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PHARMACOLOGICAL ANALYSIS OF DIRECTIONALLY SENSITIVE RABBIT RETINAL GANGLION CELLS

BY M. ARIEL* AND N. W. DAW

From the Department of Physiology and Biophysics, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110, U.S.A.

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SUMMARY

1. Cholinergic drugs were infused into the retinal circulation of the rabbit while we analysed the receptive field properties of directionally sensitive retinal ganglion cells. Physostigmine eliminated the trigger feature, directional specificity, of both types (on-centre and on–off) of these cells. In this respect the action of physostigmine (an ACh potentiator) was very like that of picrotoxin (a GABA antagonist). Therefore, a detailed analysis of the receptive field properties of directionally sensitive ganglion cells was made to analyse the effects of physostigmine and picrotoxin.

2. Size specificity and radial grating inhibition were not abolished by physostigmine, but were often affected by picrotoxin. The optimal velocity in the preferred direction (as measured by maximum firing frequency) was not much changed by physostigmine, but was higher during infusion of picrotoxin. Infusion of nicotine, a depolarizing ACh agonist which increases the activity of retinal ganglion cells, revealed the presence of inhibition to movement in the null direction. The null direction response during picrotoxin started slightly later than this inhibition. The null direction response during physostigmine was weaker and started later still. Mecamylamine and dihydro-β-erythroidine, nicrotinic receptor antagonists, totally blocked the effect of physostigmine and reduced the control light response by about half.

3. From this analysis, it appears that on–off ACh release onto directionally sensitive cells provides a substantial excitation which, when potentiated by physostigmine, overcomes or outlasts the null direction GABA inhibition within the receptive field. The spatial extent of GABA inhibition is asymmetric to and larger than the spatial extent of ACh excitation. Similar pathways appear to be involved in both the on-centre and on–off directionally sensitive ganglion cells, yet the on-centre cell pathway may receive an additional input which suppresses the ACh excitation at light offset. Possible schemes for the cellular mechanism of directional sensitivity are discussed in light of these results and recent anatomical and pharmacological findings.

* Present address: Biology Department, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, U.S.A.

INTRODUCTION

Directionally sensitive (d.s.) neurones respond best to movement in one direction ('preferred'), but have little or no response to movement in the opposite ('null') direction (Barlow & Hill, 1963). The receptive field properties of d.s. rabbit retinal ganglion cells were described in detail by Barlow, Hill & Levick (1964). These cells respond to small spots flashed within their receptive field with either an on–off or on-centre response. Spontaneous activity (if any) is suppressed during movement in the null direction, except at slow speeds. Responses are velocity-sensitive; spots moving very rapidly produce a very brief or no response whereas very slowly moving spots cause a response to movement in both directions. Responses are also size-specific; large stimuli produce less response than small ones.

The inhibition found during movement in the null direction suggested to Barlow & Levick (1965) that this is the fundamental mechanism of directional sensitivity for the rabbit retina. They showed that directional sensitivity is mainly a result of the lateral spread of inhibition in an asymmetric fashion, so that it blocks excitation which subsequently arrives on one side of it, but not on the other. This finding was supported by the observation that antagonists to the inhibitory transmitter gamma-aminobutyric acid (GABA) abolish directional sensitivity (Wyatt & Daw, 1976; Caldwell, Daw & Wyatt, 1978).

We have studied the receptive field properties of d.s. ganglion cells during the infusion of synaptic drugs into the retinal circulation. Physostigmine, an acetylcholinesterase inhibitor, and picrotoxin, a GABA receptor antagonist, abolish the directional sensitivity of these cells similarly. This is a difficult result to fit into our generalizations about the action of cholinergic cells in the rabbit retina (see Ariel & Daw, 1982). How can a drug which increases the effectiveness of the excitatory transmitter acetylcholine (ACh) abolish a trigger feature produced by null direction inhibition? The effect of physostigmine on d.s. neurones thus seems contrary to its effects on most other ganglion cells in the rabbit retina, for which trigger features were not abolished. The effects of cholinergic drugs and picrotoxin on d.s. ganglion cell responses were therefore compared in order to elucidate the synaptic pathways underlying the mechanism of directional sensitivity.

METHODS

Refer to Ariel & Daw (1982) for the general preparation and procedures. For the initial analysis of each d.s. cell, a standard stimulus was used to demonstrate directional sensitivity optimally. It was a white bar about 10 times brighter than the background, moving back and forth along the preferred/null axis. The bar was narrower than the receptive field and long enough to evoke separate leading and trailing edge responses. The bar moved at approximately 20 deg/sec for on–off d.s. cells and 2 deg/sec for on-centre d.s. cells. Using this standard stimulus, we compared the results of physostigmine and picrotoxin on different d.s. cells in different animals or different cells in the same animal.

This study used a sample of 100 on–off d.s. cells and thirty-two on-centre d.s. cells into which physostigmine was infused. The comparison of physostigmine and picrotoxin on the same cell involved a sample of thirty-two on–off d.s. cells and five on-centre d.s. cells. The abolition of directional sensitivity by picrotoxin has been observed for some 200 d.s. cells in total in this laboratory.

Statistical analyses were made from this sample by measuring the magnitude and duration of

each cell's response from peristimulus time histograms which represented the summed activity from four identical stimulus presentations. In the case of on–off d.s. cells, these histograms were generated in less than 25 sec so that several histograms could be recorded during each drug infusion. Stimuli for on-centre d.s. cells moved more slowly than for on–off cells. Therefore, 2 min or more were required for each histogram and so only one or two histograms were generated for each drug infusion. Histograms showing the maximal drug effect were used for the statistical analysis.

Due to the large variability in the response magnitude and duration for different ganglion cells, all statistics were performed by measuring the significance of the difference between the control response and the drug-affected response for each ganglion cell in the sample. The sample was much smaller for control responses in the null direction which could only be measured in a few cases. We also measured the significance of the difference between the response during physostigmine and the response during picrotoxin for each on–off d.s. ganglion cell for which both drugs were tested. Maximum firing frequency was defined as the maximum number of spikes in a single bin divided by the bin width (10 msec for on–off d.s. cells and about 50 msec for on-centre d.s. cells). The difference in response onset was computed as the difference in the bin number in which the first spike occurred multiplied by the bin width. Response onset usually was easy to identify in d.s. cells since their spontaneous activity is almost negligible.

The dose at which directional sensitivity was abolished by physostigmine was compared to that of picrotoxin. This comparison was made for d.s. neurones for all rabbits, for d.s. neurones in the same rabbit and even for the same d.s. neurone. Although we found that the effective concentration of both picrotoxin and physostigmine varied with different rabbit preparations, the effective molar concentration of picrotoxin was consistently five times the effective concentration of physostigmine within the same preparation. This ratio was found in more than eighty rabbits. This 5:1 ratio was also found when both drugs were tested on the same d.s. ganglion cell (thirty-seven times).

RESULTS

Drug effects on directional sensitivity

Infusion of physostigmine into the retinal circulation abolished the directional sensitivity of both the on-centre and on–off d.s. ganglion cells of the rabbit retina (Fig. 1). The loss of this trigger feature was often preceded by a transient increase in spontaneous activity followed by a loss of spontaneous activity. The abolition of directional sensitivity occurred using stimuli which evoked an optimal response when moving in the preferred direction, yet little or no response when moving in the null direction. The preferred direction responses were also increased by physostigmine.

Using long white bars which evoked separate leading and trailing edge stimulation, a difference was seen between the on-centre d.s. cell type and the on–off d.s. type. The on–off d.s. cell normally responds to both the leading and trailing edges. During infusion of physostigmine, the trailing edge response was affected sooner and more dramatically than the leading edge, in both the preferred and null directions. On the other hand, the on-centre d.s. neurone normally responds only to the leading edge. During physostigmine, there was a response to the trailing edge as well as the leading edge. The largest response was still to the leading edge in the preferred direction and the smallest response was during the movement of the leading edge in the null direction. The converse was true of long black bars; the largest response was to the trailing edge in the preferred direction and the smallest response was to the trailing edge in the null direction. Responses of on-centre d.s. cells to the trailing edge of a white moving stimulus is consistent with the effects of physostigmine on sluggish cells using stationary stimuli. On-centre d.s. ganglion cells become 'on–off' to flashed spots and annuli during infusion of physostigmine (see Ariel & Daw, 1982).

