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THE ROLE OF γ-AMINOBUTYRIC ACID MEDIATED INHIBITION IN THE RESPONSE PROPERTIES OF CAT LATERAL GENICULATE NUCLEUS NEURONES

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SUMMARY

1. We studied the effect of local ionophoretic application of bicuculline on the response of cat lateral geniculate nucleus (laminae A) cells to stimulation by sinusoidal gratings and spots of light.

2. Application of bicuculline produced an increase both of spontaneous and visually driven discharge of both X and Y cells.

3. On stimulation by drifting sinusoidal gratings, the average discharge of both X and Y cells remained constant with increasing contrast under normal conditions. Application of bicuculline caused the average discharge to increase with contrast, indicating that the constancy of the average discharge was maintained by γ-aminobutyric acid mediated inhibition.

4. Under normal conditions, the amplitude of response modulation of both X and Y cells to sinusoidal grating stimulation increased monotonically with stimulus contrast. During bicuculline application, the slope of the contrast–response curve for X cells but not for Y cells increased, indicating that the inhibition which dampened the modulation of X cells (but not Y cells) was contrast dependent.

5. Application of acetylcholine also increased the average discharge and the amplitude of modulation of the cell responses, but this increase did not depend on stimulus contrast.

6. Under normal conditions, X but not Y cells showed an attenuation of response and an increase in contrast threshold to low spatial frequencies. This attenuation vanished during bicuculline application. The shape of Y-cell response curves was unaffected by bicuculline.

7. Bicuculline had the same effect on the non-linear component of Y-cell response as on the linear component.

8. Although bicuculline had a different effect on the response of X and Y cells to stimulation by gratings, it reduced the antagonistic surround of both X and Y cells to a similar extent (revealed by plotting the cell receptive fields with flashed spots of light).

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INTRODUCTION

Since the work of Hubel & Wiesel (1961) the dorsal lateral geniculate nucleus (l.g.n.) has been thought of as a visual station where the information flowing from the retina is not simply relayed to the visual cortex, but is rearranged and processed. They pointed out that retinal ganglion cells and l.g.n. cells, despite having very similar receptive fields, differed in the structure of their discharge as well as in their response to a large spot of light. The receptive fields of l.g.n. cells have a more powerful antagonistic surround and this antagonism is present even at low luminance levels where the antagonistic surround of retinal ganglion cells disappear (Maffei & Fiorentini, 1972; Hammond, 1973; Virsu, Lee & Creutzfeldt, 1977). The mechanisms and the local circuitry which fortify the antagonism of the geniculate receptive field surround are not yet well understood.

Although several retinal ganglion fibres converge onto a single l.g.n. cell, often one fibre appears to provide the dominant input (Cleland, Dubin & Levick, 1971*a*, *b*). Furthermore, in most cases all the converging fibres are from the same type of retinal ganglion cells: X, Y and W ganglion cells relay to X-, Y- and W-type geniculate cells respectively. On-centre ganglion cells relay principally to on-centre l.g.n. cells and off-centre to off-centre (Lennie, 1980; Schiller, 1982). Thus it seems unlikely that the increased l.g.n. antagonism results solely from a convergence of retinal ganglion cells. Another possibility is that inhibitory mechanisms play a role in fortifying l.g.n. antagonistic surround (Singer & Creutzfeldt, 1970; Singer, Poppel & Creutzfeldt, 1972; Sillito & Kemp, 1983).

Both anatomical (Famiglietti & Peters, 1972; LeVay & Ferster, 1979) and electrophysiological (Dubin & Cleland, 1977; Lindstrom, 1982) data provide evidence for the existence of local inhibitory interneurones. Dubin & Cleland (1977) found two types of inhibitory interneurones that converge on relay cells. One type has the cell body located in the perigeniculate nucleus, the other in the l.g.n. itself. According to their and other data, (LeVay & Ferster, 1979) the intrageniculate interneurones comprise about 25% of the total population of the nucleus and could correspond to class III cells of the morphological classification of Guillery (1966) and Famiglietti & Peters (1972). This circuitry in the l.g.n. indicates that a dual inhibition converges on l.g.n. relay cells: a feed-back inhibition mediated by the perigeniculate interneurones and a feed-forward inhibition mediated by the intrageniculate interneurones. The putative transmitter of both inhibitory systems is γ-aminobutyric acid (GABA) (Curtis & Tebecis, 1972; Morgan, Sillito & Wolstencroft, 1974; Houser, Vaughn, Barbe & Roberts, 1980; Sterling & Davis, 1980; Hunt, Liebermann, Ohara & Wu, 1982).

Recent publications (Kaplan, Marcus & So, 1979; Friedlander, Lin, Stanford & Sherman, 1981; Shapley & So, 1981) have questioned the existence and the relevance of intrageniculate inhibitory circuitry. They suggest that the difference between the properties of retinal ganglion cells and l.g.n. cells are small and could follow from the excitatory convergence.

By means of ionophoretic application of bicuculline, a GABA antagonist, Sillito & Kemp (1983) have recently shown that l.g.n. cells do receive a strong visual inhibitory input and that the antagonistic strength of receptive field surround is attenuated during bicuculline application. This demonstrates that the antagonistic surround of l.g.n. cells is partly due to GABA mediated local inhibition.

One method of characterizing the spatial and temporal properties of visual neurones, which has been applied with some success in the l.g.n., is to measure cell responses to sinusoidal gratings. Geniculate cells respond well to gratings, and the amplitude of their response varies monotonically with grating contrast. X and Y cells have different spatial frequency tuning (Maffei & Fiorentini, 1973; Derrington & Fuchs, 1979; Lehmkuhle, Mangel & Sherman, 1980). The tuning curve for Y cells is low-pass, showing attenuation only for high spatial frequencies, X cells, however, have bandpass tuning curves, showing attenuation of response for frequencies both higher and lower than their preferred frequency. This bandpass tuning is not observed for the X cells' retinal input (Lee, Elepfandt & Virsu, 1981).

The aim of the present work was to study the relevance of local GABA mediated inhibitory inputs to the spatial and temporal properties of X and Y cells in laminae A and A1 of the cat l.g.n., by measuring their response to gratings with and without local application of bicuculline. Preliminary results have been reported by Berardi & Morrone (1982) and Morrone & Berardi (1983).

METHODS

Experiments were carried out on thirteen adult cats. We recorded 113 cells in laminae A and A1 of the cat l.g.n. and extensively analysed fifty-nine of them, thirty-one X and twenty-eight Y cells. Anaesthesia was induced with intramuscular injection of α-Althesin (CT1341, Glaxo), 1·5 ml/kg. A small opening in the skull was made in the region overlying the l.g.n. and at the end of the surgical procedure all the operated areas were infiltrated with local anaesthetic (Novocaine). During the recording session the animal was paralysed and anaesthesia was maintained with continuous intravenous infusion of Pavulon (pancuronium bromide, N.V. Organon) at 0·2–0·3 ml/kg per hour and Althesin at 0·1–0·4 ml/kg per hour.

End-tidal CO_2 (3·8–4·2 %), e.c.g. and e.e.g. were monitored throughout the experiment and the body temperature was maintained at 38 °C.

Pupils were dilated with atropine and optically neutral lenses with artificial pupils of 3 mm diameter were applied. The refraction of the cat's eyes was determined by means of retinoscopy and corrected with suitable spectacle lenses placed in front of the eyes. At the beginning of the experiment the position of the papillae and the area centralis was determined using either an inverting ophtalmoscope or the technique described by Fernald & Chase (1971).

Five-barrel micropipettes were used for the extracellular recording of action potentials and ionophoretic application of drugs. The centre recording barrel (which was also used to mark the recording site) was filled with a solution of 2 % Pontamine Sky Blue in 0·5M-sodium acetate. The remaining four barrels contained a selection of the following drugs made up in aqueous solution: γ-aminobutyric acid (Sigma) (0·5 M, pH 3); *N*-methylbicuculline, as chloride or iodide (Pierce) (5 mM, pH 3, in 165 mM-NaCl); acetylcholine (ACh) chloride (Sigma) (0·2 M, pH 4·5). Where necessary, pH adjustments were made with HCl or NaOH.

