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A SIGN-REVERSING PATHWAY FROM RODS TO DOUBLE AND SINGLE CONES IN THE RETINA OF THE TIGER SALAMANDER

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SUMMARY

1. Signal transmission between rods and cones was studied by passing current into a rod and recording the voltage response in a nearby double or single cone and vice versa. Two types of rod–cone interaction were found.

2. Between immediately adjacent rods and cones, passage of current into either receptor elicited in the other receptor a sustained voltage response of the same sign as the injected current. These signals were still seen in the presence of Co^{2+}, and are probably mediated by the electrical synapses which have been seen anatomically between adjacent rods and cones.

3. In addition to this short-range sign-preserving interaction, passing current into a rod elicited a transient sign-inverted signal in cones up to at least 80 μm from the injected rod. No such response was seen in rods for current injection into cones. This signal was greatly reduced by Co^{2+} ions. Hyperpolarization of the cone to about -65 mV, with about 0·1 nA current, reversed this signal, which is presumed to be mediated by a chemical synaptic input to cones.

4. Light flashes suppressed the sign-inverted signal for a period which was longer for brighter flashes. The time of reappearance of the signal was correlated with the return of the rod and horizontal cell potentials to their dark levels. This suppression could also be produced by an annulus of light which produced no light response in the receptors at the centre of the annulus, but which did polarize horizontal cells under the centre of the annulus.

5. The wave form of the sign-inverted signal was similar to that produced in horizontal cells by current injection into rods, but of opposite sign.

6. If an electrode was left in a cone for some time, the normal hyperpolarizing light response diminished, leaving a depolarizing response produced, presumably, by feed-back from horizontal cells. This signal was reversed when the cone was hyperpolarized with about 0·1 nA current.

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7. These data suggest that the sign-inverted response is mediated by feed-back from horizontal cells and, assuming that depolarization increases the rate of release of horizontal cell synaptic transmitter, then the feed-back transmitter opens channels in the cone membrane whose currents have a reversal potential around −65 mV.

INTRODUCTION

In the vertebrate retina, cones are hyperpolarized when illuminated by small spots of light, but also receive a depolarizing input when receptors some distance away are illuminated (Baylor, Fuortes & O'Bryan, 1971; O'Bryan, 1973). This antagonistic surround response is thought to be mediated by horizontal cells, through a sign-reversing synapse to the cones (often called the feed-back synapse). The surround response contributes to the centre–surround organization of the bipolar cell receptive field, and may be involved in edge enhancement and colour analysis early in the retina (Schantz & Naka, 1976; Stell, Lightfoot, Wheeler & Leeper, 1975).

Numerous studies have shown that it is difficult to evoke an explicit cone depolarization by simply illuminating the area surrounding a cone: it is thought that light scattered to impinge on the cone itself produces a hyperpolarization which can outweigh the surround-induced depolarization. To avoid this problem, a centre light spot has been used to desensitize photo-transduction in the central cone (Baylor *et al.* 1971; O'Bryan, 1973; Lasansky & Vallerga, 1975; Burkhardt, 1977). We have studied the feed-back pathway in a different way, using the fact that horizontal cells in the tiger salamander retina receive synaptic input from rods and cones (Hanani & Vallerga, 1980). By passing current into a rod, horizontal cells can be polarized (Attwell, Werblin, Wilson & Wu, 1981), and thus one might hope to elicit a feed-back response in cones.

Using this approach we have been able to examine the physiological properties of the feed-back synapse independently of the use of light stimuli. Our conclusions differ in several respects from those of Lasansky (1981), who examined the feed-back synapse in the same animal.

METHODS

Preparation

Experiments were carried out on small larval tiger salamanders, *Ambystoma tigrinum*, with eye diameters of 2–3 mm. The living retinal slice and flat-mounted retina preparations described in detail by Werblin (1978) and Attwell & Wilson (1980) were employed. In the retinal slice preparation, thin sections of retina are oriented so that all cell types are visible to the experimenter, and may be impaled at will. After setting up the preparations in dim red light, all subsequent viewing was carried out using an infra-red TV system (Cohu Inc., Palo Alto, CA, U.S.A.; Model 4415 equipped with silicon vidicon tube) attached to the microscope. Recording techniques were as described by Attwell & Wilson (1980). The micro-electrodes used had resistances of 100–300 MΩ, measured in the perfusing medium, when filled with 3 M-potassium acetate. Electrodes were inserted into cells under visual control, using modulation contrast optics (Hoffman Modulation Optics Inc., Greenvale, NY, U.S.A.).

Electrodes were inserted into the outer or inner segments of rods, but always into the inner segments of cones. Visual observation was sufficient to ensure that an electrode penetrated the desired rod in the flat-mounted retina, but was not always sufficient to guarantee that an electrode penetrated the desired cone, because the cones sit deeper in the receptor layer than the rods and are more difficult to see. Consequently, when attempting to penetrate a cone, the electrode

occasionally entered a neighbouring rod. To guard against this, we routinely recorded the light responses of all the cells penetrated, and positively identified the cells from the sensitivity and time course of the responses. Double cones are easier to penetrate than single cones and most experiments were carried out on the former, but some experiments were done on single cones. Rod to double cone transmission was studied between more than 100 pairs of cells.

Horizontal cell bodies in the retinal slice were identified initially by their position close to the outer plexiform layer, with processes extending in this layer. This identification was confirmed by recording the voltage response to light, and occasionally by filling with the fluorescent dye Lucifer Yellow CH (generously provided by Dr Walter Stewart of the N.I.H.)

When necessary, data were averaged with a Northern Scientific NS-560 signal-averaging computer and played out on a Houston Instruments Omnigraphic 2000 $X-Y$ plotter.

Solutions

Preparations were maintained at room temperature (24–27 °C), in an oxygenated medium comprising either (i) Leibovitz L15 culture medium (Gibco, Grand Island, NY) made up to 38% normal strength, to which supplementary ions were added to bring the calculated final concentrations (mM) of the major constituents to: NaCl 104; KCl 2·1; $CaCl_2$ 3·6; Na_2HPO_4 0·5; $MgCl_2$ 0·4; $MgSO_4$ 0·3; glucose 5, HEPES 5; pH adjusted to 7·6 with NaOH; or (ii) a conventional Ringer solution comprising (mM): NaCl 108; KCl 2·5; $MgCl_2$ 1·2; $CaCl_2$ 2, HEPES 5; pH 7·7. Results obtained in these solutions were not significantly different. In experiments in which the effect of cobalt was investigated, 2 mM-cobalt chloride was added to the conventional Ringer solution. The L15 culture medium could not be used for these experiments because amino acids in this medium bind cobalt and reduce its effective concentration.

Light source

The preparation was illuminated with light from a quartz halogen source, which could be passed through narrow band (10 nm half-width) interference filters and through neutral density filters. The light was transmitted to the preparation through the microscope objective, after introduction into the microscope light path through a beam-combining prism. Unless stated otherwise in the text, the illumination pattern on the preparation was a 500 μm diameter circle (the largest diameter stimulus possible with this apparatus). The light source used in these experiments was not calibrated in absolute units, and intensities are quoted in the text in $\log_{10}$ units relative to the intensity of the unattenuated beam. For comparison, in a parallel series of experiments with an absolutely calibrated light source, isolated rods were found to give a half-maximal voltage response to a step of 520 nm wave-length light of intensity $6{\cdot}1 \times 10^2$ ($\pm 1{\cdot}3 \times 10^2$ S.D., $n = 10$) photons/μm^2 per sec. The corresponding figure for isolated single cones was $1{\cdot}5 \times 10^5$ ($\pm 0{\cdot}9 \times 10^5$ S.D., $n = 6$) photons/μm^2 per sec of 620 nm light. Results for rods and cones in the intact retina, illuminated with a large spot of light, were similar to those for isolated cells.

