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TWO TYPES OF LUMINOSITY HORIZONTAL CELLS IN THE RETINA OF THE TURTLE

BY ELLIOTT J. SIMON

From the Laboratory of Neurophysiology, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

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SUMMARY

1. Two classes of luminosity horizontal cells have been identified in the retina of the turtle by intracellular recording. These cells differ in the area of retinal surface over which they are sensitive to light. For moderate light intensities the maximum response amplitude of 'type I' cells increases progressively as the radius of a circle of light is enlarged to over 750 μm. Responses of 'type II' cells instead reach maximum amplitude with circular illumination of about 300 μm radius.

2. Both types are maximally sensitive to red light and have similar action spectra.

3. The cells from which these responses originated were identified by intracellular marking with Procion Yellow dye. Both types were located in the inner nuclear layer. Type I cells had asymmetric, thick, tuberous processes and no apparent discrete somata. Type II cells had thin processes radiating symmetrically from rounded cell bodies.

4. The different properties of the two types of cells can be simply explained assuming that the cells of type I are coupled by low-resistance electrical junctions while in cells of type II the junctional resistance is higher.

5. Since type I and type II cells differ both morphologically and physiologically, they may have different functions.

INTRODUCTION

Horizontal cells are second order retinal neurones which are remarkable for the large area over which they are sensitive to light. In some instances the 'summation area' includes virtually the entire retina (Norton, Spekreijse, Wolbarsht & Wagner, 1968). That these cells respond to light well outside their anatomic extent has been explained by their interconnexion through electrical junctions (Naka & Rushton, 1967; Kaneko, 1971).

The main classification of horizontal cells distinguishes a luminosity type which responds to all colours of light by hyperpolarization and a chromaticity type which may hyperpolarize or depolarize depending on the stimulating wave-length (Svaetichin & MacNichol, 1958). A subdivision of luminosity cells was suggested by the research of Byzov (1966) which showed that the receptive field of horizontal cells in turtles could vary greatly. In addition, Dr P. O'Bryan (personal communication) noted that there are in the turtle two kinds of luminosity horizontal cells which differ in the area of retina over which they summate light.

In many animals there are several morphological kinds of horizontal cells, and these are usually stratified into layers (Ramon y Cajal, 1893). Lasansky (1971) has identified two types of horizontal cells in Golgi-impregnated turtle retinae. One type has discrete somata and thin dendritic branches; the other has broad, trunk-like main branches and no definite somatic swellings. Both types form lateral processes in cone pedicles. The two kinds are located in the same retinal layer.

In this study, I have investigated two distinct types of luminosity horizontal cells in turtles. These cells differ both in receptive field and in morphology. The hyperpolarizing response of one class ('type I') increases gradually as the size of a circular light stimulus centred on the cell is increased up to 833 μm in radius (the largest light tested). The other class ('type II') reaches maximum response amplitude for a circle of light of about 300 μm. Intracellular marking with Procion Yellow dye identified type I cells as the trunk-like cells and type II cells as those with thin branches.

METHODS

Preparation and recording

Experiments were performed on turtles, *Pseudemys scripta elegans*. After the animals were rapidly decapitated and pithed, both eyes were removed from their orbits. Each eye was bisected with a razor blade, and vitreous was drained as thoroughly as possible with tissue paper. One retinal hemisphere was immediately placed in an experimental chamber which was continuously flushed with moistened 95 % O_2, 5 % CO_2 at room temperature (21 °C). The other eyecup was refrigerated under the same oxygenated atmosphere. Refrigerated eyes could be used several hours later without noticeable deterioration of horizontal cell responses. Glass micropipettes filled with 5 % aqueous Procion Yellow M4R (Stretton & Kravitz, 1968) were advanced into the retina from the vitreal surface until characteristic horizontal cell responses (Baylor & O'Bryan, 1971) were obtained. Experiments were included only when stable recordings were maintained throughout the experimental procedure.

Light stimuli

Retinae were stimulated with pulses of light of 40 or 400 msec duration. The light source was a 30 W tungsten bulb (Wotan no. 390158) which delivered approximately 5×10^3 erg cm^{-2} sec^{-1} between 400 and 700 nM wave-length. Light intensity was

attenuated with a neutral density wedge. Action spectra were obtained by interposing in the beam narrow-band interference filters. Circles of light of 50–833 μm radius were selected by appropriate adjustment of an iris diaphragm placed in the light beam, and light spots could be positioned relative to the micro-electrode by moving the iris with micrometer adjustments.

