J. Physiol. (1987), **388**, *pp.* 233–243
With 3 text-figures
Printed in Great Britain

PUSH–PULL EFFECT OF SURROUND ILLUMINATION ON EXCITATORY AND INHIBITORY INPUTS TO MUDPUPPY RETINAL GANGLION CELLS

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(*Received 4 March 1986*)

SUMMARY

1. Changes in membrane potential and conductance were measured in on-centre and off-centre ganglion cells during the responses to illumination of different portions of the receptive field.

2. In on-centre ganglion cells the sustained depolarizing response to steady illumination of the receptive field centre was associated with a net increase in conductance. In the presence of centre illumination, stimulation of the surround with an annulus of light caused a hyperpolarization and a net decrease in conductance, and the reversal potential of the light-evoked response was shifted in a negative direction. In the absence of centre illumination the same annular stimulus caused a hyperpolarization and a net increase in conductance.

3. In off-centre ganglion cells the sustained hyperpolarizing response to centre illumination was associated with a net increase in conductance. In the presence of centre illumination, stimulation of the surround with an annulus caused a depolarization and a net decrease in conductance, and the reversal potential of the light-evoked response was shifted in a positive direction. In the absence of centre illumination the same annulus caused a depolarization and a net increase in conductance.

4. The results indicate that illumination of the receptive field surround can affect both the excitatory and inhibitory sustained inputs to a given ganglion cell in a 'push–pull' manner, by decreasing the synaptic input that was increased by centre illumination and increasing the synaptic input of opposite sign. The relative effect of a given surround illumination on these two inputs, and hence the sign and magnitude of the net conductance change, varied with the amount of centre illumination.

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INTRODUCTION

In mudpuppy retina illumination of the receptive field centre causes an increase in sustained excitatory input in on-centre ganglion cells and an increase in sustained inhibitory input in off-centre ganglion cells (Belgum, Dvorak & McReynolds, 1982). That study also showed that in darkness on-centre cells receive tonic inhibitory input and off-centre cells receive tonic excitatory input. The extent to which illumination of the receptive field centre affected these 'dark' inputs was difficult to assess, but it was suggested that in a given cell the opposing sustained inputs might operate in a synergistic manner to produce a given potential change. For example, a depolarization might be produced by a simultaneous increase in excitatory input and decrease in inhibitory input, and a hyperpolarization by an increase in inhibitory input and decrease in excitatory input. Such a 'push–pull' mechanism had previously been suggested for goldfish ganglion cells by Levine & Shefner (1977), based on a statistical analysis of firing patterns of ganglion cells. McGuire, Stevens & Sterling (1986) also postulated a push–pull mechanism for β ganglion cells in the cat retina, from anatomical studies. However, it has never been shown that a given light stimulus actually modulates sustained excitatory and inhibitory inputs to ganglion cells in such a push–pull manner. The present study presents physiological evidence for such a mechanism by showing that a given surround stimulus can produce an increase in one type of sustained synaptic input and a decrease in synaptic input of the opposite sign. However, the neurones which provide the opposing sustained inputs have not yet been identified.

METHODS

Intracellular recordings were made from ganglion cells in the eyecup preparation of the mudpuppy (*Necturus maculosus*) using micropipettes of 300–500 MΩ resistance filled with 4 M-potassium acetate. Details of the preparation, cell identification and the systems for electrical recording and optical stimulation have been described previously (Belgum *et al.* 1982). Current–voltage relations were measured by passing constant current steps through the micro-electrode and recording the resulting voltage displacements, using an active bridge circuit. Measurements made when the electrode was outside the cell were used to correct for electrode rectification. In each cell, measurements for a given condition of illumination were made at a fixed time relative to the onset of the light stimulus. When spontaneous spike-like events or other fluctuations occasionally interfered with measurement at this exact time the potential was measured from an adjacent portion of the trace, as indicated in the Figures.

