

J. Physiol. (1953) 119, 58-68

ACTION POTENTIALS FROM THE FROG'S RETINA

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(Received 18 June 1952)

The retina contains three layers of cells, of which only one is directly responsive to light. Even histological inspection suggests that the other two layers do more than simply pass on the pattern of activity aroused by the light without modifying it in any way. The work of Adrian & Matthews (1927*a, b*, 1928), Hartline (1938, 1940*a, b*), and Granit (1947), has shown that this is so. The pattern of light and shade cast on the retina by the optical system of the eye gives rise to a pattern of nerve impulses in the optic nerve, but neither the temporal nor the spatial features of this pattern of impulses are accurate copies of the pattern of light. The present work was undertaken with the idea that these distortions might amount to some integrative action of the nervous layers of the retina analogous to the integrative action of the spinal cord studied by Sherrington.

This paper is concerned mainly with the technical problems encountered in detecting the electrical activity of single retinal units in the frog's retina. The results of other experiments with this preparation are described in a second paper. Some preliminary results have already been published (Barlow, 1950).

METHOD

Preparation. The eyes of partially light-adapted frogs (*Rana temporaria* and a few *R. esculenta*) were excised and opened. The vitreous was removed and the surface of the retina explored with a micro-electrode, similar to those used by Granit & Svaetichin (1939), made of 20 μ platinum wire insulated with a transparent plastic (H.T. cement), and then cut off so that a flat surface was presented to the retina. The electrode was moved over the retina with a pantograph type of micro-manipulator, and lowered gently onto its surface with a fine adjustment screw.

Amplifiers, etc. It was convenient to lead from the small insulated dish holding the preparation to the grid of the first stage of the amplifier, so that the micro-electrode was at earth potential. The amplified action potentials were fed to a loudspeaker, and displayed on a cathode ray screen. In order to determine the size of the action potentials, a conventional calibrator was inserted between the micro-electrode and earth, and the size of the deflections produced by the calibration compared with the action potentials. The frequency response of the amplifier is not important in interpreting this work, though it was possible to improve the signal/noise ratio by cutting off both

high and low frequencies; the extent to which this was done can be judged from the shape of the calibration pulses.

Optical stimulation. The retina was illuminated by a constant background light, and upon this a spot of light variable in intensity, size, and position was superimposed. This additional stimulus light was turned on for 1 sec every 2 sec by hand, and the response of the retina observed. Fig. 1 shows the apparatus used to do this. The constant, even, background light is provided by the lamp *M* which illuminates an opal screen *O*; the objective *H* puts a defocused image of this screen reflected in the half-silvered prism *G*, onto the preparation *I*. The main stimulus light is the lamp *A*, which illuminates a condensing lens *D*, 'seen' in Maxwellian view by the objective *H*; an image of a movable stop of variable size, *C*, is thus cast on the retina, and it can be varied in intensity by the wedge *B*, and turned on and off by the shutter *F*. A second stimulus spot was added, when required, by inserting the coverslip *E*; this reflected light from the lamp *K*, reduced in intensity by the screen *L*, onto the objective, and an image of the filament was thus cast on the retina. In the experiments described in this paper the background illumination was varied from 0.1 to 0.01 f.c. by the neutral screen *N*, and a threshold discharge would be produced by a stimulus spot 0.2 mm in diameter of the same intensity falling on a sensitive region of the retina.

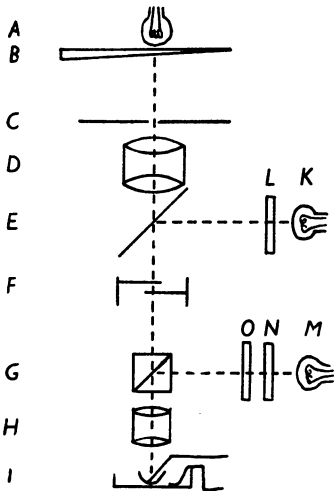


Fig. 1. Optical stimulator. Explanation in text.

