Oscillations of Potential in the Electroretinogram of the Lobster

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ABSTRACT The electroretinogram (ERG) evoked in the lobster by a short flash of light consists of a highly damped, slow oscillation of potential, triggered apparently by a single excitatory process. Near the threshold, only one wave may be evident; but as the intensity of stimulus rises, a prior wave appears, and grows so much more rapidly as to become dominant. Simultaneously third and later waves appear, so that at high intensities the response may include five to seven waves. Dark adaptation favors the second and later waves relative to the first; light adaptation tends to suppress them. On turning on a steady light the oscillations are superimposed on the early stages of development of a maintained, steady-state potential (on-response). Turning off the light causes a rapid fluctuation of potential followed by a similarly damped slow oscillation (off-response). These phenomena resemble in part oscillations recently observed in the b wave of the ERG of many vertebrates including man.

The electroretinogram (ERG) evoked by a short flash of light in the compound eye of the common lobster, *Homarus americanus*, consists of a highly damped oscillation of potential that may include as many as seven successive waves. A study of the spectral sensitivity of this eye, dark-adapted and adapted to bright colored lights, revealed a single and apparently invariant spectral sensitivity function, with λ_{max} about 520 mμ (Kennedy and Bruno, 1960–61; Wald, 1967–68). The associated visual pigment had been extracted earlier (Wald and Hubbard, 1957). The same spectral sensitivity curve was obtained whether a constant amplitude of the first or the second wave of the ERG was taken as the criterion of response. It seems therefore that a single excitatory process triggers at least the first two waves, and probably the entire ERG.

The present paper presents further observations on the form of the lobster ERG and relationships among its components.

The apparatus and procedures employed in these experiments have already been described (Wald, 1967–68). Two considerations are of special importance: (*a*) The experiments were performed with unanesthetized animals that were merely restrained,

and always survived the experiment to be used again; so that it can be taken for granted that the visual system behaved normally. (*b*) For these observations it is essential to use DC or very low frequency AC amplification, to avoid the large distortions of signal introduced by high frequency AC amplification. These measurements were made using as preamplifier either the Grass DC P6, or with the Grass AC P5 set for the frequency range from 0.1 to 100 cps.

Intensity of Stimulus Fig. 1 shows the effect of raising the intensity of stimulus on the development of the first and second waves of the ERG. The animal was dark-adapted, and exposed to 22 msec flashes at 550 mμ.

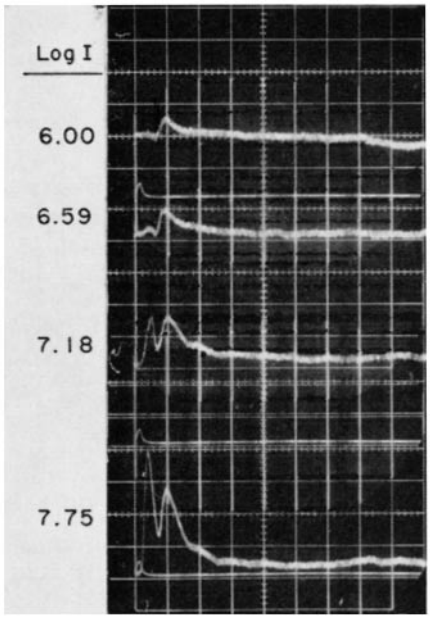

FIGURE 1. Relation between the first and second waves in the ERG of the dark-adapted lobster. Near the threshold only one, cornea-negative wave is evident rising after a latency of about 70 msec to peak at about 100 msec (top record). At a higher intensity (log I = 6.59) a prior wave enters, peaking at about 40 msec. As the intensity rises further, this first wave grows so much more rapidly than the second wave as to overtake and then greatly exceed it. Exposures, 22 msec at 550 mμ. Sensitivity 80 μv/division. Sweep 0.1 sec/division.

In this preparation, near the ERG threshold only the second wave appeared clearly. In the first record of Fig. 1 its amplitude is about 40 μv; and as yet the only sign of an earlier wave is a just perceptible inflection. On increasing the intensity of the flash, however, the earlier wave appears, and thereafter grows so much more rapidly than the first wave as to overtake and then considerably exceed it. At still higher intensities than shown in this figure the first wave increasingly dominates the second, which finally appears only as an inflection on its descending limb. Meanwhile a third wave has developed, already evident in the two lower records of Fig. 1, to be followed at still higher intensities by a succession of later waves.

