Electrophysiology of the Insect Dorsal Ocellus

I. *Origin of the components of the electroretinogram*

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ABSTRACT Dorsal ocelli are small cup-like organs containing a layer of photoreceptor cells, the short axons of which synapse at the base of the cup with dendritic terminals of ocellar nerve fibers. The ocellar ERG of dragonflies, recorded from the surface of the receptor cell layer and from the long lateral ocellar nerve, contains four components. Component 1 is a depolarizing sensory generator potential which originates in the distal ends of the receptor cells and evokes component 2. Component 2 is believed to be a depolarizing response of the receptor axons. It evokes a hyperpolarizing postsynaptic potential, component 3, which originates in the dendritic terminals of the ocellar nerve fibers. Ocellar nerve fibers in dragonflies are spontaneously active, discharging afferent nerve impulses (component 4) in the dark-adapted state. Component 3 inhibits this discharge. The ERG of the cockroach ocellus is similar. The main differences are that component 3 is not as conspicuous as in the dragonflies and that in most cases ocellar nerve impulses appear only as a brief burst at "off." In one preparation a spontaneous discharge of nerve impulses was observed. As in the dragonflies, this was inhibited by illumination.

INTRODUCTION

The insect dorsal ocellus is a relatively simple photoreceptor organ with a cuticular cornea, a retinal layer of photoreceptor cells, and a short nerve leading from the retina into the brain. The photoreceptor cells, arranged in retinulae, are typical of those found generally in arthropod eyes. Distally, each receptor cell bears a specialized light-sensitive organelle, the rhabdomere, and proximally an axon. The photoreceptor axons are quite short and synapse with dendritic processes of ocellar nerve fibers just proximal to the retina. Light stimulates the photoreceptor cells and, *via* synaptic processes, controls the rate of discharge of nerve impulses in ocellar nerve fibers.

Complex electroretinograms (ERG's), including components which originate both in the receptor cells and in the ocellar nerve fibers, are recorded with an electrode placed anywhere in the ocellus. A previous component analysis of the ocellar ERG of cockroaches and grasshoppers (Ruck, 1957) revealed that two components originate in the photoreceptor cells and that a third (off-impulses) originates in the ocellar nerve fibers. Data were not adequate in the earlier study to support a complete functional interpretation of the ocellar ERG. The present work with ocelli of cockroaches and dragonflies has provided new data and the following functional interpretation of the ocellar ERG has emerged. Light stimulation elicits a depolarizing, sensory generator potential from the distal ends of the photoreceptor cells. The generator potential evokes a depolarizing response of the receptor cell axons. This in turn evokes a hyperpolarizing, inhibitory postsynaptic potential from the ocellar nerve fibers. The result of this is inhibition of a spontaneous discharge of nerve impulses which originates in the ocellar nerve fibers.

Previous studies on the origin of the electrical activity of dorsal ocelli include those of Parry (1947), Hoyle (1955), and Burtt and Catton (1956), which are discussed by Ruck (1957). Ruck (1958 *a*, *b*) has reported on dark adaptation and other photoreceptor functions of ocelli and compound eyes of four insect species. Goldsmith and Ruck (1958) studied the spectral sensitivities of ocelli in cockroaches and honeybees.

MATERIALS AND METHODS

Dragonflies (adults) of various species were captured at local ponds. They were brought to the laboratory and used immediately or stored in a refrigerator for a day or two. Adult specimens of the cockroach, *Blaberus craniifer*, were obtained from a laboratory culture. Intact experimental animals were immobilized with tacky wax (Central Scientific Co.) on a small platform. Further details of preparation differed in each of the several kinds of experiments performed and will be described in appropriate sections of the text.

Amplifying and recording equipment included a Grass P6 preamplifier, a Dumont M333 double beam oscilloscope, and a Grass kymograph camera. The preamplifier was operated push-pull and was coupled either directly or capacitatively to the animal preparation. When capacity-coupled, a frequency band-pass of 1 cycle/sec. to 2 kc./sec. was used. Input impedance was sufficiently high so that when using stainless steel electrodes in contact with body fluids electrode polarization effects were negligible. This was ascertained by substituting Ag-AgCl electrodes for the steel ones in control experiments. Ocellar responses recorded with the two types of electrodes were indistinguishable. Stainless steel electrodes were used regularly for the sake of convenience.

When leading off responses from the corneal surface of the receptor cell layer in intact animals, the tip of a finely pointed stainless steel electrode was passed just through the cornea. A micromanipulator was used. This lead proved equivalent to

one from a saline pool covering the surface of the exposed receptor cell layer. A finely pointed pair of jeweler's forceps was used when recording from the ocellar nerves of dragonflies. Separation of the tips was controlled with a set screw. This device, similar to a ruling pen, was carried on a micromanipulator. A wire fastened to the forceps led into one side of the amplifier. A lateral ocellar nerve was exposed by dorsal dissection and grasped in the forceps at its point of entry into the brain. The nerve was sectioned at the brain with a fine scalpel. The end of the nerve was then lifted slightly above the level of the hemolymph. The crushed aerial portion of the nerve served merely as a passive extension of the forceps, connecting the latter with living nerve close to the blood-air interface.

In experiments in which ocellar responses were studied as a function of depth in the ocellus, glass micropipette electrodes were used. These were drawn to tip diameters of less than 1 μ and filled with 3 M KCl.

In all experiments except one (that of Fig. 7) a stainless steel reference electrode was placed so as to be indifferent to electrical events originating in the ocellus. In *Blaberus* an antenna was used, and generally the one on the side opposite the experimental ocellus. The flagellum was cut off about a centimeter from its base and the indifferent electrode was inserted in the stump. With dragonflies the reference electrode was passed through the skeleton of the frons in the midline making contact with hemolymph. No electrical responses could be recorded between an electrode at either of these sites and one in a leg when the preparation was illuminated. The same polarity convention was used in the display of all ocellar responses except those of Fig. 7; an upward deflection signifies negativity of the active electrode whether in the cornea or on the nerve.

A glow modulator tube (Sylvania, R1166) was used as a stimulating light source. It was housed in a flashlight barrel behind two lenses which focussed the light on the cornea. The intensity of light emitted depended on the current through the glow modulator. This current was drawn from a D.C. power supply controlled by a Grass S^4 physiological stimulator. Square light pulses of desired intensity, duration, and frequency were produced. This optical stimulator had the main advantages of compactness and mobility. Mounted in a universal clamp on a movable stand it could be positioned wherever desired in any experiment. A potential disadvantage was the variation in wave length composition of the stimulus with glow modulator current. Over the range of stimulus intensities used, the light changed in color from red to blue-white. If an ocellus contained only one kind of receptor cell with a single visual pigment, the change of color would matter little. In this case, only the total number of quanta absorbed, and not their wave length distribution, could influence the responses. If, on the other hand, an ocellus contained two or more kinds of receptor cells with as many different visual pigments, the wave length distribution of the quanta as well as their total number could become a significant property of the stimulus. The following tests of the effect of wave length were performed: (*a*) Using the glow modulator as light source, two series of ocellar responses were determined as a function of stimulus intensity. In one series, intensity was controlled by varying the current through the glow modulator and color of the stimuli varied as stated above. In the other series, light output of the glow modulator was maintained constant and maximal,

and intensity was controlled with Wratten neutral density filters. There was no qualitative difference between the two series of responses. A particular response in one series could be matched in amplitude and wave form by a response from the other series. (*b*) Again using the glow modulator as source a Wratten filter No. 25, which transmits red light, was placed in the light path. By adjusting glow modulator current, a near-threshold response was recorded. The red filter was removed and replaced by a Wratten filter No. 47, which transmits blue light. Stimulus intensity was adjusted so as to evoke a near-threshold response as nearly similar to the previous one as possible. The procedure was repeated throughout the available range of stimulus intensities. Responses to red and blue light matched almost perfectly throughout the intensity range. The main conclusion pertinent to the present investigation is that the wave form of the ocellar responses of cockroaches and dragonflies is not a hue-dependent property in the visible range of wave lengths. All evidence suggests that if two or more receptors cell types are present excitation of each is associated with the appearance of the same components in the electroretinogram (*e.g.*, see Goldsmith and Ruck, 1958).