The stimulus for the receptive field centre was a 100 μm diameter spot of white light which was centred in the cell's receptive field by positioning it so as to maximize the response amplitude. The stimulus for the receptive field surround was an annulus (inner diameter 800 μm, outer diameter 1800 μm) of white light, concentric with the spot. For a given cell, the intensity of the centre stimulus was adjusted with neutral density filters to produce a non-saturating response; the intensity of the surround stimulus was then set to a value that caused a noticeable antagonism of the centre response. This value was usually within 0·6 $\log_{10}$ units of the centre intensity. The unattenuated light intensity at the plane of the retina was equivalent, for mudpuppy cones, for $3{\cdot}25 \times 10^{15}$ photons cm^{-2} s^{-1} at their maximum wave-length, which is 572 nm (Fain, 1975). Stimulus intensities in the Figures are expressed as $\log_{10}$ units of attenuation of this value.

RESULTS

In both on-centre and off-centre ganglion cells steady illumination of the receptive field centre produced a sustained potential change which could be driven back toward the dark potential level by the addition of light in the receptive field surround. In some cells such 'antagonistic' responses could also be elicited by surround illumination alone, but usually some degree of centre illumination was necessary in order for an antagonistic surround effect to be detected.

On-centre ganglion cells

The left-hand column of Fig. 1*A* shows responses of an on-centre ganglion cell to illumination of the receptive field centre with a 100 μm diameter spot of light; during the plateau phase of this reponse the receptive field surround was illuminated with an annular stimulus. The durations of these stimuli are indicated by the upward deflections of the two horizontal lines at the top of the Figure. The different traces show responses to the same combination of light stimuli when the membrane potential was displaced by steps of current, whose intensities are indicated at the left of each trace. At resting potential (zero current) illumination of the receptive field centre caused a sustained depolarization, and the addition of the annulus caused a hyperpolarization back toward the dark level.

Current–voltage relations for this cell (Fig. 1*B*) were constructed by plotting the membrane potential under different conditions of illumination as a function of extrinsic current intensity. All potential changes are plotted relative to the resting membrane potential in darkness. Because depolarizing current usually caused significant membrane rectification in ganglion cells (see Belgum *et al.* 1982), only data obtained with hyperpolarizing currents were used in constructing the current–voltage plots; for the purpose of comparing the conductance during different conditions of illumination it was sufficient to compare the slopes of the current–voltage relations over a common voltage range. The times at which the measurements were made during the different conditions of illumination are indicated by the vertical dashed lines in Fig. 1*A*. The depolarization produced by centre illumination (squares) was associated with an increase in conductance relative to darkness (circles), indicating that this response involved an increase in excitatory synaptic input. The addition of surround illumination (triangles) drove both the membrane potential and conductance back toward their dark values, which is consistent with the idea that it acted at some earlier stage in the retina to reduce this excitatory input. If that were the only effect of adding the annulus, then the reversal potential of the net light response (the potential in light with respect to that in darkness, regardless of the pattern of illumination) should not be changed. However, when both centre and surround were illuminated the reversal potential of the net light response (extrapolated intersection of open triangles with circles) was quite different than when only the centre was illuminated (intersection of squares with circles). This shift of the reversal potential to a more negative value suggests that the addition of surround illumination caused not only a decrease in excitatory input but also an increase in inhibitory input..

An annulus-evoked increase in inhibitory input could be clearly seen when the same

