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LOCALIZATION OF ORIGINS OF ELECTRORETINOGRAM COMPONENTS BY INTRARETINAL RECORDING IN THE INTACT CAT EYE

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A large literature has developed on the electroretinogram (e.r.g.); for the most recent review see Crescitelli (1960). Structures generating the various components, however, are still not clearly identified. This problem must be solved before the significance of the e.r.g. in the visual process can be understood and before the e.r.g. can be accurately interpreted as a tool for visual research or clinical diagnosis.

A number of investigators have now attacked this problem by using micropipette electrodes to record the intraretinal potentials of the frog retina (Tomita, 1950, 1959; Tomita, Funaishi & Shino, 1951; Tomita, Mizuno & Ida, 1952; Ottoson & Svaetichin, 1953; Brindley, 1956, 1958, 1960; Tomita & Torihama, 1956; Tomita, Murakami & Hashimoto, 1960). Agreement has not resulted from these studies, however, and results as well as interpretations have sometimes differed sharply. Brindley (1956) has shown that lack of agreement is due at least partly to difficulties of working with retinas which have been removed from the animal.

The technique of using micropipette electrodes in the retina of the intact mammalian eye solves certain technical problems (Brown & Wiesel, 1959). This preparation can be maintained in normal physiological condition for one or two days. Methods have also been developed in this preparation for determining the location of the electrode tip by physiological criteria; these methods are at least as accurate as electrode staining procedures (Brown & Tasaki, 1961) and give immediate information about electrode depth.

In a previous paper the dark-adapted cat e.r.g. has been analysed into four major components (Brown & Wiesel, 1961). In addition to the well known a-, b-, and c-waves, there is a d.c. component which is positive in polarity with conventional recording methods. All these components have also been identified in the local e.r.g. (designated the l.e.r.g.) recorded by an intraretinal electrode. Each component of the e.r.g. seems to be

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generated in fundamentally the same way as its counterpart in the l.e.r.g., and detailed differences in time course of these responses may be accounted for by demonstrated differences in electrical recording conditions. Since a direct link seems established between the e.r.g. and the l.e.r.g., findings concerning origin of the local responses can be applied equally well to the more diffusely produced e.r.g. The local responses are more favourable for studying this problem and have therefore been used exclusively in our work on localization, a preliminary report of which has been given (Brown & Wiesel, 1958). The present paper will give detailed findings concerning the retinal level at which each of the four major components is generated.

METHODS

Detailed methods for using micropipette electrodes in the intact cat eye have been described previously (Brown & Wiesel, 1959). Application of this technique to e.r.g. work has also been described (Brown & Wiesel, 1961).

RESULTS

When the active electrode is in the retina, placement of the indifferent electrode in the vitreous humour makes it possible to record the l.e.r.g. in isolation from the e.r.g. (Brown & Wiesel, 1961). The l.e.r.g. may also be isolated by using retinal stimulus spots with diameters of 0·5 mm or less (Brown & Wiesel, 1961). Examples of the l.e.r.g. isolated by these methods may be seen in Figs. 2 and 6. It should be noted in Fig. 2 that with a deep intraretinal electrode there is a positive a-wave, negative b-wave, and positive c-wave. In Fig. 6, where a long stimulus was used, termination of a negative d.c. component may also be seen when the stimulus is turned off. Thus all components of the l.e.r.g. are inverted in polarity, with respect to the conventionally recorded e.r.g., except the c-wave.

Location of amplitude maxima

Each component was localized by establishing the retinal level at which it was recorded with maximum amplitude. The theory underlying the use of this criterion, and its probable significance, will be discussed later in this paper. These retinal penetrations were made on the nasal side of the retina just within the border of the tapetum lucidum; here the electrode approached the retina as perpendicularly as possible. There were technical advantages in collecting data only during electrode withdrawal, rather than during penetration. It seemed possible, however, that penetration of the retina would alter electrical recording conditions so that results during penetration and withdrawal would be different. This possibility was checked by making measurements during electrode movement in both directions, and the results for the c- and b-waves are shown in Fig. 1. The

c-wave increased steadily in amplitude as Bruch's membrane was approached on the way in, reaching a maximum just before penetration of that membrane and becoming slightly negative just after penetration. This pattern was also found during withdrawal. During penetration the b-wave reached peak amplitude just after the electrode passed the inner nuclear layer. Beyond this point the amplitude declined quickly, then

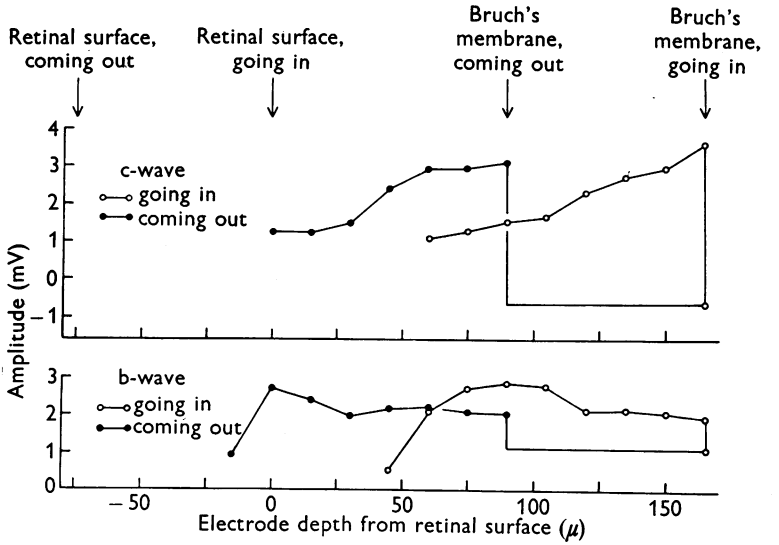

Fig. 1. Amplitudes of b- and c-waves as a function of electrode depth, during both penetration and withdrawal from the retina. Indifferent electrode on back of head. The zero point on the depth scale is where the retina was first contacted during penetration, and all electrode depths were measured from that point. Electrode advance and withdrawal was by steps of 15 μ. When the electrode was at a depth of 165 μ, records were obtained from very close to the retinal side of Bruch's membrane; then the electrode slipped through Bruch's membrane without being advanced further. Thus records from both sides of Bruch's membrane were obtained at the same electrode setting. Response amplitudes were measured from the prestimulus base line level to the peak of each wave, and each plotted point is an average value from measurements of 2–3 records. Stimulus was a 3 mm spot centred on the electrode, with a retinal illumination of 71 lm/m^2. Stimulus duration was 50 msec, and the repetition rate was one every 5 sec.