Stated values of stimulus intensity for the glow modulator were obtained by a kind of bioassay. The following procedure was carried out for one ocellus of *Blaberus craniifer* and two ocelli of the dragonfly *Libellula luctuosa*. Responses were first recorded as a function of stimulus intensity, expressed in log I units, using a tungsten lamp stimulator. This was calibrated in foot-candles of illumination produced at the cornea. Wratten neutral density filters controlled stimulus intensity. The responses of the same ocellus to glow modulator stimulation were recorded, using output voltage of the electronic stimulator as an index of stimulus intensity. For any given magnitude of response a stimulus value in log I corresponded to one in voltage units. Each value of glow modulator stimulus intensity used in this paper is given the value in log I of the stimulus from the tungsten lamp which would produce the same response. This method of calibration is sufficiently precise for the kinds of experiments to be described.

All experiments were performed at room temperature which varied through the year from about 22° to 27°C.

RESULTS

The electroretinogram of the ocellus is a complex of components originating in photoreceptor cells and ocellar nerve fibers. Resolution of the components is difficult using either dragonfly or cockroach ocellus alone, but using both preparations the data supplement each other and permit a plausible analysis. Design and interpretation of the experiments which will be described were aided considerably by the development of a simplified, model ocellus. The model ocellus embodies the main conclusions of this study and will be described before the experimental data are presented.

1. Components of the ERG of the Model Ocellus Data from cockroach and dragonfly ocelli have provided support for the functional interpretations represented in the model ocellus of Fig. 1. Real ocelli contain hundreds or thousands

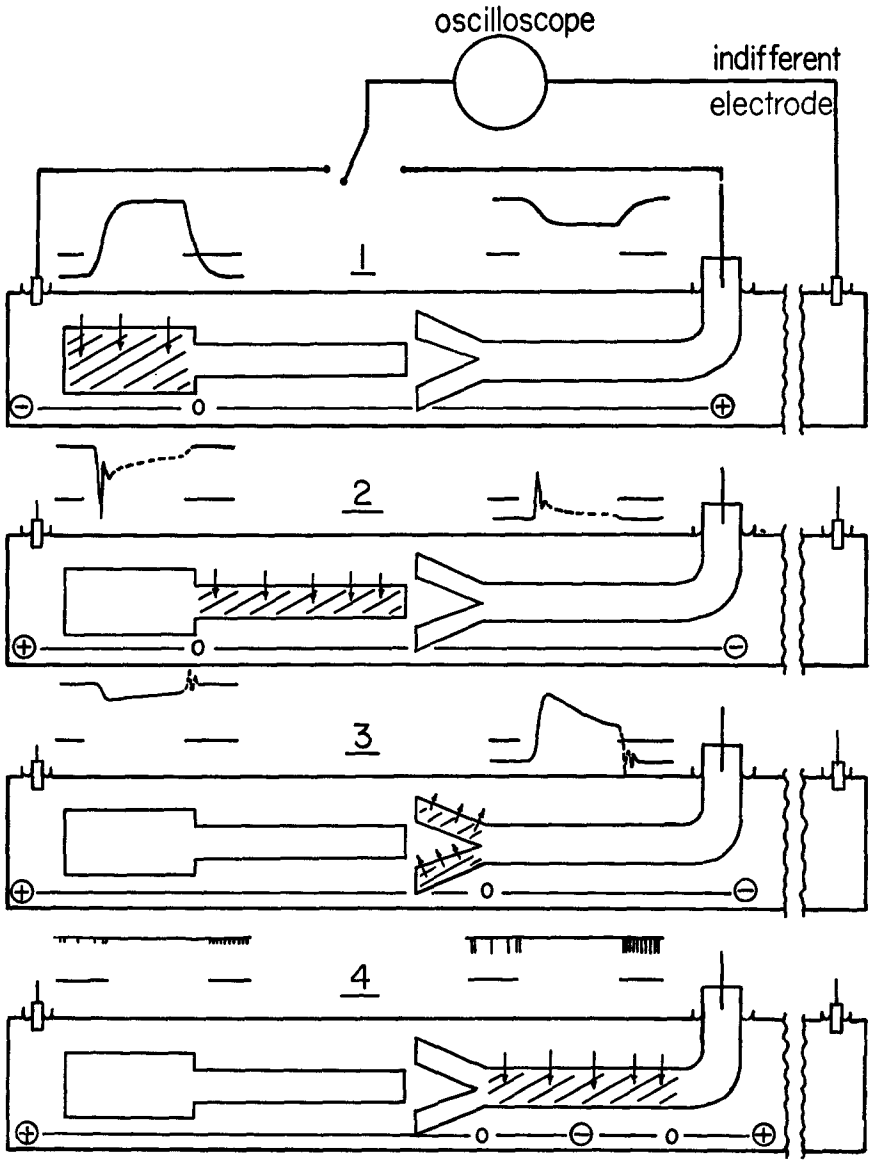

FIGURE 1. Components of the ERG of the model ocellus. One photoreceptor cell (left) with an expanded sensory ending, and an axon which makes synaptic contact with one ocellar nerve fiber. The two units are contained in an electrolyte-filled compartment. A "corneal" electrode enters at left; a "nerve" electrode lifts the nerve fiber into the air; an indifferent electrode is placed far to the right. Four components of the ERG are shown, each in one repetition of the model. Active sites for each component are shaded. Current at active sites is indicated by arrows. Each component appears at corneal and nerve electrodes. (See text.)

of receptor cells, and several ocellar nerve fibers. The model ocellus contains a single photoreceptor cell and a single ocellar nerve fiber. Both are bathed in an electrolyte solution which fills a long compartment. One electrode (comparable to the corneal electrode used in the experiments) enters the compartment at the left. Another electrode (comparable to a lead from the ocellar nerve) lifts the end of the nerve fiber into the air. The end of the nerve fiber is assumed to be dead and serves as an extension of the electrode into contact with living nerve below the air-fluid interface. An indifferent electrode (comparable to one in the frons or antenna) is placed in the compartment so far to the right that it is virtually unaffected by electrical events arising as the result of excitation of the receptor cell and the nerve fiber.

The total electrical response to a light stimulus of high intensity consists of four components, two originating in the receptor cells and two in the nerve fiber. The site of origin of each component is shaded in one of the four repetitions of the model. The corresponding electrical response is shown in the space immediately above. Each component is assigned the number appearing above the compartment in which it is considered. Each component appears at both active electrodes, components 1, 2, and 3 having opposite polarities in the two leads. Electrical sign at each electrode is indicated in the lower part of each compartment; regions of polarity reversal are indicated by zeros. The total electrical response appearing at either electrode would represent the algebraic sum of all the components. Arrows indicate the direction of current flow through active (shaded) membrane. Circuits are completed through passive (non-shaded) portions of the cell and the external volume conductor. Components 1, 2, and 3 appear in the external medium as dipolar electrical fields.

Component 1, a generator potential, is assumed to arise because of a selective change in ionic permeability restricted to membrane of the distal end of the receptor cell. Net inward current flows through active distal membrane as indicated by the arrows. This inward current is balanced by an equal outward current (not indicated) through the membrane of the receptor axon, which is passive with respect to component 1. A dipole is established in the external medium, the corneal lead lying at the negative end, the nerve lead at the positive end.

Component 2, evoked by component 1, is a depolarizing response of axonal membrane of the receptor cell. The total wave form is uncertain as indicated by the dashed line. (The nature of the unit receptor axon response is the most uncertain feature of this analysis; it will be discussed later in this paper and in the two which follow.) Net inward current through active axonal membrane is balanced by net outward current through membrane of the distal end of the receptor cell. The corneal electrode lies at the positive end of the dipole of component 2, the nerve electrode at the negative end.

