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RESPONSES TO DIRECTIONAL STIMULI IN RETINAL PREGANGLIONIC UNITS

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SUMMARY

1. Extracellular recordings were made from directionally selective ganglion cell units in the isolated frog retina and decapitated *Necturus* preparation.

2. Intracellular recordings were made from individual photoreceptor cells in the frog and *Necturus* retinae while stimuli which had evoked directionally selective responses at the ganglion cell level were presented. No evidence for inhibition of photoreceptors for any direction of movement of the light stimulus was found. This appeared to rule out a mechanism for directional selectivity involving inhibition of photoreceptor potentials.

3. Intracellular recordings were made from the nuclear layer between photoreceptors and ganglion cells in *Necturus*. The responses were of two types: either transitory or sustained.

4. The sustained type responses could be divided into two classes depending on their receptive field organization. One type of sustained potential had a large receptive field without any evidence for a centre-surround antagonism and corresponded to the luminosity type S-potential recorded in fish. The other type had a smaller receptive field and showed a difference in sign of response between centre and surround if the centre was flooded with a steady light. This is very similar to what has been described for a type of on-centre, off-surround ganglion cell.

5. The transitory type of responses showed some centre-surround antagonistic organization. Some of these transitory units also appeared to

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show some discrimination in response as a function of the distribution of light on the retina.

6. No specific directional selectivity was found from units at the inner nuclear layer. This further excluded any mechanism of directional sensitivity which involves selectivity at the photoreceptor level.

7. It was concluded that although inner nuclear layer units may play a role in the mechanism of directional selectivity, no specific directionality was found at the first synaptic level of the retina.

INTRODUCTION

The retinal mechanisms which produce directional selectivity have been sought since directionally sensitive units were first demonstrated in the optic nerve of the frog (Maturana, Lettvin, McCulloch & Pitts, 1960). Subsequently, cells which have the ability to respond differentially depending upon the axis and direction of movement of the stimulus have been reported in several animals (Maturana & Frenk, 1963; Cronly-Dillon, 1964; Barlow, Hill & Levick, 1964; Michael, 1966). In all those experiments the responses were recorded extracellularly from retinal ganglion cells, their axons, or the optic tectum. To explain these findings certain theories have been put forward as to the functional organization in the retina responsible for directional coding (Barlow & Levick, 1965; Michael, 1966). However, the question remains as to what role the different preganglionic cells of the retina play in the organization of directional selectivity.

In order to study this problem intracellular recordings were made from cells between the photoreceptor and ganglion cell layers in either the isolated retina of the frog or the eyecup of *Necturus*. One of the central questions to be examined is at what level in the visual pathway may directional selectivity first be detected. The earliest cell in the visual pathway whose response is directionally selective will have to integrate, or analyse, incoming signals from preceding sequence discriminating subunits. Since directional selectivity has been demonstrated in ganglion cells, the ganglion cell itself may be the analyser. However, it is possible some preceding unit in the chain of signal processing may fulfil this function. At this preganglionic level the relative contributions from different incoming signals would then be integrated and an evoked response which is a function of the direction of stimulus could be obtained. However, if a certain type cell does not show directional selectivity, it follows that the analyser would have to be at a succeeding point, for example at the ganglion cell level.

There are many possibilities as to the mechanism of directional selec-

tivity. Indirect evidence has been presented to show that there is no inhibitory feed-back system from stimulated photoreceptors to adjacent receptors (Barlow & Levick, 1965). By recording directly from photoreceptor cells we were able to examine this possibility.

It has been known for some time that there is a difference in sign of response between centre and periphery of the receptive field of the ganglion cell (Kuffler, 1953). No such organization was found for S-potentials, the graded, sustained, intraretinal response (Norton, Spekreijse, Wolbarsht & Wagner, 1968). Recently, it has been shown that such an antagonism exists for some units which give a slow, graded response to diffuse illumination (Werblin & Dowling, 1969). As will be described, we have found both slow and transient type responses whose form changes depending upon what portion of their receptive field is being stimulated. However, what part these units may play in the mechanism of directional selectivity is still not clear.

