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Is the input to a GABAergic synapse the sole asymmetry in turtle's retinal directional selectivity?

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Abstract

We examined the effects of picrotoxin and pentylenetetrazol (PTZ) on the responses to motions of ON-OFF directionally selective (DS) ganglion cells of the turtle's retina. These drugs are antagonists of the inhibitory neurotransmitter GABA. For continuous motions, picrotoxin markedly reduced the overall directionality of the cells. In 21% of the cells, directional selectivity was *lost* regardless of speed and contrast. However, other cells *maintained* their preferred direction despite saturating concentrations of picrotoxin. And in most cells, loss, maintenance, or even *reversal* of preferred and null directions could occur as speed and contrast were modulated. In 50% of the cells, reversal of preferred and null directions occurred at some condition of visual stimuli. However, picrotoxin did not tend to alter the preferred-null axis for directional selectivity. For apparent motions, picrotoxin made motion facilitation, which normally occurs exclusively in preferred-direction responses, to become erratic and often occur during null-direction motions. Finally, PTZ had effects similar to picrotoxin but with less potency. The results in this paper indicated that models of directional selectivity based *solely* on a GABAergic implementation of Barlow and Levick's asymmetric-inhibition model do not apply to the turtle retina. Alternative models may comprise multiple directional mechanisms and/or a symmetric inhibitory one, but not asymmetric facilitation.

Keywords: Directional selectivity, Retinal ganglion cells, GABA, Preferred-direction facilitation, Null-direction inhibition

Introduction

Barlow and Levick (1965) hypothesized a spatially asymmetric inhibitory mechanism for directional selectivity in the rabbit retina. This mechanism would inhibit the responses of ON-OFF directionally selective (DS) ganglion cells to motions in a particular (null) direction, but not to motions in the opposite (preferred) direction (Barlow & Levick, 1965; Wyatt & Daw, 1975; Werblin et al., 1988; Amthor & Grzywacz, 1993*a*). Such inhibitory mechanism was consistent with directional selectivity in other species (see Amthor & Grzywacz, 1993*b*, for a review) and in particular, turtle (Ariel & Adolph, 1985). Pharmacological studies provided evidence that γ-aminobutyric acid (GABA) is probably the neurotransmitter mediating null-direction inhibition in rabbit (Caldwell et al., 1978; Ariel & Daw, 1982) and turtle (Ariel & Adolph, 1985). Picrotoxin, a specific antagonist of GABA, reduced directional selectivity. In contrast, strychnine, an antagonist of glycine, did not affect directionality.

If a GABAergic synapse with a spatially asymmetric input (receptive field) were the *only* asymmetry in the DS pathway, then one would expect that full blockade of GABA by antagonists would eliminate motion asymmetries from the responses of DS ganglion cells regardless of the parameters of the visual stimulus.

To test this hypothesis, we measured the responses of ON-OFF DS ganglion cells of turtle to continuously moving slits of varying contrast, speed, and direction, and to apparent motions. These measurements were made in control conditions and in the presence of two specific GABAergic antagonists, picrotoxin and pentylenetetrazol (PTZ). One concern in this study was to ensure that the concentrations of the drugs were sufficiently high to block GABAergic transmission as completely as possible. A previous study in rabbit (Caldwell et al., 1978) used low concentrations of picrotoxin (estimated at $\ll 16\ \mu M$ by the authors), which might explain why GABA blockade did not seem to eliminate directional selectivity fully. Another concern was to use sufficiently low stimulus contrasts. These contrasts would control for potential blockades of directional selectivity by GABA antagonists because of response saturation following inhibition's removal. The studies of speed dependence of the GABA antagonists' effect extended the range of speeds investigated by Ariel and Adolph (1985).

To study whether another process found in rabbit responses, namely, preferred-direction facilitation (Barlow & Levick, 1965; Grzywacz & Amthor, 1993) is also present in turtle and is affected by GABA antagonists, we used apparent motions. If an asymmetric GABAergic process were the only asymmetric pro-

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cess mediating directional selectivity, then this facilitation would not be consistently spatially asymmetric in the absence of GABAergic inhibition. In this work, we examined whether facilitation also exists in turtles and whether it remains asymmetric under GABAergic antagonists.

Parts of this report appeared previously in abstract form (Smith et al., 1991*a*,*b*).

Methods

Animal preparation and dissection

We used for these experiments 10–15 cm long turtles of the species *Pseudemys scripta elegans*. The turtles were dark adapted in a light-tight box for at least 8 h prior to an experiment. Decapitation, pithing, and dissection were then performed under infrared light (Borg-Graham & Grzywacz, 1990; see also Perlman et al., 1990). An eye was enucleated, hemisected on blotter paper, and the posterior half laid face down on the paper to drain for about 20 s. We then resectioned the posterior half with a cut perpendicular to the streak, narrowly skirting the optic disk. The piece resulting from this cut was one-third to one-fourth the width of the half-eye. Two partial cuts were made through the retina but not through the sclera to divide the piece into thirds. The retina was then flattened on a glass slide (the retina was encouraged to evert through a protrusion on this slide). We held the retina to the slide by clips with the partial cuts isolating the central retinal section from the damage done by the clips. The retina was then placed under an inverted stage holding a triangle of nitrocellulose filter paper (0.45 μm), approximately 5 mm wide at the base, with an approximately 2-mm-diameter hole in its center. The hole was lined up with the center third of the eyecup section and the filter paper lowered until it rested upon the retina. After 20–40 s, the paper was raised from the eye piece, taking the retina from the central section with it. We placed the paper and retina, with photoreceptor side down, under a nylon holder in a perfusion chamber. This chamber had a constant flow of superfusate at approximately 2 ml/min. The composition of the control superfusate was the same as that used by Ariel and Adolph (1985), including the bubbling with 5% CO_2/95% O_2 gas mixture. The GABAergic antagonists were added to the control solution as described later. Recordings were begun after at least 1.5 h wait to allow the retina to recover from dissection trauma.

In total, 81 eyes were used for these experiments, from which we recorded data on 382 ganglion cells. Of these, 56 (15%) were found to be ON-OFF DS, consistent with the percentage found by Granda and Fulbrook (1989), who recorded from a smaller sample of cells. Of these 56 cells, we recorded for a sufficiently long time to perform pharmacology on 52, 43 with picrotoxin, and nine with PTZ. These 52 cells provided the data for this report.

Apparatus

The retina was placed on the stage of a Zeiss UEM microscope. This microscope was fitted with an infrared camera and its light source was covered with an infrared filter to allow visualization of the retina without bleaching it. The stimuli were presented through a Tektronix 608 monitor controlled by a Picasso waveform generator (Innisfree, Cambridge, England). The stimuli were focused by a planospherical lens, by a half-silvered mirror in the illumination path, and by the microscope's condenser onto the photoreceptor layer of the retina. By taking into account these optical components, the maximal retinal illumination was 123 lx.

Extracellular electrodes (parylene coated, 2 MΩ and 5 MΩ, A-M Electrophysiology) accessed the ganglion cell layer through the hole in the filter paper, and the spikes were isolated through a window discriminator (WPI). An IBM PC running ASYST 3.0 (Keithley Metrabyte, Tauton, MA), recorded and analyzed the data, and controlled the stimuli through the Picasso waveform generator. Spikes were taken as response to a stimulus if they occurred between stimulus start and 500 ms after the stimulus end. These extra 500 ms ensured collection of all of the responses due to the stimulus, including any delayed ones. (Virtually no spontaneous activity exists in the cells studied.)

Throughout this paper, we use as a definition of contrast $C = 100\%\ (F - B)/B$, where F represents the foreground illumination and B the background illumination. (This definition is similar to the standard definition of contrast for sinusoidal stimuli, if one equates the denominator, B, with the mean illumination. This equality is approximately correct here and would be exactly correct if our slit were infinitesimally narrow.) The highest contrast used was labeled "infinite" when the background illumination coming from the monitor was set to zero. For all other contrasts, $B = 3$ lx and F set their value. Consequently, except for the infinite contrast, the influence of a change in the adaptation level could be excluded from consideration in this paper. Furthermore, we could exclude that the infinite contrast exerted less surround inhibition than the other contrasts, since from Bowling (1980) we estimated that there could not be an antagonist effect from the surround for background illuminations below about 6 lx. (And as it will be seen in Results, there were no peculiar features in the infinite contrast data, that is, their results were "continuous" with the results of the other contrasts.) The background illumination from sources other than the monitor were below the resolution of our photometer (Minolta CS100 Colorimeter), that is, below 0.16 lx.

Identification of ON-OFF DS ganglion cells

Light-responsive cells were found by flashing a search stimulus (400-μm square flashing at 0.9 Hz) while moving the electrode down into the retina. When a cell was found, it was tested for ON-OFF responses by a 0.25-Hz, 500-μm square flashing stimulus (10 repetitions). The background activity of the cell was measured by an equal period without stimulus. If both the ON and OFF responses to this stimulus were not statistically significantly greater than the background activity of the cell at the 5% level, the cell was discarded from this study. The receptive field of the cell was then mapped by a 150-μm square flashing twice at 0.6 Hz centered at each point of a 6×6 grid. (The total stimulated area was $900 \times 900\ \mu m^2$). The stimulus center was then shifted by eye to the center of the cell's receptive field. This controlled for potential false directionality due to motions that sweep the receptive field asymmetrically. Some cells were discarded at this point if their receptive field could not be properly mapped because of excessive noise, or if their receptive-field centers were outside the spatial range of the stimuli.

The directional tuning curve of the cell was then measured by a moving slit stimulus (200 μm $\times$ 600 μm, infinite contrast—see explanation in "Apparatus"—and moving at 800 μm/s, pausing for 1 s between runs). This stimulus was moved across the

receptive field once in each direction. This procedure was repeated in 10-deg increments, 180 times, so that response in each direction of motion was tested 10 times over the course of the experiment. (In our arbitrary convention, the direction of motion was 0 deg for rightward motion, regardless of whether the eye was the right or the left one. Also, in this convention, the direction increased counterclockwise.) We accepted a cell as directional if the responses to two adjacent directions of motion were statistically significantly larger than the responses to their two diametrically opposed motions (*t*-test, 18 degrees of freedom, $P < 0.01$). The probability of incorrectly accepting as directional an isotropic cell, that is, one whose responses to all directions of motion obey the same distribution, was 0.0018. (This definition of directional selectivity was the only definition used for this property in this paper. Later, we describe other measurements of motion asymmetry, but we used them only to compare the properties of the DS cells with and without GABAergic antagonists.) The preferred direction was taken as the median of the largest set of consecutive directions showing this opposing-motion asymmetry and the null direction as the opposite of the preferred direction.