Component 2 evokes component 3, a hyperpolarizing postsynaptic potential. Net outward current flows through synaptic membrane, net inward current through more proximal regions of ocellar nerve fiber membrane. The corneal electrode lies at the positive end of the dipole of component 3, the nerve electrode at the negative end. Component 3 may decline at "off" through a series of oscillations as indicated by the dashed portion of the response in Fig. 1. Alternatively, component 3 may return to the baseline at off without oscillations or overshoot, or may overshoot the baseline as a sustained after-potential (Ruck, 1961).

In the absence of component 3 the ocellar nerve fiber discharges nerve impulses (component 4) continuously. These impulses originate in the fiber just proximal to the synaptic region. They appear monophasic and positive at both corneal and nerve electrodes because they invade neither the synaptic region nor the end of the nerve fiber, which is assumed to be dead. A tripolar field is indicated for the impulses in Fig. 1. The synaptic region and the end of the nerve act as current sources for inward current in the region of impulse generation.

2. ERG Components of the Dragonfly Ocellus Dragonflies have three dorsal ocelli, two lateral and one median. Lateral ocelli, which have longer ocellar nerves, were used exclusively in the following experiments. The diagram of Fig. 2 indicates the features of ocellar anatomy considered pertinent to the experiments. The retina is a palisade of photoreceptor cells, several of which are shown. Axons of the photoreceptor cells synapse with dendritic branches of ocellar nerve fibers, only one of which is shown. The ocellar nerve fibers pass into the brain *via* the ocellar nerve.

Fig. 2 is based upon studies (Ruck and Edwards, 1958; unpublished data) with light and electron microscope of the lateral ocellus of *Sympetrum rubicundulum*. The photoreceptor cells occur in groups, or retinulae, three of which are indicated in the diagram. Each retinula typically contains three photoreceptor cells. A rhabdome (indicated by a broad line) runs lengthwise through the center of each retinula. Nuclei of the photoreceptor cells lie basally in the region between the proximal end of the rhabdome and the axons. A lateral ocellus of *Sympetrum* contains about 225 retinulae, hence about 675 photoreceptor cells. Each receptor cell sends an axon into the synaptic region. The precise number of postsynaptic ocellar nerve fibers is uncertain. There is one very large fiber of 25 to 38 μ, and three or four fibers of from 4 to 13 μ. There may be additional ocellar nerve fibers of about 1 μ diameter but these, if they exist, are very difficult to distinguish from receptor axons in cross-sections of the nerve. The cell bodies of the ocellar nerve fibers lie in the brain (Cajal, 1918; Satija, 1958 *a, b*). Ocellar nerve fibers may therefore be regarded as dendrites. The high degree of convergence of receptor axons on ocellar nerve fibers is enhanced by two factors: (*a*) the nerve fibers branch repeatedly, each branch synapsing with many receptor axons; (*b*) many synapses occur along the course of each receptor axon.

Placements of the recording electrodes are indicated in Fig. 2. A stainless steel electrode was imbedded in the cornea. A forceps electrode grasped the end of the ocellar nerve and lifted it out of the blood into the air. Each of the active electrodes was coupled with an indifferent electrode, a stainless steel wire which was thrust through the exoskeleton of the frons.

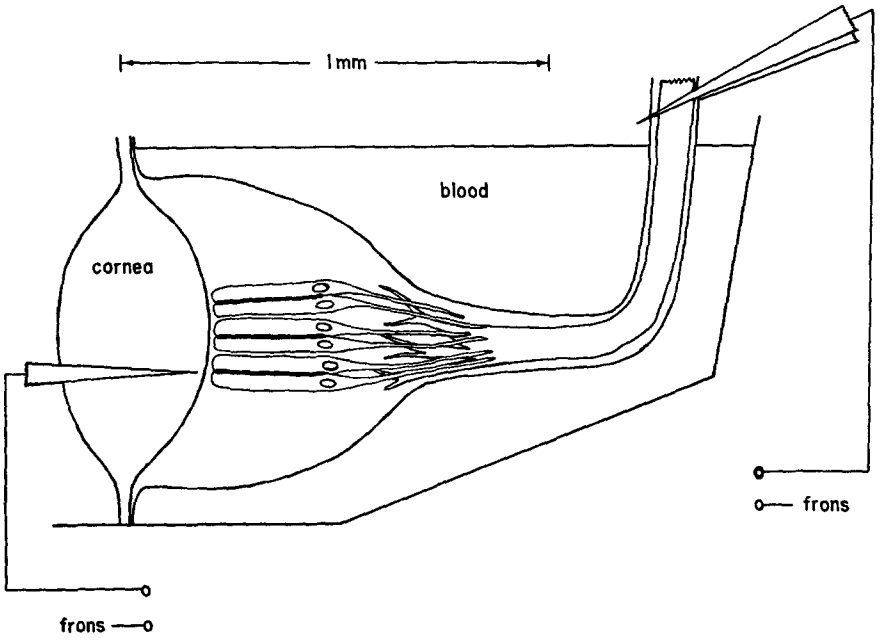

FIGURE 2. Diagram of lateral ocellus of dragonfly showing photoreceptor cells and the "giant" ocellar nerve fiber. Corneal and nerve electrodes are shown. Indifferent electrode in each case makes contact with blood beneath the exoskeleton of the frons.

It was not possible to record simultaneously from both pairs of electrodes in the same preparation. When this was attempted recordings from the corneal lead were so reduced in amplitude that much of their detail was lost in the noise level of the recording system. This was a probable consequence of shunting between corneal and indifferent electrodes in operated animals. Responses using the corneal lead were always obtained therefore from unoperated animals.

Responses of dragonfly ocelli (Fig. 3) include pre- and postsynaptic components. With constant stimulus duration each component varies in amplitude with stimulus intensity and in polarity depending on the position of the active electrode. The polarity of each component reverses between the cornea and the ocellar nerve; a component which appears positive to the corneal electrode appears negative to the nerve electrode. All records of Fig. 3 are steady state responses to light flickering at a rate of 2/sec. Each light flash of 0.25 sec. is