Sinusoidal gratings either drifting or alternating in phase were presented on a display (HP 1300 or Cambridge Electronic). Contrast, spatial frequency and temporal frequency could be varied independently. Spot stimuli were generated by placing an appropriate cut-out template on the oscilloscope screen and modulating the oscilloscope luminance. The luminance of the screen was 6 cd/m^2 for the HP 1300 display and 50 cd/m^2 for the Cambridge Electronic display; the latter was reduced, when necessary, by placing neutral-density filters in front of the cat's eyes. Spot stimuli contrasts were around 30 % and grating contrasts ranged from 0 to 65 %. All measurements were made in the mesopic to photopic range.

Cell responses to visual stimuli were suitably amplified and filtered and fed to a Digital PDP11/03 laboratory computer which displayed on-line the peri-stimulus time histogram (p.s.t.h.) and stored the sequences of discrimination spikes for further analysis.

In the course of the experiments we always took the amplitude of the fundamental harmonic as a measure of the linear part of cell-response modulation to sinusoidal gratings, and the amplitude of the second harmonic as a measure of the non-linear part.

On isolating a unit we classified it as X or Y on the basis of numerous tests, including the presence or the absence of a null point/second harmonic modulation to a counter-phased grating (Enroth-Cugell & Robson, 1966; Hochstein & Shapley, 1976), presence of absence of a shift effect (Kratz, Webb & Sherman, 1978), and the highest spatial frequency eliciting a cell response when the grating was drifted compared to the value obtained when the stimulus was modulated in counter-phase. Most cells could be unambiguously classified as X or Y.

The contrast–response curves and the spatial and temporal frequency tuning curves were measured by constructing a sequence of trials where the chosen parameter was increased or decreased step by step, and then running the trials in a quasi-random order. The steps we used were generally half an octave or less. The contrast–response curves and the tuning curves were taken in the normal condition first, then during the ionophoretic application of bicuculline and again after the cessation of bicuculline effects. To evaluate the contrast threshold of a cell for a given spatial and temporal frequency we took both the minimum stimulus contrast which still yielded an audible modulation of the discharge and the extrapolation to zero of the contrast–response curve. The thresholds given by these two methods were generally well in accordance. The experimental points of the contrast–response curves were interpolated by means of a best fit with polynomials up to the 3rd order.

The pharmacological effectiveness of bicuculline was assessed in the following manner. Initially, the GABA ejection current level necessary to completely suppress the cell response to an optimum stimulus was determined. Once the bicuculline application was initiated, it was judged to be effective when it blocked the action of this pre-determined GABA ejection current on the visual response. Generally we observed that GABA ejection current could be increased to 2 or 3 times this level during bicuculline application without having any effect. As the test sequences took some considerable time to complete, long bicuculline ejection periods (20–30 min) were necessary, and changes in cell excitability over this period due to an increasing concentration of the drug had to be considered. For this reason, critical observations were repeated several times through the sequence. In general we found it necessary to hold a cell at least 2 h to complete a set of our test and controls.

At the end of each penetration, current (10–20 μA, negative polarity) was passed continuously through the tip of the micropipette during withdrawal to mark the recording site. At the end of the experiment, which usually lasted for two days, the animal was killed with an overdose of Pentothal, perfused with 10% formaldehyde in 165 mM-NaCl, and blocks of frozen tissue were sectioned to 80 μm. Sections were mounted on gelatinized slides, stained with Neutral Red and examined for the reconstruction of the penetration.

RESULTS

Contrast–response curves of X and Y cells

Effect of bicuculline

L.g.n. cells respond to a drifting grating both with an increase in the discharge rate and with a modulation of discharge, the modulation having the temporal periodicity of the stimulus.

Figs. 1 and 2 (for an X and a Y cell respectively) show a typical example of the response of l.g.n. cells to drifting gratings at three different contrast levels.

For low contrasts (Figs. 1 and 2, bottom row) both cells responded with a slight modulation of their activity around a mean value similar to their spontaneous discharge. As the stimulus contrast increased the modulation depth also increased, while the average discharge of both cells remained constant. Only at very high contrasts did the average discharge start to increase. However, at high contrasts the cell responses, particularly those of X cells, tended to become rectified, dropping to zero for a substantial part of the p.s.t.h. This rectification could account for the slight increase of the average discharge at high contrast. The responses of the cells shown in Figs. 1 and 2 are typical of all X ($n = 15$) and Y ($n = 11$) cells we observed.

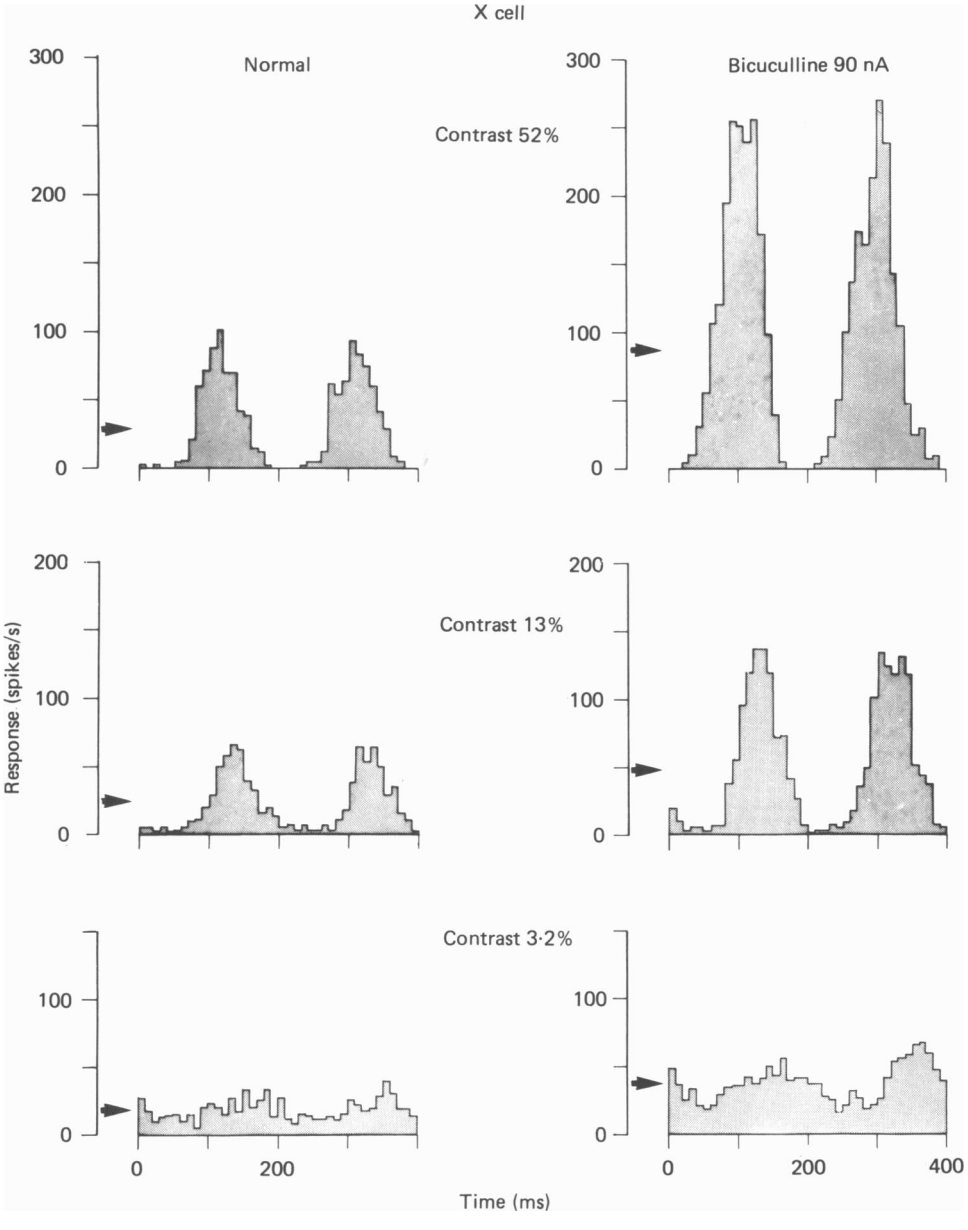

Fig. 1. Peri-stimulus time histogram of the response of an X cell to a drifting sinusoidal grating (spatial frequency 0·3 cycle/deg, temporal frequency 2·5 Hz) at three different contrast levels (52, 13 and 3·2%). The cell response was averaged over sixty periods of stimulation, and two periods of the response are shown. Bin size = 10 ms. The responses in normal condition are shown in the left column, those during bicuculline application (90 nA) in the right column. The black arrows mark the average discharge. The cell spontaneous discharge was around 17 spikes/s in normal conditions and around 24 spikes/s during bicuculline application.