remained rather stable for the remainder of the distance to Bruch's membrane, and was sharply reduced when Bruch's membrane was penetrated. This pattern was also followed closely during withdrawal. Since results during penetration and withdrawal were essentially identical, results during electrode movement in either direction are equally valid. It should also be noted that during withdrawal the entire curves, for both c- and b-waves, were shifted about 80 μ to the left on the electrode depth

scale. This is because of 'tissue lag'. The zero point on the depth scale was retinal contact on the way in; during withdrawal contact with the retina was therefore lost at a negative depth value. It should be noted especially that the retinal surface and Bruch's membrane were displaced equal distances to the left during withdrawal. Thus tissue lag seems to affect all retinal levels to an equal extent. This has been discussed previously (Brown & Wiesel, 1959) and is well illustrated by Fig. 1. For present purposes this has the consequence that electrode depth values during withdrawal have the same significance as those during penetration, providing that depth values during withdrawal are all measured from the point at which contact with the retina is lost. Figure 1 also shows that during electrode movement through the tissue both waves changed magnitude gradually, apart from the abrupt changes at Bruch's membrane. This indicates that under favourable conditions the electrode motion through the tissue was very smooth.

The results of Figs. 2–5 were all obtained during electrode withdrawal. In each penetration the electrode was introduced all the way into the choroid; this assured that tissue lag was taken up before the electrode was withdrawn through Bruch's membrane. All electrode depth values were then referred to a zero point where retinal contact was lost. This procedure of making measurements during withdrawal had several advantages. During penetration there was danger that the electrode advance would be impeded momentarily by some mechanically resistant structure; during withdrawal that danger was removed, so movement of the electrode through the tissue was usually smoother. Since there was less chance of the electrode tip pressing against a structure during withdrawal, the recording of pulse movement artifacts was also reduced. The histological depth to Bruch's membrane at the nasal border of the tapetum lucidum is about 168–176 μ (Brown & Wiesel, 1959). Electrode depth to Bruch's membrane was measured during each penetration, and data were gathered during withdrawal only if this value did not exceed 200 μ. This assured that data were taken only when the electrode passed rather directly through the retina and eliminated serious errors of absolute depth values.

Figure 2 shows records obtained at 15 μ intervals during a single electrode withdrawal. Essentially pulse-free recordings were obtained at all except the 60 μ and 90 μ electrode depths. In these cases a pulse beat was unavoidably recorded, owing to the transducer properties of micropipette electrodes (see Brown & Wiesel, 1959). Relatively good dark-adaptation was maintained, in spite of a high-intensity stimulus which was repeated at 5 sec intervals, by using a stimulus duration of only 50 msec. This procedure gave large a-, b-, and c-waves and permitted accurate measurements of these components.

In Fig. 3 the amplitudes of the a-, b-, and c-waves are plotted as a function of electrode depth for the same electrode withdrawal illustrated in Fig. 2. Curves obtained for the a- and c-waves were very similar in shape, with maxima adjacent to the retinal side of Bruch's membrane and

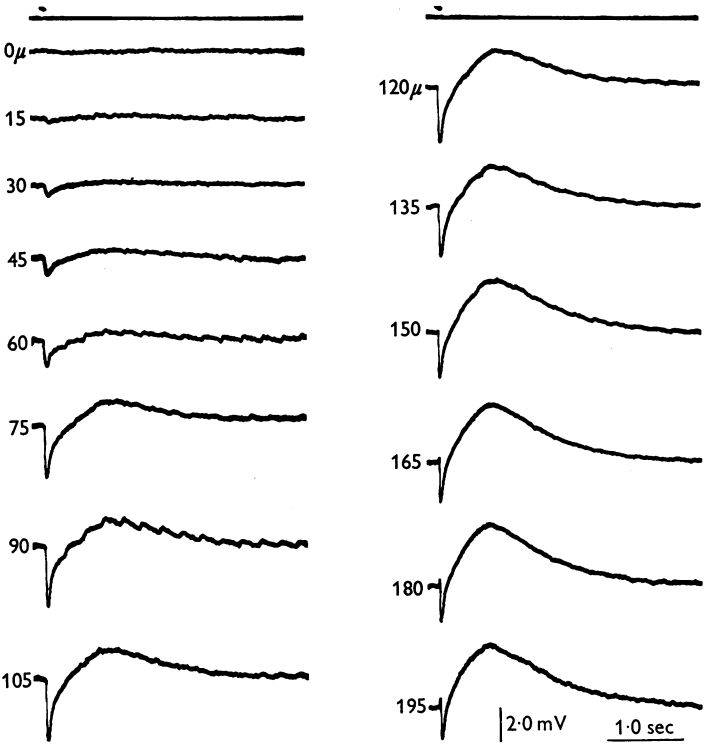

Fig. 2. Records taken at 15 μ intervals during a single electrode withdrawal. Electrode depth from the retinal surface is given in micra at left of each record. The electrode was immediately adjacent to the retinal side of Bruch's membrane at a measured depth of 195 μ, and the record labelled 0 μ was obtained just after the electrode lost contact with the retina. Indifferent electrode in vitreous humour. Stimulus records shown at the top of each column. The stimulus was a 3·0 mm spot, centred on the electrode, with a retinal illumination of 71 lm/m^2. Stimulus duration was 50 msec, and the repetition rate was one every 5 sec. No background illumination. All records in this paper obtained by d.c. amplification; positive potentials are displayed upward, in accordance with the convention for the e.r.g.

gradually decreasing amplitude as the electrode was withdrawn through the retina. The b-wave, however, increased in amplitude during withdrawal until just before the electrode entered the inner nuclear layer. As the electrode passed from the 75 μ to the 60 μ depth, it entered the inner nuclear layer. The b-wave amplitude dropped sharply at that point and

then decreased more gradually during the remainder of withdrawal. The amplitude maximum of the b-wave was therefore slightly distal (choroidal) to the inner nuclear layer.

