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CENTRIFUGAL ACTIONS ON AMACRINE AND GANGLION CELLS IN THE RETINA OF THE TURTLE

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SUMMARY

1. An electrophysiological investigation of efferent synapses in the retina of the turtle was conducted by recording intracellularly from amacrine cells. These cells have been selected because in birds they have been shown to have direct anatomical connexions with centrifugal fibre terminals.

2. Amacrine cells could be easily distinguished from most other retinal cells, except ganglion cells, by their different photo-responses. Because both amacrine and ganglion cells may generate action potentials they were distinguished by their responses to optic nerve stimulation.

3. The response of ganglion cells to single shock stimulation of the optic nerve consists of an antidromic action potential followed by a late synaptic potential.

4. Cells which did not show antidromic responses but were electrically excitable, by passing direct current through the recording electrode, were considered to be amacrine cells.

5. Amacrine cells generate an e.p.s.p. in response to optic nerve stimulation. An analysis of the e.p.s.p. suggests that it may be due to a single afferent fibre terminating in the proximity of the cell soma. By analogy to the bird, it is concluded that the amacrine cells e.p.s.p.s result from the activation of centrifugal fibres.

INTRODUCTION

The original description of centrifugal optic nerve fibres originating in the mid-brain (Perlia, 1889; Wallenberg, 1898) and directly terminating upon the amacrine cells of avian retinae (Cajal, 1889, 1892; Dogiel, 1895) has been confirmed by many authors (see for ref. Ogden, 1968, and Cowan, 1970). An important conclusion derived from the recent work is that the centrifugal fibres to the retina are the efferent path of a reflex loop whose central portion, named the isthmo-optic nucleus after Craigie (1928) and Huber & Crosby (1929), receives afferent connexions from the retinal

ganglion cells, via the optic tectum (Cowan, Adamson & Powell, 1961; Cowan & Powell, 1963; McGill, 1964; McGill, Powell & Cowan, 1966*a*, *b*; Holden, 1968*a*, *b*). More specifically, it has been found that a precise retinotopic distribution exists throughout the reflex pathway by which a centrifugal control may ultimately be exerted upon the same restricted retinal areas where the afferent path originated (McGill *et al.* 1966*a*, *b*; Holden & Powell, 1972).

The electron-microscope investigation of Dowling & Cowan (1966) has conclusively shown that, in the pigeon retina, the centrifugal fibres originating in the isthmo-optic nucleus terminate only on amacrine cells, with large, richly vesiculated endings characteristically located upon or near the cell soma. Amacrine cells in turn may transmit their signals both to bipolars and ganglion cells, to which they are extensively connected (Cajal, 1892; Dowling & Boycott, 1966; see for ref. Stell, 1972).

The present work is an attempt to define, by intracellular recording, the functional properties of the centrifugal synapses on amacrine cells, and to describe the secondary effects exerted thereby on ganglion cells. Because these two classes of cells cannot be easily distinguished by their responses to light, particular emphasis is given to some tests leading to the identification of the cell type.

The turtle has been selected for this study because the large size of its cells permits stable intracellular recordings. A drawback of this preparation is the lack of an anatomical demonstration of the centrifugal system. However, the consideration that reptiles represent the phylogenetic continuity between two species in which centrifugal retinal innervation has been found, i.e. amphibians (Maturana, 1958; Branston & Fleming, 1968; Lázár, 1969) and birds, suggests that a similar organization exists also in the turtle. It will be shown that amacrine cells receive a strong depolarizing action from the centrifugal optic nerve fibres, with one efferent fibre (or a branch thereof) for each amacrine cell.

METHODS

The experiments were performed in retinae of the turtle *Pseudemys scripta elegans*. After decapitation of the animals, the eyes were completely isolated from the surrounding tissues together with the optic nerves up to the chiasma, where they were sectioned. The eyes were cut with a razor blade, along a vertical equatorial plane at about 1 mm behind the ora serrata. The vitreous humor was drained as much as possible by positioning narrow leaflets of tissue paper upon the eye-cup for a few minutes until they firmly adhered to the retina. The eye-cup, with the attached optic nerve, was then placed in a moist chamber equipped with two silver wire electrodes connected to a Digipulser (W-P Instruments) to stimulate the central extremity of the nerve. The optic nerve fibre responses to such stimulation were then recorded at the end of each intracellular recording session by means of a silver chloride ball

electrode of small diameter (*ca.* 1 mm) placed upon the optic nerve fibres entrance in the eye, at the level of the 'papilla optica'. The chamber was perfused by a stream of 95 % O_2–5 % CO_2; the room temperature was kept around 22° C. The micropipettes for intracellular recordings usually had a resistance of about 400 MΩ. The electronic equipment used and the apparatus for illumination were of the same types as those described in a previous paper (Lasansky & Marchiafava, 1974).

RESULTS

After penetration of the most superficial layer of the retina, intracellular photoresponses may be recorded from ganglion and amacrine cells. In the few cells in which spikes cannot be evoked by flashes of light, action potentials can be induced by extrinsic currents, either during injection of depolarizing currents or at the end of hyperpolarizing steps.

Naka & Ohtsuka (1975) have already reported that available descriptions of photoresponses are not reliable criteria to distinguish amacrine from ganglion cells. An 'on–off' transient depolarization, for example, is the most frequently described 'amacrine' cell photoresponse (Werblin & Dowling, 1969; Kaneko, 1970; Matsumoto, 1975) but similar responses may be recorded also from ganglion cells, as previously observed by Schwartz (1973). Similar features are present in the 'on–off' ganglion and amacrine cells responses to brief flashes, as shown in Fig. 1*A*. Also, sustained photo responses similar to those recorded by Matsumoto & Naka (1972), Kaneko (1973) and Toyoda, Hashimoto & Ohtsu (1973) from amacrine cells, were obtained from cells which in the present work were identified as ganglion cells (Fig. 1*B*). Hyperpolarizing photoresponses have also been recorded from both ganglion and amacrine cells, identified by the criteria to be described below, without showing significant differences between their time courses (Fig. 1*C*). However, some photoresponses appeared to be unique to ganglion cells (Fig. 1*D*, *E*), since similar responses were never recorded from amacrine cells.

Amacrine cells are difficult to identify even using intracellular staining with Procion dye because the axon departing from ganglion cells is often missed in histological section (cf. Stell, 1972), and therefore ganglion cells can be incorrectly classified as amacrines. To overcome the uncertainties related to such an unfavourable anatomical situation, the two cell types were distinguished in the present work on the basis of their responses in darkness to optic nerve stimulation. These responses are described in the following sections.