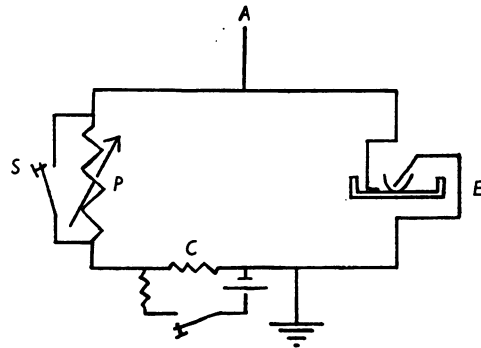


Fig. 2. Circuit for measuring resistance of preparation and electrode. Explanation in text.

RESULTS

The results reported here concern the characteristics of the potentials picked up by the micro-electrode and the general properties of the preparation itself.

Source of noise

When the electrode is in the vitreous, but not touching the retina, there is a low level of electrical disturbance which both sounds and looks like random thermal noise. Measurement of the resistance of the electrode immersed in saline confirms this, for the amplitude of the disturbance corresponds to the predicted amplitude assuming that the only source of disturbance is thermal

noise in the electrode and the first stage of the amplifier. Calculation shows that the resistance of the electrode is not much greater than that of the hemisphere of saline immediately surrounding its tip; the wire and the surface contribute a negligible fraction.

When the electrode is lowered onto the surface of the retina there is an increase in the amplitude of this noise. At first this was thought to represent some type of biological activity, but the following experiment shows that it is caused simply by an increase in the resistance of the preparation. The circuit shown in Fig. 2 was set up. The input of the amplifier *A* is connected to earth through the preparation and electrode (*E*) as usual. In parallel with the preparation is a potentiometer *P* and shorting switch *S*, and these are connected to earth through the calibrator *C*. The calibrator gives rectangular pulses of 100 or 200 μV . The value of *P* is adjusted so that 200 μV pulses with *S* open give the same deflection on the screen as 100 μV pulses with *S* closed. The resistance of *P* is then measured, and must be close to the impedance of the electrode and preparation over the frequency bandwidth of the amplifier. The internal resistance of the calibrator *C* is very low and can be neglected. At the same time the level of disturbance on the oscilloscope screen was measured with the preparation alone in the input circuit, and again with the potentiometer alone, after this had been adjusted as described. The figures obtained are shown in Table 1. Clearly there is an increase of resistance when the electrode touches the retina, and this increase is sufficient to account for the increased noise. A similar increase also occurs when the electrode tip is brought close to a block of Perspex in a saline bath. It can be concluded that the base-line disturbances in the absence of stimulation are caused by the thermal noise of the resistance of the preparation, and that there is some structure in the retina which has a high resistance compared with saline.

TABLE 1

Electrode position	Resistance (Ω)	Noise (arbitrary units)	
		Observed	Calculated
In vitreous	15,000	8.0	8.6
On retina	40,000	11.8	13.0
In saline	15,000	8.6	8.7
On perspex block	23,000	11.1	10.2

Action potentials

When the electrode is in the vitreous the only sign of activity that can be detected on illumination of the retina is the ordinary electroretinogram. With weak stimulation and the amplifier adjusted to a short time constant this is barely visible. As soon as the electrode touches the retina and the increase of resistance has occurred, two more electrical signs of activity can be picked up and differentiated; these have been described by Gernandt (1948). Adjust-

ments of the intensity, size, and location of the stimulus light sometimes enable a regular train of impulses of one or other type to be isolated. Fig. 3 shows these two types of action potential on a fast time base, and a diagram indicates the region of the retina which must be illuminated to elicit each type. One is a rapid triphasic potential which produces a high-pitched crackle in the

