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EFFECTS OF 2-AMINO-4-PHOSPHONOBUTYRIC ACID ON RESPONSIVITY AND SPATIAL SUMMATION OF X CELLS IN THE CAT RETINA

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SUMMARY

1. We studied the effect of locally applied 2-amino-4-phosphonobutyric acid (APB) on the response of X-type cat retinal ganglion cells to stimulation by sinusoidal gratings of photopic mean luminance.

2. Application of APB produced a decrease in the mean firing rate of on-centre X cells, but an increase in the mean firing rate of off-centre X cells.

3. The response and responsivity of both on- and off-centre X cells to drifting sinusoidal gratings were suppressed by APB. Under control conditions, the response amplitude was linear with grating contrast for contrasts less than 10%. During APB application, the slope of the contrast response curve was reduced, and linearity was maintained for a larger range of contrast.

4. Assuming that APB acts selectively on the cone to depolarizing bipolar cell synapse, the suppression of the response of on- and off-centre X cells suggests a functional link between depolarizing bipolar cells and both types of X cell.

5. Because APB reduces the centre response of both on- and off-centre X cells, caution should be observed in interpreting the effect of APB on higher visual centres in terms of the blockage of 'on' signals leaving the retina.

6. By analysing the spatial frequency tuning curve before and during APB application in terms of the difference-of-Gaussians model of receptive field structure, it was found that the balance between the integrated strengths of the centre and surround was not modified by APB. APB, however, had a differential effect on the summating area of the centre and surround. While the centre radius was unaffected by APB, the surround radius was reduced. This suggests that the peak sensitivity of the surround mechanism was reduced less severely than the peak sensitivity of the centre mechanism.

7. Strychnine also suppressed the response of the centre mechanism of both on- and off-centre X cells to drifting gratings.

INTRODUCTION

Synaptic transmission from cone photoreceptors to second-order neurones in the vertebrate retina is now thought to be mediated by L-glutamate or a closely related

compound (for review see Massey & Redburn, 1987). It is also known that different glutamate analogues preferentially affect receptors on different types of bipolar and horizontal cells (e.g. Slaughter & Miller, 1983). Of particular interest is a glutamate agonist, 2-amino-4-phosphonobutyric acid (APB), also known as AP4. In the retinae of mudpuppy (Slaughter & Miller, 1981), and rabbit (Bloomfield & Dowling, 1985), this has been found to act exclusively on the depolarizing bipolar cells. This selective action of APB offers a unique opportunity for studying the involvement of depolarizing cone bipolar cells in the receptive field circuitry of more proximal visual neurones, such as the retinal ganglion cells.

It is generally accepted that the centre response mechanism of an X-type retinal ganglion cell is mediated by direct synaptic input from only a few bipolar cells, which in turn receive their inputs from the photoreceptors. Since there exist both depolarizing (on) and hyperpolarizing (off) bipolar cells, as well as on- and off-centre ganglion cells, the question of whether a ganglion cell of a given centre polarity receives its input only from bipolar cells of the same response polarity has been of some interest. For the cat retina, McGuire, Stevens & Sterling (1986) argued, on anatomical grounds, that the centre response of both on- and off-centre β (X) cells is mediated by both depolarizing and hyperpolarizing cone bipolar cells. This has been called the push–pull model. That is, a bipolar and ganglion cell of the same response polarity would communicate with a sign-conserving synapse (push), while a bipolar cell of the opposite response polarity would use a sign-inverting synapse (pull). The two inputs would then act synergistically, so that at the onset of light an on-centre ganglion cell would receive excitation from depolarizing cone bipolar cells and the withdrawal of inhibition from hyperpolarizing cone bipolar cells. The inputs to the on-centre β-cells are bipolar cells ending in sublamina b of the inner plexiform layer, while off-centre ganglion cells receive inputs from a complementary pair of bipolar cells branching in sublamina a. Unfortunately, since one still cannot distinguish between depolarizing and hyperpolarizing cat bipolar cells with any certainty on morphological criteria alone, the proposal of McGuire *et al.* (1986) remains only a hypothesis. Bipolar cells that have the proper response polarity and lamination to serve as the push–pull pair for the on-centre X cells have been identified by Nelson & Kolb (1983), which strengthens the case for the push–pull model. Direct verification, however, would require current injection into physiologically identified bipolar cells while recording from a nearby ganglion cell. Since this is not feasible at present, other approaches must be taken.

Recently, Wässle and co-workers (Bolz, Wässle & Thier, 1984; Wässle, Schäfer-Trenkler & Voigt, 1986) approached the question of a push–pull mechanism pharmacologically, by making use of APB, which would be expected to block the transmission between the photoreceptors and depolarizing bipolar cells. They reported that, under mesopic conditions, APB diminished the centre light response of on- but not off-centre X and Y cells; APB only increase the mean firing rate of the off-centre cells. One way to interpret this result is to assume that the off-centre ganglion cells receive inputs from some depolarizing (i.e. APB-sensitive) bipolar cells which do not contribute to the ganglion cell's modulated response to light under mesopic conditions. Subsequently, Müller, Wässle & Voigt (1988) discovered that

APB abolished the centre light response of both on- and off-centre X and Y cells under scotopic conditions, and proposed a revision of the push–pull model. They suggested that off-centre ganglion cells receive inputs from both APB-insensitive hyperpolarizing *cone* bipolar cells (push) and APB-sensitive depolarizing *rod* bipolar cells (pull). With this circuitry, APB would be expected to increase the mean rate of off-centre ganglion cells under high mesopic, and presumably photopic conditions, but not to change their modulated response to light, since the rod bipolar half of the pathway would be saturated.

This model, however, differs from the push–pull hypothesis of McGuire *et al.* (1986), which includes a dual innervation of off-centre ganglion cells by depolarizing and hyperpolarizing *cone* bipolar cells. Part of the resolution depends on whether APB affects the cone-driven centre light response of off-centre ganglion cells. As will be elaborated in the Discussion and in the next paper (Chen & Linsenmeier, 1989), the conclusions of Wässle and co-workers regarding the photopic and mesopic states may be misleading because of the particular light stimulus, response measure, and amount of APB used. Exclusion of depolarizing cone bipolar cells from a model of the photopic retina would not be justified if APB affects the cone-driven centre light response of off-centre ganglion cells. The purpose of the present work was to reinvestigate the effects of locally applied APB and strychnine on cat retinal X cells, concentrating on the effects of moderate doses of APB on the responses to stimuli of moderate contrast. The mean luminance of the stimuli was in the photopic range, so that only the cone pathways contributed to the response.

A further reason for studying APB is that it has been used as a blocker of the 'on channel' at higher levels of the visual system (e.g. Schiller, 1982). This may be justified in some species or under some conditions, but Horton & Sherk (1984) noted that intraocular APB caused subtle effects on at least some off-centre cells in the cat lateral geniculate nucleus. That study could not investigate those effects in detail, because the same cells were not studied before and during APB application.

