The Involvement of Gamma-Aminobutyric Acid in the Organization of Cat Retinal Ganglion Cell Receptive Fields

A Study with Picrotoxin and Bicuculline

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ABSTRACT The effects of picrotoxin and bicuculline upon the discharge pattern of center-surround organized cat retinal ganglion cells of X and Y type were studied. All experiments were carried out under scotopic or possibly low mesopic conditions; mostly but not exclusively on-center cells were studied. Stimuli were chosen so that responses were either: (*a*) "purely" central; (*b*) surround dominated; or (*c*) clearly mixed but center dominated. In each case a pre-drug control response was established, the drug was administered intravenously, and its subsequent effect upon the response was observed. In Y cells both picrotoxin and bicuculline caused the center-driven component of the response to become somewhat reduced in magnitude, while the surround component was substantially reduced. There was thus a change in center-surround balance in favor of the center-driven component. Responses of X cells remained virtually unaffected by both picrotoxin and bicuculline.

INTRODUCTION

Many cat retinal ganglion cells have receptive fields whose functional organization may be understood in terms of two mutually antagonistic mechanisms, the center and the surround mechanisms (Kuffler, 1953; Rodieck and Stone, 1965). Both the on- and the off-center varieties of these cells can by physiological tests be further divided into two major classes. Within one class, X cells, spatial summation over the receptive field is approximately linear, while within the second class, Y cells, spatial summation is very nonlinear (Enroth-Cugell and Robson, 1966). It has been suggested that Cleland and Levick's (1974*a*) brisk sustained cells are the same as X cells; their brisk transient cells, the same as Y cells. A third functional group, which will not concern us in this paper, has been designated W cells by both Stone and Hoffman (1972) and Cleland and Levick (1974*b*), although they disagree as to the cells in this group.

Whether center-surround ganglion cells are of X or Y type, their total receptive fields (center plus surround), as determined by physiological methods, are

larger than any anatomically measured ganglion cell dendritic fields (Dowling and Boycott, 1969). This is commonly interpreted to mean that the extent of the dendritic field determines the size not of the total receptive field, but of its center. Bipolar cells which synapse onto ganglion cell dendrites and soma are assumed to convey signals from the center. Signals from the surround may then reach the ganglion cell from bipolars via synapses onto amacrine cells, which in turn contact the ganglion cell (see, e.g., Stell, 1972). Alternatively, center-surround organization manifest in ganglion cell receptive fields could simply reflect the fact that bipolar cell receptive fields have a center and a concentric antagonistic surround.

Recently, Boycott and Wässle (1974) divided cat retinal ganglion cells into three different morphological classes. Alpha cells are believed to correspond to Y cells, beta cells to X cells, while the third morphological group may be identified with W cells. At any one retinal eccentricity alpha cells have larger dendritic fields than beta cells (Boycott and Wässle, 1974), just as Y cells (at one location) are likely to have larger receptive field centers than X cells (Cleland et al., 1975). This is what one could expect if the neural connections were similar in the sense that within both X- and Y-type receptive fields, those bipolars which form the center contacted the ganglion cell directly, whereas those that form the surround did so via lateral elements. The functional differences between X and Y cells mean that there must be some differences in the underlying retinal circuitry, and these may have associated with them pharmacological differences.

Rather little is known about synaptic transmitters in the mammalian retina. For the rabbit (Ehinger and Falck, 1971; Ehinger, 1972) it has been suggested that different subpopulations of amacrine cells utilize different transmitters of which gamma-aminobutyric acid (GABA) is one. Recently, Marshall and Voaden (1975) have shown GABA uptake by some amacrine cells in the cat.

This study was undertaken to see if the GABA antagonists picrotoxin and bicuculline might selectively affect the center- or the surround-driven components of the ganglion cell's discharge in cat, and if X- and Y-cell behavior might be differently affected by these GABA antagonists. It will be shown that there are clear differences between the two kinds of cells and between the two mechanisms. While the center-driven component of the response of Y cells is somewhat reduced in magnitude, the surround-driven component is substantially reduced. X-cell responses, on the other hand, are virtually unaffected by GABA antagonists.

METHODS

Experiments were performed on a total of 36 ganglion cells in 27 cats (2.6–5.1/kg). Anesthesia was induced with ketamine hydrochloride (20–25 mg/kg intramuscularly) or thiamylal sodium (approximately 10 mg/kg intravenously). Light anesthesia was maintained throughout the experiment with intravenous ethyl carbamate (20–30 mg/kg·h preceded by a 200–500-mg/kg loading dose), paralysis with gallamine-triethiodide (up to 50 mg/kg·h). Mean arterial blood pressure (femoral cannula) and heart rate were continuously monitored. Subscapular temperature was kept at 38°C. Neosynephrine and atropine were instilled in the conjunctival sacs, and contact lenses containing a 4–4.8-mm artificial pupil were selected (by direct ophthalmoscopy) to yield the best possible retinal image. Auxiliary lenses were used if needed. Action potentials were recorded from single

fibers in the optic tract with stereotaxically placed tungsten microelectrodes (Hubel, 1957), amplified, displayed on an oscilloscope, monitored over a loudspeaker, and recorded on magnetic tape.

Single intravenous doses of picrotoxin (Abbott Laboratories, South Pasadena, Calif.) and bicuculline (Pierce Chemical Co., Rockford, Ill.) varied from 0.3 to 0.5 mg/kg. For gamma-aminobutyric acid (Sigma Chemcial Co., St. Louis, Mo.) doses ranged from 0.75 to 1.0 mg/kg.