Pharmacology

Choosing a narrow range of concentrations could bias the responses observed in the presence of a drug. It could produce either weak effects, because the concentrations are too low, or strong, irreversible (and potentially unspecified) effects, because the concentrations are too high. Therefore, we decided to apply a broad range of increasing concentrations of GABAergic antagonists to the DS cells. We arbitrarily began with 25 μM, 50 μM, and 100 μM concentrations of picrotoxin, and later increased to 50 μM, 100 μM, and 200 μM. These different concentrations were premixed and then exchanged for the control solution. In each experiment, once a cell was found to be DS and all other tests under control conditions completed, the lowest concentration of picrotoxin was applied to the preparation. After 2 min to allow flow of picrotoxin into the bath, a flashing stimulus (500-μm square, flashing at 0.95 Hz, infinite contrast) was presented to the cell until the response reached a plateau. To determine when this happened, the computer inspected responses to nonoverlapping sets of 120 flashes. For each such set, the computer compared the response to the nth flash to the response to the $(n + 60)$th flash. This produced a number indicating whether the later of the two responses was the greatest. This number was compared to a binomial distribution in which there was a 60% chance of a greater later response at each comparison. If the probability of this number or lower numbers was less than 0.05, we assumed that the effect of the drug reached its plateau. Otherwise, we kept delivering to the cell new sets of 120 flashes until it reached plateau. Reaching the plateau generally took between 4 and 20 min. The cell was then tested by a slit moving along the preferred-null axis. If the cell was significantly DS ($P < 0.01$), the concentration was successively increased until either (1) the directionality was blocked, or the preferred and null directions reversed; or (2) the maximum concentration was reached. (This method effectively underestimated the number of cells for which a reversal occurs. If it were possible to perform the tests with a continuous ramp of concentrations, then the transition between the original preferred direction and a reversal would likely include a concentration for which a blockade occurs. In this case, we would stop at that concentration and would possibly not see the reversal. Therefore, it is possible that some of the cells for which we see blockade could display reversal if tested at an higher concentration.) At this point, tests to be performed under pharmacological conditions were conducted; the mean and standard deviations for the concentrations used during the tests were 90 ± 50 μM. After all tests were complete, the superfusate was returned to control conditions (using the above plateau test, but with a 60% chance of a lesser later response), and the cell tested for directionality. Return to control was successful for the majority of cells, often taking as long as 1 h.

For nine cells, PTZ was used to confirm that the effects seen were not picrotoxin specific. Such confirmation was possible, since the mode and site of action of PTZ was different from that of picrotoxin. While the former blocked GABA release (Feldman & Quenzer, 1984), the latter blocked the coupling between the GABA receptor and the Cl^- channel (Olsen, 1981, 1982). In the PTZ experiments, steps of 0.5 mM, 1 mM, and 2 mM were used. The mean and standard deviations for the concentrations used during the tests were 1.3 ± 0.7 mM.

Preferred-null runs

Most tests involved a slit, moving across the receptive field along the preferred-null axis; we refer to these as "Preferred-Null Runs." In these tests, the slit was swept across 1000 μm of retinal surface, first in the null, then in the preferred direction. This range of sweep was larger than the extent of the excitatory portion of the cell's receptive field, which typically was around 500 μm. Motion was stopped for 1 s at the end of each sweep. The stimulus was presented 11 times both in the preferred and null directions for each set of parameters. The results of the first sweep were discarded to minimize transient contributions.

We studied seven of the 43 cells tested with picrotoxin only with infinite contrast, 800 μm/s motions. (This speed was in the middle of the range of speeds eliciting strong directional selectivity in control conditions.) In 14 of the other 36 cells, response to variation in contrast (at 800 μm/s and generally using steps of infinite contrast, 4000%, 2000%, 1000%, 500%, and 250%) and speed (at infinite contrast and generally using steps of 200 μm/s, 400 μm/s, 800 μm/s, 1600 μm/s, 3200 μm/s, 6400 μm/s, 12,800 μm/s) were also explored. Of these 14 cells, we stimulated one with the contrast series, three with the speed series, and 10 with both series. (More cells than these 14 were tested with the speed and contrast series, but the entire data were ignored if any of the contrast or speed conditions showed a statistically significant decrease of response upon addition of picrotoxin.)

Apparent-motion stimuli

An apparent-motion stimulus is one in which two stationary slits, at some distance from each other, are presented simulating a motion. We used a two-slit stimulus, where the first slit was not removed until after the response to the second slit has been measured. Each presentation of our stimuli consisted of the first slit alone, the second slit alone, the second slit then the first (null-direction apparent motion), and the first slit then the second (preferred-direction apparent motion). There was a delay of 1.25 s between each of these presentations, which were repeated 30 times for each parameter value. We explored varia-

tions in contrast, inter-slit distance, and delay, and in the shape of the slits.

Data analysis: The directional-selectivity index

To summarize the effect of GABAergic antagonists on the responses along the preferred-null axis, we computed the directional-selectivity index (Grzywacz & Koch, 1987). If the preferred-direction response is P and the null-direction response is N, then this index is $(P - N)/(P + N)$. It is such that if $P > N$, then the index is positive; if $P = N$, then the index is zero; and if $P < N$, then the index is negative. The maximal and minimal values that this index can reach are 1 and -1, respectively, being in this sense normalized. Moreover, the index increases as the ratio between the preferred and null responses increases. Hence, this index quantifies directionality and allows for a quantification of reversals of preferred and null directions through negative numbers.

Data analysis: The directionality statistic

To study the effect of GABAergic antagonists on the angle of the preferred direction, we computed a parameter we call the directionality statistic (also used by Sernagor & Grzywacz, 1995). From the directional tuning curve described in "Identification of ON-OFF DS Ganglion Cells," we found the average responses (R_i) of a cell to stimuli moving in each direction (θ_i), and the standard errors (se_i) of these responses. We defined $\mathbf{R}_i$ as vectors with magnitude R_i and direction θ_i, and took their vector sum ($\mathbf{D} = \sum_i \mathbf{R}_i$). This statistic was in the mean direction of the directional tuning curve and we refer to this direction as the statistical preferred direction. We were interested to obtain a measure of the uncertainty of the magnitude of this statistic, $u(|\mathbf{D}|)$, so that we could compute $|\mathbf{D}|/u(|\mathbf{D}|)$ as a measure of the certainty of the cell's asymmetry in the direction of $\mathbf{D}$. We called this measure the "Directionality Statistic" and plot it in Figure 7 against the angle of $\mathbf{D}$, and in Figure 8 against the difference in angle of $\mathbf{D}$ between control and picrotoxin conditions.

To find $u(|\mathbf{D}|)$, we measured the standard errors in the estimate of $\mathbf{D}$ along each axis, through:

$$se_x^2 = \sum_i se_i^2 \cos^2 \theta_i$$

$$se_y^2 = \sum_i se_i^2 \sin^2 \theta_i \qquad (1)$$

This formula held, since the variance of a sum of random independent variables is the sum of the variances of these variables. (Independence resulted from the variables being measured at sufficiently different instants in time—see "Identification of ON-OFF DS Ganglion Cells." In other words, because the measurements were separated in time, from the response to one direction of motion, one could not predict whether the response to another direction was above or below that response's mean.) In a similar fashion to eqn. (1), we computed the correlation between the vertical and horizontal components of $\mathbf{D}$ as

$$\rho_{xy} = \frac{\sum_i se_i^2 \cos(\theta_i)\sin(\theta_i)}{se_x se_y} \qquad (2)$$

Because $\mathbf{D}$ was the mean of a large sample of random independent two-dimensional variables, its distribution was to a good approximation Gaussian. From Johnson and Wichern (1992), this distribution is

$$N(\mathbf{D}) = \frac{1}{2\pi se_x se_y (1-\rho_{xy}^2)^{1/2}} \times e^{-\frac{1}{2(1-\rho_{xy}^2)}\left[\left(\frac{x-D_x}{se_x}\right)^2+\left(\frac{y-D_y}{se_y}\right)^2-2\rho_{xy}\left(\frac{x-D_x}{se_x}\right)\left(\frac{y-D_y}{se_y}\right)\right]}$$

where D_x and D_y are the horizontal and vertical components of $\mathbf{D}$ respectively. To examine the uncertainty in the magnitude of $\mathbf{D}$, we calculated the values of the normal distribution restricted to the direction of $\mathbf{D}$, which we called θ_D. These values defined a one-dimensional function whose independent variable was denoted r (the distance from the origin). Because $D_x = |\mathbf{D}|\cos\theta_D$, $D_y = |\mathbf{D}|\sin\theta_D$, $x = r\cos(\theta_D)$, and $y = r\sin(\theta_D)$, we got

$$p(r) = \frac{1}{2\pi se_x se_y (1-\rho_{xy}^2)^{1/2}} \times e^{-\frac{se_y^2\cos^2(\theta_D)+se_x^2\sin^2(\theta_D)-2\rho_{xy}se_x se_y\cos(\theta_D)\sin(\theta_D)}{2se_x^2 se_y^2(1-\rho_{xy}^2)}(r-|\mathbf{D}|)^2}$$

After normalization, this became the formula for a normal distribution in r, and the standard deviation, which therefore was the uncertainty of our estimate of $|\mathbf{D}|$, was:

$$u(|\mathbf{D}|) = \left(\frac{se_x^2 se_y^2(1-\rho_{xy}^2)}{se_y^2\cos^2(\theta_D)+se_x^2\sin^2(\theta_D)-2\rho_{xy}se_x se_y\cos(\theta_D)\sin(\theta_D)}\right)^{1/2} \qquad (3)$$

Results

Basic histograms

The data reported in this paper were collected from extracellular recordings from ON-OFF DS ganglion cells of the turtle retina. We obtained the majority of the data from experiments we call preferred-null runs. These experiments started by determining the preferred and null directions of the cell as described in "Methods." Then, in the preferred-null runs, a narrow slit of light first moved in the preferred and then in the null direction. This back-and-forth motion was repeated several times, and the histogram (or its temporal integral) of the times of occurrence of spikes obtained. The protocol then repeated the procedure under GABAergic antagonists. Fig. 1 illustrates histograms obtained with this protocol for one cell with and without 50 μM picrotoxin.

