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THE EFFECTIVENESS OF BICUCULLINE AS AN ANTAGONIST OF GABA AND VISUALLY EVOKED INHIBITION IN THE CAT'S STRIATE CORTEX

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SUMMARY

1. The iontophoretic application of the alkaloid bicuculline to neurones in area 17 of the cat's visual cortex effectively antagonized the inhibitory action of iontophoretically applied GABA in fifty-four out of sixty-two neurones examined. It had little or no effect on the inhibitory action of iontophoretically applied glycine.

2. At the stage that the iontophoretic application of bicuculline blocked the inhibitory action of GABA it also reduced or blocked visually evoked inhibitory influences acting on forty-three of the fifty-four cells. This effect on visually evoked inhibition was not reproduced by simply raising the neural spontaneous activity with iontophoretically applied glutamate.

3. For those seven neurones where the iontophoresis of bicuculline failed to block the inhibitory action of iontophoretically applied GABA it also failed to produce any change in visually evoked inhibition.

4. In all cases where a visually evoked inhibition of a cells resting discharge was reduced by the iontophoretic application of bicuculline, the inhibitory response was replaced by an excitatory response. The application of bicuculline also revealed excitatory responses to certain of the visual stimuli that previously appeared to exert neither inhibitory nor excitatory effects on a cell, and often where cells normally exhibited small excitatory responses it produced large increases in the magnitude of the evoked response.

5. These results indicate that the normal responses of the neurones examined in the present work, to the particular visual stimuli used, reflect an interaction between simultaneously evoked excitatory and inhibitory inputs. It is suggested that the iontophoretic application of bicuculline by blocking or reducing the inhibitory input moves the balance between the inputs in favour of the excitatory input.

6. The present results support the view that GABA is an inhibitory transmitter in the visual cortex.

INTRODUCTION

Several lines of evidence suggest that gamma-aminobutyric acid (GABA) may be an inhibitory transmitter in the visual cortex. It is spontaneously released from the visual cortex and its rate of release is increased by electrical stimulation of the lateral geniculate body or cortex with stimulus parameters that cause an inhibition of neural activity (Mitchell & Srinivasan, 1969; Iversen, Mitchell & Srinivasan, 1971). Further to this Iversen *et al.* (1971) demonstrated that exposing the cortex to a calcium free collecting fluid, whilst having no effect on the spontaneous rate of release of GABA, eliminated both the increase in the rate of release produced by electrical stimulation and the inhibition. It has also recently been reported that iontophoretically applied GABA has a strong inhibitory action on all types of neurone encountered in the visual cortex (Wallingford, Ostdahl, Zarzecki, Kaufman & Somjen, 1973). Previously it has been demonstrated that in the precruciate cortex iontophoretic application of GABA produces changes in membrane conductance that are equivalent to those associated with the normal post-synaptic inhibitory potential (Krnjević & Schwartz, 1967; Dreifuss, Kelly & Krnjević, 1969).

In many parts of the central nervous system including the cerebral cortex the inhibitory action of GABA can be reversibly antagonized by iontophoretic application of the alkaloid bicuculline (Curtis, Duggan, Felix & Johnston, 1970, 1971*a*; Curtis, Duggan, Felix, Johnston & McLennan, 1971*b*; Curtis & Felix, 1971; Dreifuss & Matthews, 1972). This antagonism appears to be relatively specific to GABA because, for example, bicuculline has little effect on the inhibitory action of glycine. However the effectiveness of bicuculline as an antagonist of GABA has been questioned and it has been further suggested that in some cases it may even potentiate the action of GABA (Godfraind, Krnjević & Pumain, 1970; Straughan, Neal, Simmonds, Collins & Hill, 1971). The over-all evidence to date none the less appears to favour the view that bicuculline is an effective antagonist of GABA (Curtis & Johnston, 1974).

In the present work bicuculline has been iontophoretically applied to neurones in area 17 of the visual cortex and its effect on the inhibitory action of iontophoretically applied GABA and visually evoked inhibition determined. The objective of this was threefold. Firstly, with reference to the possible doubt concerning the effectiveness of bicuculline (Godfraind *et al.* 1970; Straughan *et al.* 1971) to ascertain whether iontophoretically

applied bicuculline is an effective antagonist of GABA in the visual cortex. Secondly, to establish whether it can be used to block or limit naturally occurring inhibitory processes in the visual cortex, with a view to using the technique in a subsequent investigation of the role of inhibitory processes in the generation of the receptive field properties of visual cortical neurones (Sillito, 1975). Finally to provide further indirect and clearly tentative evidence concerning the role of GABA as a possible inhibitory transmitter in the visual cortex.