The stimulator (Fig. 1 A) utilized two sources, S_1 and S_2, superimposed with a half-

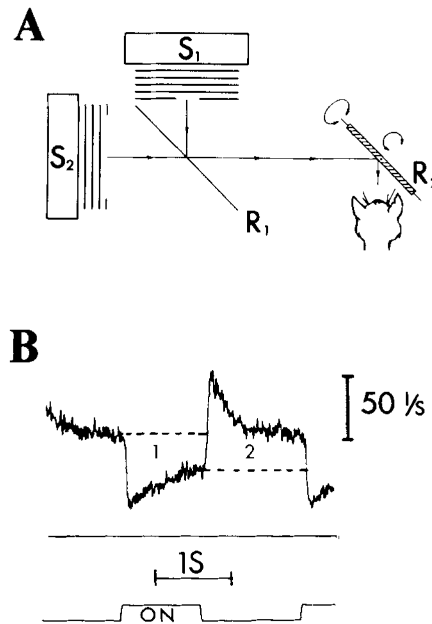

FIGURE 1. A. Plan of stimulator used in all experiments except cell 70-1. S_1 and S_2, light sources; R_1, half-silvered mirror; R_2, front-surface mirror for centering the optic axis of the stimulator on the receptive field. B. Response to flashing annulus. Areas 1 and 2 were measured with planimeter to obtain response magnitude expressed as total number of spikes. Horizontal bar 1 s. This averaged response was obtained from an on-center X cell with a flashing annulus (4°–15°), while the center's sensitivity was depressed with a small steady bright spot located in the middle of the receptive field center. Hence, the time course of the "inhibitory" (1) and the "excitatory" (2) phases together approximates that of the input to an on-center cell from its surround mechanism.

silvered mirror, R_1. Each consisted of a bank of fluorescent tubes (cool white) behind opal glass with an iris diaphragm in front of the glass. Spot sizes for S_1 could be adjusted to subtend angles from 0.05° to 2.65°; for S_2, from 0.5° to 9.4°; and S_2 also provided annuli of varying inner and outer diameter (maximum OD, 9.4°). The unattenuated luminance of S_1 was 137; that of S_2 was 29 scotopic cd/m^2. Neutral density filters provided coarse and crossed polaroids fine attenuation for both sources. S_1 was mounted on an indexing head so that the spot could be positioned along a vertical or horizontal axis across the receptive field. Both sources could be electronically 100% square-wave modulated, and unless otherwise stated, averaged responses were always elicited by 0.4 Hz stimuli. Thresholds

by listening were determined at 4 Hz. A first-surface mirror (R_2) mounted on gimbals was used to center the optic axis of the stimulator on the receptive field. In the experiment on cell 70-1 a Maxwellian view stimulator was used. It will be described in detail elsewhere.[1]

The sensitivity profile of the center was plotted for each cell against a dark background. A 0.1° stimulus was placed in several locations along two perpendicular receptive field diameters and spot luminance was adjusted until the experimenter could just barely hear that the cell fired in synchrony with the flashing spot.

The central mechanism sums light over a considerable retinal area (Barlow et al., 1957; Wiesel, 1960). When one centers a series of circular stimuli of increasing area on the receptive field and for each of them determines the illumination required for "threshold" (constant small response), illumination is first inversely proportional to area. That is, for small stimuli log illumination plotted against log diameter is a straight line of slope -2. As area is further increased, the slope first decreases, assumes a minimum value, and then may again increase. The diameter at the intersection of the extension of the line of slope -2 and a horizontal line drawn through the minimum criterion illumination is a measure of effective center size (D_t of Cleland and Enroth-Cugell, 1968; equivalent center of Cleland et al., 1973). D_t was also determined for all cells.

In addition to being classified as on- or off-center, all cells were also diagnosed as X or Y cells, on the basis of at least two of several tests. The "windmill" test was used routinely, always supplemented by observing the cell's response to a narrow slit of light moved through the receptive field at different velocities (Cleland et al., 1973). Near symmetry (X cells) at "on" and "off," or lack thereof (Y cells), of "pure" central square-wave responses of moderate magnitude provides good supportive evidence as to cell type as does the decay time of the on-transient of these responses. When responses are of equal magnitude at the same level of background illumination, the peak decays faster for Y cells than for X cells (Jakiela et al., 1976). Finally, for X cells it is very rare that D_t equals the width of the center's sensitivity profile (determined as described above) at a level where the sensitivity has declined by more than 0.35–0.5 log units from its peak value. In Y cells, on the other hand, sensitivity has declined by a full log unit or more at the point where the width of the profile equals D_t.[2]

All responses are presented as pulse-density tracings (Enroth-Cugell and Robson, 1966) with each tracing being the average of 32 individual responses to identical square-wave stimuli. Estimates of the effectiveness of the surround in suppressing the cell's discharge (see Results) were obtained by subtracting one response from another in the averaging computer. To obtain the magnitude of a response such as the off- or on-transient (Fig. 1 B) the area bounded by the pulse-density tracing and a horizontal line drawn at the level of firing during the end of the preceding stimulus half-cycle was measured with a planimeter and converted into total number of spikes.

RESULTS

General

In all experiments from which detailed results are reported the pharmacological agents were administered intravenously. In a few preliminary experiments they were introduced directly into an opened eye preparation of the Granit type (1947). This method was however not feasible in this study where sharp imagery of stimuli of different geometry was required to stimulate selectively one or the

[1] Enroth-Cugell, Hertz, and Lennie. Submitted for publication.

[2] Bonds, Jakiela, Kirby, Shapley, and Enroth-Cugell. Manuscript in preparation.

other of the two response mechanisms. Alternatively, drugs could be deposited close to the retina through a transcleral needle, retaining the image-forming apparatus intact. This method, too, was attempted, but often resulted in loss of the unit and/or disturbed intraocular pressure and decreased sensitivity.

Both picrotoxin and bicuculline, administered systemically, can have a considerable effect on the peripheral circulation and hence on the systemic blood pressure. One may therefore ask what evidence there is that the observed changes in ganglion cell behavior actually reflect alterations in retinal synaptic activity rather than secondary drug effects due to changes in the general condition of the animal.