METHODS

Retinas were dissected from enucleated eyes of bullfrogs (*Rana catesbiana*). The retina was placed receptor side up in a water-cooled chamber. The chamber was kept at 16° C and moist oxygen passed over the preparation. Light was projected through the retina from below passing from ganglion cell to photoreceptor layer. The mudpuppy (*Necturus maculosus*) was decapitated and pithed. The head was pinned to a paraffin block and the overlying skin together with the cornea, iris and lens was removed. Absorbent tissue paper, soaked in frog Ringer solution, was placed over the *Necturus* preparation to prevent drying. The stimulating light in this case was projected down upon the eye from above, passing first through partially drained vitreous and then the ganglion cell layer. The retinas were dark adapted and not exposed to extraneous light or background illumination except where otherwise noted.

A 100 W tungsten projection lamp served as a source for two independent light stimuli which could be focused upon the preparation. The wave-length, stimulus duration and intensity could be varied independently in either channel. A detailed description of the optical stimulator, including its spectral distribution, has been made by Wagner, MacNichol & Wolbarsht (1960). However, in one optical pathway a modification of the original design was made in the following way. A rigid arm attached to an x–y recorder was placed in a plane of focus. This arm could accept different apertures and opaque spots with diameters ranging from 40 μ to 1·5 mm, as well as various-size slits. The slits varied in width from 40 μ to 1 mm and were of two types. One produced a band of light on the retina, while the other type was an opaque band moving across an illuminated field. The x and y axes of the recorder were driven by a function generator through an operational amplifier so that the stimulation light could be moved at various speeds ranging from 0·1 to 100 mm/sec over the retina. The path-length of the moving stimulus was linearly proportional to the amplitude of the triangular wave-form generated by the function generator. There were four different axes along which the stimulus could move across the preparation in both directions (see Fig. 8). Therefore, a variety of different moving stimuli, light or dark, could be imaged on the preparation. The stimulating light could be centred with respect to the micro-electrode by means of the adjustable

zero setting of the x–y recorder. This setting served as a reference voltage about which the function generator oscillated. Therefore the moving stimulus always passed directly over the recording electrode independent of the direction and speed of the stimulus spot. The exact position of the moving stimulus in relationship to the triangular wave-form could be ascertained by calibration before the experiment. Figure 1 depicts the relation between the signal trace of the driving voltage and the moving stimulus across the retina. At both the apex and base of each triangle the stimulus spot is fully displaced in one direction and has completed a single sweep across the retina. At the midpoint of the side of the triangle the stimulus is directly over the electrode.

Although stimulus wave-length was varied between 400 and 750 nm, no response studied in the frog or *Necturus* was found to differ from a monotonic function of wave-length. Unless otherwise noted, white light of maximum intensity 350 lm/m^2 was used experimentally.

The recording electrodes were glass micropipettes filled with 2 M-KCl and had a resistance of about 200 MΩ. The response signals were led to a high input impedance, capacitance compensated d.c. amplifier via chlorided silver wires. The responses were displayed on an oscilloscope and stored on magnetic tape as well as recorded with a strip chart pen recorder. The extracellular experiments performed on the ganglion cells were obtained by glass-insulated platinum electrodes (Wolbarsht, MacNichol & Wagner, 1960) and a capacitor coupled amplifier. A chlorided silver wire placed up the vertebral canal in the *Necturus* preparation and directly on the isolated retina served as the reference electrode.

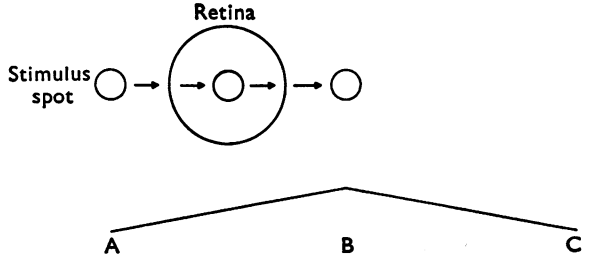

Fig. 1. A representation of the stimulus light traversing the retina in relation to the calibrated driving triangular waveform. The triangular voltage drives the x–y recorder which in turn moves the light stimulus. At point A the stimulus has just finished a sweep across the retina and is about to begin the return sweep along the same axis. At the midpoint of the side of the triangle, half-way between segment A–B, the stimulus is directly over the recording micro-electrode. At point B it has finished the traversal across the retina and is ready to return, which it does during segment B–C. At point C, the stimulus is exactly where it was at point A. By prior calibration one knows where the stimulus light is at any instant by its relationship to the triangular signal trace. This arrangement is used in subsequent figures.