RESULTS

Light responses

The resting potentials of rods, single cones and double cones in the isolated flat-mounted retina ranged between −35 and −50 mV. Fig. 1 shows light responses recorded simultaneously from a rod and one member of a double cone. The peak light responses which could be elicited were typically 20 mV in rods and 15 mV in cones. No significant difference was observed between the sensitivity and time course of the light responses of single and double cones. Spectral sensitivity measurements (D. Attwell, F. S. Werblin, M. Wilson & S. M. Wu, in preparation), showed that almost all the rods recorded from were so-called 'red' rods, with a peak sensitivity in the green at $\lambda_{max} = 520$ nm, while all the single and double cones studied had a peak sensitivity near $\lambda_{max} = 620$ nm (Attwell, Werblin & Wilson, 1982*a*). Simultaneous recording from both halves of double cones showed that each half had the same

spectral sensitivity, but that the two halves were not electrically coupled (D. Attwell, F. W. Werblin, M. Wilson & S. M. Wu, in preparation).

Response of double cones to current injection into rods

The standard protocol used was to insert one electrode into a rod and another into a double cone in the isolated flat-mounted retina. Current steps were then passed into the rod, and the response in the cone recorded (and vice versa). Since the experiments

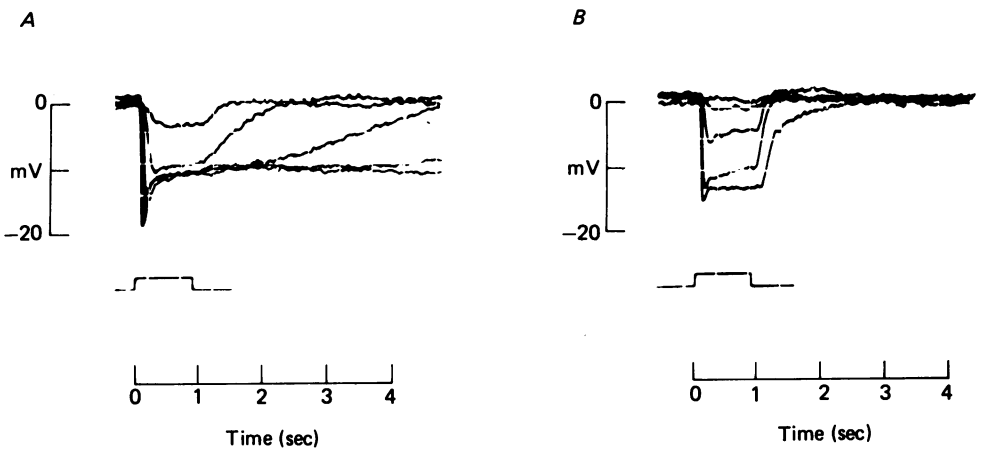

Fig. 1. Simultaneously recorded light responses of a rod (*A*) and one member of a double cone (*B*) in the isolated flat-mounted retina. These cells were immediately adjacent (about 10 μm apart). Rod resting potential −50 mV; cone resting potential −40 mV. Timing of broad-field flash shown by lower trace. Intensities of white light flashes used, given in $\log_{10}$ units relative to the intensity of the unattenuated beam (log (intensity) = 0) were: −5, −4, −3, −2, −1. The rod response to bright flashes shows a pronounced peak–plateau sequence and a prolonged tail after the flash, while the cone shows almost no peak–plateau sequence and a faster return to base line after the flash.

were carried out under visual (infra-red) control, the relative positions of the cells studied was known and the distance separating them could be measured directly from the TV monitor. Fig. 2 shows the results obtained for a pair of next neighbour cells (approximately 10 μm apart). When current was injected into the rod, there were two components to the cone response. These were seen most clearly in the response to hyperpolarizing current (Fig. 2*A*, second trace). First, there was a sustained component of the same sign as the injected current (Attwell & Wilson, 1980, Fig. 3). Such a response might be expected from the evidence that there are electrical (gap) junctions between neighbouring rods and cones (Custer, 1973; Gold & Dowling, 1979), and is in accord with observations in the toad (Fain, 1976) and turtle (Schwartz, 1975; Owen & Copenhagen, 1977) that an attenuated cone light response can, under favourable circumstances, be seen in rods. Secondly, at the termination of the hyperpolarizing pulse, there was initially a repolarization of the cone followed by a transient hyperpolarization (arrow). A similar transient hyperpolarization was seen at the onset of a depolarizing pulse (Fig. 2*A*, upper trace, arrow). For depolarizing pulses, the sustained component with the same sign as the injected current was

smaller than for hyperpolarizing pulses, and in Fig. 2*A* its onset is obscured by the transient hyperpolarization.

Fig. 2*B* shows the response of the same rod when current was injected into the cone. No sign-inverted signal was seen. However, there was a sustained hyperpolarizing response to hyperpolarizing current, and a smaller depolarizing response to

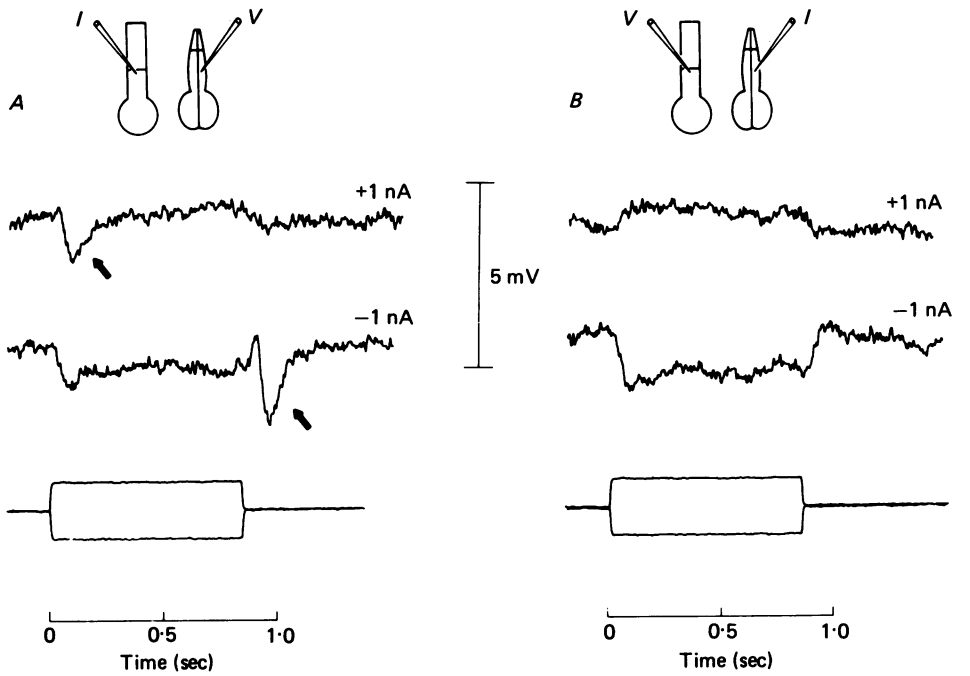

Fig. 2. The sustained sign-preserving component, and the transient sign-inverting feed-back component, of rod–double cone transmission. This rod and double cone were next neighbours (10 μm apart) in the isolated flat-mounted retina. Rod and cone resting potentials were both −40 mV. All traces are the average of four responses. *A*, the response of one member of the double cone to +1 nA (upper trace) and −1 nA (middle trace) injected into the rod. Current is shown as the lower trace. The slight 'non-squareness' of the current trace is the result of sampling the signal at discrete times in the averaging procedure (i.e. due to the low temporal resolution of the averager). Passing current into the rod is found to elicit both a sign-preserving response in the cone, probably mediated by electrical coupling, and a transient sign-inverted response (arrows), probably mediated by feed-back from horizontal cells. *B*, response of the rod to +1 nA (upper trace) and −1 nA (middle trace) injected into the cone. Only a sustained sign-preserving response is seen, probably because there is no horizontal cell feed-back to rods.

depolarizing current. The asymmetry of the sustained response in Figs. 2*A* and *B*, to depolarizing and hyperpolarizing currents of equal magnitude, is similar to that seen for electrical coupling between rods, and is probably due to the outward rectification of the receptor membrane current–voltage relations (Attwell & Wilson, 1980; Attwell *et al.* 1982*a*).