It should be noted that all three components in Fig. 3 were plotted during a single electrode withdrawal and therefore on a single depth scale. It follows that relationships between the three curves must be accurate. Also the retinal locations of these maxima have now been confirmed by electrode staining (Brown & Tasaki, 1961). It therefore seems established

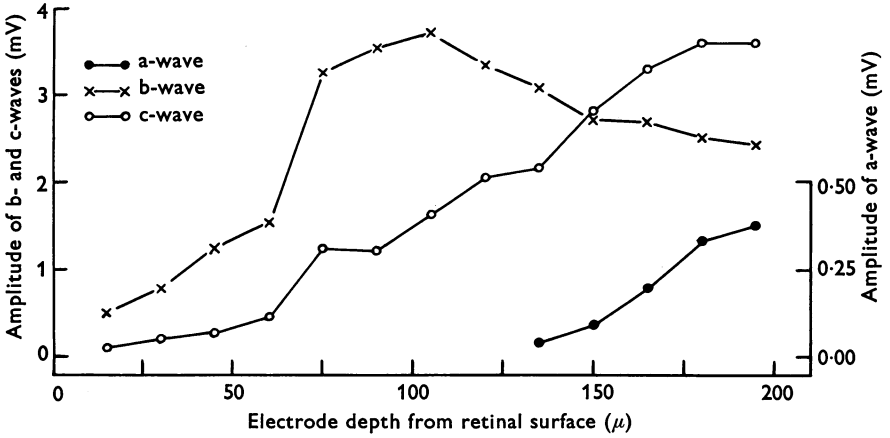

Fig. 3. Amplitudes of a-, b- and c-waves as a function of electrode depth. Results are from the same electrode withdrawal illustrated in Fig. 2. Indifferent electrode in vitreous humour. Amplitude of each wave measured from the pre-stimulus base line to the peak of the wave, and each plotted point is the average obtained by measuring 4–6 records. Stimulus spot was 3·0 mm in diameter, centred on the electrode, with a retinal illumination of 71 lm/m². Stimulus was 50 msec in duration and repeated every 5 sec.

that whereas the a- and c-waves have amplitude maxima immediately adjacent to the retinal side of Bruch's membrane, the maximum amplitude of the b-wave is in the external plexiform layer.

We may now consider possibilities of error in Fig. 3 due to electrical summation between different components of the complex response. In Fig. 3 the b-wave was measured from the base line to the peak. The a-wave may not terminate before the peak of the b-wave, in which case the a-wave would affect measurements of the b-wave. The time of onset of the d.c. component is unknown, so it may also affect measurements of the b-wave. The time from beginning of the stimulus to the peak of the b-wave (the peak delay) decreases as the electrode approaches Bruch's membrane during penetration. This appears to be because the c-wave begins before the peak of the b-wave, and since the c-wave increases in amplitude while

approaching Bruch's membrane the peak of the b-wave is cut off earlier as Bruch's membrane is approached. Hence there are possibilities that measurements of the b-wave are interfered with by all three of the other components. This problem was handled by two different approaches.

The data in Fig. 4 were gathered with a stimulus intensity low enough to eliminate the a-wave (see Brown & Wiesel, 1961). The b-wave was then measured from the base line, and plots are shown at several intervals of time from the beginning of the stimulus. These intervals were chosen to terminate on the rising slope of the b-wave, and thus give depth plots of the b-wave which are free from any effects due to cutting off of its peak by the

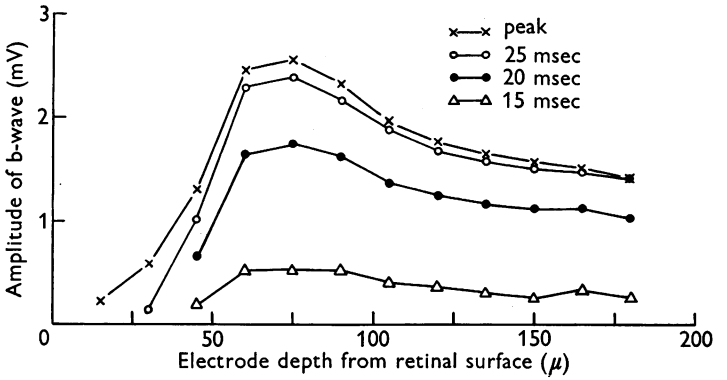

Fig. 4. Amplitude of b-wave as a function of electrode depth, plotted for both the peak and for several constant time intervals after beginning of the stimulus. These time intervals all terminated on the rising slope of the b-wave. Indifferent electrode in vitreous humour. Amplitudes were measured from the pre-stimulus base line, and all plotted values were averages from measurements on 3 or 4 records. Stimulus was a 1·5 mm spot, centred on the electrode, with a retinal illumination of 7·1 lm/m^2. Stimulus duration was 100 msec, and the repetition rate was one every 5 sec.

c-wave. Peak amplitude of the b-wave is also shown. In the vicinity of Bruch's membrane the curve for peak amplitude approached and coincided with that for a 25 msec interval. This is because of decreased peak delay of the b-wave near Bruch's membrane. The position of the amplitude maximum was the same in all curves, however, and is therefore independent of interfering effects from the c-wave. This approach eliminates interference from both the a- and c-waves, and since the d.c. component is small by comparison with the b-wave any interfering effects from this component should be negligible. This possibility was checked, however, by a second method.

The data in Fig. 5 were obtained by a stimulus intensity so low that both a- and c-waves were eliminated, leaving only the b-wave and d.c. component (see Brown & Wiesel, 1961). The curve for the uncorrected b-wave

shows the amplitude of the b-wave peak from the base line. The curve for the d.c. component was obtained by measuring the amplitude of this component at its termination, when there was no longer any sign of the b-wave, so this curve should represent amplitude of the d.c. component with no interference from other components. On the extreme assumption that the entire magnitude of the d.c. component was represented in peak measurements of the b-wave, a corrected curve for the b-wave was then obtained by subtracting the magnitude of the d.c. component from the peak magnitude of the b-wave. The corrected curve for the b-wave shows the position

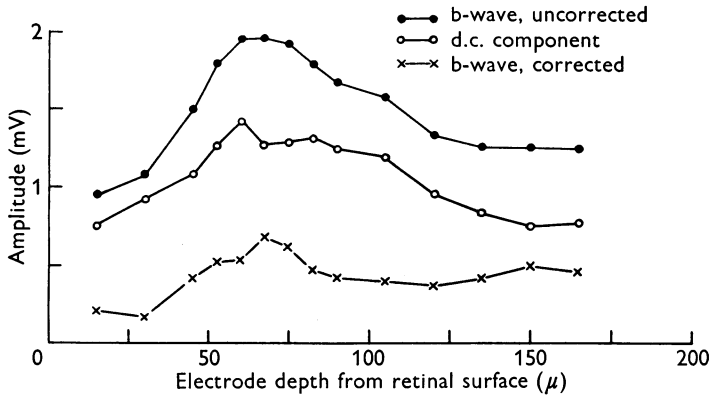

Fig. 5. Amplitudes of b-wave and d.c. component as a function of electrode depth. The uncorrected b-wave was measured from the pre-stimulus base line to the peak of the wave, while the d.c. component was plotted by the magnitude of the deflexion at termination of the stimulus. The corrected b-wave was then plotted by subtracting the curve for the d.c. component from that for the uncorrected b-wave. All plotted points are average values from measurements on 4–6 records. Indifferent electrode in vitreous humour. Stimulus was a 1·0 mm spot, centred on the electrode, with a retinal illumination of 0·022 lm/m^2. Stimulus duration was 0·8 sec, with a repetition rate of one every 5 sec.

of maximum amplitude unchanged. Thus in measurements to the peak of the b-wave the amplitude falls off too sharply, on either side of the maximum, to be accounted for by any contribution of the d.c. component. These two approaches therefore complement one another and indicate that the position of maximum amplitude of the b-wave has been localized without error due to other components. Figure 5 also shows that the position of maximum amplitude of the d.c. component is very close or identical to that of the b-wave.