On–off d.s. ganglion cells

The mechanism by which directional sensitivity was abolished by physostigmine was enigmatic since directional sensitivity has been shown to be a result of null direction inhibition (Barlow & Levick, 1965). We first considered whether the abolition of directional sensitivity was simply a result of depolarization of the d.s. ganglion cell membrane, since one of the effects of physostigmine may be such a depolarization (Masland & Ames, 1976; see also Ariel & Daw, 1982). If so, nicotine

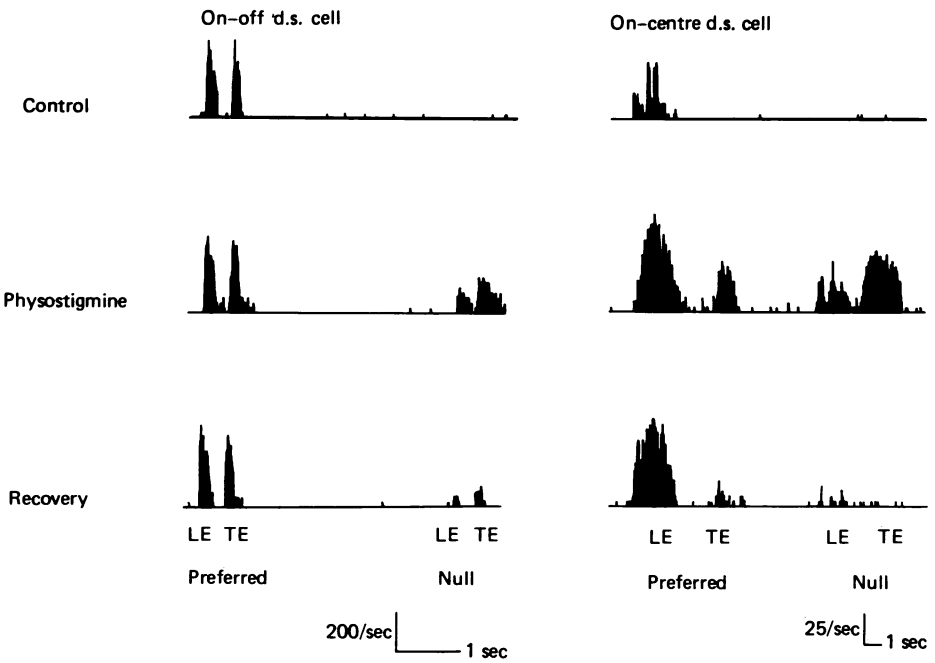

Fig. 1. On–off d.s. cell (left) and on-centre d.s. cell (right). Responses to long narrow white bars, moving in the preferred direction and then in the null direction. During infusion of physostigmine, responses occurred to the movement in the null direction, yet they were not as large as the preferred direction response. The preferred direction responses became larger and more sustained. Responses occurred to both the leading and trailing edges in both directions for both types of d.s. cell. Bar velocity was 24 deg/sec for the on–off d.s. cell and 1 deg/sec for the on-centre d.s. cell. Preferred direction was anterior for both cells.

should also abolish directional sensitivity: nicotine presumably depolarizes the d.s. ganglion cell membrane directly, since ACh itself has that effect (Masland & Ames, 1976). In fact, infusion of nicotine initially raised the spontaneous activity of both the on-centre and on–off d.s. cells, during which time an inhibition in the null direction was revealed (see arrow, Fig. 2). Further infusion of nicotine resulted in a loss of all spike activity, a result which is presumed to be a depolarizing block of the ganglion cell membrane since it follows a dramatic increase in spontaneous activity. It appears then that a *tonic* release of ACh onto d.s. cells would increase their spontaneous activity and thereby enhance directional sensitivity, if the amount

released is not so much that it causes a depolarizing block. This possibility therefore cannot account for the abolition of direction sensitivity by physostigmine.

Mecamylamine and dihydro-β-erythroidine were next tested on d.s. cells to measure the extent of cholinergic input in the light response. These drugs are nicotinic receptor antagonists which have been shown to block ACh responses onto rabbit retinal ganglion cells (Masland & Ames, 1976). Mecamylamine, infused alone, reduced

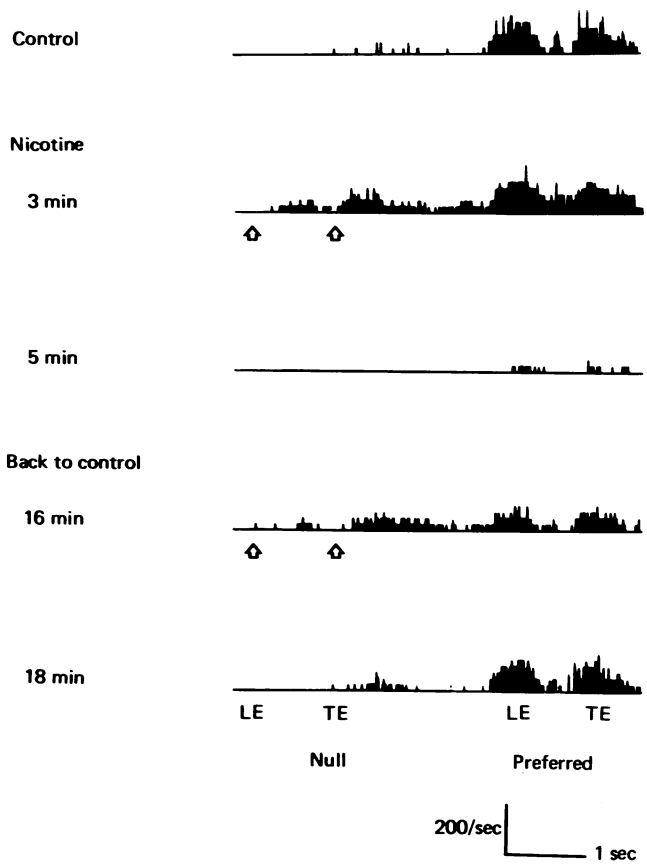

Fig. 2. On–off d.s. cell. Responses to a long narrow white bar, moving first in the null direction and then in the preferred direction. Nicotine initially increased the spontaneous activity of the cell which revealed inhibition to the leading and trailing edges in the null direction (see arrows). Continued infusion of nicotine eliminated all spontaneous activity and reduced the preferred direction response considerably. After returning to the infusion of saline, the spontaneous activity gradually increased and then decreased for full recovery. Bar velocity was 12 deg/sec. Preferred direction was downward.

both the on and off response of the on–off d.s. cell by about 50% (Fig. 3). It also reduced the response to the leading and trailing edge of a bar moving in the preferred direction by about one half. Dihydro-β-erythroidine had a similar effect to mecamylamine. One may then conclude that ACh does not account for all the light-evoked excitation of the on–off d.s. neurone. When mecamylamine was added to the infusion of physostigmine, d.s. neurones lost their null direction response (Fig. 4).

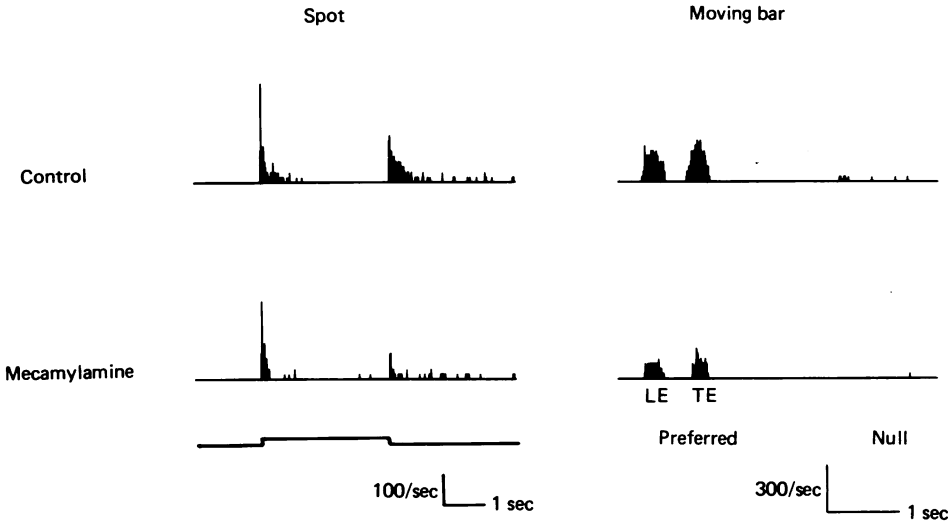

Fig. 3. On–off d.s. cell. Responses to a white stationary spot (left) and a long narrow white bar, moving in the preferred direction and then in the null direction. During infusion of mecamylamine, responses became smaller and more transient. Higher concentrations of mecamylamine did not further decrease these responses. Bar velocity was 16 deg/sec. Preferred direction was upward.