Off line analysis and averaging of the taped records was done with a Computer of Average Transients (CAT). Averaging time, number of responses averaged, as well as number of intervals averaged per period were controlled externally and synchronized with the function generator. A final analogue record was made with an x–y recorder except for the ganglion cell responses which were photographed from an oscilloscope.

In our series we recorded 33 photoreceptor units, 74 S-potentials, 47 other slow sustained potentials with a centre-surround field organization, and 29 transitory type units (17 'on' and 12 'off').

RESULTS

Extracellular recordings

Figure 2*A* depicts the response for a non-directionally sensitive ganglion cell in the isolated frog retina. The triangular signal trace in this and subsequent figures represents the movement of the stimulus across the retina. Between the apex and base of each triangle the stimulus has passed over the retina. The stimulus is directly over the electrode at the

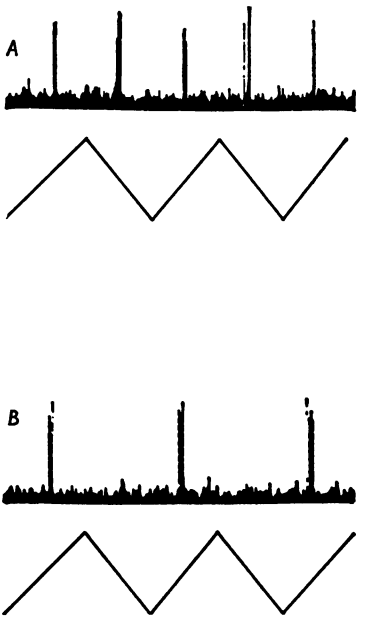

Fig. 2. *A*, Responses of a non-directionally sensitive ganglion cell in the isolated frog retina to a moving 50 μ diameter stimulus light. Each burst of spikes corresponds to one traversal of the stimulus across the receptive field. The lower trace is the triangular voltage driving the stimulus light. At the apex and base of each triangle the stimulus has reached the end of a sweep across the retina and commences to return along the same path (axis) in the opposite direction. At the midpoint of each side of the triangular waveform the stimulus is directly over the recording micro-electrode. *B*, Responses of a directionally sensitive ganglion cell. Responses for a particular axis occur only every second sweep of the stimulus light across the receptive field. There are brisk responses when the 50 μ diameter light is moving in one direction (preferred direction) and no response in the opposite direction (null direction). Signal trace is exactly as in *A*. Velocity of stimulus is 10 mm/sec.

half amplitude point of the triangular trace. Therefore, a single sweep of the stimulus across the retina is represented by one side of a triangle. For the non-directionally selective unit in Fig. 2*A*, each sweep of the stimulus spot across the receptive field evoked a response. For a directionally selective unit (Fig. 2*B*), a sweep across the receptive field in one direction, for a specific axis, produced a brisk response. This was the