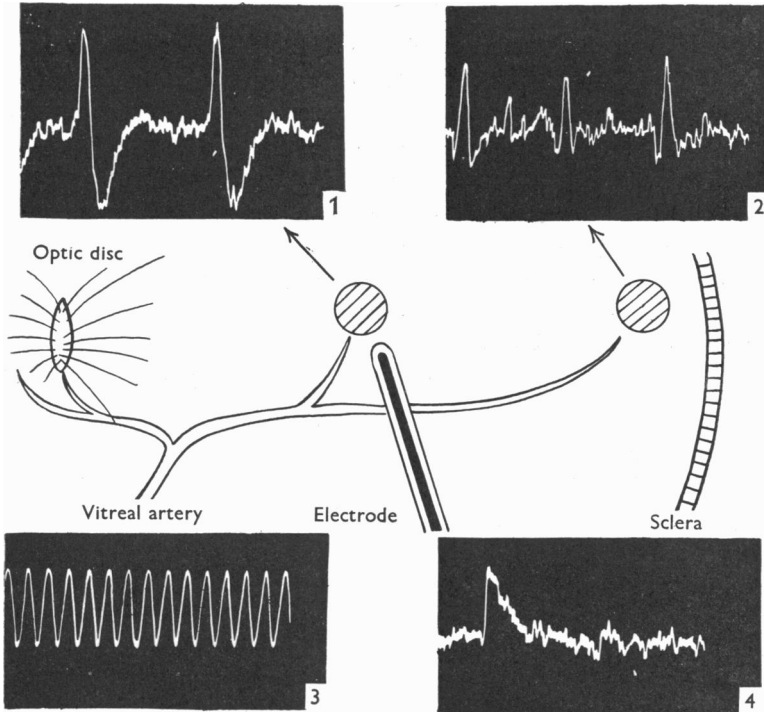


Fig. 3. Diagrammatic drawing of retina and electrode showing position of stimulus and records of responses for (inset 1) slow type, (2) fast type of action potential. (3) 1000 c/s time base. (4) $20\mu\text{V}$ positive-going pulse, equivalent to electrode negative-going.

loudspeaker. It is only elicited by illumination of the region of the retina lying roughly on a line from the optic disc produced beyond the electrode. It can reasonably be concluded that this type represents the action potentials in nerve fibres passing from peripheral ganglion cells to the optic disc. It is very hard to isolate single units of this type of potential, and the few that have been isolated have failed very rapidly.

The other type is a rather slower diphasic potential audible as a lower-pitched thump through the loudspeaker. This is more often single, and sometimes of greater amplitude. It is only elicited by light falling within a few tenths of a millimetre of the tip of the electrode. This is the type of potential

used in the subsequent work, and its site of origin is of considerable importance. The location of the receptive field suggests that the potential arises from a ganglion cell, or from a point on the nerve fibre very close to the ganglion cell. This confirms Granit's (1947) impression, and fits in with Rushton's (1949) conclusions about the site of origin of these potentials. Rushton also showed that a special type of large cell was responsible for the large potentials observed in the cat's retina. The possibility exists that special cells are responsible for the potentials in the frog's retina as well, but the evidence is rather against it. In the cat the potentials are large (300–500 μ V) and can be picked up over a circle of about 100 μ radius. In the frog the potentials are usually about 50 μ V, and a very small movement of the electrode causes one unit to disappear, and sometimes substitutes another of a different type. Frequently a 20 μ electrode picks up two or three units of about the same amplitude. In the cat there are large cells corresponding to these large potentials. In the frog there are a few large cells in the extreme periphery, but the sites from which potentials can be recorded are very much more frequent, and extend inwards towards the optic disc much further. With a good electrode a single unit can be isolated at almost any place where the electrode is lowered onto the retina. It does not appear likely that an unusually large or infrequent type of ganglion cell is being picked out by this technique, though it would of course be unsafe to assume that the technique detects the activity of all types of ganglion cell, or that it yields a fair sample of the types of ganglion cell which can be isolated.