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 and 2·0–3·5% Halothane. It was subsequently maintained throughout the course of the experiment by a 70% N_2O, 30% O_2 mixture with the addition of 0·1–0·5% Halothane as necessary to maintain an adequate depth of anaesthesia. A solution of 2% (w/v) procaine hydrochloride was applied to all wound areas. The animals were immobilized by a continuous intravenous infusion of gallamine triethiodide at a rate of 10 mg/kg per hour in a solution of 4·3% dextrose saline to give a total volume of 3·0 ml./hr. They were artificially ventilated and the end-tidal CO_2 and e.e.g. were monitored throughout the course of each experiment. The respiratory rate was adjusted to maintain an end-tidal CO_2 of 4·0–4·5%. The eyes were atropinized, the nictitating membranes retracted by the administration of phenylephrine and the corneas protected with contact lenses. Prior to administering atropine to the eyes but after administering Flaxedil the percentage of Halothane was adjusted with reference to the state of the pupils (i.e. using level of constriction as an indication of depth of anaesthesia) and the e.e.g. Subsequent changes in the depth of anaesthesia were judged by changes in the e.e.g. In general the halothane was initially set at a level in the region of 0·5% and reduced to a lower figure over the course of 36 hr.

Five barrel micropipettes with tip diameters from 3 to 7 μm 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% pontamine blue which enabled the recording site to be marked by the technique described by Hellon (1971). The other four barrels contained respectively, glutamate, bicuculline, GABA and glycine. The drugs were made up in aqueous solution as follows: L-glutamic acid (Sigma Chemical Company), 0·2 M adjusted to pH 8 with NaOH; bicuculline (Fluka Chemicals Ltd), 5 mM in 165 mM-NaCl, solution adjusted to pH 3 with HCl, bicuculline initially dissolved in several drops N/10 HCl; gamma-aminobutyric acid, GABA (Sigma Chemical Company), 0·5 M solution, adjusted to pH 3 with HCl, glycine (Sigma Chemical Company), 0·5 M solution adjusted to pH 3 with HCl. The retaining and ejecting currents used for controlling the release of the drugs were respectively: glutamate + 12 nA (retaining) and −10–50 nA (ejecting); bicuculline −10 nA (retaining) and +20 to +160 nA (ejecting); GABA −18 nA (retaining) and +1–30 nA (ejecting); glycine −15 nA (retaining) and +20–60 nA (ejecting). In some experiments an additional barrel was filled with bicuculline in place of either the glycine or glutamate. This procedure was adopted 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.

Penetrations were made through 4 mm diameter apertures in the skull overlying area 17. A very small area of the dura so exposed was dissected apart with watchmakers forceps, leaving the arachnoid intact. This enabled the micro-electrode to be positioned without damaging it on the tough superficial layer of dura. Generally four such apertures were made. A metal chamber 1·8 cm diameter and 1·0 cm deep was positioned over the apertures. The micro-electrode was inserted into the cortex underlying one of the apertures and sealed in position by filling the chamber with a solution of agar. Once the agar solidified it served to reduce both brain pulsations and the cooling of the exposed cortex but at the same time it permitted movement of the fine shaft of the micro-electrode. The electrode recording sites were marked by passing a current of 8 μA for 2–4 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 under each aperture was lightly marked with black dye. The brain was then fixed in a solution of 10% formol saline. A photographic record was made of the location of the marks on the surface of the cortex. Serial sections 50 μ thick were taken on a freezing microtome through the marked regions of the cortex. The sections were then stained with 1% neutral red, mounted and the location of the blue spots marking the recording sites determined. All the observations made in the present and the subsequent paper have been restricted to recording sites located within area 17.

The visual stimuli were projected on a target screen situated 1·0 m in front of the animal. The stimuli used were moving slits of light passing over the receptive field of the neurones examined at orientations or directions of motion that were found to produce an inhibition of the neural spontaneous discharge. With respect to the known properties of visual neurones (Hubel & Wiesel, 1962; Creutzfeldt & Ito, 1968; Bishop, Coombs & Henry, 1973) these represent fairly 'natural' stimuli in contrast to the electrical stimulation procedures often used to produce synaptically evoked inhibition (Curtis & Tebecis, 1972; McLennan & Miller, 1974; Curtis & Felix, 1971). Further details of the visual stimulation techniques and an account of the optical procedures are given in the subsequent paper (Sillito, 1975). The term 'optimal orientation' used in the test refers to that orientation of the testing stimulus that will evoke the largest excitatory response in the cell. An orientation other than the optimal is referred to as being so many degrees from the optimal.

As the iontophoretic application of bicuculline invariably caused an increase in neural spontaneous activity and this alone could produce some apparent modification of a visually evoked inhibitory input to the neurone, the effect of raising neural spontaneous activity with iontophoretically applied glutamate was compared with that of bicuculline. The inhibitory effect of iontophoretically applied glycine was also tested and compared with that of GABA before and during the application of bicuculline.

The neural responses to the moving visual stimuli were analysed by constructing post-stimulus histograms on line using a small purpose built computer (Lewin, 1974). Similarly, using either the computer or a spike counter, histograms were constructed for the response of the cells examined to the iontophoretic application of drugs. All the experimental data were recorded on an Ampex P.R. 500 instrumentation recorder for further evaluation of the results.