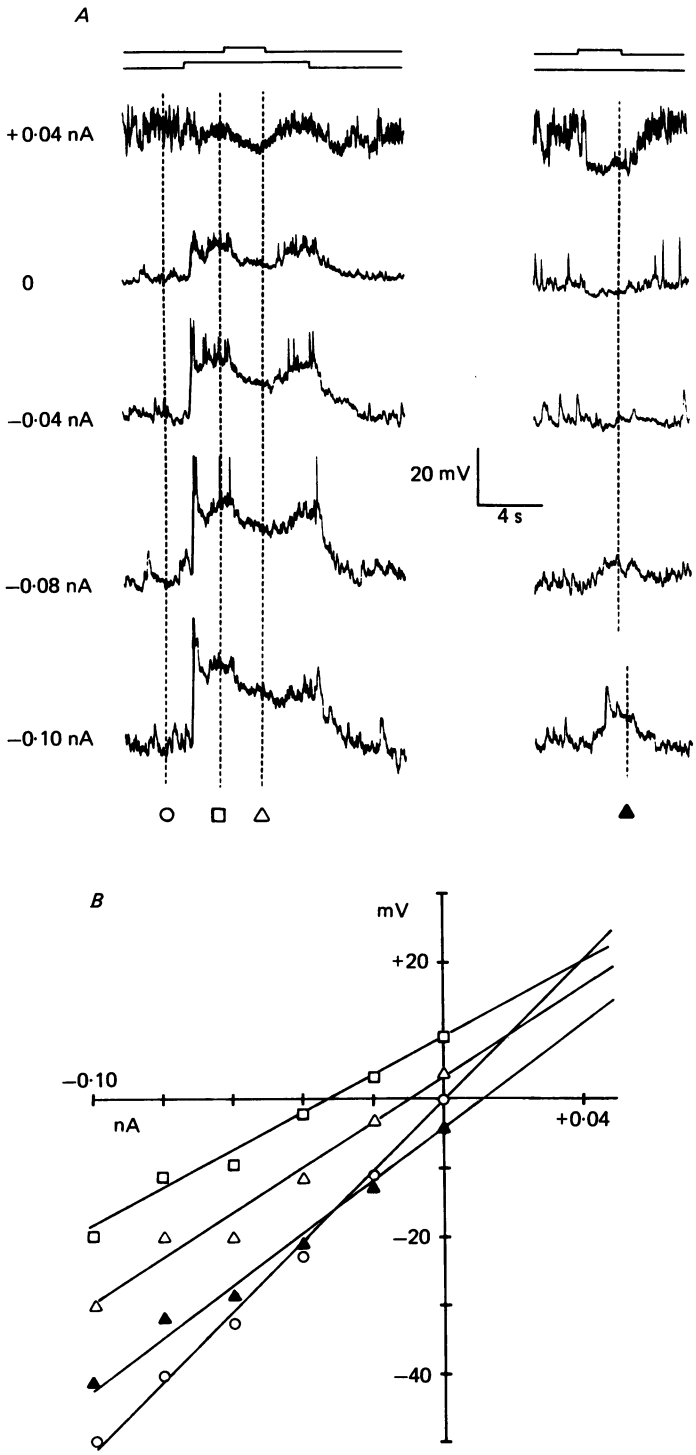

Fig. 1. For legend see opposite.

annular stimulus was presented in the absence of centre illumination (right-hand column of Fig. 1 *A*). Under these conditions the size of the hyperpolarization produced by the annulus became smaller when the cell was hyperpolarized with current and was reversed in polarity by hyperpolarizing currents greater than −0·05 nA. The current–voltage relations (Fig. 1 *B*) show that in the absence of centre illumination the hyperpolarizing response to the annulus (filled triangles) was associated with an *increase* in conductance relative to darkness (circles) and had a reversal potential more negative than the dark potential, indicating that this response was dominated by an increase in inhibitory input.

The results obtained from the twenty-six on-centre ganglion cells studied can be summarized as follows. In the presence of the centre illumination used in these experiments the hyperpolarization produced by the addition of surround illumination was always associated with a net decrease in conductance, indicating a decrease in excitatory input. In twenty-four of these cells the addition of surround illumination caused the reversal potential of the net light-evoked response to become more negative, indicating that it also caused an increase in inhibitory input. In seven on-centre ganglion cells a hyperpolarizing response could be evoked by the annulus in the absence of centre illumination; in every case this response was associated with an increase in conductance, indicating an increase in inhibitory input.

Off-centre ganglion cells

The results from off-centre ganglion cells were similar to those found in on-centre cells, except that the polarities of the responses to centre and surround illumination were reversed. Fig. 2 *A* shows the effect of extrinsic current on the hyperpolarizing response to centre illumination and the depolarizing response to the addition of an annulus. In this cell, which was primarily the subject of a different kind of experiment, the response to surround illumination alone was not studied. However, the responses show very clearly that the degree to which the surround illumination antagonized the centre response varied with membrane potential; in the uppermost trace (+0·04 nA) the addition of the annulus drove the membrane potential only about one-third of the way back to the dark level, whereas in the bottom trace (−0·1 nA) it drove the potential beyond the dark level. This behaviour indicates that