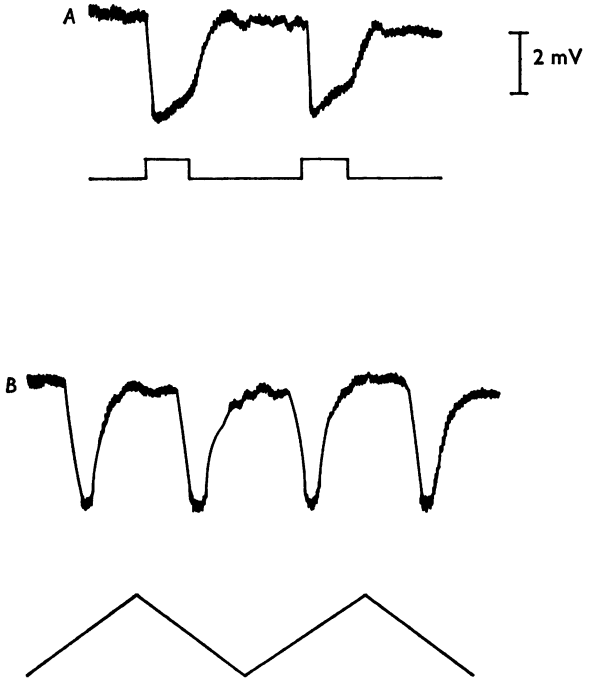

Fig. 3. *A*, Photoreceptor response in isolated frog retina. Each stimulus of white light is 0·5 sec in duration. The first stimulus is of greater diameter than the retina. The second is 40 μ in diameter. These potentials show no area effect in distinction to S-potentials which possess large area effects. *B*, Photoreceptor responses to moving stimuli. The responses were averaged over five sweeps across the retina in both directions along a single axis. Therefore, the first and third potentials would be responses to stimuli moving in one direction while the second and fourth potentials are responses to the stimulus light returning in the opposite direction. No evidence of directional selectivity is seen. This experiment was repeated for all four axes at velocities ranging from 0·1 mm/sec up to 100 mm/sec with similar results. The velocity used in the figure was 15 mm/sec.

preferred direction. When the stimulus returned back across the retina in the opposite direction there was no response. This indicated the null direction as described by Barlow & Levick (1965). Therefore, a response

is seen only for every other stimulus sweep. If the axis was changed some response, although a weak one, was obtained for both directions from the directionally sensitive cells. Similar results were obtained with the *Necturus* preparation. The repetition of stimulus back and forth across the preparation, as well as the variations in velocity, make it unlikely that the directionally selective responses are an artifact of adaptation.

Intracellular recordings

Photoreceptor potentials

Recordings were made from frog photoreceptor cells. The photoreceptor potentials fulfilled the various criteria proposed by Tomita, Kaneko, Murakami & Pautler (1967), such as relatively small size (less than 5 mV), closeness of recording micropipette to the distal retinal surface and always hyperpolarization, regardless of stimulus wave-length. However, the one criterion which we felt most useful was the absence of an area effect. As seen in Fig. 3*A*, when stimulated with a flash of white light, the photoreceptor potential was of nearly equal size whether a 40 μ diameter spot or diffuse light of greater diameter than the retina was used.

Figure 3*B* shows the averaged responses of a photoreceptor cell to a 40 μ stimulus light passing over the retina through the micro-electrode recording site, first in one direction and then in the opposite one for a given axis. This was repeated for all four axes in which the stimulus could be moved. Similar results were obtained with various size stimuli of black spots and opaque slits on an illuminated field as well as with slits of light. These stimuli ranged from a diameter or width of 40 μ to 1·0 mm. In no case was any difference in response shape, amplitude, or latency seen. Barlow & Levick (1965) raise the question of whether directional specificity might depend on receptor inhibition in the null direction. Our experiments provide direct evidence that no such inhibition exists with regard to the photoreceptor potential in frog. To rule out all types of inhibition of photoreceptors is difficult. The results would depend on where the interaction occurs and from what part of the cell one is recording. For instance, the photoreceptor potentials most likely are recorded from the inner segment of the cell (Kaneko & Hashimoto, 1967), but photoreceptor interaction may be taking place at the photoreceptor synaptic endings. However, evidence that photoreceptor inhibition is not the primary mechanism of directional selectivity may be implied from succeeding experiments where the lack of directional selectivity at the next synaptic level is demonstrated.

S-potentials

The usual criteria for S-potentials are that they be of greater amplitude than photoreceptor potentials and show a marked area effect. The S-potentials were always hyperpolarizing in the frog and *Necturus* regardless of stimulus wave-length and summated over a large portion of the retina. Therefore, they corresponded to the luminosity type S-potentials recorded in fish (MacNichol & Svaetichin, 1958). The same constancy of results pertained to these responses as to the photoreceptors utilizing the same stimulus parameters. There was no evidence of a preferred or null stimulus direction.

In addition, in both the frog and *Necturus*, different size stimulus annuli and stimulus spots centred in the receptive fields of the sustained, hyperpolarizing S-potential were used. This was done in an attempt to find some comparable type of centre-surround field organization in these S-potentials analogous to that of ganglion cells. Either a centre-surround antagonism or some evidence that the strictly hyperpolarizing type of potential received an input signal of more than one sign was sought. No such evidence was found in the frog or *Necturus*.