RESULTS

The results are based on the study of sixty-two neurones recorded from area 17 of the visual cortex. All but one of these neurones were inhibited by iontophoretic application of GABA with currents from 2 to 40 nA. In many cases simply switching off the backing current would produce inhibition of the spontaneous activity. Generally currents from 10 to 20 nA produced a complete suppression of both spontaneous and visually driven activity. Glycine was found to be less effective in producing inhibition than GABA insofar as higher ejection currents were necessary to produce a notable inhibition of spontaneous activity. Iontophoretic application of bicuculline with ejecting currents from 20 to 160 nA produced within 2–15 min a marked block of the inhibitory action of GABA in all but seven of the neurones tested. A block of the inhibitory action of iontophoretically applied GABA was generally accompanied by an increase in the neural spontaneous activity. The histograms in Fig. 1 show for a complex type visual cell (Hubel & Wiesel, 1962) the effect of bicuculline on the inhibitory action of iontophoretically applied GABA. In this example, prior to the bicuculline application the neural discharge rate was maintained at a steady high level by the continuous iontophoretic application of glutamate with a +20 nA ejecting current. Under these conditions even switching off the GABA backing current resulted in a suppression of the neurones' glutamate induced discharge, as illustrated in Fig. 1*a*. This shows very clearly the inhibitory effect of three consecutive 5 sec periods with the GABA backing current off. The continued application of glutamate to this neurone produced a steady increase in the discharge rate and the histogram in Fig. 1*b* shows the effect on this higher level of activity of three consecutive applications of GABA with a 4 nA ejecting current. Once more there was a marked inhibition of the neural activity. During the iontophoretic administration of bicuculline with an ejecting current of 80 nA the neural spontaneous activity increased and it was no longer necessary to maintain it with glutamate. As shown in Fig. 1*c* and *d* after 8 min continuous bicuculline application neither switching the GABA backing current off nor using a 4 nA ejecting current had much effect on the neural discharge. This was clearly not simply a product of the increased spontaneous discharge associated with the bicuculline as the discharge rate was very similar to that observed during the application of glutamate. Following termination of the bicuculline application there was a decrease in the neural spontaneous activity. The histogram in Fig. 1*f* shows the recovery of the inhibitory effect of 4 nA of GABA 10 min after the termination of the bicuculline application. Raising the neural discharge with iontophoretically applied glutamate and testing