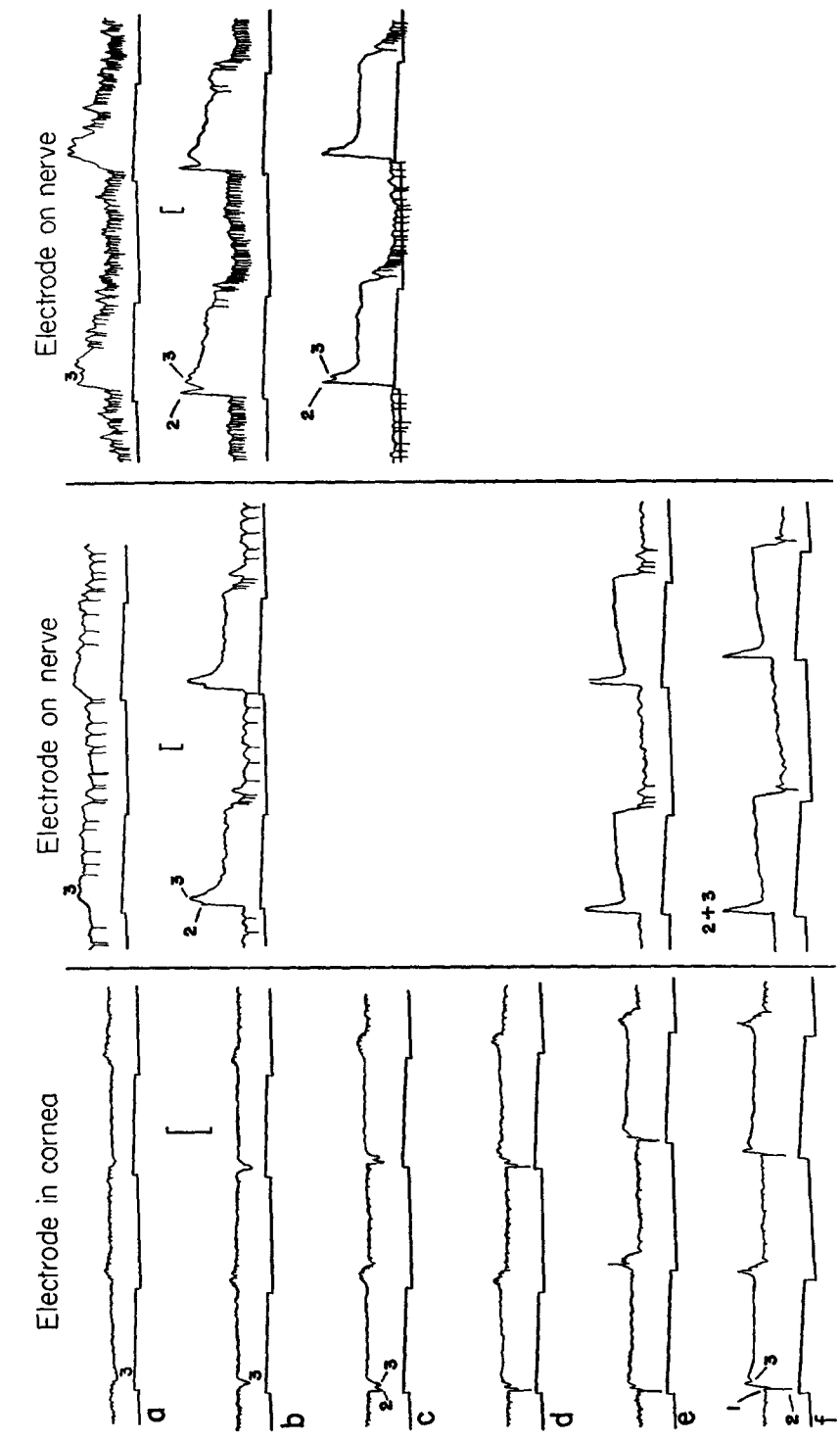

FIGURE 3. ERG's of lateral ocellus of *Symphetrum rubicundulum*. Leads as shown in Fig. 2. Signal marker in lower trace. Stimulus duration, 0.25 sec.; stimulus flickers at 2/sec. Stimulus intensities the same in any row: *a*, log $I = -6.0$; *b*, -5.5; *c*, -4.5; *d*, -3.3; *e*, -2.0; *f*, -1.0. ERG components numbered as in Fig. 1. Negativity of either corneal or nerve electrode gives upward deflection. Calibration marks indicate 2 mv.

followed by a dark period of 0.25 sec. Stimulus intensity increases from *a* through *f*, and has the same value for records in the same horizontal row.

Spike potentials (component 4), the afferent output of the largest ocellar nerve fiber, appear in the two columns of ocellar nerve responses. These nerve impulses, present in the dark phase of the flicker cycle, are inhibited by illumination. Corresponding spike potentials of much smaller amplitude are visible in responses from the corneal lead. Associated with inhibition of nerve impulses in the giant fiber is a complex of slow potentials. There appear to be three components, corresponding to components 1, 2, and 3 of the model ocellus (Fig. 1), which sum to produce the total response recorded from either lead. All three components can be discriminated only when high intensity stimuli are used (Fig. 3 *e, f*). Near threshold, component 3 and nerve impulses appear to be the only components present (Fig. 3 *a*). Components 1 and 2 are assumed to be present but of such low amplitude that they are masked by component 3.

The following evidence, which involves the relative magnitudes of the ERG components as recorded from the two ends of the ocellus, supports the hypothesis that components 1 and 2 originate in the receptor cells and component 3 in the ocellar nerve fibers. Receptor cells lie nearest the cornea. Accordingly components originating in them would be expected to appear relatively larger at the corneal electrode. Conversely, components originating in the postsynaptic units would be expected to appear relatively larger at the nerve electrode. These relationships are indicated for the model ocellus in Fig. 1. Comparisons of relative magnitudes in responses of real ocelli are complicated by the fact that threshold responses of the receptor cells are adequate to evoke nearly maximal postsynaptic responses. Thus at low intensities, postsynaptic responses dominate in both corneal and nerve leads (Fig. 3). With increase of stimulus intensity the amplitudes of receptor cell components increase steadily. At high intensities they become large enough, in the corneal lead, almost to obscure the postsynaptic responses. In the nerve lead, though receptor cell components become increasingly prominent, the postsynaptic responses continue to dominate even at high intensity. These relationships are illustrated in the records of Fig. 4.

Each row of responses of Fig. 4 was obtained from a separate lateral ocellus of *Sympetrum rubicundulum*. Stimulus intensity increased for both ocelli through the same series of values, *a* through *h*. Nerve impulses (component 4) were initially present in the preparation supplying the lower records but dropped out shortly before recordings were made. Components 1, 2, and 3 survived throughout the experiment in normal condition. Component 3 appears as the dominant threshold response in both leads. From *a* through *h* in the corneal lead, components 1 and 2 increase steadily until they dominate the total re-

sponse. In *h*, component 3 appears as a mere notch on the ascending limb of component 1. Component 1 determines the polarity of the sustained negative wave in the corneal lead. From *a* to *h* in the nerve lead, components 1 and 2 also become increasingly prominent, but component 3 continues to dominate the total response. Component 2 may become somewhat larger than component 3 in the nerve lead (*f* to *h*), but never so much larger as in the corneal lead. The presence of component 1, opposite in polarity to component 3, is inferred in the nerve lead from the return of the sustained portion of the response toward the baseline during illumination (*d* to *h*). In summary, when

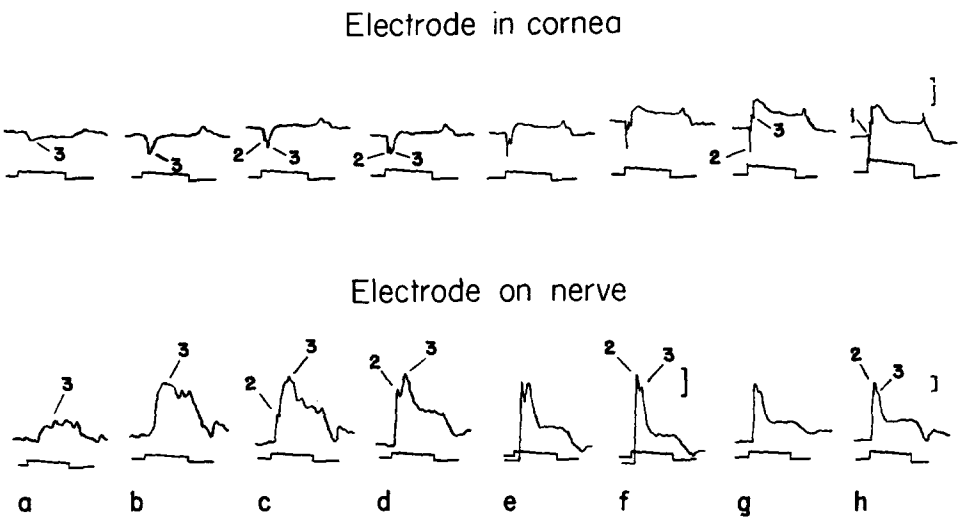

FIGURE 4. ERG's of lateral ocellus of *Sympetrum rubicundulum*. Leads as in Fig. 2. Stimulus duration 0.125 sec. D. C. amplification. Negativity at either electrode gives upward deflection. Stimulus intensities: *a*, $\log I = -6.5$; *b*, -5.5; *c*, -5.0; *d*, -4.5; *e*, -4.0; *f*, -3.0; *g*, -2.0; *h*, -1.0. Components numbered as in Fig. 1. Calibration marks indicate 1 mv. in upper records, 2 mv. in lower records.

stimulus intensity is high enough to evoke maximal responses of all components, components 1 and 2 are larger, relative to 3, when recorded from the cornea. Conversely, 3 is larger, relative to components 1 and 2, when recorded from the nerve. This is considered to be evidence that 1 and 2 originate in the receptor cells, component 3 in the ocellar nerve fibers.

Better evidence for the origin of components 1 and 2 in the photoreceptor cells has been obtained from the ocellus of the cockroach *Blaberus craniifer*.

3. ERG Components of the Cockroach Ocellus The ocellus of *Blaberus craniifer* is similar in structure to that of *Periplaneta americana*, which has been described, but twice as large (Ruck, 1957). Pertinent histological details for *Blaberus* are shown in Fig. 6.