First, in experiments where GABA antagonists were introduced directly into the opened eye, or through a fine transcleral needle into the unopened eye, changes in center-surround balance similar to those that will be reported below occurred in the absence of pronounced changes in arterial blood pressure. However, pressure changes in the retinal vascular bed would not have been detected had they occurred. It was therefore satisfying that on the occasions when intravenously administered GABA antagonists resulted in clear changes in systemic blood pressure, the observed effects upon X- and Y-cell responses were very different.

Second, after intravenous injection of GABA antagonists, the changes in ganglion cell behavior came either at the same time as the blood pressure increase or several minutes later. In neither case was the return of blood pressure to its control level synchronous with the recovery of the ganglion cell response.

Third, arterial blood pressure may become rhythmic after picrotoxin or bicuculline, showing slow fluctuations in mean level with each cycle lasting up to several minutes. Ganglion cell responses under these conditions remained identical whether they were recorded during a peak or a trough of the arterial blood pressure.

Finally, methoxyamine hydrochloride, which maintains systemic blood pressure by stimulating alpha-receptors, was used to raise arterial blood pressure by about 80 mm Hg, the maximum ever observed after administration of picrotoxin or bicuculline. In these tests, where GABA antagonists were not given, no difference in X- and Y-cell behavior was noted.

There is one more piece of evidence that it was changes in synaptic transmission rather than various stages of detrimental effect of the drugs upon the cat's general condition that were observed. This is discussed below.

Experiments on Responses to Central Flashing Spots

In this section it will be shown that there are differences between the manners in which GABA antagonists affect the discharge of X and Y cells, respectively, driven by stimuli smaller than the center and placed in the field middle. Here the center's sensitivity is much higher than the surround's, when the center is not selectively adapted. A well-centered, flashing, small spot will then generate a response which is predominantly due to inputs from the central mechanism ("pure" central response), provided the luminance is no more than one to two

log units above the threshold (Rodieck and Stone, 1965; Stone and Fabian, 1968; Cleland and Enroth-Cugell, 1968). To study the effect of GABA antagonists upon the central mechanism, "pure" central responses were generated in this way. The general procedure (in this and all following experiments) was first to obtain a pre-drug control response (averaged over 32 stimulus cycles) and then retain all stimulus conditions during and after drug administration. Leaving the tape recorder on for continuous monitoring of the cell's response, the effect of the drug upon the discharge pattern was then observed by averaging at intervals. Recording was continued until either the unit was lost or a changing response had fully recovered. This experiment was done with picrotoxin (0.4 mg/kg) on three Y cells of which one had an off-center. Fig. 2 shows the results from one such experiment where the cell was held for 17 min after drug injection, during which time the response decreased in size. All five responses have the same time course because they can all be superimposed by vertical scaling. This suggests that the change in the cell's response was due to a decrease in the input from the center mechanism rather than due to an increase in the surround mechanism's contribution (Stone and Fabian, 1968; Cleland and Enroth-Cugell, 1968). The unit was lost soon after the last response shown in Fig. 2 was obtained so we do not know if the response had reached its minimum. Most probably it was close to it, since the decrease during the last 8 min is less pronounced than during the preceding 5 min. Moreover, in experiments on two more Y cells, the response reduction slowed down or turned into recovery about 20 min after picrotoxin had been injected. One of these cells is shown in Fig. 3. Note that again the response time course remained remarkably constant all the time.

If the magnitude of central responses depends upon GABA concentration and if picrotoxin is a competitive antagonist, then a large response should decrease less than a small one after picrotoxin administration, for the relative concentration (in the synapse) will then determine response size. That is, a stronger stimulus would cause more GABA to be released so that a given amount of picrotoxin would be less effective as an antagonist than it would if less GABA were present (weaker stimulus). This was tested in three Y cells by using central responses of different magnitudes. Response magnitude was measured as indicated in Methods and is expressed in terms of total number of spikes during the 1.25-s response. For one cell (36-5) three stimulus luminances (0.5 log units apart) were used to elicit pre-drug control responses. After picrotoxin administration the response to each stimulus was followed until reduction had ceased. The largest response (44.1 impulses) decreased by 10.6 impulses; the smallest (32.7) decreased by 16.3. The medium response fell in between. For each of the other two Y cells the fate after picrotoxin of two different initial magnitudes was followed. Again, the large responses decreased by a smaller number of spikes than the small responses. It might be argued that picrotoxin adversely affects the cat's general condition, thus leading to decreased ganglion cell sensitivity. If this were to express itself in a shift of the response vs. log-stimulus curve to the right along the stimulus axis, then small responses would suffer a greater decrease in magnitude than large ones only if all the control responses were on the saturating part of the response vs. log-stimulus curve. But the pre-drug control

responses never exceeded 125 imp/s in peak magnitude and were thus well below the saturating portion of the curve.

Bicuculline, which is more specifically antagonistic to GABA than picrotoxin (Curtis et al., 1971) was also used in the type of experiment described above. Seven Y cells (one off-center) were studied with doses ranging from 0.3 to 0.4 mg/kg. As with picrotoxin, the amplitude decreased but the response retained