Fig. 2 shows examples of the relationships between the peak amplitude of the first and second waves and the intensity of the stimulus. In such a log-log

plot the slope for the first wave over the range of low intensities is nearly 45°: the amplitude rises almost linearly with intensity to 400-600 μv. The amplitude of the second wave rises almost linearly with *log* intensity to 80–120 μv. The second wave always grows much more slowly with intensity than the first wave, and is more difficult to measure adequately because it is so small at low intensities and is absorbed into the first wave at high intensities. There is considerable variation in these relationships from one preparation to

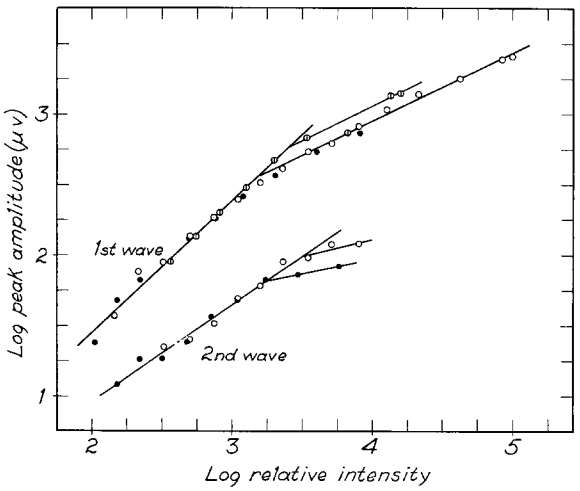

FIGURE 2. Relationship between log intensity of stimulus and log amplitude of the first and second waves in the lobster ERG. Stimulus at 520 mμ, 20°C. Open and solid circles, 18 msec flashes. Barred circles, strobe flashes, 0.5 msec. The peak amplitude of the first wave is roughly linear with intensity to amplitudes of 300-500 μv, then rises more slowly. That of the second wave is roughly linear with log intensity to amplitudes of 80-120 μv. The data shown with open circles are in their correct relationship as measured; the others have been shifted somewhat on the log intensity axis so as to fall together with them. I am indebted to E. B. Seldin for help with these measurements.

another; in some preparations the first and second waves appear together and with about the same amplitude near the ERG threshold.

Time Relationships These again vary considerably in different preparations, but can be summarized approximately as follows: Following a short flash—e.g., a strobe flash of about 0.5 msec—there is a latency of about 30 msec at low intensities declining to about 15 msec for bright stimuli. The first wave peaks at 40–80 msec (average 59), the second wave at 100–200 msec (average 171), at 18°–20°C. Subsequent peaks appear at intervals that tend to lengthen out somewhat; a typical series of times to the third to seventh peaks ran 190, 315, 443, 623, and 823 msec. At higher temperatures these times are shortened, at lower temperatures lengthened.

Duration of Exposure Fig. 3 shows the effect of lengthening the exposure to light at 520 mμ. At each duration the intensity was adjusted so as to yield about the same height of first wave.

Flashes short relative to the development of the first wave (16–48 msec) all yielded about the same type of ERG. Its oscillatory nature was particularly

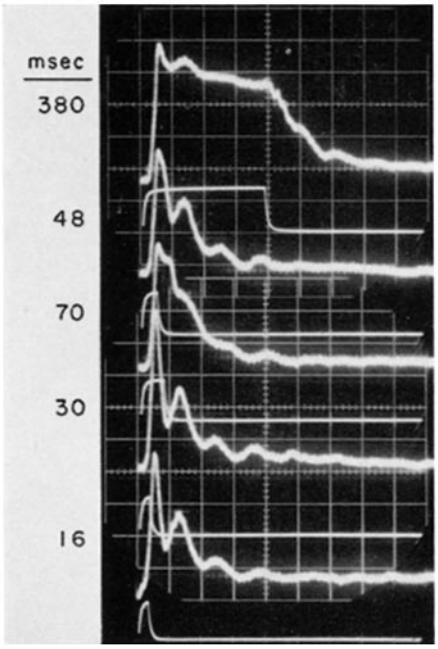

FIGURE 3. Effect of duration of exposure on the lobster ERG. Dark-adapted animal, 520 mμ. At each duration of stimulus the intensity was adjusted so as to yield about the same height of first wave. At 16, 30, and 48 msec exposures, the response is a damped oscillation falling rapidly back to the base line. At 70 msec the downsweep is slowed, and the oscillations are less prominent. At 380 msec the potential falls only slightly while the stimulus remains on, with a few small oscillations superimposed on it. As the light goes off (see signal below top record), there is a rapid fluctuation (two cornea-negative cusps) followed by two to three slow oscillations. Sensitivity 80 μv/division. Sweep 0.1 sec/division. DC recording.

evident in this preparation, which regularly displayed four to seven waves. At exposures of 70 msec and longer, the response decayed more slowly, so that the second and later waves were drawn increasingly into the descending limb. Finally all the later waves appeared only as fluctuations on the development of a maintained steady potential. The longest exposure shown in Fig. 3 has almost achieved this condition. Now turning off the light evokes a complex and oscillatory off-response.