The cornea is a flat transparent disc. Beneath it lie thousands of small retinulae arranged in somewhat less orderly fashion than Fig. 6 suggests. Each retinula contains three, four, or five photoreceptor cells each of which sends an axon into the synaptic region. (In the diagram only a single axon is drawn for each retinula.) Four or five large ocellar nerve fibers of 4 to 15 μ diameter enter *via* the ocellar nerve, branch, and make synaptic contacts with the receptor axons. An undetermined number of smaller axons accompanies the large fibers. All synapses lie within the ocellar cup in cockroaches.

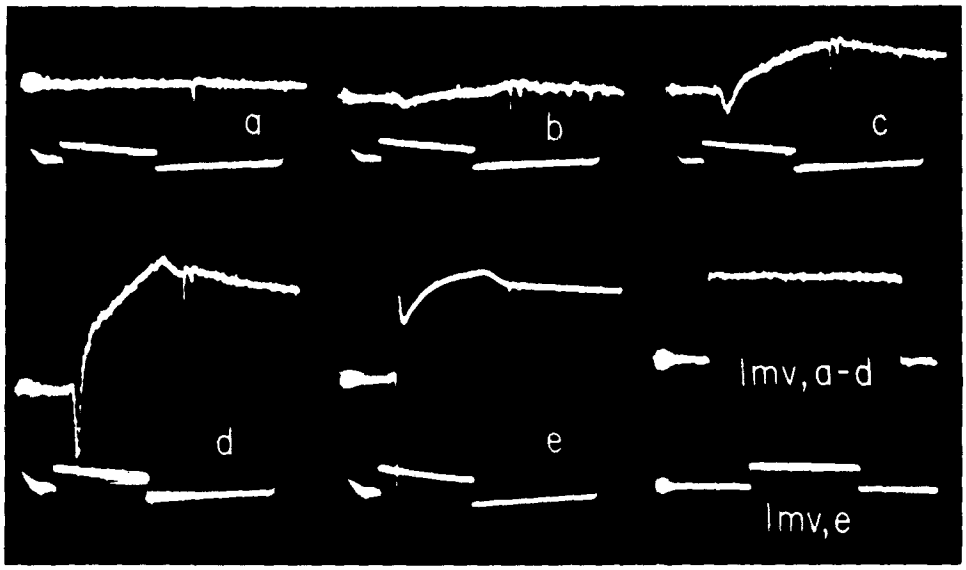

FIGURE 5. ERG's of ocellus in *Blaberus craniifer*. Corneal lead. Negativity of corneal electrode gives upward deflection. D.C. amplification. Stimulus duration, 0.125 sec. Stimulus intensities: *a*, $\log I = -7.0$; *b*, -6.5; *c*, -5.5; *d*, -4.0; *e*, -0.5.

The responses of the *Blaberus* ocellus shown in Fig. 5 were recorded with an active electrode in the cornea and with an indifferent electrode in an antenna. Stimulus durations were all $\frac{1}{8}$ sec.; stimulus intensity increased from *a* to *e*. The threshold response is an off-effect (*a*) believed to represent nerve impulses in the largest ocellar nerve fibers. Above threshold components 1 and 2 appear and increase in amplitude. Component 3, which is very prominent in dragonfly ocelli, is rarely discernible in responses of cockroach ocelli recorded with a corneal lead.

The preparation of Fig. 6 was designed to isolate the response of the photoreceptor cell layer and to determine whether components 1 and 2 do in fact originate there as argued in the previous section. ERG's were recorded between a microelectrode which was positioned at different depths in the ocellus and a fixed reference electrode placed at the surface of the receptor cell layer.

An animal was immobilized, attached to a micromanipulator, and positioned so that the cornea faced directly upward. A wax cup was built up about the cornea. A small piece of cornea was dissected away, exposing the surface of the receptor layer. A very slender stainless steel reference electrode coated to the tip with an insulating varnish, was located with its tip at the surface of the receptor layer. This electrode was fixed

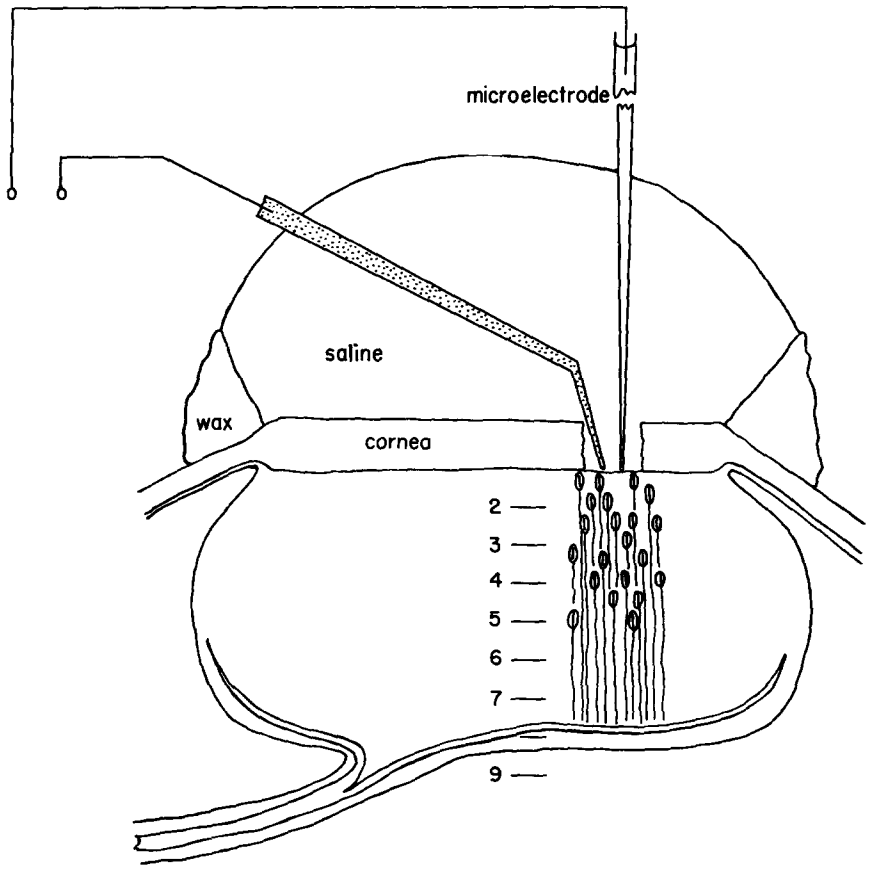

FIGURE 6. Preparation of *Blaberus* ocellus used in recording ERG's at successively greater depths. Fixed reference electrode at left. ERG's recorded with tip of microelectrode at each numbered position. Responses shown in Fig. 7.

to the micromanipulator bearing the animal and was not moved throughout the experiment. The microelectrode, a glass pipette filled with 3 M KCl, was attached to a second micromanipulator. The tip of the microelectrode was brought into the focus of the stimulating light. The preparation was moved upward until the hole in the cornea surrounded the tip of the microelectrode, which was brought just into contact with the exposed receptor layer. A drop of physiological saline was added to the wax cup and the preparation appeared as shown in Fig. 6. The microelectrode manipulator was adjusted for a total vertical excursion of 0.5 to 0.6 mm. This was sufficient

to move the electrode tip from the surface of the receptor layer just through the ocellar sheath. A scale mounted by the end of the lever arm which controlled the movement divided the total excursion into eight equal parts. Corresponding positions in the ocellus are indicated in Fig. 6. In a preparation of this sort one cannot visualize directly the position of the electrode tip at any time, and consequently there is some uncertainty regarding precise localization. However, positions 1 and 8 are fairly easy to establish as "landmarks." Position 1 is established the instant that electrical contact is made in the recording circuit as the microelectrode moves into the hole in the cornea. Position 8, or thereabouts, corresponds to the ocellar sheath. When the electrode penetrates it, responses change in a characteristic manner. With considerable regularity ocelli may be prepared so that, with total electrode excursion set at 0.5 to 0.6 mm., the electrode penetrates the ocellar sheath between positions 8 and 9.