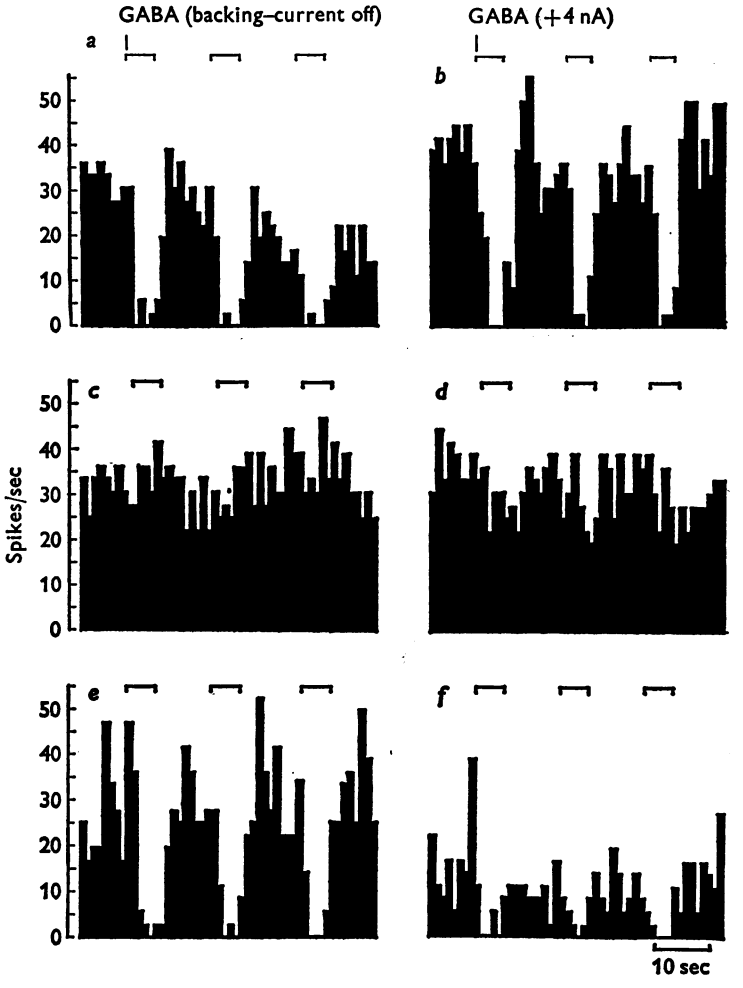

Fig. 1. Action of iontophoretically applied bicuculline on the GABA induced inhibition of the discharge of a complex cell in area 17 of the visual cortex. Histograms indicate number of spikes (ordinate) discharged by the cell in consecutive 1 sec periods. Bars above group of histograms on left indicate 5 sec periods with GABA backing current (-18 nA) off, bars above histograms on the right indicate 5 sec periods of electrophoresis of GABA with $+4$ nA. (*a*) effect of switching off GABA backing current on the background firing level induced by iontophoresis of glutamate with -20 nA. (*b*) effect of application of GABA with $+4$ nA on higher level of background activity induced by iontophoresis of glutamate (-20 nA) following an initial 3 min application period. (*c*) Switching GABA backing current off during the iontophoretic application of bicuculline with $+80$ nA. Bicuculline administered for 8 min period prior to test. Background firing not induced by glutamate. (*d*) as for *c* but GABA applied with $+4$ nA. (*e*) record taken 12 min after terminating bicuculline application. Effect of switching GABA backing-current off on background firing induced with -20 nA glutamate. (*f*) record taken before *e* (10 min after termination of bicuculline application) shows inhibitory action of $+4$ nA GABA on normal spontaneous activity of cell.

the effect of switching the GABA backing current off as shown in the histogram in Fig. 1*e* demonstrated that the effectiveness of the GABA had completely recovered.

The relative effectiveness of bicuculline in blocking the inhibitory action of iontophoretically applied GABA and glycine is illustrated in Fig. 2. This is constructed from the responses of another complex type visual cell and the histograms in Fig. 2*a* and *b* show the action respectively of 10 nA GABA and 40 nA glycine on the cell's spontaneous activity. Whilst both exerted a definite inhibitory effect on the neurones activity GABA appeared to have a rather stronger action. However, after a 15 min period of bicuculline application with 100 nA ejecting current the GABA had little effect on the cell's activity (Fig. 2*c*) while glycine still had a clear inhibitory action (Fig. 2*d*). There was a recovery of the inhibitory action of GABA 10 min after terminating the bicuculline application (Fig. 2*f*). For all the neurones where the comparison between glycine and GABA was made (twenty), bicuculline selectively antagonized the inhibitory action of GABA as compared to glycine.

At the stage that bicuculline blocked the inhibitory action of iontophoretically applied GABA it was also observed in forty-three neurones to produce modifications of receptive field properties consistent with a block of visually evoked inhibitory influences. An example of the effect of bicuculline on visually evoked inhibition is shown in Fig. 3. The records in this figure are post-stimulus histograms showing the response of the cell to the motion of a vertically oriented slit forwards and backwards over the receptive field. This stimulus exerted a small but clear inhibition of the neurone's spontaneous discharge.

The starting point of each histogram represents the time of initiation of the slit motion such that the histogram up to the dotted line corresponds to a movement of the slit from a region outside the receptive field forwards over the receptive field and beyond the receptive field. The histogram to the right of the dashed line corresponds to the return of the slit 'backwards' over the receptive field to the starting point. Each point on the histogram thus represents a particular point in time with respect to the start of the stimulus cycle and a spatial position with respect to the movement of the slit over the receptive field. Prior to the application of bicuculline, as shown in Fig. 3*a*, the time at which the slit passed over the receptive field is quite apparent from the inhibition produced in the neurone's spontaneous discharge. The cell illustrated in this example was atypical because it was apparently either unaffected or inhibited by all visual stimuli whether presented to the ipsilateral or the contralateral eye. Most cells in the visual cortex show an excitatory response to some visual stimuli. During the iontophoretic administration

