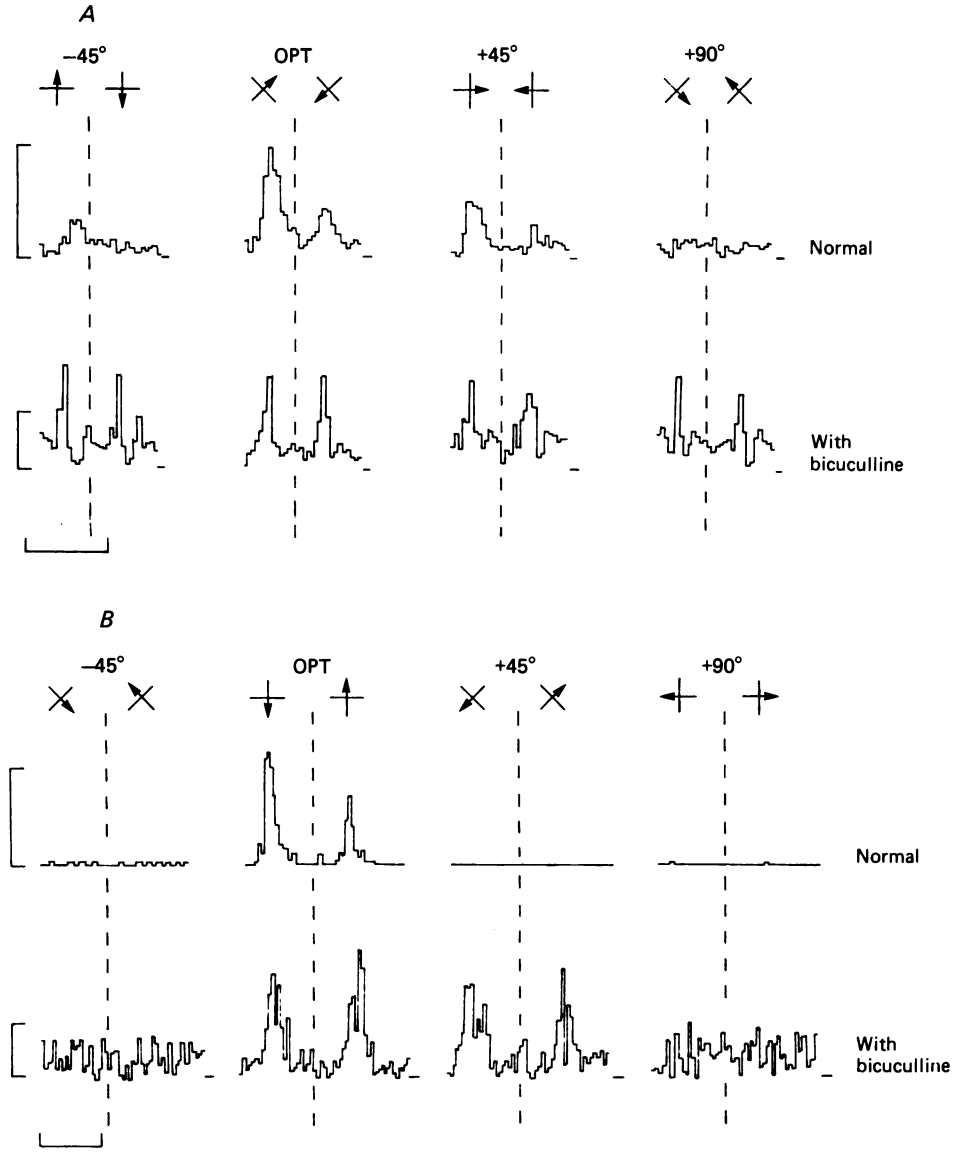

Fig. 1. *A*, action of ionophoretically applied bicuculline on complex cell orientation selectivity. The testing orientation is indicated above each set of p.s.t.h.s. Optimal is arbitrarily referred to as zero, ' − ' indicates anticlockwise rotation from optimal, ' + ' indicates clockwise rotation from optimal. P.s.t.h.s show response to both directions of stimulus motion over the receptive field at each orientation. Response to forwards motion to left of dotted line, response to return to right of dotted line. P.s.t.h.s constructed from response to twenty-five complete cycles of stimulus motions. Bin size, 50 msec. Vertical calibration indicates range corresponding to 0–100 counts/bin (0–80 impulses/sec). Horizontal calibration, 1 sec. Upper records refer to normal response, lower records to response during the ionophoretic application of bicuculline (50 nA ejecting current). *B*, complex cell showing more restricted reduction in orientation selectivity during the application of bicuculline. All details as for 1*A* except that vertical calibration indicates range corresponding to 0–50 counts/bin (0–40 impulses/sec) and bicuculline ejecting current, 70 nA.

Modification of complex cell orientation tuning during ionophoretic application of bicucuttine

All cells examined showed a decrease in their orientation selectivity during ionophoretic application of bicuculline. They appeared to fall into two categories on the basis of the changes observed in their orientation selectivity during the application of bicuculline. One group exhibited a loss of their orientation selectivity. The