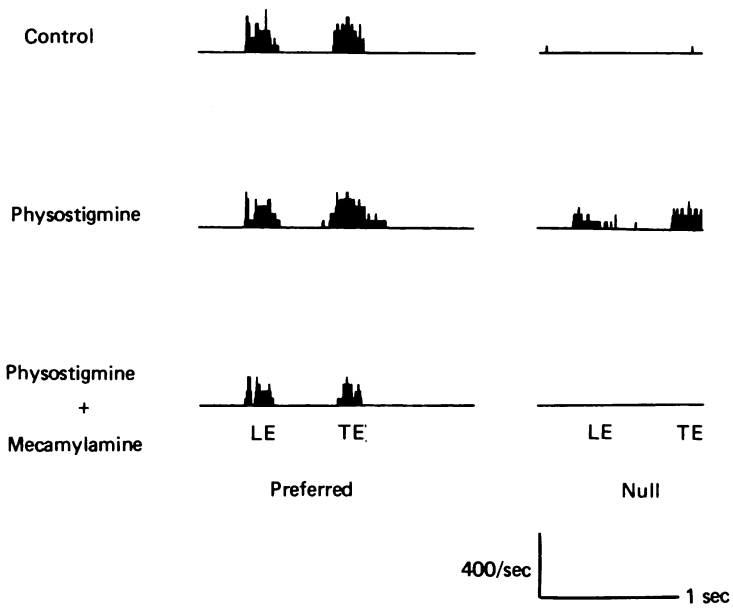

Fig. 4. On–off d.s. cell. Responses to a long narrow white bar, moving in the preferred and null directions. Infusion of physostigmine caused the cell to respond to both the preferred and null directions. Simultaneous infusion of mecamylamine completely antagonized the effects of physostigmine, abolishing both the null direction response and the increase in the preferred direction response. The number of spikes during preferred direction movement was reduced to about one half of the control value. Bar velocity was 24 deg/sec. Preferred direction was upward.

Mecamylamine also reduced the preferred direction response dramatically, again to about 50 % of its control value. Dihydro-β-erythroidine also blocked physostigmine's effect. It therefore appears that the response to ACh by on–off cells is mediated by nicotinic-like receptors.

Comparison of physostigmine and picrotoxin. Wyatt & Daw (1976) have previously reported that picrotoxin abolishes the directional sensitivity of d.s. ganglion cells. In order to understand the effect of physostigmine, it was clearly important to compare the effects of these two drugs on the responses of d.s. cells to similar stimuli (Fig. 5).

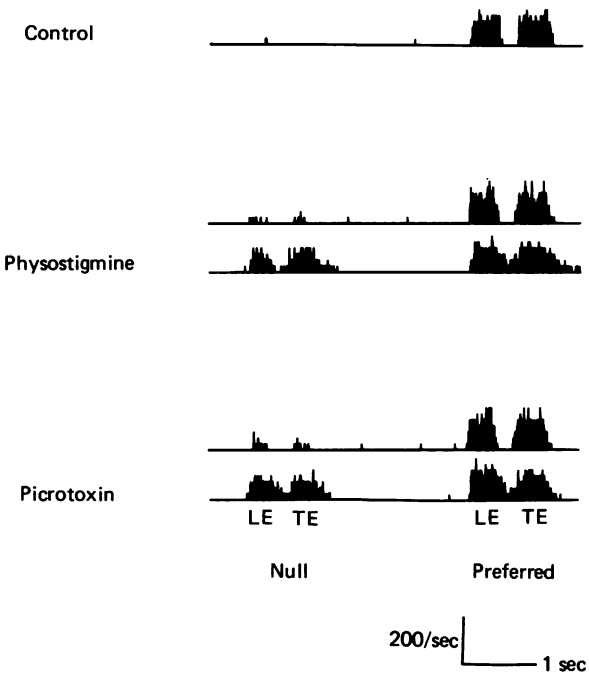

Fig. 5. On–off d.s. cell. Responses to a long narrow white bar, moving first in the null direction and then in the preferred direction. The top histogram in each pair shows drug effects close to threshold concentrations, at which time a few spontaneous spikes occurred. During suprathreshold concentrations of each drug (lower histograms of each pair), the cell responded to movement in both directions, and spontaneous activity was absent. Note that the effects of infusion of physostigmine and picrotoxin on this stimulus are very similar. The trailing edge response was more affected than the leading edge response. Bar velocity was 17 deg/sec. Preferred direction was downward.

Qualitatively, physostigmine and picrotoxin had very similar effects on the on–off d.s. neurones. Both drugs first caused a small transient increase in the spontaneous activity of the cell (top histograms of each pair; Fig. 5). The response to the trailing edge was affected sooner for both drugs. Yet the most obvious similarity of physostigmine and picrotoxin was their effect on directional sensitivity (bottom histograms of each pair; Fig. 5).

Due to the abundance of positive drug effects obtained with on–off d.s. neurones, it was possible to quantify the effects on the responses to the standard stimulus (see Methods) by statistical analysis (see Table 1 for means, numbers of cells used,

standard errors of the mean and P values). The preferred direction responses during infusion of either physostigmine or picrotoxin was significantly increased relative to control responses in total spikes/response, maximum firing frequency and response duration. The leading edge response began significantly earlier than the control response, by an average of 79 msec during picrotoxin and 40 msec during physostigmine. This difference between the two drugs was also significant. No other parameters tested (maximum firing frequency, total spikes/response and response duration) could distinguish these two drug effects statistically, i.e. $P < 0{\cdot}05$.

The maximum null response during physostigmine and during picrotoxin was surprisingly variable. This may indicate some variability in the effectiveness of the doses given. However, the evidence in many cases suggests that neither physostigmine nor picrotoxin was able to cause equal responses to movement in the preferred and null directions even in saturating doses.

Increases in the null direction responses (total spikes/response, maximum firing frequency and response duration) were highly significant, with the trailing edge response being affected more dramatically than the leading edge response. The responses to the null direction stimulus during picrotoxin and physostigmine were statistically distinguished in two ways. First, although both drugs increased the ratio of null responses to preferred responses for the trailing edge (physostigmine: 0·20 higher $\pm$ 0·06, $N = 22$, $P < 0{\cdot}01$; picrotoxin: 0·33 higher $\pm$ 0·07, $N = 21$, $P < 0{\cdot}001$), picrotoxin increased this ratio 38% more than physostigmine ($P < 0{\cdot}01$). Secondly, the null response onset was significantly earlier for picrotoxin than physostigmine by an average of 131 msec for both the leading and trailing edges. Earlier null responses during picrotoxin were found in almost every cell in which both drugs were infused and was thus the most striking difference of picrotoxin and physostigmine using this standard stimulus.

Analysis of drug effects on response timing. When the result of applying a drug is to make the response to a moving stimulus earlier, this may be a function of one or both of two effects. The retinal processing time (the time from light stimulation until ganglion cell response) may become shorter and/or the excitatory area of the receptive field may become larger. Since the timing of the responses to moving stimuli represented a major difference in the effects of physostigmine and picrotoxin on d.s. ganglion cells, it was important to distinguish between these two possibilities.

A direct way to monitor changes in retinal processing time is to flash a small spot onto the centre of the receptive field. An analogous situation for moving stimuli is to place a mask in the visual field so that the moving bar only appears when it is within a small area of the centre of the receptive field. Using such a mask, response onset did not change during infusion of picrotoxin as it did without the mask (Fig. 6). If the response timing shift was due to a shortening of retinal processing time, response onset should also be earlier for the masked stimulus, which was not the case. The response timing shift may therefore indicate a larger excitatory receptive field area.

It is difficult to monitor the spatial extent of the receptive field directly using small spots since d.s. cells respond poorly to flashed spots. An alternative method is to determine the position of the moving bar in visual space when the response begins. However, these results have to be corrected for the latency of the retinal response

TABLE 1. Statistics of on–off d.s. cells

	Preferred leading edge				Preferred trailing edge				Null leading edge				Null trailing edge			
	x	$\pm s_x$	,N	P	x	$\pm s_x$	,N	P	x	$\pm s_x$	,N	P	x	$\pm s_x$	,N	P
Spikes/response																
Control	26	11	,22	—	28	2·8	,22	—	0·75	0·21	,22	—	2·3	0·45	,22	—
Increases with physostigmine	13	15	,22	< 0·001	17	3·8	,22	< 0·001	15	3·0	,22	< 0·001	23	3·8	,22	< 0·001
Increases with picrotoxin	16	22	,21	< 0·01	13	3·7	,21	< 0·01	17	3·7	,21	< 0·001	25	3·3	,21	< 0·001
Maximum firing frequency (spikes/sec)																
Control	215	16	,22	—	210	14	,22	—	13	5	,22	—	35	6	,22	—
Increases with physostigmine	25	10	,22	< 0·05	30	10	,22	< 0·01	90	10	,22	< 0·001	99	10	,22	< 0·001
Increases with picrotoxin	43	19	,22	< 0·05	23	13	,21	= 0·05	123	14	,21	< 0·001	130	16	,21	< 0·001
Response duration (msec)																
Control	271	42	,17	—	295	60	,18	—	65	23	,4	—	299	93	,10	—
Increases with physostigmine	95	16	,18	< 0·001	145	47	,18	< 0·01	159	35	,4	< 0·02	157	71	,12	< 0·05
Increases with picrotoxin	92	22	,17	< 0·001	102	45	,18	< 0·05	339	49	,4	< 0·01	195	52	,10	< 0·005
Response onset (msec earlier than control response)																
Physostigmine	40	17	,19	< 0·05	30	33	,17	> 0·9	133	52	,5	< 0·05	118	20	,11	< 0·001
Picrotoxin	79	23	,22	< 0·005	−0·95	34	,20	> 0·9	403	107	,6	< 0·02	321	72	,12	< 0·001
Response onset (msec earlier than physostigmine response)																
Picrotoxin	35	13	,22	< 0·02	−41	40	,20	> 0·2	135	29	,22	< 0·001	128	24	,20	< 0·001