The sustained component of the response was usually only seen for rod–cone pairs that were immediately adjacent (about 10 μm apart). However, the sign-inverted response was recorded at rod–cone separations up to 80 μm (i.e. about 5 times the

distance between neighbouring rods), and its wave form and amplitude did not appear to depend significantly on the distance between the cells. For technical reasons we could not investigate transmission between cells separated by more than 80 μm. The

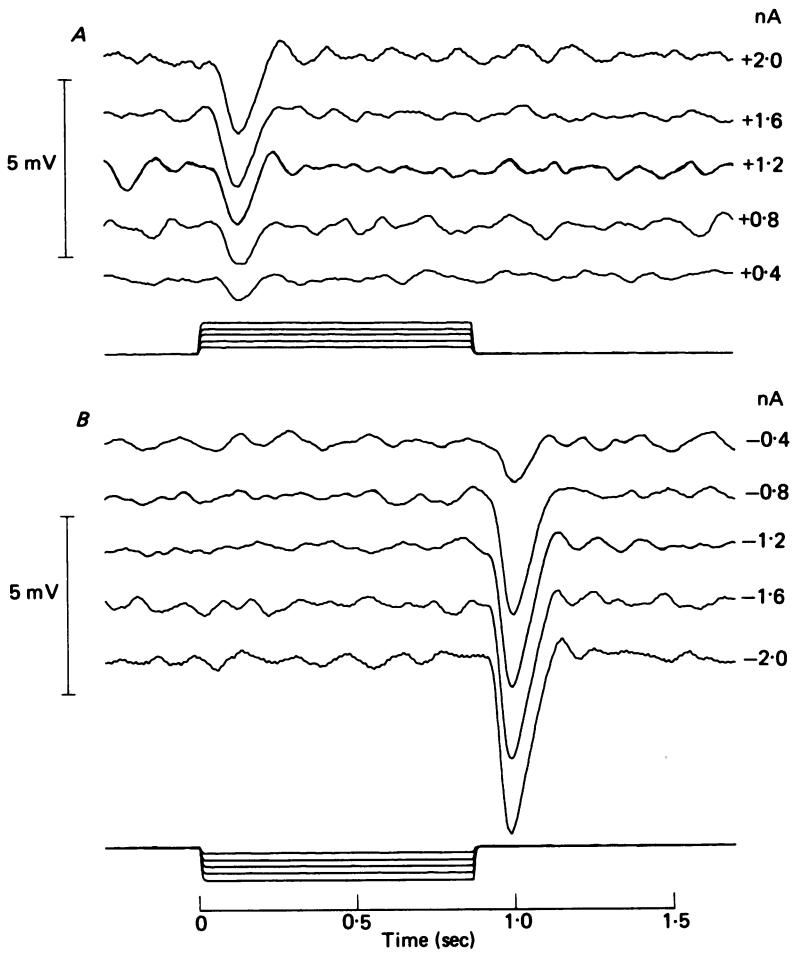

Fig. 3. The response of one member of a double cone (resting potential −40 mV) to current injected into a rod (resting potential −50 mV). Cell separation about 25 μm in the isolated flat-mounted retina. Only a sign-inverted response is seen at this separation, at the onset of the depolarizing pulse, and at the termination of the hyperpolarizing pulse. All traces are the average of four responses. *A*, voltage responses (upper traces) to depolarizing currents (lower traces). The injected current is given by each trace. *B*, the responses to hyperpolarizing currents. Slight 'non-squareness' of the current traces is due to sampling of the signal at discrete times during the averaging procedure.

maximum sign-inverted response recorded in a double cone, at the termination of a 1 nA hyperpolarizing current pulse into a rod, was −4·5 mV. The size of the sign-inverted response was graded with the injected current, and could be detected for hyperpolarizing currents as small as 0·2 nA injected into a rod: this current polarizes the rod into which current is injected by about 20 mV (Attwell & Wilson,

1980). Fig. 3 shows the dependence of the sign-inverted response on injected current for a rod and double cone separated by about 25 μm.

In approximately 30% of double cones no sign-inverted response was seen. This might be due to some cones not receiving the synaptic input underlying the response, but might alternatively reflect disruption of the synapse during impalement with the recording electrode.

The responses of cones which showed only a sign-inverted component to the signal (Fig. 3) were strikingly similar in wave form to horizontal cell responses elicited by passing current into a rod, although of the opposite sign (Fig. 4: these data were obtained using the retinal slice preparation). The termination of a hyperpolarizing pulse produced in both cells a larger response than did the onset of a depolarizing pulse, and the onset of a hyperpolarizing pulse produced a small or negligible response.

Strictly speaking, the responses in Fig. 3 (obtained in the flat-mounted retina) cannot be compared directly with those in Fig. 4 (obtained in the retinal slice). Slicing of the retina will alter the electrical geometry of the rod network and thus make the presynaptic rod voltages produced by current injection different in the two cases. Furthermore, the slicing will cut off horizontal cell processes, removing synaptic input to the horizontal cells from the rods and possibly altering the horizontal cell input resistance. Nevertheless, for rough comparison of the response wave forms these are probably not serious problems because: (i) when current is injected into a rod the voltage responses in rods at various distances away are similar in the retinal slice to those in the flat-mounted retina, i.e. in addition to the response becoming smaller with distance it also becomes more transient (Attwell & Wilson, 1980, fig. 2); (ii) the light responses of horizontal cells in the retinal slice are similar to those recorded in the flat-mounted retina or eyecup, with peak amplitudes up to 50 mV (D. Attwell, F. S. Werblin, M. Wilson & S. M. Wu, in preparation), suggesting that the cut processes of horizontal cells seal over and do not greatly reduce the input resistance.

The responses in Figs. 3 and 4 are remarkably similar in both being much more transient than the square pulse of current injected into the rod. They show similar asymmetries in the magnitudes of the responses to depolarizing and hyperpolarizing current, and to the onset and termination of hyperpolarizing current. However, some systematic differences were observed between the horizontal cell and double cone responses. Firstly, the ratio of the response at the onset of a hyperpolarizing current pulse to the response at the end of the pulse was larger in magnitude for the horizontal cell ($-0{\cdot}27 \pm 0{\cdot}09$ S.D., $n = 15$) than for the cone (0, i.e. no response detectable at the onset of the hyperpolarizing current pulse for all but one of the approximately seventy cones showing the sign-inverted response only). Secondly, the times to the peaks of the responses were different. For the response at the end of a -1 nA pulse into the rod, the horizontal cell depolarization peaked 101 (± 8 S.D., $n = 15$) msec after the current pulse ended, while the cone hyperpolarization peaked 119 (± 23 S.D., $n = 15$) msec after the pulse.

In the remainder of this paper we present evidence that the transient sign-inverted component of the cone response is a feed-back signal from horizontal cells, which are polarized by rod input. In particular, we shall try to rule out the possibility that the sign-inverted response is mediated via a direct chemical synapse from rods to cones, although one is described anatomically by Lasansky (1973). A detailed analysis of rod–horizontal cell transmission will be published later.