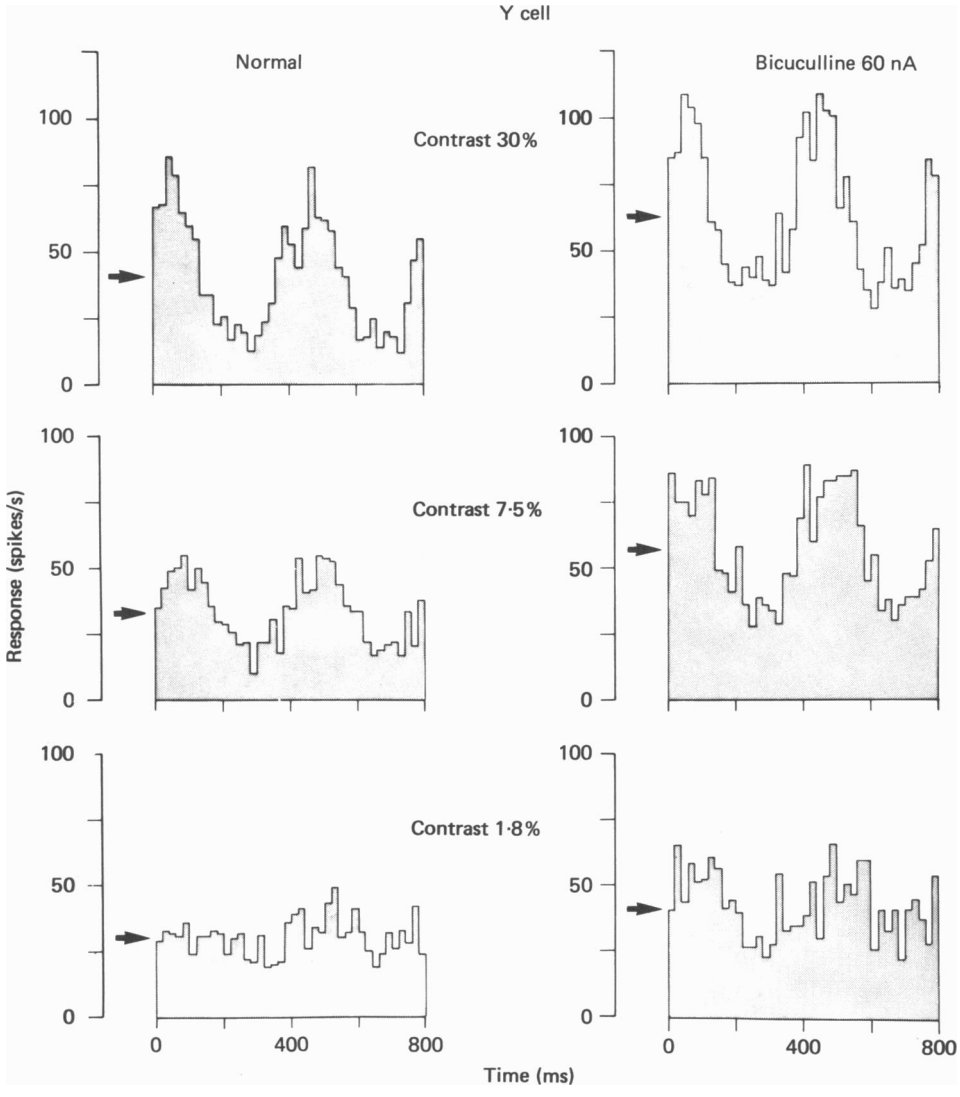

Fig. 2. Peri-stimulus time histogram of the response of a Y cell to a sinusoidal drifting grating (spatial frequency 0·3 cycle/deg, temporal frequency 2·5 Hz) at three different contrast levels (30, 7·5 and 1·8%). The cell response is averaged over sixty periods of stimulation, and two periods of the response are shown. Bin size = 10 ms. Conventions as in Fig. 1. The cell spontaneous discharge was 22 spikes/s in normal conditions and 36 spikes/s under bicuculline application.

Application of bicuculline increases the cell responsiveness both during visual stimulation and when it is unstimulated (spontaneous activity). The cell response to sinusoidal gratings during bicuculline application still shows the characteristic modulation shown by the response in the normal condition, with little change of temporal phase. However, at each contrast level bicuculline induces an increase both

in the amplitude of modulation and in the average discharge. After a suitable period of recovery, the cell responsiveness returns to normal.

(The phase of the 1st harmonic of the modulation is a monotonically decreasing function of contrast for Y cells, with a phase advance up to 60 deg/log unit, while the phase advance of X cells is much less pronounced, and is sometimes appreciable only when saturation of the cell response with contrast is reached. Bicuculline application does not alter the phase advance of Y cells appreciably, and induces a small phase shift in X cells. The phase of the response to a given contrast, on average, does not shift during bicuculline application by more than 30 deg.)

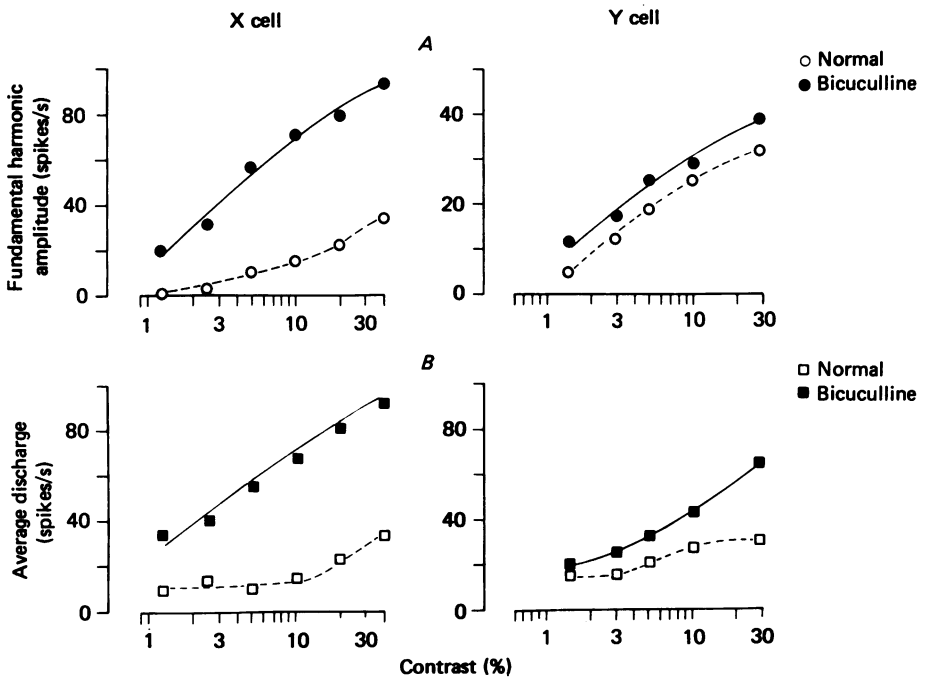

Fig. 3. *A* and *B*, contrast–response curves for an X cell (left) and a Y cell (right) under normal conditions (open symbols) and during bicuculline application (filled symbols). The cell response is evaluated in terms of the first harmonic of the temporal modulation of the discharge (*A*) and in terms of the average discharge (*B*). Bicuculline ejection currents: 90 nA for the X cell and 60 nA for the Y cell. Spatial frequency 0·3 cycle/deg for X cell and 0·5 cycle/deg for Y cell, temporal frequency 5 Hz for the X cell and 4 Hz for the Y cell. The polynomial fit of the data is up to the third order except for the filled squares in Fig. 3*B*, right, where the third order was no improvement with respect to the second-order fit.

The effect of bicuculline is best documented by the contrast–response curves of Fig. 3. Consider first the amplitude of modulation (Fig. 3*A*). For the X cell, (Fig. 3*A*, left) the effect of bicuculline application is not only to shift the intercept of the curve, implying a lower contrast threshold, but also to change the slope of the contrast–response curve, implying higher gain. Response still increases monotonically with

stimulus contrast, as it does in normal conditions, but the differential increase is greater. This suggests that the effect of bicuculline on the cell responsiveness progressively increases with contrast. The contrast–response curve of the Y cell, (Fig. 3*A*, right) also exhibits a shift of threshold, but not a change in slope. The two contrast–response curves are virtually parallel.