Other inner nucleus layer responses

Due to the relatively large size of the cells at the inner nuclear layer, the *Necturus* afforded an opportunity to record intracellularly from a variety of units at this level (Bortoff, 1964). The responses of these units to white light were of two types. One was a slow, sustained depolarizing or hyperpolarizing type of potential, the other was of a non-sustained transitory nature.

Sustained responses. Considering the former type first, there were recordings of sustained hyperpolarizing responses to diffuse illumination (stimulus light of greater diameter than the retina) which did give an indication of a more complex receptive field with a centre-surround organization. Responses which gave a depolarizing response to diffuse illumination were also recorded and similarly had centre-surround antagonistic receptive fields. Figure 4*A* gives an example of the hyperpolarizing case. By using the appropriate stimulus a centre-surround organization can be demonstrated for this type unit. To a flash of diffuse stimulation the response appears as a sustained hyperpolarizing type potential. However, if a small steady spot (less than 100 μ) of light is centred on the recording micropipette a change in d.c. level corresponding to the response to diffuse light is found as expected (segment A–B of lower signal trace). But if now an annulus of light about 200 μ inside diameter and 300 μ outside diameter is superimposed, a reversal may be seen, with the

potential becoming less negative. This effect disappears quickly as the annulus becomes larger in outer or inner diameter. The important point to note is that the surround response in the depolarizing direction only comes back to the original resting potential, i.e. where the d.c. level was in the dark, non-stimulated state. In no case did the depolarizing surround component ever rise above this d.c. level, nor could such a response ever be elicited by a purely surround stimulus without first stimulating the centre. Exactly corresponding results were obtained for the depolarizing response (Fig. 4*B*). Steady illumination of the centre caused depolarization while superimposed annular stimulation caused a return to the resting d.c. level.

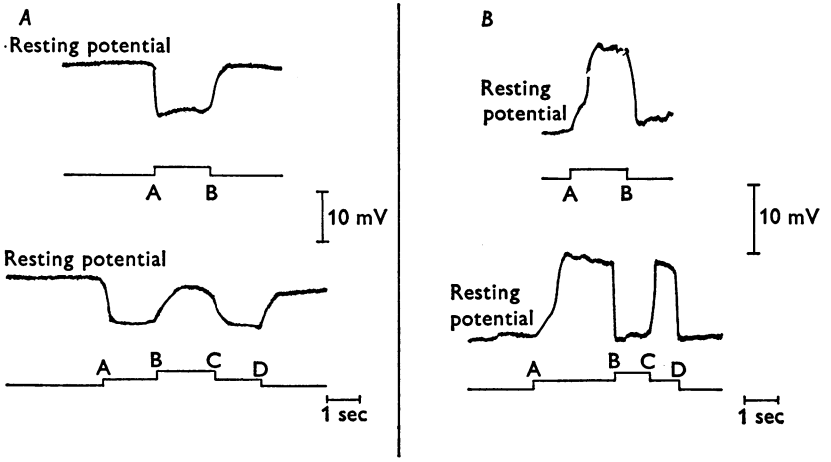

Fig. 4. *A*, Hyperpolarizing response recorded from the inner nuclear layer of *Necturus*. Upper recording is the response to a 0·5 sec flash of white light. In contrast to the luminosity type hyperpolarizing S-potential which was also recorded, the receptive fields of these potentials showed a distinct centre-surround organization. During segment A–B on the lower signal trace a 100 μ diameter light stimulus is imaged on the recording electrode site, causing a hyperpolarizing response. During segment B–C an annulus of light 200 μ inner and 300 μ outer diameter is added, and then removed during segment C–D. After D all stimulus light is removed from the retina and the potential returns to its resting level. *B*, Repetition of the experimental sequence for a depolarizing potential produced the same results. In this case the potential deflexions are of course in the opposite direction.

This effect is very similar to one described by Barlow *et al.* (1964) for an on-centre, off-surround ganglion cell response in the rabbit. They found that the opposite phase response in the surround could not be elicited unless the centre was simultaneously flooded with constant light.

