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ORGANIZATION OF ON–OFF CELLS IN THE RETINA OF THE TURTLE

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SUMMARY

1. The organization of central and peripheral responses for one type of spike-producing cell in the retina of the turtle (*Pseudemys scripta elegans*) has been studied. These cells produced a short burst of spikes following the onset and offset of a small spot of illumination (i.e. ON–OFF cells). The effect of increasing the area of illumination was to include a peripheral inhibition. Intracellular ON and OFF responses were, however, affected differently.

2. ON–OFF cells marked intracellularly with Procion Yellow M4R had somata in the inner nuclear and ganglion cell layers.

3. Peripheral inhibition could be evoked without a change in the membrane potential of horizontal cells.

4. Passing 5 nA hyperpolarizing or depolarizing current through a micropipette within a horizontal cell elicited in ON–OFF cells a transient excitation following the onset and offset of current.

5. It is concluded that inhibition from the periphery of an ON–OFF cell receptive field is not mediated by luminosity horizontal cells but more probably by peripheral bipolar cells.

INTRODUCTION

Retinal ganglion cells produce responses which are determined by the distribution of incident illumination. Their receptive fields are frequently divided into concentric central and peripheral areas which each give different responses (Kuffler, 1953; Barlow, 1953). How retinal interneurones contribute to each of these responses is not known. Retinal interneurones interact in two synaptic layers: the outer synaptic layer is formed by the interconnexions of receptor, horizontal and bipolar cells; the inner synaptic layer is formed by the interconnexions of bipolar, amacrine and ganglion cells. Maksimova (1969) and Naka & Nye (1970,

1971) have shown that polarizing horizontal cells with extrinsic current can elicit activity in ganglion cells. They have suggested that horizontal cells contribute to ganglion cells the responses of peripheral illumination.

In this work, I have investigated the organization of one type of spike-producing cell. This cell produced a burst of spikes following the onset and offset of illumination incident within the centre of its receptive field and was inhibited by light incident within the periphery. Inhibition from the periphery could be evoked by stimuli which did not affect horizontal cells. Polarizing horizontal cells elicited ON–OFF bursts similar to those produced by central illumination. It is concluded that the response of the periphery is not mediated by horizontal cells.

Cells producing ON–OFF responses were injected with dye and found to have somata in either ganglion cell or inner nuclear layers. It is possible that those in the inner nuclear layer were amacrine cells even though their responses were indistinguishable from those of positively identified ganglion cells. For this reason these spike-producing cells are collectively referred to as ON–OFF cells.

METHODS

Preparation

Experiments were carried out on the isolated eyecups of turtles, *Pseudemys scripta elegans*, whose carapace lengths were 7–12 in. An animal was decapitated, one eye enucleated and bisected with a razor blade along the greatest diameter, and vitreous humour removed from the posterior half by dissection and drainage with absorbent paper. The eyecup was then placed into a chamber at room temperature (20° C) continuously flushed with moist 95% O_2, 5% CO_2.

Light stimuli

The retina was stimulated by a light which passed through four lenses. A quartz-iodine lamp (Sylvania 100 W, type FAV) was imaged on to the plane of an electromagnetic shutter; light from the plane of the shutter was collimated and then passed through a spherically corrected field lens; the image of the field lens was focused on to the retina by a Leitz U-O 6·5X/0·18 microscope objective with an optical reduction of 20 to 1. Apertures of calibrated size or patterns could be introduced at the field lens for projection on to the retina. In all experiments a monochromatic light was obtained by inserting into the beam a narrow-band interference filter with a transmission maximum at 615 nm. The maximum irradiance delivered to the retina was 8×10^{14} photons cm^{-2} sec^{-1} and was attenuated with neutral density filters calibrated in optical density units (O.D.). In some experiments the retina was stimulated by two independent beams combined by a prism.

Recording

Intra- and extracellular recording probes were advanced from the vitreal surface dorsal to the linear *area centralis*. Extracellular probes were sharply pointed Pt-Ir wire insulated with Corning Type 7574 glass except for the platinum blacked tip. The passband of the amplifier was 200-3k Hz. Micropipettes for intracellular recording were filled with 4M-KAc for recording from ON–OFF cells, with 3 M-KCl

for recording and passing current in horizontal cells, or with 4% Procion Yellow M4R for recording and subsequently iontophoretically injecting dye.