A. *Ganglion cells responses*

Fig. 2 shows that a 1 msec electric shock applied to the optic nerve induces an action potential in the penetrated cell. The spike shows an

inflexion along its rising phase similar to the segmentation described by Eccles (1955) and by Fuortes, Frank & Becker (1957) in motoneurones, and hyperpolarization reduces the spike amplitude in an 'all or none' fashion. These events are similar to those occurring following antidromic activation of other nerve cells and can be interpreted assuming that the two components of the responses of Fig. 2 are antidromic spikes blocked at

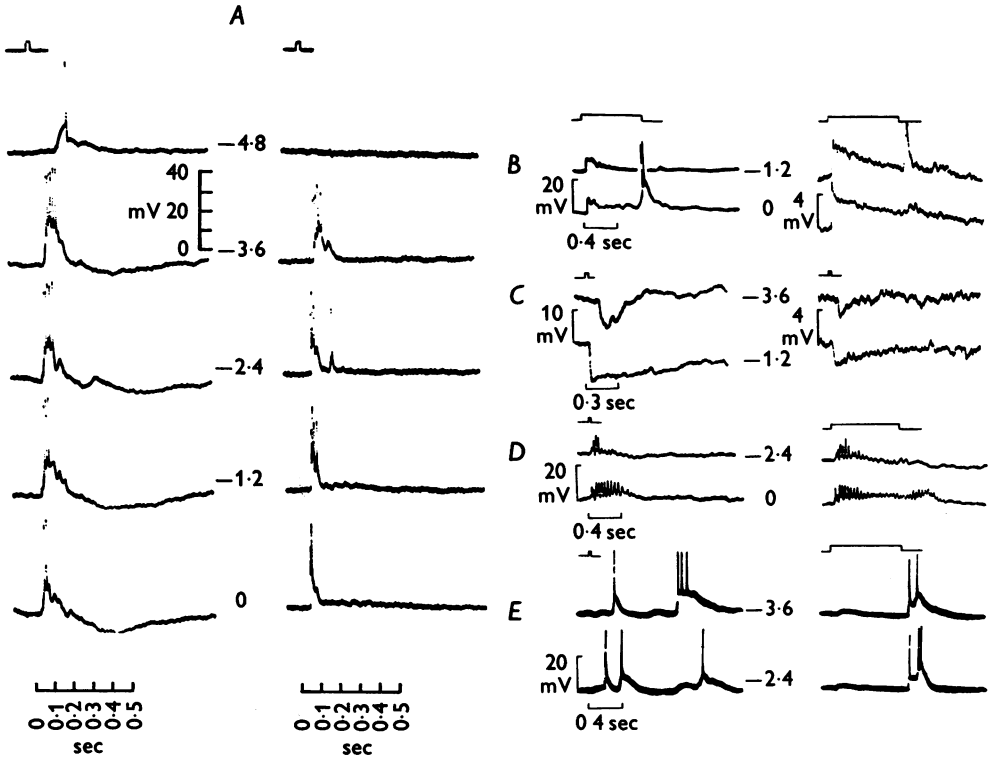

Fig. 1. Intracellular recordings of ganglion and amacrine cell photoresponses. The cells have been identified by using the discrimination criteria described in the text. *A*, ganglion (left column) and amacrine cell responses (right column) to brief flashes (20 msec) of increasing intensity. The flashes started at time zero, as indicated on the upper line. The light intensities are represented by logarithmic units of attenuation, at the middle of each line. Note the similarity between the two series of photoresponses. *B*, ganglion (left column) and amacrine cell (right column) sustained responses to a 920 msec flashes of increasing intensity. Both cells attained the same amount of steady-state depolarization during illumination. *C*, hyperpolarizing responses of a ganglion (left) and amacrine cell (right) to a 20 msec flash. *D* and *E*, ganglion cells responses to a 20 msec (left column) and a 920 msec (right column) flashes of increasing intensity. These photoresponses, in contrast with those shown from *A* to *C* (left column), show a time course which has never been observed in amacrine cells. The flashes are indicated above each pair of responses.

different distances from the recording electrode. It is important, however, to demonstrate antidromic invasion unequivocally since it is possible that the cell is activated orthodromically, producing a large depolarizing synaptic potential with a superposed spike. A useful test to distinguish between these two possibilities is the study of the interactions between the direct spike evoked by injection of current in the soma and the potentials induced by stimulation of the optic nerve. If the small response of Fig. 2 is an axonal spike it will be abolished by collision or refractoriness when the optic nerve is stimulated at a suitable interval after evoking a direct spike in the soma. It will not be abolished if, instead, it is a synaptic potential. In Fig. 3*A*, first line, the direct spike (marked by a circle) precedes the action potential evoked by the nerve stimulation (marked by a square). By

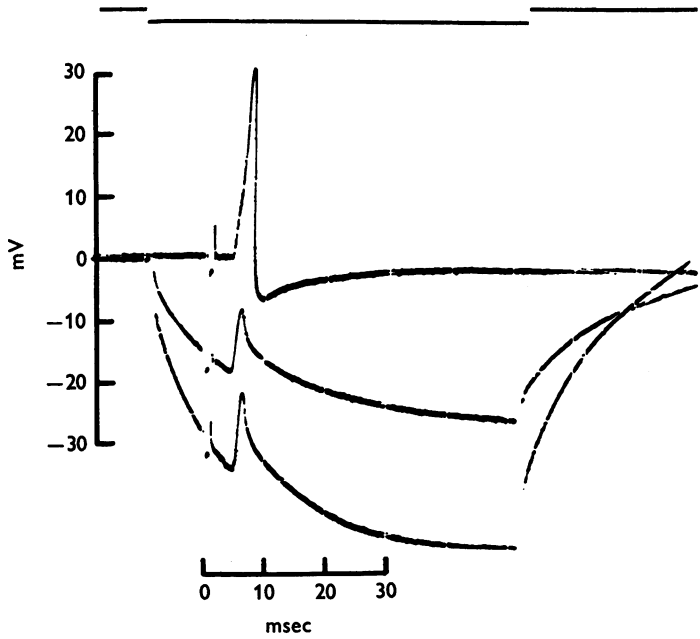

Fig. 2. Intracellular recordings of ganglion cell responses to single shock stimulation of the optic nerve. Superimposed recordings. Stimulus parameters: 1 msec, 1·25 mA. The stimulus current used is just above threshold. The responses were taken in darkness, at resting potential (first trace) and during periods of hyperpolarization induced by injecting current through the micro-electrode (second and third trace). The current injection is indicated in the upper line. Note that hyperpolarization of 20 mV below the resting potential reduced the size of the spike to a smaller response which is not appreciably altered by further hyperpolarization (lower trace). Zero in the voltage scale represents the membrane potential in darkness. The optic nerve stimulation starts at time zero.