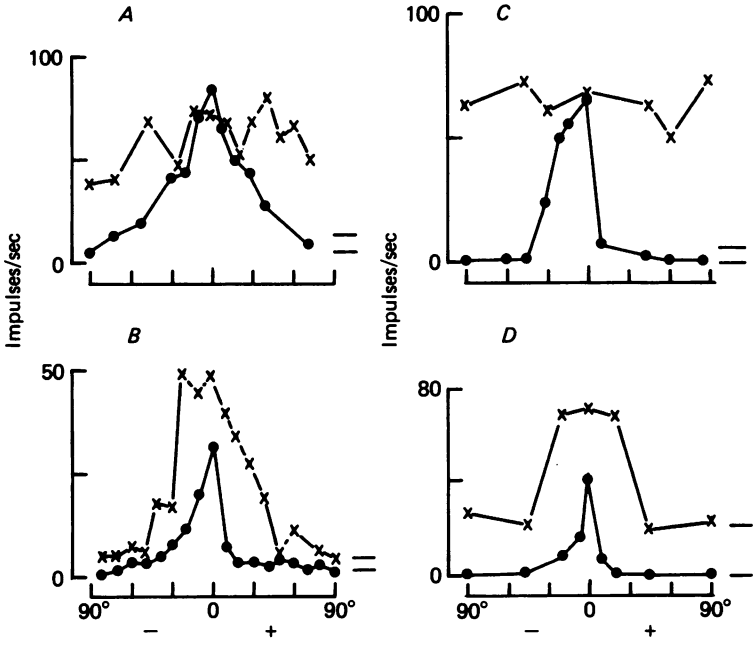

Fig. 2. Orientation tuning curves illustrating action of ionophoretically applied bicuculline on complex cell orientation selectivity. Continuous lines indicate normal tuning curves. Interrupted lines indicate tuning curves during application of bicuculline. Each point shows the average response per trial (impulses/sec), for the duration of each response, for twenty-five trials. Response assessed from preferred direction of motion at optimal orientation and equivalent at non-optimal orientations. Bars to the right of the curves indicate spontaneous activity level. *A* and *B*, complex cells showing loss of orientation selectivity with bicuculline. Bicuculline application currents 70 and 100 nA, respectively. *C* and *D*, cells showing reduction but not loss of orientation selectivity. Bicuculline application currents 90 and 100 nA. See Methods for further details.

other showed a reduction in their orientation selectivity, but retained an over-all preference to a range of orientations that was generally centred on the original optimal. These effects are illustrated in Figs. 1 and 2. The p.s.t.h.s. in Fig. 1*A* and *B* show the response of two complex cells, both exhibiting directional selectivity as well as orientation selectivity. In each case, the ionophoretic application of bicuculline resulted in a loss of directional selectivity and a similar increase in response amplitude. The cell in Fig. 1*A* exhibited an almost equal response to all the testing orientations during the application of bicuculline. Conversely, the cell in Fig. 1*B*, although showing a response to the testing orientation at + 45° where previously there had been no response, failed to respond to the other testing orientations. The pharmacological

effectiveness of bicuculline in terms of its capability to block the action of ionophoretically applied GABA (see Methods) was equal in both cases. Functionally its effect was also equal as judged by its action on directional specificity and response magnitude, yet the effects on orientation selectivity are clearly different.

The tuning curves in Fig. 2*A–D* illustrate the same effects for another four complex cells over a larger number of testing orientations. The rather erratic profiles of the curves in Fig. 2*A–C* during bicuculline application reflect small variations in neuronal excitability. It was very difficult to maintain a completely stable level of neuronal excitability during the long bicuculline application period necessary to complete the tests. There was an elimination of the original orientation selectivity of the cells in Fig. 2*A* and *B*. In both cases this was produced without a significant increase in response magnitude beyond that previously observed at the optimal orientation. Moreover, in both cases it was possible to drive the cell at approximately double the response magnitude. Thus a saturation of the excitatory response was not a contributory factor to the 'flattening' of these orientation tuning curves during bicuculline application. For the cells illustrated in Fig. 2*C* and *D*, the application of bicuculline resulted in a broadening of the orientation tuning curve but not a loss of the orientation bias of the cell. Although for both cells there was an increase in response magnitude during the application of bicuculline their responses remained below the saturation level.

For some cells it was possible to produce an elimination of orientation selectivity (as in Figs. 1*A*, 2*A*, *B*) by a short period of bicuculline application (3–15 min) with fairly low ejecting currents (50–70 nA). However, for others a similar application produced only a small reduction in orientation selectivity (as in Figs. 1*B*, 2*C*, *D*), which was not effectively increased by extended periods of application (up to 1 hr) with high ejecting currents (up to 160 nA). Indeed, what was notable for many cells in this latter category, was that even when the level of increased excitation during bicuculline application was allowed to reach the point where the excitatory responses to preferred orientations were saturating, significant responses to orientations approaching 90° to the optimal were still not observed. This information, together with the fact that for some cells in both groups there was an elimination of directional selectivity and a similar change in response magnitude (as in Fig. 1), suggests that variation in effect on orientation selectivity reflects a difference in the contribution made by GABA-mediated inhibitory processes to the orientation tuning, rather than a difference in the effectiveness of bicuculline.

It must be emphasized that the application of bicuculline was always pharmacologically effective, 'variations in its effectiveness' in this context refers to variations in its ability to diffuse to, and block, the inhibitory synapses acting on the cell under examination. The present data suggest that for cells of both groups bicuculline application was effective in blocking inhibitory synapses controlling directional selectivity. Hence its distribution would appear to be similar, yet the effect on orientation tuning is different. It is just plausible that the synapses controlling orientation tuning do not have the same location in the two groups, although those controlling directional selectivity do. However, the orientation tuning of cells in both groups was modified, showing that in each case bicuculline was reaching the synapses controlling orientation selectivity. If the limited reduction in orientation selectivity in the one group of cells reflected a limited diffusion to the inhibitory synapses controlling orientation selectivity, one would anticipate that the very high ejecting currents and extended application periods would produce a more effective distribution of the drug and further reductions in orientation selectivity. This did

not occur, and it thus seems that the most likely explanation for the two types of effect is a difference in the contribution of inhibitory and excitatory mechanisms to orientation tuning.

The percentage of cells showing the two types of effect was approximately equal, with 52 % showing a loss of orientation selectivity and 48 % a reduction only. This is summarized in Fig. 3, which shows the distribution of orientation selectivity in the population of cells before and during bicuculline application.