In Fig. 3 the a-wave measurements were made from the base line to the peak of the wave. It will be shown later in this paper that elimination of the b-wave greatly increases the amplitude of the a-wave. Hence the peak of the a-wave occurs where it is cut off by the b-wave, and amplitude

measurements to the peak of the a-wave could be affected by changes in amplitude of the b-wave. Figure 3 shows that amplitude of the b-wave is rather constant over the range where the a-wave is changing rapidly, so interfering effects from the b-wave should be small. This possible source of error was checked, however, by making amplitude-versus-depth curves for the a-wave at several constant time intervals terminating between the initial rise and peak of the a-wave. The method of plotting was similar to that shown in Fig. 4 for the b-wave. These curves all showed the a-wave decreasing steadily in amplitude as the electrode was withdrawn from the retinal surface of Bruch's membrane. Thus the amplitude maximum of the a-wave seems to have been localized accurately without interference from the immediately following b-wave. The c-wave was measured from the base line to its peak, which occurred after termination of all other components (see Fig. 2). Measurements of the c-wave should therefore be free of distortion from other components.

It may also be noted that positions of amplitude maxima were the same whether the indifferent electrode was in the vitreous humour or on the back of the head. The obtained positions of amplitude maxima are therefore not dependent upon a unique location of the indifferent electrode. This may be seen from Fig. 1, obtained with the indifferent electrode on the back of the head, since the locations of amplitude maxima in Fig. 1 agree with our other findings. This may be predicted from analysis of the intraretinal potentials (Brown & Wiesel, 1961). The two positions of the indifferent electrode are equivalent with respect to the local potentials. The diffuse e.r.g. is constant in magnitude throughout the retina when the indifferent electrode is on the back of the head, so it is absent from intraretinal recordings when the indifferent electrode is in the vitreous humour. Positions of amplitude maxima should therefore be unaffected by the diffuse e.r.g. in either case, and identical positions of amplitude maxima should be found with either location of the indifferent electrode.

In summary, the four major components were localized by their amplitude maxima at two clearly established levels. The a- and c-waves were found close to the retinal surface of Bruch's membrane, while the b-wave and d.c. component were just distal to the outer margin of the inner nuclear layer.

The effect of local anaesthetic

In these experiments a glass pipette for drug injections was inserted into the eye through the second needle, and the tip of this pipette was placed in the vitreous humour as close as possible to where the electrode entered the retina. Local anaesthetics were injected very slowly by a micrometer-driven syringe. The slow rate of injection was to prevent sudden changes of intraocular pressure, which could cause retinal movements or produce

effects on the l.e.r.g. due to pressure on the retina. The active electrode was located close to the retinal side of Bruch's membrane; here large responses were recorded which were free from movement artifacts. Since contact with Bruch's membrane was readily detected, this electrode position could also be checked periodically to eliminate the possibility of electrode movement affecting the results. Since the indifferent electrode could not be in the vitreous humour it was placed on the back of the head, and local responses were elicited by a small stimulus spot carefully focused on the point where the electrode entered the retina.

Complete failure of local anaesthetic to produce an effect was never regarded as significant in this work, since this could result from failure of the anaesthetic to penetrate to the origin of the recorded potentials. When an effect was obtained, it was not considered significant unless reversible. This was to guard against results which could be due to toxic actions of the anaesthetic. Several local anaesthetics were tried. The effects of xylocaine (lignocaine B.P., lidocaine U.S.P.) were clear, reliable, and reversible. Cocaine did not produce any effect, even with large amounts and at high concentrations (0·1 ml. of a 10 % solution), so it cannot have diffused to the origins of the potentials and was probably blocked by the internal limiting membrane. Procaine sometimes seemed to produce an effect; these effects were qualitatively like those of xylocaine but were not as clear or reliable. Thus effects of procaine were intermediate between those of xylocaine and cocaine.

The effects of xylocaine are illustrated in Fig. 6. By the termination of the injection, which required 1 min, the b-wave was already greatly reduced. The a-wave was then more apparent. This was probably because the b-wave normally cuts off the recorded magnitude of the a-wave; depression of the b-wave by xylocaine would then allow the a-wave to be recorded at increased amplitude. Hence the effect of xylocaine on recorded magnitude of the a-wave was probably secondary to its effect upon the b-wave. The c-wave and d.c. component were unaffected. Maximum effects were obtained after about 2·5 min. At this time there was a large initial positive deflexion, identified by its latency as the a-wave. Then both the beginning and termination of the negative d.c. component were superimposed upon the slow positive c-wave, with no indication of a b-wave. At the time of maximum effect there was no clear change in either the c-wave or d.c. component, by reference to the normal recording before beginning the injection. The lack of effect upon the d.c. component may be seen by observing the magnitude of this potential at its termination. After 6·0 min the b-wave was clearly recovering, with consequent decrease in recorded magnitude of the a-wave, and after 56 min recovery was almost complete.

Interpretation of the initial part of the 2·5 min record was further clarified in other experiments. Figure 3 shows that the a-wave disappears when the electrode is withdrawn about 75 μ from Bruch's membrane. This change of electrode position increases both the b-wave and d.c. component. When maximum xylocaine effects were obtained, similar to the 2·5 min record in Fig. 6, the electrode was withdrawn quickly about 75 μ. This manoeuvre abolished the initial positive deflexion, further identifying it

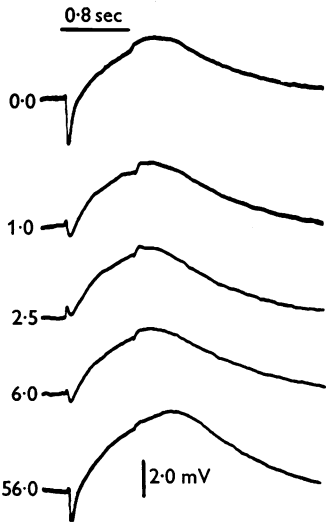

Fig. 6. Selective and reversible block of the b-wave by local anaesthetic. Electrode was on the border of the area centralis, close to the retinal surface of Bruch's membrane, and the indifferent electrode was on the back of the head. Then 0·01 ml. of a 2 % solution of xylocaine was injected slowly into the vitreous humour close to the point where the electrode entered the retina. The injection required 1 min, and at left-hand side is shown the time of each record in minutes after starting the injection. The maximum effect occurred after about 2·5 min, and recovery was essentially complete after 56 min. Stimulus was a 0·5 mm spot, centred on the electrode, with a retinal illumination of 71 lm/m^2 and repeated at 5 sec intervals.

as the a-wave. The record then showed only the onset and termination of the d.c. component, superimposed upon the c-wave. The negative deflexion of the d.c. component at 'on' was equal in magnitude to the positive deflexion at 'off'. Thus there was no sign that the initial negative deflexion contained any contribution from the b-wave, in spite of the favourable electrode location for recording this potential. It is therefore concluded that xylocaine completely abolished the b-wave, producing a secondary increase in the a-wave, while the c-wave and d.c. components were unaffected.