In the absence of picrotoxin, the total number of extracellular spikes (223) elicited during preferred-direction motions was higher than the number of spikes (86) elicited during null-direction motions. This difference indicates that this ganglion cell was DS. Furthermore, this was an ON-OFF cell, as shown by the two bursts of spikes that started during the preferred-direction motion at about 70 ms and 530 ms after the motion onset. After application of picrotoxin, the total number of spikes increased for both the preferred- and the null-direction motions, as would be expected by the elimination of an inhibitory mechanism. In this particular cell, and under these particular stimulus conditions, the total number of spikes elicited during preferred- and null-direction motions became similar (346 and

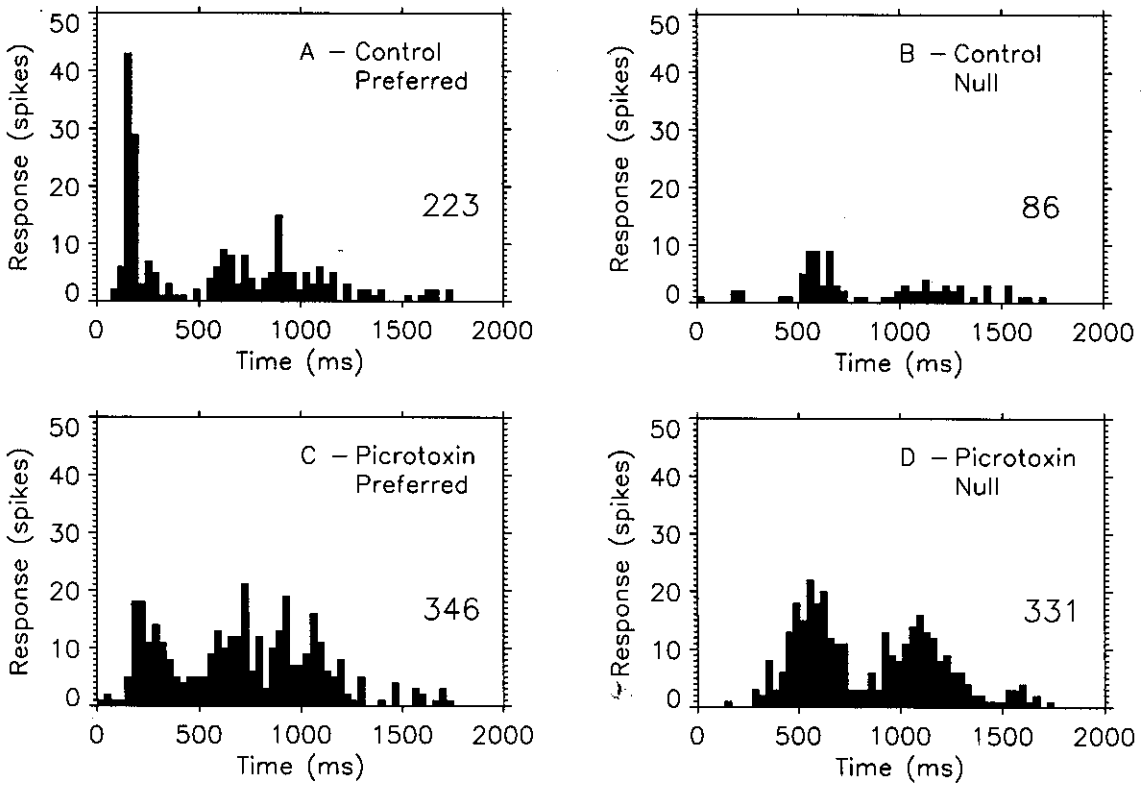

Fig. 1. Response histograms for a slit moving along the preferred-null axis. The histograms for the control condition (without picrotoxin) appear in A and B. The histograms for the condition with 50 μM picrotoxin in the superfusate appear in C and D. In all cases, we computed the histograms from the response to a 200 μm × 600 μm slit moving perpendicularly to its long side at 800μm/s. The slit's contrast was infinite (see explanation in "Methods"). The slit swept across 1000 μm of the retinal surface crossing the center of the receptive field. Time zero in the histograms corresponds to the onset of the motion and the histogram's bins are 33 ms wide. The numbers printed on the right side of the histograms correspond to their integrals, that is, the total number of spikes. The effect of picrotoxin was to increase the total number of spikes and, in this cell, to block directional selectivity.

331, respectively). (The apparent reduction of the initial transient response in this cell did not occur in other cells.) This indicated a blockade of directional selectivity, following a blockade of GABAergic receptors.

Another set of basic measurements did not focus on the preferred-null axis, but rather on several motion axes. In these measurements, we recorded responses to motions in 36 directions, 10 deg apart, over several cycles. Histograms were built from the total number of spikes in all of the cycles. Examples of these histograms for a cell superfused with control solution and with a solution containing 100 μM picrotoxin appear in Fig. 2. Each histogram in this figure combines the data of three adjacent directions for purposes of avoiding clutter and improving the signal-to-noise ratio. For further ease of visualization, we included at the center of each set of histograms a normalized polar plot with radiuses corresponding to the integrals of the histograms.

In this example, although the picrotoxin reduced the sharpness of the directional tuning, the cell remained significantly DS and roughly kept its preferred direction. Before application of the drug, the direction eliciting the strongest response (preferred direction) was roughly at 60 deg and the direction eliciting the weakest response (null direction) was roughly 240 deg. After application of the drug, the responses became larger and the preferred direction shifted to 30 deg from the rightward direction. Later in the paper, we will quantify this type of shift and show that preferred directions tend to remain close to the preferred-null axis.

Blockade, maintenance, and reversal of preferred and null directions

We sought to determine whether the maintenance of preferred direction observed in cells like the one in Fig. 2 was because the concentration of picrotoxin was not sufficient to block GABA-activated channels fully. To control for this possibility, we measured the integral of the responses to preferred-null runs (Fig. 1) divided by the motion's duration, as a function of picrotoxin concentration. (This division normalized the response to be per unit of time of stimulation, that is, per unit of time that the slit spent in the receptive field, thus removing the effect of stimulus duration on the strength of the response.) This concentration was increased until one of three conditions held: (1) Directional selectivity was statistically significantly blocked; (2) The preferred and null directions were statistically significantly reversed; and (3) The picrotoxin concentration reached the highest concentration investigated (see "Methods" for more detail). In these concentration experiments, bright slits on a

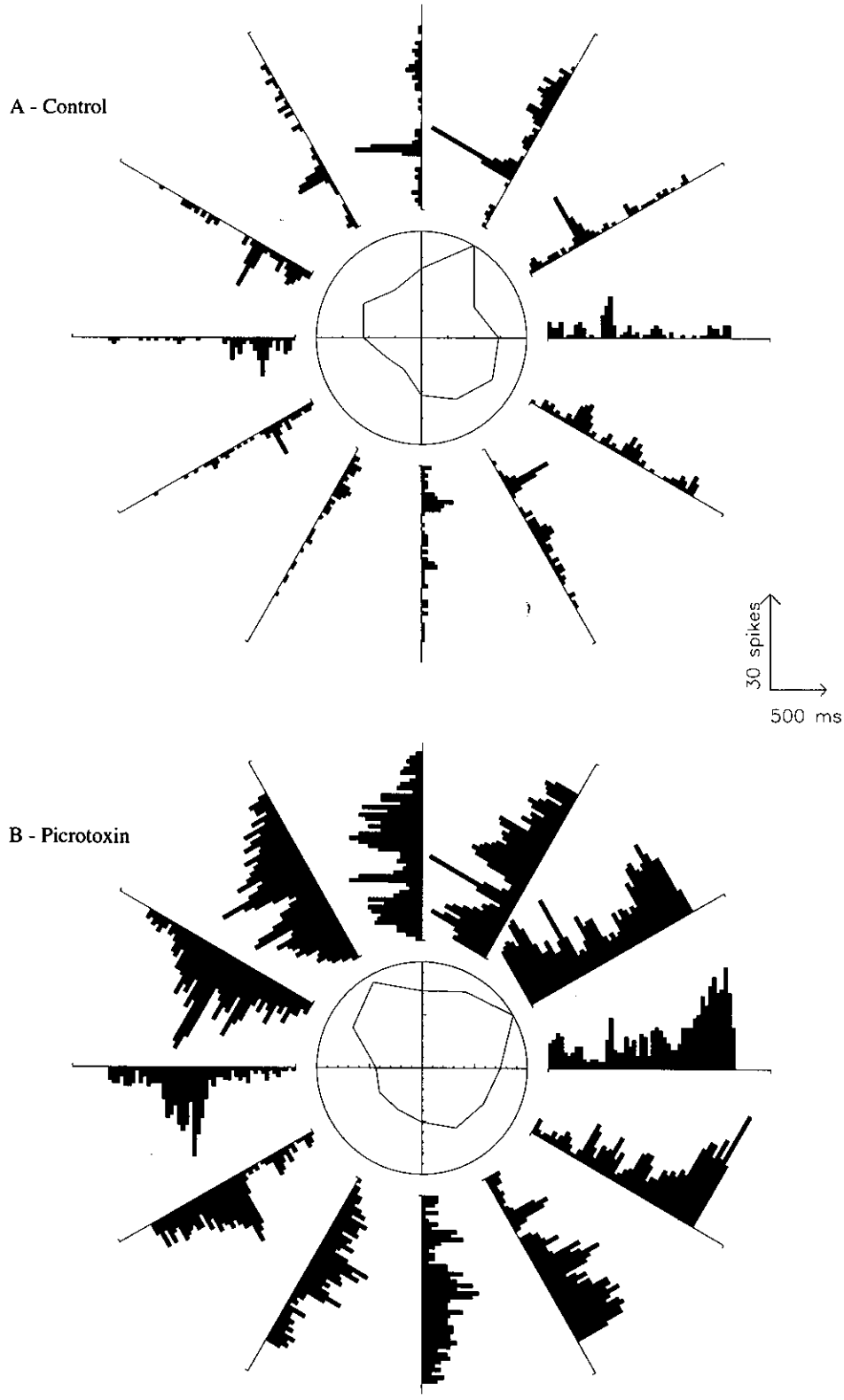

Fig. 2. Response histograms under control and pharmacological conditions for movement at different directions through the receptive field. A, B: The responses to motion with no picrotoxin and 100 μM picrotoxin in the superfusate, respectively. Each displayed histogram is the sum of three response histograms taken at 10-deg increments and is oriented at the median angle of the three. The motions used to obtain these histograms were similar to those in Fig. 1, except that here we used multiple directions of motion. The histograms represent 1.75 s of data collection and each bin holds data collected for 35 ms. At the center of A and B are the normalized polar plots of the integral of the histograms versus direction of motion. For this cell, although the responses to the null direction increased, directional selectivity was maintained as evidenced by the polar plots. The original preferred-null axis did not shift by more than 30 deg.

black background moved at 800 or 470 μm/s. Fig. 3 shows the results of this experiment for three cells.