In this paper we describe the effect of locally applied APB on the mean rate and responsivity of the centre and surround of on- and off-centre X cells to sinusoidal gratings. APB reduced the response of both types of cells. In the next paper, we present results obtained with stimuli designed to separate the push and pull components of the responses. Portions of this work have been presented at the Society for Neuroscience meeting (Chen & Linsenmeier, 1987).

METHODS

Preparation. Acute experiments were performed on adult cats weighing between 2·5 and 5 kg. Anaesthesia was induced with an intravenous injection of sodium thiamyal (Surital; 15–20 mg kg^{-1}). Additional Surital was given as necessary during surgery, which included insertion of two venous and one arterial catheters, a tracheotomy and eye surgery. During experiments, anaesthesia was maintained with urethane given intravenously at 100–200 mg h^{-1} (following a loading dose of 100 mg kg^{-1}). The animal was paralysed with pancuronium bromide (Pavulon, 0·2–0·3 mg kg^{-1} h^{-1}) to minimize residual eye movements. Arterial blood samples were taken for determination of pH, P_{CO_2}, and P_{O_2} with a blood gas analyser (model 158, Corning Medical and Scientific, Medfield, MA, USA), and the respirator was adjusted so that these values were maintained within the physiological range. Body temperature was maintained at 38·5 °C. Stability

of femoral arterial pressure and heart rate were monitored continuously as an indication of the level of anaesthesia. Also, in similar experiments without muscle relaxants, lower doses of urethane have been shown to suffice for light anaesthesia (Enroth-Cugell & Pinto, 1970).

The animal's head was mounted in a head holder. Bone and soft tissue from the temporal side of the right orbit were cleared so that the right eye could be attached to a stainless-steel stabilizing ring with a flap of conjunctiva. The eye was then penetrated with a 15-gauge hypodermic needle about 1 cm behind the limbus. This needle served as a guide tube through which the microelectrodes were introduced into the eye. The needle and microelectrode were held in a manipulator similar to that described by Steinberg, Walker & Johnson (1968). The electrode was sealed into the needle with a device that allowed movement, but prevented the leakage of vitreous humor (Yancey, 1989). The pupil was dilated with 1% atropine and 1% neosynephrine. The cornea was protected with a contact lens having a 4 or 5 mm diameter artificial pupil. The lens power required to bring the stimulus pattern into focus on the retina was determined by direct ophthalmoscopy.

Visual stimulus. Visual stimulation was provided by a cathode ray tube (CRT) with a P-31 phosphor (Joyce Electronics, Cambridge, England) controlled by a PDP-11/04 or 11/34 computer (Digital Equipment Corp., Maynard, MA, USA). The mean luminance was generally about 400 cd m^{-2}, but in some later experiments, a mean of 250 cd m^{-2} was used. These are in the photopic range (Enroth-Cugell, Hertz & Lennie, 1977). A mirror between the CRT and the cat's eye was used to position the receptive field at the centre of the CRT which subtended 13·4 (horizontal) by 10·7 (vertical) deg. Both drifting and sinusoidally modulated stationary gratings were used in the experiments. A detailed description of the stimuli can be found in Enroth-Cugell, Robson, Schweitzer-Tong & Watson (1983). Contrast, spatial frequency, spatial phase, and temporal frequency could be controlled by the computer.

Electrodes and recording. Double-barrelled glass electrodes were used in order to apply drugs locally as well as record from cells. The two barrels were pulled separately with a vertical pipette puller (model 700C, David Kopf Instruments, Tujunga, CA, USA) from 0·7 mm o.d. glass containing a fibre (Omega Dot, Glass Co. of America, Bargaintown, NJ, USA) and glued together with cyanoacrylate. One of the barrels was used to record action potentials from ganglion cell bodies. The other was used to deliver drugs by pressure ejection. The tip of the ejection barrel was broken to a diameter of 3–5 μm and its shank was bent slightly under heat so that the lateral separation of the tips was about 30 μm. The axial separation was adjusted before gluing so that the ejection barrel was behind the recording tip by 100–150 μm, a separation which allowed the tip of the ejection barrel to be just outside of the retina during recording. This was necessary since pressure ejection of fluid within the retina could have reduced stability or caused tissue damage. The recording barrel was filled with 5 M-potassium acetate the night before the experiment and bevelled before use to an impedance of 15–25 MΩ (model BV-10, Sutter Instrument Co., San Francisco, CA, USA). The reference electrode was a chlorided silver plate tied into soft tissue near the orbit. Action potentials were amplified and filtered by an AC-coupled preamplifier (model 2400, Dagan, Minneapolis, MN, USA). A spike discriminator was used to generate standard pulses of 0·4 ms for each action potential, which were fed to the computer for further analysis. The standard pulses were also smoothed and sent to a chart recorder to provide a continuous record of mean firing rate.

Most recordings were made from cells near the area centralis. When a single unit was isolated, the receptive field mid-point was hand mapped on a tangent screen. Recordings were made only from cell bodies so that the drug ejected could reach the synaptic sites of the cell as uniformly as possible. The recording site could usually be judged from the shape of the action potentials. The slow potential rise before the spike can only be observed from recordings made at the cell body (Cooper, Robson & Waldron, 1969). One can also identify an axonal recording from the separation between the location of the electrode tip and the mapped receptive field.

Pressure ejection. Solutions of 20 mM-DL-APB and 2·5 mM-strychnine sulphate (Sigma Chemical Company, St Louis, MO, USA) were prepared in a phosphate-buffered Ringer solution (pH 7·4). The back end of the ejection barrel was connected to a pressure ejection system (Picospritzer II, General Valve Corp., Fairfield, NJ, USA) by heat-shrinkable tubing. In these experiments it was necessary to maintain the drug effects for many minutes. This could be achieved by controlling the ejection pressure (5–80 lbf in^{-2}; 35–550 kPa) and duration and frequency of pulse. Pulses of 100–1000 ms were given at 2 s intervals by triggering the Picospritzer from a stimulator (model S8, Grass Instruments, Quincy, MA, USA). Although the drugs were ejected in pulses, the effect

produced on all the cells was steady rather than being time-locked to the pulses. Ejection of phosphate buffer alone had no effect on the mean rate and fundamental response (defined below) in five cells.

Data analysis. The standard pulses provided by the spike discriminator were compiled into peristimulus time histograms by the computer. Each histogram contained 128 bins and was compiled over a period of 10·24 seconds. For a grating modulated or drifting at 1·954 Hz (rounded to 2 Hz for discussion), the stimuli that were most often used, there were twenty repetitions of the stimulus in each histogram. Mean firing rate and modulated responses to either the drifting or modulated gratings were determined on-line by computing the amplitude and phase of the zeroth (mean rate) and first (fundamental) Fourier components in the histograms. A modified null test (Hochstein & Shapley, 1976) was used to determine whether a cell was an X cell.