This recording arrangement permitted discrimination between components originating in the receptor cells and those originating postsynaptically. With the reference electrode fixed at position 1 and the microelectrode anywhere from 1 to 8 along the path indicated in Fig. 6 one would predict minor contributions, if any, of the postsynaptic units to the recorded responses. Unlike the dragonfly, the synapsing branches of the ocellar nerve fibers are oriented transversely with respect to the electrode path. Differences of potential between the synaptic regions and more central portions of the nerve fibers would not be expected to appear along the electrode path. On the other hand, the receptor cells are wholly contained within the ocellus and are oriented mainly parallel with the electrode path. Maximal receptor cell responses should be recorded between positions 1 and 8. With the microelectrode at position 1 no response should be recorded under any conditions, but as the tip is advanced from 1 to 8 the response of the receptor cells should appear. If components 1 and 2 originate in the receptor cells, both should appear at about the same depth. If, on the other hand, one of these components originates in the postsynaptic units it should not appear at all, or should appear feebly developed and at greater depth than that at which the receptor cell response first appears.

A steady state response was established during 5 minutes of repetitive stimulation with flashes of $\frac{1}{8}$ sec. duration delivered at a rate of 1 flash per 3 sec. Recordings (Fig. 7) of the steady state response were made with the microelectrode at successively greater depths in the ocellus. No response was recorded at position 1. Between positions 1 and 3 components 1 and 2 appeared simultaneously. With further advances to position 8 components 1 and 2 increased in amplitude. At position 8 the response appears quite similar to one recorded with the reference electrode placed at an indifferent site (Fig. 5), except for absence of the off-effect. When the tip was advanced from 8 to 9, passing through the ocellar sheath into the head cavity, two changes occurred. The off-effect appeared abruptly and the amplitudes of

components 1 and 2 decreased. The recording situation was then essentially the same as in the experiment of Fig. 5 with one electrode in the cornea and an indifferent electrode in antennal blood. Clearly, the off-effect arises at some site other than those at which components 1 and 2 originate. Therefore, if 1 and 2 originate in the receptor cells the most likely source of the off-effect

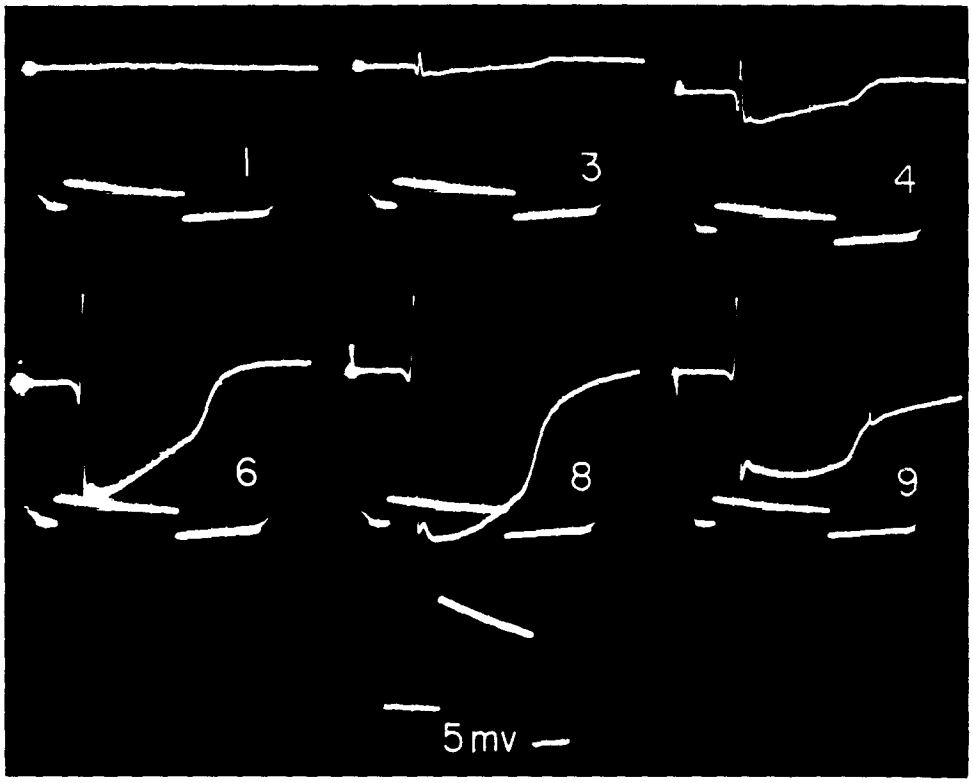

FIGURE 7. ERG's of *Blaberus* ocellus recorded at successively greater depths in the ocellus. Electrodes as in Fig. 6. Stimulus conditions constant throughout: $\log I = -1.0$; duration = 0.125 sec.; stimulus rate, 1 flash/3 sec. Numbers correspond to positions of the microelectrode tip indicated in Fig. 6. A.C. amplification; frequency band pass of 0.5 cycle/sec. to 2 kilocycles/sec. Negativity of microelectrode gives downward deflection.

seems to be the postsynaptic units. The decrease in amplitude of 1 and 2 on passage through the ocellar sheath indicates that the sheath has significant electrical resistance.

If the electrode were moved along the same path as in Fig. 7 but with the reference electrode removed from the surface of the receptor layer and placed at an indifferent site, one would predict quite different results. In this case, only a single electrode would lie within the electrical fields es-

tablished on excitation of the receptor cells and postsynaptic fibers. On passage from position 1 to 8 the microelectrode tip should move from one "pole" of a dipolar receptor field to the other, while remaining in the same pole of a dipolar postsynaptic field. Components originating in the receptor cells should reverse polarity while those originating postsynaptically should

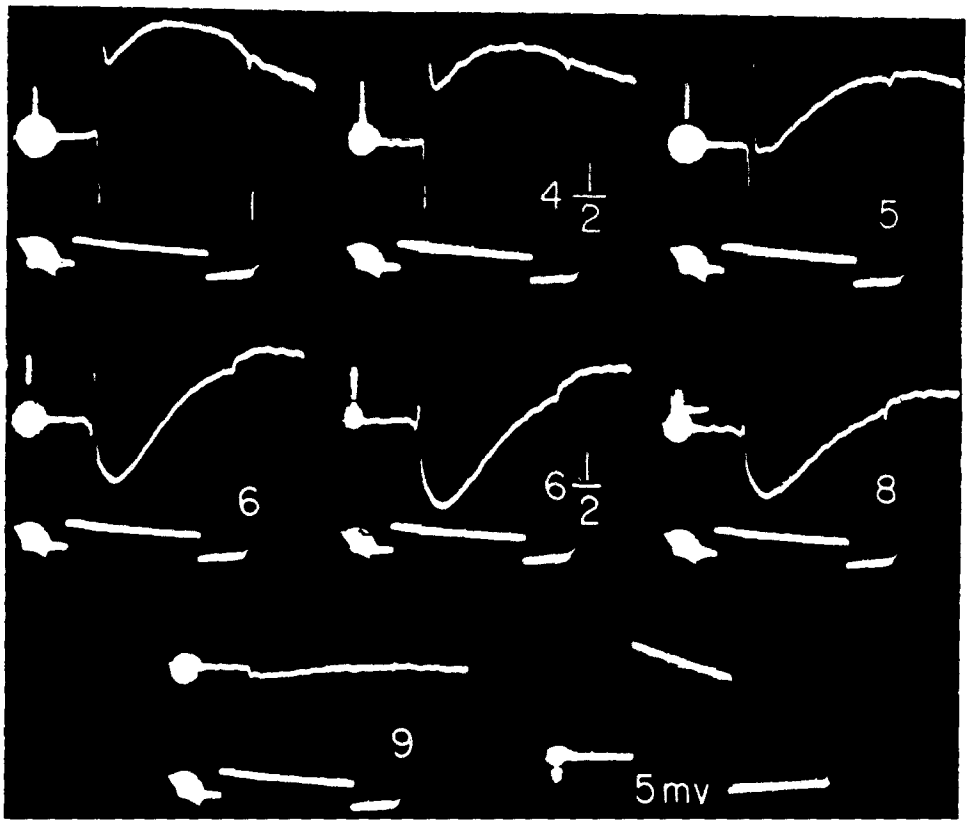

FIGURE 8. ERG's of *Blaberus* ocellus recorded at successively greater depths in ocellus. Microelectrode as in Figs. 6 and 7. Reference electrode in base of antenna. Otherwise as in Fig. 7.

not. A preparation similar to that of Fig. 6 was used except that the reference electrode was moved to an antenna. Fig. 8 shows the responses obtained. Stimulus conditions were the same as in the preceding experiment. Between positions 1 and 8 components 1 and 2 reversed polarity. The off-effect did not. Therefore components 1 and 2 have different sites of origin than the off-effect. The experiment favors assignment of components 1 and 2 to the receptor cells, the off-effect to the ocellar nerve fibers. On passage from position 8, which is inside the ocellus, to position 9, which lies outside the ocellar

sheath, the total response almost disappears. This sudden decrease in amplitude implies that the sheath has significant electrical resistance.