For the cell in Fig. 3A, addition of 25 μM of picrotoxin was sufficient to block directional selectivity completely. Removal of the drug led to the return of the normal directional selectivity in about 45 min. In contrast, an addition of 200 μM of the drug was not sufficient to block directionality of the cell in Fig. 3B, even though the responses increased dramatically. That the effect of the drug was saturated is indicated by the constancy of the response for concentrations above 50 μM. Thus, it cannot be claimed that the maintenance of preferred direction observed here was due to insufficient concentration of the drug. But perhaps the most surprising result in these experiments was the reversal of preferred and null directions observed in some cells such as that in Fig. 3C. In 50% of the cells, reversal occurred under at least one condition of visual stimulation (see "Contrast and speed dependencies" below). Such reversal induced by GABAergic antagonists was reported before in DS cells of fly (Bülthoff & Bülthoff, 1987) and was evident (but not mentioned by the authors) in some of the histograms of Ariel and Daw (1982) and Ariel and Adolph (1985).

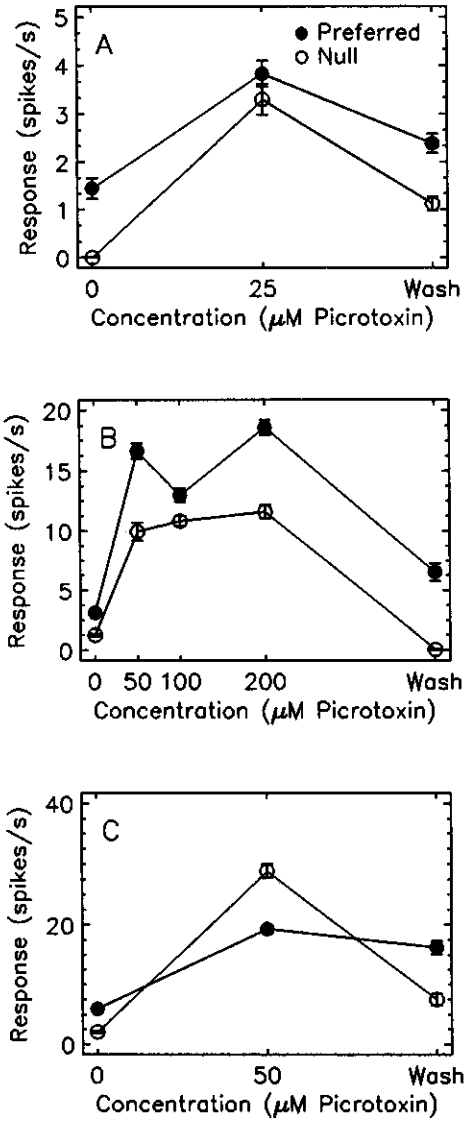

Fig. 3. Examples of cellular activity as a function of concentration of picrotoxin. A: An example of blockade of directional selectivity. B, C: Examples in which the preferred and null directions were maintained and reversed, respectively. For each figure, the stimulus was a moving slit with infinite contrast, 200 μm × 600 μm, sweeping through 1000 μm of the retinal surface along the preferred-null axis. For B and C, the stimulus moved at 800 μm/s, while it moved at 470 μm/s for A. The filled circles represent response to motion in the preferred direction and the empty circles response to motion in the null direction. The error bars stand for standard error of the response. The response data labeled "wash" correspond to a return to 0 μM picrotoxin.

Fig. 4 summarizes the results obtained with the highest concentrations of picrotoxin in experiments similar to the one illustrated in Fig. 3. The summary, based on 43 cells, uses the directional-selectivity index (see "Methods"). This index runs from −1 to 1 and assumes negative values when the null response becomes larger than the preferred response, and positive values in the opposite case.

The picrotoxin-induced shifts towards zero directional-selectivity indices in Fig. 4 show that GABAergic antagonists

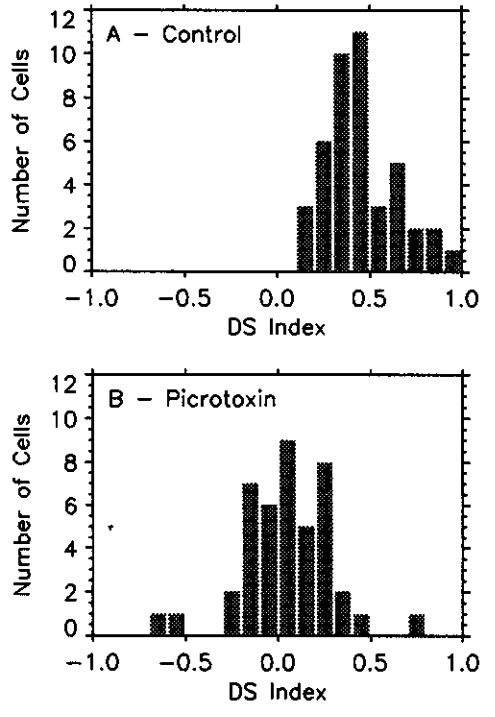

Fig. 4. Population histograms of directional selectivity indices of cells. A, B: The conditions without and with picrotoxin in the superfusate, respectively. The concentration of picrotoxin for the cells shown in B varied; in each case, it was the final pharmacological concentration as described in "Methods." For each figure, the directional-selectivity index was computed by the formula $DS = (P - N)/(P + N)$ (see "Methods"). Positive values of the directional-selectivity index correspond to responses to the control preferred direction that were stronger than those to the null direction. On the other hand, negative values correspond to stronger responses to the null direction. The absolute value of the index increases with directionality. Each bin in this figure has the width of 0.1 of the directional-selectivity index. All indices were derived from the response to stimuli similar to those in Fig. 1. Picrotoxin reduced the directionality of the cells, as evidenced by the shift towards zero index, but could not block directional selectivity in all of them, even reversing the preferred and null directions in some cells.

affected directional selectivity adversely. Out of the 43 cells, 37 (86%) showed a decrease in the absolute value of the directional-selectivity index, whereas six showed the opposite effect. The mean absolute values of the directional-selectivity index without and with picrotoxin were 0.456 ± 0.030 (standard error) and 0.185 ± 0.024, respectively. (There was no statistically significant correlation between the directional-selectivity indices in these two conditions.) However, the adverse effect of the drug was not simply a blockade of directional selectivity. In Fig. 4B, reversal of preferred direction was readily evident in cells with directional-selectivity indices smaller than −0.5, whereas maintenance of preferred direction was evident in cells with indices around 0.5 and higher. (One should not interpret the value 0.5 used in these arguments as our criterion for directional selectivity. Our criterion was statistical as explained in "Methods." But the value of 0.5 indicated a strong directionality with the ratio between the preferred and null responses being equal to three.) In a statistical analysis of the responses to the specific visual condition of infinite contrast and 800 μm/s speed, 24 (56%) of the 43 cells tested with picrotoxin showed blockade, ten (23%) showed maintenance, and nine (20%) showed reversal. However, true blockades over all stimuli are less common than these numbers indicate, since, as shown in the next section, only 21% of the cells showed blockade over the full range of contrasts and speeds tested. For the ease of reference, Table 1 summarizes these results and those of the other experiments in this paper.

Contrast and speed dependencies

The initial motivation to study the contrast dependence of the effects of GABAergic antagonists on directional selectivity was to control for the possibility that response saturation mediated blockade of directionality. Because blockade of GABA removes an inhibitory drive from the retinal network, neural responses to both preferred and null direction increase. If this increase is sufficiently large, then some retinal process might saturate, making the final preferred and null responses similar. However,

Table 1. *Summary of the effects of GABAergic antagonists on ON-OFF directional selectivity*[a]

	Control	Picrotoxin	Pentylenetetrazol
Standard motion: C = ∞, S = 800 μm/s	52	43	9
Maintenance	–	10 (23%)	6 (67%)
Blockade	–	24 (56%)	2 (22%)
Reversal	–	9 (21%)	1 (11%)
Speed modulations	13	13	–
Maintenance	–	3 (23%)	–
Blockade	–	4 (31%)	–
Reversal	–	6 (46%)	–
Contrast modulations	11	11	–
Maintenance	–	1 (9%)	–
Blockade	–	6 (55%)	–
Reversal	–	4 (36%)	–
Overall speed and contrast	**14**	**14**	–
Maintenance	–	**4 (29%)**	–
Blockade	–	**3 (21%)**	–
Reversal	–	**7 (50%)**	–
Shifts of preferred direction	25	16	9
<45 deg of control preferred direction	–	10 (63%)	7 (78%)
<45 deg of preferred-null axis	–	12 (75%)	8 (89%)
>45 deg of preferred-null axis	–	3 (19%)	1 (11%)
Insignificant directionality statistic	–	1 (6%)	0 (0%)
Preferred direction apparent motion	6	6	–
Facilitation	5 (83%)	3 (50%)	–
Inhibition	0 (0%)	1 (17%)	–
Insignificant	1 (17%)	2 (33%)	–
Null-direction apparent motion	6	6	–
Facilitation	0 (0%)	4 (67%)	–
Inhibition	5 (83%)	1 (17%)	–
Insignificant	1 (17%)	1 (17%)	–

[a]Double horizontal lines separate different experiments. Single horizontal lines separate conditions within these experiments. The first row within each experiment and condition gives the total number of cells. The other rows give the breakdown of these cells in the various experimental categories. The numbers in parentheses correspond to the percentage of cells calculated from this breakdown. Columns 2, 3, and 4 list the number of cells tested under control, picrotoxin, and pentylenetetrazol, respectively. In the contrast and speed experiments, the reversal category contained all cells which exhibited a statistically significant reversal of preferred and null directions for one or more conditions of visual stimulation. In the same experiments, the blockade category contained all cells which lost directionality at all conditions of visual stimulation. C = contrast and S = speed.

if one uses low-contrast stimuli, the retinal signals become smaller, avoiding saturation. Consequently, if blockade of directional selectivity by GABAergic antagonists were due to saturation in an artifactual manner, then one would expect that at low contrasts directional selectivity would be restored. To test this prediction, we measured the integral of the responses to preferred-null runs (as in Fig. 1) divided by the motion's duration of slits moving at 800 μm/s at various contrasts. The contrast-dependence plots of Fig. 5 show that the prediction that low-contrast stimuli could restore directional selectivity blocked at high contrasts does not hold.