The spatial summation of X cells was characterized by measuring their responsitivity to sinusoidal gratings of various spatial frequencies. Figure 1 shows an X cell's responsivity as a function of spatial frequency (Enroth-Cugell *et al.* 1983). Responsivity, which is the slope of the linear portion of the function relating response amplitude to grating contrast, was measured by adjusting the contrast of the grating to produce a fundamental Fourier response of 8–12 impulses s^{-1}. Responsivity is then the ratio between this response and the contrast that produced it. In this study the receptive field profile of X cells was assumed to follow the difference-of-Gaussians model (Rodieck & Stone, 1965) from which the spatial frequency tuning curve (eqn (1)) was derived in Enroth-Cugell & Robson (1966):

$$R(u) = S_c \exp[-(\pi r_c u)^2] - S_s \exp[-(\pi r_s u)^2], \quad (1)$$

where R is the responsivity at spatial frequency u, S_c and S_s are integrated strengths of the centre and surround, and r_c and r_s are the radii of centre and surround. By fitting the difference-of-Guassians model (continuous line in Fig. 1) to the experimental values, the radii and integrated strengths of centre and surround mechanisms of a cell could be estimated. The fitting was achieved by minimizing the root mean square error between the predicted and measured responsivities using the quasi-Newton algorithm BFGS (Dennis & Schnabel, 1983). The two terms of eqn (1) then give the dashed centre and surround curves of Fig. 1 and the inset shows the spatial domain representation of the receptive field derived from these parameters.

RESULTS

In the first set of experiments, we measured the cell's responsivity to gratings of various spatial frequencies as a preliminary step, and then investigated the effects of APB on responses to only one grating whose spatial frequency was slightly above the peak. The response to such a stimulus is almost purely due to the centre mechanism (Linsenmeier, Frishman, Jakiela & Enroth-Cugell, 1982). Through these experiments it was discovered that the influence of APB could be maintained at a constant level for a period of time that would be sufficient to complete a spatial frequency tuning curve. In addition, by stimulating with different contrasts at this one spatial frequency, it was found that an X cell would still have a linear range in the contrast–response relationship during APB application, so that its responsivity could be meaningfully measured. These findings together enabled us to study the entire spatial frequency tuning curve during APB administration in the second set of experiments.

Effects of APB on centre response

Responses to 2 Hz drifting gratings of spatial frequency slightly higher than peak were monitored before, during and after the application of APB. The response of the cell was measured as often as possible, generally at about 30 s intervals. The contrast

used was low enough to yield an unsaturated pre-drug response and to prevent the firing rate from falling to zero during any part of the stimulus cycle (i.e. no 'clipping').

The effects of APB on the mean rate and on the centre-dominated fundamental

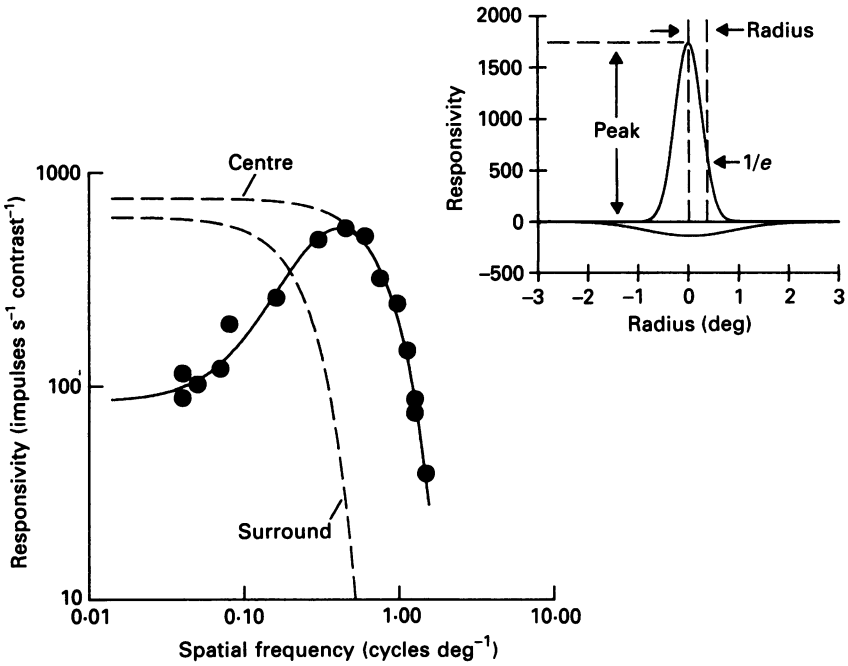

Fig. 1. Spatial frequency tuning curve of an off-centre X cell to a sinusoidal grating drifting at 2 Hz. Responsivity was measured (●) from an average of three histograms, each collected over twenty stimulus repetitions. The continuous line is a fit to the difference-of-Gaussians model (eqn (1)) and the dashed lines are the first (centre) and second (surround) terms of the model. The inset shows the spatial representation of the receptive field derived from this tuning curve, with the centre plotted at positive responsivities and the surround at negative responsivities. The radius, r_c, and peak responsivity of the centre mechanism are indicated.

response of an on-centre X cell are shown in Fig. 2, along with histograms obtained before and during APB application. Both the mean rate (○) and the fundamental response (●) were reduced almost immediately after the onset of APB ejection. Within a couple of minutes, the effects of the drug reached a steady level, and the response was reasonably stable for the entire duration of ejection (5 min). A constant effect on the response could be maintained for up to 30 min for both on- and off-centre cells. When the ejection was terminated, the drug effects were completely reversible within 2 min.

The action of APB on the centre mechanism of an off-centre X cell is shown in Fig. 3. For this cell the responses before APB were known to be in the linear range, so responsivity is plotted instead of response amplitude. The validity of measuring responsivity during APB application will be demonstrated below. As shown in Fig. 3*A*, APB suppressed the off-centre cell's responsivity. Unlike its action on the on-centre X cells, however, APB elevated the mean rate of the off-centre cells

(Fig. 3*A*). The cell recovered from these effects within 2 min of the ejection being terminated.

Due to the method of drug delivery, the concentration of APB at the action site could not be determined. Pressure and duration of the ejection pulses were typically