Intracellular marking

After recording the electrical responses of an impaled cell, 10 nA of hyperpolarizing current were passed in pulses of 3–6 sec duration for up to 3×10^{-6} C. If the resistance of the electrode became greater than 1200 MΩ during current passage, the electrode was discarded and the cell was not marked.

The response of the cell to light was tested after each current pulse. If the characteristic cell response was lost, injection was terminated immediately. This precaution was necessary since on several occasions the electrode abruptly moved during injection from one cell to another with a different characteristic response (these experiments were discarded). Thus, without careful monitoring it is possible to inject dye into a cell other than that from which initial records had been obtained.

After a cell had been injected, the retina was allowed to incubate for at least 1 hr before histological processing for fluorescent microscopy.

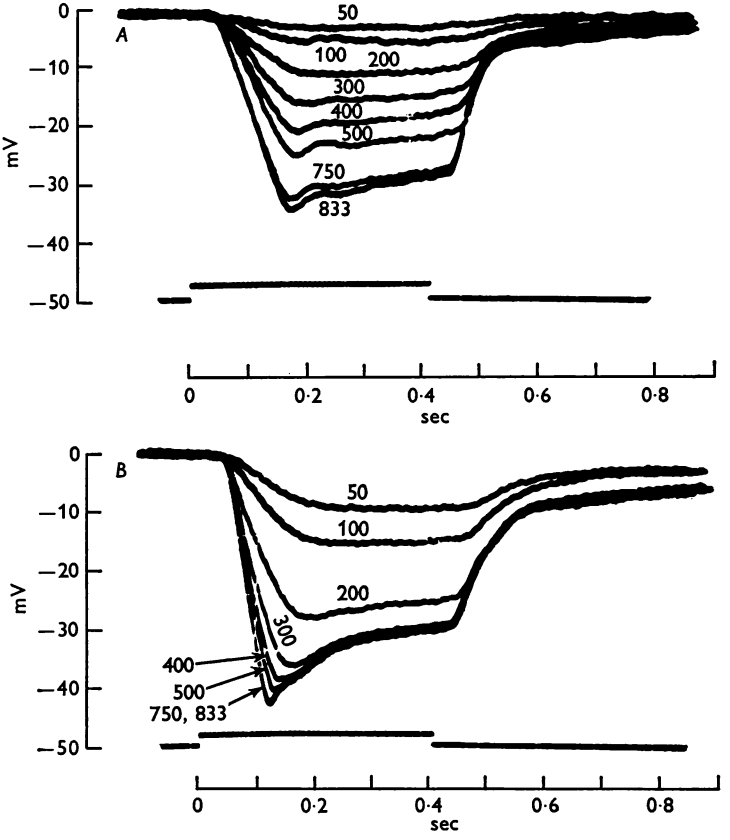
RESULTS

Effect of area of illumination on horizontal cell responses

Responses of the two types of horizontal cells to increasing areas of illumination are illustrated in the records of Text-fig. 1. Responses of the cell designated 'type I' continue to increase as the radius of a circle of light of moderate intensity is enlarged beyond 750 μm. This property holds for intermediate and dim lights as shown in plots of response amplitudes (peak and plateau) as a function of area of illumination for several intensities (Text-fig. 2*A*). By contrast, in 'type II' the plateau of the response reached maximum with spots of 300 μm radius (Text-fig. 2*B*).

The majority of horizontal cells penetrated at random were type I. Sixty-two cells with type I properties were studied. The response amplitude of all continued to increase for dim light stimuli up to 750 μm radius, and in most cases it increased up to 833 μm, the largest light used. Although 13% of these cells reached maximum response amplitude for 500 μm, moderately bright (o.d. 1·2) lights, light scatter was so extensive in these conditions that effective stimulus size was much greater than that of the image. Eighteen type II cells were penetrated. Only rarely did response amplitude grow by more than a few per cent when the circle of light was increased beyond 300 μm radius; and none increased beyond 500 μm for any intensity. In some experiments two electrodes were advanced into the same area of retina. On two occasions cells with type I and type II properties were penetrated simultaneously illustrating that their differences do not reflect differences in retinal topography. The maximum hyperpolarizing response could be 60–70 mV for either cell type.