Control experiments eliminated the possibility that effects of xylocaine were due to increased intraocular pressure rather than to the drug itself. In these experiments normal saline, or a cocaine solution, was injected to simulate the effect of the xylocaine solution upon intraocular pressure. When injection rates and amounts were similar to those with xylocaine, the control injections had no effect.

Local anaesthetics in low concentration abolish impulse activity but not graded slow potentials such as receptor potentials (see Gray, 1959). Since the effect in this study was selective for the b-wave, it seems almost certain that the result of eliminating impulse activity was observed without the more drastic abolition of receptor potentials. Elimination of the b-wave by xylocaine therefore agrees with other evidence that the b-wave is generated some distance back in the retina; it also indicates that the b-wave is dependent upon conducted impulses. Since the only structures which are clearly distal to the origin of the b-wave are the receptors themselves, this observation likewise indicates that conduction through receptor fibres is by nerve impulses. Failure of xylocaine to affect the d.c. component probably means that although this response occurs at the same retinal level as the b-wave, it is dependent only upon electrotonically conducted activity. Failure of xylocaine to decrease either the a- or c-wave agrees with other evidence in this paper on the origins of these components.

Intracellular recording

Certain recordings were obtained which appeared to be intracellular potentials from cells which generate components of the l.e.r.g. When the electrode was very close to the retinal surface of Bruch's membrane, a negative d.c. shift of 40–60 mV sometimes occurred which was not correlated with the usual signs of penetrating Bruch's membrane. After such a d.c. shift a light response was recorded, with time course identical to that of the c-wave, but it was negative in polarity and had a peak magnitude of 20–30 mV; thus it was opposite in polarity and much larger than the extracellular c-wave. Such recordings were extremely rare and usually lasted only a few seconds, after which the negative d.c. potential disappeared and recordings typical of the retinal side of Bruch's membrane reappeared. Hence they seemed to be fragmentary intracellular records, and although satisfactory photographs were not obtained the observations were quite clear. Since the time course of the c-wave is unique among retinal potentials, and since the time course of the intracellular response was identical to that of the c-wave, it seems very likely that these were intracellular responses from cells which generate the c-wave. It also seems significant that these intracellular responses were obtained at the same retinal level where the c-wave had maximum amplitude.

Since these recordings were near the retinal side of Bruch's membrane, the only structures from which they may have been obtained are the pigment epithelium cells or outer receptor segments. It is very unlikely that outer receptor segments were ever penetrated, since they are so small. Also, the electrode was oriented along the long axis of the outer segments, which are relatively free to move when contacted by an electrode. The pigment cells of the cat retina are roughly rectangular in cross-section, with a thickness of about 5 μ and a width of about 8 μ. Since they present better targets than the outer receptor segments and are attached to

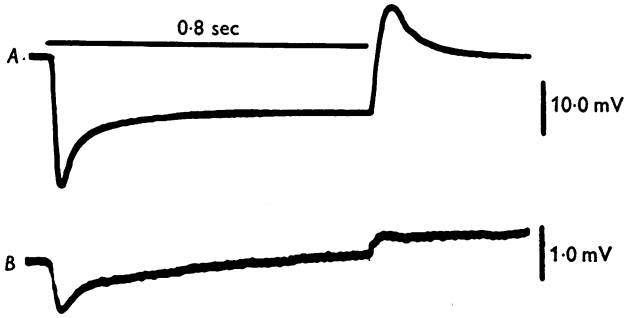

Fig. 7. Intra- and extracellular recordings of slow retinal potentials. Electrode in area centralis at depth of outer plexiform layer. Record *A* is an intracellular record from a single unit which responded to light with slow potentials but no impulse activity. A stable membrane potential of 50 mV was maintained during about 30 min of intracellular recording. Record *B* was then made just after the membrane potential suddenly disappeared. Thus the intracellular single unit response and the l.e.r.g. at the same retinal level may be compared. Record *B* undoubtedly contains some contamination from the diffuse e.r.g., since the indifferent electrode was on the back of the head, but such effects were negligible for purposes of comparing the local b-wave and local d.c. component with the intracellular record. Stimulus was a 3·0 mm spot, centred on the electrode, with a retinal illumination of 71 lm/m^2. Stimulus was repeated every 10 sec and was superimposed on a background illuminated area with a diameter of 4·0 mm and a retinal illumination of 2·19 lm/m^2.

Bruch's membrane, they should be much easier to penetrate. These cells are still small for intracellular recording, however, and the rarity and brevity of the recordings probably reflect that fact. It therefore seems likely that these intracellular recordings of the c-wave were obtained from the pigment epithelium.

Intracellular recordings have also been obtained just distal to the inner nuclear layer (Brown & Wiesel, 1958, 1959). These single units respond to light with slow potentials but never with impulse activity. Figure 7 shows an intracellular response from such a unit and also the l.e.r.g. recorded near the unit. It is suggested that this type of intracellular response is

from the single units in the cat which generate both the b-wave and d.c. component. The intracellular response exhibits what appear to be both rapid and d.c. components, and the time courses of the two intracellular responses are quite comparable to those of the local b-wave and d.c. component, respectively. Also the b-wave and d.c. component are localized by their amplitude maxima at the same retinal level, which is identical to that at which the type of intracellular response shown in Fig. 7 is found. It will be noted that although the intracellular response in Fig. 7 is much larger than the l.e.r.g., the polarities of the two responses are identical. Failure of the intracellular response to invert polarity could be a serious objection to our hypothesis, but a possible explanation of this result will be discussed later in this paper.

DISCUSSION

Present status of evidence locating origins of major e.r.g. components

The main types of evidence concerning location of the origin of each component will now be critically considered. Evidence obtained by others with micro-electrodes has been primarily from the frog retina, and it will be assumed that the frog and cat are alike with respect to the origin of any given component. This assumption seems reasonable and there is no evidence to the contrary. On this assumption it has proved possible to resolve existing conflicts of evidence.