The picrotoxin-induced dependence of preferred and null responses on contrast turned out to be very complex and cell specific. Of the 11 cells for which full contrast dependence was measured with and without picrotoxin, six (55%) showed a blockade of directional selectivity independent of contrast as in Fig. 5A. (Many of these cells did not show blockade when tested at other speeds, thus explaining the 21% overall blockade figure mentioned earlier—more on this below.) For one of the cells (9%), the original preferred direction was maintained at intermediate contrasts, but blocked at the highest contrast (Fig. 5B). Three other cells (27%) responded with reversal at intermediate contrasts and maintenance at high contrasts (for example, Fig. 5C). One cell (9%) showed the opposite phenomenon, that is, maintenance at intermediate contrasts and reversal at the highest contrast (Fig. 5D). The only case we could account for with the saturation explanation provided above is the single cell in Fig. 5B.

Besides contrast, we also varied speed to study its influence on the effects of GABAergic antagonists on directional selectivity. As the contrast experiments, the speed experiments were also of the preferred-null-runs type (Fig. 1). However, in the speed experiments, the contrast was always infinity (bright slit on black background). We measured from the speed data the integral of the response divided by the motion's duration for speeds ranging from 200–12,800 μm/s. Fig. 6 illustrates the types of behavior observed in the speed data.

We determined the speed dependence of picrotoxin effects in 13 cells. Picrotoxin strongly affected their speed tuning and the effects showed a complexity similar to that observed in the contrast data. At 200 μm/s, directional selectivity was never statistically significant both in the presence and absence of the drugs. At the highest speed tested (12,800 μm/s), directional selectivity appeared in 10 (77%) of the cells with picrotoxin and in three (23%) of the cells without picrotoxin. At intermediate speeds, one could roughly classify the cells' behavior into four categories: In the first category, there were four cells (31%) such as that in Fig. 6A for which the blockade of directional selectivity was independent of speed. For another three cells (23%), the preferred direction was maintained at intermediate speeds (Fig. 6B) and in two of these cases, at the highest speed tested. In a third category, three cells (23%) responded with reversal

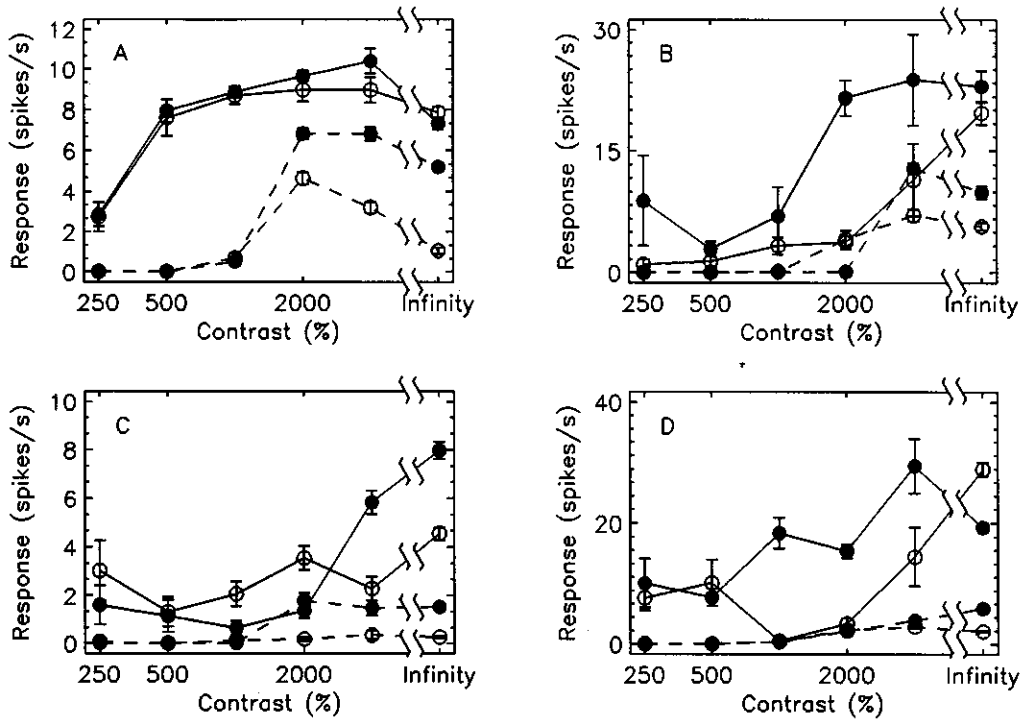

Fig. 5. Examples of cellular activity as a function of contrast. The effects of picrotoxin fell basically into four categories: A: Blockade of directionality at all contrasts. B: Predominant maintenance of preferred and null directions. C: Crossover from reversal of preferred and null directions at low contrasts to maintenance at high contrasts. D: Crossover from maintenance at low contrasts to reversal at high contrasts. The concentrations of picrotoxin for the four examples shown were (A) 100 μM, (B) 200 μM, (C) 200 μM, and (D) 50 μM. Control results are shown by dotted lines and results obtained in the presence of picrotoxin by solid lines. Solid and open circles represent responses to the preferred and null direction, respectively. The stimuli eliciting these responses were similar to those in Fig. 1 except for the contrast variation. Each point, except for the value shown for infinite contrast, represents the average of the responses to a single preferred-null run as described in "Methods." For the infinite-contrast response, several preferred-null runs were averaged to reduce noise.

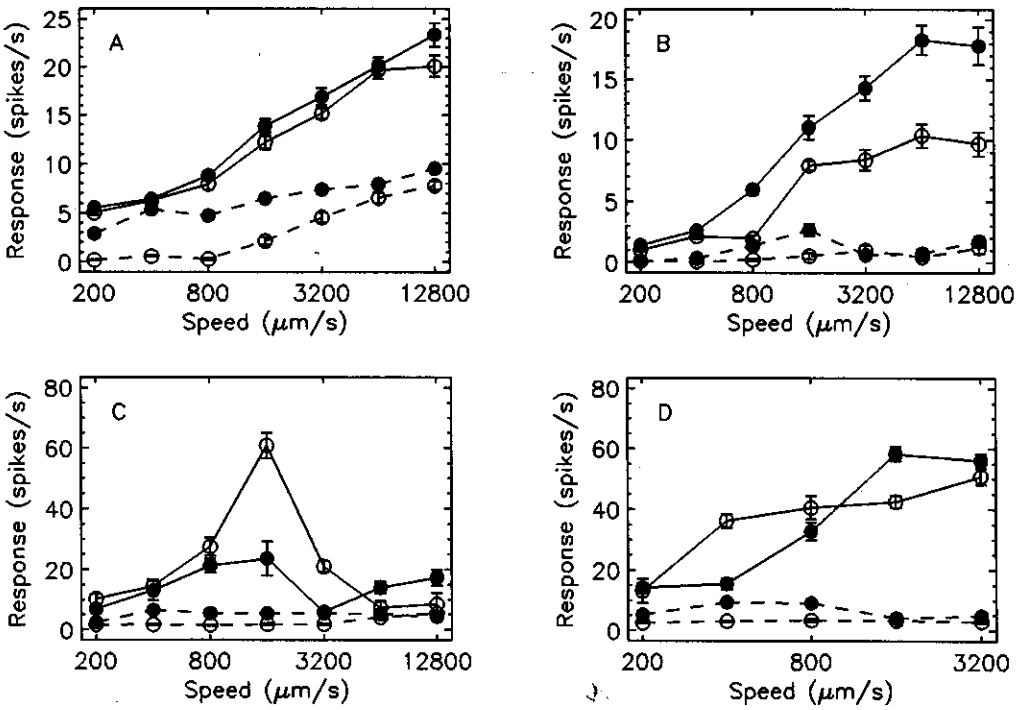

Fig. 6. Examples of cellular activity as a function of speed. The effects of picrotoxin fell basically into four categories: A: Blockade of directionality at all speeds. B: Predominant maintenance of preferred and null directions. C: Predominant reversal of preferred and null directions. D: Crossover from reversal at low speeds to maintenance at high speeds. The lines and symbols have the same meaning as in Fig. 5. The stimuli eliciting these responses were similar to those in Fig. 1 except for the speed variation.

at speeds ranging between 400 and 3200 μm/s. Finally, in three cells (23%), there was a crossover from reversal at speeds ranging from 400–1600 μm/s to maintenance at speeds ranging from 3200–12,800 μm/s.

Overall, we studied contrast and speed dependence of picrotoxin effects in 14 cells (see "Methods"). Of these 14 cells, only in three (21%) was directional selectivity blocked independently of contrast and speed. In seven cells (50%), the preferred and null directions reversed significantly in at least one condition of visual stimulation. The other four cells (29%) showed maintenance of the original preferred direction, despite strong effects of picrotoxin on the overall responsiveness of the cells.

Shifts of preferred directions

Because the above experiments indicated possible reversals of preferred and null directions, we wondered whether sometimes GABAergic antagonists change the preferred direction to axes other than the original preferred-null axis. To examine this issue, we performed experiments of the type illustrated in Fig. 2 with and without GABAergic antagonists. These experiments were always performed at infinite contrast (bright slit on black background) and with a speed of 800 μm/s. From the results of these experiments, we built polar plots with the integral of the responses divided by the motion's duration as the radius and the direction of motion as the angle (Fig. 2). These polar plots were used to compute a statistical preferred direction (the mean direction of the directional tuning curve). In addition, we computed a statistic indicating the confidence on the computed preferred direction (the directionality statistic, see eqns. (1), (2), and (3) in "Methods"). Fig. 7 shows an example of these computations for one cell, with the direction of arrows indicating the computed preferred direction and the length of the arrow indicating the directionality statistic.