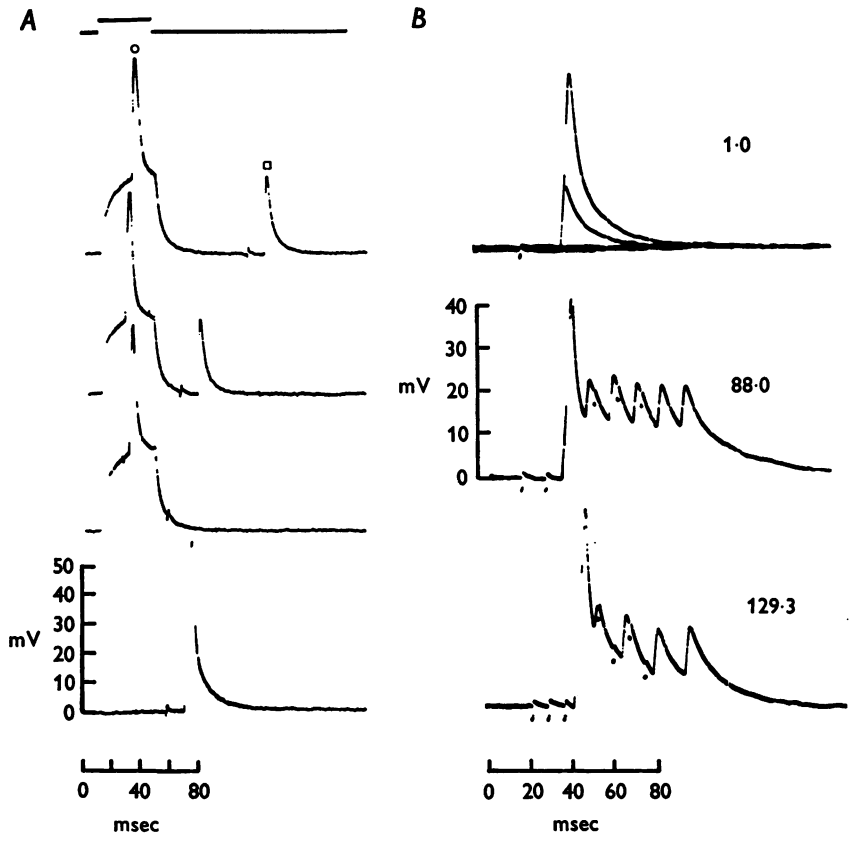

Fig. 3. Abolition by refractoriness of the ganglion cell spike response to optic nerve stimulation. *A*, interaction between a direct spike (indicated by a circle at left of the upper trace) superimposed on a brief depolarizing step, and the spike response to single shock stimulation (1 msec, 2·2 mA) of the optic nerve (indicated by the square at right). The depolarizing step is produced by intracellular current injection, at the time indicated in the upper line. In the upper two traces the second spike fails to invade the soma. When the interval between the two spikes is reduced below a critical value, the spike response to optic nerve stimulation disappears altogether (third trace). The fourth trace shows the full spike response. *B*, spike responses recorded from a ganglion cell during repetitive stimulation of the optic nerve at increasing frequencies. The repetition rate of the stimulus is indicated above each line (impulse/sec). The superimposed responses at 1/sec were obtained with a stimulus intensity at threshold (1 msec, 1·6 mA). At higher stimulus repetition rates, second and third traces, an intensity of 2·8 mA was used.

progressively decreasing the interval between the direct spike and the optic nerve shock the second spike eventually disappears altogether. Since there are not graded synaptic responses replacing the spike which has failed, as one expects to occur in the case of a synaptically induced spike, it may be concluded that the second action potential is antidromically propagated from the optic nerve to the cell body in the retina and disappears as it collides with the direct spike travelling othodromically along the same nerve fibre. The collision interval found in different ganglion cells approximately corresponds to the values calculated from conduction times and refractoriness. The refractory period of the axonal membrane, as determined by repetitive stimulation of the optic nerve, was found in most ganglion cells to vary between 8 and 15 msec (Fig. 3*B*).

In many ganglion cells the antidromic action potential is followed by a graded depolarizing wave, which is not affected by collision. This late response usually has higher threshold than the antidromic response, and gradually augments with the stimulus intensity (Fig. 4*A*). With strong stimuli the late potentials may give rise to local responses (Fig. 4*A*, fifth line) or to action potentials. The synaptic origin of these potentials is indicated by a lower following frequency with respect to the antidromic spike and by their amplitude modulation during repetitive stimulation of the optic nerve (Fig. 4*C*).

The amplitude of the synaptic responses differs from the antidromic response because it depends strongly, but in a graded fashion, upon the level of membrane polarization. Fig. 4*B* illustrates the effects of extrinsic currents upon a ganglion cell response to a 3·0 mA, single shock applied to the optic nerve (the response at resting potential is shown in Fig. 4*A*, fifth line). The antidromic component of the response (indicated by an arrow on the first trace) is not markedly affected by either depolarizing (upper two traces) or hyperpolarizing the cell membrane. The same artificial depolarization, however, is sufficient to bring the peak of the late response to the threshold for a local response or for a full spike in the soma. This is probably due to summation of the artificial depolarization with the late response which does have a greater amplitude than the axonal spike (see Fig. 4*B*, second trace). The artificial hyperpolarization abolishes the regenerative component of the late response (Fig. 4*B*, third trace) which then reflects the time course of an e.p.s.p. In fact, the latter response becomes larger by further hyperpolarizing the membrane, as expected when artificially increasing the voltage difference between the membrane potential and the equilibrium potential of the e.p.s.p.

A slight increase of the axonal spike, as shown in the fourth trace, is sometimes observed. In cells showing this increase the existence of a simultaneous e.p.s.p. can be demonstrated (manuscript in preparation).

The late graded synaptic response of ganglion cells may be interpreted as a signal transmitted by amacrine cells (see Discussion).