Repeated passage of a microelectrode through the ocellus produced essentially the same results with each repetition. The original electrode tip, however, did not survive the first penetration of the ocellar sheath.

4. Reversible Elimination of Component 2 with High Concentrations of K Ion

Having assigned components 1 and 2 to the receptor cells one may ask what functional relationships exist between the two. Some evidence bearing on this question comes from work with dragonfly ocelli undergoing physiological deterioration. As preparations age, component 1 eventually remains in isolation, except occasionally for the presence of nerve impulses. In the latter case, when only component 1 and nerve impulses remain, illumination no longer inhibits or has any other effect on the impulse discharge. This suggests that component 2 is an essential link between component 1 and synaptic inhibition.

Experiments in which the ocellar tissues were exposed to solutions containing high concentrations of K^+ corroborate this suggestion. Component 2 of the *Blaberus* ocellus drops out of the ERG on exposure of the ocellus to 70 mM $[K^+]$. As component 2 disappears so does the off-effect, a postsynaptic response. Component 1 then remains in isolation. The experimental arrangement was similar to that of Fig. 6 with the following differences. The cornea was almost completely dissected away except for a narrow central strip which was left in place to prevent pressure changes in the head from ejecting the soft tissues of the ocellus. A drop of physiological (control) saline, or of a test saline containing high $[K^+]$ was placed in the wax cup. Responses were recorded between an active electrode placed in the drop and an indifferent electrode placed in an antenna.

Hoyle's (1953) locust saline, used as the control saline, has the following composition: NaCl, 140 mM; $MgCl_2$, 2 mM; $KHCO_3$, 4 mM; KH_2PO_4, 6 mM. The test saline consisted of the control saline plus 60 mM KCl; the K ion concentration of the test saline was 70 mM. At the beginning of an experiment control saline was placed in the cup and half an hour or so allowed to elapse while the ocellar tissues equilibrated with this solution. Throughout the equilibration period and the remainder of the experiment steady state responses were maintained with stimuli of constant rate, intensity, and duration. At the end of the equilibration period several responses were recorded. Then the control saline was replaced with test saline. Responses were recorded as component 2 declined in the presence of the test saline.

Fig. 9 *a* illustrates a steady state response in the presence of the control saline. Within 2 minutes of replacing the control saline with a test saline

containing 70 mM [K^+], component 2 began to recede up the ascending limb of component 1 (Fig. 9 *b*). After 3 minutes component 2 was just visible as an inflection point on the ascending limb of component 1 (Fig. 9 *c*). After 4½ minutes component 2 had disappeared (Fig. 9 *d*). At about the same time the off-effect dropped out and component 1 remained in isolation. The effect of the added KCl was attributable to an increase of [K^+] and not to osmotic effects or to increased [Cl^-], for when the test saline was replaced by

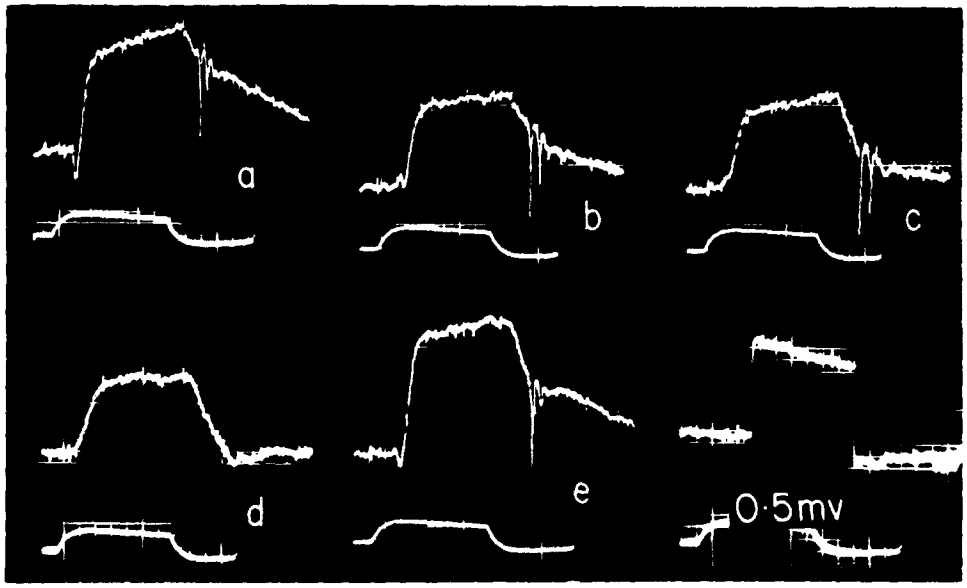

FIGURE 9. Effects of high K ion concentration on ERG of *Blaberus* ocellus. Preparation similar to that of Fig. 6 except that most of cornea has been removed. Active electrode is placed in drop of saline; indifferent electrode in antenna. Stimulus conditions constant throughout: $\log I = -3.0$; duration = 0.125 sec.; rate, 1 flash/2 sec. Record *a*, ERG in physiological saline (control); *b*, 2 min. after change to control saline plus 60 mM KCl; *c*, 3 min. after; *d*, 4½ min. after; *e*, 2 min. after change to control plus 60 mM NaCl.

another with 60 mM NaCl substituted for the 60 mM KCl, component 2 and the off-effect reappeared in a few minutes (Fig. 9 *e*).

Component 1 is comparatively little affected by high [K^+]. Exposed to 70 mM [K^+] it may decrease to a third of its original amplitude in 5 to 10 minutes but retain approximately this level for 3 to 4 hours.

In the presence of the control saline, component 2 may appear to have the shortest latency, as in Fig. 9 *a*. After application of 70 mM [K^+], however, component 1 has the shorter latency, as in Fig. 9 *c* and *d*. This, coupled with the fact that component 1 is the only one which may exist in the absence of any other component, leads to the interpretation that component 1 is the

sensory generator potential. It is reasonable to allocate it to the rhabdomere-bearing ends of the receptor cells. There is indirect support for this interpretation in experimental data from compound eyes. Several investigators (Bernhard, 1942; Jahn and Wulff, 1942; Autrum and Gallwitz, 1951; Hartline, Wagner, and MacNichol, 1952) have simplified the responses of compound eyes by cutting away the optic ganglion at the level of the basement membrane. The receptor axons are sectioned at the bases of the ommatidia in this operation. There is general agreement that the intraommatidial portions or rhabdomere-bearing ends of the receptor cells produce only a monophasic, cornea-negative potential. This probably corresponds to component 1 of the ocellar response.

Component 2 of the ocellar response is evoked by component 1, originates in the receptor cells, and must be present if synaptic transmission is to occur. For these reasons it may be assigned tentatively to the receptor cell axons.