Identification of retinal cells

Luminosity horizontal cells were identified by their responses to light according to the criteria of Baylor & Fuortes (1970). ON–OFF cells were marked for subsequent histology with Procion Yellow M4R (Stretton & Kravitz, 1968). Dye was passed from the micropipette with 8 nA hyperpolarizing current for approximately 2 min. Iontophoresis was terminated if the intracellular response was lost. After 2 hr in the dark, the preparation was fixed in 4% formaldehyde pH4, dehydrated through acetone and embedded in Epon 812. 50 μm sections were viewed by dark field illumination with an Osram 200 W Hg lamp, Schott BG 12 3 mm excitation filter and GG 530 3 mm barrier filter.

RESULTS

Properties of ON–OFF cells

This study was restricted to cells which produced a short burst of spikes following both the onset and offset of a 10 μm diameter spot projected anywhere within their receptive fields.

These cells only produced spikes following a change in the stimulus (spontaneous firing was less than 0·1/sec). The receptive field for excitation by a 10 μ spot was usually a circular area 400–600 μm in diameter. Threshold for the OFF response was frequently lower than for the ON response. After mapping the receptive field, stimuli were applied to the geometric centre.

Response pattern

The firing pattern of cells was recorded in scattergrams constructed on an oscilloscope during the experiment. Each spike intensified a point on the oscilloscope at an abscissa which measured real time and an ordinate which measured the reciprocal of the interval from the preceding spike in logarithmic scale. Records of responses to successive stimuli were superimposed by multiple exposure of a single frame of photographic film. Scattergrams which presented the result of ten responses indicated the range in spike distribution elicited by a given stimulus.

ON–OFF cells gave rather similar responses to stimuli varying over a large range of intensity and area. The cells shown in Text-fig. 1*A* and *B* are typical of the extremes in response pattern of ON–OFF cells. The four scattergrams of each set are taken from a single cell. For the cell of Text-fig. 1*A*: a 3·6 O.D., 10 μm spot was below threshold; when the spot diameter was increased to 1000 μm this light was sufficient to evoke an ON–OFF response; a 1·2 O.D., 10 μm spot produced brief ON and OFF responses; when the spot diameter was increased to 1000 μm there occurred in addition a few delayed spikes at an interval of more than 0·5 sec after the