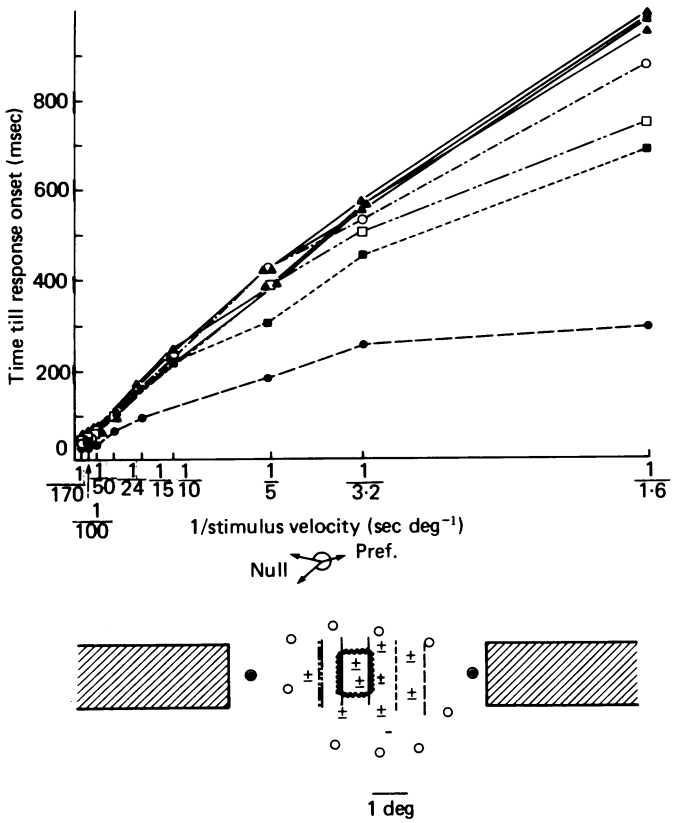

Fig. 6. On–off d.s. cell, before and during picrotoxin, using only leading edge responses. Graph of the time-till-response-onset in msec plotted as a function of the inverse of stimulus velocity (see text). The stimulus was a narrow white bar moving between 1·6 and 170 deg/sec in the preferred and null directions starting from outside the receptive field. Points falling on a straight line correspond to a response which begins in the same position in the receptive field for all speeds. The continuous lines connect the points (▲) determined when the stimulus was masked so that its light was only projected onto the centre of the receptive field (see boxed area in lower half of Figure). Note that the lines for control and picrotoxin coincide for both the preferred and null directions. On the other hand, the unmasked stimuli in the preferred direction (irregular dashed lines) evoked an earlier response. The response during picrotoxin (line of long dashes) began earlier than the control response (line of short dashes) which happened to occur to the bar moving in the null direction for this on–off d.s. cell. The receptive field map (below) demonstrates the position of the bar in the receptive field. On, off and no responses (+, − and ○, respectively) were roughly determined by flashing small spots on the receptive field prior to infusion of picrotoxin. A light sensor (◉) was placed in the path of the moving bar (hatched rectangle) to measure the time-till-response-onset. The vertical lines (below) correspond to the position of response onset calculated from the slopes of the lines in the graph (above).

by making measurements at a variety of stimulus velocities. We determined the time-till-response-onset from the number of p.s.t. histogram bins between an artifact produced by a light sensor placed near the receptive field and the spike responses.

The time-till-response-onset equals the distance the stimulus travelled divided by the speed of movement plus the retinal processing time,

$$\text{Time} = (\text{distance})\ (1/\text{speed}) + \text{retinal delay}$$

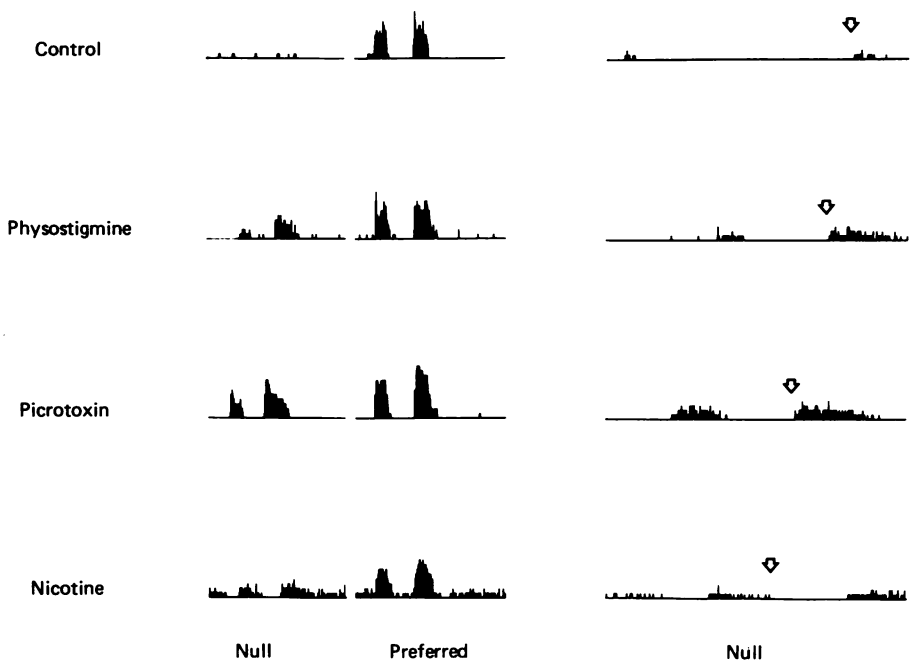

Fig. 7. On–off d.s. cell. Responses to a long narrow white bar, moving in the null direction and in the preferred direction. Histograms on the left demonstrate the effects of the drugs on directional sensitivity. Histograms on the right were recorded with a shorter bin width to reveal timing differences during null direction movement. The arrows indicate the null response onset during physostigmine and picrotoxin and the onset of the null inhibition during nicotine. These arrows correspond to the arrows in Fig. 13. Bar velocity was 14 deg/sec. Preferred direction was anterior.

which is the equation for a straight line with time on the ordinate, 1/speed on the abscissa, distance as the slope and retinal delay as the Y-intercept. We therefore chose to plot these time points as a function of the inverse of the speed (Fig. 6). A curve which falls on a straight line represents a stimulus which evokes a response at the same position in the receptive field for all speeds. In Fig. 6, the points for each stimulus did in fact fall roughly in a straight line, and the slope of the lines represented the distances which each stimulus travelled beyond the light sensor before a response occurred. These distances were plotted on the receptive field map shown in the lower half of Fig. 6.

The distances travelled by the stimulus before response onset, when a mask was present, correlated approximately with the edge of the mask. The position of the unmasked stimulus at response onset coincided roughly with (somewhat inside) the receptive field borders as estimated by small flashing spots. When picrotoxin was infused, the response onset time for movement in the null direction was dramatically shorter than the control figures (which happened to occur in this cell), indicating that the responsive area of the receptive field was enlarged. For the d.s. cell studied in Fig. 6, the preferred direction was to the right. A null direction response began as the stimulus approached the right side of the receptive field, when moving left. Calculations showed that the receptive field was enlarged on its right side by at least 0·8 deg. The graph also demonstrates that the retinal processing time (the extrapolated Y-intercept) for this cell was approximately 40 msec, which is consistent with latency measurements of other d.s. cells using flashed stimuli (M. Ariel & N. W. Daw, unpublished results). This latency is not dramatically affected by picrotoxin.

The correlation between response onset times and receptive field position suggests that response timing may be used to compare the spatial extent of excitatory (ACh) and inhibitory (GABA) inputs to the ganglion cell. Consequently we examined the response timing of these cells by expanding the response histograms for movement in the null direction, where such responses occurred (Fig. 7). Infusion of nicotine revealed inhibition to movement in the null direction (the arrow above the lowest right histogram indicates the onset of inhibition). The null direction response during picrotoxin began during this period of inhibition (the arrows above the three top-right histograms indicate the onset of the responses for movement in the null direction). During physostigmine, the null direction response began consistently later than the response during picrotoxin. The interpretation of these results will be taken up in the Discussion.