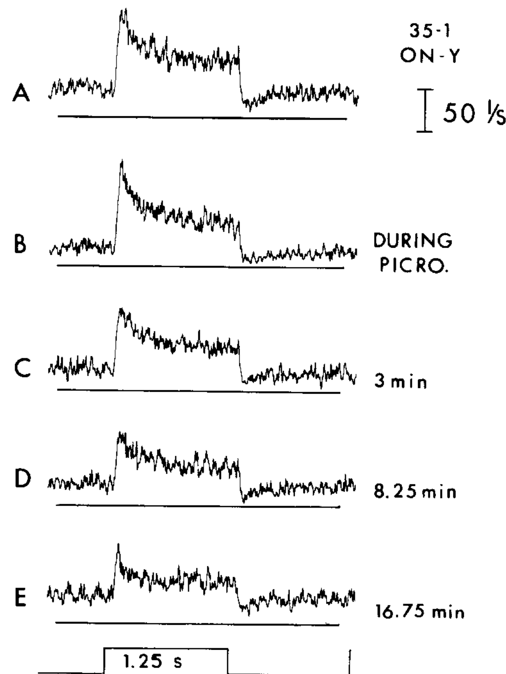

FIGURE 2. Reduction in magnitude of "pure" central response of on-center Y cell after intravenous injection of 0.4 mg/kg of picrotoxin. A is the control response to 0.13°-diam spot flashing on and off at 0.4 Hz in receptive field middle. In E the response to the same stimulus was about half the original size but the time course remained unchanged (B–E superimpose nicely on A if vertically scaled). General background (9.4° diam) 1.28×10^{-5}, stimulus 6.9×10^{-2} scotopic cd/m^2. Diameter of equivalent center D_l is 4.0°. Luminances throughout this paper given in scot. cd/m^2; time course of stimulus in this and following figures given below pulse density tracings which all are averages of 32 stimulus cycles. Horizontal line under each pulse density tracing indicates 0 impulses/s level in this and following figures.

its time course. In the case of those cells where control responses of two sizes were followed, the smaller one decreased by a larger number of impulses.

In summary, then, both picrotoxin and bicuculline injections are followed by some decrease in the center's contribution to Y-cell responses and it seems certain that the observed effect is due to interference with retinal synaptic activity. In contrast to this, responses elicited with central spots from X cells seem to remain unaffected by the administration of GABA antagonists. This was

tested on a total of four X cells. Bicuculline alone (doses 0.3 and 0.4 mg/kg) was used on three of them. The fourth was studied first with picrotoxin, then with bicuculline. The responses in Fig. 4 are from this cell and were obtained after picrotoxin but before bicuculline had been given. The picrotoxin dose (0.5 mg/kg) was larger than in any of the Y-cell experiments, yet 32 min later (E) there was no measurable effect. When 7 more min had passed, i.e. while there was still picrotoxin in the bloodstream, 0.4 mg/kg bicuculline was given and the response to the same fixed stimulus observed for 31 more min. Still there was no change in magnitude or time course of the response.

For Y cells the response reduction (in absolute terms) after picrotoxin and bicuculline varied inversely with the magnitude of the control response. The X

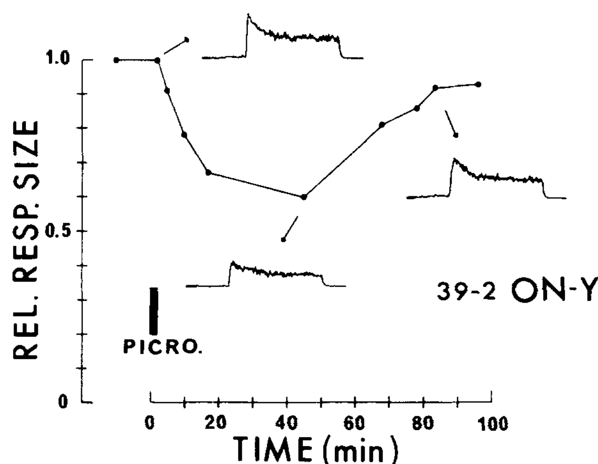

FIGURE 3. Relative response magnitude (control = 1.0) from on-center Y cell as a function of time after administration of 0.4 mg/kg picrotoxin. All 10 responses on which curve is based superimpose well after vertical scaling. Stimulus 2.5° in receptive field middle; 0.4 Hz square-wave 2.0×10^{-4} cd/m^2. Zero background. D_t = 4.2°. Height of response peak in uppermost pulse density tracing is 50 impulses/s.

cell response in Fig. 4 was quite large, so it is conceivable that the reduction was so small as to escape detection. Two of the four X cells were studied with more than one stimulus strength and one of these control responses is shown in Fig. 5. This response was of the same magnitude as Y cell responses whose reduction was easily detectable. Yet the X cell response did not become smaller after picrotoxin.

Selective Adaptation of the Center and Subtraction Experiments

The goal of the experiments described in the next two sections was to isolate as well as possible the surround's contribution to the cell's discharge in order to study the effect of picrotoxin and bicuculline upon the surround mechanism. Two techniques which work better on X than on Y cells were used.

The first consisted of selectively adapting the center with a centrally located, steady light while stimulating the surround with a flashing annulus. This technique was first used on cat retinal ganglion cells by Bishop and Rodieck (1965)

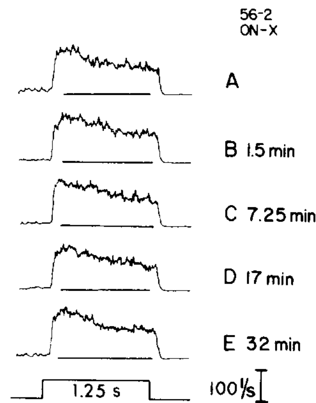

FIGURE 4. Response from on-center X cell to 1.5°-diam centered spot to show constancy of response magnitude and time course after 0.5 mg/kg picrotoxin. A is the control. Stimulus luminance 8.35 × 10^{-4} cd/m^2. Zero background. D_t = 1.4°, i.e. only slightly smaller than stimulus diameter which makes it probable that surround mechanisms contribute somewhat to the cell's discharge. At 39 min after picrotoxin administration 0.4 mg/kg of bicuculline was given and 31 min later response was still the same as in E.