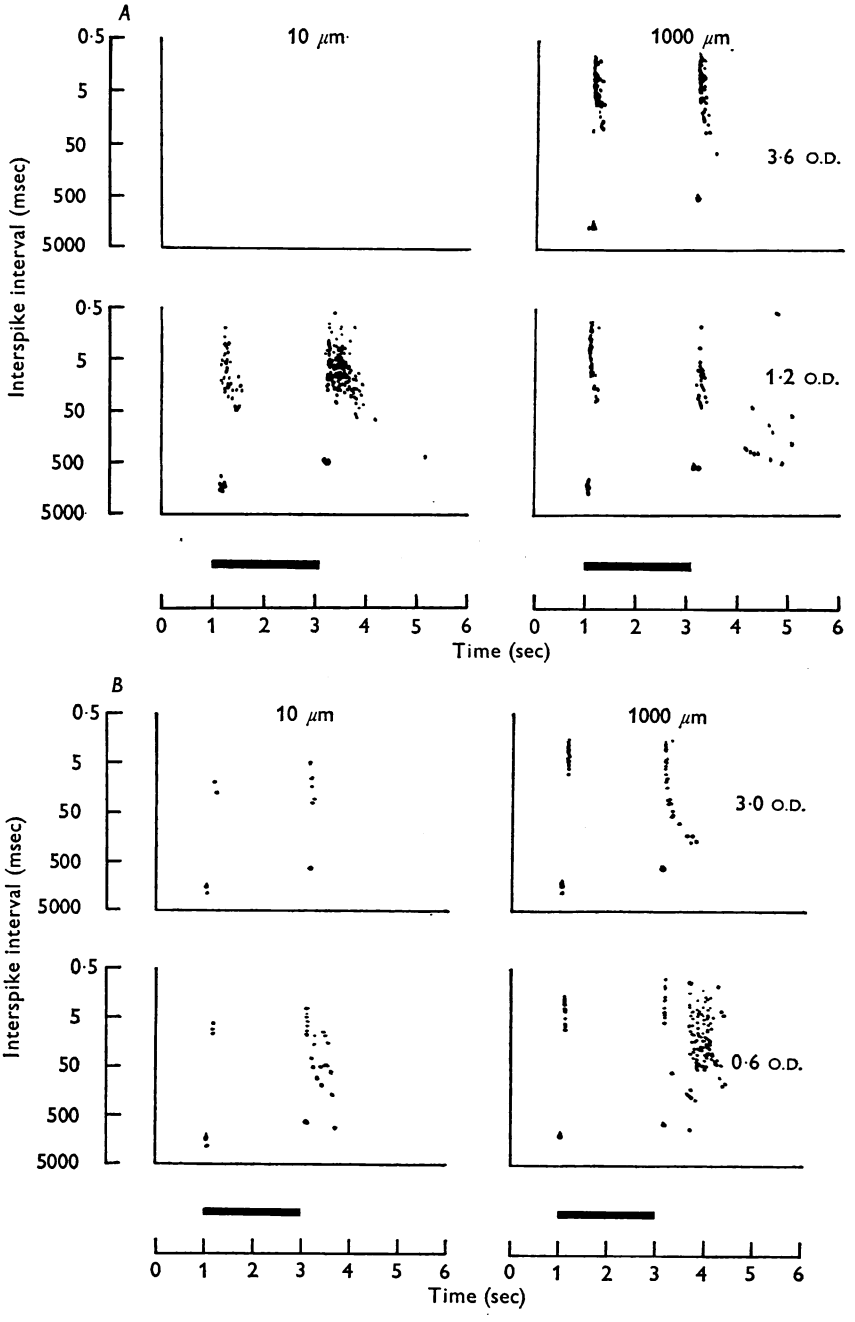
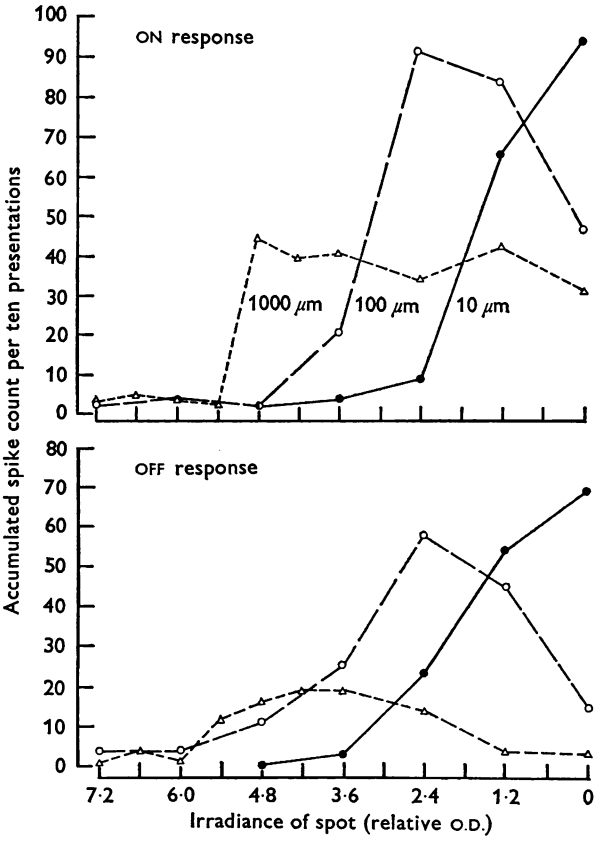


Fig. 1. For legend see facing page.



Text-fig. 2. Stimulus–response relationships for different sized spots. The accumulated number of spikes following ON or OFF in ten consecutive stimuli is plotted as ordinate against irradiance as abscissae for 10, 100 and 1000 μm diameter spots.

Text-fig. 1. Scattergrams to illustrate the response characteristics of ON–OFF cells. Each scattergram is constructed from ten stimulus presentations. The four frames of each set are from a single cell and show the response characteristics to spots of varying size and irradiance. The two sets indicate the extremes in response pattern of this class of cell. *A*: a 10 μm, 3·6 O.D. spot was below threshold; a 10 μm, 1·2 O.D. spot gave an ON–OFF response; enlarging the spot decreased threshold, shortened ON and OFF responses, and gave a few delayed spikes following a moderately bright light. *B*: the responses are similar to the responses of *A* except for a more prominent delayed OFF response following the 1000 μm, moderately bright spot; a 10 μm, 3·0 O.D. spot was at threshold.

stimulus was terminated. The cell of Text-fig. 1*B* behaved similarly; it differed only by having fewer spikes at ON and OFF and a more prominent delayed OFF burst. Progressive inhibition of the immediate OFF response and increasing latency for the delayed OFF response occurred as the 1000 μm spot was further brightened. The irradiance at which the delayed OFF response could be best obtained varied between 2·4 and 0 O.D.