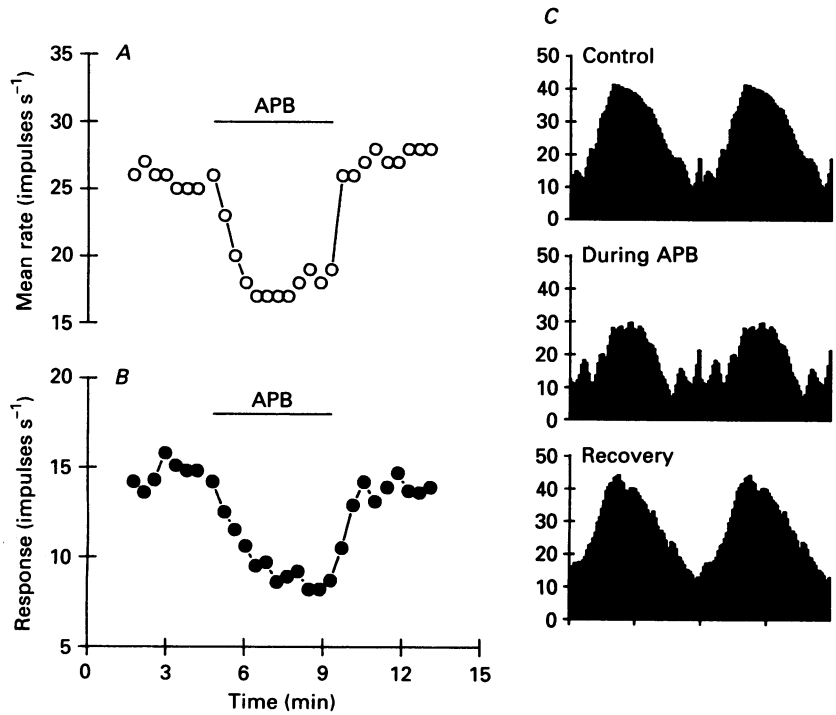

Fig. 2. Time course of the effect of APB on the mean rate (*A*) and fundamental response (*B*) of an on-centre X cell. The cell was stimulated with a grating drifting at 2 Hz (0·75 cycles deg^{-1}, 50 % contrast with a background of 400 cd m^{-2}). Each pair of points (mean rate and response) was measured from the same histogram. Each histogram was compiled over 10·24 s, and the points are plotted at a time corresponding to the beginning of histogram accumulation. In this case APB was ejected in pulses of 1 s every 2 s at 70 lbf in^{-2} (480 kPa) over the time indicated by the horizontal bar. Histograms collected before, during and after APB are shown in *C*. The histograms shown are each averages of four histograms obtained just before the APB ejection, just before the end of the ejection and 3 min after the end of the ejection. Each histogram is smoothed with 3-point smoothing and is placed twice to indicate better the time course of the response.

selected to elicit a significant but moderate change in the mean firing rate. For off-centre cells, the mean firing rate was not elevated above 80 impulses s^{-1}. For on-centre cells, it was often possible to find ejection parameters that would completely silence the cell. The absence of a mean rate, however, would have prevented the measurement of the responses in any meaningful way. The ejection rate was therefore chosen so that during APB the cell had a mean rate of at least 10–15 impulses s^{-1}.

The effects of APB described above were observed in most of the twenty-two on-centre and eighteen off-centre cells studied. In Fig. 4 the mean rates of both on- and off-centre X cells measured before and during APB are plotted against each other.

Note that all of the points from the on-centre cells (○) fall below the 45 deg line, confirming the effect of APB in reducing the mean rates of these cells (Bolz *et al.* 1984). On the other hand, the points from all but two of the off-centre cells (●) lie above the 45 deg line, indicating that APB elevated the mean rate of these cells. It is not clear why two off-centre cells were exceptional.

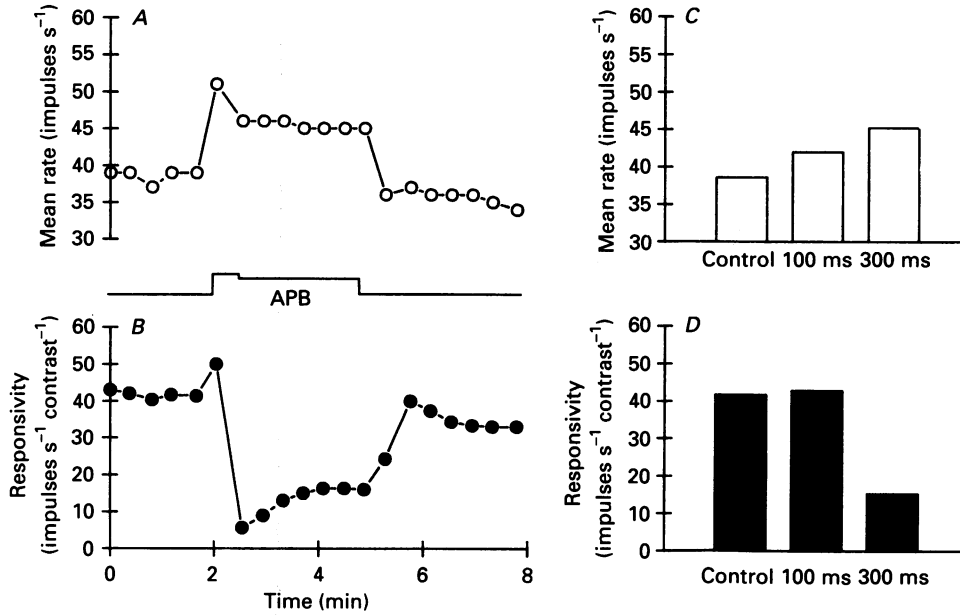

Fig. 3. Time course of the effect of APB on the mean rate (*A*) and responsivity (*B*) of an off-centre X cell, stimulated with a 2 Hz drifting grating (0·71 cycles deg^{-1}, 50% contrast with a background of 400 cd m^{-2}). APB was ejected with 30 lbf in^{-2} (200 kPa) pulses at 2 s intervals. For approximately the first ten seconds, the pulse duration was 400 ms. This was shortened to 300 ms thereafter, as indicated by the bar, and this is responsible for the initial transients in the observed effects. The bar histograms on the right show the effect on mean rate (*C*) and responsivity (*D*) of different pulse durations on this cell. The 300 ms bars were obtained by averaging the last four points in the ejection episode in (*A*) and (*B*). The results of the 100 ms pulse ejection were averaged values obtained from ejection episodes both before and after the 300 ms episode, but the effect would clearly be larger in terms of both mean rate and fundamental.

In Fig. 5*A* the fundamental response for fifteen on- and ten off-centre cells before and during APB are plotted against each other, and Fig. 5*B* is a similar plot for the responsivity of a separate group of seven on- and seven off-centre cells. With the exception of two off-centre cells, the fundamental response of every cell studied was suppressed by APB irrespective of its polarity. The lack of effect on these two off-centre cells cannot be attributed to ejection failure, since the mean rate of both cells was significantly elevated by APB. It is possible that the fundamental response could have been suppressed if the optimal ejection rate had been used, but these cells could not be held long enough to try different ejection rates. This possibility was demonstrated in two other off-centre cells including the one shown in Fig. 3. In this cell the first ejection attempt, with pressure pulses of 100 ms duration, yielded no noticeable effects on the fundamental responses (Fig. 3*D*), but a higher ejection rate

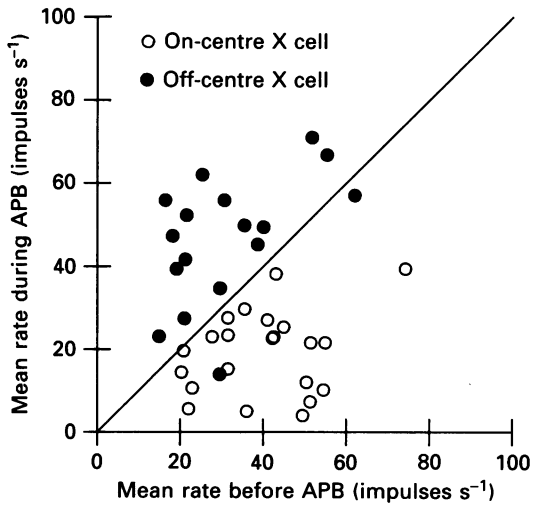

Fig. 4. Effect of APB on the mean firing rate of twenty-two on-centre and seventeen off-centre X cells to drifting sinusoidal gratings. Since the grating contrast was low in these experiments, the mean rate was essentially the same as the cells' maintained firing rate. Except for five on-centre and five off-centre X cells, the mean rate before and during APB ejection was obtained from experiments like those shown in Figs 2 and 3. For each condition, four histograms from a section of apparent stationariness were averaged to produce the mean rate estimate. The exceptions were from later experiments in which the effect of APB on the spatial frequency tuning curve was investigated. For these cells, mean rate was estimated by averaging all histograms collected at all spatial frequencies.