On-centre directionally sensitive ganglion cells

The responses of on-centre d.s. ganglion cells during infusion of physostigmine were clearly different from those during picrotoxin. It has been reported that picrotoxin abolishes the directional sensitivity of on-centre d.s. cells (Wyatt & Daw, 1976). When these cells were tested with long bars which can evoke separate leading and trailing edge responses, equal leading edge responses occurred in both the preferred and null directions during picrotoxin, yet the trailing edge responses were comparatively small (the trailing edge response of the example in Fig. 8 may have been partly due to a subthreshold effect of physostigmine, which persisted during the infusion of picrotoxin, since when picrotoxin was infused first, hardly any trailing edge response was seen (Fig. 9)).

On the other hand, the edge responses during physostigmine remained directionally asymmetric yet clearly occurred to both the leading and trailing edges (Fig. 8). The responses to each edge were significantly increased in total spikes/response, maximum firing frequency and response duration (Table 2). During physostigmine, the leading edge responses in the preferred direction were significantly larger than the leading edge responses in the null direction ($P < 0·001$) but the trailing edge responses were statistically indistinguishable from one another at a 80% confidence level. The leading edge response was also significantly larger than the trailing edge response in

the preferred direction ($P < 0{\cdot}001$). The average response to the leading edge (183 spikes) in the preferred direction was twice as great as the average response to either of the trailing edges (preferred = 86 spikes, null = 97 spikes), whereas the average leading edge response in the null direction was smaller (60 spikes). These results suggest that, in addition to the inhibition evoked by movement in the null direction,

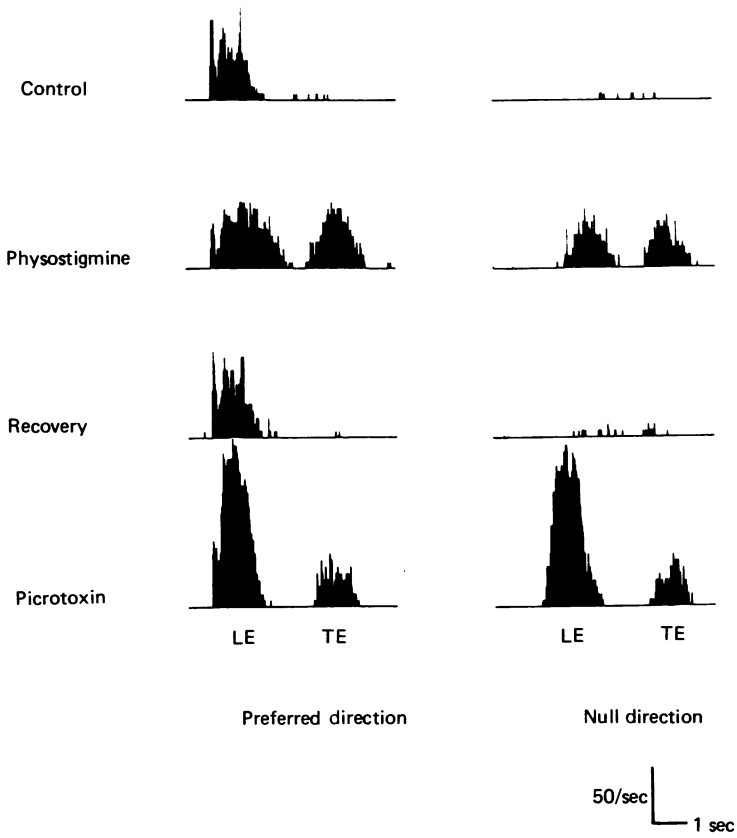

Fig. 8. On-centre d.s. cell. Responses to a long narrow white bar, moving in the preferred and null directions. During infusion of physostigmine, responses occurred to all edges, the preferred leading edge evoking the largest response and the null leading edge causing the smaller response. Directional asymmetry remained for the leading edge response. During infusion of picrotoxin, both responses were directionally symmetric. The leading edge responses were much stronger than the trailing edge responses. Bar velocity was 3 deg/sec. Preferred direction was up-posterior.

there may be inhibition which suppresses the trailing edge response more than the leading edge response. Infusion of physostigmine presumably augments the effectiveness of ACh so that inhibition is partially overcome and the neurone responds to both edges in both directions.

When physostigmine and picrotoxin were infused simultaneously (Fig. 9), the on-centre d.s. neurone responded equally to both edges moving in either the preferred or null directions. Since picrotoxin alone did not cause equal responses to both the

leading and trailing edges, GABA is not normally responsible for suppressing the off-centre ACh excitation. It is possible that another mechanism suppresses the trailing edge responses of on-centre d.s. cells. If so, it is difficult to understand why simultaneous physostigmine and picrotoxin overcomes it. Perhaps the removal of tonic GABA inhibition and the augmentation of ACh effectiveness combine to saturate the light responsiveness and give equal responses to both edges when picrotoxin and physostigmine are infused together.

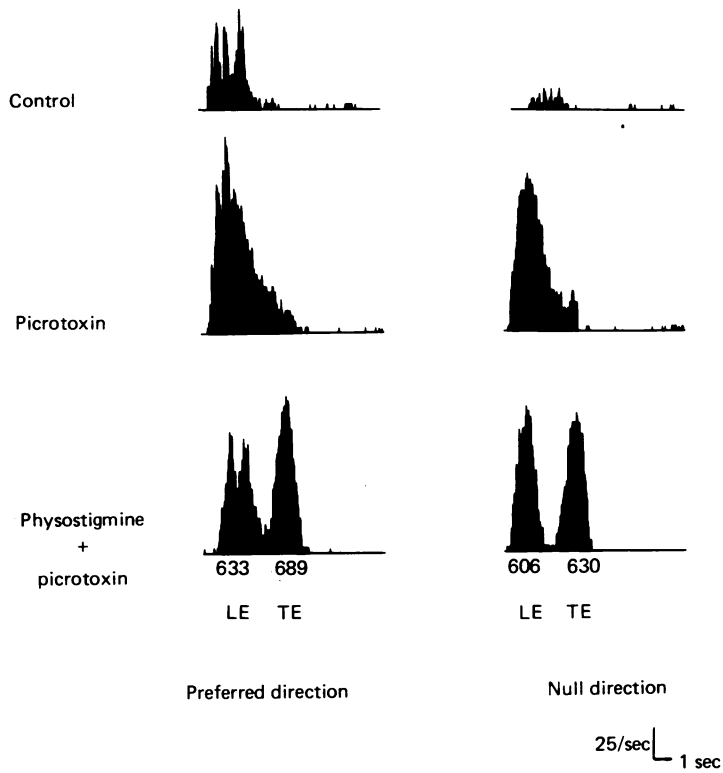

Fig. 9. On-centre d.s. cell. Responses to a long narrow white bar, moving in the preferred and null directions. During infusion of picrotoxin, equal responses occurred to the leading edge in both the preferred and null directions. During simultaneous infusion of physostigmine and picrotoxin, equal responses occurred to both the leading and trailing edges in both the preferred and null directions. The numbers beneath the lower histogram represent the number of spikes of each response of the histogram (i.e. the sum of four stimulus presentations). Bar velocity was 2·5 deg/sec. Preferred direction was up-posterior.

Drug effects on other inhibitory properties

Picrotoxin blocks the size specificity and radial grating inhibition found in d.s. ganglion cells, and alters the speed specificity, as well as eliminating the directional sensitivity (Caldwell *et al.* 1978). In order to compare the actions of GABA and ACh, we next tested whether physostigmine affected these other properties in a fashion similar to picrotoxin.

TABLE 2. Statistics for on-centre d.s. cells

	Preferred leading edge				Preferred trailing edge				Null leading edge				Null trailing edge			
	x	$\pm s_x$	,N	P	x	$\pm s_x$	,N	P	x	$\pm s_x$	,N	P	x	$\pm s_x$	,N	P
Spikes/response																
Control	78	10	,14	—	3·4	1·3	,14	—	5·4	2·0	,14	—	3·5	1·4	,14	—
Increase with physostigmine	105	18	,14	< 0·001	82	11	,14	< 0·001	55	11	,14	< 0·001	94	18	,14	< 0·001
Maximum firing frequency (spikes/sec)																
Control	60	4·9	,15	—	9·0	2·5	,15	—	12	4·1	,15	—	7·3	2·6	,15	—
Increase with physostigmine	26	6·5	,15	< 0·01	40	3·5	,14	< 0·001	33	4·1	,15	< 0·001	40	2·7	,14	< 0·001
Response duration (msec)																
Control	3945	374	,14	—	2417	552	,7	—	2149	545	,6	—	2385	590	,6	—
Increase with physostigmine	924	341	,14	< 0·02	1580	336	,7	< 0·01	944	266	,6	< 0·02	1988	719	,6	< 0·05
Response onset (msec earlier than control)																
Physostigmine	−146	118	,14	> 0·2	655	312	,6	< 0·05	545	182	,6	> 0·5	309	262	,6	> 0·2