A different type of phenomena is depicted in Fig. 5. A sustained hyperpolarizing response, which if examined closely may be seen to have a rapid component soon after onset of stimulation, underwent a profound

change with background illumination. If a diffuse background light, of 1·0 log. unit less intensity than the stimulating light, is turned on there is the expected d.c. shift in the negative direction (point C on the signal trace). Now, if a flash of diffuse light is superimposed, only transient responses are observed at both on and off. The d.c. component has been fixed at a negative level with respect to the resting potential but responses to changes in illumination, light on or off, are still present. Over a range of decreasing intensity of background illumination the d.c. component was altered in a graded fashion, becoming more prominent while the transients decreased in amplitude. With the background illumination removed the response reverted to its original hyperpolarizing form.

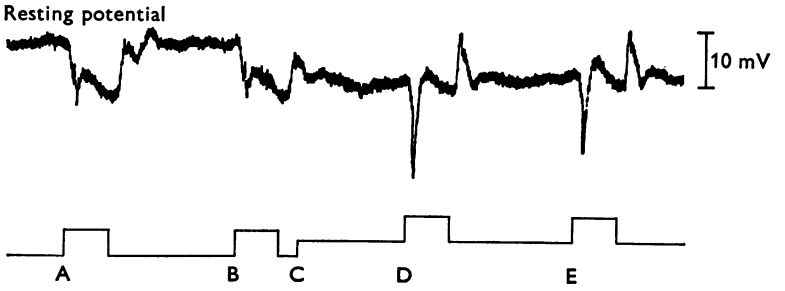

Fig. 5. Hyperpolarizing response to white light recorded from *Necturus* retina (monochromatic stimuli of wave-lengths between 400 and 750 nm did not alter the response shape). Signal trace shows a 0·5 sec flash of diffuse white light at A and B. At point C background illumination one log unit less intense than the stimulus flash is directed onto the retina. When the stimulus is flashed on for 0·5 sec at D and E, the d.c. component all but disappears leaving only transient potential changes with onset and termination of the light stimulus. When the background illumination was removed the potential reverted to its original sustained form.

The question is, did these sustained type units or their components show any evidence of directional selectivity? The answer was no to all sizes and forms of stimuli tried over a 0·1–100 mm/sec range of velocities.

Transitory responses. The transitory units showed a rapid non-sustained depolarizing response either to onset or termination of stimulation. The 'on' type response was followed by a smaller, slower, non-sustained depolarizing component while the 'off' response was preceded by a similar type of component. However, the larger component of the 'off' type response was more complex than the 'on' type (see Fig. 6).

The receptive fields of the 'on' units appeared to have some complexity. As shown in Fig. 7, if the stimulus light spot is changed from a diameter of 1·5 mm, flashes A, B, and E, to a spot of 150 μ flash D, the evoked response becomes a sustained, purely hyperpolarizing potential. If a

slightly larger stimulus spot of 300 μ is used, flash C, an intermediate response is obtained which is transiently hyperpolarizing followed by a more sustained depolarization. For this type of response the peripheral effect dominates for diffuse light stimulation. Annuli produced responses similar to those found with larger spots of light. There was no evidence observed for directionality in the 'on' type unit.

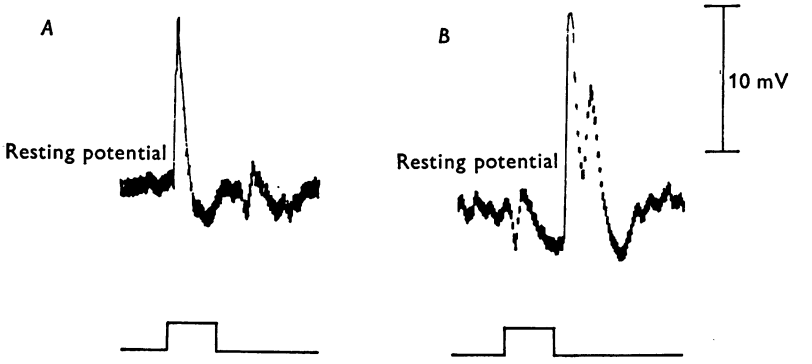

Fig. 6. Two types of transient potentials recorded intracellularly from the *Necturus* retina. Potential *A* represents an 'on' type response to the onset of diffuse illumination, whereas potential *B* is a more complex response to the termination of illumination and therefore is an 'off' type response. Both potentials have similar smaller components occurring respectively at termination of stimulation for the 'on' potential and at onset of stimulation for the 'off' potential. The stimulus light flashes were 0·5 sec duration.