The sustained and sign-inverted components of the cone responses were dissociated

in experiments like those in Fig. 2 carried out in the presence of 2 mM-cobalt chloride. It was not practical to change the perfusing solution, and thus record the response of one cone in the presence and absence of cobalt. For cones in the presence of cobalt, the sign-inverted signal was greatly reduced. In fifteen of the sixteen cells studied, no sign-inverted response could be seen. (In the other cell no sign-inverted response could be seen for hyperpolarization of an immediately adjacent rod, however

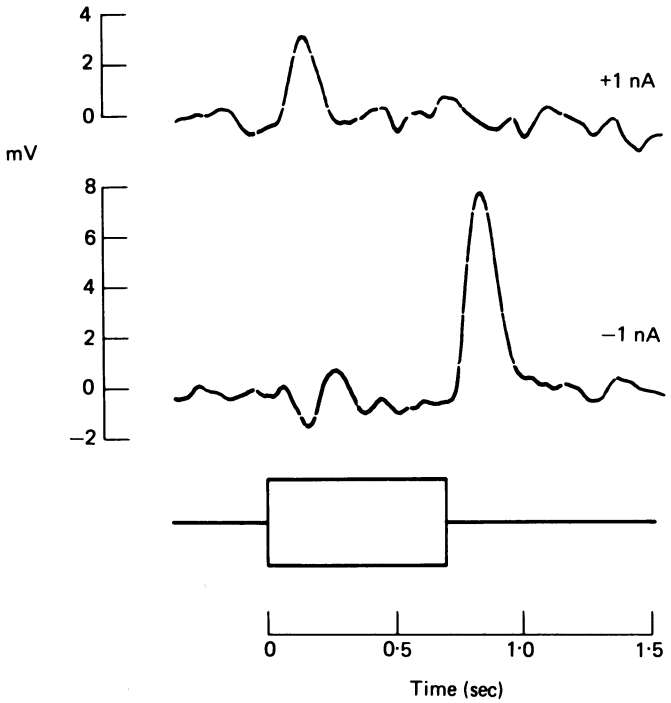

Fig. 4. responses from a horizontal cell body in a retinal slice to current steps of ±1 nA (bottom trace) injected into a rod. The onset of the hyperpolarizing current was followed by a small hyperpolarizing transient and, after an oscillation, a very small sustained hyperpolarization. The termination of hyperpolarizing current generated a transient depolarization of about 8 mV. The upper trace shows a depolarizing transient of less than half this magnitude produced at the onset of a depolarizing current into the rod. Resting potential in this horizontal cell was −35 mV.

depolarization of the rod with 1 nA produced a small (0·3 mV) hyperpolarization in the cone, following the onset of the current step.) However, the sign-preserving response in cones in cobalt was as seen in cones in normal solution. This is consistent with the hypothesis that the sustained signal is mediated via electrical synapses, while the sign-inverted signal passes via at least one chemical synapse (Weakly, 1973; Marshall & Werblin, 1978).

The majority of the experiments were carried out on double cones, since these were easier than single cones to penetrate with the electrodes. Nevertheless, some experiments were performed on single cones. Fig. 5 shows the response of a single cone in the isolated flat-mounted retina when current was injected into a rod about

25 μm away. A sign-inverted response is seen, similar to that seen in double cones (experiment performed in nine single cones).

In some experiments both electrodes were inserted into cones in the isolated flat-mounted retina, either both in (separate) double cones, or one in a single cone and one in a double cone. When current was passed into one cone, a sign-inverted response was only rarely recorded in the other cone (two out of thirty-two experi-

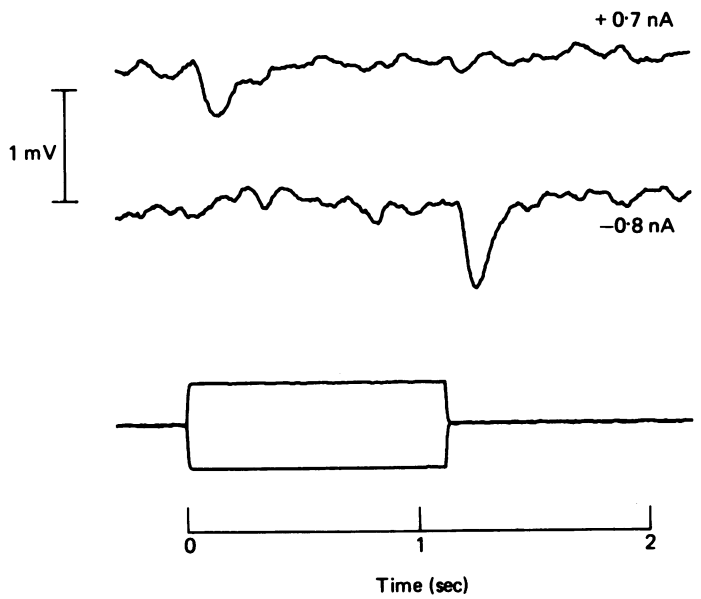

Fig. 5. The sign-inverted response in a single cone in the isolated flat-mounted retina. Voltage responses in a single cone when +0·7 nA (upper trace) or −0·8 nA (middle trace) were injected into a rod 25 μm away from the cone. Rod resting potential −40 mV. Cone resting potential −35 mV. Current shown as bottom trace. All traces are averages of four responses.

ments), although both cells exhibited good light responses. Polarization of a cone also occasionally evoked a small *sign-preserving* response in another cone. This is attributable to transmission via rods, through electrical junctions (Attwell, Werblin, Wilson & Wu, 1982*b*). No direct electrical junctions have been observed anatomically between salamander cones (Custer, 1973).

Reversal of the sign-inverted response

An important test for the existence of a conventional chemical synapse is to see whether the synaptic potential can be reversed by polarization of the post-synaptic membrane. While recording the sign-inverted response in a double cone as in Fig. 3, we polarized the cone by passing current through the recording electrode with a bridge circuit. In fourteen double cones the sign-inverted response was reversed by hyperpolarization of the cone (Fig. 6). The reversal occurred for an injected current of approximately −0·1 nA. An injected current of −0·2 nA always converted the transient sign-inverted hyperpolarization into a transient depolarization. A detailed

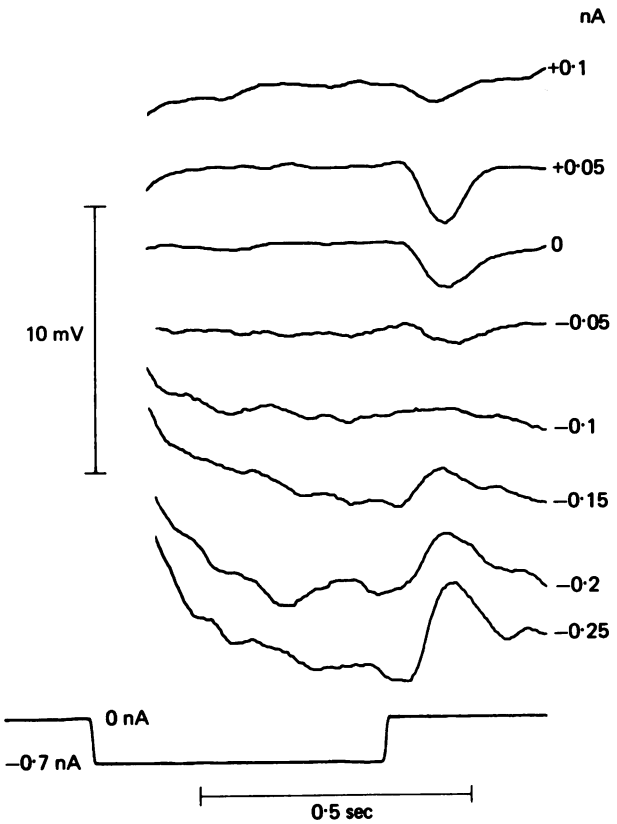

Fig. 6. Reversal of the sign-inverted response by polarization of the double cone. Experiment performed on the isolated flat-mounted retina. Rod resting potential −50 mV. Cone resting potential −40 mV. These cells were next neighbours which happened to show only a sign-inverted response with no sustained sign-preserving component. Hyperpolarizing current pulses (−0·7 nA, lower trace) were repeatedly passed into the rod to elicit a transient hyperpolarization at the end of the pulse. The double cone was then polarized by passing current through the recording electrode. This polarizing current was turned on just before the start of the records shown, and turned off just after the end of the records. The polarizing current is given by each trace. The transient hyperpolarization is reduced in magnitude for −0·05 nA hyperpolarizing current, is essentially flat for −0·1 nA current, and is reversed for −0·15 nA current. Further hyperpolarization increased the magnitude of the inverted response. The drift in potential seen at the start of these records for large currents is an artifact generated by the passage of current across the recording electrode. Depolarizing current of +0·05 nA increased the response; further depolarization reduced it. Traces for hyperpolarizing currents are averages of ten responses. Traces for zero and depolarizing currents are averages of four. Vertical separation of these traces is of no significance.

investigation of the time course of the response near its reversal was prevented by the noise induced in the recording electrode by the polarizing current. However, it appeared that the response consisted of one component which reversed at a single potential, rather than two components with different reversal potentials as suggested by O'Bryan (1973). Small depolarizing currents into the cone increased the sign-

inverted response; larger depolarizing currents reduced, but never reversed, the response.