Scattergrams indicated that with increasing area (1) threshold decreased and (2) at high irradiance inhibition increased. A quantitative measure of these two properties was obtained by counting the total number of spikes

TABLE 1. The means for n cells are given with S.E. indicated within parentheses.

A, the number of spikes in ten presentations of a 100 or 1000 μm spot is expressed as a fraction of the number of spikes in ten presentations of a 10 μm spot.

B, tabulated are the mean accumulated spike count to ten repeated presentations of a test spot while a broken annulus was stationary or rotated at 2–4 Hz (see Text-fig. 4).

C, tabulated are the mean accumulated spike count to ten repeated presentations of a stimulus. Horizontal and ON–OFF cells were separated by less than 100 μm. In all pairs in which a horizontal cell responded to a 1000 μm, 1·2 O.D. light with a hyperpolarization greater than 30 mV, a 5 nA current within the horizontal cell was excitatory to the associated ON–OFF cell. In the fifteen consecutively studied pairs, the maximum horizontal cell responses were 28–56 mV. When the pipette was withdrawn from the horizontal cell and current passed into the extracellular space, only a few spontaneous spikes occurred which were not synchronous with current passage

A. Progressive inhibition on enlarging a bright light (n = 10)

Fraction of response present			
100 μm		1000 μm	
ON	OFF	ON	OFF
0·63 (0·05)	0·71 (0·11)	0·32 (0·07)	0·06 (0·01)

B. Inhibition of the centre by peripheral stimulation (n = 15)

10 μm spot and stationary periphery		10 μm spot and rotated periphery	
ON	OFF	ON	OFF
47 (8·2)	93 (15·4)	20 (5·8)	43 (8·6)

C. Transient excitation with current passage in a horizontal cell (n = 15)

100 μm, 2·4 O.D. spot		Intracellular current (−5nA)		Extracellular current (−5nA)	
ON	OFF	ON	OFF	ON	OFF
56 (10·4)	74 (10·7)	64 (13·0)	30 (8·6)	2 (0·4)	4 (0·9)

after the onset and offset of illumination in ten consecutive responses. An example is presented in Text-fig. 2 where the accumulated number of spikes is plotted as ordinate against irradiance as abscissa for 10, 100 and 1000 μm diameter spots. Progressive inhibition on enlarging a 0 O.D. light is shown for a series of ten cells in Table 1*A*.

The plot of Text-fig. 2 does not take into account the attenuation of light due to scatter. The responses of the receptors are determined by the light impinging directly upon them, which may be reduced by scatter, and interactions from neighbouring cells. To obtain approximately equal receptor responses, the 10 μm response curves should be shifted to the left approximately 1·3 O.D. and the 100 μm response curves should be shifted to the left approximately 0·3 O.D. (Baylor, Fuortes & O'Bryan, 1971).

Intracellular records

Intracellular records show that ON and OFF responses were each generated by depolarizing events which were individually modified by brightening or enlarging the area of illumination. Text-fig. 3*A* shows intracellular responses to 10, 100 and 1000 μm diameter, 0 O.D. spots. The cells responded to a 10 μm spot with simple transient depolarizations at ON and OFF. When the spot was enlarged to 100 μm there was a shortening of the ON response while the OFF response was little affected. After enlarging the spot to 1000 μm, the initial ON depolarization was further shortened, while the OFF response was smaller, had a slightly increased latency and was followed by a late depolarizing component. The same sequence of responses could be obtained by progressively brightening a 1000 μm spot. In addition, for some cells the brightest light did not give an immediate OFF response and the interval to the onset of the delayed depolarization became longer. Superimposed on the slow potentials were fast spikes which could be 70 mV in amplitude (Text-fig. 3*B*).

From the intracellular records it is seen that the ON and the OFF spikes evoked by illuminating a very small retinal area were each produced by a simple depolarization. Since no significant hyperpolarizing component occurred, generation of the OFF response cannot be attributed to post-inhibitory rebound of the ON–OFF cell membrane but must rather be attributed to a separate period of synaptic depolarization. The characteristic delayed OFF response to bright, large diameter spots must also be due to properties of the input.