Electrodes varied a great deal in their abilities to pick up isolated units of the second type. A good deal of time was spent in trying to decide what features were necessary in a good electrode, but the matter was never satisfactorily settled. The insulation had to extend to the tip and encircle it completely, and the cut surface had to be so orientated that it descended flat onto the retina, but many electrodes which apparently fulfilled these requirements were nevertheless failures.

On first placing the electrode, action potentials were usually picked up from several units. With a good electrode adjusted so that the cut surface descended flat onto the retina, gently screwing down the electrode usually reduced the number of action potentials to one or two. Excessive pressure on the electrode made the part of the retina surrounding the tip change colour from grey-black to a reddish pink and a further increase usually caused the ganglion cells to start discharging rhythmically, often at a high rate. The first change in colour was avoided if possible, but some preparations lasted well even if there was a slight bleaching round the tip of the electrode. If the pressure started spontaneous discharges the preparation failed quickly.

When there was more than one action potential it was sometimes easy to distinguish them by their amplitude or the quality of sound they made, and

in these cases attention could be confined to whichever was most easily distinguished from the background noise. It was much harder to be certain that the same unit was responsible for the action potentials occurring under different conditions of stimulation, for example at onset and cessation of illumination, or from stimulation of different parts of the receptive field. Occasionally increasing the intensity of the stimulus, or changing the area of the retina illuminated, showed that two rather similar units were being confused, but this was comparatively rare, for it was unusual for two units to appear the same on the screen, and sound the same through the loudspeaker.

Characteristics of the discharge

Of the eighty-nine preparations which gave good single unit discharges five (6%) were classified as 'on' units, forty-five (50%) as 'on-off' units, and thirty-nine (44%) as 'off' units. The comparable figures obtained by Hartline (1938) were: 'on' 20%, 'on-off' 50%, and 'off' 30%. The small number of 'on' units in this series is accounted for partly by the fact that they were deliberately avoided, but they were only rarely encountered. Some of those classified as 'on-off' units gave only an 'on' discharge when the whole retina was illuminated, but gave 'off' discharges as well when the stimulus spot was confined to the receptive field; such units might have been classified as 'on' type by Hartline. The proportion of the off and 'on-off' types is also slightly distorted by the fact that some experiments had to be done on one particular type, and in these cases a promising preparation of the other type might be rejected. This was not done very often because a good preparation of either type was sufficiently rare to be valued quite highly. Two other factors may distort the proportions. First, the technique may favour the isolation of one type, and secondly the periphery of the retina was used more often than the central regions. Taking all these disturbing factors into account it is surprising that the percentages of each type isolated agree as well as they do with those obtained by Hartline, using a totally different method of isolation. Though it is conceivable that both techniques favour the isolation of the larger cells with larger nerve-fibres, the agreement adds some weight to the conclusion that the types of ganglion cell isolated are not particularly rare or infrequent.

Hartline's description of the rate of adaptation of each type of discharge has also been confirmed by listening and observing the trace. The 'on' type gives a maintained discharge which builds up rather slowly. On reducing the intensity of illumination there is often a short period of silence before impulses reappear at a slower rate. The 'on-off' type gives a brief and rapidly adapting discharge, especially at high intensities. The 'off' type is intermediate, and the discharge often persists for several seconds after extinguishing a light.

Distribution of 'off' and 'on-off' units

'Off' and 'on-off' units discharge in response to different stimuli, and this suggests that they fulfil different functions. The frog's eye is not highly mobile, so that a given region of the retina corresponds roughly to a region of the visual field fixed in relation to the head. The optic axes of the two eyes make an angle of about 110° , and there is a small overlap of visual fields in a region close to the midline of the head and in front of the frog. This corresponds approximately to the region of the visual field within which a frog will strike at a small moving object; if it is presented elsewhere the frog turns towards it, and then approaches to within striking distance before attacking. If the suggestion that 'off' and 'on-off' units have different functions is correct, one might expect them to occur in different proportions in different retinal regions, because the frog uses these regions for different purposes. The particular region that is likely to be most specialized is the posterior retina, corresponding to the anterior visual field, and it was of interest to see whether either of these types of unit was particularly common or uncommon in this region.