This is shown better in Fig. 4. The stimulus was left on for 5 sec. Following

the oscillatory on-response, as shown in the upper part of the record, the potential fell to a steady level in about 1 sec. Turning off the light (lower record) triggered a complex off-response: a rapid pair of waves followed by a slow oscillation that included at least six waves at intervals resembling those of the on-response.

Light Adaptation Fig. 5 shows the effect of light adaptation on the ERG. The top record shows the response of the dark-adapted eye to a flash of light of 22 msec duration at 510 mμ. Then the eye was exposed to a continuous

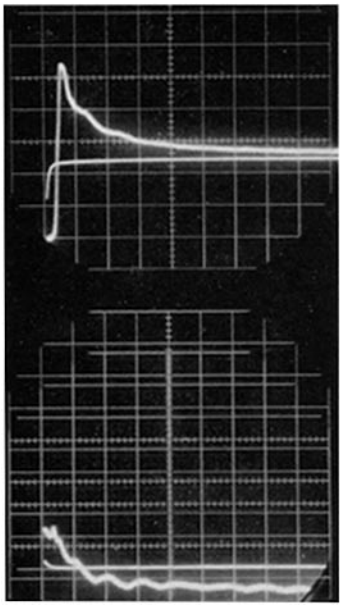

FIGURE 4. On- and off-responses in the lobster ERG. Upper record: turning on of the light, as shown by the signal, causes a rapid, cornea-negative peaking of potential, falling back with slow oscillations to a steady level, achieved in about 1 sec. Lower record: after the light had been on for 5 sec, turning it off causes a rapid fluctuation (two cornea-negative cusps) followed by six to seven slow oscillations as the potential falls to the base line. Stimulus 520 mμ. Sensitivity 200 μv/division. Sweep 0.1 sec/division. DC recording.

white background light (intensity not measured), and the test flash, superimposed on this, was raised in intensity 0.47 log unit, in order to yield the same height of first wave. Then the background light was increased in brightness, so that the test flash, to evoke the same height of first wave, had to be raised in intensity another 0.49 log unit.

With increasing light adaptation, the first wave, though kept at the same height, peaks more quickly. The second wave also peaks sooner, and is considerably reduced in amplitude. The third and fourth waves, evident in the dark-adapted condition, no longer appear in the bottom record.

Dark Adaptation As Fig. 6 shows, these changes are reversed during dark adaptation. The top record was made with the adapting light still on. Then it was turned off, and the intensity of the test light was adjusted periodically so as to yield the same height of first wave. As dark adaptation proceeded, the first wave peaked a little later, the second wave considerably later, and the

second wave grew markedly in height. Also third and fourth waves appeared that were absent from the light-adapted ERG.

Fig. 7 shows the first 50 sec of dark adaptation in another preparation. Again the top record was made with the adapting light still on, the others after various intervals in the dark. Again dark adaptation is seen to increase considerably the heights of the second and later waves, and to delay somewhat their times to peak.

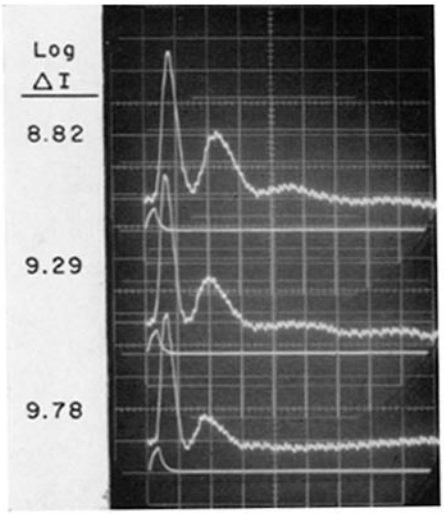

FIGURE 5. Effects of light adaptation on the lobster ERG. The top record shows the response of the dark-adapted eye to a 22 msec flash at 510 mμ (relative log I = 8.82). The eye was then exposed for a time to a continuous white light. To yield the same height of first wave, the test flash, superimposed on this background, had to be raised to log I = 9.29 (middle record). When the background brightness was raised further, the test flash had to be increased to log I = 9.78 to keep the height of the first wave the same (bottom record). As the eye is light-adapted, the first and second waves peak sooner, and the second and later waves are increasingly suppressed. Sensitivity 72 μv/division. Sweep 50 msec/division. AC recording.