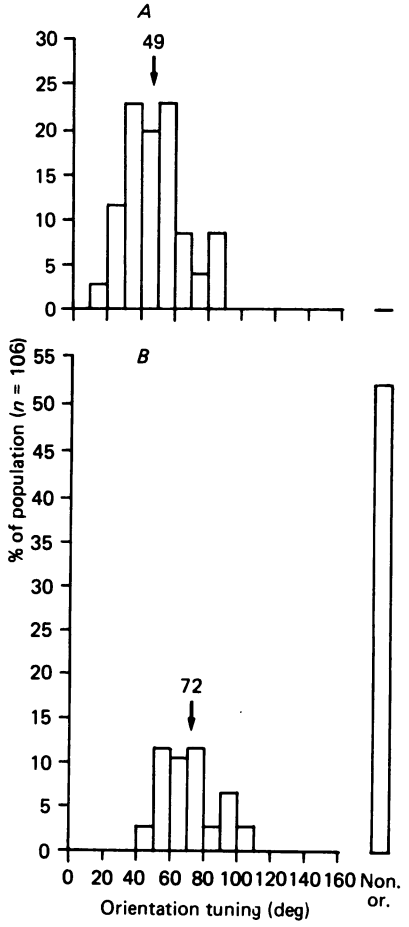

Fig. 3. Histograms showing the distribution of orientation tuning in the population of cells (n = 106) before and during bicuculline application. The orientation tuning is derived from the width of the tuning curve at half-height. The 'Non. or' column refers to cells without clear orientation selectivity, as seen for example during bicuculline application in Fig. 2*A* and *B*. Arrows show median orientation tuning for those cells showing selectivity. *A*, normal distribution. *B*, distribution during bicuculline application.

The contribution of the inhibitory input to complex cell orientation tuning

If it is assumed that bicuculline application produces a block of inhibitory inputs acting on a complex cell, then the difference between the normal orientation tuning curve and the curve plotted during bicuculline application, provides an indication of the distribution of effectiveness of the inhibitory input in the orientation domain. The

curves in Fig. 4 were constructed from data obtained by subtracting the values on normal orientation tuning curves from those on the curves plotted during bicuculline application. In Fig. 4*A* and *B* the curves are derived from cells showing a loss of selectivity without an increase in response magnitude at the optimal orientation, whilst in the example in Fig. 4*C*, the loss of selectivity was associated with a large

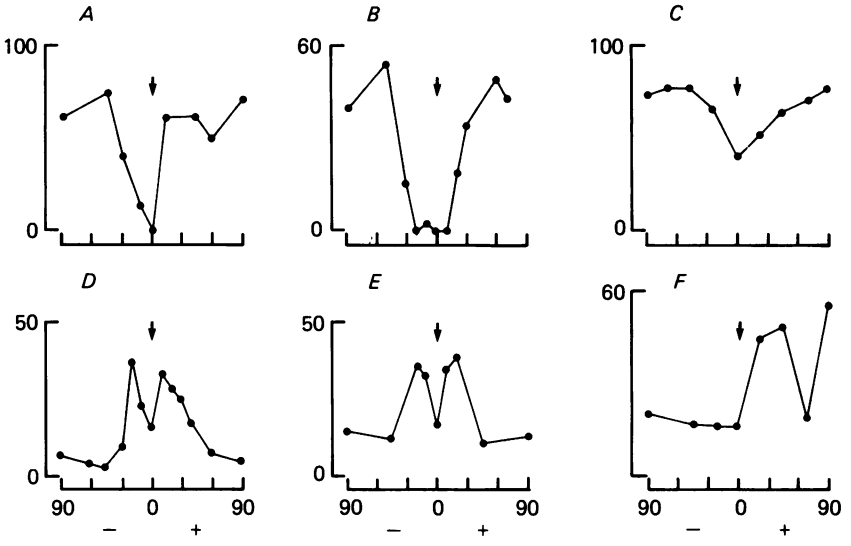

Fig. 4. Curves illustrating distribution of effectiveness of the inhibitory input in the orientation domain for six complete cells. Each curve constructed by subtracting values on the normal orientation tuning curve from those during bicuculline application (details of construction of original curves as in Fig. 2). Values on ordinate in impulses/sec therefore refer to change in average response relating to the block of the action of the inhibitory input. Largest changes are consequently seen at orientations where the inhibitory input normally exerts the largest effect. See text for further details.

increase in response magnitude. In Fig. 4*D* and *E* the curves refer to cells exhibiting a reduction in orientation selectivity only. The asymmetric curve in Fig. 4*F* illustrates data from a cell that was unusual insofar as it showed a loss of selectivity on one side of the optimal but not on the other. What is most notable for all six curves is that in no case does the inhibitory input appear to be maximally effective at the cell's optimal orientation. None of the data obtained from the use of ionophoretically applied bicuculline in the present population of cells (106), provides any support for the view that maximal inhibitory effects are normally exerted at the optimal orientation.

Effect of increased resting discharge level on complex cell response pattern

The initial objective of the tests described in this section was to ascertain the effect on complex cell responses of increasing excitability and resting discharge level, without blocking the action of GABA, and to compare this with the situation during the ionophoretic application of bicuculline. The results were clear but puzzling. In the absence of a block of the action of GABA, an increase in complex cell resting discharge level to a value approximating that seen during bicuculline application tended to cause a decrease, not an increase, in responsiveness. This change in respon-

siveness appeared to relate to an over-all enhancement of the effectiveness of the inhibitory inputs to the cell. This is illustrated in Figs. 5–9.

The peristimulus histograms in Fig. 5 show the response of a complex cell to a testing slit at +20°. The cell normally gave only a residual response to this stimulus as shown in Fig. 5*A* but gave a vigorous response during the application of bicuculline. When the resting discharge level was raised by ionophoretic application of DLH (DL-homocysteic acid) as shown in Fig. 5*B*, the magnitude of the response was not