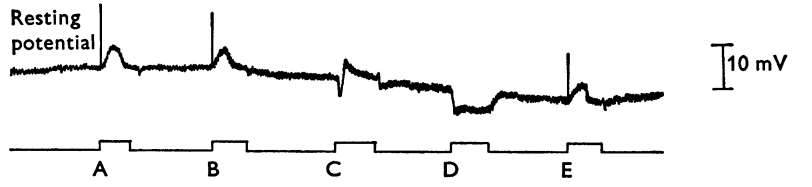

Fig. 7. An 'on' type unit similar to that shown in the Fig. 6A record. Stimulus flashes A, B and E had a diameter of 1·5 mm and evoked the same response as did diffuse light which covered the entire retina. A smaller 300 μ flash at C caused a transient hyperpolarization followed by a sustained depolarization. If a 150 μ spot of light was used as at D then a purely hyperpolarizing sustained response is found. Stimuli are approximately 0·5 sec in duration.

The units with a large transitory 'off' response did show differences in response to moving stimuli. Averaged responses were obtained from only two 'off' units. This was due to the difficulty of maintaining this type unit long enough for several repetitive sweeps of the stimulus along each axis. Four of the ten other 'off' type units studied showed similar

results when stimulated with only two or three non-averaged sweeps along each axis. Figure 8 shows a series of averaged responses from an 'off' unit. There are a pair of responses for each of four different axes. Each pair consists of an averaged response to one sweep of the stimulus across the receptive field and back. The lower signal traces are as described before.

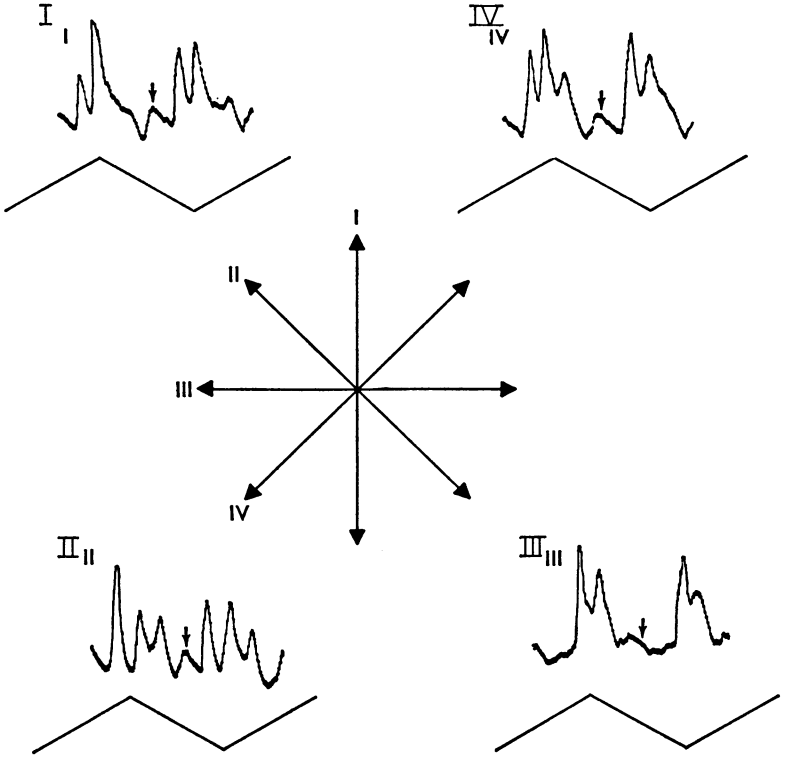

Fig. 8. The four records shown here are from an 'off' unit of the type shown in the Fig. 6*B* record. Each record consists of a pair of averaged responses to the sweep of a 100 μ diameter light stimulus along the designated axis first in one direction and then back in the opposite one. There are examples for all four axes. As the figure demonstrates, for any one of the axes the response is different for the stimulus light moving in one direction than for another direction. This is the case for all four axes. The individual signal traces are as described before. The arrows indicate the small on component. Similar results were obtained with velocities varying between 0·1 and 100 mm/sec. The velocity shown in this figure was 15 mm/sec.