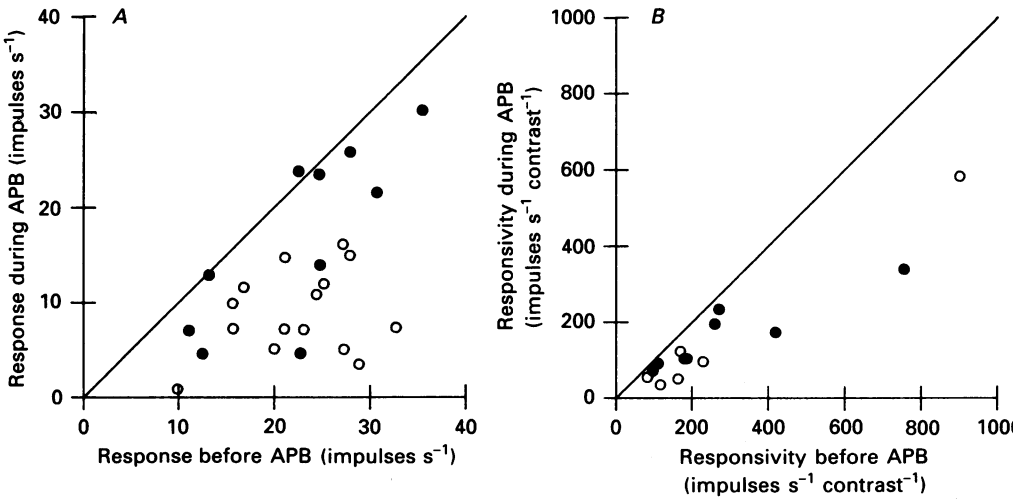

Fig. 5. Effect of APB on the centre-dominated response (*A*) and responsivity (*B*) of the same groups of cells shown in Fig. 4. The same averaging protocol as in Fig. 4 was used for the seventeen on- and twelve off-centre X cells studied at only one spatial frequency. For those cells involved in the tuning curve experiments, the centre responsivity was estimated by fitting the tuning curve data to the difference-of-Gaussians model as described in Methods.

(300 ms pulse duration) resulted in the suppression of the fundamental response. The lack of effect of the first ejection on the fundamental response cannot be attributed to ejection failure, since the mean rate was elevated the first time APB was ejected (Fig. 3*C*).

The effects of APB were reproducible in the same cells when the same ejection settings were used. Increased pressure and duration of pressure pulses also led to an enhancement of the effects. There was, however, no correlation between the magnitude of the effects on mean rate and fundamental response.

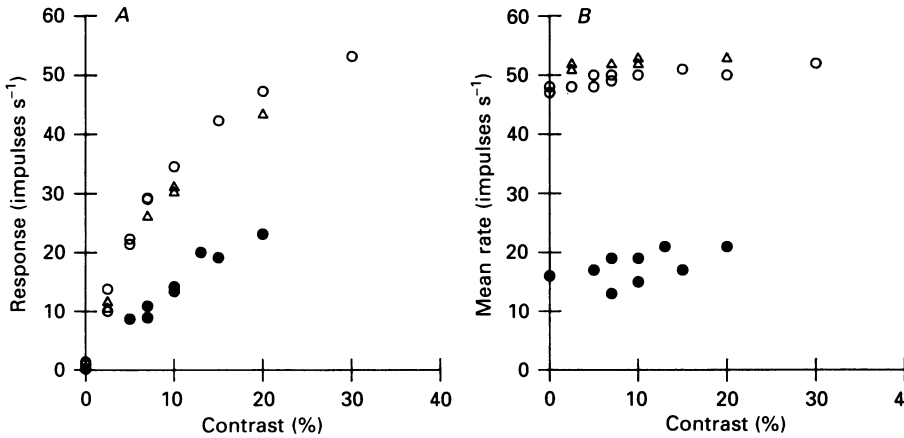

Fig. 6. Contrast–response curves for an on-centre X cell under normal conditions (○), during APB application (●) and during recovery (△). The cell's response to a drifting grating (2 Hz, 1·15 cycles deg^{-1}) of various contrasts was evaluated in terms of the fundamental response (top) and mean discharge rate (bottom). APB was ejected with 350 ms pulses every 2 s at 45 lbf in^{-2} (310 kPa).

Contrast–response relation

The contrast–response curves shown in Fig. 6*A* were obtained from an on-centre X cell before (○), during (●), and 10 min after (△) the ejection of APB. The basic characteristics of the contrast–response curve were not altered by APB. Response increased linearly with stimulus contrast over the range shown, justifying measurement of responsivity under APB. The slope of the curve was reduced by APB, however, implying a lowered contrast gain. For the control curve, the response began to saturate at about 15% contrast. During APB ejection, probably simply due to a lowered contrast gain, the cell remained linear up to 20% contrast. The mean rate of the cell was independent of contrast both before and during the application of APB, as shown in Fig. 6B.

Effects of APB on the spatial summation of X cells

A typical example of the effect of APB on the spatial tuning curve of an off-centre cell is shown in Fig. 7. The open and filled circles are responsivity measurements before and during the application of APB, respectively. The open squares are

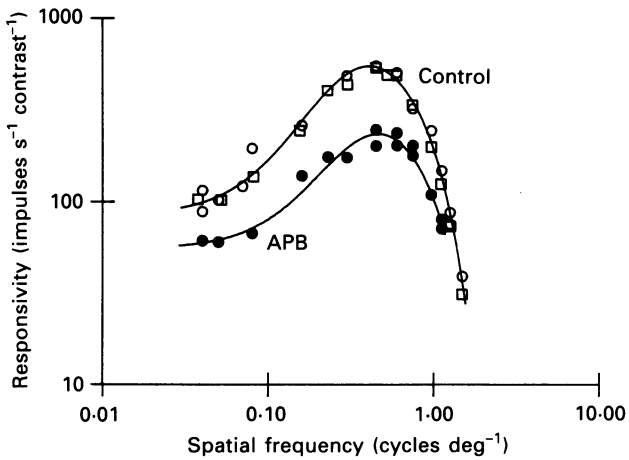

Fig. 7. Spatial frequency tuning curve for an off-centre X cell under normal conditions (○), during APB application (●) and after it recovered from the application (□). The smooth curves are the best-fitting difference-of-Gaussians models (eqn (1)). Under the normal conditions, the parameters of the model that produced the smooth curves were: centre radius (r_c) = 0·372 deg, surround radius (r_s) = 1·238 deg, integrated centre strength (S_c) = 755·4,and integrated surround strength (S_s) = 670·5. The root mean square (r.m.s.) error was 0·08. During APB application, r_c = 0·343 deg, r_s = 1·040 deg, S_c = 338·8, S_s = 283·4 and r.m.s. error = 0·067.

responsivity made 15 min after the ejection was terminated. To verify the constancy of the effect, the responsivity at one or more frequencies was repeated at various times during the ejection. An experiment of this type took about 30 min.