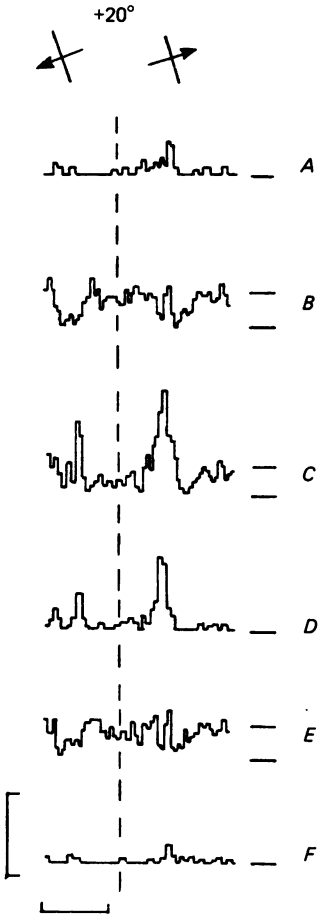

Fig. 5. Influence of resting discharge level on complex cell response pattern. *A*, normal response of complex cell to a testing slit at +20° to optimal. *B*, response in presence of high background resting discharge level maintained by ionophoretic application of DLH with 2 nA ejecting current. *C*, as for *B* but during application of bicuculline with 40 nA ejecting current. *D*, testing slit alone immediately after cessation of bicuculline application. *E*, response to testing slit after recovery from effects of bicuculline with resting discharge once more maintained by ionophoretic application of DLH (2 nA). *F*, effect of ionophoretic application of strychnine (40 nA) on response of cell to testing slit alone. Horizontal bars to right of records show level corresponding to zero counts per bin (lower bar) and where it is markedly greater than zero the mean resting discharge level (upper bar). One bar only is used where resting discharge levels are very low and indicates position corresponding to zero counts per bin. Vertical calibration indicates range corresponding to 0–100 counts/bin. Bin size 50 msecs. Horizontal calibration, 1 sec.

increased and in fact the stimulus could be seen to produce a suppression of the resting discharge as it passed over the receptive field. However, at the same resting discharge level but with a simultaneous application of bicuculline (Fig. 5*C*), the cell gave a clear excitatory response to the stimulus. Immediately after cessation of drug application the excitatory response was still present (Fig. 5*D*). A subsequent application of DLH (Fig. 5*E*) produced the same effect as the first. It is to be noted that the DLH was

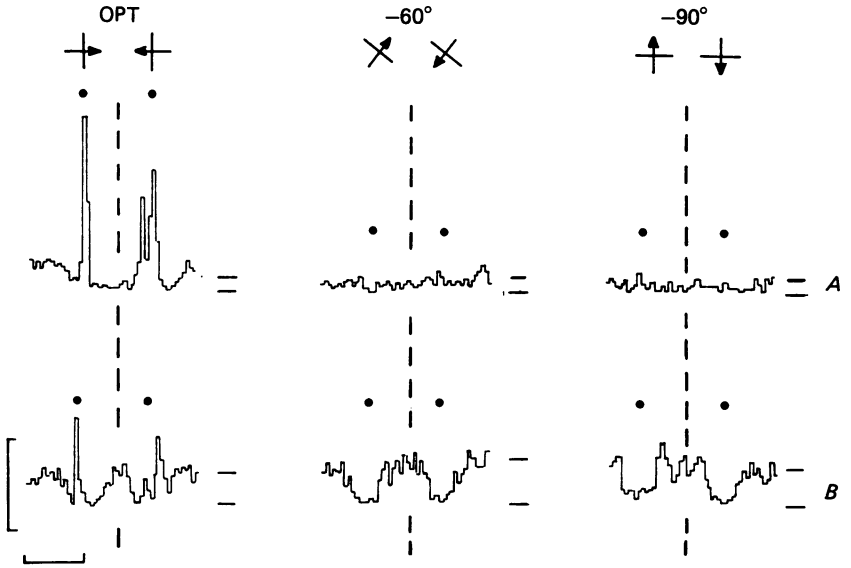

Fig. 6. Influence of resting discharge level on complex cell response pattern. *A*, normal responses to testing slit at orientations indicated above records. *B*, responses to testing slit whilst resting discharge maintained at artificially high level by small visual conditioning stimulus oscillating independently in the receptive field discharge centre. P.s.t.h.s constructed from twenty-five cycles of stimulus motion. Bin size, 50 msec. Vertical calibration indicates range corresponding to 0–100 counts/bin. Horizontal calibration, 1 sec. Black dots above records indicate approximate point at which the stimulus crossed receptive field centre.

applied with a very low ejecting current, 2nA, which would suggest that its main effect would be restricted to the vicinity of the cell under examination. Ionophoretic application of strychnine produced no effect on the response of the cell, supporting the view that the action of biculline on the response was primarily due to an antagonism of the action of GABA.

The effects shown in Fig. 5*B* and *C* were also seen if resting discharge levels were increased with a small visual conditioning stimulus oscillating independently of the testing slit motion in the receptive field discharge centre. This is illustrated in Fig. 6 which shows the response of a complex cell to a testing slit at the optimal orientation, −60 and −90°. When the background discharge level was increased, the excitatory response to the optimal stimulus was greatly reduced, and the non optimal stimuli suppressed the cells firing as they passed over the receptive field. Whilst these effects appear identical to those produced when the resting discharge was increased by application of an excitatory amino acid, the experimental situation here is rather more complex. It is possible in this case, that the synaptic interactions resulting in an increased effectiveness of inhibitory inputs, could also be occuring on the excitatory

input neurones driving the complex cell, because their resting discharge level would be higher (driven by the conditioning stimulus). Consequently, the loss of complex cell response to the testing stimulus could reflect a loss of its excitatory drive as the

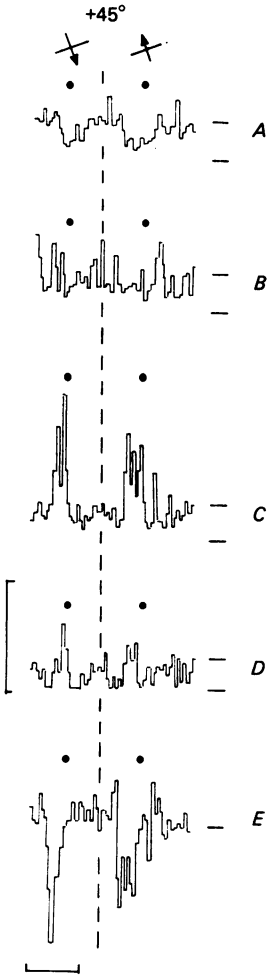

Fig. 7. Effect of ionophoretically applied bicuculline on the inhibitory response of a complex cell at high resting discharge levels. P.s.t.h.s show response of cell to testing slit at + 45° to the optimal. *A*, response to testing slit in presence of visually driven resting discharge. *B*, as for *A* but after 5 min. bicuculline application with 140 nA ejecting current. *C*, as for *A* but after 30 min. application of bicuculline with 140 nA ejecting current. *D*, response to testing slit in presence of visually driven resting discharge, but 3 min. after termination of bicuculline application. *E*, record produced by subtraction of p.s.t.h. in *C* from that in *A*. See text for comment. Calibration details as for Fig. 5.