Consider now the curves plotting the average discharge (Fig. 3*B*). As mentioned earlier, in the normal condition the average discharge varies little with contrast variation, at least for low contrasts. During bicuculline application, however, the response increases greatly with increasing contrast even at low contrast levels. Now the increase can no longer be attributed to the rectification of the cell response, as at no point does the cell response reach zero. The difference between the response in the normal condition and during bicuculline application is mainly noticeable at low contrast levels (less than 15%), where the average discharge curves are flat in normal conditions, but show a response increase of a factor of two during bicuculline application. This behaviour is typical for both the X and Y cells we tested (fourteen X and ten Y cells).

Controls: application of ACh

From the previous results alone it is not clear whether the described changes in the contrast–response curve result from the bicuculline block of the GABA mediated inputs, or from a non-specific excitatory effect. To distinguish between these two hypotheses, we increased cell responsiveness without removing the inhibitory input, by application of ACh, and remeasured the contrast–response curves. ACh is a transmitter that possibly mediates the excitatory component of the mesencephalic reticular formation of l.g.n. (Curtis & Davis, 1963; Phillis, Tebecis & York, 1967; Hoover & Jacobowitz, 1979; Kemp & Sillito, 1981).

The spontaneous discharge, the amplitude of modulation and the average discharge all increase during ACh application (Fig. 4*A* and *B*) as they do during bicuculline application. However, the changes in the contrast–response curve induced by ACh are different from those induced by bicuculline (Fig. 3*A* and *B*). The increase in the average discharge of neither X nor Y cells varies with contrast. The curves of Fig. 4*B* are completely flat, not even showing the slight increase at high contrasts that occurs in the normal condition, and certainly not exhibiting the uniform increase observed during bicuculline application. Only when the inhibitory input is removed by means of bicuculline is the average discharge dependent upon stimulus contrast.

When the amplitude of modulation is considered (Fig. 4*A*), the effects of ACh are to facilitate the cell response for both X and Y cells, without inducing a change of slope of the contrast–response curve.

The results of this and of the previous section (which were observed for all the X and Y cells we tested) suggest that the inhibitory input is different for X and Y cells. For Y cells the inhibitory input seems to be related only to the control of the cell average discharge. The parallel shift in the amplitude of modulation curves could follow from the general increase in cell responsiveness. The inhibition on X cells, however, affects the slope of both average discharge and amplitude of modulation. These changes in curve slope suggest the presence of an inhibitory input related to gain control.

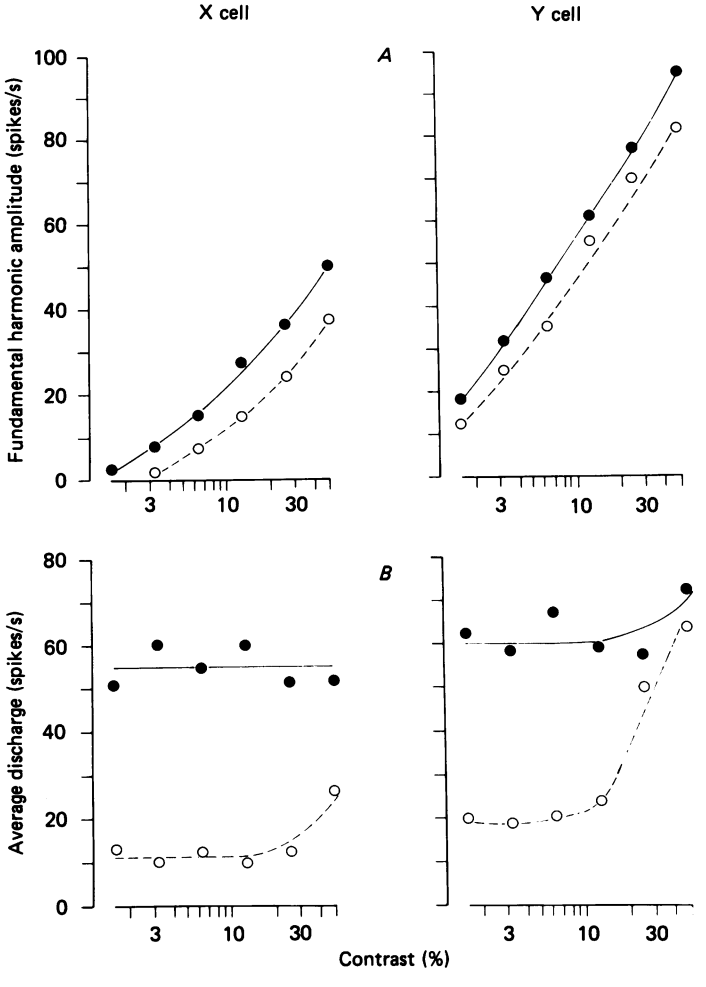

Fig. 4. Contrast–response curves for an X cell (left) and a Y cell (right) under normal conditions (open symbols) and during ACh application (filled symbols). The cell response is evaluated in terms of the fundamental harmonic amplitude (*A*) and in terms of the average discharge (*B*). The ACh ejection current was 40 nA for the X cell and 60 nA for the Y cell. Spatial frequency was 0·2 cycle/deg and temporal frequency 5 Hz for both cells. These results have been confirmed on six X cells and two Y cells.

Spatial frequency tuning curves of X and Y cells

Effect of bicuculline

The major difference between retinal ganglion cells and l.g.n. X cells is that l.g.n. X cells show a stronger attenuation of their spatial tuning at low spatial frequencies (Maffei & Fiorentini, 1973; Lee *et al.* 1981). By means of ionophoretic application of bicuculline, we investigated whether this difference might result from local GABA mediated inhibitory mechanisms.

A typical example of the results obtained for X cells is shown in Fig. 5, left column. On bicuculline application (continuous curves) the low spatial frequency attenuation

was greatly diminished. However, the optimum spatial frequency, and the cell acuity (highest spatial frequency to which the cell responds) remained virtually unchanged. After 15 min of recovery, the cell responses returned to normal.

The reduction in the attenuation rate for the low spatial frequency range is nearly a factor of 10 (0·3 db/octave *versus* 3·3 db/octave) for the cell in Fig. 5*A* and a factor

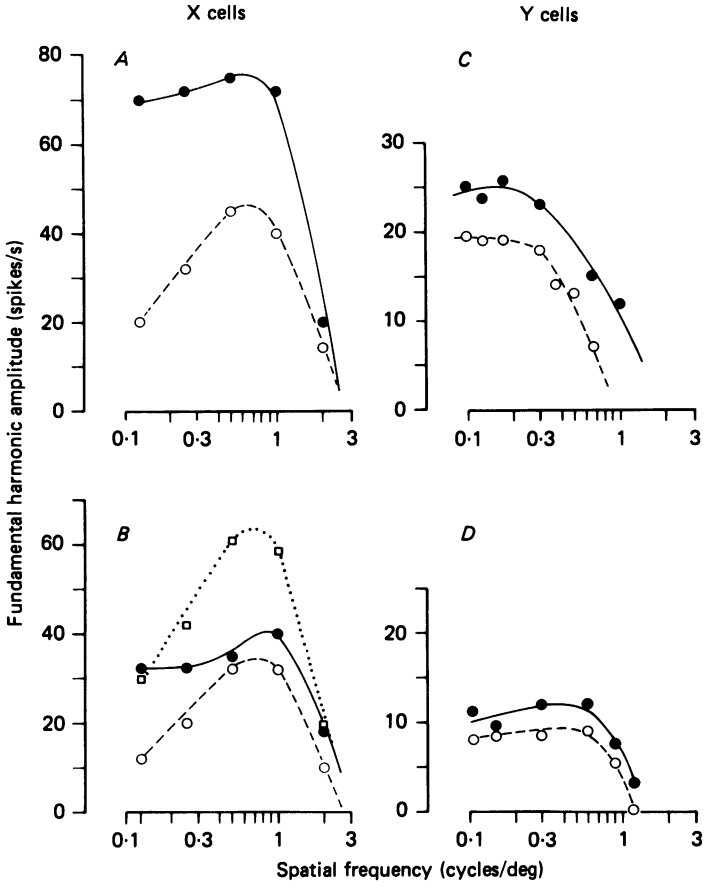

Fig. 5. Spatial frequency tuning curves for two X cells (left column) and two Y cells (right column). Open symbols, normal conditions; filled symbols, bicuculline application. The set of data indicated by the open squares in *B* was taken at a very high contrast (see text). The other contrasts used were all approximately 10%, and the temporal frequency was the optimum for each cell. Bicuculline ejection currents: *A*, 60 nA; *B*, 80 nA; *C*, 50 nA; *D*, 30 nA.

of 4 (1·2 db/octave *versus* 4·5 db/octave) for the cell in Fig. 5*B*. Values of this order have been obtained for all the other X cells we tested ($n = 15$).