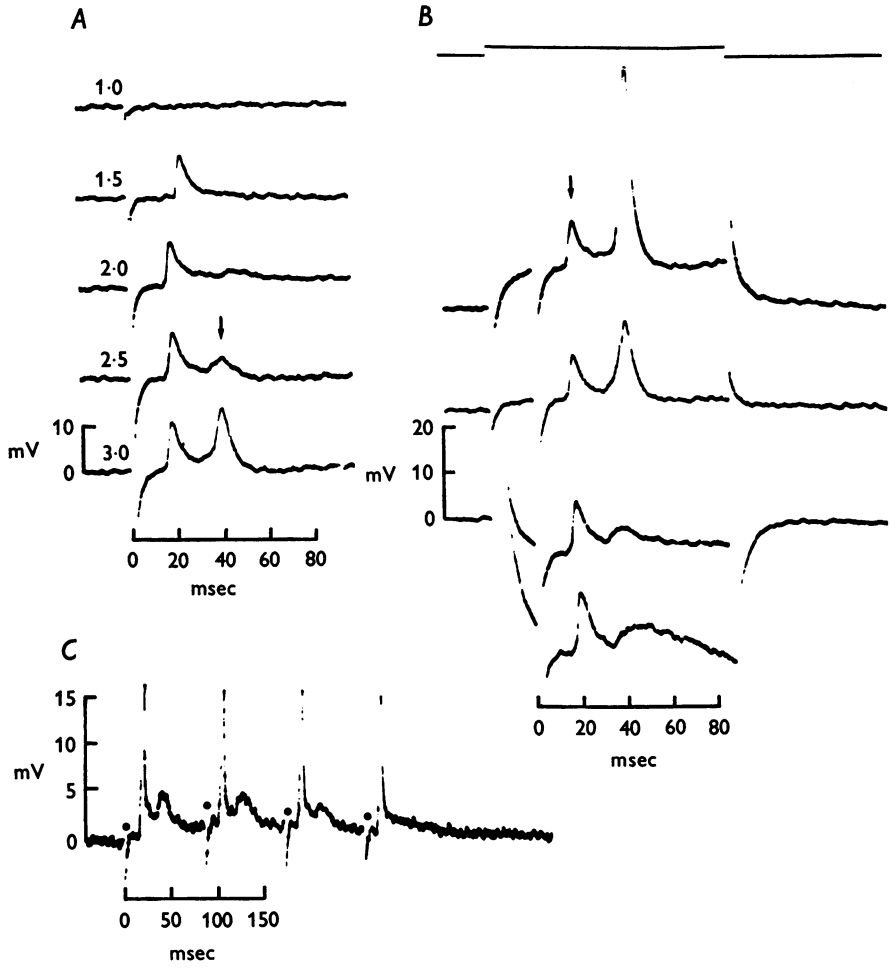

Fig. 4. For legend see opposite page.

B. *Amacrine cells responses*

All cells which upon penetration generated action potentials in response to light or to intracellularly applied currents, but not following electrical stimulation of the optic nerve were identified as amacrine cells.

It may be supposed that at least some of these cells were in fact ganglion cells whose axons remained unexcited by the optic nerve stimulation. This interpretation, however, is not in good agreement with the results of Fig. 5. Here the response to the optic nerve stimulation was recorded at the level

of the papilla optica by means of a small silver ball (diameter *ca.* 1 mm). It includes two negative deflexions corresponding to two distinct groups of fibres conducting impulses at a mean velocity of about 8·5 and 3 m/sec, respectively. (The distance between the stimulating and the recording electrode was about 8 mm.) With a stimulus intensity of about 3 mA, which is about 3 times the threshold (T) for the fastest conducting fibres, the nerve response attains a maximal amplitude which is not modified by further increasing the stimulus strength. Barring serious experimental damage to the nerve, this result shows that all optic nerve fibres are excited with an intensity of about 3 mA. However, the cells to be identified as amacrines, did not show any antidromic response with stimulus intensities up to $5T$.

The amacrine cell response to 1/sec stimulation of the optic nerve consists of a large, fast rising depolarization, but at threshold stimulus intensity sporadic failures are also observed, as shown in Fig. 6, upper left. By increasing the stimulus intensity to $1{\cdot}2$–$1{\cdot}5T$ the response occurs regularly and it maintains the same amplitude even after the optic nerve stimulus is further increased to $2{\cdot}0$–$2{\cdot}5T$. This 'all or none' behaviour

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Legend to Fig. 4.

Fig. 4. Antidromic and synaptic responses of ganglion cells to optic nerve stimulation. *A*, effect of increasing the intensity of the optic nerve stimulus (single shock) upon the ganglion cell response. The stimulus, as in *B*, starts at time zero, and is indicated by the artifacts in the first and subsequent traces. The stimulus intensities (mA) are indicated at the left, above each line. A single shock at low intensity (1 msec, 1·5 mA) induces the antidromic response alone. The antidromic spike fails to invade the soma. A late synaptic response (marked by an arrow on the fourth trace) is elicited with stimulus intensities about 1·5-times the threshold for the antidromic response (2·5 mA). By further increasing the stimulus intensity the e.p.s.p. gives rise to a local response (fifth trace). *B*, the effect of membrane polarizaiton on the ganglion cell responses to high intensity, single shock stimulation of the optic nerve (1 msec, 3 mA). The response at resting potential is shown in *A*, fifth line. The membrane potential is artificially driven by injecting steps of current through the micro-electrode, at the time indicated in the upper line. During current injection the bridge circuit was not compensated. The antidromic response (indicated by the arrow in the first trace) is not appreciably altered with either depolarizing (upper two traces) or hyperpolarizing the membrane (lower traces), but the synaptic response appears to be strongly dependent, in a graded manner, on the level of membrane polarization. *C*, comparison between the antidromic and the synaptic reponses of ganglion cells to repetitive, high intensity stimulation of the optic nerve (1 msec, 4·5 mA). The stimulus artifact is marked by a black dot. The antidromic response follows the stimulus delivered at 11/sec, but the synaptic response is greatly depressed.

suggests that the amacrine response is a 'unitary e.p.s.p.' evoked by the action potential of only one afferent fibre.

During repetitive stimulation of the optic nerve the amplitude of the amacrine cell response shows remarkable fluctuations (Fig. 6) which may be interpreted, as in other synapses, as a consequence of variability in both the amount of transmitter available at the presynaptic terminals and in the probability of release of the individual quanta (Betz, 1970; Christensen & Martin, 1970). These amplitude fluctuations may be due also to the failure of the action potential to invade all the afferent terminals.

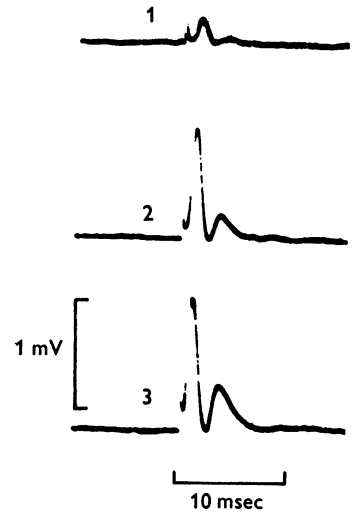

Fig. 5. Extracellular responses to optic nerve stimulation recorded from the papilla optica. The responses were recorded with a small chlorided silver ball (diameter about 1 mm) lying directly over the papilla, against a similar indifferent electrode placed on the sclera. The numbers above each line indicate the stimulus intensity used, expressed in mA. The maximal response was obtained with about 3 mA and its amplitude remained constant when the pulse strength was increased further.