Unfortunately the position of the receptive field on the retina was not always recorded, and most of the experiments were done on the dorsal or ventral regions. In twelve recent experiments done only on the anterior or posterior retina, there were four 'off' units and these were all posterior; the remaining eight were 'on-off' units, and four of these lay in the anterior retina, the remaining four being posterior. This suggests that 'off' units are more commonly found in the posterior retina, and this conclusion is confirmed by observing the massed discharge in the optic nerve fibres following illumination, of anterior and posterior retina. If an electrode is placed on the anterior or posterior lip of the optic disc, and the anterior or posterior retina is illuminated a confused hiss or rattle is heard. The size or intensity of this noise depends very much on the exact placing of the electrode, as well as on the intensity of illumination, but comparison of the discharges from anterior and posterior retina reveals two features which support the idea that 'off' units are more frequent in the posterior retina. First, the 'off' discharge is considerably greater than the 'on' discharge when the posterior region is stimulated, whereas it is only slightly greater when the anterior region is used; and secondly the 'off' discharge is more prolonged from the posterior region. The 'off' units adapt more slowly than the 'on-off' units, so the prolonged 'off' discharge from the posterior retina also supports the idea that 'off' units are more frequent in this retinal region. Both these features occur consistently and reproducibly, and are not critically dependent on the exact placing of the electrode or the intensity or size of the stimulus light.

Dark adaptation

When an action potential has been isolated, the threshold, under uniform conditions, often remains stable for an hour or more, but this is hardly an adequate criterion for judging that a piece of nervous tissue cut off from its blood supply and exposed to the air is in good condition. It was therefore decided to test two characteristic responses of a retina which certainly should

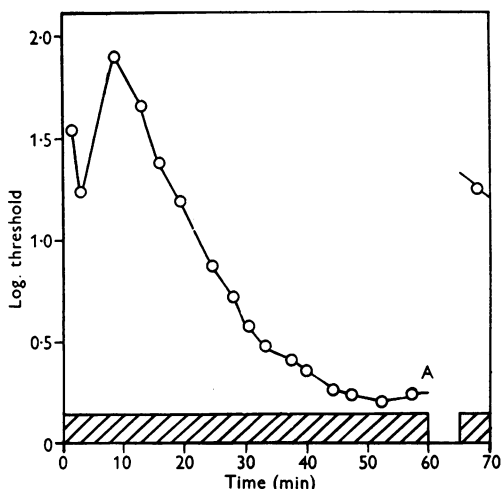


Fig. 4. Dark adaptation in an 'off' unit. At A the retina was re-illuminated for 5 min.

occur, and which would give an indication of whether both rods and cones were still active. Fig. 4 shows the result of an experiment in which the retina was exposed to a bright light for a few minutes, and then placed in total darkness, the threshold being tested at intervals. It will be seen that dark adaptation occurs, and this may be taken as an indication that the rods are capable of something approaching their normal activity. The same can be said of the cones, since the Purkinje shift occurs at high levels of illumination. This was shown by measuring the threshold with the Ilford 'spectral' range of filters, and finding that dark adaptation caused a much greater lowering of the threshold to blue than to red light.

Rhythmic activity

One other type of activity is of interest, though it was not an index of the normal state of the retina, but rather tended to occur when the retina was damaged or drying. This was the appearance of spontaneous rhythmic activity in the retina. The ganglion cells discharged groups of impulses with a well-marked periodicity, and at the same time a slow oscillating potential of the

same period appeared. This had many of the characteristics of such potentials in other nervous ganglia, such as the optic ganglion of *Dytiscus* (Adrian, 1931) and the human electro-encephalogram, but was much slower. It occurred when the retina was left alone, and especially if it was allowed to dark-adapt. It stopped if the retina was illuminated, but occasionally it started up again even if the illumination was maintained. The normal speed of the rhythm was about 90/min and, if the electrode picked up from several cells, a remarkable sound, like marching feet, was produced. This waxed and waned as the units joined or left the rhythmic discharge.