Some low frequency attenuation is still apparent during bicuculline application, but it is no more than is typically observed in retinal ganglion cells. These results with bicuculline suggest that the low spatial frequency attenuation exhibited by l.g.n. cells is enhanced by local GABA mediated inhibitory processes.

For the Y cells studied ($n = 6$), bicuculline does not produce dramatic changes in spatial frequency tuning curves (Fig. 5, right column), as it does for X cells, but produces only an upward shift of the curves. The lack of any change in the tuning curve shape is not surprising, since Y retinal ganglion cells and Y l.g.n. cells have very similar spatial frequency tuning curves (Maffei & Fiorentini, 1973).

Controls for X cells

It may be argued that the flattening of the X cells' tuning curve could be an artifact resulting simply from saturation of the cell response. To rule out this possibility we performed two controls. First, we remeasured the spatial frequency tuning curve of the X cell in Fig. 5*B* at very high contrast (30 times threshold) without any drug

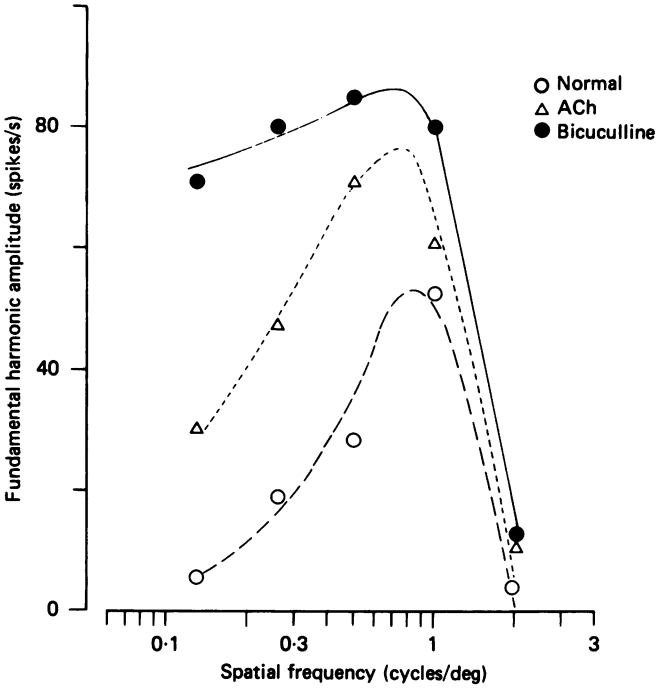

Fig. 6. Spatial frequency tuning curves of an X cell in normal conditions (○), during ACh (40 nA) application (△) and during bicuculline (120 nA) application (●). The contrast is equal to 15 % and the temporal frequency to 4 Hz. These results have been confirmed for other three X cells.

application (Fig. 5*B*, □). At this contrast the response was higher than that depicted by the continuous line, showing that the cell was not saturated during bicuculline application. Note also that the response function still showed the low frequency attenuation, even though the over-all level of the response was greatly increased.

As a further control, we measured the spatial frequency response curve during ACh application, which increases cell responsiveness (see previous section). In this case also, the curve retains its characteristic low spatial frequency attenuation (Fig. 6, △, dotted curve). Thus, the loss in attenuation in the low spatial frequency range

obtained during bicuculline application cannot simply be ascribed to either saturation of the cell response or an aspecific increase in cell responsiveness.

Threshold measures for X cells

The spatial frequency tuning curves shown in the previous section were obtained with relatively high contrasts (6–8 times threshold). However, the low frequency attentuation is also observed in contrast sensitivity curves (see for instance Lehmkuhle

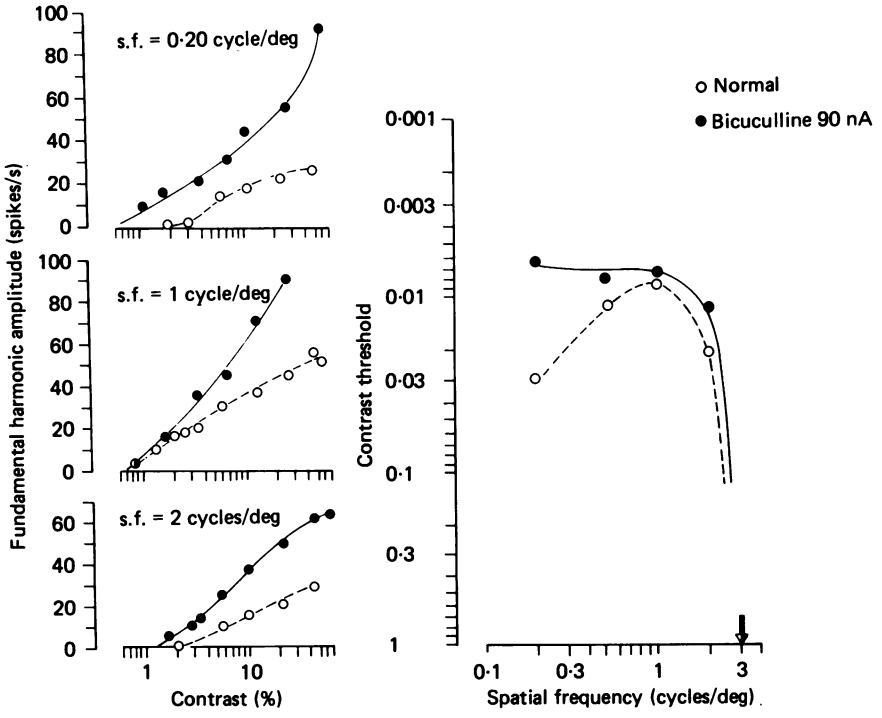

Fig. 7. Right: contrast sensitivity curve of an X cell under normal conditions (○) and during bicuculline application (90 nA, ●). The arrow points to the cell acuity, which is unaffected by bicuculline application. Left: contrast–response curves for the same cell, taken at three different spatial frequencies (s.f.; 0·2, 1 and 2 cycles/deg, respectively, from top to bottom, temporal frequency 3·5 Hz). Symbols and bicuculline ejection current as above. The linear fit of each curve, where the curve slope has been taken from is as follows:
0·2 cycle/deg: normal, $y = -2{\cdot}8 + 17{\cdot}8x$; bicuculline application, $y = 2{\cdot}6 + 44x$.
1 cycle/deg: normal, $y = 7 + 27{\cdot}3x$; bicuculline application, $y = 5 + 57x$.
2 cycles/deg: normal, $y = -5{\cdot}7 + 20x$; bicuculline application, $y = -3{\cdot}6 + 38x$,
where y is the fundamental harmonic amplitude and x is the logarithm of contrast. The correlation coefficient was greater than 0·97 for each fit.

et al. 1980). We also investigated low frequency attenuation of cells' contrast sensitivity (the inverse of contrast threshold) during bicuculline application. Contrast thresholds were measured as a function of spatial frequency both by extrapolation of contrast–response curves and by increasing the stimulus contrast step by step until the cell response was just audible. Both methods gave the same result. Virtually all the low frequency attenuation of the contrast sensitivity for all X cells tested ($n = 7$) vanished during bicuculline application. Fig. 7 (right) shows an example of the

results. The thresholds for this cell were measured using the extrapolation technique (see Fig. 7, left part).

Fig. 7 shows three of the contrast–response curves used to estimate the contrast thresholds. Note that all three curves undergo a change of slope during bicuculline application. This change is more pronounced for the lowest spatial frequency tested, as is to be expected, given that the stronger inhibition occurs in the low spatial frequency range. A change of the contrast threshold is also induced by bicuculline, and this threshold shift is greater for the low spatial frequencies.