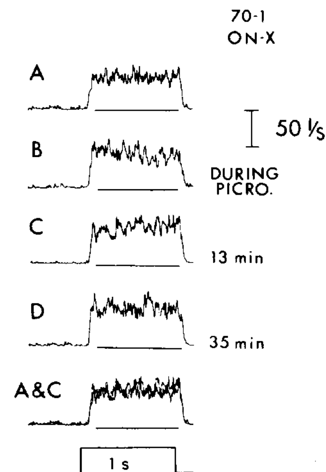

FIGURE 5. Responses from an on-center X cell to a centered stimulus spot of 0.8° diam, i.e. considerably smaller than the equivalent center (D_t = 1.2°). Stimulus luminance 1.62 × 10^{-3} cd/m^2. Amplitude of control response (A) about one-third of that yielded by the X cell in Fig. 4. This response is probably "pure" central. At the bottom the control and the 13-min response are superimposed to show constancy of magnitude and time course. Zero background. Note that vertical scale is different from that in Fig. 4.

and it relatively easily yields a surround-dominated response from some cells but not from others (Enroth-Cugell and Pinto, 1972). The former are most likely X cells, the latter Y cells. Even when selectively adapted the Y cell center has significant sensitivity in the region stimulated by the annulus (e.g., Ikeda and

Wright, 1972; Winters et al., 1973). This is probably why it is more difficult to evoke surround-dominated responses from Y cells.

This annulus technique yielded results (to be described in detail below) which together with the earlier ones on central responses (see above) suggested that GABA antagonists reduce a Y cell's surround-driven response-component considerably more than its center-driven component. Our stimulator did not permit a "pure" central and a surround-dominated annulus response to be observed in parallel during the same experiment. This made it difficult to obtain information from a large number of cells about the effect of GABA antagonists upon the two response mechanisms belonging to the same receptive field, for only twice (see below) was a cell held so long that first a pure central and then a surround-dominated response could be observed.

We therefore turned to a second technique for estimating the surround's contribution to a cell's discharge. This technique also monitors the fate of the center's contribution to the discharge of that same cell. This is a subtraction technique (Enroth-Cugell and Lennie, 1975) which isolates reasonably well the surround-driven response component. It is particularly useful for assessing the surround's effectiveness in suppressing a cell's discharge when the retina is well dark adapted, which was the condition under which the pure central responses were obtained. The principle of the subtraction technique is as follows: when determining threshold illumination for stimuli of increasing area (see Methods) the added light will sooner or later fall outside the central summing area and hence threshold illumination falls no more. The largest spot still resulting in a decreased threshold and small enough not to stimulate the surround substantially is the optimal spot. It is large enough so that when, at constant luminance, it is expanded to cover the entire receptive field, the center receives only minimal additional light. The difference between the response to the optimal spot and the response to diffuse illumination provides an estimate of the surround component. For X cells it is rather easy to find an optimal spot, because the sensitivity profile of the center falls off steeply within the profile of a considerably larger surround (Ikeda and Wright, 1972; Enroth-Cugell and Lennie, 1975). So when a stimulus becomes large enough to extend beyond the center, the added light falls on surround regions with appreciable sensitivity. Hence surround antagonism sets in rather abruptly in X cells. This is not true for Y cells, because here it seems that the center's sensitivity profile has wider skirts and the extent of the center is more closely matched to that of the surround. For Y cells as well as X cells the center mechanism is more sensitive in the middle of the receptive field. However, further out, over an annular region, center and surround sensitivities of a Y cell are more evenly balanced. The result is that a stimulus which covers most, but not all, of the center also stimulates the surround. Expansion of such a spot to diffuse illumination not only increases the surround's input substantially, but to some extent also augments the center component. In Y cells, therefore, the subtraction technique always tends to underestimate the surround's contribution. That neither of the two techniques described above accomplishes a neat separation of center and surround in Y cells, as it does for X cells, is thus a consequence of receptive field properties typical of Y cells.

Adaptation of Center

The sensitivity profile for the central mechanism was first determined. Then a combination was found of area and luminance for a central steady adapting spot and a flashing annulus, such that the cell was driven strongly by the surround. This response was averaged to serve as a control. The drug was then given and its effect upon the response observed as usual.

The results from one Y cell are shown in Fig. 6. Since this was an on-center

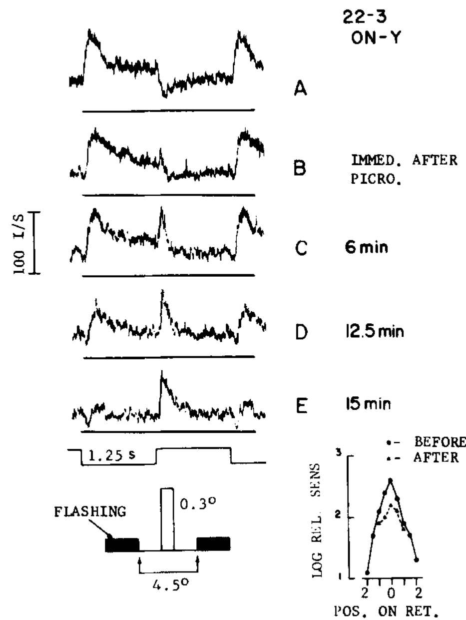

FIGURE 6. Transformation of surround-dominated response from on-center Y cell into center-dominated response after administration of 0.4 mg/kg picrotoxin. Steady 0.3°-diam spot in receptive field middle; luminance 3.48 cd/m^2. Flashing 4.5°–9.4° annulus; luminance 5.08 × 10^{-4} cd/m^2. Zero general background. Lower right: sensitivity profile before (●—●) and after (▲—▲) picrotoxin. The clearly surround-dominated control response (A) begins to show signs of more prominent center inputs (note small peak at "on" in B) very soon after the picrotoxin administration and 15 min after it the response is dominated by center inputs.