DISCUSSION

Slow oscillatory responses from retinas have frequently been observed since Fröhlich (1914) measured oscillatory electroretinograms in three octopods. Adrian and Matthews (1928) observed rhythmic bursts of impulses in the optic nerve of the eel that had much the same characteristics. Hartline (1928) found similar responses in the distal branch of the optic nerve of the scallop, *Pecten*. Adrian (1937) showed that such rhythmic volleys in the optic nerve of the water beetle, *Dytiscus* were correlated with rhythmic oscillations of potential in the optic ganglion. Adrian thought the latter to be abnormal responses of injured ganglia; but shortly afterward Roeder (1939, 1940) found

similar rhythms in the ERG of intact grasshoppers, originating apparently in the optic ganglion. Crescitelli and Jahn (1942) carried out an extensive comparative study of such phenomena in the compound eyes of grasshoppers, moths, and butterflies, and tried to bring them into some order.

These rhythms, though comparable in frequency with the transitory responses in the lobster ERG, are for the most part quite different in character:

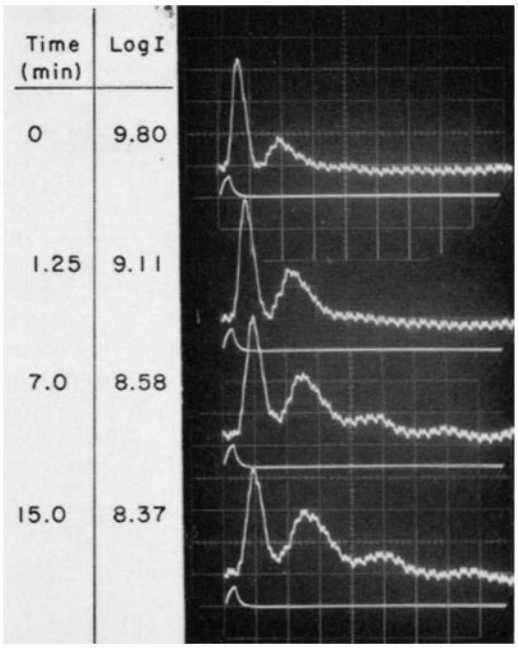

FIGURE 6. Effects of dark adaptation on the lobster ERG. The top record was made with the eye light-adapted to a continuous white background, as in the lowest record of Fig. 5. Then the light was turned off, and the eye was periodically stimulated in the dark with test flashes (22 msec, 510 mμ), adjusted in intensity so as to yield the same height of first wave. As dark adaptation progresses, the changes due to light adaptation shown in Fig. 5 are reversed. The first and second waves peak later, and the second and subsequent waves grow more prominent. Sensitivity 72 μv/division. Sweep 50 msec/division. AC recording.

they tend to come in relatively slowly after the onset or cessation of light, to be relatively regular in frequency and amplitude once established, and usually to persist throughout relatively long periods of light or subsequent darkness.

The oscillatory ERG of the lobster seems to be much more closely related to the slow oscillations of potential that have recently been observed in the vertebrate ERG—by now in a sufficient variety of vertebrates as to make this seem a general phenomenon. The first such report seems to have been that of Cobb and Morton (1954) on the human ERG. On stimulation with short, intense flashes of light, these workers saw as many as four to six small oscilla-

tions of potential superimposed on the cornea-positive b wave. They were about equal in amplitude and had a period of about 7 msec. Pressure on the eye, inducing partial anoxia by occluding the flow of blood, had no effect on the a wave, which is apparently a receptor potential, but abolished the b wave and the oscillations. This report contains also a curious parallel with the present observations, in that the first oscillation seemed to be missing at lower intensities, appearing only with the brightest flashes. These observations on