This finding suggests that the inhibitory input also operates at low contrast, even at threshold.

Effect of bicuculline on temporal tuning

L.g.n. cells usually prefer visual stimuli moving at a particular velocity. Selectivity for velocity is reflected in the temporal frequency tuning curves. Both X and Y cells show a high temporal frequency cut-off and a majority of them show also a low

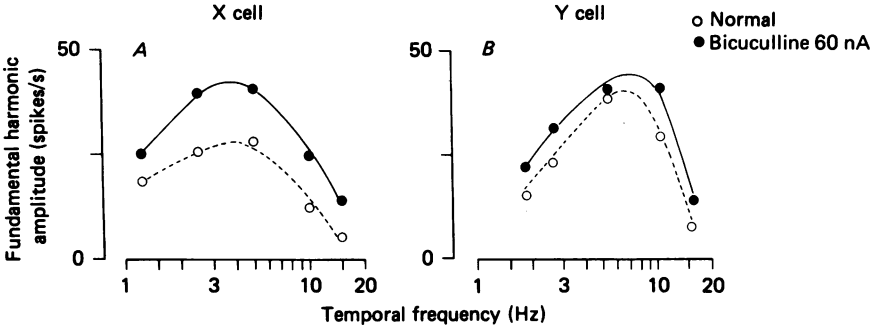

Fig. 8. Temporal frequency tuning curve for an X and a Y cell, both under normal conditions (○) and during bicuculline application (●). The stimulus was a drifting sinusoidal grating of spatial frequency equal to 1 cycle/deg for the X cell and 0·8 cycle/deg for the Y cell and of 20% contrast.

temporal frequency attenuation. However, the precise cut-off values and the tuning curve shape depend on the spatial frequency used to measure it and on the cell type (Derrington & Fuchs, 1979; Lehmkuhle *et al.* 1980; S. Bisti, unpublished results). An example of temporal tuning curves for one X out of seven and one Y out of three and of the effect of bicuculline application is shown in Fig. 8.

During bicuculline application, the low and high cut-offs of both cells' temporal frequency tuning curves remain practically unaffected. For the Y cell bicuculline produces an over-all response increase at all temporal frequencies (parallel shift). For the X cell the increase is greater at the preferred temporal frequency.

Effect of bicuculline on the non-linear response of Y cells

One of the defining characteristics of Y cells is their non-linear response to stationary gratings modulated sinusoidally in contrast (Hochstein & Shapley, 1976). We studied the non-linear behaviour of Y cells when the GABA mediated inhibition is blocked.

The type of cells response does not change substantially during bicuculline application (Fig. 9). The spatial frequency tuning curve of the non-linear part (second harmonic modulation) of the response maintains the same shape (apart from an upward shift), and the contrast–response curves remain practically parallel.

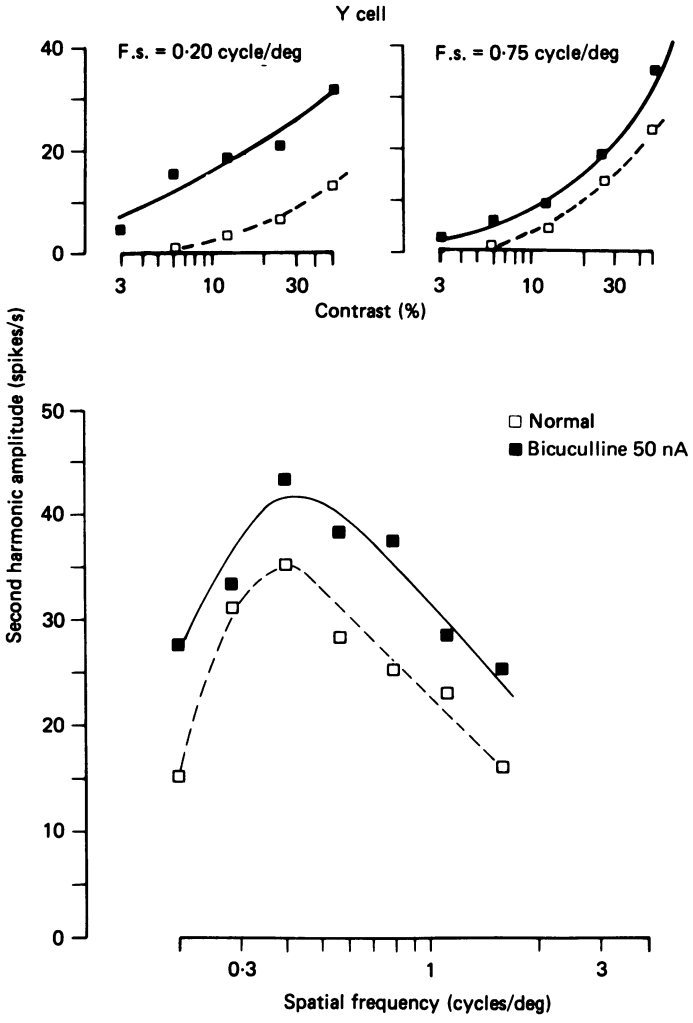

Fig. 9. Top row: contrast dependence of the second harmonic amplitude for a Y cell at two spatial frequencies (0·2 and 0·75 cycle/deg, temporal frequency 6 Hz). □, normal conditions, ■, bicuculline application (60 nA). Bottom row: spatial frequency tuning curve for the second harmonic amplitude of the cell response, in normal conditions (□) and during bicuculline application (■).

These results, confirmed in eight other cells, suggest that the local inhibitory circuits do not influence the characteristics of this non-linear response. This is not surprising, since this non-linearity is already present at the level of Y retinal ganglion cells (Hochstein & Shapley, 1976).

Effect of bicuculline on the receptive fields

The results of the previous section show that GABA mediated inhibition exerts an effect on the activity of geniculate cells, particularly X cells. We also studied the effect of bicuculline on the receptive fields of four X and four Y cells, by stimulating the cell with spots of light of increasing diameter. As noted by Sillito & Kemp (1983), this procedure reveals no major difference between X and Y cells in the alteration of the receptive field during bicuculline application.