Size specificity is revealed by a comparison of the response to narrow and wide moving bars, stimulus width being the dimension perpendicular to the axis of movement (Daw & Wyatt, 1974; Wyatt & Daw, 1975). As has been shown previously for the on-centre d.s. cell (Wyatt & Daw, 1976), on–off d.s. cells respond equally to narrow and wide bars during infusion of picrotoxin (Fig. 10). However, during physostigmine the wide bars were still less effective even though the narrow bar

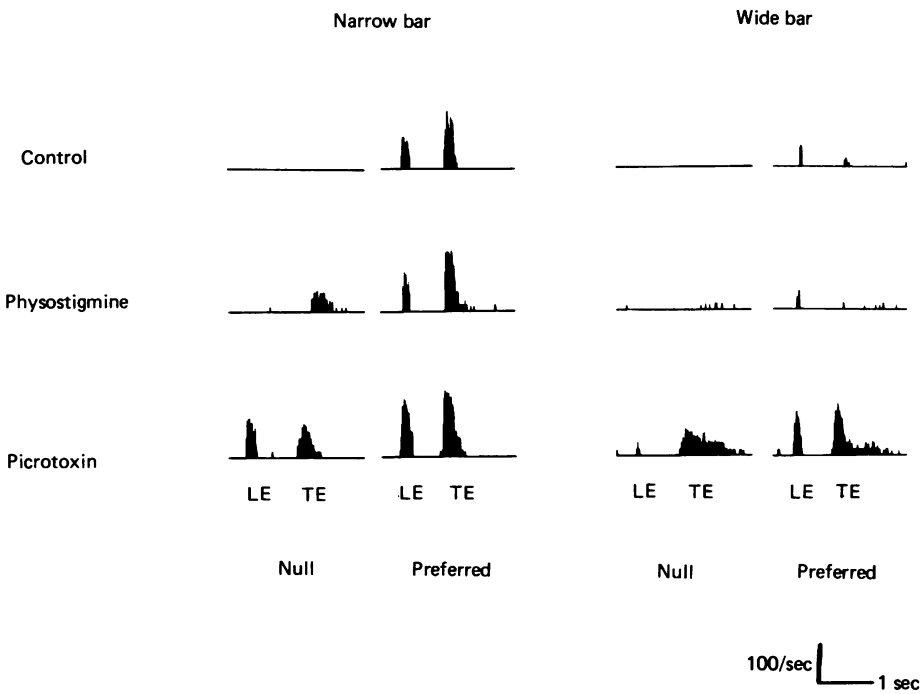

Fig. 10. On–off d.s. cell. Responses to a narrow (0·6 deg) and wide (7·9 deg) long white bar moving in the null and preferred directions. The wide bar (right) evoked smaller responses than the narrow bar (left). For the narrow bar, both physostigmine and picrotoxin allowed responses in the null direction. Physostigmine had little effect on the wide bar responses, yet picrotoxin abolished both the directional sensitivity and the inhibition due to the wide bar. Bar velocity was 14 deg/sec. Preferred direction was upward. Receptive field was approximately 1·6 deg across.

response was enhanced in both the preferred and null directions (observed in eight on–off d.s. cells and nine on-centre d.s. cells).

Reduction of the response to a spot is produced by the rotation of a radial grating outside the receptive field, as defined by flashing spots of light (Werblin, 1972; Caldwell & Daw, 1978*a*, *b*). During physostigmine, responses to stationary stimuli were increased and prolonged (Fig. 11), which was the opposite of the effect of mecamylamine (see Fig. 3). Yet inhibition remained when the radial grating was rotated during physostigmine for the nine on–off cells and four on-centre d.s. cells tested, in contrast to the effect of picrotoxin.

Directionally sensitive ganglion cells respond to stimuli moving in the preferred direction at a wide range of speeds. The optimal speed in the preferred direction (as measured by maximum firing frequency) is dependent on the stimulus and cell type. On-centre d.s. cells prefer slow speeds, rarely responding to movements higher than 40 deg/sec (Oyster, 1968). In contrast, on–off d.s. cells often respond to speeds of 300 deg/sec or more. Infusion of picrotoxin reduces the speed specificity of on-centre d.s. cells (Caldwell *et al.* 1978). In contrast, infusion of physostigmine did not alter the speed tuning of these cells (ten on-centre d.s. cells and seven on–off d.s. cells tested).

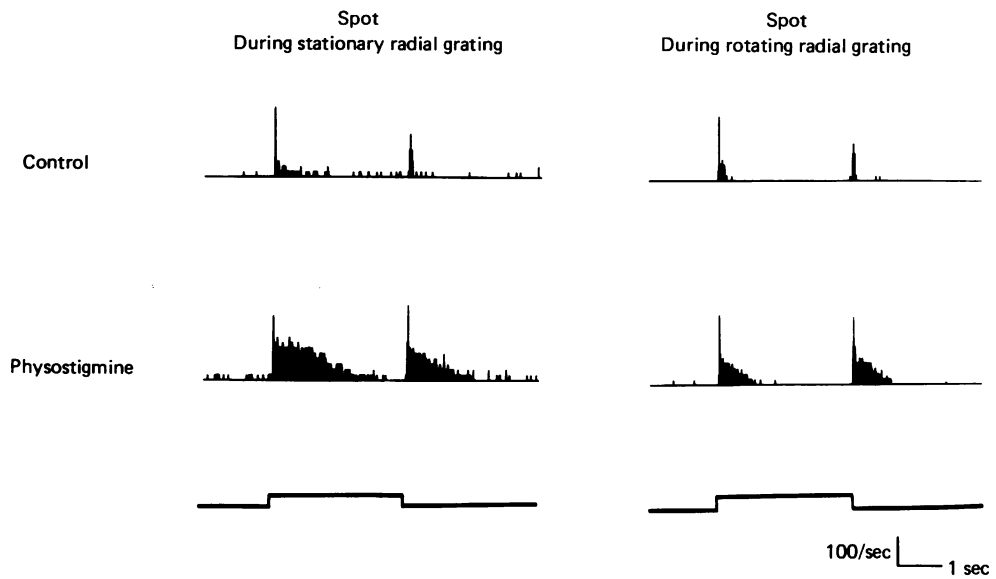

Fig. 11. On–off d.s. cell. Responses to a flashed white spot, during either a stationary or continuously rotating radial grating. Physostigmine made the responses more sustained at both the onset and offset of light. During physostigmine, rotation of the radial grating still dramatically reduced the responses. The radial grating had sixteen white sectors extending from 10 to 20 deg around the receptive field. It rotated at 10 revolutions/min.

We found that picrotoxin also dramatically reduced the speed sensitivity of most on–off d.s. cells (Fig. 12). This result reveals the presence of GABA inhibition of responses to fast stimuli for the on–off d.s. cells as well as the on-centre d.s. cells. This form of inhibition onto on–off d.s. cells is presumably due in part to lateral inhibition from the surround since masking the moving bar from the surround also increases the cut-off velocity of d.s. cells (Oyster, Takahashi & Collewijn, 1972). Physostigmine does not affect this form of lateral inhibition as was the case with size specificity and radial gating inhibition, again contrasting with the effect of picrotoxin. These differences between the effects of physostigmine and picrotoxin indicate that the spatial extent of ACh and GABA input to d.s. cells may be different, as was suggested by the response onset timing differences shown in Fig. 7.

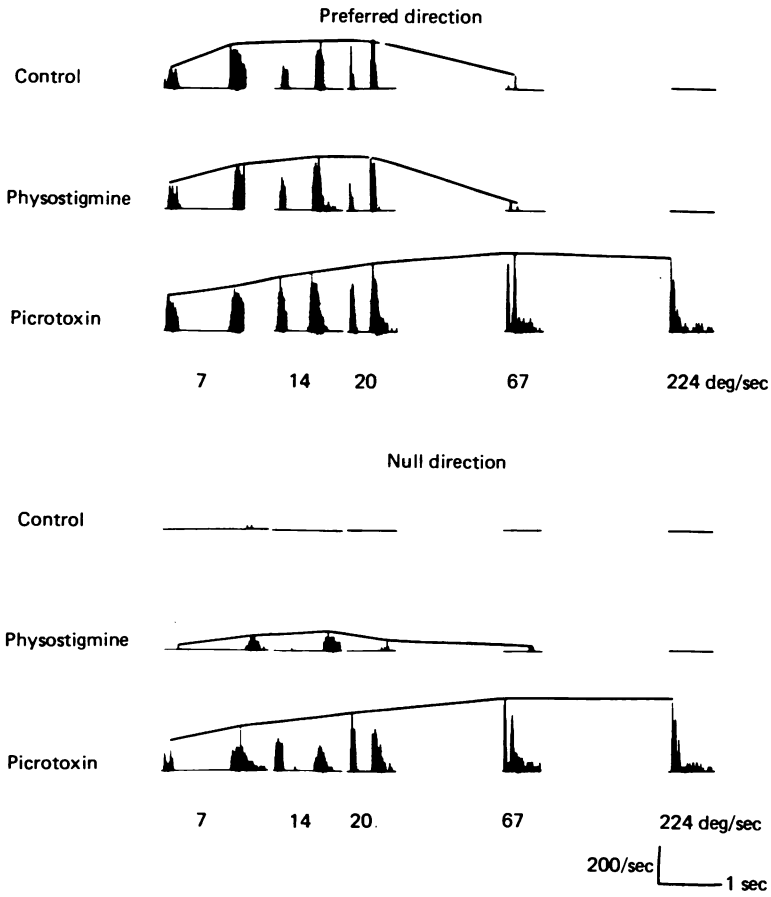

Fig. 12. On–off d.s. cell. Responses to a long narrow white bar, moving in the preferred direction (top) and in the null direction (bottom). Stimulus speed is positioned logarithmically along the abscissa. Physostigmine caused the cell to respond in the null direction (bottom) but not to fast speeds (right). Picrotoxin abolished both the directional sensitivity and the speed specificity. Preferred direction was upward.