The patterns of responses of type I and type II cells to a 300 μm light spot displaced from the receptive field centre are illustrated in the records of Text-fig. 3. Responses of the type I cell decrease gradually as the stimulus is displaced over a distance of 1 mm; responses of the type II cell, however, diminish abruptly as the edge of the light is moved across that cell (from 200 to 400 μm).



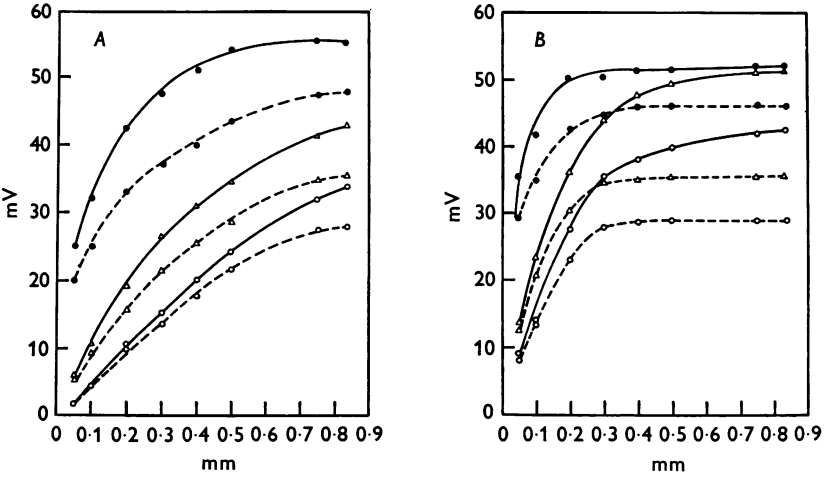
Text-fig. 1. Horizontal cell responses to increasing area of illumination (indicated by stimulus radius in μm) for constant stimulus intensity (optical density (o.d.) 3·0). Light duration is shown in the lower trace.

A, responses of type I cell continue to grow as the radius of a circular light stimulus is increased to 833 μm (the largest stimulus used).

B, responses of type II cell reach maximum plateau amplitude for a 300 μm stimulus.

The two classes of cells differ also in their recovery from bright illumination. Text-fig. 4 shows superimposed responses to steps of light covering an area of 500 μm radius. The type I cell starts to recover abruptly less

than 200 msec after the end of the step; in the type II cell, instead, the recovery is delayed. In both cells the recovery phase includes a hyperpolarizing inflexion (arrows) which can be interpreted as an oscillation generated by the negative feed-back from horizontal cells to cones (Baylor, Fuortes & O'Bryan, 1971; Fuortes, 1972). Responses to brief flashes showing analogous features are illustrated in the inset of Text-fig. 4 where it is seen that the hyperpolarization is greatly prolonged only in the cell classified as type II. An interpretation of these events will be proposed below.



Text-fig. 2. Graphs of horizontal cell responses to increasing area of illumination for 400 msec light pulses at three different intensities (filled circles, o.d. 1·2; triangles, o.d. 2·4; open circles, o.d. 3·0). Continuous curves represent transient responses; dotted curves represent plateau responses.

A, type I cell responses continue to grow when the stimulus radius (abscissae) is increased to 833 μm.

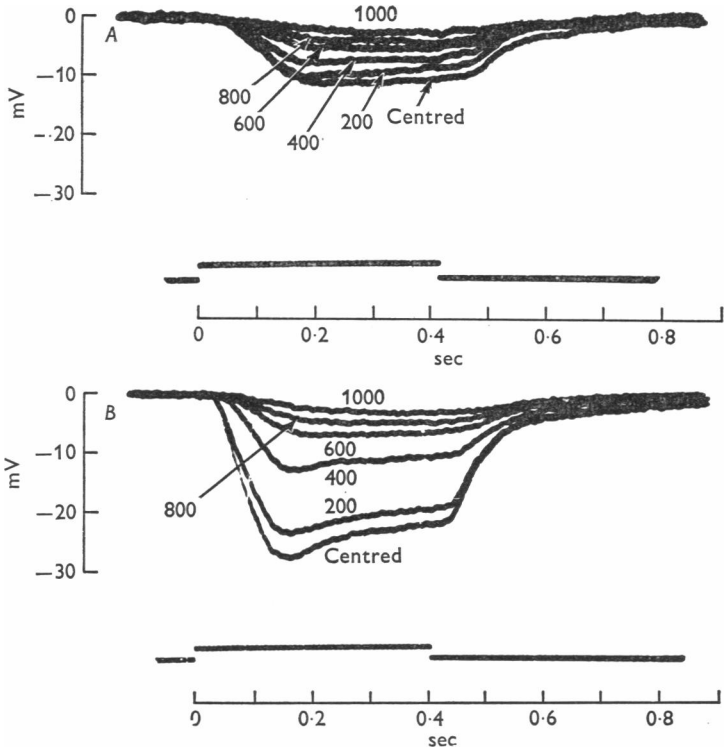
B, steady-state responses in type II cells are essentially maximal for 300 μm lights although the transient responses do change slightly for dim lights larger than this. Type II responses are proportionately greater for very small stimuli and increase in size to saturation more rapidly with enlarging stimuli than do type I responses.