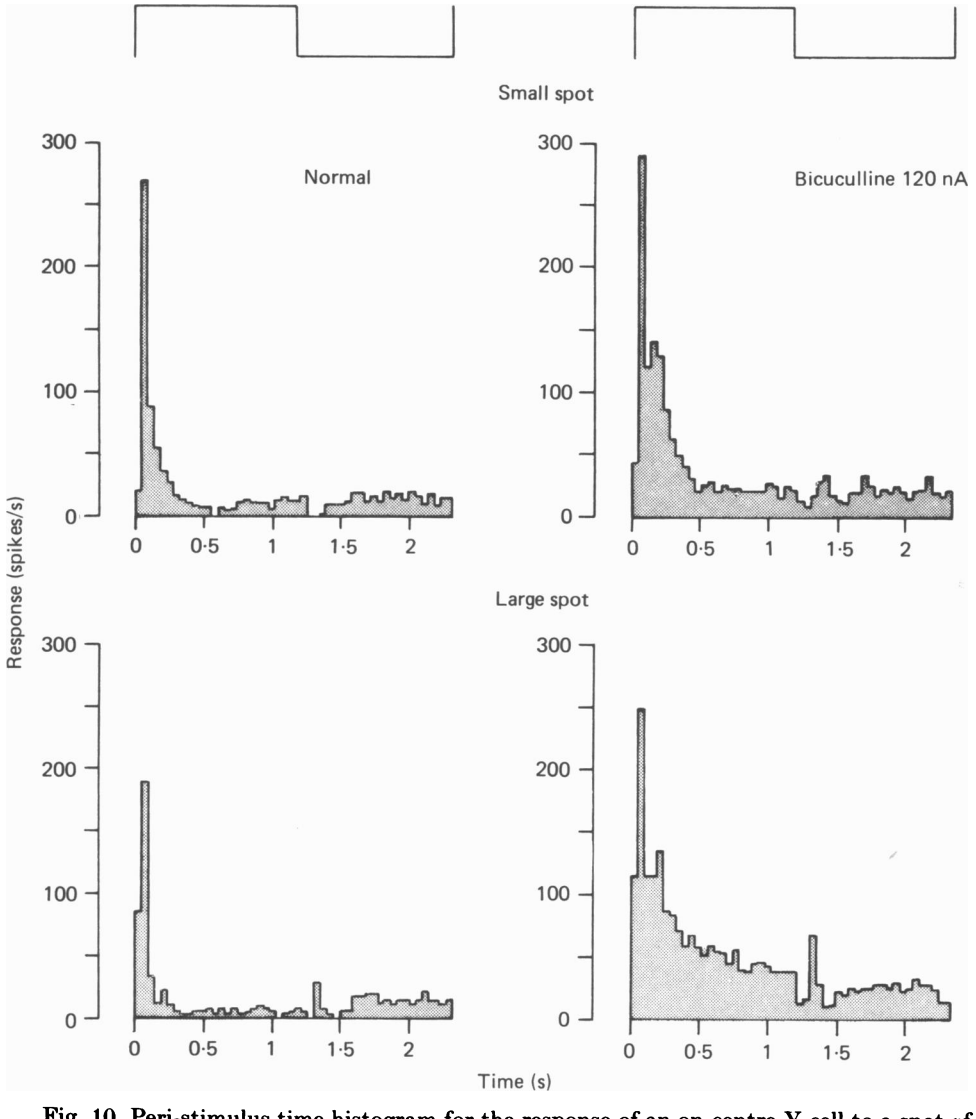

Fig. 10. Peri-stimulus time histogram for the response of an on-centre Y cell to a spot of light flashed on the receptive field. The time course of the stimulus is indicated at the top. Top row, spot diameter 1 deg; bottom row, spot diameter 4 deg. Left column, normal conditions; right column, bicuculline application (120 nA). Bin, 45 ms; thirty trials.

Fig. 10 shows an example of the response of an l.g.n. cell, in this case a Y cell. The left column of the Figure shows the standard cell response to a small and a large spot of light flashed on the receptive field, in normal conditions. When the spot is small (about the size of the centre of the cell receptive field), the cell shows a phasic increase of its discharge, when the light is turned on, and a decrease when it is turned off. As the size of the spot increases to encompass the whole receptive field, the 'on' phasic response decreases, as a result of the surround antagonism, and a small phasic response of the surround itself related to the 'off' of the stimulus appears.

Application of bicuculline affects the response to both small and large spots. The response to a small spot, which stimulates only the on-centre, is slightly increased during bicuculline application. The response to a large spot, which stimulates both the on-centre and the antagonistic 'off' surround, is increased even more during bicuculline application, implying that bicuculline dampens the antagonism.

For two of the cells in which we studied the receptive field properties (one X and one Y cell), we remeasured the contrast–response curve with and without bicuculline. In both cases the results were the same as observed before (Fig. 3): a change in slope for the X cell but not for the Y cell of the amplitude of modulation curves. For the same cell, with exactly the same drug dosage, we observed a difference in the effect of bicuculline on the amplitude of the modulation of the X and Y cells, but not on their response to flashes of light.

DISCUSSION

Our experiments confirm those of Sillito & Kemp (1983) in showing that local application of bicuculline, which produces a pharmacological block of GABA, removes a visually driven inhibitory input to l.g.n. cells. We also confirm Sillito & Kemp's (1983) findings that bicuculline reduces the effectiveness of the antagonistic surround of l.g.n. receptive fields of X and Y cells to a similar extent.

When the response to sinusoidal gratings was studied, however, there were some interesting differences in the effect of bicuculline on X and Y cells. While it increased the contrast dependence of the average visually elicited discharge for both X and Y cells, only for X cells did it increase the slope of the amplitude of modulation as a function of contrast (gain). The spatial selectivity of X cells is modified by bicuculline (both at threshold and suprathreshold contrasts), while that of Y cells remains unchanged.

At first glance the effects of bicuculline on receptive field plots and on grating response may seem at variance. However, one would expect complete agreement between the two results only for a perfect linear system, which the visual system, particularly the Y system, is not. Indeed one form of non-linearity may arise from inhibitory mechanisms. A further difficulty arises from the fact that the response to gratings can be measured by two parameters, average discharge and modulation amplitude, and it is not clear which of these is more appropriate for the comparison with receptive field plots.

One may suggest that the difference between the results for X and Y cells could be that the spread of bicuculline is sufficient to block more GABA mediated synapses for X cells than for Y cells (which have larger dendritic arborization). It would be strange, however, if the number of blocked synapses necessary to affect the

dependence of Y cell average discharge on contrast and the receptive field inhibitory interactions were insufficient to give a visible effect on the dependence amplitude of modulation upon contrast.

Inhibitory effects in the l.g.n. seems to be mediated by two types of interneurones: one type has the cell body located in the perigeniculate nucleus and one is intrinsic to the l.g.n. The intrinsic interneurones have a receptive field organization similar to relay cells and have X- or Y-like properties, depending on their retinal input (Dubin & Cleland, 1977). The perigeniculate interneurones which receive their primary visual input from l.g.n. relay cells (Ahlsen, Lindstrom & Sybirska 1978; Friedlander *et al.* 1981) have larger and diffuse receptive fields and respond to drifting gratings with an unmodulated increase of discharge rate (Shapley & So, 1981). It is possible that these two different inhibitory systems serve different roles. The properties of the perigeniculate neurones make them candidate mediators for moderating the average discharge of l.g.n. cells, maintaining it at a roughly constant level. Intrinsic interneurones could mediate the antagonistic surround of both X and Y cells, and also shape spatial frequency selectivity of X cells.

Our data are consistent with Singer & Bedworth's (1973) suggestion that an inhibitory input of X cell is mediated by the Y system. Y cells have lower contrast thresholds than X cells, and spatial frequency tuning curves with no attenuation in the low spatial frequency region. Y-type interneurones could therefore inhibit X cells at threshold, producing the low frequency attenuation in the X-cell contrast sensitivity curve. In addition Y cells are more resistant to dark adaptation, and the spatial frequency tuning curves of X cells do not change their shape very much during dark adaptation conditions (Bisti, Clement, Maffei & Mecacci, 1977).

The inhibitory input at the l.g.n. level probably serves to reduce the amount of information transmitted to further visual stations. The control of the average discharge could privilege the information contained in the change of firing rate. The enhancement of low frequency attenuation of X cells would start the tuning for spatial frequency, a process which is continued in the visual cortex. It is interesting that this is accomplished without loss of spatial localization, while if the system were inhibition-free and linear a narrow band in the spatial tuning should correspond to a broad receptive field and therefore to a loss of the spatial localization (see for example Marr, 1976 and Marr & Hildreth, 1980).

It is also interesting that the information relayed via the X pathway is more subject to a local inhibitory control than the Y pathway (our results, Fukuda & Stone, 1976). This could support the view that X and Y pathways have a different role in visual stations higher than the l.g.n. (see Lennie, 1980).

In addition, the availability of inhibitory mechanisms at the level of the l.g.n. provides the possibility of control of the activity of relay cells by non-retinal inputs, such as the visual cortex and the brain-stem projections (Singer, 1977; Geisert, Langsetmo & Spear, 1981; Ahlsen, Grant & Lindstrom, 1982; Sillito, Kemp & Berardi, 1983), which would relate the visual information transmitted to the cortex to other parameters, such as the behavioural state of the animal.

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REFERENCES

AHLSEN, G., GRANT, K. & LINDSTROM, S. (1982). Monosynaptic excitation of principal cells in the lateral geniculate nucleus by corticofugal fibres. *Brain Research* **234**, 454–458.

AHLSEN, G., LINDSTROM, S. & SYBIRSKA, E. (1978). Subcortical axon collaterals of principal cells in the lateral geniculate body of the cat. *Brain Research* **156**, 106–109.

BERARDI, N. & MORRONE, M. C. (1982). Visual inhibition on X and Y cells of the cat LGN. *Behaviour and Brain Research* **5**, 33.

BISTI, S., CLEMENT, R., MAFFEI, L. & MECACCI, L. (1977). Spatial frequency and orientation tuning curves of visual neurones in the cat: effect of mean luminance. *Experimental Brain Research* **27**, 335–345.

CLELAND, B. G., DUBIN, M. & LEVICK, W. (1971*a*). Sustained and transient neurons in the cat's retina and lateral geniculate nucleus. *Journal of Physiology* **217**, 473–496.

CLELAND, B. G., DUBIN, M. W. & LEVICK, W. R. (1971*b*). Simultaneous recordings of input and output of lateral geniculate neurones. *Nature* **231**, 191–192.