APB reduced the cell's responsivity across all spatial frequencies, implying that both centre and surround sensitivities were reduced. The spatial resolution, as defined by the high spatial frequency cut-off, appeared not to change. The difference-of-Gaussians model (eqn (1)) was fitted to the data using the non-linear regression algorithm, BFGS. There are four parameters in this model: centre radius (r_c), integrated centre strength (S_c), surround radius (r_s), and integrated surround strength (S_s). The fits of eqn (1) to the data are shown by the continuous lines. The fits showed that the integrated surround strength (S_s) was reduced by the same proportion as the integrated centre strength (S_c). On the other hand, the surround radius was reduced, while the centre radius was unchanged.

These results were confirmed in the ten cells from which recordings could be maintained long enough and APB effects were stable enough to yield data across the full range of spatial frequencies. When the percentage reduction in the integrated centre strength was plotted against percentage reduction in the integrated surround

strength (Fig. 8*A*), the points did not deviate in any consistent way from the 45 deg line, indicating that the centre and surround balance was not altered by APB. Furthermore, when centre radius estimated before APB application was plotted against centre radius estimated during APB ejection (Fig. 8*B*), all the points fell on

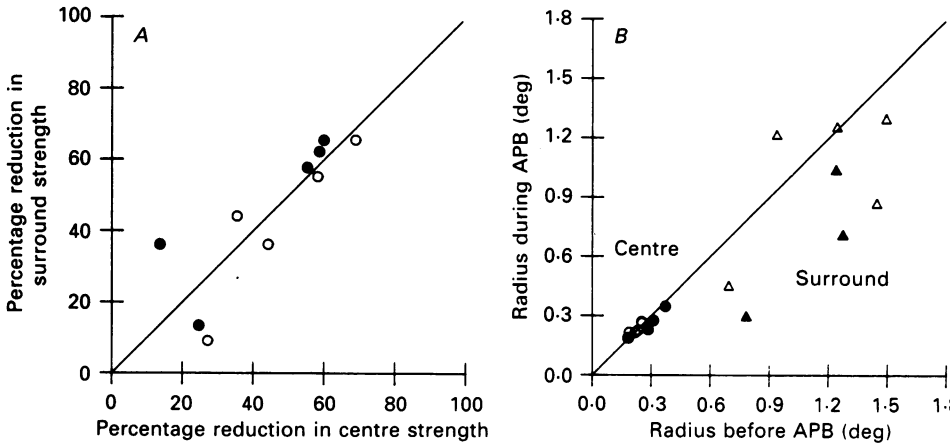

Fig. 8. Effects of APB on receptive field parameters. *A*, percentage reduction of the integrated surround strength (S_s) as a function of the percentage reduction of integrated centre strength (S_c) for five on-centre (○) and five off-centre (●) X cells. *B*, the absolute values of the centre radius (circles) and surround radius (triangles) during APB administration *vs.* these values under normal conditions. On-centre and off-centre cells are represented by open and filled symbols respectively.

the 45 deg line, indicating that APB did not alter the centre radius. Interestingly, when the surround radius before and during APB were plotted against each other, the points fell below the 45 deg line, showing that the surround radius was reduced by APB. The effects on ganglion cell spatial summation were similar in both on- and off-centre X cells.

Effects of strychnine on centre response

In their formulation of the push–pull model, Wässle and his co-workers (Wässle *et al.* 1986; Müller *et al.* 1988) have suggested that the pull action for the on-centre ganglion cells and the push action for the off-centre ganglion cells might be mediated by glycinergic synapses. This necessarily leads to the prediction that strychnine would reduce the responsiveness of the centre mechanism of both on- and off-centre ganglion cells. However, Bolz, Thier, Voigt & Wässle (1985) found that strychnine *increased* the centre's light-evoked response in both on- and off-centre ganglion cells under photopic conditions. Effects of strychnine were therefore examined in the present study, but not very extensively (three on- and two off-centre cells in two cats), because of the potential for an action at so many sites in the retina (McGuire, Stevens & Sterling, 1980; Pourcho, 1980; Pourcho & Goebel, 1985; Bolz *et al.* 1985; Wässle *et al.* 1986).

Figure 9 shows the effects of strychnine on the centre response of an on- (*A*) and an off-center (*B*) X cell. The stimulus was a 2 Hz drifting grating of low contrast

(10%). Strychnine, like APB, reduced the fundamental response of both on- and off-centre X cells. There were, however, a number of distinctions between the effects of the two drugs. First, the onset of the strychnine effect on both the mean rate and the fundamental response was slower than that of APB. The recovery from strychnine

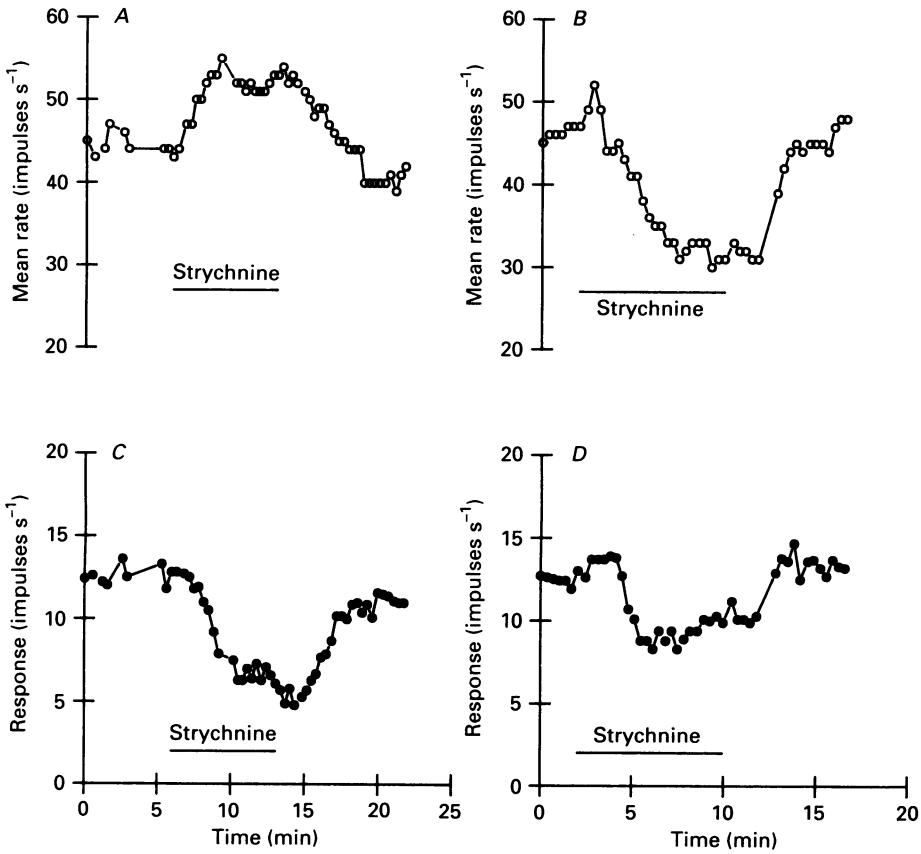

Fig. 9. The effect of strychnine on the mean rate (*A* and *C*) and fundamental response (*B* and *D*) of an on- and an off-centre X cell. Both cells were stimulated with a 2 Hz drifting grating (0·71 cycles deg^{-1}, 50% contrast with a background of 400 cd m^{-2}). Strychnine was ejected wtih 30 ms pulses for the on-centre cell (*A* and *B*), and with 50 ms pulses for the off-centre cell (*C* and *D*). The pressure in both cases was 30 lbf in^{-2} (200 kPa). The periods of application are marked with horizontal bars.