This rhythmic activity sometimes made it necessary to abandon an experiment, for it became impossible to judge a threshold in its presence. More often the action potentials got smaller and smaller until they could no longer be detected, though usually there was not great change in threshold up to the last moment. At other times the threshold became very unstable; it was often possible to drive the threshold up by giving a succession of sub-threshold stimuli, or to drive it down with supra-threshold stimuli. Occasionally preparations showed another type of spontaneous activity. Impulses appeared initially at 1/sec or less; the rate increased slowly to a climax, and then stopped abruptly, leaving the preparation inexcitable.

One or other of these events usually stopped an experiment in $\frac{1}{2}$ -4 hr, but there were no other progressive changes up to this point.

DISCUSSION

The preparation is very similar to Hartline's, and though it is inferior in having the shadow of the electrode obscuring part of the receptive field, it is almost certainly easier to prepare, and appears to last as well as his. There is, however, some uncertainty about the source of the action potentials. It does not matter very much if the potential is derived from the ganglion cell itself, the fine processes of the ganglion cell, the axon hillock, or from the nerve fibre as it passes out of the layer of high resistance responsible for the increased electrode noise observed when the electrode touches the retina; in any of these cases the volley of action potentials would be the same as in the nerve fibre leading to the central nervous system. The alarming possibilities are that the technique isolates a highly specialized type of ganglion cell, or even a cell in the ganglion cell layer which does not send a fibre to the central nervous system. This last possibility is very unlikely, because the results fit in well with Hartline's and because action potentials have occasionally been isolated from nerve fibres (Fig. 3.2) and the properties of their discharge are similar to those of the potentials commonly isolated. The other possibility has already been discussed; there are no grounds for believing that the units isolated belong to a rare or infrequent type, but, equally, one cannot neglect the possibility that there are ganglion cells which cannot be isolated by this technique, and

there is certainly one type—the 'on' unit—which is only rarely isolated. This possibility must be remembered, but it does not prevent one from using those units which can be isolated.

The fact that dark adaptation and the Purkinje shift occur is some evidence that the retina is in a fairly normal functional state. There was a great deal of variation in the time for which preparations lasted, and the cause of this was never discovered. Attempts to keep them in good condition for longer periods by washing with oxygenated Ringer were complete failures; indeed the addition of any Ringer to the vitreous appeared to have a devastating effect, for the preparations promptly became inexcitable.

Though the preparation is unreliable, both because an electrode which looked perfectly good often failed to isolate single units, and because an apparently good preparation often died before an experiment was complete, it was possible, with patience, to use it for the experiments planned.

SUMMARY

1. Electrodes similar to those used by Granit pick up three types of potential when brought close to the isolated frog's retina:

(i) An increased thermal noise, caused by the higher resistance of the retina compared with the vitreous.

(ii) Rapid triphasic action potentials. These are thought to originate in nerve fibres, because they are only excited by light falling in the retinal region from which the fibres under the electrode are coming.

(iii) Slower diphasic action potentials. These are thought to originate in ganglion cells, because they are only elicited by light falling close to the electrode tip.

2. Units of the third type were isolated which discharged at 'on', 'on and off', and 'off'. The relative frequencies of occurrence of these units, and rates at which their discharges adapted, were approximately as described by Hartline.

3. 'Off' units are more frequent in the posterior retina.

4. Single fibre preparations show dark adaptation and the Purkinje shift.

This work was done while I held a Research Studentship, under Professor E. D. Adrian, from the Medical Research Council. I am also very much indebted to the Medical Research Council Neurological Research Unit, Queen Square, who gave me much help with the equipment.

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