A large, significant shift in preferred direction emerged from the data used in Fig. 7. The directional tuning with no picrotoxin was relatively sharp, resulting in a large arrow 3 deg counterclockwise from rightward direction. The directional tuning with 100 μM picrotoxin was less sharp but statistically significantly asymmetric. It resulted in a smaller arrow with direction 198 deg. Therefore, this calculation indicated a statistically significant shift of preferred direction of 195 deg, which was consistent with a reversal of preferred and null directions.

Fig. 8 shows in a polar plot the behavior of 16 cells for which we measured the preferred-direction shifts due to picrotoxin. This figure was built for each cell from calculations as the one illustrated in Fig. 7. The total directional shift was the difference in angles between the preferred directions before and after application of the drug. For example, this shift was 195 deg for the cell in Fig. 7. The shift appears in Fig. 8 as the polar plot angle of each point with zero shift arbitrarily defined as the rightward direction. The distance of each point from the origin is the directionality statistic of the response under picrotoxin. The small dashed circle is the limit of statistical significance at the 1% level. Therefore, even after the application of the drug, 15 out of 16 cells (94%) showed statistically significant directionality under this new test of directionality. (No cells showed an increase in their directionality statistics with picrotoxin. The figure's dashed outer circle, whose radius is the average direc-

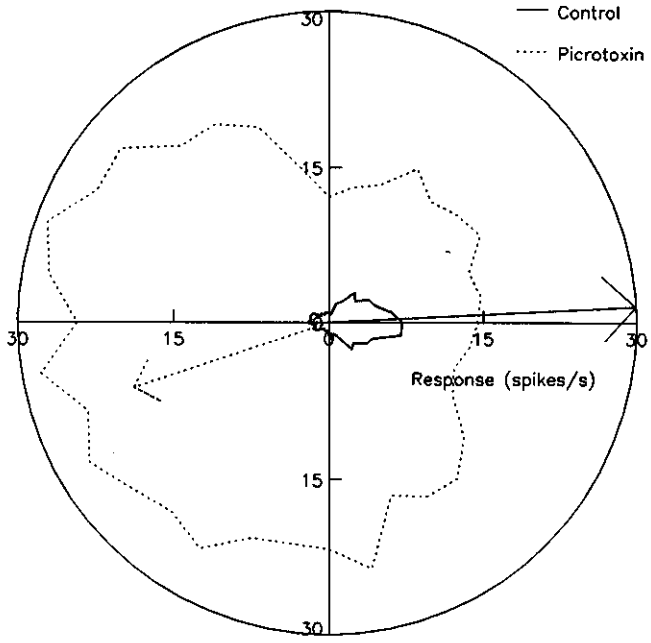

Fig. 7. Example of directional tuning curve and directionality statistic, with and without picrotoxin. The polar plots are the mean responses of the cell as a function of direction of motion (gathered as in Fig. 2). Responses without picrotoxin are represented by the solid plot, while responses with 100 μM picrotoxin by the dotted plot. From these plots, we computed the solid and dotted arrows, respectively. The lengths of the arrows are proportional to the directionality statistics and the angles of the arrows are the mean directions of motion of the directional tuning curves. The directionality statistic is a dimensionless variable corresponding, in units of standard errors, to our confidence of the degree of directionality of the cell (see "Methods"). The dotted and the solid arrows were 9.9 and 15.0 standard errors in length, respectively. Hence, for this cell, the arrows essentially indicate a statistically significant directionality both with and without picrotoxin, and a reversal of preferred and null directions.

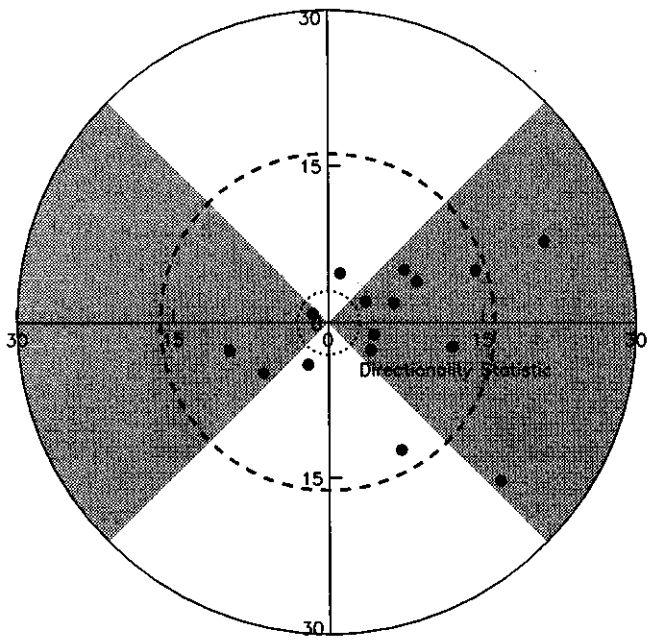

Fig. 8. Shifts of preferred direction due to picrotoxin. The different points represent data obtained from different cells (16 cells). The concentration of picrotoxin for each point varied, but was in each case the final concentration as described in "Methods." The angle of each point was the subtraction of the angle of the picrotoxin arrow of Fig. 7 from the angle of the control arrow of the same figure. Therefore, the angles of the points correspond to the shifts in the preferred direction of motion. In this figure, the 0-deg shift is the rightward direction. Also, increasing shifts of the points are represented in an anticlockwise manner. The distance of each point from the origin corresponds to the directionality statistic in the picrotoxin condition (see Fig. 7). The inner dashed circle is set at a distance such that if this directionality statistic falls outside the circle, then the probability that the directionality happened by chance is smaller than 0.01. Thus, the data show that 94% of the cells maintain directionality in the presence of picrotoxin. (No cells showed an increase in directionality or their directionality statistics with picrotoxin. The outer dashed circle, whose diameter is the average directionality statistic of the cells without picrotoxin, shows that picrotoxin tends to reduce the directionality statistic.) Moreover, as indicated by the shadowed area, 81% of the cells have their preferred direction in the presence of picrotoxin within 45 deg of the original preferred-null axis.

tionality statistic of the cells without picrotoxin, shows that picrotoxin tends to reduce the directionality statistic.)

For 10 out of the 16 cells (63%), the directional shift was less than 45 deg of the rightward direction (right shaded area). Given their relatively wide directional tuning under normal and picrotoxin conditions (Figs. 2 and 7), such small directional shifts indicated a tendency of the cell to maintain their preferred direction. Overall, 12 cells (75%) had statistically significant preferred directions within 45 deg of the preferred-null axis under picrotoxin (shaded area). Thus, cells tended to maintain their original preferred-null axis under this drug. However, not all cells did so, as three cells (19%) fell out of the shaded area.

Facilitation

The shifts in preferred direction, the blockades of directional selectivity in preferred-null runs, and the reversals of preferred and null directions argue against the mechanism of preferred-direction facilitation being spatially asymmetric in a consistent manner in the absence of inhibition. To test this prediction, we stimulated six cells with apparent motions in the presence and absence of picrotoxin. In these apparent-motion protocols, two slits of light appeared in two positions of the receptive field. Each slit was first presented in isolation and then in two temporal sequences simulating preferred- and null-direction apparent motions. In all cases, the slits on the field were not briefly flashed, but sustained until the end of the data recording (500 ms after the onset of the second slit—as in Amthor & Grzywacz, 1991, 1993*a*). This two-slit procedure was repeated 30 times and the average number of spikes computed for each condition. The results for one cell with and without 50 μM picrotoxin appear in Fig. 9.

This figure demonstrates for the first time the existence of preferred-direction facilitation in turtle and that this facilitation is not consistently asymmetric in the absence of inhibition. To demonstrate facilitation, we first summed the responses to the first and second slits (bin labeled *combined* in Fig. 9). This sum was then statistically compared to the total response to the preferred-direction motion. In the control condition (Fig. 9A), the apparent motion elicited more spikes than the sum, demonstrating preferred-direction facilitation. In contrast, for the

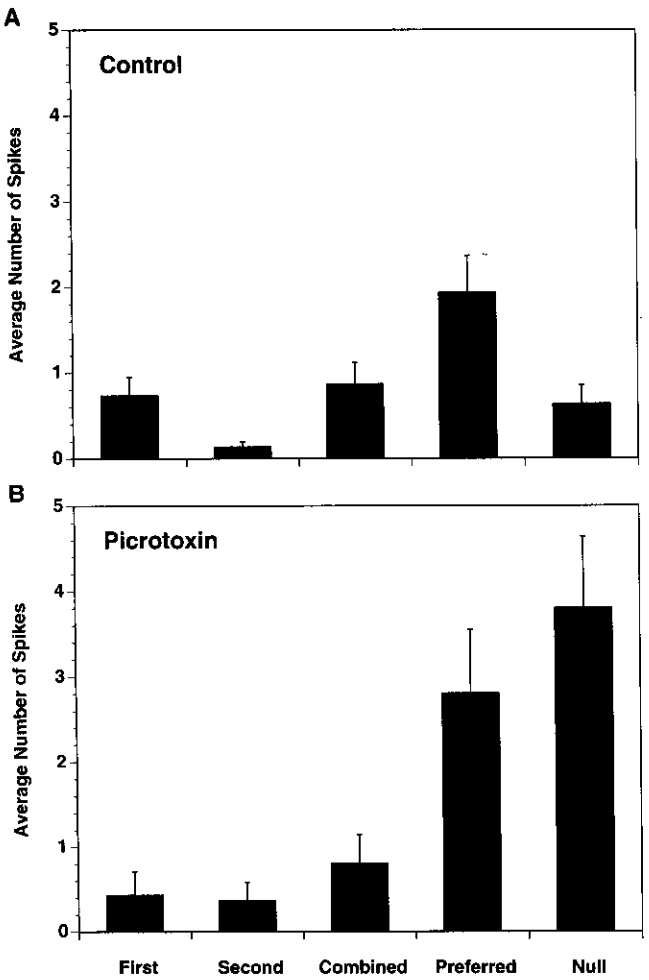

Fig. 9. Example of two-slit apparent-motion responses with and without picrotoxin. A, B: The responses in the control conditions, and with 50 μM picrotoxin in the superfusate, respectively. Each bar represents the averaged responses to 30 presentations of the labeled stimulus. The bars labeled "First" and "Second" correspond to the first and second slits of the apparent motion being delivered individually to the receptive field. The bar labeled "Combined" is the sum of the bars labeled "First" and "Second." The bars labeled "Preferred" and "Null" correspond to the response elicited by the apparent motions in the preferred and null directions, respectively. For this figure, the responses were to slits of 100 μm $\times$ 500 μm, 6400% contrast, and with a spatial separation of 50 μm. During the apparent motion the slits had a temporal separation of 100 ms. The apparent-motion data revealed preferred-direction facilitation ("Preferred" bar larger than "Combined" bar) and null-direction inhibition ("Null" bar smaller than "Combined" bar) in the control condition. However, in the presence of picrotoxin, this cell showed both preferred and null-direction facilitation.

null-direction motion, the response to the motion was smaller than the sum, indicating inhibition (Fig. 9A – not statistically significant for this cell, but significant for all of the other cells tested – Table 1). The preferred-direction motion still elicited facilitation under picrotoxin (Fig. 9B). However, the null-direction motion now elicited facilitation instead of inhibition, with this facilitation slightly surpassing that of the preferred-direction motion (not statistically significant). Although the result illustrated in Fig. 9B is the most typical, over the six cells tested with the apparent-motion protocol, the apparent-motion behavior was erratic under picrotoxin (Table 1), with results showing facilitation and inhibition for both directions. These results contrast with the no-picrotoxin condition, for which null-direction facilitation and preferred-direction inhibition were never observed.