Anatomical identification

Six ON–OFF cells were marked intracellularly with Procion Yellow M4R. Four had somata at the inner margin of the inner nuclear layer and two had somata in the ganglion cell layer. All had a dendritic tree which was stratified within the inner plexiform layer. One example of each type is

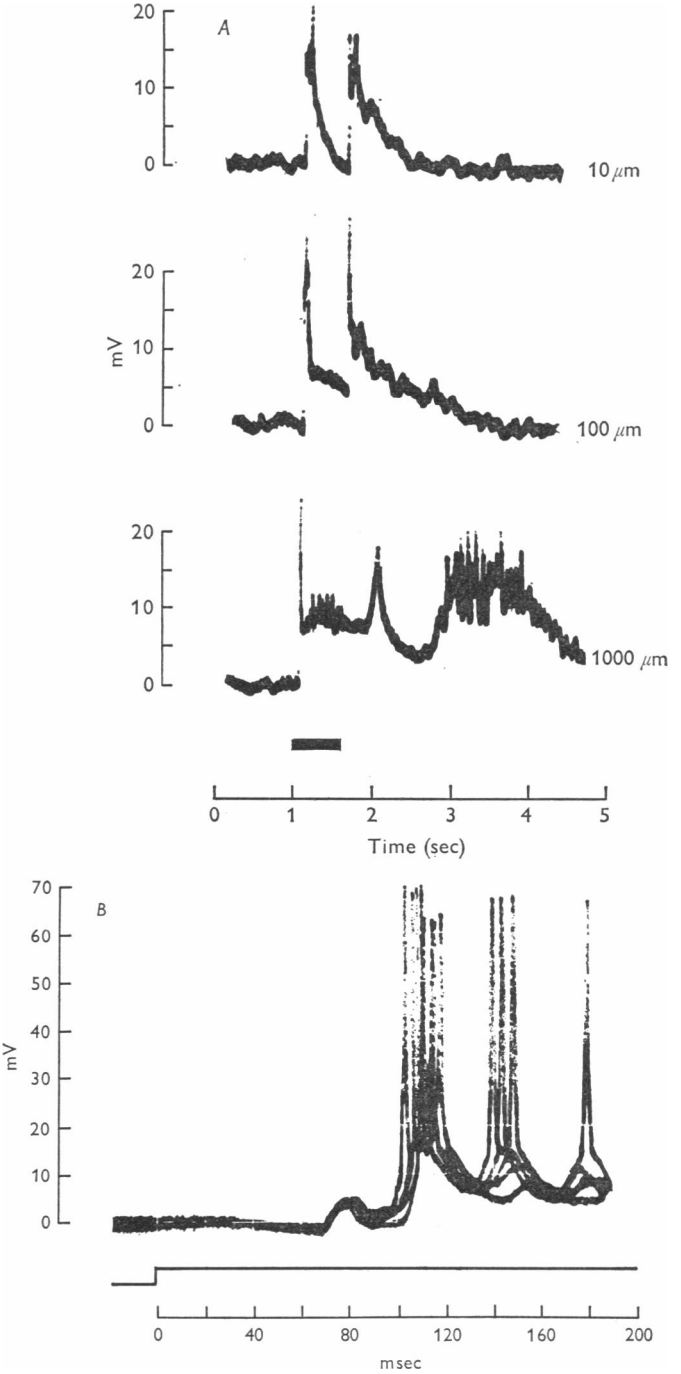


Fig. 3. For legend see facing page.

given in Pl. 1. An axon could not be identified for the four cells with somata in the inner nuclear layer. It was not possible to determine if these were amacrine or displaced ganglion cells. An axon might not have been identified when a dendritic tree was incompletely filled with dye; in a series of marked cells of another physiologic type (not reported here) with somata in the ganglion cell layer an axon could not always be identified. This must be considered a limitation of the technique. However, it is certain that cells giving indistinguishable responses had somata in two different retinal locations; these may have been either ganglion and displaced ganglion or ganglion and amacrine cells.

Origin of peripheral inhibition

Maksimova (1969) and Naka & Nye (1971) have suggested that horizontal cells contribute to ganglion cells a response of peripheral illumination. In the previous section it was demonstrated for ON–OFF cells that increasing the area of illumination included an inhibition from the periphery. In the following section it is shown that inhibition from the periphery could be obtained without a horizontal cell response. It is further shown that extrinsic current in a horizontal cell did not produce inhibition but rather a transient excitation following its onset and offset.