Fig. 1. Effect of extrinsic current on responses of an on-centre ganglion cell to illumination of the receptive field centre and surround. *A*, responses to the same annular stimulus in the presence (left) and absence (right) of centre illumination when membrane potential was displaced to different levels with steady currents (indicated at left of each trace). Duration of annulus and spot stimuli indicated by upward deflections of the two horizontal lines above the response traces (upper line = annulus, lower line = spot). Spot intensity −4·8, annulus intensity −5·1. The vertical dashed lines indicate the time at which the current–voltage measurements were made. *B*, current–voltage relations measured in darkness (○) and during the responses to illumination of the centre alone (□), centre plus surround (△), and surround alone (▲). The times at which the measurements were made are indicated by the vertical dashed lines in *A*. Depolarizing currents were not used in constructing current–voltage plots. In this and all subsequent graphs voltage changes are plotted relative to the resting membrane potential in darkness, and straight lines were drawn by eye through the data points. The slopes of the lines are 520 MΩ (○), 270 MΩ (□), 330 MΩ (△) and 380 MΩ (▲). Resting potential was −51 mV.

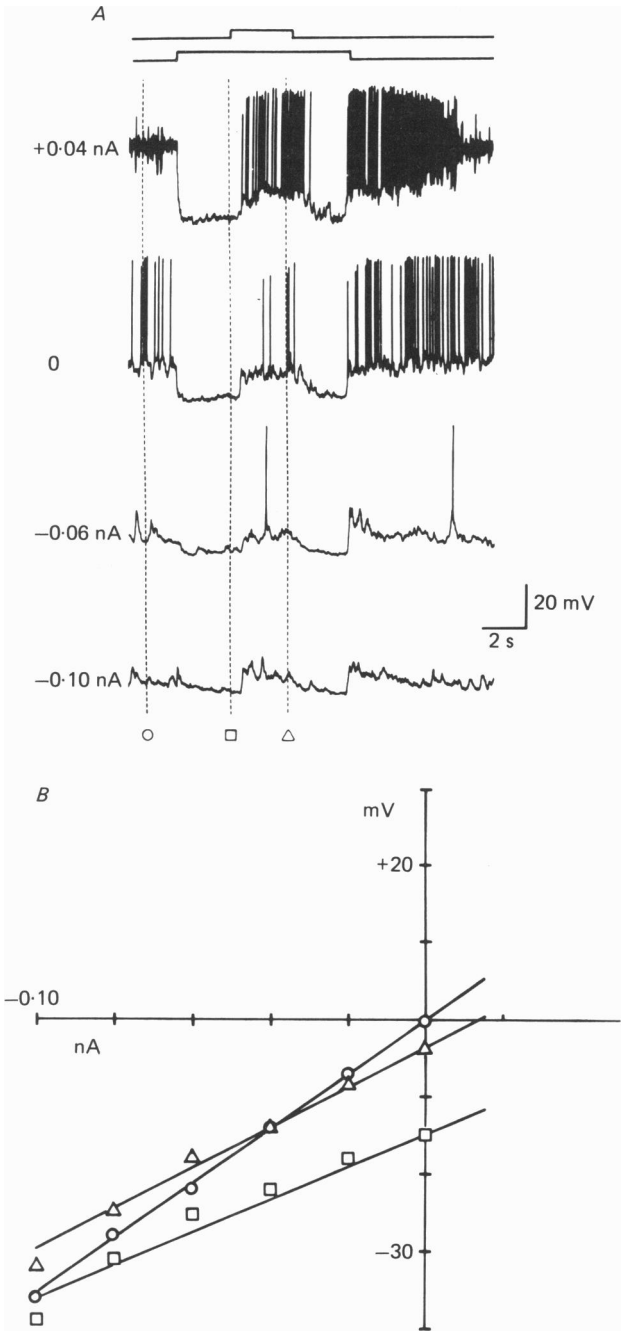

Fig. 2. Off-centre ganglion cell. *A*, effect of current on responses to illumination of the receptive field centre and surround. Intensity of current steps indicated at the left of each trace. Spot intensity −5·7, annulus intensity −5·1. *B*, current–voltage relations measured in darkness (○) and during illumination of the centre along (□) and centre plus surround (△). The slopes of the lines are 360 MΩ (○), 210 MΩ (□) and 260 MΩ (△). Resting potential was −49 mV. Other details as in Fig. 1.