Both cell types respond only by hyperpolarization to all wave-lengths of light and have similar action spectra (Text-fig. 5). They are maximally sensitive to light at about 600 $\pm$ 15 nM, a value close to the peak absorption of a red cone photopigment (Liebman & Granda, 1971).

Histological identification of horizontal cell types

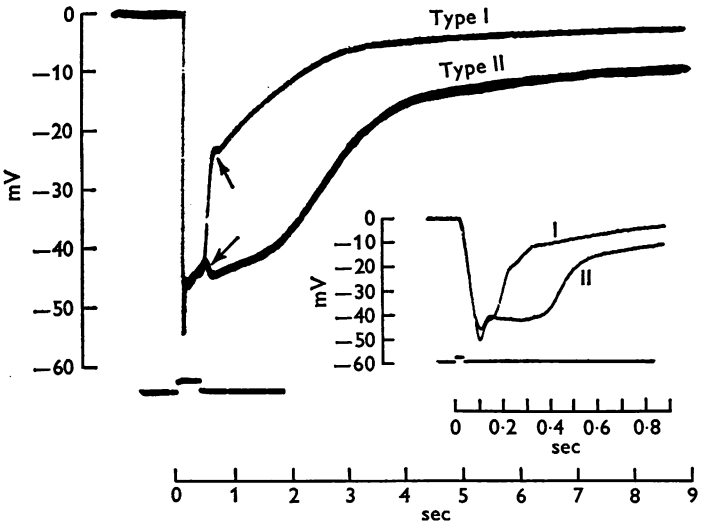
Intracellular marking with Procion Yellow dye was attempted in fifty-four cells and was successful in forty cases. In spite of careful monitoring,

cells other than horizontal cells were often marked in addition to the horizontal cells. Presumably this was caused by leakage of dye along the electrode track. With smaller quantities of dye injected, marking of additional cells could be prevented, but horizontal cell processes were less adequately outlined.



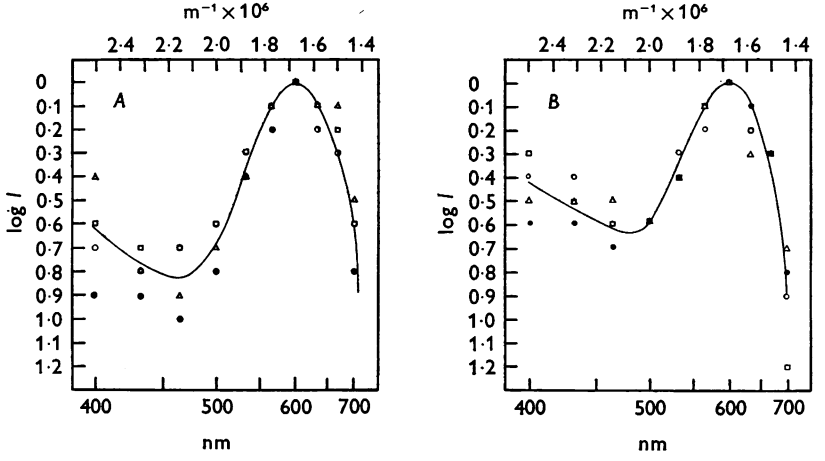
Text-fig. 3. Horizontal cell responses to a 300 μm, o.d. 3·3 stimulus displaced at 200 μm increments from a position centred about the recording electrode. The maximum amplitude of the type I cell (*A*) was the same as that of the type II cell (*B*) when the stimulus was large (833 μm) and bright (o.d. 1·2). Type I responses decrease gradually, whereas type II responses decrease sharply as the light edge is moved across the cell (from 200 to 400 μm off centre).