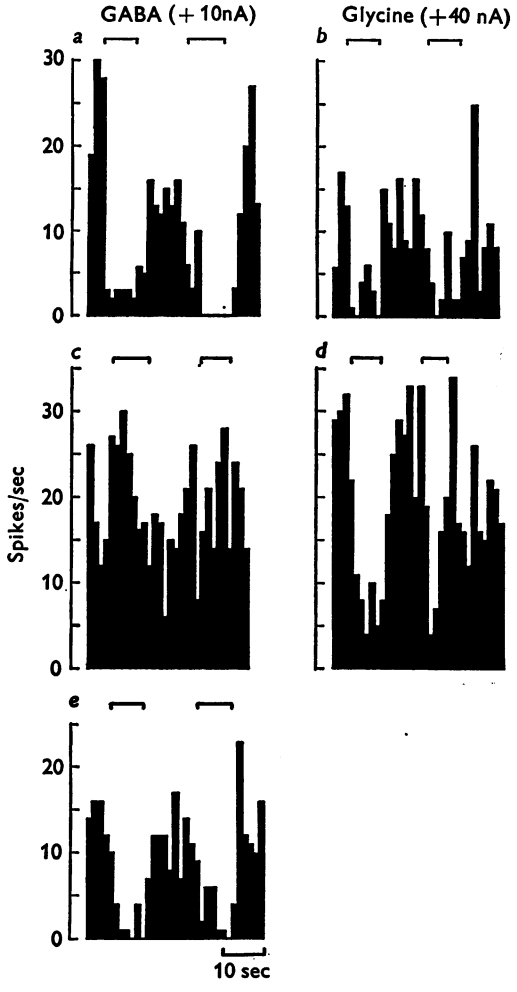

Fig. 2. Relative action of iontophoretically applied bicuculline on the GABA and glycine induced inhibition of the spontaneous discharge of a complex type visual cell. Histograms indicate number of spikes (ordinate) discharged by the cell in consecutive 1 sec periods. (*a*) effect of two consecutive applications of +10 nA GABA on the normal spontaneous discharge. Period of GABA application indicated by horizontal bars above records. (*b*) effect of two consecutive applications of glycine with a +40 nA ejecting current on the cells normal spontaneous discharge. Period of glycine application indicated by horizontal bars above records. (*c*) effect of +10 nA GABA during the iontophoresis of bicuculline (+100 nA). Records taken after an initial 15 min period bicuculline application. (*d*) effect of 40 nA glycine during the iontophoresis of bicuculline (+100 nA). Record taken 1·0 min after record *c*. (*e*) Effect of 10 nA GABA 10 min after termination of bicuculline application.

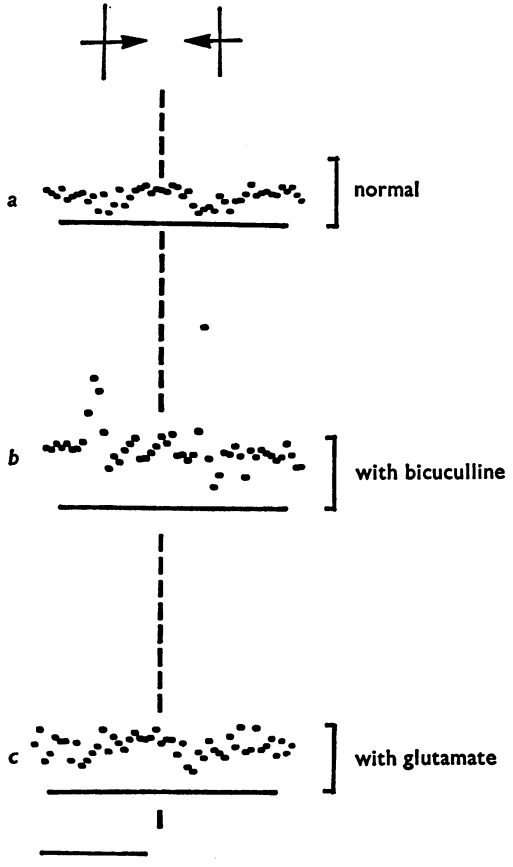

Fig. 3. Action of iontophoretically applied bicuculline on visually evoked inhibition. See text for comments on type of cell. Post-stimulus histograms show response of the cell to the motion of a vertically oriented luminous slit forwards (record to left of dashed line) and then backwards (record to the right of the dashed line) over the receptive field. Each post-stimulus histogram is constructed from twenty-five consecutive complete cycles of the stimulus motion over the receptive field. Height of dots above the base line represent the total number of action potentials discharged in a particular 50 msec period (bin). Vertical calibration scale to the right of the record indicates range corresponding to 0–80 spikes/sec. Horizontal calibration bar indicates 1·0 sec. (*a*) normal response of cell. (*b*) Response of cell during iontophoretic application of bicuculline (+70 nA). Record taken after 9 min continuous application. (*c*) response of cell after recovery from effects of bicuculline but with background firing level increased by iontophoresis of glutamate (−25 nA).

of bicuculline (70 nA) there was an increase in the over-all neural discharge rate and the appearance of an excitatory response to both directions of motion of the slit (Fig. 3*b*) where previously there had been apparently only inhibition. This effect could be thought to relate to an increase in the neurones discharge rate and excitability secondary to some excitatory action of bicuculline rather than to a block of inhibition. The histogram in Fig. 3*c* shows an attempt to answer this question after the recovery

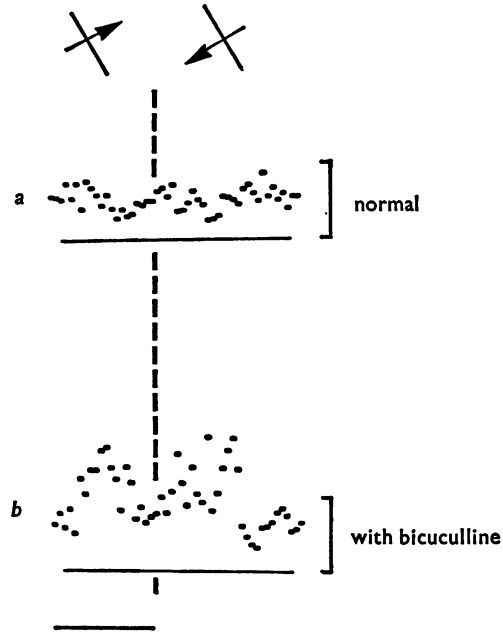

Fig. 4. Further example of action of iontophoretically applied bicuculline on visually evoked inhibition. Post-stimulus histograms show the response of a complex cell to a luminous slit at 90° to the optimal orientation moving over the receptive field. Each record constructed from twenty-five consecutive stimulus cycles. Bin size 50 msec. Vertical and horizontal calibrations as for Fig. 3. (*a*) Normal response of the cell. (*b*) response of cell during iontophoretic application of bicuculline (120 nA). Record taken after initial 12 min period of bicuculline application.