These fluctuations contrast with the stability of antidromic ganglion cell responses to similar stimuli (Fig. 3*B*). Thus, the different behaviour of the two responses to repetitive stimulation becomes a practical, if incomplete, criterion for discrimination of the two cell types whenever their responses to single optic nerve stimuli have similar time courses. A further discrimination test is the absence of collision between the amacrine cell response and the direct spike generated at the same cell. The direct spikes recorded from amacrine cells did not differ significantly from ganglion cells, either in amplitude or in duration. Thus, the observation by Kaneko & Hashimoto (1969) that amacrine's spikes have longer duration than

ganglion cell's was not confirmed. In fact, the antidromic spikes shown in Fig. 3*A* and *B* are longer than the amacrine's spike shown in Fig. 7. Fig. 7 shows that the amacrine cell response to the optic nerve stimulation (marked by the thick arrow in the first trace) never fails at any interval after the amacrine cell action potential. On the contrary, it increases when it occurs during the after-hyperpolarization of the spike. This dependence

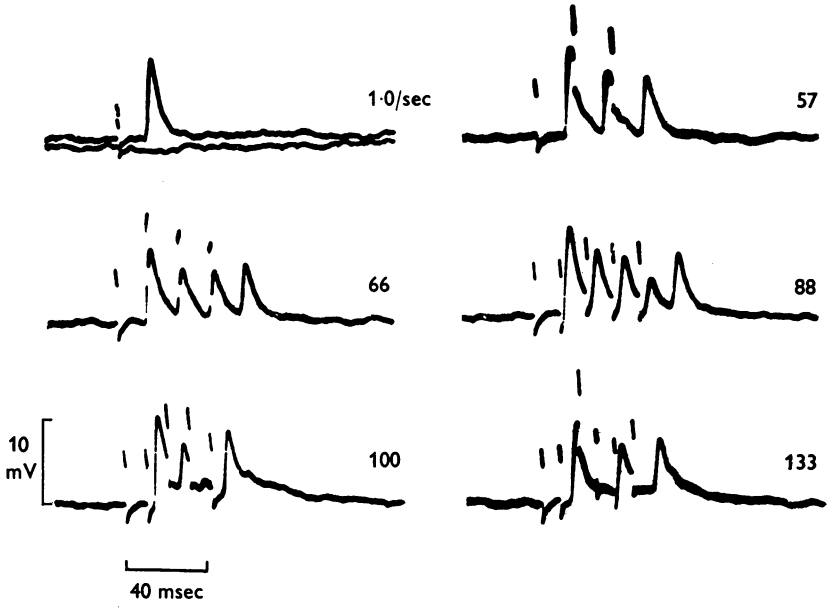

Fig. 6. Intracellular recordings of amacrine cell responses to single shock and repetitive stimulation of the optic nerve. The stimulus repetition rate (impulses/sec) is indicated by the numbers to the right of each line. The response at 1/sec (first trace) was obtained with threshold intensity (2·4 mA). At 1/sec two traces are superimposed to show the 'all-or-none' character of the response. The stimulus artifact is the fast, upward deflexion preceeding the response. With repetitive stimulation the intensity used was 3·5 mA. Stimulus duration: 1 msec. The series of responses to different frequencies of stimulation show the variability of the response amplitude. Note that at the stimulation frequency of 100 and 133 impulses/sec, some responses fail to appear.

of the amacrine response upon the level of membrane potential is better illustrated in Fig. 8. Here, the amplitude of the cell response is decreased or increased, in a graded manner, by artificially depolarizing or hyperpolarizing the cell membrane respectively. With large hyperpolarizations (about 40 mV more negative than the resting potential in darkness), the response amplitude may become more than twice that observed in the resting conditions. These results indicate that the amacrine cell response is

an e.p.s.p., which originates at sites readily accessible to the applied current and therefore is effectively controlled in its amplitude by the current.

It has been found that the times to peak of the e.p.s.p. obtained during repetitive stimulation (as shown in Fig. 6) are all within a narrow range of

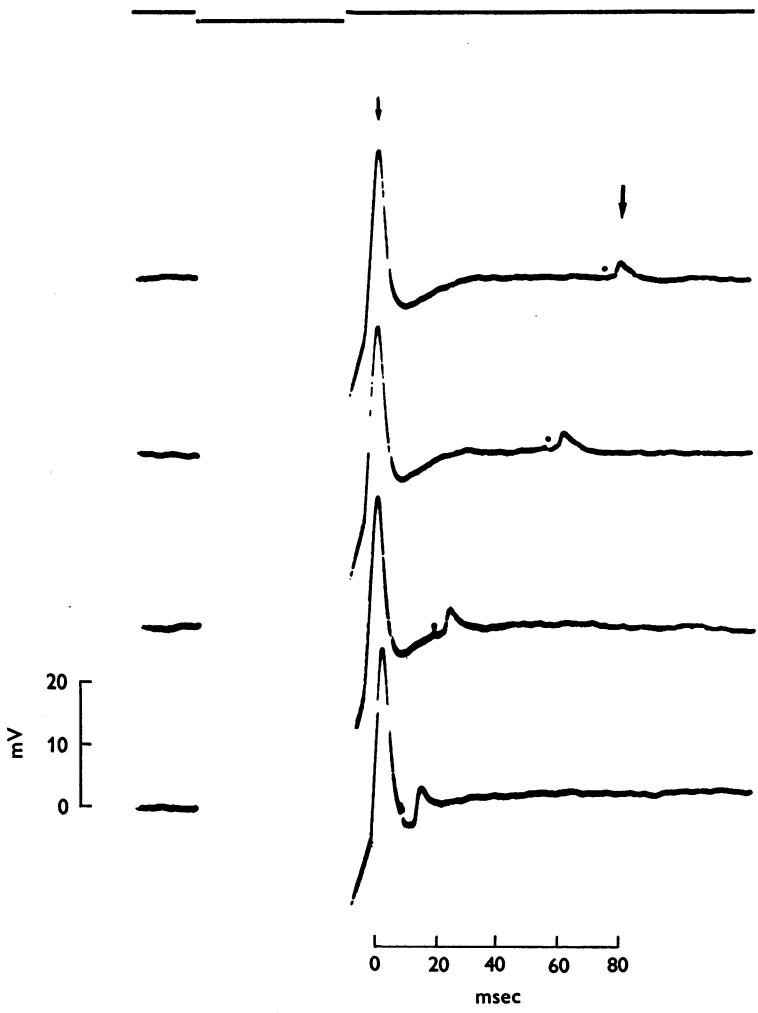

Fig. 7. Interaction between the direct spike and the synaptic response of an amacrine cell. The spike, indicated by the thin arrow at left on the first trace, originates at the end of a large hyperpolarizing deflexion induced by intracellular injection of current (indicated by the upper line). Hyperpolarizing potential is not illustrated. Note that the synaptic response, indicated by the thick arrow at right on the first trace, is not prevented from occurring at any interval from the spike.