The c-wave. In our work the c-wave had maximum amplitude adjacent to the retinal side of Bruch's membrane and was unaffected by local anaesthetic; also intracellular recordings of the c-wave seemed to be obtained from the pigment epithelium. Hence our evidence points to the pigment epithelium as the origin of this component. This conclusion has also been drawn by Noell (1954) who showed selective abolition of the c-wave in rabbits when the pigment epithelium was damaged by sodium iodate poisoning, and by Yamashita (1959), who showed that the c-wave is selectively eliminated when the toad retina is removed from the pigment epithelium. Tomita (1950) found the c-wave 'very small or missing' in the isolated retina and allocated it to the receptor layer, but now considers his evidence to agree with an origin of the c-wave in the pigment epithelium (Tomita, 1959). Thus there is good agreement on the origin of this component.

Intracellular organelles which have structural similarities to light-sensitive organs have been described by electron microscopy in retinal pigment cells of the frog (Porter, 1955–56) and bat (Yamada, 1958). J. E. Dowling and I. R. Gibbons (personal communication) find similar organelles in pigment cells of the rat retina and have observed that they

degenerate, like rod outer segments, when the animal is maintained on vitamin A acid. The light-sensitivity of the pigment cell may therefore depend upon these intracellular structures. The c-wave has not been recorded from isolated pigment cells, however, so the light response of these cells may require contact with the receptor outer segments.

The a-wave. The local a-wave in this work had maximum amplitude at the same retinal level as the c-wave and was likewise undiminished by local anaesthetic. The a-wave cannot originate from the pigment cells, however, because it can be recorded from an isolated retina (Ottoson & Svaetichin, 1953; Brindley, 1956; Tomita & Torihama, 1956; Yamashita, 1959). Thus the outer segments of receptors appear the most likely origin of the a-wave; this view is supported by a number of other observations. It seems particularly significant that in mouse and rat retinas, where the receptor layer is intact, but where development of the ganglionic and inner nuclear layers has been impaired by administration of sodium glutamate, the a-wave is normal but the b-wave is entirely absent (A. M. Potts, personal communication). This finding is consistent with other evidence assigning the b-wave to the region of the inner nuclear layer and strongly favours an origin of the a-wave distal to the inner nuclear layer. The a-wave has the shortest latency of any electrical response of the vertebrate retina, and in this study a-wave latencies as short as 3·5 msec were found reliably. This also suggests that the a-wave results from electrical activity at a more distal level than the b-wave.

The b-wave can be reduced independently of the a-wave by several methods (Granit, 1933; Noell, 1954), and the effect of xylocaine in this study was especially selective. On the other hand, there is no clear evidence that the a-wave can be reduced without affecting the b-wave. The earlier disappearance of the a-wave as stimulus intensity is reduced (Brown & Wiesel, 1961) may be expected since the a-wave is so much smaller than the b-wave. As the a-wave is reduced in amplitude there is a corresponding reduction of the b-wave, and when the a-wave is no longer recorded this probably does not mean it is no longer generated.

Alcohol reduces the recorded magnitude of the a-wave, increases the b-wave, and eliminates the slow negative potential which may be recorded from the frog retina with conventional leads (Bernhard & Skoglund, 1941). This decrease of the recorded a-wave has been interpreted as due to elimination of the slow negative potential, which has been assumed to give rise to the a-wave. An alternative possibility is that the size of the a-wave which is generated is unchanged by alcohol, but that the recorded magnitude of the a-wave is reduced because of the effect of alcohol upon the b-wave. Since elimination of the b-wave by xylocaine produces a secondary increase in the recorded a-wave, an increase of the b-wave by alcohol would

reduce the observed magnitude of the a-wave. Interpretation of the effect of alcohol on the a-wave is therefore equivocal. Hence the b-wave can vary independently of the a-wave, but there is no clear evidence that the a-wave can vary without affecting the b-wave. This suggests that the b-wave occurs proximally to the a-wave and is dependent upon the electrical activity which has been designated the a-wave.

The effects of local anaesthetic upon the e.r.g. of the frog retina have varied to some extent. Ottoson & Svaetichin (1953) noted that cocaine diminished the b-wave and enhanced the a-wave. Since no components were completely abolished, however, they interpreted both the a- and b-waves to originate from receptors. Their differential effects agree with ours, and since the b-wave was abolished in this work it seems likely that their anaesthetic did not diffuse in sufficient quantity to the site of origin of the b-wave. On the other hand, the records of Tomita *et al.* (1960) show that cocaine eliminated the local b-wave within 30 sec and after 2 min the local a-wave was also abolished. This finding is consistent with the view of Tomita (1959) that the a- and b-waves both originate in the inner nuclear layer. It has been shown in a variety of receptors, however, that local anaesthetics which block nerve impulses will also reduce receptor potentials later if the concentration is sufficiently great (see Gray, 1959). It seems quite likely, therefore, that the later elimination of the a-wave was due to the delayed effect upon receptor potentials. Since elimination of the entire response cannot be interpreted in terms of origins of the various components, the important information in this case is probably the time sequence, which agrees with our findings. Since the effect of xylocaine in this study was *selective* for the b-wave, it appears almost certain that it was due only to blocking of nerve impulses. More drastic effects upon receptor potentials should have abolished the a-wave in any case. Hence the b-wave appears to depend upon conducted impulses, whereas the other components, including the a-wave, do not.

Tomita & Torihama (1956) find that the a-wave changes its amplitude most rapidly in the inner nuclear layer. In their work the focal potentials were deliberately minimized by ageing the preparation, so they recorded only the diffusely produced intraretinal e.r.g. It has been shown that the intraretinal e.r.g. is due to electrical activity generated in retinal areas at considerable distance from the electrode (Brown & Wiesel, 1961). This means that an electrode penetrating the retina does not pass the origin of current for the diffuse e.r.g. It merely passes along lines of radial current flow produced by origins of current flow which are remote from the electrode. This conclusion has also been made recently for the frog (Tomita *et al.* 1960). Under these conditions the radial current flow should be uniform through the various layers of the retina. Hence an active electrode