For one particular cone, a significant sustained depolarization was elicited in the cone during a hyperpolarizing pulse into the rod, in addition to the larger transient hyperpolarization seen at the end of the pulse (cf. the very small sustained hyperpolarizing response recorded in horizontal cells during rod hyperpolarization: Fig. 4). These two phases of the response reversed together when the cone was hyperpolarized with the bridge circuit, suggesting that they were mediated by the same synaptic input rather than two separate inputs.

These data strongly support the idea that the sign-inverted response is mediated by a conventional chemical synaptic input to cones. The reversal potential of the synaptic current can be estimated if we assume that members of double cones have membrane properties similar to those of single cones. Attwell *et al.* (1982*a*) found that isolated single cones have membrane resistances around 250 MΩ near the resting potential. If the resting potential is −40 mV, a hyperpolarizing current of 0·1 nA will thus maintain the cone potential at about −65 mV. The true reversal potential may be less negative than this, however, because: (i) the input resistance of cones in the retina may be lower than that of isolated cones because of the weak electrical coupling to neighbouring rods, and the possible presence of a tonic synaptic input from horizontal cells raising the membrane conductance; (ii) there may be some voltage decrement between the cone inner segment and the site of synaptic input at the axon terminal. The initial increase, and subsequent reduction of the sign-inverted response by depolarization of the cone are also explained by the data of Attwell *et al.* (1982*a*, Fig. 3). For small depolarizations from the resting potential the cone resistance stays roughly constant, and the potential is further from the reversal potential, so the response increases. For larger depolarizations, although the driving force on the synaptic current is larger, the cone slope resistance is much lower, and hence the voltage response is smaller.

The synaptic conductance activated following a hyperpolarizing current pulse into a rod can be estimated if the cone membrane is treated as ohmic near the resting potentials. (This is only an approximation because there is a time-dependent gated current in the cone membrane – see Attwell *et al.* (1982*a*, Fig. 3) for data from single cones.) Suppose that the cone I–V relation with no synaptic input is expressed as $g_m(V-V_{dark})$, where g_m is the conductance and V_{dark} the resting potential, and the synaptic current is written as $g_s(V-V_s)$, where g_s is the synaptic conductance and V_s is the reversal potential. Then, if we ignore the cone capacitance, for an injected depolarizing bridge current, I_{inj}, the voltage, V, is given by

$$I_{inj} = g_m(V-V_{dark}) + g_s(V-V_s).$$

When the synapse is activated, the change of potential from its initial value, at this injected bridge current (with $g_s = 0$), is thus

$$V-\{V_{dark}+I_{inj}/g_m\} = \frac{g_s\{g_m(V_s-V_{dark})-I_{inj}\}}{g_m\{g_m+g_s\}}. \quad (1)$$

This reverses when $I_{inj} = g_m(V_s - V_{dark})$, so $g_m(V_s - V_{dark}) = -0{\cdot}1$ nA. For no injected current ($I_{inj} = 0$ in eqn. (1)), therefore, the potential change produced by the synapse is

$$V - V_{dark} = \frac{-0{\cdot}1\ nA}{g_m}\left(\frac{g_s/g_m}{1+g_s/g_m}\right). \quad (2)$$

For our best cell, the peak hyperpolarization produced (with no injected bridge current) after a 1 nA hyperpolarizing pulse into a rod was $V - V_{dark} = -4{\cdot}5$ mV. Using this in eqn. (2), with $g_m = 1/(250\ M\Omega)$, gives $g_s = 0{\cdot}88$ nS $= 1/(1139\ M\Omega)$ for

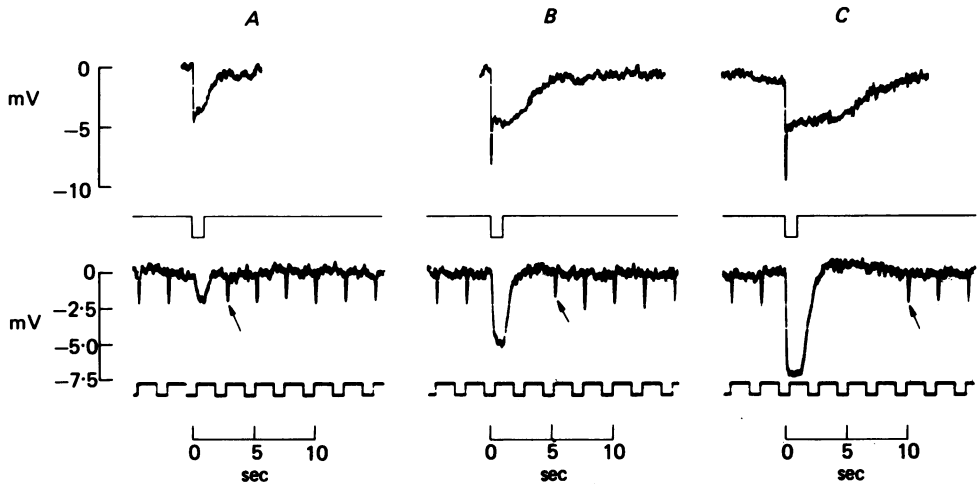

Fig. 7. Suppression of the sign-inverted response by light. The top trace shows the response of a rod in the isolated flat-mounted retina to broad-field white light flashes of $\log_{10}$ (intensity) $= -3$ (A), -2 (B) and -1 (C) (intensity relative to that of the unattenuated beam). Rod resting potential -50 mV. Flash timing shown by the depression in the second trace. The third trace shows the potential of one member of a double cone (resting potential -40 mV), recorded while a -1 nA hyperpolarizing pulse was repeatedly passed into the rod (bottom trace). At the termination of each -1 nA pulse a transient hyperpolarization of about 2·5 mV is seen in the cone potential. While repeatedly eliciting this sign-inverted response, the light flashes were again applied (timing given by second trace). The cone hyperpolarizes in response to light, as expected, but in addition the sign-inverted signal is suppressed for a time which depends on the flash intensity. The arrows show the first full-size sign-inverted response to reappear after the flash. Rod and double cone separated by about 25 μm.

the peak synaptic conductance. Including the membrane time constant and the gated current in the cone would increase the calculated synaptic conductance. Note that the current needed to reverse the synaptic potential, $I_{inj} = g_m(V_s - V_{dark})$ in eqn. (1), does not depend on g_s. Thus, the activation of g_s during the experiment of Fig. 6 does not alter the reversal potential estimated above from that experiment, as

$$V_s = V_{dark} + I_{inj}/g_m.$$

Suppression of the sign-inverted response by light

We investigated how the sign-inverted response, elicited by current passed into a rod in the isolated flat-mounted retina, is affected by light. The protocol was first

to record the rod and double cone responses to light flashes of various intensities, without passing current into the rod. Then a current pulse was repeatedly passed into the rod, and the same light stimuli were applied. Fig. 7 shows that the sign-inverted response is suppressed by light. For bright flashes the period of suppression extends to times when the cone potential has returned to its original (dark) level, so the suppression is not simply due to the cone potential approaching the reversal potential for the synaptic input. For the same reason, the suppression cannot be due to the cone input resistance being lower during the light response (and, in fact, the resistance is likely to be higher (Attwell *et al.* 1982*a*)). Bright flashes suppress the response for