was also more sluggish. It took 5–15 min before the mean rate and fundamental response returned to their pre-strychnine levels. Second, the effect of APB on mean rate and fundamental response occurred in synchrony, but with strychnine, the effect on the mean rate led the effect on the fundamental response. The suppression of the fundamental response by strychnine usually did not become prominent until 2–3 min after the onset of ejection, while the effect on the mean rate appeared about 1 min after the onset of ejection. Third, the effect of strychnine on the mean rate was often transient. For both on- and off-centre X cells, the mean rate first increased about 1 min into the ejection, and then declined to a lower level which could be at, above or

below the control level. This transience could not be attributed to a reduction in the amount of strychnine delivered (i.e. by a blocked electrode), since the suppression of the fundamental response remained.

DISCUSSION

The results of this study confirm the findings of Bolz *et al.* (1984) that local application of APB suppresses the maintained firing rate and the centre response of on-centre X cells. If APB is assumed to act like the cone transmitter on the depolarizing bipolar cells (Slaughter & Miller, 1981; Bloomfield & Dowling, 1985), then this is not a surprising observation, since the centre mechanism of the on-centre X cells is mediated at least by these bipolar cells through sign-conserving synapses.

Our findings on off-centre X cells, however, differ partly from those of Bolz *et al.* (1984) and Müller *et al.* (1988). While both studies have shown an APB-induced elevation of mean rate, only the present results show clearly that APB also reduced the centre response of off-centre cells under photopic conditions. This suppression is an integral part of the original push–pull hypothesis, as it points to a functional connection between depolarizing cone bipolar cells and off-centre ganglion cells. A failure in demonstrating such an effect under mesopic conditions led Müller *et al.* (1988) to reformulate the hypothesis by excluding from their model the depolarizing cone bipolar inputs to off-centre ganglion cells. It should be pointed out that, although this study supports the notion that the off-centre X cells receive inputs from the depolarizing cone bipolar cells, it is not possible to tell whether such inputs are direct as in the original hypothesis or whether they go through amacrine cells. Neither does our study rule out the possibility of an input to ganglion cells from depolarizing rod bipolar cells as suggested by Müller *et al.* (1988) under photopic conditions. A block of this input by APB may contribute to the change in mean rate, but it cannot influence the modulated response to photopic stimuli as reported in this study.

In the next paper (Chen & Linsenmeier, 1989) the reason why Bolz *et al.* (1984) and Müller *et al.* (1988) failed to observe an APB-induced suppression of cone-driven centre responses in off-centre cells will be explained. There we show that APB acts selectively on various response components of the off-centre ganglion cells. Specifically, the transient increase in firing rate at the offset of light stimuli, which was used by Bolz *et al.* as the sole estimate of centre response, is resistant to APB.

If the depolarizing bipolar cells contribute to both on- and off-centre ganglion cells, then the effective dose of APB for on- and off-centre ganglion cells should be the same, though the reduction in the sensitivity of on- and off-centre cells need not be equal. The relative amount of APB needed for the two types of cells was difficult to estimate, since ejection pressure and duration would be expected to vary with at least the electrode tip size, and its distance from the retina. Within the limit of this uncertainty, it seems that both types of cells required, on the average, about the same amount of APB.

Whether or not on-centre X cells receive inputs from hyperpolarizing bipolar cells cannot be determined by studying the effects of APB alone. The finding that strychnine suppressed the centre response of both on- and off-centre X cells suggests

that a glycinergic synapse may be involved in the centre mechanism of both types of cells either in the direct pathway or at a synapse serving a modulatory role. For on-centre X cells, the glycinergic pathway is not sensitive to APB (Wässle *et al.* 1986), so glycine may be involved in a pathway that does not involve a depolarizing bipolar cell. This is likely to be mediated by a hyperpolarizing bipolar cell, acting either directly on the ganglion cell or through an amacrine cell. For off-centre cells, Wässle *et al.* (1986) showed that the synapses sensitive to APB and glycine are in series. Surprisingly, Bolz *et al.* (1985) found that strychnine enhanced the centre responses, which is not consistent with this model and disagrees with the results of studies, including the present one, that have shown modest reductions in centre responses with strychnine (Kirby, 1979; Saito, 1981; Frishman & Linsenmeier, 1982). A possible explanation for this difference can be proposed. Inspection of the response histograms in the study by Bolz *et al.* (1985) shows that the firing rate was usually zero over part of the stimulus cycle. Under these conditions there is no baseline against which response amplitude can be measured, and a change in firing rate does not necessarily give a good representation of the underlying change in ganglion cell membrane potential. Part of the actual response may be essentially clipped off by the limitation that the firing rate cannot be less than zero. Strychnine led to an increase in mean firing rate, so that response amplitude could be measured accurately during strychnine application. It is possible, however, that strychnine actually led to a small decrease in the size of the membrane potential change evoked by light.

The finding that APB also reduces the surround responsivity of both on- and off-centre X cells leads to the conclusion that depolarizing bipolar cells contribute to the surround pathways. It is difficult, however, to deduce the details of their involvement. The ganglion cell surround is possibly mediated by the same bipolars that make up the centre via horizontal cell feedback at the outer plexiform layer. But the surround may also utilize a bipolar-to-amacrine cell pathway. Either way, the suppression of depolarizing bipolar input could lead to a reduction in surround responsivity. It should be noted that, although APB did not alter centre and surround balance, surround radius was reduced in the presence of APB. This means that a significant portion of the reduction in integrated surround strength occurs because the mechanism is summating inputs over a smaller region. In other words, the peak sensitivity of the surround mechanism may be less severely suppressed by APB than the peak sensitivity of the centre mechanism. This suggests a more complex design for the surround. For example, the surround may be composed of a larger APB-sensitive mechanism and a smaller APB-resistant mechanism.