In a sample of thirty-nine cells identified as type I by their area of summation, six were stained in isolation and had the morphological characteristics illustrated in Pl. 1*A*; fourteen had a similar appearance, but a Müller cell was stained as well; two showed thick processes but were incompletely filled with the dye; in five cases numerous additional cells were stained including bipolar and Müller cells; finally, in twelve instances



Text-fig. 4. Recovery of type I and type II horizontal cells to large, bright stimuli (radius 500 μm, o.d. ○). Both cell types have identical responses during a 400 msec light, but the recovery in the type II cell is greatly delayed after the light is extinguished. The arrows point out an inflexion in the recovery phase of both types.

Inset: A 40 msec flash elicits maintained hyperpolarization in the type II cell only.



Text-fig. 5. Action spectra for type I (*A*) and type II (*B*) horizontal cells. Symbols represent experimental points for four different cells of each type. These points were obtained by determining the relative intensity (ordinates) of a 300 μm, 400 msec stimulus needed to produce a criterion response at each of ten wave-lengths. Abscissae are calibrated in wave-length and wave number. Both cell types were maximally sensitive at about 600 ± 15 nm. Within experimental error there is no significant difference in the shape of the continuous curves which best fit the data.

no mark was recovered. It should be noted that no units identified as type II horizontal cells were stained in this sampling.

Procion injections were performed also in fifteen cells identified electrically as type II. In three cases a cell having all the morphologial features of type II cells as shown in Pl. 1B was the only cell stained; three were similar but a Müller cell was stained in addition; in four cases round cell bodies resembling type II somata were found but the dendritic trees were not sufficiently well filled for identification; in three retinae there were one to three small bipolar cells in addition to a type II horizontal cell, and no mark was recovered in two instances. Thick processes which could be ascribed to type I cells were never found.

As shown in Pl. 1, type I cells have broad, tuberous, asymmetric processes with short, fine terminal branches. There are no discrete swellings that could be identified as somata. Type II cells have discrete, oval cell bodies of about 15 μm in greatest diameter. Processes are much finer (less than 1 μm) than the main tuberous ones of type I cells (about 6 μm) and radiate like spokes from the cell body to the receptor layer. The horizontal spread of well marked type I cells was about 150 μm and that of type II cells was about 110 μm. There was no stratification of the two types into internal and external layers as has been described in other species (Ramon y Cajal, 1893). Type I cells run serpentine courses through many depths of the inner nuclear layer. Type II cells are located in either the middle or outer portion of the inner nuclear layer. Electrical measurements reflected this finding since the type II cell responses were encountered both superficial and deep to type I responses.

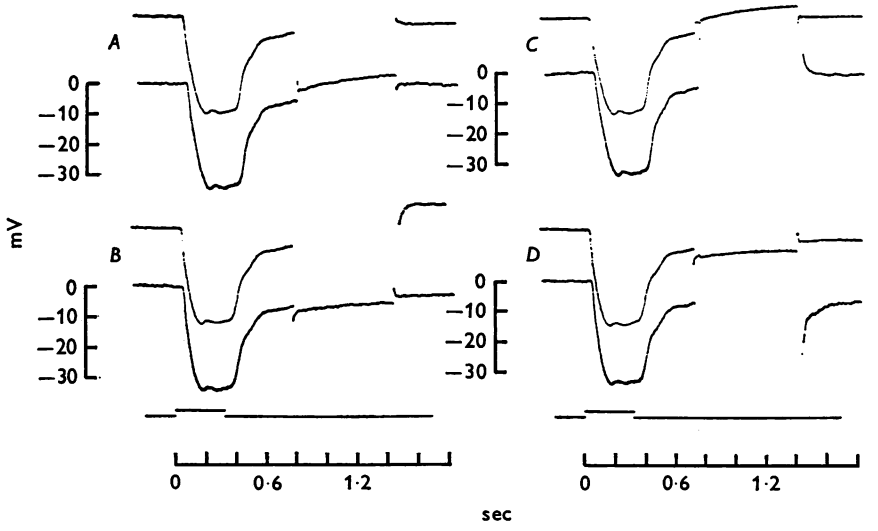
In spite of the limitations of the Procion Yellow technique, it is clear that type I and type II cells are distinct morphologically as well as physiologically.