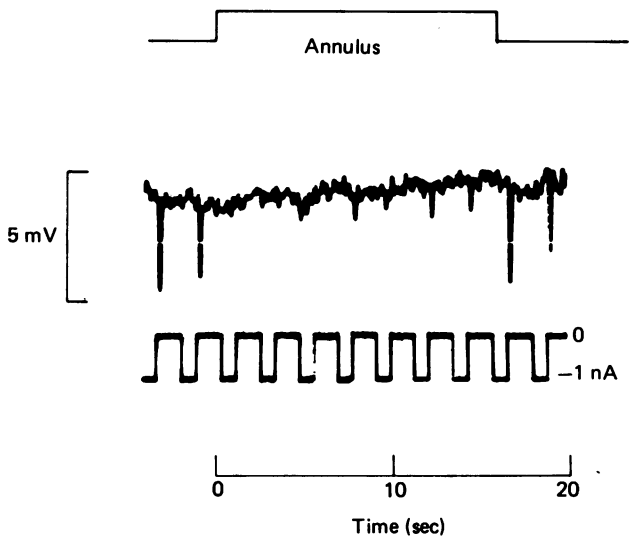

Fig. 8. Suppression of the sign-inverted response by an annulus of light. A 1 nA hyperpolarizing pulse (lower trace) was repeatedly passed into a rod (resting potential −45 mV) to elicit a transient hyperpolarization, at the termination of each pulse, in a double cone (resting potential −40 mV, about 40 μm away from the rod). An annulus of white light, with inside diameter 300 μm and outside diameter 500 μm, reduced the sign-inverted response, although it gave no detectable response in the rod. Experiment performed in the isolated flat-mounted retina.

a longer period than dim flashes do. Comparing the time course of suppression with the time course of the rod response to the flashes (Fig. 7) reveals a close correlation between the two: the sign-inverted response returns to its full amplitude just as the rod potential returns to its dark level. Recordings made in the retinal slice showed that the potential of horizontal cells also returned to its dark level at about this time.

One hypothesis for the mechanism of this suppression is that a synapse mediating the signal from the rod to the double cone is hyperpolarized out of its operating range by the light stimulus. If the sign-inverted signal is transmitted via the horizontal cells (see later), this could be either the synapse from rods to horizontal cells, or the synapse from horizontal cells to double cones. Alternatively, if the sign-inverted signal is transmitted by a direct synapse from rods to double cones (see Discussion), then this would be the one which is hyperpolarized out of its operating range.

Pathway for the sign-inverted signal

In order to distinguish whether the sign-inverted signal passes via horizontal cells or via a direct pathway from rods to double cones, an annulus of light, centred on the cone, was used to polarize horizontal cells. These experiments were carried out in the isolated flat-mounted retina preparation. The intensity of the annulus was adjusted so that it hyperpolarized horizontal cells, as verified by direct recording from horizontal cells at the centre of the annulus, but did not elicit any measurable response in either rods or cones at the centre of the annulus.

Fig. 8 shows that the sign-inverted signal from rods to double cones was suppressed in the presence of the annulus (ten cells). Since horizontal cells, but neither the rod nor the cone, were hyperpolarized by the annulus, the experiment suggests that horizontal cells are involved in the transmission pathway from rods to double cones. We cannot distinguish here whether horizontal cells carry the sign-inverted signal, or simply modulate a more direct pathway.

The light-elicited feed-back signal in cones

To test further whether the sign-inverted signal evoked by rod polarization passes via horizontal cells, we investigated whether the feed-back signal elicited in cones by light absorption in surrounding receptors had the same reversal potential as the sign-inverted response.

To do this, while avoiding the necessity of using an annulus of light to evoke a feed-back signal (with the concomitant problem of light scatter to the recorded cone), we made use of a fortuitous discovery. If an electrode was left in a double cone for a long time (or if the cone outer segment was deliberately damaged before impalement), the normal hyperpolarizing light response to broad-field illumination (500 μm diameter) decreased. The mechanism by which this occurred is uncertain, although it presumably involved some degradation of the transduction machinery, perhaps by ions diffusing into the cell from the recording electrode, or from the extra-cellular fluid through the leak introduced by the electrode. The result was that, for dim illumination, the cone light response became depolarizing (Fig. 9*A*) because the feed-back input dominated the response (see below). As the light intensity was increased, the light response developed a characteristic transient hyperpolarization when the light was turned off. At higher intensities, the initial phase of the light response became hyperpolarizing for the cell of Fig. 9*A* (although with much slower kinetics than usual), presumably because the normal light response of the cell outweighed the depolarizing input (see below) at these intensities. In other cells the normal hyperpolarizing response was lost completely, even at the highest intensities.

We interpret the responses of Fig. 9*A* as being the sum of (i) a feed-back input (producing a depolarization for the lower intensities and a depolarization followed by a transient hyperpolarization at the end of the light step for log $(I) = -2{\cdot}5$ and $-2{\cdot}0$ in Fig. 9*A*), and (ii) a hyperpolarizing component, due to the photocurrent in the outer segment of the cone from which recordings were being made, which is only seen at higher intensities because of damage to the outer segment. To support this interpretation, Fig. 9*B* shows responses to dim broad-field (500 μm diameter) light recorded simultaneously from a double cone and a horizontal cell body in the retinal