Peripheral inhibition not mediated by horizontal cells

To demonstrate that inhibition is independent of the horizontal cell response, it is necessary to obtain peripheral inhibition under conditions in which the horizontal cell membrane potential is constant. The strategy and results for this are shown in Text-fig. 4. A broken annulus continuously illuminated the retina. In the left trace a small centred spot evoked an ON–OFF response. The annulus was then alternately stepped clockwise–counterclockwise and the centred test spot was repeated. During the period of dithering the annulus the response to the test spot was decreased or abolished. In the central trace the response of the nearest ON–OFF cell was

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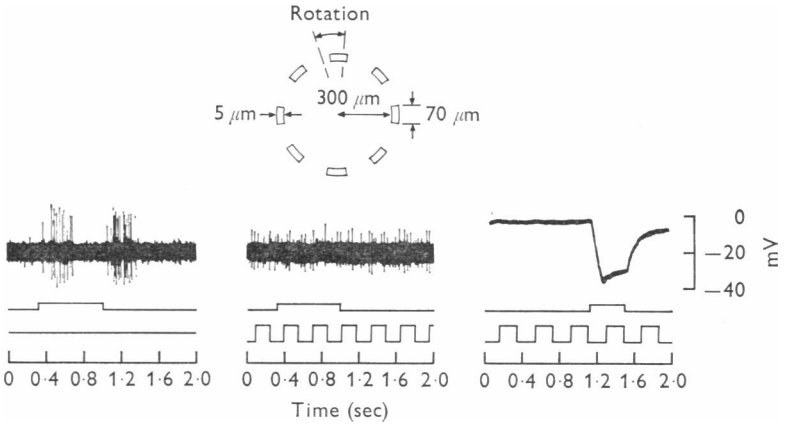
Text-fig. 3. Intracellular records. *A*: the change in response with increasing area of illumination. The stimuli are from above downward 10, 100 and 1000 μm concentrically centred spots of constant irradiance of duration indicated by the solid bar. 50 mV spikes superimposed on the slow potentials are lost in photographic reproduction; only slow potentials are recorded at the very slow sweep speed. The cell responded to a 10 μm spot with transient depolarizations at ON and OFF. When the spot was enlarged to 100 μm there was a shortening of the ON response while the OFF response was little affected. After enlarging the spot to 1000 μm the OFF response was smaller, had a slightly increased latency and was followed by a late depolarizing component. *B*: five superimposed responses to the onset of a 1000 μm, 1·2 O.D. light.

inhibited while there appeared smaller spikes from distant cells which presumably had excitatory areas under the dithering annulus. The total light from the dithering annulus was constant and because of radial symmetry the light scattered to the receptors under the test spot was approximately constant. Horizontal cells, which produce a maintained hyperpolarization that approximately indicates the total light incident over a large retinal area, were not affected by periodic change in position of the broken annulus. This is shown in the intracellular recording from a horizontal cell in the right trace. Turning on the annulus hyperpolarized the horizontal cell 3 mV; dithering the annulus had no additional effect; a 1000 μm spot evoked the characteristic intracellular response.

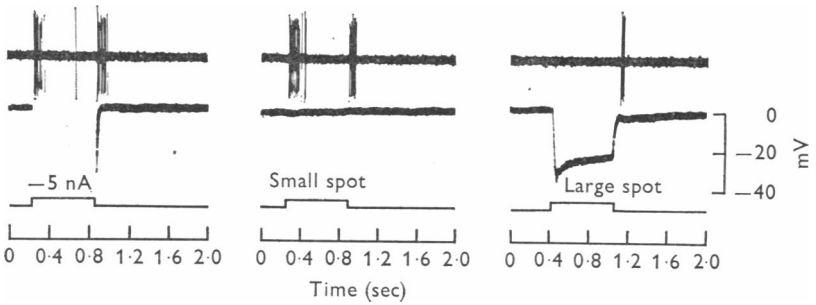


Text-fig. 4. Inhibition of the central response by peripheral elements with small receptive fields. For the left and centre records the upper timing trace indicates the duration of a 10 μm spot. The lower timing trace indicates the position of a broken annulus (with the dimensions indicated above) which is continuously on and stationary at the left and rotated clockwise–counterclockwise in the centre. During rotation of the annulus the ON–OFF cell was inhibited and there appeared smaller spikes from distant cells which presumably had excitatory areas under the dithering annulus. The right record shows that horizontal cells did not respond to the dithering annulus. The upper timing trace indicates the duration of a 1000 μm, 0·6 O.D. light and the lower trace the position of the same broken annulus as used in the left two Figures. Turning on the annulus (before the start of the record shown) hyperpolarized the horizontal cell 3 mV. Dithering the annulus as shown in the left half of the trace had no significanct effect.