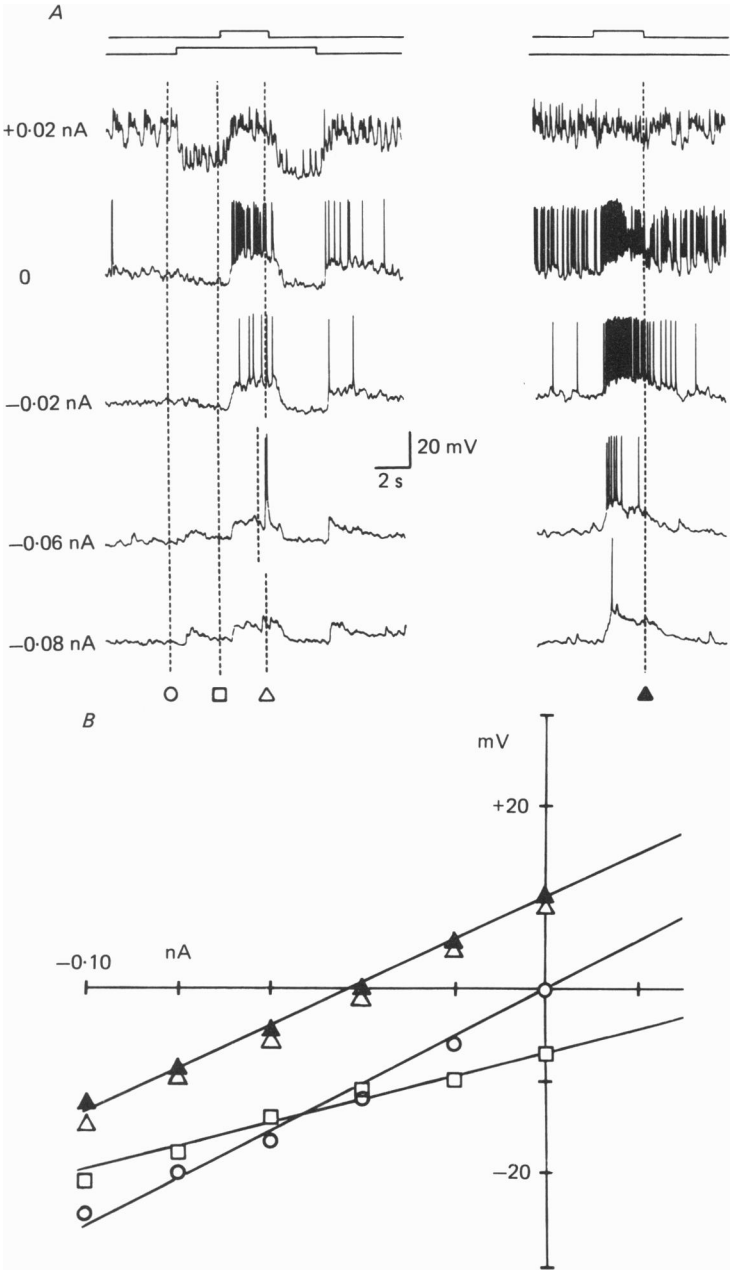

Fig. 3. Effect of current on the responses of an off-centre ganglion cell to illumination of the receptive field centre and surround. *A*, responses to the same annular stimulus in the presence (left) and absence (right) of the centre illumination, at different levels of steady current indicated at left of each trace. Spot intensity −4·8, annulus intensity −4·8. *B*, current–voltage relations measured in darkness (○) and during illumination of the centre alone (□), centre plus surround (△), and surround alone (▲). The slopes of the lines are 260 MΩ (○), 130 MΩ (□) and 230 MΩ (▲). A separate line was not drawn through the open triangles, but its slope should be the same as that of the line through the filled triangles. Resting potential was −50 mV. Other details as in Fig. 1.

at least two different synaptic inputs with different reversal potentials were involved in the light response. The current–voltage relations for this cell (Fig. 2*B*) show that the depolarization produced by the addition of surround illumination (triangles) was associated with a decrease in conductance relative to centre illumination alone (squares), which is consistent with a decrease in inhibitory input. However, the addition of surround illumination caused the reversal potential of the net light-evoked response to become more positive than when only the centre was illuminated, suggesting that surround illumination also caused an increase in excitatory input.