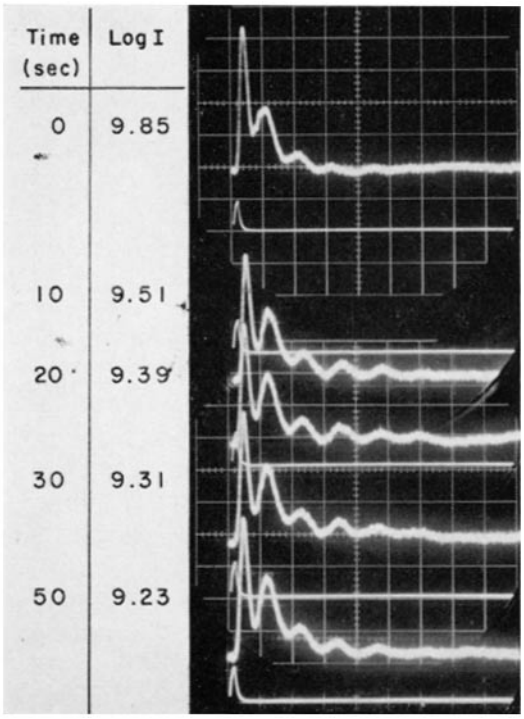

FIGURE 7. Effects of short periods of dark adaptation on the lobster ERG. The top record was made with the adapting light still on, the others after various times in the dark. At each time the intensity of test flash (22 msec duration, 520 mμ) was adjusted in preliminary trials so as to yield about the same height of first wave. As dark adaptation progresses, the second and later waves become more prominent. In this preparation these changes are almost complete within about 20 sec in the dark. Sensitivity 80 μv/division. Sweep 0.1 sec/division. DC recording

the human ERG have since been largely confirmed by Bornschein and Goodman (1957), Heck and Rendahl (1957), and Yonemura, Aoki, and Tsuzuki (1962).

Yonemura, Masuda, and Hatta (1963) have since extended such observations to the cat, rabbit, guinea pig, and chicken. As in man, the successive oscillations were found to be spaced at nearly equal intervals of 5–10 msec (cf. also Auerbach et al. (1964) on the rat). In cold-blooded vertebrates at 18°–22°C., as expected, the oscillations are slower—the period is about 20 msec in the tortoise and 45 msec in the bullfrog. Comparable oscillations in the ERG have also been observed in the turtle (Armington, 1954), fish (Konishi, 1960), the all-rod eye of an owl (Bornschein and Tansley, 1961), and the all-cone eye of the ground squirrel (Crescitelli, 1961). Yonemura et al. (1963), however, could find no indication of them in the lamprey.

This type of response in vertebrate eyes displays certain general features: (*a*) It is closely associated with the b wave, and treatments (e.g., anoxia) that depress the b wave also abolish the slow oscillations. (*b*) It is evoked only at high intensities, though once the oscillation is well-established, the number of waves and their period are relatively independent of intensity. (*c*) Successive waves in the slow oscillation may be almost equal in amplitude, or may decline or increase in amplitude; damping of the oscillation occurs only in isolated instances. (*d*) Like the b wave, these oscillations appear to arise at loci more central than the receptors. Yonemura et al. (1963) argue that they arise in the bipolar layer.

Some features of the oscillation in the lobster ERG resemble that in vertebrates; for example, the period is comparable with those in cold-blooded vertebrates at the same temperature, and as in vertebrates is relatively independent of the intensity.

The lobster ERG, however, also presents distinct features not yet reported in these other forms: (*a*) The special relations between the first and later waves, particularly the much more rapid rise of the first as compared with later waves with increasing intensity of the stimulus. (*b*) The marked damping in amplitude of successive waves after the first. (*c*) The special relationships to light and dark adaptation. I do not know of comparable observations in a vertebrate eye. (*d*) The oscillatory off-response, similar in period, number of waves, and damping with the on-response; again I do not know of comparable observations in vertebrate eyes (cf. however, Armington (1954) on the turtle *Pseudemys*).

Without further analysis it hardly pays to discuss possible mechanisms of the oscillatory response. I thought at first that it might represent a train of responses from successive levels in the optic radiation, declining in amplitude with distance from the receptors. (This, of course, would not account for the relasions between the first and second waves.) The anatomy of the visual pathways in the lobster, however, hardly offers a long enough chain of neurons to account for six to seven waves in the ERG (Hámori and Horridge, 1966). One thinks also of a reverberating circuit—some feedback chain in which the response might reecho and gradually die away. A third possibility involves the interplay among nearby receptors of reciprocal inhibition and rebound. Hartline et al. (1961; pp. 280–281) have demonstrated an oscillatory response of this nature in *Limulus*. A fourth possibility is that the oscillation has its origin in the receptor cells themselves, each of which may respond to the onset or cessation of light with an oscillation of membrane potential. That single cells can produce such slow oscillatory potentials has been demonstrated in lobster muscle (Werman and Grundfest, 1961) and smooth muscle cells of cat intestine (Nagai and Prosser, 1963). Of course, whatever their mechanism, for such responses to appear in the ERG requires the synchronized

activity of large numbers of units. The observed damping of successive waves in either the on- or off-response could therefore be due either to a falling-off in the numbers of responding units, or to a decline in the potentials evoked in individual cells, or to combinations of both effects.

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