values ($3{\cdot}4 \pm 0{\cdot}4$ msec), independently of the e.p.s.p. amplitude. Since the amplitude variations of the e.p.s.p. are probably the consequence of random failures of the component quantal e.p.s.p. (cf. Martin, 1966), a change in the response rise time should be observed if any of the quantal e.p.s.p. were generated by inputs from terminals located at varying distances from the recording site. In fact, according to Rall (1967), the potential transients are affected by electrotonic distortion as a function of the distance between

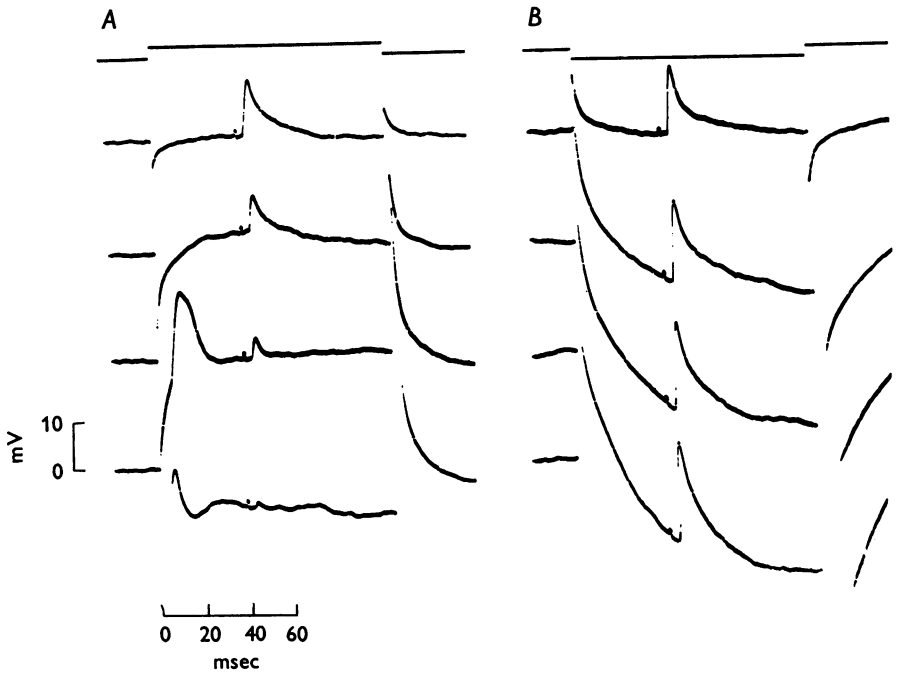

Fig. 8. The effect of membrane polarization on an amacrine cell synaptic response to single shock stimulation of the optic nerve. The cell membrane was alternatively depolarized (*A*) or hyperpolarized (*B*) by injecting current steps of increasing intensities (from top to bottom trace) through the microelectrode. During current injection (indicated by the upper lines in *A* and *B*) the bridge circuit of the recording amplifier was not balanced. Note the gradual decrease in amplitude of the synaptic response with increasing the level of depolarization, and the response increase with hyperpolarization.

the generation site of the e.p.s.p. and the recording electrode location. It may be concluded that the amacrine cell e.p.s.p. is generated by either one or more synaptic terminals closely grouped together; furthermore, the short time constant of the e.p.s.p. and its sensitivity to extrinsic currents suggest that the synaptic endings on the cell membrane should be close to the electrode recording site (cf. also Rall, Burke, Smith, Nelson & Frank, 1967).

C. Recording from other retinal cells

Cells which do not generate spikes either directly, or after a flash of light are penetrated at deeper retinal levels than ganglion and amacrine cells. Some of these cells show graded photoresponses with antagonistic concentric receptive fields, strongly resembling those of bipolar cells (Kaneko, 1973; Werblin & Dowling, 1969; Toyoda, 1973; Schwartz, 1974; Naka & Ohtsuka, 1975), others were recognized as either horizontal or receptor cells

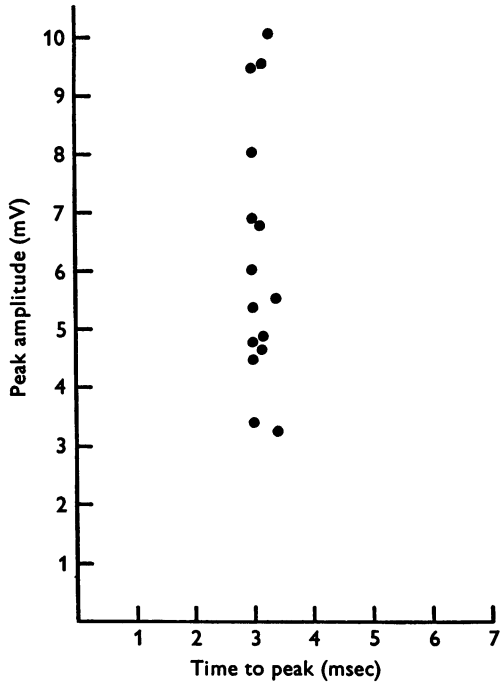

Fig. 9. Graph of the relationship between the time to peak and the amplitude of an amacrine cell response to optic nerve stimulation. The data were obtained from the same cell illustrated in Fig. 6.

in accordance with previous reports (Baylor & Fuortes, 1970; Fuortes, Schwartz & Simon, 1973; Fuortes & Simon, 1974; Simon, 1973; see for ref. Simon, 1974). In receptors and horizontal cells the stimulation of the optic nerve never produced an effect, confirming the negative evidence reported by Gliozzi (1966) in the fish. One may suppose that the 'reciprocal' type of synaptic arrangement described by Dowling & Boycott (1966) may transmit the amacrine synaptic response to bipolar cells, but bipolar cells recordings have not been numerous enough to confidently draw any conclusion.

DISCUSSION

The photoresponses of amacrine or ganglion cells can be easily distinguished from those of other retinal cells, since photoreceptors, horizontal and bipolar cells generate only slow, graded potentials (Baylor & Fuortes, 1970; Kaneko, 1973; Toyoda, 1973; Simon, 1973; Schwartz, 1974; Fuortes & Simon, 1974; Naka & Ohtsuka, 1975) while amacrine and ganglion cells generate in addition action potentials (Werblin & Dowling, 1969; Kaneko, 1973; Schwartz, 1974; Naka & Ohtsuka, 1975). It is difficult to distinguish amacrine from ganglion cells by the features of their photoresponses since there are ganglion cells whose photoresponses, either transient or sustained cannot be recognized from those of amacrine cells.