Pentylenetetrazol

To control for actions of picrotoxin not specific to GABA receptors, we investigated whether the effects of this drug on preferred direction could be replicated with another GABAergic antagonist. We used pentylenetetrazol (PTZ), a GABAergic antagonist that is widely used in pharmacological and clinically related studies (Nicoll & Padjen, 1976; Kalichman, 1982; Allan & Harris, 1986). With PTZ, we repeated the concentration and shift-of-directional axis experiments previously performed with picrotoxin (Figs. 3 and 8). The results for both experiments were similar to those obtained with picrotoxin (Table 1), except that PTZ was less potent than picrotoxin, consistent with previous studies of the action of PTZ on GABAergic transmission (Simmonds, 1982). Fig. 10 illustrates the results of two concentration data sets.

Consistent with its role as a GABAergic antagonist and with the role of GABA on directional selectivity, PTZ increased responses and blocked directionality in the cell of Fig. 10A. After removal of the drug, the original directionality returned (statistically significantly) in approximately 30 min. In contrast, for the cell in Fig. 10B, PTZ could not block directionality, resulting in the maintenance of preferred direction despite high concentrations of the drug (2 mM). Of the nine cells tested with 2 mM PTZ, six (67%) showed maintenance, two (33%) showed blockade, and one (11%) showed reversal of preferred and null directions when tested with preferred-null runs (bright slits moving on a black background at 800 μm/s). These results demonstrated that PTZ has effects similar to picrotoxin but with less potency, since with similar stimulus conditions, only 23% of the cells showed maintenance under picrotoxin.

Discussion

Summary

In summary, saturating concentrations of GABAergic antagonists did not always eliminate directional selectivity from ON-OFF DS ganglion cells of the turtle retina. Rather, these drugs had complex effects (see Table 1 for a summary of the results), which included changes of preferred direction. In only 21% of the cells (see bolded entries in Table 1), directional selectivity disappeared regardless of speed and contrast. However, other cells maintained their preferred direction. And in most cells, loss, maintenance, or reversal of preferred and null directions could occur as speed and contrast were modulated. In particular, when stimulated with high contrasts, the cells typically had their directionality blocked (Figs. 3A, 4, 5A, 5B, and 10A) or their preferred direction maintained (Figs. 8 and 10B). In contrast, with relatively slow stimuli moving along the preferred-null axis, the preferred and null direction often reversed (Figs. 6C and 6D). At higher speeds, the normal preferred direction tended to be maintained (Figs. 6B and 6D). This complex dependence on speed and contrast reflected itself in apparent-motion protocols. For them, facilitation, which normally only appears in preferred-direction motions, became erratic under picrotoxin (Table 1)

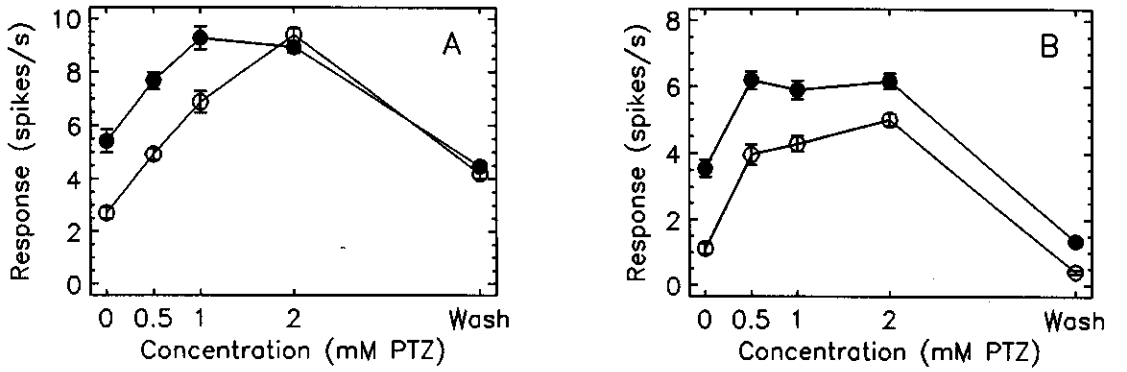

Fig. 10. Examples of cellular activity as a function of concentration of PTZ. A, B: Examples of blockade of directionality and maintenance of preferred and null directions, respectively. The stimuli were identical to those in Fig. 1, and the symbols and labels are identical to those in Fig. 3.

and could also occur during null-direction motions (Fig. 9B). Finally, despite the complexity of the motion responses, picrotoxin did not tend to alter the preferred-null axis for directional selectivity (Fig. 8).

What does such complex data teach us about GABA's role for directional selectivity?

The data reveal a wide range of effects of GABAergic antagonists on DS cells. This is not surprising, considering the complex circuitry in the inner retina (Dowling, 1987; Vaney, 1990) and that GABA is one of the dominant neurotransmitters there (Vaney, 1990; Criswell & Brandon, 1992). In addition, GABA may be released by horizontal cells in the outer retina (Lam & Steinman, 1971; Tachibana & Kaneko, 1984; Criswell & Brandon, 1992). Consequently, it is not clear at a first sight whether the multiple effects of GABAergic antagonists reflect differences in circuitry subserving different DS cells and/or differences in the effectiveness of these antagonists on different synapses. Nevertheless, although effects unrelated to directional selectivity probably occur, we now argue that the data have strong implications for directional selectivity.

That about 21% of the cells had their directionality blocked despite varying contrasts and speeds suggests that some cells' directional symmetry may depend exclusively on a GABAergic synapse with an asymmetric input (receptive field). However, the other 79% did not appear to depend solely on such a mechanism. For them, directional selectivity did not disappear and in 50% of all cells (bolded entries in Table 1), there was a reversal of preferred direction. Could it be that, in 79% of the cells, accidental wiring unrelated to directional selectivity gave rise to artifactual directionality? This is unlikely, since the majority of the cells tended to maintain their preferred-null axis (Fig. 8). If the remaining directionally were artifactual, then the new preferred direction should have been independent of the preferred-null axis. Hence, even after blocking GABAergic inhibition, asymmetries with axes essentially parallel to the preferred-null axis remained.

Our data thus argue against a GABAergic implementation of the asymmetric-inhibition model of Barlow and Levick (1965) being the *only* mechanism of retinal directional selectivity.

But this conclusion does not mean that GABA is unimportant for directional selectivity. On the contrary, about 21% of the cells may depend exclusively on an asymmetric GABAergic mechanism. And even those cells that do not seem to depend exclusively on GABA can only maintain their preferred direction for a relatively small range of contrasts and speeds under GABAergic antagonists. Consequently, GABAergic action seems crucial for retinal directional selectivity. But do the effects of GABAergic antagonists reflect an action of GABA on the DS pathway or a saturation of excitatory pathways in the retina?

In the "Introduction," we were concerned that the previously observed effects of GABAergic antagonists (Caldwell et al., 1978; Ariel & Daw, 1982; Ariel & Adolph, 1985) resulted from saturation in some retinal process. The reasoning was that without a major inhibitory mechanism, retinal responses would become too large. In this case, preferred and null responses could become similar even though GABA might not have a direct role in directional selectivity. But if so, one would not expect reversals of preferred direction. Moreover, one would expect that low contrasts would take the retinal signal out of saturation, restoring normal preferred direction. However, it was observed that in many cells, reducing contrast had the opposite effect, namely, a reversal of preferred and null directions (Fig. 5C). Hence, the effects of the GABAergic antagonists were not just due to saturation, and probably reflect a direct GABAergic action on DS pathways.

Several separate mechanisms of directional selectivity?

GABAergic antagonists seem to block directional selectivity in some cells (≈21%) regardless of contrast and speed, but do not do so in the other cells (≈79%). This suggests that there are *at least* two separate mechanisms of directional selectivity: One that is exclusively dependent on an asymmetric GABAergic mechanism and the other which is not. Could it be that there are more than two mechanisms? In support of this possibility is the observation that DS ganglion cells of turtle have a wide variety of dendritic tree stratifications (Jensen & DeVoe, 1983). Moreover, several types of retinal cells other than the ganglion cells might possess some directional selectivity (Adolph, 1988; DeVoe et al., 1989). If there were many mechanisms of directional selectivity, this could help to account for part of the complex behavior we observe under GABAergic antagonists. In this case, it would be important to find out whether DS ganglion cells exist in a continuum or a discrete number of subtypes.

Against asymmetric facilitation as a mechanism of retinal directional selectivity

If a GABAergic synapse with an asymmetric input is not the *only* mechanism of retinal directional selectivity, then what could other mechanisms of directional selectivity be?

One possibility would be an asymmetric facilitatory mechanism. In rabbit, Grzywacz and Amthor (1993) expanded earlier experiments by Barlow and Levick (1965) to find that if the spatio-temporal parameters of apparent motions are appropriate, then preferred-direction facilitation is as strong as null-direction inhibition. In this paper, we report that turtle also exhibits preferred-direction facilitation. If the facilitatory process were asymmetric, then it would implement a mechanism of directional selectivity similar to the Reichardt model (Fig. 11A). This model contrasts with that of Barlow and Levick, which uses an asymmetric inhibitory mechanism (Fig. 11B).