testing stimulus passed over the receptive field, not an increase in the direct inhibitory input to the complex cell. However, as in the cases where the resting discharge was maintained by an excitatory amino acid, the 'inhibitory' effects seen in the presence of a visually evoked resting discharge were antagonized by the ionophoretic application of bicuculline. This is illustrated in Fig. 7 which shows the response of a complex cell to a testing slit at 45° to the optimal. The cell normally gave no response

to this stimulus. When the resting discharge was increased with a visual conditioning stimulus, the testing slit caused a suppression of unit activity as it passed over the receptive field (Fig 7*A*). Simultaneous application of bicuculline initially blocked the suppression of the resting discharge (Fig. 7*B*) and then revealed a large excitatory response (Fig. 7*C*). Three minutes after cessation of bicuculline application the

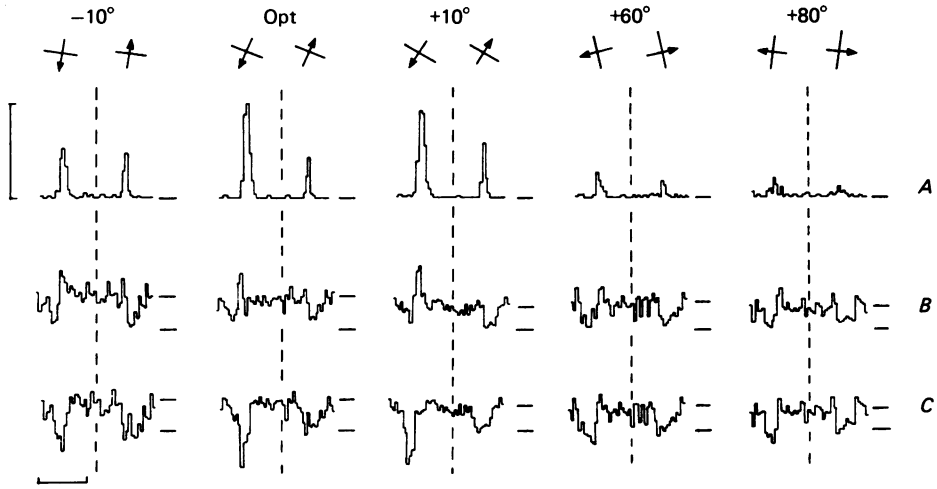

Fig. 8. Further example of the modification of complex cell response pattern at high resting discharge levels. *A*, normal response to the testing slit. *B*, response in presence of visually maintained resting discharge. *C*, records obtained by subtracting records in *A* from those in *B*. All other details as for Fig. 5.

excitatory response was greatly reduced (Fig. 7*D*). The inhibitory effect of the stimulus was thus greater than one would expect from the suppression of the resting discharge shown in Fig. 7*A*, because an underlying excitatory response was also apparently blocked. Some impression of this is given if the response pattern in Fig. 7*C* is subtracted from that in Fig. 7*A* as shown in the lower record (Fig. 7*E*). The same argument applies to the inhibitory effect seen in Fig. 5*B* in the context of the excitatory response in Fig. 5*C*. As the inhibitory effects seen in the presence of chemically or visually maintained resting discharge levels are blocked by ionophoretic application of bicuculline, it seems that they reflect the action of a local GABA-mediated inhibitory input.

The effect of a high resting discharge on the response of a complex cell to a range of testing orientations is further illustrated in Fig. 8. As the upper records show, although orientation tuned, this cell normally also gave some excitatory response to the non-preferred orientations. In the presence of a high resting discharge the responses to the non-preferred orientations appear to be primarily inhibitory, and to the preferred orientations a mixture of excitation and inhibition with the excitatory component greatly reduced in comparison to the normal. The reduction of the normal excitatory responses suggests that there may have been an over-all increase in the effectiveness of the inhibitory input at all orientations. Subtracting the normal records from those in the presence of the increased resting discharge gives some indication of the magnitude of this effect. This is illustrated in the lower records. In

fact these indicate that maximal inhibitory effects occur at the optimal orientation. However, as the data obtained with bicuculline show, the normally poor responses of complex cells at non-optimal orientations may not reflect the absence of an excitatory input, but the action of a powerful inhibitory input suppressing the excitatory input. Hence in Fig. 8*C* the inhibitory effects at non-optimal orientations