In the present experiments the working hypothesis for identification of ganglion cells is the presence of an antidromic response to stimulation of the optic nerve fibres. The crucial test leading to the identification of the antidromic nature of the ganglion cell response consisted in its complete suppression due to collision with a 'direct' spike. Accordingly, the cells which do not fire antidromically, even though generating spikes either in response to illumination or during intracellular injection of current, were identified as amacrines. It is unlikely that a ganglion cell would not be antidromically excited by the optic nerve shock and consequently mistaken for an amacrine cell, since the testing stimulus intensity used was about twice that required to excite all optic nerve fibres. This conclusion is supported by the observation that those cells which did not fire antidromically all showed a characteristic synaptic response which was never observed in the ganglion cells identified by their antidromic activation.

The anatomical demonstration of centrifugal fibre endings on the amacrine cells of the pigeon (Maturana & Frenk, 1965; Dowling & Cowan, 1966) and the conspicuous size of the reptile nucleus isthmo-opticus (Ariens-Kappers, Huber & Crosby, 1936), i.e. the site of origin of the centrifugal optic fibres in birds, support the interpretation that the observed synaptic responses in the turtle amacrine cells may indeed be due to centrifugal actions. Furthermore, it is clear that these synapses exert a strong depolarizing action on the cell membrane and thus provide an important mechanism for the control of amacrine cells excitability.

The amplitude of the centrifugally induced e.p.s.p. can be greatly affected by displacing the membrane potential with intracellular current injection, suggesting that the responsible synapses are close to the electrode (cf. Rall *et al.* 1967), which is presumably located at the soma. Such synaptic location of centrifugal endings suggests a further analogy with the avian synaptic organization reported by Dowling & Cowan (1966).

An interpretation of the amacrine cells synaptic responses to optic nerve

stimulation in frogs has been recently proposed by Matsumoto (1975) as an alternative to the 'centrifugal' hypothesis forwarded by Byzov & Utina (1971) in the same animal. Matsumoto assumes that the antidromic activation of ganglion cells induces dendritic spikes which are transmitted to amacrine cells through dendrosomatic synapses. The negative anatomical evidence for this type of retinal synapses, however, and the observation that in the present experiments the ganglion cell antidromic spikes were never followed by the 'delayed depolarization' often indicative of dendritic activation (Granit, Kernell & Smith, 1963; Kernell, 1964; Nelson & Burke, 1967), suggests that the interpretation of Matsumoto is not entirely satisfactory.

The evidence of a late e.p.s.p. in ganglion cells following single shock stimulation of the optic nerve shows that the centrifugal system may ultimately exert a significant influence upon the retinal output. The origin of the ganglion cell's synaptic responses can be explained as a reverberation of the centrifugal effects exerted upon amacrines. This interpretation rests upon the overwhelming anatomical evidence that ganglion cells do not send collaterals to other ganglion cells (cf. Stell, 1972) nor are they presynaptic to amacrine cells, or in fact to any other retinal cell (Dowling & Boycott, 1966; Dubin, 1970; Wong-Riley, 1974). An exception represented by the associational cells in the ganglion cell layer of mammals, described by Gallego & Cruz (1965) is not relevant to the present results because their axons terminate in the retina. Finally, there is no electron-microscopic evidence of centrifugal fibres terminating upon ganglion cells (Dowling & Cowan, 1966).

From the present results it is not possible to ascribe a functional role to the centrifugal system, but a more general conclusion may be drawn that the depolarizing synaptic responses recorded from ganglion cells may add to the photoresponses, thus facilitating them. A similar operation may explain the potentiation of ganglion cell activity recorded in the chick by Miles (1970) following electrical stimulation of the nucleus isthmo-opticus.

Note added in proof. While this manuscript was in preparation, L. Cervetto (personal communication) has obtained anatomical evidence for the existence in the turtle mid-brain of neurones projecting to the retina. The neurones were selectively stained with Procion yellow by soaking the proximal end of the cut optic nerve in a solution of the dye. Presumably the staining was due to diffusion of the dye along the cell axons.

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REFERENCES

ARIENS-KAPPERS, C. U., HUBER, G. C. & CROSBY, E. C. (1936). *The Comparative Anatomy of the Nervous System of Vertebrates, including Man*, vol. 2. New York: Macmillan.

BAYLOR, D. A. & FUORTES, M. G. F. (1970). Electrical responses of single cones in the retina of the turtle. *J. Physiol* **207**, 77–92.

BETZ, W. J. (1970). Depression of transmitter release at neuromuscular junction of the frog. *J. Physiol.* **206**, 629–644.

BRANSTON, N. M. & FLEMING, D. G. (1968). Efferent fibers in the frog optic nerve. *Expl Neurol.* **20**, 611–623.

BYZOV, A. L. & UTINA, I. A. (1971). Centrifugal influence on amacrine cells in frog retina (in Russian). *Neurophysiology* **3**, 293–300.

CAJAL, S. R. Y (1889). Sur la morphologie et les connexions des éléments de la rétine des oiseaux. *Anat. Anz.* **4**, 111–121.

CAJAL, S. R. Y (1892). La rétine des vertébrés. *Cellule* **9**, 121–225.

CHRISTENSEN, B. N. & MARTIN, A. R. (1970). Estimates of probability of transmitter release at the mammalian neuromuscular junction. *J. Physiol.* **210**, 933–945.

COWAN, W. M. (1970). Centrifugal fibres to the avian retina. *Br. med. Bull.* **26**, 112–118.

COWAN, W. M., ADAMSON, L. & POWELL, T. P. S. (1961). An experimental study of the avian visual system. *J. Anat.* **95**, 545–562.

COWAN, W. M. & POWELL, T. P. S. (1963). Centrifugal fibres in the avian visual system. *Proc. R. Soc.* B **158**, 232–252.

CRAIGIE, E. H. (1928). Observations on the brain of the humming bird (*Chrysolampis mosquitus* Linn. and *Chlorostilbon caribaeus* Lawr.). *J. comp. Neurol.* **45**, 377–481.

DOGIEL, A. S. (1895). Die Retina der Vogel. *Arch. microsk. Anat. EntwMech.* **44**, 622–648.

DOWLING, J. E. & BOYCOTT, B. B. (1966). Organization of the primate retina: electron microscopy. *Proc. R. Soc.* B **166**, 80–111.

DOWLING, J. E. & COWAN, W. M. (1966). An electron microscope study of normal and degenerating centrifugal fiber terminals in the pigeon retina. *Z. Zellforsch. mikrosk. Anat.* **71**, 14–28.

DUBIN, M. W. (1970). The inner plexiform layer of the vertebrate retina: a quantitative and comparative electron microscopic analysis. *J. comp. Neurol.* **140**, 479–506.

ECCLES, J. C. (1955). The central action of antidromic impulses in motor nerve fibres. *Pflügers Arch. ges. Physiol.* **260**, 385–415.