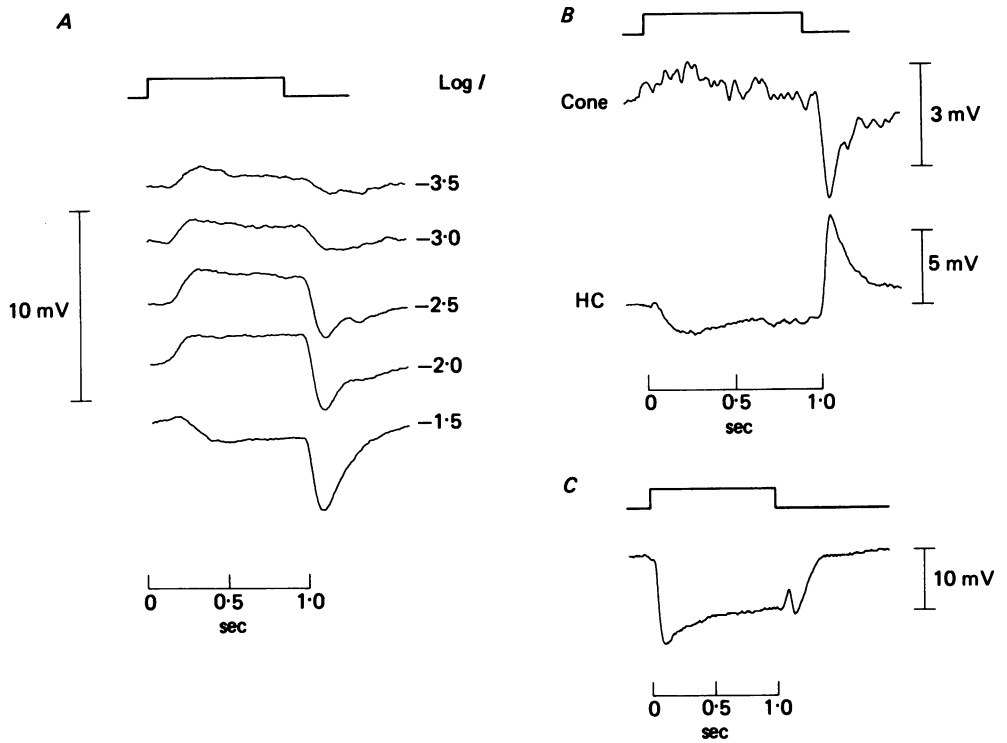

Fig. 9. The light-evoked feed-back signal. *A*, response of a double cone (resting potential −46 mV) in the isolated flat-mounted retina to broad-field illumination (of wave-length 636 nm). Flash timing shown by upper trace. Log_{10} (intensity) values, to the right of each trace, give the intensity relative to that of the unattenuated beam. When the electrode was first put into the cone flashes of these intensities all gave hyperpolarizing light responses, but after a time the hyperpolarizing response diminished, leaving a depolarizing response for dim flashes. Each trace an average of fifteen responses. *B*, simultaneously recorded light responses from a double cone (resting potential −40 mV) and horizontal cell body (HC) (resting potential −23 mV) in the retinal slice, to broad-field light (wave-length 636 nm; flash timing shown by lower trace). The cone response is similar to the horizontal cell response, but inverted. This horizontal cell had an atypically small light response probably caused by electrode damage. Other horizontal cells (recorded from other retinae) gave light responses similar in wave form to that shown here, but larger in amplitude, with an initial hyperpolarization of up to 50 mV and a transient depolarization after the flash of up to 20 mV. *C*, response of a double cone in the flat-mounted retina to broad-field white light (flash timing shown by upper trace), showing a transient hyperpolarization after the light is turned off. Average of twenty sweeps. Resting potential −39 mV.

slice preparation. As for Fig. 9*A*, the electrode was left in the cone until the normal hyperpolarizing light response was lost. The wave form of the cone response is similar in shape (although inverted) to that of the horizontal cell response. The depolarizing overshoot in the horizontal cell response at the end of the flash (which may reflect light adaptation: see Werblin, 1974; Normann & Perlman, 1979) contrasts with the rod response at the end of a similar light flash, which does not show a fast depolarization at all. Thus, we attribute the depolarizing responses (and transient

hyperpolarizing 'off-responses') in Fig. 9*A* and *B* to synaptic input from horizontal cells, rather than input via the direct synapse from rods to cones (Lasansky, 1973). To make this conclusion more rigorous, it would be desirable to show that horizontal cell axon terminals, as well as cell bodies, show the depolarizing overshoot of Fig. 9*B* (see Discussion).

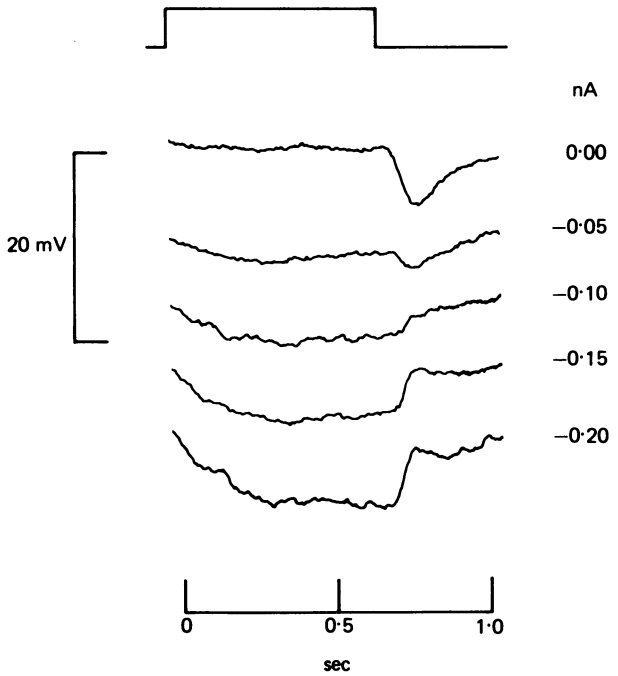

Fig. 10. Reversal of the light-elicited feed-back signal by polarization of the double cone (resting potential −40 mV). Experiment performed in the isolated flat-mounted retina. While a dim broad-field white light flash was applied (upper trace) to evoke a transient hyperpolarization following the flash, the cone was polarized with current through the recording electrode (current given by each trace). Hyperpolarization of the cone inverted the response. Each trace an average of between three and ten responses. The polarizing current was turned on just before the start of the records shown, and turned off just after the end of the records. The drift in potential seen at the start of the records for large currents is an artifact generated by the passage of current across the recording electrode. Vertical separation of these traces is of no significance.

It is possible that these depolarizing responses have been recorded by other workers from damaged cones in the eyecup preparation, but were not recognized as coming from cones. Since our experiments were carried out under visual control, we know these responses are produced in cones. Such depolarizing responses are never seen in single or double cones isolated from the retina (Attwell *et al.* 1982*a*).

Some cones, in the isolated flat-mounted retina preparation, showed a normal hyperpolarizing light response during a step of light, but the normal repolarization at the end of the light step was followed by a transient hyperpolarization (Fig. 9*C*). We attribute this, as for the responses in Fig. 9*A* and *B*, to a feed-back signal produced by the transient depolarization seen in horizontal cells at the end of a light step. Thus, significant feed-back signals can be elicited in cones showing a normal

hyperpolarizing light response. Of the cones which showed a hyperpolarizing response of normal magnitude *during* a step of light, only a small proportion (about 10%) showed the transient hyperpolarization when the light was turned off (cf. Fig. 1*B*). Since the transient horizontal cell depolarization following a light step is apparently only seen in light adapted preparations (Werblin, 1974, Fig. 3; Norman & Perlman, 1979, Fig. 3), it is likely that the transient cone hyperpolarization after the light step only occurs when the retina has become somewhat light adapted. Alternatively, the infrequent observation of the signal might reflect variations in the strength of the synaptic input to individual cones, or may be due to the synapse being easily disrupted during impalement of the cone with the recording electrode. Since the transient hyperpolarization was seen far less often than the feed-back response evoked by current injection into rods (seen in 70% of double cones: see p. 319), we favour the light adaptation hypothesis.

Reversal of the light-evoked feed-back signal

While evoking a transient hyperpolarizing feed-back response at the termination of a light step, as in Fig. 9*A*, the cone being recorded from was hyperpolarized with current through the recording electrode (using a bridge circuit, experiment performed in six cells). Hyperpolarizing currents reduced, and then reversed the feed-back response (Fig. 10). The reversal occurred when about −0·1 nA current was passed into the cone, as for the sign-inverted signal elicited by rod polarization (Fig. 6), consistent with the notion that the same synapse is responsible for both signals. As in the case of the transient cone signal elicited by rod polarization, a critical examination of the response time course near the reversal potential was not possible, although the response apparently consisted of a single component only.

DISCUSSION

Synaptic pathway from rods to cones

Two anatomically defined pathways might mediate the sign-inverting influence of rods on cones. One possible pathway is via the direct chemical synapses from rods to cones shown by Lasansky (1973), and the other is via horizontal cell processes that appear to connect rods to cones. Three lines of evidence support our contention that the sign-inverted response is mediated by horizontal cell processes. (1) The sign-inverted response evoked by current injection into rods can be suppressed by an annulus of light which has no effect upon the potential of the rod and cone under study at the center of the annulus, but which does polarize horizontal cells, thus implicating them in the signal pathway. (2) The response in the cone closely resembles that of the horizontal cell response, but is of opposite polarity. This is true for the light-elicited responses (Fig. 9) and the current-elicited responses (Figs. 3 and 4). Yet both cone and horizontal cell response wave forms differ from those of the rods, suggesting that synaptic input to the cones comes from horizontal cells rather than rods. (3) The reversal potential for the current-evoked sign-inverted response and for the light-evoked synaptic response in cones appear to be the same; both signals reverse when about −0·1 nA is injected into the cone to hyperpolarize it. Since the light-evoked synaptic response probably involves horizontal cell input, the

similar reversal potential for the current-evoked response is consistent with a common pathway for both signals through horizontal cells.

We cannot rule out a contribution of the direct rod–cone synapses observed by Lasansky (1973) to the sign-inverted responses measured in double cones adjacent to an impaled rod. Alternatively these synapses could mediate the sign-preserving signals between rods and cones, although our results showing that the sign-preserving signals persist in the presence of cobalt ions argue against this. Furthermore the putative electrical synapses observed anatomically between neighouring rods and cones (Custer, 1973; Gold & Dowling, 1979) provide a plausible pathway for transmission of this signal.