As shown, the 'off' type unit appeared to vary its response for each axis tested with the moving light stimulus. Furthermore, for a given axis, there was a consistent difference in the shape of the response for each direction. For instance, if one examines response number III in Fig. 8,

it may be seen that the response to the stimulus moving from right to left is different than when the stimulus moves from left to right. The difference in directions was more pronounced for some axes than for others. This is certainly not specific directional selectivity but it does suggest that a unit at this level of the retinal organization is affected by the distribution and direction of light on the retina.

DISCUSSION

When one penetrates a retina with a micropipette whose tip measures about 0·1 μ (Tomita *et al.* 1967), the first question is, from what structures are the recordings being made. There are two general approaches to this question. One is a physiological approach based on the particular electrophysiological characteristics typical of a specific class of unit, and the other is by use of a dye-injection technique to stain and identify histologically the cell from which the recordings were made.

The first approach applies to the two classes of cells lying on opposite sides of the retina, the photoreceptors and the ganglion cells. The area effect, as described earlier, presumably allows one to differentiate photoreceptor potentials from other sustained graded potentials (Tomita *et al.* 1967). The ganglion cell can generally be identified by stimulating antidromically fibres of the optic nerve and driving the unit being recorded from (Kaneko & Hashimoto, 1968). There is no such physiologically specific criterion for identifying intervening cells such as those lying in the inner nuclear layer. For these units, dyes have been injected from the micropipette into the different cells after recording. That this technique is less than ideal can be seen from the variety of results published (MacNichol & Svaetichin, 1958; Mitarai, 1958; Oikawa, Ogawa & Motokawa, 1959; Tomita, Kaneko, Murakami, Sato & Hashimoto, 1959; Bortoff, 1964; Werblin & Dowling, 1969). What there does seem to be agreement about is that, except where noted, the types of responses reported here were not from photoreceptors or ganglion cells. Therefore, these intracellular responses were probably from the horizontal-bipolar cell level. If one accepts that the purely hyperpolarizing S-potential originates in the horizontal cell (Svaetichin, 1967; Byzov & Trifonov, 1968; Kaneko & Hashimoto, 1969), then the transient potentials as well as slow graded responses with centre-surround receptive fields most likely come from amacrine and bipolar cells. In fact, two recent investigations have stated this to be the case (Werblin & Dowling, 1969; Kaneko & Hashimoto, 1969).

The results reported here indicate that at the level of the inner nuclear layer, no definitive retinal organization of directional selectivity has taken place. This is not unexpected in the case of those sustained slow S-potentials

which have a simple field organization. It has been shown that their receptive field extends over a wide area and is of a type of organization unlikely to select small differences in direction of stimulus (Norton *et al.* 1968).

The sustained slow potentials which have a more complex antagonistic type of organization seem likely candidates to play a more direct role in directional selectivity. As stated, they did not show any directional selectivity either, but this certainly does not rule out the possibility of their being a component of the directional selectivity mechanism. It is apparent that the term S-potential applied to slow, sustained intraretinal responses really describes a family of such responses. The shape of the particular potential depends not only on the wave-length of stimulus but also on the intensity of stimulus, level of background illumination, and receptive field organization of the particular unit being recorded.

The transient-type responses, with their more complex receptive field organization and faster response times, appear to be good possibilities to play a part in the directional selectivity mechanism. Although no null direction was present in this group, in some units moving stimuli evoked different responses in all axes, along both directions. This might represent a preganglion cell discrimination which by interaction between several similar units becomes specific at the later level.

The results described here do not give any specific answers to how the coding of the sensory information takes place to produce a directionally sensitive ganglion cell. What part the different units at the inner nuclear layer level play is still speculative (Barlow & Levick, 1965; Michael, 1968). However, it appears that both the sustained and transitory type potentials have begun to show some discrimination in regard to their receptive field organization as well as to the intensity and distribution of illumination on the retina.

Due to the lack of directional selectivity at the preganglionic level, photoreceptor inhibition is probably not the mechanism of directional selectivity and the analyser is most likely the ganglion cell itself.

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