DISCUSSION

Masland & Ames (1976) reported that physostigmine increases the spontaneous and light-evoked spike activity of d.s. cells in the rabbit retina. We have found that physostigmine also abolishes the directional sensitivity of these cells, yet does not affect other properties such as speed specificity, size specificity and radial grating inhibition.

The effect of physostigmine on directional sensitivity is atypical of the effects of physostigmine on other retinal ganglion cells (see Ariel & Daw, 1982). Why do d.s. cells lose their trigger feature during physostigmine, whereas other more complex ganglion cells do not? Perhaps d.s. ganglion cells receive a larger amount of light-driven

ACh release than other ganglion cells. This high level of excitation may then allow the ganglion cell to overcome inhibition and reach spike threshold to elicit a response. We can support this hypothesis with the following observations.

First, d.s. ganglion cells receive very potent input from ACh amacrine cells (Masland & Ames, 1976). The response of these cells to flashed spots of light are the most increased by physostigmine of all the ganglion cell types in the rabbit retina (see Ariel & Daw, 1982). In fact, on two occasions, physostigmine by itself was sufficient to cause a presumable depolarizing block of the spike activity of an on–off d.s. cell. Also the time course of the effect of nicotine, from an increase in spontaneous activity till the depolarizing block in activity, is very rapid (see Fig. 2), suggesting that the ACh receptors can dominate the d.s. ganglion cell membrane potential. Other ganglion cells were never sufficiently depolarized by nicotine to produce a depolarizing block.

Secondly, one can see in certain circumstances that inhibition is still present during the infusion of physostigmine. When a small moving grating was placed in the receptive field to raise the spontaneous activity, a short period of inhibition occurred as the bar entered the receptive field in the null direction. This period of inhibition remained during physostigmine. Moreover, the null direction response during physostigmine started after the onset of inhibition revealed when the spontaneous activity is increased by nicotine.

Spatial extent of ACh and GABA input

If directional sensitivity is overcome, yet not abolished, during physostigmine, why then are size specificity and speed specificity not also overcome during physostigmine? The answer to this question apparently lies in the balance between the ACh excitation and the GABA inhibition, i.e. the spatial differences in the extent of ACh and GABA input to the receptive field, and the relative local strength of each transmitter at every point within the dendritic field.

It is not easy to define the normal extent of neurotransmitter input to a ganglion cell; excitatory influences may be either subthreshold or suppressed by inhibitory transmitters and inhibitory influences may be overlooked due to a lack of excitation. We used a stimulus which minimizes the light-evoked inhibition for the statistical analysis of the on–off d.s. cell (a narrow bar moving slowly in the preferred direction) to estimate the spatial extent of light evoked ACh excitation to this cell. Since this stimulus moved at 20 deg/sec, the leading edge response, which occurs 40 msec earlier during physostigmine, corresponds to an extension of the receptive field border on the preferred side by 0·8 deg. Therefore, the spatial extent of ACh input to the d.s. ganglion cell may be slightly larger than the receptive field borders (as measured by moving stimuli).

GABA input to d.s. ganglion cells has been suggested to be asymmetric (see the cardioid shaped model of Wyatt & Daw, 1975). This conclusion is consistent with our measurement of the borders of the light-responsive area during picrotoxin. The border extends further on the null side (2·7 deg) than on the preferred side (0·8 deg), relative to the borders measured during physostigmine. An asymmetry of inhibitory input is also revealed during infusion of nicotine or by using a light stimulus which raises the spontaneous activity of the d.s. cell.

We therefore hypothesize that the ACh and GABA inputs are as shown in the model at the bottom of Fig. 13, with the ACh input coming from a symmetric area in the centre of the receptive field, and the GABA input coming from a wider, asymmetric, cardioid shaped area. The relative strengths of input along the central

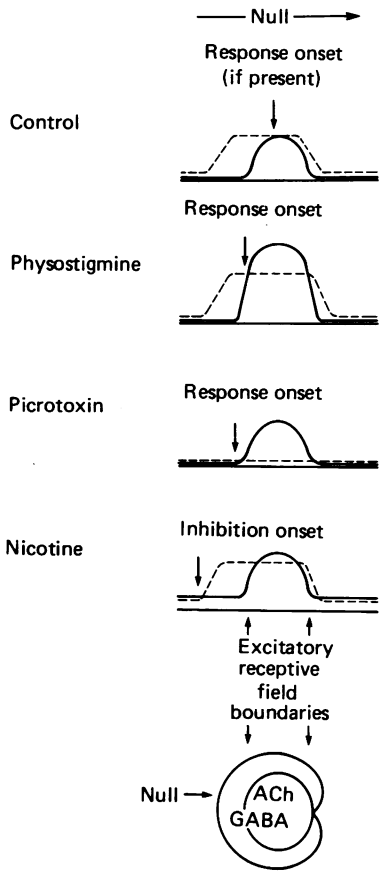

Fig. 13. Possible model for null direction timing differences. Response onset and inhibition onset are indicated by the position in the receptive field where the null moving edge is found at that moment in time. Continuous black lines represent the local strength of excitation for the post-synaptic ACh receptor at each point along the path of the moving edge. Dashed lines represent the strength of GABA inhibition along the same path. Null response onset is shown to occur (arrows) when the light-evoked ACh excitation overcomes the GABA inhibition. Note that the asymmetry of the GABA inhibition is consistent with the cardioid shaped inhibitory surround (below) along its central axis (Wyatt & Daw, 1975).

axis of the receptive field are shown by continuous lines (cholinergic input) and dashed lines (GABAergic input), and can be tested by a narrow long bar moving slowly to the right along the central axis of the cardioid shaped inhibitory field shown on the bottom of Fig. 13.

Normally, d.s. ganglion cells respond vigorously to stimulus movement in the

preferred direction since the excitation presumably arrives earlier and is stronger than the inhibition. Null direction responses, if any, occur when the stimulus is well within the receptive field. They occur when the ACh excitation (continuous line) which is strongest in the centre of the receptive field overcomes GABA inhibition (dashed line) which arrives laterally from points previously stimulated in visual space. The response onset is demonstrated graphically (arrows) as the point at which the continuous (ACh) line first becomes higher than the dashed (GABA) line as the stimulus moves from left to right. Light responses are dependent on the relative balance between excitatory and inhibitory pathways, and are relatively weak and brief, if they occur at all, because the inhibitory influences are more powerful.

During infusion of physostigmine, the ACh esterase is inhibited and the effect of ACh is strengthened and prolonged at the ACh receptor. This is represented graphically in the figure by expanding the continuous line upwards. The continuous ACh line then crosses the dashed GABA line to the left, earlier in time than it does without the infusion of physostigmine (see Fig. 13; the arrows enable an easy comparison with the response onset shown in Fig. 7).

During infusion of picrotoxin, GABA receptors are blocked so that inhibition is abolished. This is represented graphically by flattening the dashed line downwards. The null direction response then occurs with an onset earlier than null responses during physostigmine. According to the model, the null direction response during picrotoxin begins as soon as the cell receives any excitatory ACh input (continuous line), whereas during physostigmine, the response onset is delayed by the presence of GABA inhibition (dashed line).

Accordingly, the null direction response during picrotoxin begins during the period when normally GABA is strongly inhibiting the cell (Fig. 13, control). This period of inhibition may be revealed by tonically depolarizing the cell with nicotine. In Fig. 13, this is represented by shifting the continuous line upwards throughout the receptive field, raising the level of excitation above the level of tonic inhibition (dashed line). In this way, the place where GABA inhibition is most effective is revealed by nicotine, and occurs where the dashed line is higher than the continuous line.

The model explains why the standard stimulus we used resulted in the strikingly similar effects of physostigmine and picrotoxin. After each d.s. cell was characterized, we chose a narrow bar to produce the best response in the preferred direction. Presumably, this bar optimized the central excitation relative to the lateral inhibition and movement in the null direction produced the largest amount of ACh release compared to GABA release. Physostigmine was therefore very effective in enabling ACh excitation to overcome the null direction GABA inhibition due to the stimulus. Both the bar size and its position were factors: if the same sized bar was placed slightly off centre, then it did not evoke a null direction response during physostigmine yet did so during picrotoxin.