If horizontal cells are indeed producing the sign-reversed signal seen in the cones, one might expect to elicit this signal in cones by hyperpolarizing horizontal cells directly with injected current. (Baylor *et al.* (1971) have carried out this experiment in the turtle). We have never been able to elicit a response in the cones by polarizing *horizontal cell bodies* in the retinal slice preparation. This might suggest that some lateral process other than the horizontal cell body and dendrites carries the signal to the cones, the most likely candidate being the horizontal cell *axon terminals* (Piccolino, Neyton & Gerschenfeld, 1981). Horizontal cell bodies and axon terminals have been shown to act as independent processing units (Lasansky & Vallerga, 1975). We have been unable to test the effect on cones of polarizing the horizontal cell axon terminals, because they are too difficult to impale reliably under visual control in the slice preparation. An alternative explanation for the negative result might be that a measurable synaptic input to cones can only be produced by the synchronous activity of a larger number of horizontal cells than can be polarized by impalement of a single horizontal cell body.

Reversal of the feed-back response

Our demonstration that the feed-back response can be reversed (Figs. 6 and 10) strongly supports the notion that it is mediated by a conventional chemical synapse. However, Byzov, Golubtzov & Trifonov (1977) have proposed that the feed-back signal is mediated by a novel kind of electrical synapse. During hyperpolarization of the horizontal cell, current flow from the horizontal cell to the cone pedicle was suggested to produce a small depolarization of the cone pedicle membrane, activating a voltage-dependent (calcium) current. (Activation of a voltage-gated current was an essential part of this proposal because the feed-back potential recorded at the cone soma would otherwise have the same sign as the change in horizontal cell potential, rather than being sign-inverted.) Byzov *et al.* (1977) proposed this mechanism because no clear reversal potential had been demonstrated for the feed-back input.

Our demonstration that the sign-inverted response is blocked by Co^{2+} (p. 320) does not rule out the hypothesis of Byzov *et al.* (1977). This block is expected to occur if the sign-inverted response passes via horizontal cells, whether the feed-back synapse to cones is chemical *or* electrical, because Co^{2+} blocks transmission from rods to horizontal cells (Cervetto & Piccolino, 1974; Trifonov, Byzov and Chailakhyan, 1974; Kaneko & Shimazaki, 1975; Marshall & Werblin, 1978). However, our demonstration that the sign-inverted response can be reversed by hyperpolarizing the cone does at least rule out the specific suggestion of Byzov *et al.* (1977) that a voltage-gated Ca^{2+}

current produces the feed-back signal. For their hypothesis to be consistent with our data, the voltage-gated current, rather than being a Ca^{2+} current activated by depolarization, would have to be a current with a reversal potential around −65 mV which is activated by hyperpolarization. Figs. 6 and 10 would then demonstrate the reversal potential of this current. No such current is found in isolated cones (Attwell *et al.* 1982*a*) but it is possible to postulate a current of this sort in the synaptic terminals (which are probably lost in the isolated cones). We assume in what follows, for simplicity, that the feed-back response is mediated by a conventional chemical synapse.

Assuming that hyperpolarization of horizontal cells reduces transmitter release to cones, our results imply that the feed-back transmitter opens channels with a reversal potential more negative than the dark potential. The effect of an annulus of light is to reduce release of transmitter, closing these channels, and thus depolarizing the cone.

These conclusions are in disagreement with the work of Lasansky (1981) in the tiger salamander, and O'Bryan (1973) in the turtle. Lasansky (1981, Fig. 2) claimed that by using micro-electrodes filled with potassium chloride, instead of potassium acetate, the feed-back input to cones could be enhanced. This was attributed to Cl^- ions leaking into the cell and displacing the reversal potential for the feed-back input: Cl^- ions were thus assumed to carry some of the synaptic current, and the reversal potential for this input was proposed to be more positive than the resting potential. This would imply that the horizontal cell transmitter *closes* synaptic channels, or that hyperpolarization of the horizontal cell increases transmitter release.

We were unable to reproduce the results of Lasansky's (1981) Fig. 2. The use of potassium-chloride electrodes never gave a depolarizing cone light response to an annulus (outside diameter 500 μm, inside diameter 300 μm) or large spot of light (diameter 500 μm) in our experiments. (Our light source did not allow the use of stimuli larger than 500 μm, so these stimulus configurations are not precisely comparable to those used by Lasansky.) Furthermore, when the current injection experiment of Fig. 3 was carried out using potassium-chloride electrodes, the transient cone hyperpolarization seen at the end of a hyperpolarizing current pulse into the rod was replaced by a transient depolarization (experiment performed in two cones). This was suppressed by a spot or annulus of light, as in Figs. 7 and 8. Hyperpolarization of the cone with a bridge circuit (cf. Fig. 6) increased the magnitude of the transient depolarization; depolarization of the cone reduced the response, but no reversal could be demonstrated, possibly because the outward rectification of the cone membrane (Attwell *et al.* 1982*a*) made the signal very small. These data are consistent with the feed-back input being mediated partly via Cl^- ions with potassium chloride electrodes displacing the reversal potential from *below* the dark potential to a level *depolarized* from the dark potential. However, the feed-back response must still be mediated via channels which *open* when the horizontal cells depolarize, and which normally have a reversal potential below the dark potential. Consequently, with potassium chloride electrodes we would predict the response to an annulus of light to be hyperpolarizing (and thus not distinguishable from the response to light scattered to the centre of the annulus). We are, therefore, unable to account for the apparent reversal of the *depolarizing* feed-back response at a depolarized potential seen by Lasansky (1981, Fig. 5) when using potassium chloride electrodes.

O'Bryan (1973) concluded that the feed-back input to turtle cones consisted of two components, one of which reversed at a potential depolarized from the resting potential. His data are difficult to interpret, however, because: (i) the time dependence of the voltage drop induced across the recording electrode in his bridge experiments seems to have been highly variable (see the responses in his Fig. 5 to 'spot' and

'spot + annulus' for $+6 \times 10^{-10}$A injected current); (ii) there is strong cone coupling in the turtle retina (Baylor *et al.* 1971) which could account for his observation that the feed-back response was reduced, but not reversed, when the cone was hyperpolarized with current; (iii) a feed-back-activated calcium current may contribute to the response in turtle cones (Piccolino & Gerschenfeld, 1978). We never observed any 'feed-back spikes' of the type attributed to a calcium current by Piccolino & Gerschenfeld (1978). In salamander cones, the reversal of the feedback response (Figs. 6 and 10) gave no indication that more than one synaptic input contributes to the response.

Implications for vision

The antagonistic surround response in conès and bipolar cells is best seen when an annulus of light is applied during illumination of the receptive field centre by a spot of light (Baylor *et al.* 1971; O'Bryan, 1973; Burkhardt, 1977; Werblin, 1977). This is because the presence of the centre spot greatly reduces the effect of light scattered to the centre of the receptive field when the annulus is applied. For two reasons, the surround response is expected to be *decreased* in the presence of *strong* central illumination. First, the feed-back response is reduced by hyperpolarization of the cone, because its reversal potential is negative to the resting potential. Secondly, synaptic transmission between rods, horizontal cells and cones is reduced by light, perhaps because the presynaptic membranes are hyperpolarized towards the end of the potential range over which they can release transmitter. Abolition of the antagonistic surround response has recently been demonstrated, for cones and bipolar cells in the tiger salamander retina, in the presence of strong central illumination (J. Skrzypek & F. S. Werblin, in preparation). It appears, therefore, that the contribution of feed-back in the outer plexiform layer to edge detection and colour analysis (Schantz & Naka, 1976; Stell *et al.* 1975) must depend, via the potential of the cones on the level of illumination on the retina.

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