The effect of peripheral inhibition as an antagonist to illumination of the receptive field centre is tabulated in Table 1*B*. Inhibition maintained by dithering must have been mediated by elements with a receptive field centre not much larger than the distance of occluded segments in the broken annulus, 165 μm.

Transient excitation from horizontal cells

Artificially hyperpolarizing a horizontal cell evoked transient excitation in an associated ON–OFF cell. This is shown in Text-fig. 5. The upper traces are extracellular recordings from an ON–OFF cell; the lower traces are simultaneous intracellular recordings from a horizontal cell. The intracellular pipette and extracellular wire were located within 100 μm of each other. The pair of traces on the left shows the effect of passing a hyperpolarizing current in the horizontal cell. Assuming in the turtle the maximum resistance from within a horizontal cell to the extracellular space to be 2 MΩ (Baylor, Fuortes & O'Bryan, 1971) a 5 nA current could change membrane potential by less than 10 mV or about 20 % of the dynamic range of these cells. This small current in the horizontal cell evoked a



Text-fig. 5. Effect of horizontal cell polarization on an ON–OFF cell; simultaneous intracellular recording from a horizontal cell and extracellular recording from an ON–OFF cell separated by less than 100 μm. At the left is shown the transient activity associated with passing a 5 nA hyperpolarizing current through the intracellular pipette indicated by bridge imbalance. In the centre is the response to a 100 μm, 2·4 O.D. spot which gave similar ON and OFF bursts and no discernable response in the horizontal cell. To the right is the response to a 1000 μm, 0 O.D. spot which gave a characteristic hyperpolarizing response in the horizontal cell and in this record abolished the ON response and decreased the OFF response of the ON–OFF cell. Both stimulation with a small spot or a small voltage change in a horizontal cell were excitatory to ON–OFF cells.

vigorous excitation following both its onset and offset. The centre pair of records shows the effect of a 100 μm, 2·4 O.D. spot which evoked ON–OFF activity and no discernible response in the horizontal cell. Finally, in the pair on the right the spot was enlarged and brightened to 1000 μm, 0 O.D. which evoked a smaller ON–OFF cell response and the characteristic hyperpolarizing response in the horizontal cell.

In Table 1*C* it may be noticed that the response to horizontal cell

polarization was greater at ON (when the membrane potential was moving in the hyperpolarizing direction). Similarly in four experiments it was found that a 5 nA depolarizing current gave an ON–OFF response and that the response was greater at OFF (when the membrane potential was moving in the hyperpolarizing direction). This was not further investigated.

Both stimulation with a small spot or a small voltage change in a horizontal cell were excitatory to ON–OFF cells. As shown in the previous section, inhibition from the periphery could be obtained without a change in the horizontal cell membrane potential. The inhibition of a large spot was only coincident with and not caused by the horizontal cell response.

DISCUSSION

This study of ON–OFF cells in the turtle retina indicates central excitatory pathways for ON and OFF responses which are preferentially activated by small fields and a peripheral inhibitory pathway which does not include horizontal cells.

Ganglion cells with ON–OFF responses which are inhibited by peripheral illumination are widely distributed among vertebrates and have been studied in the retinae of frogs (Hartline, 1940; Barlow, 1953), rabbits (Barlow, Hill & Levick, 1964), pigeons (Maturana, 1964), squirrels (Michael, 1968), and lizards (Dejours, 1965). To emphasize their possible importance in organizing animal behaviour, ON–OFF ganglion cells have been designated 'changing contrast detectors' (Maturana, Lettvin, McCulloch & Pitts, 1960).

It was surprising to find that ON–OFF cells have somata in both inner nuclear and ganglion cell layers. Marked cells with somata in the inner nuclear layer may have been amacrine or displaced ganglion cells. Ganglion cells with somata in the inner nuclear layer are known to exist in elasmobranchs, birds, amphibians, reptiles and mammals (Dogiel, 1895). It is possible, however, that a class of ganglion and amacrine cell both have the same response pattern. Bursts of fast spikes at ON and OFF have been attributed to amacrine cells in goldfish (Kaneko, 1970). The possibility of a class of amacrine and ganglion cell having a similar physiology should be further investigated.