Fig. 3*A* shows responses from another off-centre cell in which surround illumination produced a depolarizing response in both the presence and absence of centre illumination. The left-hand column shows the response to an annulus presented during centre illumination, at different levels of extrinsic current. The current–voltage plots (Fig. 3*B*) show that the depolarization produced by the addition of the annulus (open triangles) was associated with a decrease in conductance relative to centre illumination (squares), indicating that there was a decrease in inhibitory input. However, the conductance during combined centre and surround illumination was not less than the conductance in darkness, even though the membrane was depolarized 10 mV beyond the dark potential. This suggests that the addition of the annulus also caused an increase in excitatory input. An annulus-evoked increase in excitatory input can be seen more directly by observing the responses of this cell to the same annulus in the absence of centre illumination (right-hand column of Fig. 3*A*). The depolarizing response to the annulus alone became smaller with depolarizing current and slightly larger with hyperpolarizing current. The current–voltage relations (Fig. 3*B*) show that the depolarizing response to surround illumination alone (filled triangles) was associated with only a slight increase in conductance relative to darkness (circles), suggesting that it involved both an increase in excitatory input and a decrease in inhibitory input. It is unlikely that the inability to detect a significant conductance change during this response was the result of inaccurate bridge balance, since the response to centre illumination alone, measured during the same current steps, was associated with a significant increase in conductance.

The results from the twenty-four off-centre ganglion cells studied are summarized as follows. In the presence of the centre illumination used in these experiments the depolarizing response to the addition of an annulus was always associated with a decrease in conductance, indicating that it involved a decrease in inhibitory input. In twenty of these cells the addition of surround illumination caused the reversal potential of the light-evoked response to become more positive, indicating that it also caused an increase in excitatory input. In eight off-centre ganglion cells depolarizing responses could be produced by an annulus in the absence of centre illumination, and in every case the response was associated with an increase in conductance.

DISCUSSION

The results of this study show that in on-centre ganglion cells illumination of the receptive field surround can cause a hyperpolarization by simultaneously decreasing excitation and increasing inhibition, and that in off-centre ganglion cells it can cause

a depolarization by simultaneously decreasing inhibition and increasing excitation. These results thus provide physiological evidence for a push–pull control of ganglion cells, as suggested in previous studies (Levine & Shefner, 1977; Belgum *et al.* 1982; McGuire *et al.* 1986), although the presynaptic cells providing the opposing inputs may not be the same in all cases (see below). The experiments reported here show that the relative effect of a given annulus on the excitatory and inhibitory sustained inputs was different in the presence and absence of a given centre stimulus; it was also apparent that the relative effect of a given annulus on the two inputs varied continuously with the amount of centre illumination, but this was not studied in detail.

Although difficult to demonstrate directly, it is likely that centre illumination also acts in a push–pull manner. For example, in Fig. 1*B* it can be seen that the conductance during combined centre plus surround illumination was not significantly greater than during surround illumination alone, even though the cell was 10 mV more depolarized when both centre and surround were illuminated. This is consistent with the idea that centre illumination caused both an increase in excitation and a decrease in inhibition, with the latter effect being enhanced in the presence of surround illumination.

It is not known whether the sustained excitatory and inhibitory inputs are located at significantly different electrotonic distances from the recording site; if they were, the net conductance change measured in the cell soma may not accurately reflect the relative amounts of change that a given stimulus produces in the two inputs. Nevertheless, it can still be concluded that a given stimulus affects the excitatory and inhibitory sustained inputs in opposite directions. It was observed that the reversal potential of the net light-evoked response shifted under different conditions of illumination due to changes in the balance of the synaptic inputs. However, since any light stimulus may cause changes in more than one input, the actual reversal potentials of the synaptic inputs themselves cannot be inferred from the reversal potentials of any particular light-evoked response. When there is a simultaneous increase in excitatory input and decrease in inhibitory input, or vice versa, the reversal potentials of the resulting responses may even be outside the range of the reversal potentials of the actual synaptic inputs (Brown, Muller & Murray, 1971).