cell, signals from the surround tend to decrease the cell's firing rate during the on-phase of the annular stimulus, and to increase it at off-set of the annulus. The control response in A is thus clearly surround dominated, although the central mechanism, too, presumably contributes to the cell's discharge. During the 15 min that elapsed between the administration of picrotoxin (Fig. 6 B) and the response shown in Fig. 6 E, there was a successive change in the character of the cell's firing towards lesser surround dominance. Finally (Fig. 6 E), the firing pattern of the cell suggests that it is largely driven by the central mechanism, for it is during the on-phase that there now is increased firing. The most striking

feature during the off-phase is the short dip in firing just after the annulus is extinguished. After E the sensitivity profile of the center was again determined in the dark (see lower right of Fig. 6). The absolute sensitivity of the center was lower than before the picrotoxin injection, but the shape of the profile shows that there had been no eye movement. This is an important point, because a shift of the steady adapting spot and annulus, relative to the center of the receptive field, might cause drastic changes in the response pattern. Because the unit was lost shortly thereafter, no averaged response showing the recovery of the cell's discharge pattern towards that of the pre-drug control was obtained.

A total of 14 Y cells were studied with a steady central adapting spot combined with a flashing annulus. Seven of them (one with an off-center) were studied with picrotoxin, six with bicuculline, and one with picrotoxin first, then bicuculline. Some cells could be observed until the surround response was almost abolished. It is the modest reduction of the central response of one of these cells that is shown in Fig. 3. In all cases the same type of shift from surround dominance towards center dominance occurred. Five units were held long enought to permit recovery to a response pattern quite similar to that seen before the drug administration. This generally took between 40 and 70 min.

If the kind of model originally proposed by Rodieck and Stone (1965) for center-surround interaction holds, then, at first glance, the sequence of events in Fig. 6 might suggest that picrotoxin selectively almost abolished the surround's contribution to the response. But it should be borne in mind that the observed shift in center-surround balance could come about in one of three ways: (*a*) the center's contribution to the cell's discharge remains unchanged while the surround's is reduced; (*b*) the surround's contribution remains unchanged while the center's increases; (*c*) the magnitude of both the center's and the surround's contribution is affected by picrotoxin and bicuculline. We know from the previous section that the center's contribution neither remains unchanged nor increases. This suggests that the shift from a surround-dominated response in A of Fig. 6 to one which is rather center dominated (E) came about because picrotoxin resulted in a pronounced decrease in the surround's, and a lesser decrease in the center's input to the cell. The transformation from a surround-dominated response in A to one with as large a center component as in E may seem strange until one considers how selective center adaptation works in Y cells. Before picrotoxin the annular stimulus probably generates a very large surround component and a moderately large center component, which combine to yield a medium-sized, surround-dominated, mixed response. Picrotoxin thus largely eliminates the surround component while affecting the center mechanism less. Virtually all that is finally left (Fig. 6 E) is most of the original center component.

In many X cells a flashing annulus whose inner diameter is about 4° combined with a very small central (steady) adapting spot readily yields a surround response. In Fig. 7, 0.4 mg/kg bicuculline was injected immediately after a control response had been obtained. Responses B–E, all elicited after bicuculline administration, obviously show no shift in center-surround balance. During this experiment the mean firing rate fluctuated slowly (between 70 and 50 impulses/s

with a several-minute period) and responses averaged during periods of low mean firing showed a lesser depth of modulation of the cell's firing. However, when these smaller responses were vertically scaled, they superimposed perfectly on those shown in Fig. 7. That is, no shift in center-surround balance was evident during low mean firing rate either. This type of experiment was carried out on two more X cells with bicuculline and on one with picrotoxin. In no case did the response shape change. In two of these experiments (one bicuculline and the picrotoxin cell) the last response which was obtained about 30 min after drug administration was minimally larger than the control. We are uncertain whether this slight change in magnitude is of any significance, but if so, the direction of

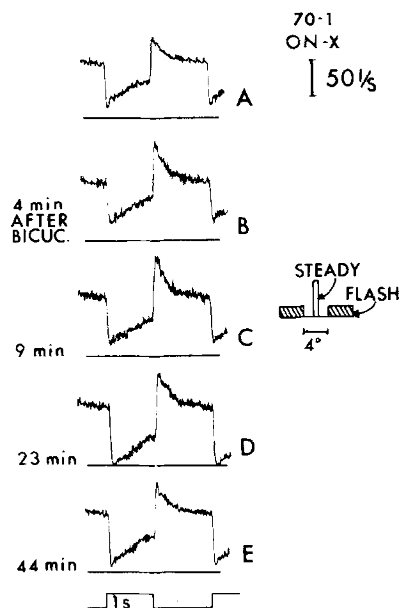

FIGURE 7. Same type of experiment as in Fig. 6 to show that GABA antagonists do not affect center-surround balance in X cells. Steady spot in receptive field middle is 0.2° in diameter; luminance 8.12×10^2 cd/m^2. Flashing annulus 4°–13° diam.; luminance 6.6×10^{-2} cd/m^2. Zero general background.

change would support our belief that GABA antagonists do not decrease the surround component of X cell responses.

Administration of GABA

Since the functional properties of Y, but not of X cell receptive fields are affected by GABA antagonists, one would expect the two cell types to behave differently after administration of GABA itself. Surround-dominated responses (peak-trough amplitude about 200 imp/s) were elicited with a flashing annulus (and steady central depressing spot) from four Y cells and one X cell, in five cats. These responses were then observed during and after injection of GABA (0.75–

1.0 mg/kg).[3] Although the annulus kept flashing, it ceased to modulate the discharge of all four Y cells within 5–6 min after onset of GABA administration. Instead the cells fired at a steady rate in the order of 50 impulses/s with only a barely detectable ripple in the pulse density tracing each time the annulus went on or off. After a few additional minutes all four Y cells were still unresponsive to the flashing annulus. For two of the cells the annulus was now turned off. In both cases the discharge continued at about 50 impulses/s. The only noticeable difference was that the ripple disappeared. Finally, the depressing spot, too, was extinguished. This left the receptive field in complete darkness, but both cells still discharged steadily at about 50 impulses/s. One of these cells was held for 16 min after the injection of the GABA. At the time the cell was lost its discharge rate had begun to decrease slowly.