Electrical coupling

Electrical coupling between horizontal cells of fish has been proposed by Naka & Rushton (1967) and demonstrated by Kaneko (1971). In the present research pairs of horizontal cells were penetrated in eleven experiments. In nine instances both cells were type I, and seven of these pairs were electrically coupled as illustrated in Text-fig. 6. In two experiments one cell was type I and the other was type II. No coupling could be demonstrated in these pairs. Simultaneous impalement of two type II cells was never obtained.

Based on these results I have assumed that only cells of the same type are electrically coupled, and I have tried to reproduce the experimental results on an electrical network representing an array of coupled cells (see Fuortes, 1972). The horizontal cell network is represented by a central cell surrounded by concentric

annuli. The thickness of each annulus is equal to a cell diameter, and the number of cells in each annulus is proportional to its area: thus, the first annulus contains eight cells, the second sixteen, etc. (Text-fig. 7*A*). Since the stimuli are circles of light concentric with the horizontal cell network, all cells in the same annulus are equipotential and their interconnexions may be disregarded. The network can therefore be represented as a ladder in which the first node on the left represents the central cell and the other nodes represent the successive annuli. Conductance of one node to ground is proportional to the area of the corresponding annulus, and the conductance from the node to the next is proportional to the circumference separating successive annuli. Cell diameters were taken from the anatomical measurements: type I, 150 μm, type II, 110 μm.



Text-fig. 6. Electrical coupling between type I horizontal cells. Electrodes were inserted simultaneously into two neighbouring large-field horizontal cells. Each trace illustrates the light response to a large, o.d. 2·4 light step (indicated in lowest traces) followed by the effect of passage of 10 nA current steps into one of the cells. Because of bridge unbalance the voltage drop could not be recorded from the cell receiving the current directly. It produced a maintained potential deflexion of the same polarity in the neighbouring cell. The effects of depolarizing and hyperpolarizing current are illustrated for one cell in *A* and *B* and the other cell in *C* and *D*. When one electrode was removed from its cell and placed in the adjacent extracellular space, current injected into either electrode did not change the voltage recorded by the other.

To simulate the effects of illumination in the form of circles of different radii, currents were injected into one or more annuli (each annulus being represented by a node). Current intensities were made proportional to the height of the receptor responses at different distances from the stimulus centre as determined in previous studies by Fuortes, Lasansky & O'Bryan as reported by Fuortes (1972).

The model includes a parameter, K, which represents the tightness of the coupling between adjacent cells. The parameter was adjusted for best fit of the voltage change

obtained in the model by means of current injection with the distribution of horizontal cell responses following illumination. With $K = 2{\cdot}0$ the voltage drop at node 0 (representing the central horizontal cell) increases with the extension of the stimulus as the responses of type I horizontal cells increase with area of illumination (Text-fig. 7*B*). A similar fit of the responses of type II cells is obtained with $K = 0{\cdot}4$.

The same two values of K were used to compute the distribution of voltages in the network for stimuli of different displacements along the network. Text-fig. 7*C* shows the results of this computation (continuous curves) together with the experimental measurements obtained from type I or type II horizontal cells by displacing a circle of light of constant size. The computed curves reproduce the main features of the experimental distribution of responses. Given the obvious over-simplification of the model, this result may be regarded as satisfactory even if the fit is only very rough. The different properties of the two types of horizontal cells may thus be interpreted simply assuming that type I cells are more tightly coupled than type II cells.