penetrating the retina should record any given component at constant-voltage amplitude unless the inter-electrode resistance varies with depth of the active electrode. The shape of this voltage-versus-depth curve is therefore determined entirely by the passive electrical resistance of the tissue and can have no significance for locating the depth at which a given component is generated. In the intact retina of either the cat (Brown & Wiesel, 1961) or frog (Tomita *et al.* 1960) the electrical resistance of Bruch's membrane appears to be so great in relation to that of the retina that changes in magnitude of the intraretinal e.r.g. through the retina are either negligible (cat) or small (frog). In the isolated retina, however, surface electrodes will record the voltage drop only across the electrical resistance of the retina itself; the voltage-versus-depth curve should then reflect faithfully the resistance of various layers of the retina, and by differentiation it should give the tissue-resistance-versus-depth curve. Byzov (1959) finds that most of the electrical resistance of the retina is in its central layers. The intraretinal e.r.g. should therefore change most rapidly in the region of the inner nuclear layer. With an indifferent electrode upon the internal limiting membrane, Tomita & Torihama found that the a-wave appeared at the inner nuclear layer, increased rapidly in amplitude while passing through that layer, and showed no further increase during penetration of the receptor layer. During penetration from the receptor side, with the indifferent electrode on the receptor surface, no a-wave was recorded until the active electrode reached the inner nuclear layer. Then the a-wave increased rapidly to a maximum while penetrating that layer, and thereafter remained constant. Polarity of the a-wave was appropriately reversed for the two positions of the indifferent electrode. These findings are therefore explicable in terms of Byzov's data on electrical resistance of the various layers of the retina. Since the reasoning developed here does not apply selectively to the a-wave, it may be predicted that in the isolated retina all components which are generated within the retina would change amplitude most rapidly in the region of the inner nuclear layer. Of course this applies only to the diffuse response. Tomita & Torihama found that changes in magnitude of the a-, b- and d-waves went closely in parallel during retinal penetration in either direction; thus the prediction has been confirmed. These findings have been interpreted by Tomita & Torihama to indicate that these components all originate in the inner nuclear layer.

In summary, many lines of evidence point clearly to an origin of the a-wave in the outer segments of receptors. Types of evidence which have been differently interpreted may be resolved with this concept on the basis of considerations which seem quite reasonable in light of recent findings.

The b-wave and d.c. component. Whereas we have located the b-wave

slightly distal to the inner nuclear layer, Tomita & Torihama (1956) and Tomita (1959) have located it in the inner nuclear layer. This difference is undoubtedly related to the different methods employed for locating the origin of the b-wave. We have used as criterion the position of maximum voltage of the local response, which agrees with our intracellular evidence. Tomita & Torihama have used the depth of most rapid voltage change of the diffuse response in the isolated retina. Several types of evidence, already mentioned in connexion with the a-wave, indicate that the b-wave is generated more proximally than the a-wave. Hence there is agreement that the b-wave is generated at some rather proximal level of the retina, but agreement has not been reached on its exact origin.

We have advanced the hypothesis that the b-wave and d.c. component of the cat are both generated by the cells which respond to light only with slow potentials. Since intracellular responses from these single units have the same polarity as the extracellular b-wave and d.c. component, our hypothesis cannot be upheld unless the failure of the intracellular response to invert polarity can be explained. This seems part of the more general problem that in the cat retina single units of this type can be detected with both extra- and intracellular leads, but the polarity of the single-unit response to light is the same in both cases. When a membrane potential is recorded, the light response is simply larger in magnitude than the extracellular single-unit response (Brown & Wiesel, 1958, 1959). Tasaki (1960) has shown that in fish the size of the membrane potential is not correlated with size of the light response and that electrical current through the electrode does not modify size of the light response. The latter finding has also been made in fish by Watanabe, Tosaka & Yokota (1960). These findings can all be explained on the basis of a general hypothesis which seems to have been considered by both Tasaki (1960) and Watanabe *et al.* (1960). It is assumed that the membrane which is penetrated supports a membrane potential but does not respond to light. The structure which responds to light is assumed to be internal to this membrane. Penetration of the outer membrane would give a membrane potential, but the electrode would still be external to the structure giving the light response. The light response would therefore be of the same polarity with both extra- and intracellular leads, but the intracellular lead would record it at larger magnitude because of the more favourable recording condition. Since the membrane supporting the membrane potential is different from that giving the light response, no correlation between size of the membrane potential and light response is expected; alteration of the membrane potential by electrical current also would not affect the light response. Since this general hypothesis can account for all major evidence on this subject, it seems to merit further investigation. In any case it shows that the similar

polarity of the l.e.r.g. and intracellular response does not preclude the view that the b-wave and d.c. component both originate from the single units which respond to light only with slow potentials. This general hypothesis could be developed in more detail in several ways. One possibility is that the light response is due to an intracellular organelle.

The positive d.c. component of the cat e.r.g. has not been clearly isolated in cold-blooded vertebrate retinas, so comparative evidence on localization of this component is not available. On the other hand, the d-wave has been found in the cat retina only in the light-adapted state (Brown & Wiesel, 1961); it is very small by comparison with that of cold-blooded vertebrates and its origin in the cat retina has not been investigated. Considerations in this paper concerning criteria for localizing components indicate, however, that the origin of the d-wave requires further study.

Technical problems in locating origins of e.r.g. components by micro-electrodes

Tomita (1959) has considered disagreements in localizing origins of e.r.g. components due to difficulties in locating the electrode tip. The locations of all amplitude maxima in this work have been confirmed by an electrode marking method (Brown & Tasaki, 1961). The methods of electrode location employed by Tomita's group have likewise proved accurate, so this factor is probably not critical.

A more crucial problem now is selection of criteria. Although many have felt that intraretinal electrodes would yield information on origins of the e.r.g., there has been no agreement on how an intraretinal lead should be used for this purpose. This problem has been complicated by the view, which prevailed until recently, that the e.r.g. and l.e.r.g. of the frog were basically different potentials (Tomita & Torihama, 1956; Brindley, 1956). The fundamental identity of these is now recognized in both cat (Brown & Wiesel, 1957, 1958, 1961) and frog (Tomita *et al.* 1960). It also seems clear that intraretinal recording of the e.r.g. itself gives little or no information for locating the origins of components. From this point of view the amplitude-versus-depth curves of both Ottoson & Svaetichin (1953) and Tomita & Torihama (1956) are probably not significant for localizing components. On the other hand, intraretinal recording of the l.e.r.g. seems to give critical information which may be applied equally well to locating origins of the e.r.g. (Brown & Wiesel, 1961). The problem is therefore reduced to the question of what criteria should be applied for locating the origins of components of the l.e.r.g.

In the case of the local response the penetrating electrode passes directly through the portion of the retina which generates the response. Thus the electrode passes through the origin of the recorded response. The radial

current flow of the local response should not be constant for all levels of the retina and should be maximum at the origin of this current. What seems desired, therefore, is determination of the retinal level of maximum current flow. Since the radial voltage drop between any two points is determined by both current flow and tissue resistance between these two points, measurements of voltage alone assume uniform resistance of all layers of the retina. It has now been shown in the frog that this is not the case (Byzov, 1959). This possibility was considered and guarded against in this work by the choice of criterion. Since the local potential shows no inversion of polarity at its origin, current must flow from both directions to the origin or in both directions away from the origin. Under these conditions a maximum negative or positive potential should be recorded at the origin, declining in magnitude on either side. A high local tissue resistance could give a deceptively large slope at any given point on either side of the maximum but would not seem to displace the position of maximum voltage. This is why the criterion of maximum voltage was used. It seems significant that this criterion gives results which agree with intracellular recording, while the maximum slope criterion would not have yielded such consistency of results. It might also be mentioned that if the maximum slope criterion were applied rigorously the origins of all components in this work would have been assigned to Bruch's membrane; this would clearly be an error resulting from the high resistance of that membrane. Hence the position of maximum voltage probably indicates accurately the origin, in the centre of the region of maximum radial current flow, independently of considerations of tissue resistance. The technique of Byzov (1959) of combining voltage and tissue-resistance measurements in the frog retina to determine patterns of radial current flow appears very promising. Results obtained thus far by this method are difficult to interpret, because local responses have not been studied in isolation from the diffuse e.r.g.; there are also problems of electrode location, since Byzov identifies the 'R membrane' with the external limiting membrane. Refinement of this technique, however, would seem quite fruitful.