Our data show that an asymmetric facilitatory mechanism is probably not directly fundamental for turtle directional selectivity. This is because under picrotoxin, facilitation becomes erratic, often occurring during null-direction motions and sometimes being stronger for the null than for the preferred direction. Furthermore, these results do not even support an extension of the Barlow and Levick model, including both asymmetric inhibitory and asymmetric facilitatory mechanisms (Fig. 11C).

Symmetric-inhibition mechanism of directional selectivity

Because an asymmetric GABAergic mechanism appears not to be the *only* mechanism of directional selectivity, and because an asymmetric facilitatory mechanism can be ruled out, we must look for other mechanisms. Such a mechanism should account for much of the data's complexities. In particular, it should explain null-direction facilitation and the reversal of preferred direction.

Recently, models for retinal directional selectivity based on nonasymmetric inhibitory mechanisms were advanced (Vaney,

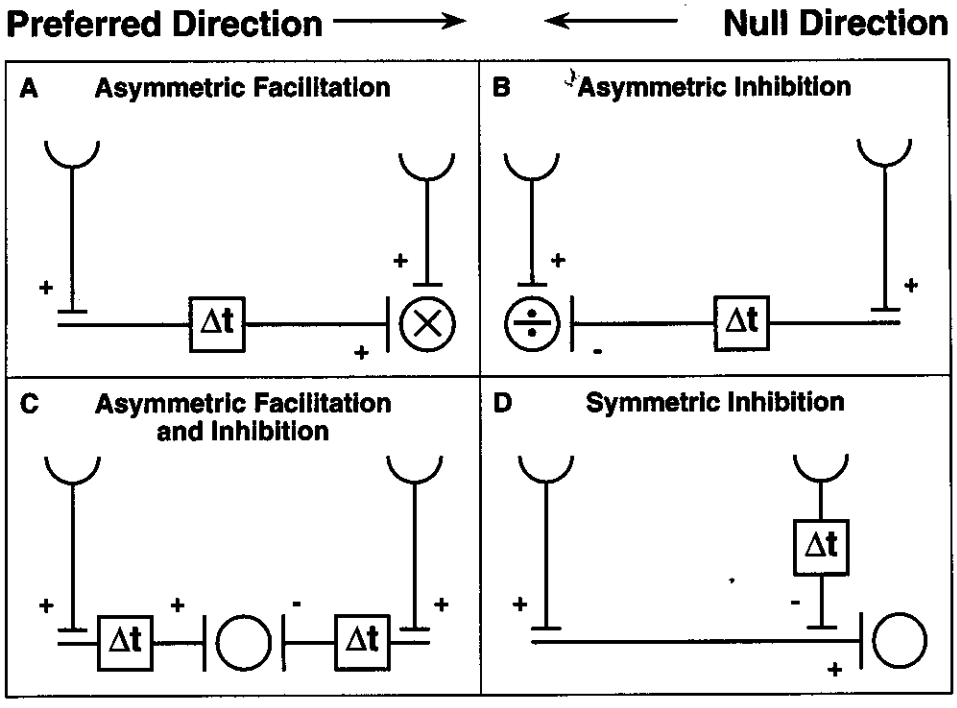

Fig. 11. Models of retinal directional selectivity. A: The asymmetric facilitation model is based on the Reichardt model (for a review see Poggio & Reichardt, 1976). It postulates that for preferred-direction motions, but not for null-direction motions, an asymmetric facilitatory signal is sufficiently slowed down to compensate for the delay of activation of spatially separated inputs. The temporal coincidence for the preferred direction would then be exploited by a multiplication-like or expansive interaction. (Reichardt and his colleagues used exact multiplication or second-order nonlinearities, but their model was computational and not physiological.) This facilitatory temporal-coincidence model was ruled out for the rabbit retina by Barlow and Levick (1965). B: The asymmetric inhibition model was first proposed by Barlow and Levick for the rabbit retina. It postulates that for null-direction motions, but not for preferred-direction motions, an asymmetric inhibitory signal is sufficiently slowed down to compensate for the delay of activation of spatially separated inputs. The temporal coincidence for the null direction would then be exploited by a veto mechanism, such as a division-like shunting inhibition (Torre & Poggio, 1978). Our data argue against an exclusive GABAergic implementation of this model, because, among other findings, blockade of GABAergic inhibition can cause the reversal of preferred and null directions. C: The asymmetric facilitation and inhibition model combines the asymmetries of both models above. Our data argue against a GABAergic implementation of this model, since blockade of GABAergic inhibition does not reveal an asymmetric facilitation. D: The symmetric-inhibition model is a hybrid between the Reichardt (Fig. 11A) and the Barlow and Levick (Fig. 11B) models. This hybrid model is similar to the Reichardt model in that the underlying spatial asymmetry is excitatory. However, the hybrid shares with the Barlow and Levick model the nonlinearity underlying the computation, namely, a veto-like inhibitory mechanism. We argue that the symmetric-inhibition model is consistent with some of the complex data presented in this paper.

1990; Borg-Graham & Grzywacz, 1992). One of these models appears schematically in Fig. 11D. The model is similar to the Reichardt model (Fig. 11A) in that the spatial asymmetry is excitatory (the inhibitory mechanism thus being symmetric*). However, different than that model, the nonlinear mechanism mediating directional selectivity is inhibitory, as in the Barlow and Levick model, and of the shunting type (Torre & Poggio, 1978), consistent with rabbit data (Amthor & Grzywacz, 1991).

An implementation in ganglion cells of a-symmetric-inhibition model as the one in Fig. 11D has been discussed elsewhere (Koch et al., 1982). However, recent data in turtle argued against an (exclusive) implementation of mechanisms of directional selectivity in ganglion cells (Borg-Graham & Grzywacz, 1991, 1992). Whole-cell patch recordings that eliminated direct inhibitory input onto the ganglion cells did not eliminate directional selectivity. Hence, one could rule out an implementation of Fig. 11D's scheme in ganglion cells of turtles. Accordingly, Borg-Graham and Grzywacz (1992) suggested that a key inhibition acts on an amacrine cell's dendrite. Vaney (1990; see also Poznanski, 1992), based on his anatomical findings and those of others (Famiglietti, 1991; Masland et al., 1984; Brandon, 1987; Amthor et al., 1989), suggested that in rabbit, dendrites of the cholinergic starburst amacrine cell could implement a model like the one in Fig. 11D (see Guiloff & Kolb, 1992, for similar evidence in turtle). In support of this idea is the finding that this cell's dendrites have GABA receptors (Brandstatter et al., 1995—but see Linn & Massey, 1992).

It is possible to show that symmetric-inhibition models can account for some of the complex features of the data under GABAergic antagonists. This is because these drugs do not eliminate the basic asymmetry. Computer simulations with models like the one in Fig. 11D have replicated some of the complexity of the data reported in this paper. These simulations demonstrated reversal of preferred direction at a synapse at the end of the asymmetric process (Ögmen, 1991; Borg-Graham & Grzywacz, 1992). In addition, if that synapse can undergo synaptic facilitation (Katz & Miledi, 1968; Parnas & Parnas, 1986), then the model predicts null-direction facilitation as in Fig. 9 (Grzywacz et al., 1993).

GABA receptors

There are at least three types of GABA receptors in the retina, $GABA_A$, $GABA_B$, and $GABA_C$ (Friedman & Redburn, 1990; Feigenspan et al., 1993; Qian & Dowling, 1993; Lukasiewicz & Werblin, 1994). Pan and Slaughter (1991) reported that $GABA_B$ receptors contribute to directional responses in tiger salamander. Baclofen, a $GABA_B$ agonist, enhanced normal directional responses in some DS cells and induced DS responses in 30% of the cells that are not normally directional. However, strychnine (a glycinergic antagonist) and picrotoxin together, but not in isolation, blocked directionality. Hence, the salamander's directionality seemed to be based on glycine and GABA-activated Cl^- channels (picrotoxin blocks the coupling between GABA receptors and the Cl^- channel—Olsen, 1981, 1982). (In this regard, the salamander's directionality is different from that of turtle and rabbit, since glycine does not contribute to directional selectivity in the latter two animals—Caldwell et al., 1978; Ariel & Adolph, 1985.) As a result, $GABA_B$ receptors did not seem to participate in the salamander's directionality directly, since their action does not involve Cl^- channels (Newberry & Nicoll, 1984, 1985). For turtle, the picrotoxin's effects reported here probably did not result from $GABA_B$ receptors. This is because they are insensitive to this antagonist. In turn, $GABA_C$ receptors could be affected by our antagonists, since these receptors are antagonized by picrotoxin (Qian & Dowling, 1993; Lukasiewicz & Werblin, 1994) and should be antagonized by PTZ, which is a GABA-release blocker (Feldman & Quenzer, 1984). However, bicuculline's effect on directional selectivity of turtle is very much like that of picrotoxin (Ariel & Adolph, 1985). This suggests that $GABA_C$ receptors do not contribute significantly to retinal directional selectivity in turtle, since they are bicuculline-insensitive (Feigenspan et al., 1993; Qian & Dowling, 1993; Lukasiewicz & Werblin, 1994).

Therefore, it is likely that the effects reported here are mostly due to blockade of $GABA_A$ receptors. More pharmacological studies are necessary to confirm this conclusion.

*This statement merits further discussion, since one can argue that the inhibitory pathway is also asymmetric in the sense that it is asymmetrically displaced relative to the excitatory pathway. This argument would be correct and would be basically the explanation for the existence of null, but not preferred, direction inhibition. But what we mean by excitatory asymmetry and inhibitory symmetry has to do with the functional anatomy of the implementation of the model in Fig. 11 in the retina. With a few exceptions, receptive fields in the retina obey a "receptor-topic" organization in the sense that they are roughly concentric with the associated anatomical process. However, in the model of Fig. 11D, the second synapse in the excitatory pathway (that is, the right excitatory synapse) has an asymmetrically displaced receptive field (the receptive field is to the left of the synapse). On the other hand, this displacement is inexistent for the inhibitory synapse and therefore, its receptive field is symmetric relative to the synapse.

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