In the push–pull system postulated for cat ganglion cells (McGuire *et al.* 1986) the opposing inputs are thought to come from excitatory and inhibitory bipolar cells with light responses of opposite polarity. The present results indicate that there is a push–pull relationship between sustained excitatory and inhibitory inputs to ganglion cells, but the sources of these inputs in the mudpuppy retina are not necessarily the same as in the cat. It has generally been assumed that sustained excitatory input to ganglion cells comes from bipolar cells of the same polarity, but it is possible that some sustained amacrine cells may also mediate this kind of input. Conversely, although inhibitory input is often attributed to amacrine cells, bipolar cells have also been considered as possible sources of inhibitory input to ganglion cells (McGuire *et al.* 1986; Wässle, Schäfer-Trenkler & Voigt, 1986). In mudpuppy, pharmacological studies suggest that the sustained inhibitory input to off-centre ganglion cells comes from amacrine cells rather than bipolar cells (Lukasiewicz & McReynolds, 1985).

The finding that illumination of the receptive field surround can cause a decrease

in the same kind of sustained input that is increased by centre illumination is not surprising, since the responses of bipolar cells, which either directly or through amacrine cells provide the centre-activated input to ganglion cells, already show a centre–surround receptive field organization. This is at least in part due to lateral interactions mediated by horizontal cells in the outer plexiform layer (Werblin & Dowling, 1969; Baylor, Fuortes & O'Bryan, 1971; Naka & Witkovsky, 1972; Werblin, 1974; Thibos & Werblin, 1978*a*; Gerschenfeld, Piccolino & Neyton, 1980; Lasansky, 1981; Murakami, Shimoda, Nakatani, Miyachi & Watanabe, 1982). A surround-induced reduction in synaptic input to ganglion cells could also be the result of some lateral interaction in the inner plexiform layer, such as presynaptic inhibition of transmitter release from bipolar or amacrine cell terminals, but at present there is no evidence that such a mechanism contributes to centre–surround antagonism in ganglion cells.

On the other hand, it has not been shown previously that steady surround illumination can cause an increase in sustained synpatic input of opposite sign to that which is increased by centre illumination, although this has been suggested to occur in cat ganglion cells (Ikeda & Sheardown, 1983). The integration of sustained excitatory and inhibitory inputs could play an important role in the receptive field organization of ganglion cells if the opposing inputs had different receptive field properties. For example, the ganglion cells inputs that are increased by surround illumination might be from wide-field amacrine cells driven by both peripheral and central bipolar cells. Alternatively, both the excitatory and inhibitory sustained inputs may have similar receptive field organizations, in which case their integration may not significantly alter the basic receptive field organization of the ganglion cell. The latter arrangement is implied in the push–pull hypothesis for β ganglion cells in cat (McGuire *et al.* 1986), in which the opposing sustained inputs are from depolarizing and hyperpolarizing bipolar cells. However, McGuire *et al.* (1986) noted that there was still some uncertainty as to whether all of the bipolar cells involved in the push–pull mechanism have antagonistic surrounds, and that inputs from amacrine cells may also affect the receptive field organization of the ganglion cells. At present, the extent to which integration of the sustained excitatory and inhibitory inputs contributes to the receptive field organization of ganglion cells in mudpuppy is also unresolved since there is insufficient information about the receptive fields of the inputs themselves.

The steady centre–surround antagonism described in this paper is quite different from another type of lateral interaction in the inner retina, namely the wide-field, change-sensitive (transient) inhibitory input to ganglion cells. This type of input has been described in mudpuppy, tiger salamander and turtle (Werblin, 1972; Schwartz, 1973; Werblin & Copenhagen, 1974; Thibos & Werblin, 1968*b*; Marchiafava, 1979; Wunk & Werblin, 1979, Belgum, Dvorak & McReynolds 1984), and is thought to be mediated by on–off (transient) amacrine cells. However, transient inhibition is active only at the onset and termination of stationary stimuli and thus does not contribute to steady centre–surround antagonism in ganglion cells.

In summary, even though the circuitry is still unresolved, it seems clear that illumination of the receptive field surround, and probably also of the centre, can

modulate the sustained excitatory and inhibitory inputs to ganglion cells in a push–pull manner.

We thank Dr Daniel Green for helpful comments on the manuscript. This work was supported by NIH Grant EY01653.

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