from the effects of bicuculline, by repeating the visual stimulation with the spontaneous activity increased to the bicuculline level by iontophoretic applied glutamate (25 nA). Despite the increase in spontaneous activity the cell did not show an excitatory response and still appeared to be slightly inhibited by the motion of the slit over the receptive field. It appears that the effect of bicuculline in this example, at least in part, related to a block or reduction of a visually evoked inhibitory input

to the neurone. Further to this, the block of the inhibition revealed the presence of a visually evoked excitatory input to the neurone, something which was not at all apparent when the neural discharge rate was raised with glutamate. Only during the application of bicuculline was an excitatory input to this cell revealed.

A further example of the effect of bicuculline on visually evoked inhibition is shown in Fig. 4. The histograms here represent the response of a complete cell to a luminous slit moving over its receptive field at an orientation 90° from its optimal. This cell showed clear excitatory responses to orientations in the region of the optimal but was inhibited by moving stimuli at orientations approaching and including 90° to the optimal. The histogram in Fig. 4*a* shows the normal response of the cell, there was a definite inhibition of the spontaneous discharge as the slit passed over the receptive field in either direction. Applying bicuculline to this neurone with a 120 nA ejecting current for 12 min resulted in a large visually evoked excitatory response in place of the inhibitory response (Fig. 4*b*). In all the instances where bicuculline was effective in blocking the visually evoked inhibition of a cell's resting discharge an excitatory response was revealed in place of the original inhibitory response.

The iontophoretic application of bicuculline also resulted in cells showing excitatory responses to stimuli that did not previously evoke any obvious response in the cell either excitatory or inhibitory. This effect was not reproduced by raising the neurone's discharge rate with glutamate. In fact when the resting discharge rate of cells, which had previously exhibited either no spontaneous activity or a very low level, was increased with glutamate, the stimulus could often (but not always) be seen to produce a suppression of the activity. It was thus possible to demonstrate that in these cases there was an inhibitory input to the cell normally blocking the excitatory response and that the action of bicuculline was consistent with a block of this inhibitory input. An example of this is provided by the responses of the simple cell shown in Fig. 5. The cell normally responded only to the forward motion of the slit over the receptive field (Fig. 5*a*). The spontaneous activity was too low to ascertain whether there was any inhibition evoked by the slit as it passed in a backwards direction over the receptive field. The iontophoretic application of bicuculline with a 60 nA current for 8 min increased the neural spontaneous activity and revealed a small excitatory response to the backwards motion of the slit over the receptive field (Fig. 5*b*). After the recovery of the cell from bicuculline, raising the spontaneous activity to the bicuculline level by iontophoretically applied glutamate revealed in place of the excitatory response to the backwards motion of the slit

seen with bicuculline, a definite but small inhibitory dip in the neurone's spontaneous discharge (Fig. 5*c*). The lower histogram in Fig. 5 shows the 'normal' response of the cell at the end of the series of tests. Although the spontaneous activity is rather higher than it was at the beginning

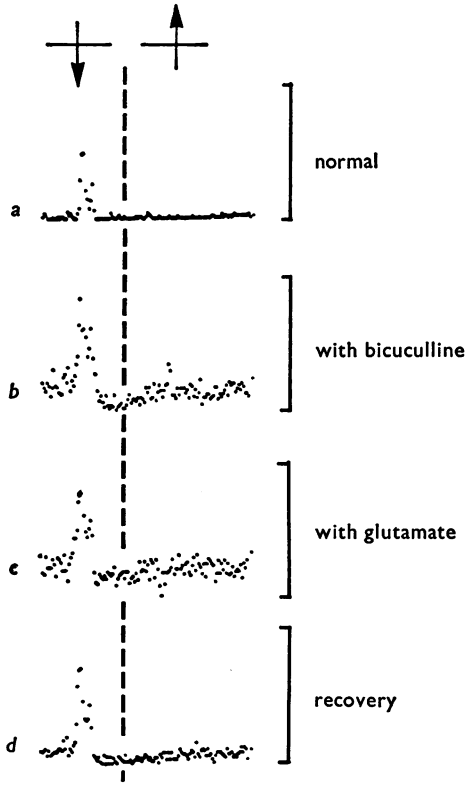

Fig. 5. Response of directionally specific simple cell to a luminous slit at the optimal orientation moving over the receptive field. Each post-stimulus histogram constructed from thirty-two cycles of stimulus motion. Bin size 20 msec. Vertical calibration indicates range corresponding to 0–156 spikes/sec. Horizontal calibration bar corresponds to 1·0 sec. (*a*) normal response of cell. (*b*) effect of iontophoresis of bicuculline (60 nA) on the response of the cell. Record taken after initial 8 min application period. (*c*) after recovery from bicuculline but with background firing raised by iontophoretic application of glutamate (30 nA). (*d*) response at end of series of tests.