CURTIS, D. R. & DAVIS, R. (1963). Excitation of lateral geniculate neurones by quaternary ammonium derivatives. *Journal of Physiology* **165**, 62–82.

CURTIS, D. R. & TEBECIS, A. K. (1972). Bicuculline and thalamic inhibition. *Experimental Brain Research* **16**, 210–218.

DERRINGTON, A. M. & FUCHS, A. F. (1979). Spatial and temporal properties of X and Y cells in the lateral geniculate nucleus. *Journal of Neurophysiology* **293**, 347–364.

DUBIN, M. W. & CLELAND, J. G. (1977). Organization of visual inputs to interneurones of lateral geniculate nucleus of the cat. *Journal of Neurophysiology* **40**, 410–427.

ENROTH-CUGELL, C. & ROBSON, J. G. (1966). The contrast sensitivity of retinal ganglion cells of the cat. *Journal of Physiology* **187**, 517–552.

FAMIGLIETTI, E. N. & PETERS, A. (1972). The synaptic glomerulus and the intrinsic neuron in the dorsal lateral geniculate nucleus in the cat. *Journal of Comparative Neurology* **144**, 285–334.

FERNALD, R. & CHASE, R. (1971). An improved method for plotting retinal landmarks and focusing the eyes. *Vision Research* **11**, 95–96.

FRIEDLANDER, M. J., LIN, C. S., STANFORD, L. R. & SHERMAN, MURRAY S. (1981). Morphology of functionally identified neurons in lateral geniculate nucleus of the cat. *Journal of Neurophysiology* **46**, 80–129.

FUKUDA, Y. & STONE, J. (1976). Evidence of differential inhibitory influences of X- and Y-type relay cells in cat's lateral geniculate nucleus. *Brain Research* **113**, 188–196.

GEISERT, E. E., LANGSETMO, A. & SPEAR, P. D. (1981). Influence of the cortico-geniculate pathway on response properties of cat lateral geniculate neurons. *Brain Research* **208**, 409–415.

GUILLERY, R. W. (1966). A study of Golgi preparations from the dorsal lateral geniculate nucleus of the adult cat. *Journal of Comparative Neurology* **128**, 21–50.

HAMMOND, P. (1973). Contrasts in spatial organization of receptive fields at geniculate and retinal levels: centre, surround and outer surround. *Journal of Physiology* **228**, 115–137.

HOCHSTEIN, S. & SHAPLEY, R. M. (1976). Quantitative analysis of retinal ganglion cell classifications. *Journal of Physiology* **262**, 237–264.

HOOVER, D. B. & JACOBOWITZ, D. M. (1979). Neurochemical and histochemical studies of the effects of a lesion of nucleus cuneiformis on the cholinergic innervation of discrete areas of rat brain. *Brain Research* **170**, 133–122.

HOUSER, C. R., VAUGHN, J. E., BARBE, R. P. & ROBERTS, E. (1980). GABA neurones are the major cell type of the nucleus reticularis thalami. *Brain Research* **200**, 341–354.

HUBEL, D. H. & WIESEL, T. N. (1961). Integrative action in the cat's lateral geniculate body. *Journal of Physiology* **155**, 385–398.

HUNT, S. P., LIEBERMANN, A. R., OHARA, P. T. & WU, J. Y. (1982). Interneurones in the dorsal lateral geniculate nucleus (LGd) of the adult rat are GABAergic: evidence from immunocytochemistry. *Journal of Physiology* **332**, 61*P*.

KAPLAN, E., MARCUS, S. & SO, Y. T. (1979). Effects of dark adaptation on spatial and temporal properties of receptive fields in cat lateral geniculate nucleus. *Journal of Physiology* **294**, 561–580.

KEMP, J. A. & SILLITO, A. M. (1981). The nature of the excitatory transmitter mediating X and Y cell inputs to the cat dorsal lateral geniculate nucleus. *Journal of Physiology* **323**, 377–391.

KRATZ, K. E., WEBB, S. V. & SHERMAN, MURRAY S. (1978). Electrophysiological classification of X and Y cells in cats lateral geniculate nucleus. *Vision Research* **18**, 1261–1264.

LEE, B. B., ELEPFANDT, A. & VIRSU, V. (1981). Phase of responses to moving sinusoidal gratings in cells of cat retina and lateral geniculate nucleus. *Journal of Neurophysiology* **45**, 807–817.

LEHMKUHLE, S., KENNETH, E. K., MANGEL, S. C. & SHERMAN, MURRAY S. (1980). Spatial and temporal sensitivity of X- and Y-cells in dorsal lateral geniculate nucleus of the cat. *Journal of Neurophysiology* **43**, 520–541.

LENNIE, P. (1980). Parallel visual pathways: a review. *Vision Research* **20**, 561–594.

LEVAY, S. & FERSTER, D. (1979). Proportions of the interneurones in the cat's lateral geniculate nucleus. *Brain Research* **164**, 304–308.

LINDSTROM, S. (1982). Synpatic organization of inhibitory pathways to principal cells in the lateral geniculate nucleus of the cat. *Brain Research* **234**, 447–453.

MAFFEI, L. & FIORENTINI, A. (1972). Retinogeniculate convergence and analysis of contrast. *Journal of Neurophysiology* **35**, 65–72.

MAFFEI, L. & FIORENTINI, A. (1973). The visual cortex as a spatial frequency analyser. *Vision Research* **13**, 1255–1267.

MARR, D. (1976). Early processing of visual information. *Philosophical Transaction of the Royal Society, Series B* **275**, 483–524.

MARR, D. & HILDRETH, E. (1980). Theory of edge detection. *Proceedings of the Royal Society* B **207**, 187–217.

MORGAN, R., SILLITO, A. M. & WOLSTENCROFT, J. H. (1974). A pharmacological investigation of inhibition in the lateral geniculate nucleus. *Journal of Physiology* **246**, 93–94*P*.

MORRONE, M. C. & BERARDI, N. (1983). Visual inhibition of X- and Y-LGN cells of cat and kitten. *Proceedings of the International Union of Physiological Sciences* XV, 596.09.

PHILLIS, J. W., TEBECIS, A. K. & YORK, D. N. (1967). A study of the cholinoreceptive cells in the lateral geniculate nucleus. *Journal of Physiology* **192** 695–713.

SHAPLEY, R. & SO, T. S. (1981). Spatial tuning of cells in and around lateral geniculate nucleus of the cat: X and Y relay cells and perigeniculate interneurons. *Journal of Neurophysiology* **45**, 107–120.

SCHILLER, P. H. (1982). Central convergence of visual ON and OFF pathways. *Nature* **297**, 580–583.

SILLITO, A. M. & KEMP, J. A. (1983). The influence of GABAergic inhibitory processes on the receptive field structure of X and Y cells in cat dorsolateral geniculate nucleus (dLGN). *Brain Research* **277**, 63–77.

SILLITO, A. M., KEMP, J. A. & BERARDI, N. (1983). The cholinergic influence on the function of the cat dorsolateral geniculate nucleus (dLGN). *Brain Research* **280**, 299–307.

SINGER, W. (1977). Control of thalamic transmission by corticofugal and ascending reticular pathways. *Physiological Reviews* **57**, 386–442.

SINGER, W. & BEDWORTH, N. (1973). Inhibitory interactions between X and Y units in the cat lateral geniculate nucleus. *Brain Research* **49**, 291–307.

SINGER, W. & CREUTZFELDT, O. D. (1970). Reciprocal lateral inhibition of on- and off-centre neurones in the lateral geniculate body of the cat. *Experimental Brain Research* **10**, 311–330.

SINGER, W., POPPEL, E. & CREUTZFELDT, O. D. (1972). Inhibitory interaction in the cat's lateral geniculate nucleus. *Experimental Brain Research* **14**, 210–226.

STERLING, P. & DAVIS, T. L. (1980). Neurons in cat lateral geniculate nucleus that concentrate exogenous [^{3}H]γ-aminobutyric acid (GABA). *Journal of Comparative Neurology* **192**, 737–769.

VIRSU, V., LEE, B. B. & CREUTZFELDT, O. D. (1977). Dark adaptation and receptive fields of cells in the cat Lateral Geniculate Nucleus. *Experimental Brain Research* **27**, 35–50.