This same experiment was done on one X cell and that cell did indeed behave very differently. During the 27 min after the GABA injection that this cell was followed, its response remained unchanged in amplitude and shape. Straschill (1968) and Straschill and Perwein (1969) observed the light-evoked activity of cat retinal ganglion cells after intra-arterial and iontophoretically administered GABA. In the first study two out of four cells, in the latter study all cells (number not given) showed depressed light-evoked activity. Whether these investigators studied X or Y cells, or both, is not indicated.

In conclusion, experiments where the center's sensitivity was selectively depressed show that GABA-antagonists profoundly affect the functional properties of Y-cell receptive fields, leaving those of X cells virtually unaffected. Although it is difficult to known just how much importance can be attached to our experiments with GABA because of the enormous dose, their outcome was compatible with the idea that the role of GABA is very different in X and Y cells.

Subtraction Experiments

The subtraction technique was applied to two X and seven Y cells. The outcome of these experiments did indeed confirm the earlier results that Y cell, but not X cell behavior is affected by GABA antagonists.

Fig. 8 is from a Y cell, and the two upper responses were obtained before drug administration. A was elicited with a stimulus of optimal diameter and B with a 9.4° diameter field ("diffuse" illumination) flashing on and off at the same luminance and frequency. The response to the optimal spot was then subtracted (in the computer) from the response to diffuse light to obtain the pre-drug estimate (C) of the surround's suppression of the cell's discharge during the on-phase. The discharge burst at "on" in C arises because the diffuse flash generated a larger center component than the optimal spot, and, when the summing areas of both mechanisms are completely filled with light of the same luminance, the surround's latency is a little longer than the center's (Enroth-Cugell and Lennie, 1975). Comparison of Fig. 8 with Fig. 9 shows that there is a clear difference between the effect upon Y cells and that upon X cells. The depressing effect that the Y cell surround exerted on the cell's firing during the on-phase is considerably reduced after bicuculline administration (D and E of Fig. 8).

[3] Very large doses are necessary to ensure passage through the blood-brain barrier.

Because the surround's contribution in Y cells tends to be underestimated (compare optimal spot diameter with sensitivity profile of the center in lower right corner), the tracings in Fig. 8D and E do not necessarily mean that there was no surround antagonism at all during the on-phase. But, clearly, the surround's ability to suppress the cell's discharge was considerably weakened for a time after bicuculline, returning to the pre-drug level at 38 min. Once again, X cells behave quite differently from Y cells after administration of GABA antago-

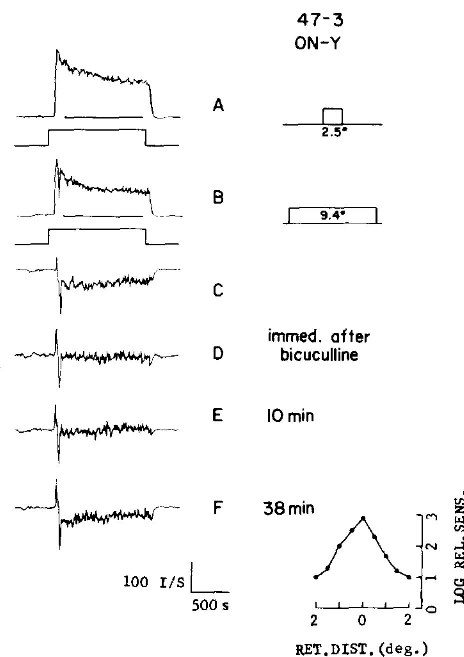

FIGURE 8. Subtraction experiment from on-center Y cell. A is pre-drug response to optimum spot whose diameter is 2.5°; luminance 1.61×10^{-3} cd/m^2. Luminance and time course of "diffuse" (9.4° diam) flashing field same as optimum spot. C obtained by subtracting A from B (in computer). At three different times after bicuculline administration a response to optimum spot and diffuse light were obtained and the latter was again subtracted from the corresponding response (D–F). Note decrease of surround's ability to depress firing during on-phase in D and E. In lower right, sensitivity profile of center.

nists. The surround's capacity to suppress the cell's firing during the on-phase was not changed. The first subtraction done after the drug was given (Fig. 9D) yielded a minimally smaller difference than the control (C), but this was probably due to a temporary shift in eye position. The 7-min response to the optimal spot (not included in Fig. 9) showed a slight shape change such as one sees after small eye movements, while the response to diffuse light, which is less sensitive to eye movements, did not.

Although only the pre-drug responses to the optimal spot and to diffuse light are shown for the Y cell in Fig. 8, the two responses whose difference yielded the

estimate of the surround's suppression of discharge were measured also after drug administration (i.e. each time a subtraction was performed). In all subtraction experiments on Y cells, the response to the optimal spot decreased after drug administration, while the response to diffuse light increased. This is what should happen if picrotoxin and bicuculline decrease the center's contribution to the cell's discharge to a lesser extent than they decrease the surround's contribution. Thus, the results from the subtraction experiments strongly support the interpretation of the previous experiments on Y cells.