(i.e. Fig. 5*a*) there was little sign of either excitation or inhibition to the backwards motion of the slit over the receptive field. Presumably in the normal state, the excitatory and inhibitory inputs evoked by a slit

passing over the receptive field in this direction, cancel each others effect.

Even where visual stimuli evoked excitatory responses the iontophoretic application of bicuculline often produced large increases in the magnitude of the evoked response that were consistent with the release of the cell from a powerful inhibitory influence. This type of effect was most notable on the responses of cells to orientations up to 30° either side of the optimal and was observed in forty-two of the cells examined. It was also seen in the response to the optimal orientation but generally the over-all increase at the optimal was smaller. The effect is well illustrated by the response of the simple cell in Fig. 6 to the motion of a slit, at an orientation 22·5° clockwise to the optimal, over the receptive field. The spontaneous activity of the cell was very low and it gave a residual excitatory response to both directions of motion of the slit over the receptive field (Fig. 6*a*). During the application of bicuculline, although there was a small increase in the spontaneous activity, the evoked response to both directions of motion showed a many fold increase in magnitude (Fig. 6*b*). The response reverted to the original 10 min after cessation of the iontophoresis of bicuculline, as shown in Fig. 6*c*.

An effective block of the inhibitory action of iontophoretically applied GABA was produced in eleven of the neurones examined without resulting in any detectable alteration in receptive field properties either with respect to inhibition or excitation. This could have resulted from the fact that although the bicuculline was reaching the cell membrane in the immediate vicinity of the electrode it was not effectively diffusing to inhibitory synapses acting on the neurone. Alternatively some other inhibitory transmitter could be involved in regulating the cell's responses. In the case of the seven neurones in which the iontophoretic application of bicuculline failed to block the inhibitory action of iontophoretically applied GABA it also failed to block visually evoked inhibitory inputs and failed to produce any modification of the excitatory responses of the cell.

DISCUSSION

The results indicate that iontophoretically applied bicuculline will block the inhibitory action of iontophoretically applied GABA on neurones in the striate cortex of the cat. In no case was bicuculline found to potentiate the inhibitory action of iontophoretically applied GABA (Straughan *et al.* 1971; Godfraind *et al.* 1970) and the results support the conclusion of Curtis *et al.* (1970, 1971*a*, *b*) that bicuculline is an effective antagonist of GABA in the central nervous system.

Iontophoretically applied glycine was found to have a less potent inhibitory action than GABA which is in accord with previous observations of the comparative effectiveness of GABA and glycine on cortical neurones (Kelly & Krnjević, 1969). The effects of glycine were not abolished by bicuculline.

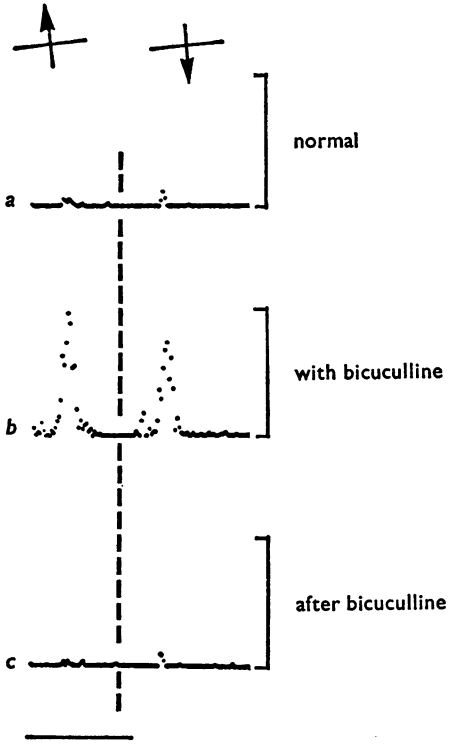

Fig. 6. Action of iontophoretically applied bicuculline on the magnitude of the evoked excitatory response in a simple cell. Post-stimulus histograms show the response of the cell to a luminous slit at an orientation 22·5° clockwise to the optimal orientation. Each histogram constructed from twenty-five cycles of the stimulus motion. Bin size 15 msec. Vertical calibration indicates range corresponding to 0–266 spikes/sec. Horizontal calibration bar corresponds to 1·0 sec. (*a*) a response of cell prior to application of bicuculline. (*b*) effect of iontophoretic application of bicuculline (100 nA) on response. Record taken after 15 min continuous application. (*c*) response of cell 15 min after termination of bicuculline application.