The present study also influences the view that visual information about light and dark are channelled through strictly segregated 'on' and 'off' pathways. At the retina, this means that depolarizing bipolar cells communicate only with the on-centre ganglion cells and hyperpolarizing bipolar cells contact only off-centre ganglion cells. Our results show that this cannot be true, at least in the cat retina, since APB depresses the response of even off-centre X cells. This conclusion, however, is based heavily on the assumption that APB is selective to depolarizing bipolar cells in cat retina as it is in the mudpuppy and rabbit retinae. While this seems reasonable, Nawy, Copenhagen & Lisberger (1988) have suggested that APB

may also act presynaptically on the goldfish cones. Until the specificity of APB in the cat retina is demonstrated, deductions about retinal circuitry from the APB results must be treated with caution. One should also exert caution in using APB to study the contribution of this 'on' channel to psychophysically measured visual processes (e.g. Smith, Duncan & Harwerth, 1987). Regardless of its site or sites of action, since APB suppresses the response of off-centre cat retinal ganglion cells, it cannot be used without reservation as an 'on' channel blocker at higher visual centres of the cat.

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REFERENCES

BLOOMFIELD, S. A. & DOWLING, J. E. (1985). Roles of aspartate and glutamate in synaptic transmission in rabbit retina. I. Outer-plexiform layer. *Journal of Neurophysiology* **53**, 699–713.

BOLZ, J., THIER, P., VOIGT, T. & WÄSSLE, H. (1985). Action and localization of glycine and taurine in the cat retina. *Journal of Physiology* **362**, 395–441.

BOLZ, J., WÄSSLE, H. & THIER, P. (1984). Pharmacological modulation of ON and OFF ganglion cells in the cat retina. *Neuroscience* **12**, 875–885.

CHEN, E. P. & LINSENMEIER, R. A. (1987). The effects of APB on the contrast sensitivity of cat retinal X cells. *Society for Neuroscience Abstracts* **13**, 381.

CHEN, E. P.-C. & LINSENMEIER, R. A. (1989). Centre components of cone-driven retinal ganglion cells: differential sensitivity to 2-amino-4-phosphonobutyric acid. *Journal of Physiology* **419**, 77–93.

COOPER, G. F., ROBSON, J. G. & WALDRON, I. (1969). The action potentials recorded from undamaged nerve fibres with micro-electrodes. *Journal of Physiology* **200**, 9–11*P*.

DENNIS, J. E. & SCHNABEL, R. B. (1983). *Numerical Methods for Unconstrained Optimization and Nonlinear Equations*. Englewood Cliffs, NJ, USA: Prentice-Hall, Inc.

ENROTH-CUGELL, C., HERTZ, B. & LENNIE, P. (1977). Convergence of rod and cone signals in the cat's retina. *Journal of Physiology* **269**, 297–318.

ENROTH-CUGELL, C. & PINTO, L. P. (1970). Gallamine triethiodide (Flaxedil) and cat retinal ganglion cell responses. *Journal of Physiology* **208**, 677–689.

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

ENROTH-CUGELL, C., ROBSON, J. G., SCHWEITZER-TONG, D. E. & WATSON, A. B. (1983). Spatio-temporal interaction in cat retinal ganglion cells showing linear spatial summation. *Journal of Physiology* **341**, 279–307.

FRISHMAN, L. J. & LINSENMEIER, R. A. (1972). Effects of picrotoxin and strychnine on non-linear responses of Y-type cat retinal ganglion cells. *Journal of Physiology* **324**, 347–363.

HOCHSTEIN, S. & SHAPLEY, R. (1976). Linear and nonlinear spatial subunits in Y cat retinal ganglion cells. *Journal of Physiology* **262**, 265–284.

HORTON, J. C. & SHERK, H. 1984). Receptive field properties in the cat's lateral geniculate nucleus in the absence of on center retinal input. *Journal of Neuroscience* **4**, 374–380.

KIRBY, A. W. (1979). The effect of strychnine, bicuculline, and picrotoxin on X and Y cells in the cat retina. *Journal of General Physiology* **74**, 71–84.

LINSENMEIER, R. A., FRISHMAN, L. J., JAKIELA, H. G. & ENROTH-CUGELL, C. (1982). Receptive field properties of X and Y cells in the cat retina derived from contrast sensitivity measurements. *Vision Research* **22**, 1173–1183.

MCGUIRE, B. A., STEVENS, J. K. & STERLING, P. (1980). Beta ganglion cells receive convergent input from 2 types of cone bipolar cells. *Society for Neuroscience Abstracts* **6**, 347.

MCGUIRE, B. A., STEVENS, J. K. & STERLING, P. (1986). Microcircuitry of Beta ganglion cells in cat retina. *Journal of Neuroscience* **6**, 907–918.

MASSEY, S. C. & REDBURN, D. A. (1987). Transmitter circuits in the vertebrate retina. *Progress in Neurobiology* **28**, 55–96.

MÜLLER, F., WÄSSLE, H. & VOIGT, T. (1988). Pharmacological modulation of the rod pathway in the cat retina. *Journal of Neurophysiology* **59**, 1657–1672.

NAWY, S., COPENHAGEN, D. R. & LISBERGER, S. G. (1988). Evidence for a presynaptic effect of APB on the cone pathway in the goldfish retina. *Investigative Ophthalmology and Visual Science Supplement* **29**, 224.

NELSON, R. & KOLB, H. (1983). Synaptic pattern and response properties of bipolar and ganglion cells in the cat retina. *Vision Research* **23**, 1183–1195.

POURCHO, R. G. (1980). Uptake of [^{3}H]glycine and [^{3}H]GABA by amacrine cells in the cat retina. *Brain Research* **198**, 333–346.

POURCHO, R. G. & GOEBEL, D. J. (1985). A combined Golgi and autoradiographic study of [^{3}H]glycine-accumulating amacrine cells in the cat retina. *Journal of Comparative Neurology* **233**, 473–480.

SAITO, H. (1981). The effects of strychnine and bicuculline on the responses of X- and Y-cells of the isolated eye-cup preparation of the cat. *Brain Research* **212**, 243–248.

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

SLAUGHTER, M. M. & MILLER, R. F. (1981). 2-Amino-4-phosphonobutyric acid: A new pharmacological tool for retinal research. *Science* **211**, 182–184.

SLAUGHTER, M. M. & MILLER, R. F. (1983). The role of excitatory amino acid transmitters in the mudpuppy retina: an analysis with kainic acid and *N*-methyl aspartate. *Journal of Neuroscience* **3**, 1701–1711.

SMITH, E. L. III, DUNCAN, G. C. & HARWERTH, R. S. (1987). Spatial contrast sensitivity deficits produced by ON-channel blockade in the Rhesus monkey. *Society for Neuroscience Abstracts* **13**, 381.

STEINBERG, R. H., WALKER, M. L. & JOHNSON, W. M. (1968). A new microelectrode positioner for intraretinal recording from the intact mammalian eye. *Vision Research* **8**, 1521–1523.

WÄSSLE, H., SCHÄFER-TRENKLER, I. & VOIGT, T. (1986). Analysis of a glycinergic inhibitory pathway in the cat retina. *Journal of Neurophysiology* **32**, 339–355.

YANCEY, C. M. (1989). Oxygen distribution and electrophysiology of the cat retina at elevated intraocular pressure. PhD Thesis, Northwestern University, Evanston, IL, USA.