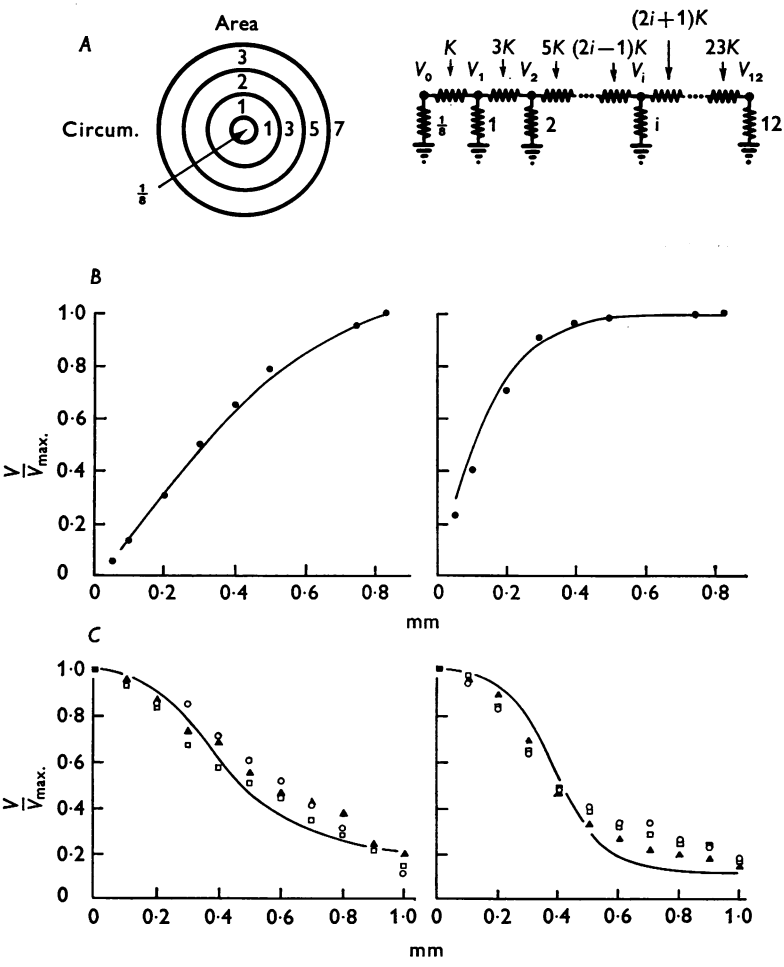


Fig. 7. For legend see facing page.

DISCUSSION

Two types of luminosity horizontal cells have been distinguished in the turtle retina on both physiological and morphological grounds. Anatomical differences between the two are inadequate to explain their different receptive field properties. Instead, a difference in the tightness of electrical coupling between cells of each type can explain their disparate properties.

Because of this difference in coupling, horizontal cell current generated by receptors far from any given horizontal cell will have much less influence on a type II response than on a type I response. When a bright (o.d. = 0), 500 μm radius light is focused on the retina, all the cones under that spot will remain hyperpolarized long after the light is extinguished (Fuortes, 1972). Cones peripheral to the spot however will not show prolonged hyperpolarization because their stimulus is only dim scattered light. Since the overwhelming contribution to type II cells would come from cones under such a 500 μm spot, one expects prolongation of their responses as was observed (Text-fig. 4). Conversely, the response of type I cells would be controlled largely by peripheral cones which generate shorter responses. The recovery is therefore faster for type I than type II cells for illuminations covering 500 μm radius or less and starts to become similar in the two cell types when the illuminated area is further enlarged.

Text-fig. 7. Electrical network representing each horizontal cell type.

A, the illustration on the left shows the presumed arrangement of interconnected horizontal cells formed by one cell in the centre and concentric annuli of surrounding cells (only a few annuli are drawn). Due to radial symmetry, this scheme can be represented by the simple electrical network on the right. Nodes 1–12 represent corresponding annuli. For the sake of simplicity the network was terminated at the twelfth annulus. The distribution of responses (V_i) can be calculated for different stimulus patterns by injecting current into the various annuli. See text for further explanation.

B, comparison of best fit of the voltage changes obtained by current injection into the network (continuous curves) with horizontal cell responses to lights of increasing radii as indicted by abscissae (points represent the mean of plateau responses to dim stimuli for fourteen cells of each type). The model fits type I responses best with $K = 2{\cdot}0$ (left) and type II responses with $K = 0{\cdot}4$ (right).

C, comparison of horizontal cell responses (symbols represent three experiments) to voltages calculated from the electrical network (continuous curves). Responses were evoked by a dim, 300 μm light displaced at various distances (abscissae) from the impaled cell. Voltages were obtained from the model by injecting current along a segment of the network located at different distances from the central node. The distribution of current along this segment was the same as the distribution of cone responses to a dim, 300 μm stimulus (Fuortes, 1972, Fig. 5). Again, best fit of the model for type I cells (left) required $K = 2{\cdot}0$ and for type II cells (right) required $K = 0{\cdot}4$.

Several important functions have been ascribed to horizontal cells that have large areas of integration as do type I cells. Baylor *et al.* (1971) have shown that horizontal cells with large receptive fields mediate negative feed-back on to cones in the turtle. In addition, Schwartz (1973) found that type I horizontal cells can contribute excitation to retinal ganglion cells in turtles, a finding similar to that in fish (Maksimova, 1969; Naka & Nye, 1970). Neither authors studied type II cells in turtles (personal communication).