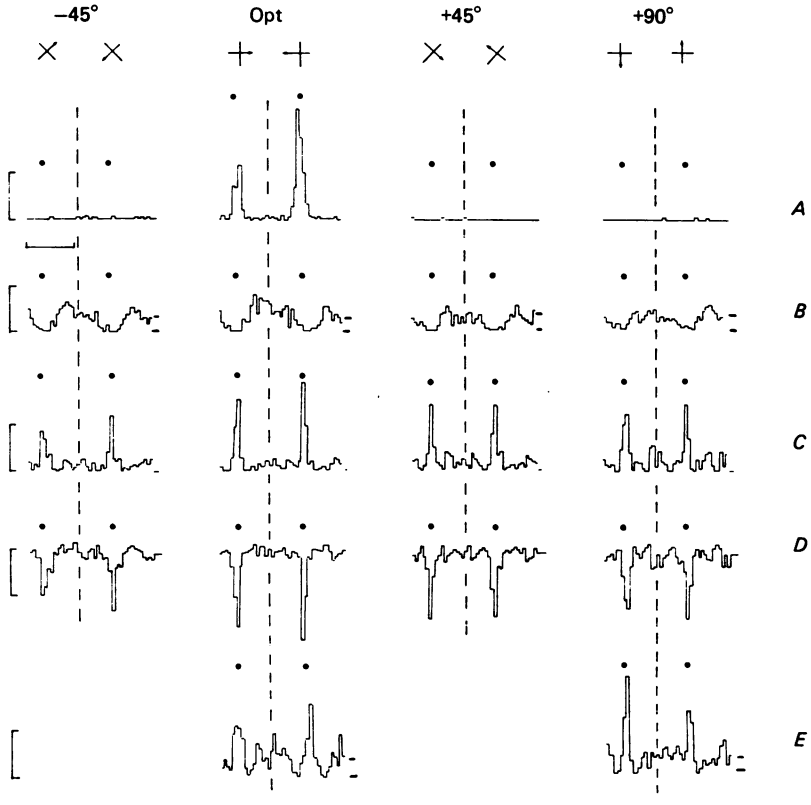

Fig. 9. *A*, normal complex cell responses to testing slit at the orientation indicated above each set of records. *B*, response to the same testing slit in the presence of a visually maintained discharge. *C*, response to testing slit alone during the application of bicuculline with 60 nA ejecting current. *D*, result of subtracting records in *C* from those in *B*, see text for comment. *E*, response to testing slit in the presence of visually maintained resting discharge but during the application of bicuculline (100 nA). Vertical calibration indicates range corresponding to 0–25 counts/50 msec bin for *A* and *B* and 0–50 for *C*, *D* and *E*. Horizontal calibration, 1 sec.

may be greater than they seem. These points are examined further by the tests carried out on the cell illustrated in Fig. 9. The records of Fig. 9*A* show the normal responses of the cell to the testing slit alone and those in Fig. 9*B* show the response in the presence of a high resting discharge. In this example the excitatory response at the optimal orientation was eliminated leaving an inhibitory effect only. Considering the records in Fig. 9*A* and *B*, the general impression is that maximal inhibitory effect occurred at the optimal orientation. However, the excitatory responses revealed during bicuculline application (Fig. 9*C*) indicate the presence of a powerful inhibitory effect at non-optimal orientations. Subtracting the data for each record in Fig. 9*C*

from that in Fig. 9*B* shows that in comparison to the situation illustrated in Fig. 8*C* the inhibitory influence is more uniformly distributed across the range of orientations, although still slightly biased towards the optimal. As an additional control the lower records in this Figure show the effect of the ionophoretic application of bicuculline on the response situation in Fig. 9*B*. The inhibitory responses are replaced by excitatory responses and the response at 90° to the optimal in this instance exceeds that to the optimal. This once more illustrates that 'suppressive' effects in the presence of visually induced resting discharge levels are not the result of a reduction of the excitatory input (p. 44), but of the activity of a local GABA-mediated inhibitory influence that acts to limit complex cell responsiveness.

The data in Fig. 9 bring together the observations made in the previous section with those under review here. At the normal resting discharge level in Fig. 9*A*, inhibitory influences acting on the complex cell appear to be strongest at non-optimal orientations, i.e. they seem to suppress the excitatory input elicited by stimuli at these orientations (as revealed in Fig. 9*C* by the application of bicuculline). However, if resting discharge level is increased, this bias in the action of the inhibitory influence is apparently changed, and powerful inhibitory effects are seen at the optimal orientation.

The actual level of increased background discharge at which complex cell responsiveness changed varied from cell to cell, and appeared to depend on the normal resting discharge level. In cells with virtually no spontaneous activity, these effects were seen at rates in the range of 15–30 impulses/sec, whilst for cells with initially higher resting discharge levels it was necessary to induce rates up to 50 impulses/sec. Chemical or visual means of increasing resting discharge levels appeared to be completely interchangeable, as judged by the modifications produced in the complex cell response pattern. However, it was much easier to produce repeated and stable levels of resting discharge with a visual conditioning stimulus. This was mainly due to the fact that for a given ejection current level, the effectiveness of an excitatory amino acid tended to increase through an application period lasting 1 or 2 min, and it was necessary constantly to adjust the ejecting current; also a series of repetitive applications generally became less effective in driving a cell. For these reasons comparative tests involving a series of visual stimuli were normally carried out in the presence of a visually induced resting discharge. Whether visual or chemical means of inducing the resting discharge were used, maximal inhibitory effects were only observed in the presence of a high resting discharge level if the testing slit passed over the receptive field centre. That is, 'gap slits', interrupted midway along their length so as to exclude the receptive field excitatory discharge zone, were ineffective in producing a suppression of an artificially maintained discharge. This suggests that an important component of the inhibitory input under these conditions was dependent on simultaneous stimulation of the region of visual space represented by the cell's excitatory discharge centre. The inhibitory effects were thus not simply a reflection of the activation of inhibitory flanking regions. These observations are very similar to those described for hypercomplex cells in the superficial layers of the cat's striate cortex (Sillito, 1977*b*), where maximal inhibitory effects from regions contributing to length preference were only produced when the central zone of the receptive field was synchronously stimulated.

DISCUSSION

The results obtained here emphasize the role of GABA-mediated inhibitory processes in generating complex cell orientation selectivity. The changes observed in orientation selectivity during bicuculline application indicated without exception that maximal inhibitory effects were normally exerted at orientations either side of the optimal, not those centred on the optimal. Consequently it appears that the GABA-mediated inhibitory input normally serves to limit the range of orientations to which a complex cell will fire, by selectively suppressing the responses to orientations away from the optimal. This stands in contrast to the view that the orientation selectivity of complex cells is 'enhanced' by an inhibitory mechanism affecting all orientations, but exerting a maximal influence at the cell's optimal orientation (Blakemore & Tobin, 1972; Rose & Blakemore, 1974).