Maksimova (1969) and Naka & Nye (1971) have suggested that the effect of peripheral illumination is mediated by horizontal cells. The present work demonstrates that for ON–OFF cells in the turtle an inhibitory interaction localized in the periphery is not mediated by horizontal cells. More than one pathway from the periphery may contribute to a ganglion cell response. Horizontal cells with their large area of summation are

themselves unlikely to contribute information concerning the spatial distribution of light. Other peripheral elements must contribute information about the geometric distribution of light. It is, therefore, necessary to make a distinction between the response to a small spot in the periphery and a large spot or annulus which includes the periphery. A small spot reveals a local property while a large spot may reveal a peculiarity of large area stimulation. Horizontal cells respond best to large diameter spots and are unlikely to contribute to a ganglion cell the response of a small spot. It would be better to consider them as mediating a difference between small and large field illumination with no reference to centre or periphery. Inhibition from a small spot in the periphery is probably mediated by peripheral bipolar cells.

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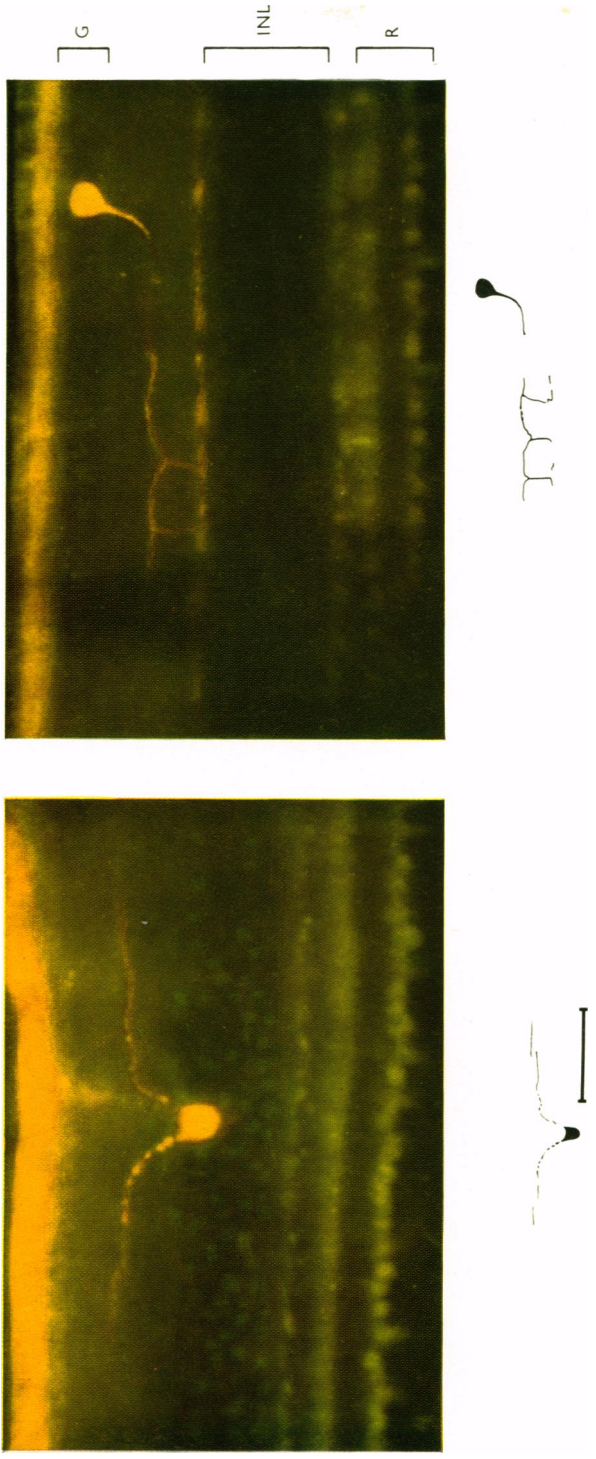
EXPLANATION OF PLATE

Photomicrographs of cross-sections of retinae to show ON–OFF cells injected with the fluorescent dye Procion Yellow M4R. One cell body is in the inner nuclear layer; the other is in the ganglion cell layer. The inserts give the outline of the cell in each photomicrograph. R: receptors; INL: inner nuclear layer; G: ganglion cell layer. The calibration mark applies to the camera lucida drawings and indicates 100 μm.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