FUORTES, M. G. F., FRANK, K. & BECKER, M. C. (1957). Steps in the production of motoneuron spikes. *J. gen. Physiol.* **40**, 735–752.

FUORTES, M. G. F., SCHWARTZ, E. A. & SIMON, E. J. (1973). Colour-dependence of cone responses in the turtle retina. *J. Physiol.* **234**, 199–216.

FUORTES, M. G. F. & SIMON, E. J. (1974). Interactions leading to horizontal cell responses in the turtle retina. *J. Physiol.* **240**, 177–198.

GALLEGO, A. & CRUZ, J. (1965). Mammalian retina: associational nerve cells in ganglion cell layer. *Science, N.Y.* **150**, 1313–1314.

GLIOZZI, A. (1966). Effect of electrical stimulation of the optic nerve on retinal potentials. *Archs ital. Biol.* **104**, 511–515.

GRANIT, R., KERNELL, D. & SMITH, R. S. (1963). Delayed depolarization and the repetitive response to intracellular stimulation of mammalian motoneurones. *J. Physiol.* **168**, 890–910.

HOLDEN, A. L. (1968*a*). Antidromic activation of the isthmo-optic nucleus. *J. Physiol.* **197**, 183–198.

HOLDEN, A. L. (1968*b*). The centrifugal system running to the pigeon retina. *J. Physiol.* **197**, 199–219.

HOLDEN, A. L. & POWELL, T. P. S. (1972). The functional organization of the isthmo-optic nucleus in the pigeon. *J. Physiol.* **223**, 419–447.

HUBER, G. C. & CROSBY, E. C. (1929). The nuclei and fiber paths of the avian diencephalon, with consideration of telencephalic and certain mesencephalic centers and connections. *J. comp. Neurol.* **48**, 1–225.

KANEKO, A. (1970). Physiological and morphological identification of horizontal, bipolar and amacrine cells in the goldfish retina. *J. Physiol.* **207**, 623–633.

KANEKO, A. (1973). Receptive field organization of bipolar and amacrine cells in the goldfish retina. *J. Physiol.* **235**, 133–153.

KANEKO, A. & HASHIMOTO, N. (1969). Electrophysiological studies of single neurones in the inner nuclear layer of the carp retina. *Vision Res.* **9**, 37–55.

KERNELL, D. (1964). The delayed depolarization in cat and rat motoneurones. In *Progress in Brain Research*, vol. 12, pp. 42–55. Amsterdam: Elsevier.

LASANSKY, A. & MARCHIAFAVA, P. L. (1974). Light-induced resistance changes in retinal rods and cones of the tiger salamander. *J. Physiol.* **236**, 171–192.

LAZAR, G. (1969). Efferent paths of the frog's optic centre. *Acta morph. hung.* **17**, 341.

MCGILL, J. I. (1964). Organization within the central and centrifugal fibre pathways in the avian visual system. *Nature, Lond.* **204**, 395–396.

MCGILL, J. I., POWELL, T. P. S. & COWAN, W. M. (1966*a*). The retinal representation upon the optic tectum and isthmo-optic nucleus in the pigeon. *J. Anat.* **100**, 5–33.

MCGILL, J. I., POWELL, T. P. S. & COWAN, W. M. (1966*b*). The organization of the projection of the centrifugal fibres to the retina in the pigeon. *J. Anat.* **100**, 35–49.

MARTIN, A. R. (1966). Quantal nature of synaptic transmission. *Physiol. Rev.* **46**, 51–66.

MATSUMOTO, N. (1975). Responses of the amacrine cells to optic nerve stimulation in the frog retina. *Vision Res.* **15**, 509–514.

MATSUMOTO, N. & NAKA, K.-I. (1972). Identification of intracellular responses in the frog retina. *Brain Res.* **42**, 59–71.

MATURANA, H. R. (1958). Efferent fibres in the optic nerve of the toad (*Bufo bufo*). *J. Anat.* **92**, 21–27.

MATURANA, H. R. & FRENK, S. (1965). Synaptic connections of the centrifugal fibers in the pigeon retina. *Science, N.Y.* **150**, 359–361.

MILES, F. A. (1970). Centrifugal effects in the avian retina. *Science, N.Y.* **170**, 992–995.

NAKA, K-I. & OHTSUKA, T. (1975). Morphological and functional identifications of catfish retinal neurons. II. Morphological identification. *J. Neurophysiol.* **38**, 72–91.

NELSON, P. G. & BURKE, R. E. (1967). Delayed depolarization in cat spinal motoneurons. *Expl Neurol.* **17**, 16–26.

OGDEN, T. E. (1968). On the function of efferent retinal fibres. In *Structure and Function of Inhibitory Neuronal Mechanisms*, ed. VON EULER, C., SKOGLUND, S. & SODEBERG, U. Oxford: Pergamon Press.

PERLIA, R. (1889). Ueber ein neues Opticuscentrum beim Hühne. Albrecht v Graefes. *Arch. Ophthal., N.Y.* **35**, Abt. 1, 20–24.

RALL, W. (1967). Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *J. Neurophysiol.* **30**, 1138–1168.

RALL, W., BURKE, R. E., SMITH, T. G., NELSON, P. G. & FRANK K. (1967). Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. *J. Neurophysiol.* **30**, 1169–1193.

SCHWARTZ, E. A. (1973). Organization of on–off cells in the retina of the turtle. *J. Physiol.* **230**, 1–14.

SCHWARTZ, E. A. (1974). Responses of bipolar cells in the retina of the turtle. *J. Physiol.* **236**, 211–224.

SIMON, E. J. (1973). Two types of luminosity horizontal cells in the retina of the turtle. *J. Physiol.* **230**, 199–211.

SIMON, E. J. (1974). Feedback loop between cones and horizontal cells in the turtle retina. *Fedn Proc.* **33**, 1078–1082.

STELL, W. K. (1972). The morphological organization of the vertebrate retina. In *Handbook of Sensory Physiology*, VII/1B, ed. FUORTES, M. G. F., pp. 111–213. Berlin, Heidelberg, New York: Springer-Verlag.

TOYODA, J.-I. (1973). Membrane resistance changes underlying the bipolar cell response in the carp retina. *Vision Res.* **13**, 283–294.

TOYODA, J.-I., HASHIMOTO, H. & OHTSU, K. (1973). Bipolar-amacrine transmission in the carp retina. *Vision Res.* **13**, 295–307.

WALLENBERG, A. (1898). Das mediale Opticusbündel der Taube. *Neurol. Zent'bl.* **17**, 532–537.

WERBLIN, F. S. & DOWLING, J. E. (1969). Organization of the retina of the mud-puppy *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**, 339–355.

WONG-RILEY, M. T. T. (1974). Synaptic organization of the inner plexiform layer in the retina of the tiger salamander. *J. Neurocytol.* **3**, 1–33.