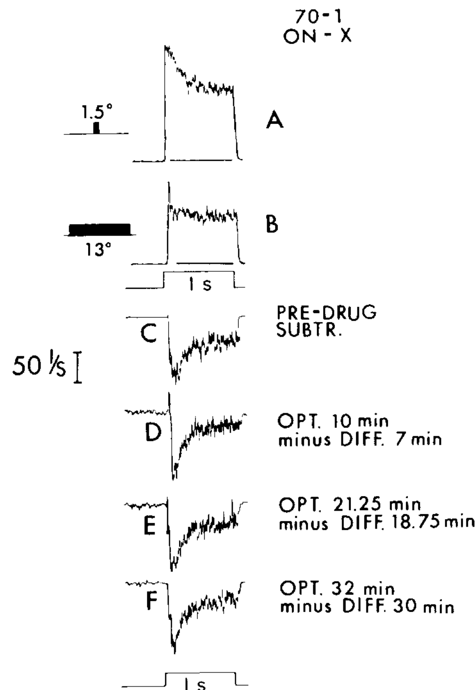

FIGURE 9. Subtraction experiment on on-center X cell. A, B, and C are pre-drug responses to optimal spot, to "diffuse" (13° diam) light, and subtraction, respectively. Optimum spot diameter 1.5°; D_t 1.2°. Luminance of optimum spot and "diffuse" light 1.62×10^{-2} cd/m². Zero general background. D, E, and F are subtractions after administration of 0.4 mg/kg bicuculline. Note that the surround's capacity to depress the cell's discharge during the on-phase remained virtually constant during the 32 min this response was observed.

DISCUSSION

The effect of GABA antagonists upon the activity of cat retinal ganglion cells has been studied before (e.g., Heiss, 1967; Chu, 1968). Previously, no attempts were made to judge separately their action upon the center and the surround mechanisms. Nor has X and Y cell behavior after administration of GABA antagonists been previously differentiated. The most important and clearcut conclusion which can be drawn from the results presented in this paper is that X and Y cells are pharmacologically different. For we have seen that the discharge pattern of

all 30 Y cells was affected, in a consistent way, by intravenous administration of picrotoxin and bicuculline. On the other hand, the discharge pattern of all six X cells remained virtually untouched whatever the type of experiment done on the cell. Our 10 off-center Y cells and the single off-center X cell were no exceptions. Daniels and Pettigrew (1975) observed the response of cat geniculate cells after intravenous administration of bicuculline and remarked that their "finding in the LGN that transient, but not sustained, cells are affected by bicuculline" agrees with our results.

The observed change in the response of Y cells to a fixed, slow, square-wave stimulus consisted of a shift in the balance between center and surround mechanisms in the direction of a relative decrease in the surround's contribution to the cell's discharge. The outcome of every type of experiment performed was compatible with this shift being caused by a pronounced reduction of the input provided by the surround mechanism paired with a lesser reduction of the center mechanism's contribution to the cell's discharge. This suggests that in Y cells, GABA is quite importantly involved in mediating the surround's influence upon the cell's discharge frequency, and that at least a portion of the center mechanism's influence also depends upon GABA.

According to the model derived from the work of Kuffler (1953), Rodieck and Stone (1965), and others, each mechanism expresses itself in two ways: (*a*) during light-on, one mechanism strives to increase the cell's discharge (the center in on-center cells, the surround in off-center cells) while the other mechanism has the opposite effect; (*b*) during light-off the mechanism which during light-on tended to increase the ganglion cell's firing, now tends to decrease it while the other mechanism again does the opposite. Thus, during both phases of on-off illumination the two mechanisms antagonize each other. One mechanism strives to depolarize the cell, the other strives to hyperpolarize it. It might therefore be thought that GABA could mediate the action of the center or of the surround, but not both. However, the ganglion cell membrane may be depolarized either by an increase in the concentration of a depolarizing transmitter or by a decrease in the concentration of a hyperpolarizing one. The corresponding holds true for membrane hyperpolarization.

At present there seems to be no convincing evidence as to whether GABA release in the cat retina causes depolarization or hyperpolarization. Some studies on the cat retina suggest that GABA is hyperpolarizing (see review by Tebēcis, 1974). On the other hand GABA has also been shown to depolarize postsynaptic neurons in mammals, including the cat (Tebēcis, 1974; Levy, 1974; Yu and Avery, 1974; Obata, 1976). Our results cannot settle whether GABA depolarizes or hyperpolarizes the membrane of the neurons upon which it acts, but are entirely compatible with both. Neither is there any contradiction in our finding that GABA antagonists influence not only the surround's contribution to Y cells but also to some extent the center's contribution. For whatever the action of GABA at the ganglion cell, we need only suppose that center signals and surround signals have opposite effects upon its concentration at synaptic terminals.

If, as Marshall and Voaden's (1975) uptake experiments suggest, amacrine cells are indeed the only neurons in the cat retina which utilize GABA, then our findings mean that the circuitry of Y cell centers cannot be as simple as is often

assumed. Some of the center's information must reach the ganglion cell via amacrine cells. Beyond this we believe that any statement, based on our results, about possible and impossible signal pathways within Y-receptive fields would be so wildly speculative as not to serve any useful purpose, particularly not in an era when cat retinal anatomy "changes from month to month." The following example illustrates the problems involved.

There are several morphological types of amacrine cells in the cat retina as, for example, pointed out by Famiglietti and Kolb (1975) who have described the synaptic connections of two amacrine types (AI and AII) in some detail (Kolb and Famiglietti, 1974). If GABA were the transmitter for one only, say that AI type, one would have to consider a different functional organization of the Y-receptive field than if GABA served the AII type only, or served both of these cell types. As mentioned in the Introduction, it has been suggested for the rabbit that a *sub*population, rather than all amacrine types, are GABA cells.

Finally, the striking difference between X and Y cell behavior after administration of GABA antagonists makes quite interesting the statement by Marshall and Voaden (1975) that GABA never has been found in mammalian horizontal cells. Perhaps part of the reason that X and Y cells are so different pharmacologically is because X cell surround signals are mediated by horizontal cells as suggested by Rodieck (1973), while those of Y cell surrounds are mediated by amacrines.

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