The block of the inhibitory action of iontophoretically applied GABA was associated with a block of visually evoked suppression of the neuronal resting discharge and modifications of receptive field properties consistent with the elimination or reduction of inhibitory inputs to the cell. It is

plausible that a visually evoked suppression of the neuronal resting discharge might not derive from a post-synaptic inhibitory process but derive instead from either a presynaptic inhibitory process or from disfacilitation via a reduction in activity of a tonic excitatory input. However the fact that the visually evoked suppression was apparent when the resting discharge was maintained at an artificially high level with iontophoretically applied glutamate but was blocked by bicuculline suggests that neither disfacilitation nor presynaptic inhibition underlie the effect. A further complicating factor in the interpretation of the present results is that in all cases where inhibition of the resting discharge was blocked an excitatory response was revealed. This introduces the possibility that the application of bicuculline not only blocked a post-synaptic inhibitory process acting on the cells examined, but in addition released an excitatory input either by blocking a presynaptic inhibitory process, or by blocking a post-synaptic inhibitory process acting on adjacent excitatory interneurones. Evidence obtained from intracellular and quasi-intracellular recordings of visual cortical cells is important in this context. It appears that most visual stimuli whether producing an inhibition or an excitation of the extracellularly recorded action potential discharge, evoke both inhibitory and excitatory post-synaptic potentials (Creutzfeldt & Ito, 1968; Benevento, Creutzfeldt & Kuhnt, 1972; Innocenti & Fiore, 1974). Thus even where stimuli evoke an inhibition of the extracellular response, a block of the post-synaptic inhibitory processes acting on a cell is highly likely to reveal an excitatory input. It is consequently unnecessary to postulate additional effects on adjacent excitatory interneurones or presynaptic inhibition to explain the excitation released by bicuculline in the present work.

Although the application of bicuculline invariably produces an increase in neuronal resting discharge and excitability, the possibility that this alone represents a significant contribution to its apparent effects on post-synaptic inhibition can largely be excluded, on the basis of the fact that iontophoretically applied glutamate does not mimic its action. However it has been suggested that bicuculline potentiates the excitatory action of synaptically released acetylcholine by acting as an anticholinesterase (Svenneby & Roberts, 1973; Miller & McLennan, 1974). With reference to the fact that the mechanism of the excitatory action of acetylcholine is rather different to that of glutamate (Krnjević, Pumain & Renaud, 1971) this could result in an excitatory effect which is not replicated by the application of glutamate. Curtis, Johnston, Game & McCullogh (1974), however, after comparing the action of bicuculline with physostigmine, contest the view that the effect of bicuculline reflects an action on acetylcholinesterase and maintain that its primary effect

is related to its action as a GABA antagonist. With reference to the sensitivity of cells in the visual cortex to acetylcholine, only about 20% of the cells appear to show an increase in their resting discharge to iontophoretically applied acetylcholine (Spehlman, Daniels & Smathers, 1971; Wallingford *et al.* 1973). Thus considering the available evidence it seems unlikely that an action on acetylcholinesterase represents a significant contribution to the effects of bicuculline as observed in the present work.

Comparable results to the present ones were obtained by Curtis & Felix (1971) in the post-cruciate cortex of the cat. They found that bicuculline blocked the inhibitory action of iontophoretically applied GABA and reduced synaptically evoked inhibition following electrical stimulation of the cortex or medullary pyramid and following chemical stimulation of intracortical neurones. Iontophoretically applied bicuculline has also been found to antagonize synaptically evoked inhibition in other parts of the central nervous system including the lateral geniculate body (Curtis & Tebecis, 1972) and the septal nuclei (McLennan & Miller, 1974). In the visual cortex modifications of receptive field properties of single neurones consistent with a block of intracortical inhibitory processes have been reported following intravenous injection or topical application of bicuculline (Daniels & Pettigrew, 1973; Rose & Blakemore, 1974). There is some difficulty however in interpreting the functional effects of intravenously or topically applied bicuculline on the responses of single cells in the visual cortex (see Sillito, 1975, for further discussion of this problem).

An important objective of the present investigation was to examine in the light of previous evidence on the matter, whether iontophoretically applied bicuculline could be used to block naturally occurring inhibitory processes in the visual cortex. The results obtained here indicate that iontophoretically applied bicuculline can be used for this purpose and hence it provides a useful experimental method for investigating the contribution made by inhibitory processes to the receptive field properties of visual cortical neurones. The specific effects of iontophoretically applied bicuculline on the receptive field of cells in the visual cortex is the subject of a further paper (Sillito, 1975). The present observations support previous evidence (Mitchell & Srinivasan, 1969; Iversen, Mitchell & Srinivasan, 1971) favouring the role of GABA as an inhibitory transmitter in the visual cortex.

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