It seems clear that those complex cells showing a loss of orientation selectivity during bicuculline application must receive an excitatory input that is not orientation selective. On the other hand, the second group of complex cells, which exhibited a decrease but not a loss of orientation selectivity, would appear to receive an excitatory input that is broadly orientation specific. The limited changes in orientation selectivity in these latter cells did not seem to reflect a limited distribution of bicuculline to the inhibitory synapses, because increasing ejecting current levels and application times did not produce further changes in their selectivity. However, this possibility cannot be totally excluded. The idea of two groups of complex cells, one receiving a non-orientation specific excitatory input and the other receiving an orientation specific input, is consistent with the presence of complex cells driven either directly by lateral geniculate neurones, or indirectly via other cortical cells (possibly simple cells, Hubel & Wiesel, 1962). There is neurophysiological evidence for both these types of input to complex cells (Hoffman & Stone, 1971; Singer, Tretter & Cynadar, 1975; Toyama, Maekawa & Takeda, 1977; Toyama, Kimura, Shiida & Takeda, 1977) and assuming complex cells to be pyramidal cells (Kelly & Van Essen, 1974), there is also anatomical evidence (Garey & Powell, 1971; Fisken, Garey & Powell, 1975; Winfield & Powell, 1976). On anatomical grounds it seems unlikely that the dendrites of a given pyramidal cell are completely restricted to one orientation column, and consequently it is likely to 'sample' excitatory inputs from a number of columns with differing orientation selectivity. This means that even complex cells driven by other cortical cells may not receive a highly orientation selective excitatory input. The inhibitory mechanism could then be viewed as compensating for this lack of 'precision' in the excitatory input. With respect to some of the larger pyramidal cells it seems plausible to speculate that they would 'sample' enough columns via their basal dendrites to derive an essentially non-orientation specific excitatory input. Hence this convergence of input from a number of columns rather than a direct geniculate input could possibly account for the excitatory drive to the first category of complex cells discussed above.

Increasing the resting discharge of complex cells, without a concomitant block of the action of GABA, does not appear to increase their excitability to a visual stimulus, rather it seems to decrease it. The effect appears to be the same if the resting discharge is increased either 'chemically', by local ionophoretic application of an

excitatory amino acid, or visually, by a small conditioning stimulus oscillating in the receptive field centre. Thus a normally highly effective testing stimulus at the optimal orientation in the presence of an increased background resting discharge, produces either a greatly reduced excitatory response or an inhibitory response (e.g. Fig. 9). These modified responses do seem to reflect the action of an inhibitory input on the cell, rather than a reduction in the excitatory drive, because they are blocked by bicuculline application. Thus in the presence of a high resting discharge level optimal stimuli still appear to elicit the same excitatory input, but the cell's response to this is masked or reduced by a simultaneously elicited inhibitory input. Considered from a slightly different viewpoint, it would seem that the selectivity of the inhibitory input to orientations either side of the optimal may be lost when the resting discharge level is raised. This is well summarized by the data in Fig. 9.

Although the findings reported in this paper concerning the nature of the inhibitory processes influencing complex cell orientation tuning seem somewhat controversial, in many ways they reflect what is already known of the organization of the visual cortex. Following the elegant work of Hubel & Wiesel (1974) it is clear that the input referring to a particular region in visual space is represented by a group of columns, with each column sensitive to a different orientation and the orientation varying in an apparently stepwise function from one column to the next. The group of columns together classify the range of orientations for that position in visual space (they are sometimes collectively referred to as hypercolumn if columns dominated by both eyes are considered), and this process is then repeated for adjacent although overlapping regions of visual space. There are extensive lateral interconnexions between the columns of cells in area 17 (Fisken, Garey & Powell, 1975) and a major effect of stimulating (chemically) one point in the visual cortex is to cause inhibition of cells in columns in a range of 100–400 μm from the point stimulated (Hess, Negishi & Creutzfeldt, 1975). Similar results have been obtained using electrical microstimulation techniques in the motor cortex (Asanuma & Rosen, 1973), where the spread of inhibitory effects was found to be wider than that of excitatory effects, and inhibitory effects predominated over excitatory effects following stimulation of layers V and VI. This suggests that an important component of the lateral interactions in the cortex is inhibitory in nature. Consequently, in the visual cortex, a given column of cells tuned to a particular orientation is likely to receive an inhibitory input from other columns sensitive to different orientations. That is, the cells will be inhibited by stimulus orientations away from the particular optimal value in question. This is precisely the prediction that derives from the data obtained here with bicuculline.

Despite the evidence that a very significant component of the functional interaction between columns appears to be inhibitory in nature, morphologically it seems that the majority of the lateral connexions may be made by excitatory fibres (Fisken *et al.* 1975). This suggests that inhibitory effects are mediated locally by inhibitory interneurones, possibly non-spiny stellate cells following the classification scheme of Le Vay (1973), or basket or chandelier cells following Szentágothai (1973). In this context it is important to note that in the neocortex there is evidence for a recurrent collateral feed-back from pyramidal cells to the inhibitory interneurone synapsing on their cell body (Szentágothai, 1973; Szentágothai & Arbib, 1975). Following the argument that in the visual complex cells are pyramidal cells (Kelly & Van Essen,

1974), the possibility is introduced that the activity levels of complex cells will influence the excitability of the inhibitory intereurones. An inhibitory interneurone influencing complex cell responses may be tentatively viewed to receive its excitatory drive from a number of sources. These are likely to include neighbouring columns,