Tomita *et al.* (1960) stress the criterion of polarity inversion during penetration. This criterion is based on the assumption that the origin of the e.r.g. is an electrical dipole, analogous to a battery, oriented perpendicular to the retinal surface. In the cat it is clear, particularly in the case of the b-wave, that the polarity inversion which does occur is due only to the different polarities of the diffuse and local responses recorded by an intraretinal lead. The positive diffuse b-wave is constant in magnitude throughout the retina, while the negative local b-wave increases from zero at the internal limiting membrane to considerable size at the outer margin

of the inner nuclear layer. The summed record is therefore positive in surface layers of the retina and becomes negative during penetration, and the position of this polarity inversion only indicates the retinal level where the local negative b-wave becomes large enough to mask the diffuse positive one by electrical summation (Brown & Wiesel, 1961). It appears that the only polarity inversion of significance would be an inversion of the local potential during penetration, as demanded by the concept that the origin is a radially oriented dipole. It is also clear that in the cat, particularly in the case of the b-wave, the local potential does not invert polarity during penetration. Thus the assumption underlying the criterion of polarity inversion appears to be invalid, and the concept of a radially oriented dipole seems to be untenable. These considerations hold equally well for the other three major components of the dark-adapted cat retina. Our preliminary interpretation that the local c-wave inverts polarity during penetration of Bruch's membrane has proved incorrect (Brown & Wiesel, 1961). There is no evidence that local potentials of the frog retina invert polarity during penetration, and results with micro-illumination by both Brindley (1956) and Tomita & Torihama (1956) indicate that the frog and cat are alike in this respect.

The nature of the origin of an e.r.g. component

On the basis of present evidence it seems unlikely that e.r.g. components are generated by radially oriented dipoles. An alternate suggestion is that the origin of the b-wave, for example, is a sink of current at a given level of the retina. This sink of current may be considered to be due to depolarization at an active site on a cell membrane, which draws extracellular current from both deeper and shallower layers of the retina. According to this hypothesis an electrode passing through the retina would record a negative voltage which would increase to a maximum at the sink of current and then decline. This assumption can therefore explain failure of the local b-wave to invert polarity as its origin is passed. The origin of the b-wave maintains a constant position and is not propagated radially through the retina. This is illustrated by Fig. 4, which shows amplitude of the b-wave as a function of retinal depth for various times after onset of the stimulus. If the origin of the b-wave were propagated radially, the peaks of the curves for different time values should be found at different retinal depths. Since the curves for all time values have peaks at the same retinal depth, the origin of the b-wave is clearly fixed. Similar evidence has been obtained that origins of the a- and c-waves occupy fixed positions. Byzov (1959) also found that the patterns of radial current flow in the frog retina did not move with respect to time. Thus the possibility is suggested that all components are due to restricted and fixed sites on cell membranes

at which depolarizations or hyperpolarizations produce sinks or sources of extracellular current flow.

Although the concept of a radially oriented dipole is adequate as a theoretical origin for current of the intraretinal diffuse e.r.g., generated by retinal areas distant from the electrode, it seems unable to account for the pattern of radial current flow associated with the l.e.r.g. The concept of a current source or sink accounts more adequately for findings on the local response, but it is not clear how this type of origin gives rise to the current of the diffuse response. Although many aspects of the relation between the l.e.r.g. and e.r.g. now seem explicable (Brown & Wiesel, 1961), this remaining problem is crucial. A formal theoretical treatment of the retinal circuits will not be attempted until this problem is solved.

SUMMARY

1. The four major components of the dark-adapted cat e.r.g. are the a-, b-, and c-waves plus a d.c. component. The local e.r.g. (l.e.r.g.) recorded by an intraretinal lead also exhibits these components and was used exclusively in the present work. Three techniques were used to determine the origins of components, and good agreement was obtained between results.

2. The amplitude of each component was plotted as a function of electrode depth. The a- and c-waves had maximum amplitudes immediately adjacent to the retinal side of Bruch's membrane, while the b-wave and d.c. component had amplitude maxima slightly distal (choroidal) to the outer margin of the inner nuclear layer. Thus the four major components appear to be generated at two distinctly different retinal levels.

3. Xylocaine (lignocaine) abolished the b-wave completely, selectively, and reversibly. The b-wave therefore depends upon conducted impulses. Since the only structures clearly distal to the origin of the b-wave are the receptors, this also indicates that receptor fibres conduct impulses. Although the d.c. component seems to originate at the same retinal level as the b-wave, it was unaffected by xylocaine and appears to depend only upon electrotonically conducted activity. Failure of xylocaine to reduce the a- or c-wave agrees with other evidence on the origins of these components.

4. Rare intracellular recordings were obtained very close to the retinal side of Bruch's membrane and were believed to be derived from single cells of the pigment epithelium. These records showed light responses with time course identical to that of the c-wave, but opposite in polarity and much larger in magnitude. Hence they appeared to be intracellular records from the cells which produce the c-wave.

5. Intracellular records were also obtained from single cells just distal

to the inner nuclear layer which responded to light only with slow potentials. The response contained both a rapid and a d.c. phase, and the time courses of these appeared identical with the b-wave and d.c. component, respectively, of the l.e.r.g. Since these intracellular records were at the same retinal level where the b-wave and d.c. components were maximum in amplitude, it is suggested that the b-wave and d.c. component are both generated by these cells.

6. The findings of this paper, in conjunction with several other lines of evidence, point to an origin of the a-wave in the outer segments of receptors. Types of evidence which have been differently interpreted may be resolved with this concept by considerations which seem reasonable in light of recent findings.

7. The problem of criteria for locating origins of e.r.g. components by means of amplitude-versus-depth curves is discussed. The maximum amplitude of the local response appears highly significant, whereas other proposed criteria do not.

8. The nature of the origin of an e.r.g. component is considered. The concept of a radially oriented dipole appears untenable, and the concept of a sink or source of extracellular current flow at a restricted and fixed site on a cell membrane is suggested.

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