There is no information about the function of type II horizontal cells. In fish 'external' horizontal cells are morphologically similar to type II cells, and 'internal' horizontal cells are similar to type I cells (Ramon y Cajal, 1893). However, the receptive field properties of these two types have been inadequately studied (Kaneko, 1970, 1971) to determine if they are similar to those in turtles. Type II horizontal cells differ physiologically and morphologically from type I cells and should be considered to be a different group of interneurones. The function of these cells is presently unknown, but those functions ascribed to type I cells cannot be applied to them *a priori*.

I am greatly indebted to Drs M. G. F. Fuortes, P. M. O'Bryan, and E. A. Schwartz for their help with all aspects of this work.

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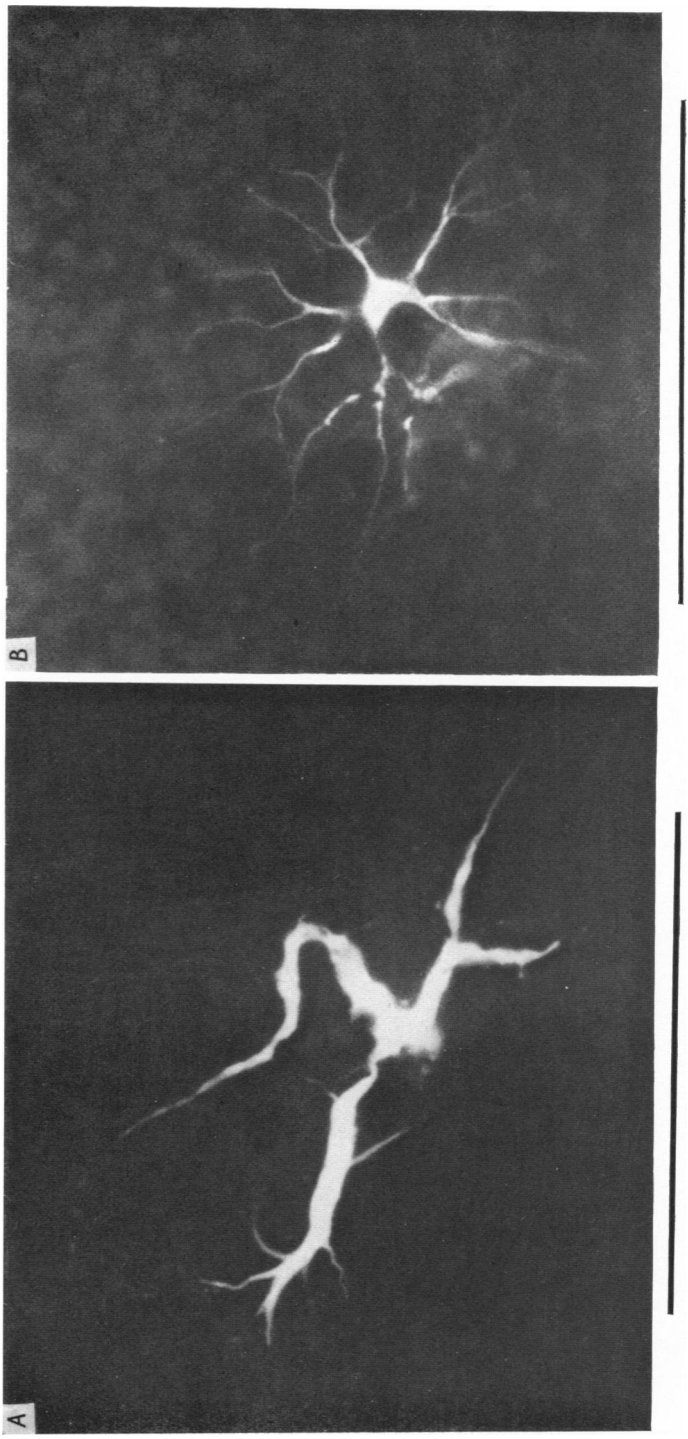

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EXPLANATION OF PLATE

Fluorescent photomicrographs of retinal flat mounts of type I (*A*) and type II (*B*) horizontal cells that have been injected with Procion Yellow. The type I cell has thick, asymmetric processes up to 6 μm in diameter and no discrete swelling that can be identified as a cell body. It extends about 150 μm in horizontal spread. The type II cell has a discrete rounded soma about 15 μm in greatest diameter and thin processes less than 1 μm in diameter radiating symmetrically to spread about 110 μm. Calibration bars are 100 μm.