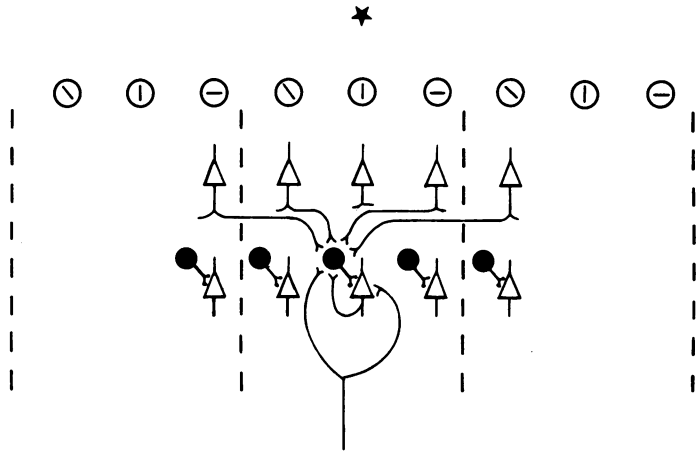

Fig. 10. Highly diagrammatic model summarizing the synaptic arrangement suggested to contribute to the inhibitory influences modifying complex cell responses in the orientation domain. Cells are represented as being in columns each headed by a bar in a circle indicating the optimal orientation for that column. Each group of three columns represents one hypercolumn processing the input from a particular point in visual space (in fact, of course, there would be many more than three columns per hypercolumn). Complex cells are represented as open triangles and the inhibitory interneurones as closed circles. Connexions are only shown with reference to the lower complex cell in the central column. The interneurone mediating the inhibitory input to this cell is represented as receiving an excitatory input from adjacent columns in the same hypercolumn, from columns contained in neighbouring hypercolumns, from a recurrent collateral from the recipient complex cell and from the afferent input (possibly non-orientation specific) to the parent column. All the laterally directed connexions to the inhibitory interneurones are from columns with preferred orientations away from the optimal of the parent column of the cell in question. An important assumption is that normally the inhibitory interneurone only exerts a significant effect on the complex cell when two or more of its inputs are simultaneously activated.

the parent column and the recurrent collateral from the recipient complex cell. In terms of orientation selectivity this suggests that there is a potential inhibitory input at all orientations for a given complex cell. This viewpoint is supported by intracellular studies of visual cortical responses (Creutzfeldt & Ito, 1968; Benevento *et al.* 1972). The further implication of the present suggestions is that the effectiveness of these inputs will be enhanced if the excitability of the inhibitory interneurone is increased by a high level drive from the recurrent collateral. The major question under review here concerns the 'normal' bias of the inhibitory input in the orientation domain, and the way in which the input may derive its selectivity for orientations either side of the optimal.

The orientation tuning of complex cells varies with stimulus length (Henry *et al.* 1974), with cells showing greater orientation selectivity to longer stimuli as opposed

to shorter stimuli. In fact some of the orientation tuning curves produced by Henry *et al.* using short slits are not unlike those seen during the application of bicuculline. This suggests that longer stimuli may bring in an additional inhibitory input, or in some way enhance the effectiveness of the existing inhibitory input, so that more powerful inhibitory effects are exerted at orientations either side of the cell's excitatory optimal. A simple suggestion is to assume that there are additional connexions to a complex cell inhibitory interneurone from cells in columns contained within adjacent hypercolumns (Hubel & Wiesel, 1974), which process the input from an adjacent, but overlapping region of visual space. These additional inputs then reinforce the lateral interactions from the immediately adjacent columns.

The ideas discussed here concerning the organization of the inhibitory input to complex cells are embodied in the diagrammatic model in Fig. 10. This is to be seen as a highly simplified representation of the concepts involved and in no way is it to be regarded as an attempt to define the actual connexions generating the complex cell inhibitory input. In this model the inhibitory interneurone is only judged to respond when there is an adequate level of summation deriving from the activation of several of the excitatory inputs. Further to this it seems reasonable to assume that in the presence of a high resting discharge in a complex cell, inputs to its inhibitory interneurone that would normally be ineffective, become effective because of the large increase in excitability induced via the recurrent collateral input. In suggesting an equal synaptic weighting from each of the inputs to the inhibitory interneurone the diagram is clearly oversimplified. It is most likely that there would be differences. More effective connexions from a particular group of columns could account for the fact that the orientation tuning curves of some complex cells shows a greater decline in responsiveness on one side than on the other. Discrete connexions from a few adjacent columns could generate inhibitory function curves of the type in Fig. 4*D* and *E*. The immediate problem raised by this model is that it suggests the presence of cells within or close to an orientation column that are tuned to orientations away from the column optimal, and in some cases up to 90° away, as for example in Fig. 4*A* and *B*. These curves could reflect the orientation tuning of bidirectional cells broadly selective to an orientation 90° away from the optimal values represented by the arrows. That is, they could be fairly normal orientation tuning curves plotted from the 'wrong' viewpoint. Cells with optimal orientations up to 90° away from that for their apparent 'column' have been described by Lee, Albus, Heggelund, Hume & Creutzfeldt (1977). They suggested that the relatively large numbers of cells to be found with preferred orientations away from the over-all optimal for any particular cortical locus constitute an argument against a strict columnar representation of orientation in the cat's striate cortex. It is interesting to speculate that an alternative interpretation for at least some of their data is that they were obtaining recordings from inhibitory interneurones. Unfortunately, there is no direct evidence concerning either the responses to visual stimuli of inhibitory interneurones, or the inputs converging on them. A further factor to be considered is that more than one inhibitory interneurone may be involved in relaying the inhibitory input to a given complex cell and thus the orientation tuning curve of the conjectured inhibitory input could be a composite of several cells. What does seem clear however, is that the response properties of inhibitory interneurones generating the stimulus specific

features of a given complex cell, must be 'out of phase' with those of the recipient cell.

In summary, the present data support the view that complex cell orientation selectivity is dependent on the action of lateral inhibitory interactions in the orientation domain mediated by inhibitory interneurones releasing GABA. These interactions appear to be truly 'lateral' insofar as they involve inputs from cells in adjacent columns and possibly hypercolumns with preferred orientations either side of the optimal for the cell in question. They do not appear to derive predominantly from a mechanism which involves an input tuned to the same optimal as the cell's excitatory response, although there is a clear component of the inhibitory input present at the optimal. In the way the inhibitory input is viewed to act here, it would enhance the contrast in the orientation domain between appropriate neural channels (columns) and non-appropriate channels for the stimulus in question. This is consistent with the view of the functional role of lateral inhibition elsewhere in the nervous system. The change in response at high resting discharge levels seems to be well explained by the possibility of a recurrent feed-back from complex cells to the inhibitory interneurones impinging on them.

I am most grateful for the helpful comments of Dr K. M. Spyer. The support of the Wellcome Trust is gratefully acknowledged.

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