The model also explains why other inhibitory effects are less affected by physostigmine than directional sensitivity. This is simply because properties such as size specificity and radial grating inhibition are due in part to GABA inhibition from the surround where we have postulated that there is no ACh excitation.

Same inhibitory and excitatory systems for both d.s. cell types?

It has been suggested that size and speed specificity are produced by the same inhibitory system which produces directional sensitivity (Wyatt & Daw, 1975; Caldwell *et al.* 1978). This is further supported by our findings that all three properties are abolished by picrotoxin for the on–off d.s. cell as well as the on-centre d.s. cell. Clearly though, these cell types normally differ in two respects. First, on-centre d.s. cells are more size and speed specific than on–off d.s. cells (Oyster, 1968). To explain the speed specificity of on-centre d.s. cells, Caldwell *et al.* (1978) postulated that there is inhibition in the preferred direction which is both powerful and rapidly decaying. The higher cut-off velocities of on–off d.s. cells may be due to (1) a less powerful inhibition and/or (2) a more rapidly decaying inhibition. It is not known whether separate GABA amacrine cells are required for each d.s. cell type but our results suggest that inhibitory influences other than GABA are not needed.

The second major difference in these two d.s. ganglion cell types is the centre response: on–off in one case and on in the other. Our results using physostigmine suggest that both types of d.s. cell receive excitation at the offset as well as the onset of light. The off input to the on-centre d.s. cells may be weak, or it may be pushed below threshold by inhibitory influences. In summary, it appears that both cell types receive similar GABA inputs responsible for their directional specificity and other inhibitory properties, and both receive similar ACh inputs which provide light-driven excitation at on and off.

Cellular mechanisms of directional sensitivity

These results clearly show an interaction between the excitatory and inhibitory pathways that influence d.s. ganglion cells. One may ask: what is the cellular mechanism underlying the integration of these inputs? Clearly, the ACh amacrine cell cannot be excitatory and presynaptic to the GABA amacrine cell. If it were, infusion of physostigmine would increase the null direction inhibition. Although many other schemes are possible, the most likely are: (1) that the GABA amacrine cell is presynaptic to the ACh amacrine cell which is presynaptic to the d.s. ganglion cell, or (2) that the GABA and ACh amacrine cells are both directly presynaptic to the d.s. ganglion cell (Torre & Poggio, 1978). These two arrangements also may exist in parallel with each other.

The first scheme (top half of Fig. 14) predicts that GABA modulates ACh release onto the d.s. ganglion cells. This is consistent with the general pharmacological results on ACh release. GABA inhibits the light-evoked release and dark release of ACh from the rabbit retina *in vivo* and this effect is blocked by picrotoxin and bicuculline (Massey & Neal, 1979; Neal & Massey, 1980). Both GABA and ACh appear to be released in the dark and GABA modulates the ACh release. However, these pharmacological results may be due to GABA and ACh influences in pathways leading to ganglion cells that are not directionally sensitive.

The first scheme requires that ACh release must be directionally sensitive itself. Also another excitatory interneurone (that is responsible for the light-evoked excitation during the infusion of mecamylamine) must also be directionally sensitive. However, no d.s. neurones other than ganglion cells have yet been reported in

vertebrate retina (Werblin, 1970; Marchiafava, 1979). Furthermore, if ACh release were directionally sensitive, physostigmine (which increases the cholinergic amacrine cell's effectiveness) would result in clearly asymmetric responses, which is often not the case.

There is evidence for the second scheme of direct GABA inhibition and ACh

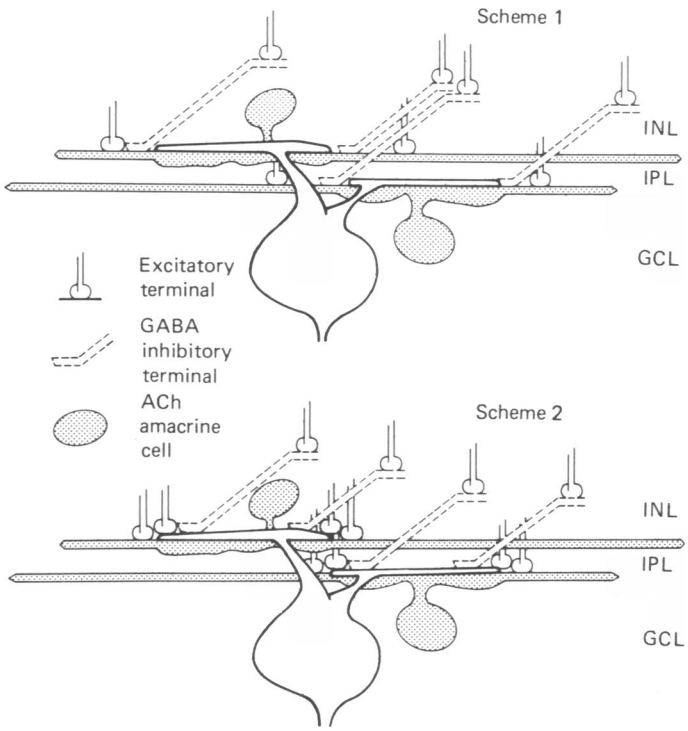

Fig. 14. Two possible schemes for the wiring of the d.s. cell. The anatomical forms shown are not intended to represent actual cell shapes or sizes (see Ariel & Daw, 1982). The small round synaptic boutons represent light-evoked excitatory input to cholinergic amacrine cells (stippled cells), lateral GABA terminals (dashed cells) and on–off d.s. ganglion cell (with bold outline). In the top half of the Figure (scheme 1), the cholinergic amacrine cells receive null direction inhibition from lateral GABA terminals and local light excitation by an unknown neurotransmitter (presumably of a bipolar cell). Since the ACh cell receives both direct excitation and asymmetric inhibition, the ACh release will be directionally sensitive. In the bottom half of the Figure (scheme 2), the cholinergic amacrine cells receive only local light-evoked excitation. It therefore serves to modulate local light evoked excitation onto the d.s. ganglion cell, which is also inhibited by an asymmetric GABA release. INL = inner nuclear layer, IPL = inner plexiform layer, GCL = ganglion cell layer.

excitation onto ganglion cells. Infusion of nicotine, which presumably depolarizes the ganglion cell membrane directly, reveals null direction inhibition, implying that GABA also acts there directly. In mudpuppy, GABA causes a chloride-dependent conductance increase of the ganglion cell membrane (Miller & Dacheux, 1976). Also, amacrine cells stained for glutamic acid decarboxylase have been seen presynaptic

to ganglion cell dendrites in the rabbit retina (Brandon, Lam, Su & Wu, 1980). ACh has been shown to excite d.s. ganglion cells directly in the rabbit retina, even after blocking synaptic transmission with high Mg^{2+}/low Ca^{2+} (Masland & Ames, 1976). It therefore seems plausible that the interaction between cholinergic excitation and GABAergic inhibition occurs at the d.s. ganglion cell, as in the second scheme (bottom half of Fig. 14). Intracellular recordings from d.s. cells in the rabbit retina are consistent with this scheme. Dacheux (1977) recorded e.p.s.p.s during stimulus movement in the preferred direction and i.p.s.p.s during null direction movement.

Directionally sensitive cells in the turtle retina have also been recorded intracellularly (Baylor & Fettiplace, 1979; Marchiafava, 1979; Jensen & DeVoe, 1980). Unlike the rabbit, null direction movement evoked a membrane depolarization which lacked spikes. One explanation for this species difference is technical. The depolarization during null direction movement may in fact be a result of an inhibitory conductance change whose reversal potential is positive relative to the resting membrane potential. The evidence for this explanation is that when injection of current depolarizes the resting membrane potential, the cell hyperpolarizes to null direction movement (Marchiafava, 1979).

Identity of the d.s. subunit

Such depolarizing (ACh) and hyperpolarizing (GABA) conductances occurring at adjacent patches of dendritic membrane would account for the mathematical non-linearities described for directional sensitivity (Torre & Poggio, 1978). Our result that physostigmine and picrotoxin are approximately equally effective on directional sensitivity within the receptive field centre suggests that the cholinergic and GABAergic systems may underly this subunit mechanism at the on–off d.s. ganglion cell dendritic membrane. However, evidence for the ACh and GABA role in the d.s. subunit will require detailed histochemistry at the electron microscopic level. It is also not known whether other synaptic transmitters are involved in directional sensitivity. Our finding that light-evoked responses still occur during infusion of the ACh receptor antagonists mecamylamine and dihydro-β-erythroidine suggests that ACh may not be the only excitatory transmitter onto these cells. The identity and role of this other transmitter requires further investigation.

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