DISCUSSION

The relatively simple structure of the insect dorsal ocellus permits a more complete analysis of photoreceptor function than has been possible with any other type of insect eye. The evidence presented above strongly supports an earlier conclusion (Ruck, 1957) that light stimulation evokes two electrical events from the receptor cells. The first of these (component 1) is interpreted as a generator potential arising in the sensory endings, the second (component 2) as a depolarizing response of the receptor axons. Components 1 and 2 have been found in cockroaches, dragonflies and, in all, about twenty-five species of insects which have been studied. A third event (component 3) is resolvable in some but not in all ocelli. It is particularly prominent in dragonfly ocelli. Component 3 is interpreted as an inhibitory hyperpolarizing postsynaptic potential. Its appearance coincides with inhibition of a spontaneous discharge of nerve impulses (component 4) carried by ocellar nerve fibers.

The precise localization and nature of the process which underlies component 1 are far from clear. In Fig. 3 component 1 is shown as a simple depolarization of a homogeneous membrane bounding the distal end of the receptor cell. This is an oversimplification. The outer boundary of a real receptor cell includes rhabdomere and non-rhabdomere membrane; in the interior of the cell there are additional membrane systems (see *e.g.*, Fernández-Morán, 1958). The specific membrane across which component 1 is generated has not been identified.

A depolarizing ommatidial potential similar to ocellar component 1 has been studied in the eye of *Limulus* with intracellular microelectrodes (Hartline, Wagner, and MacNichol, 1952; Tomita, 1956; Yeandle, 1958; Fuortes,

1958). A *Limulus* ommatidium, however, contains two kinds of neural elements, typical retinula cells with rhabdomeres and an "eccentric cell" which has no rhabdomere. It has proved difficult to visualize which kind of cell a microelectrode has entered because of dense melanin pigments in the ommatidium. For the present at least the exact localization of the *Limulus* ommatidial potential appears uncertain. Eccentric cells are absent in retinulae of ocelli. Therefore, ocellar component 1 must be produced in ordinary retinula cells; *i.e.*, those with rhabdomeres.

If component 1 of the ocelli is restricted to the rhabdomere-bearing ends of the receptor cells, as suggested above, there are two general mechanisms by which it might influence synaptic events occurring some distance away. In the first, component 1 would be the only presynaptic electrical event; in this case component 1 would be conducted electrotonically with decrement to the synaptic terminals where it would evoke synaptic transmission. Alternatively, component 1 would evoke a response in the receptor axons and this response in turn would evoke synaptic transmission. The following argument favors the second alternative. The fraction of component 1 effective at the synapses must be strongly influenced by the dimensions of the receptor axons. Large axonal diameter and short length would increase the effectiveness of component 1. But receptor axons in the *Blaberus* ocellus, *e.g.* are only about 0.5 μ in diameter, and those originating nearest the cornea may be about 300 μ long. High axoplasmic resistance in the case of such small fibers must cause rapid attentuation of component 1. An estimate of the length constant of a 0.5 μ axon was made by extrapolation from data obtained for giant axons of the abdominal nerve cord in *Periplaneta americana* (Yamasaki and Narahashi, 1959). Assumptions were that the specific resistances of axonal membrane are the same for the giant axons and those of the photoreceptor cells. A D. C. potential applied across the receptor axons would decline to $1/e$ at a distance of about 150 μ from point of application. Rather large errors may exist in this extrapolation but the qualitative argument nevertheless appears sound: near threshold responses of the rhabdomere-bearing ends of the most distally situated receptor cells could exert very little influence at the synapses unless they evoked some kind of relay mechanism in the receptor axons. Component 2 is a likely candidate for the role of relay mechanism. It originates in the receptor cells but never appears unless component 1 precedes it. It may be removed reversibly with excess K^+, an axonal blocking agent. Synaptic transmission appears to depend upon component 2. When component 2 is removed from cockroach ocelli with high $[K^+]$, the off-effect disappears. Occasionally during physiological deterioration of dragonfly preparations, component 2 drops out, leaving component 1 and ocellar nerve impulses; component 1 under these conditions has no control over the nerve impulses. Data from the dragonfly suggest that component 2 evokes

component 3, a postsynaptic potential, which exerts direct inhibitory control over the discharge of ocellar nerve impulses.

Component 3, which is prominent in dragonfly ocelli, is rarely discernible in cockroach ocelli. An anatomical difference may account for this. In the roach ocellus, unlike the situation in the dragonfly, the synapsing dendrites of the ocellar nerve fibers run transversely across and lie wholly within the ocellar cup. Perhaps the dipolar electrical field associated with component 3 is oriented transversely across the ocellus, with "source" and "sink" lying

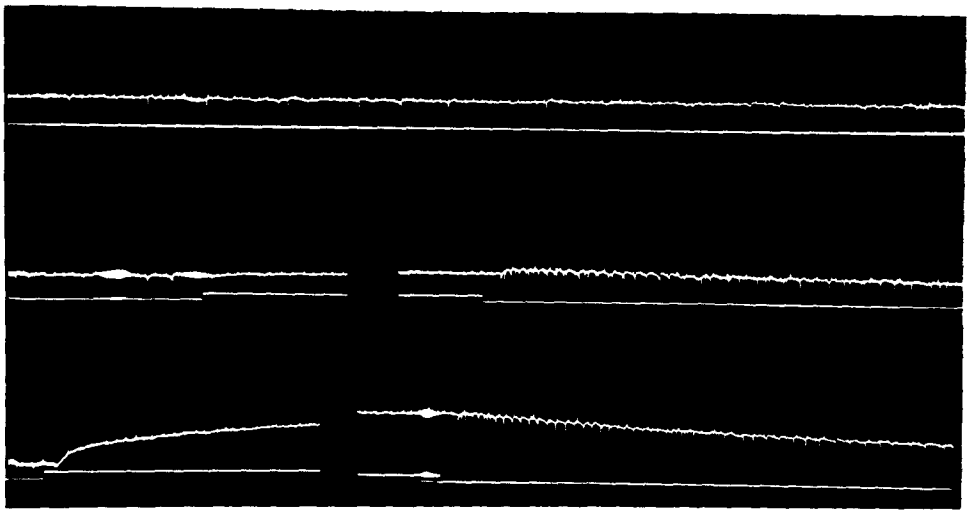

FIGURE 10. ERG's of the ocellus in *Periplaneta americana*. Active electrode just beneath cornea; indifferent electrode in antenna. D.C. amplification. Upper record, 2½ sec. of spontaneous discharge of ocellar nerve impulses in dark-adapted state. Middle, light flash of about 2 sec. duration at $\log I = -7.0$. Lower, stimulus of about 2 sec. duration at $\log I = -5.0$. Spontaneous discharge is inhibited by light. Off-impulses vary in amplitude indicating that several ocellar nerve fibers contribute spikes. Sometimes they fire synchronously as in the upper record.

largely within the ocellus; in this case a corneal electrode in the roach would record little or no sign of component 3.

The possibility that component 3 actually originates in the photoreceptor cells was ruled out in the following way. The cornea of a lateral ocellus of a dragonfly was removed and the ERG was recorded with a microelectrode at different depths in the ocellus. The experimental arrangement was similar to that shown for the roach ocellus in Fig. 6, except that the reference electrode was placed at an indifferent site. Components 1 and 2 reversed polarity near the base of the ocellar cup, but component 3 did not. In the three experiments of this type which were performed, the electrode broke before it could be passed through the synaptic layer into the ocellar nerve. The data

however, clearly indicated an origin for component 3 deeper than the layer of photoreceptor cells, which are the source of components 1 and 2. This, together with the positive sign of component 3 in the synaptic region, and the observed inhibition of ocellar nerve impulses, support the interpretation that component 3 is a hyperpolarizing, inhibitory postsynaptic potential.

There are interesting points of comparison between responses of cockroach and dragonfly ocelli. The off-effect of the cockroach ocellus probably represents nerve impulses in the largest ocellar nerve fibers (Ruck, 1957). In all cockroach ocelli which have been studied, except one, these fibers have produced pure off-effects only. In the one exceptional case (Fig. 10) the fibers were spontaneously active in the dark, and were inhibited by light. This is similar to the typical situation in dragonfly ocelli, in which the largest ocellar nerve fiber, at least, has been spontaneously active in all preparations. Under appropriate experimental conditions (Ruck, 1961) dragonfly ocellar nerve fibers can be made to discharge impulses at off only, and thus to resemble the units responsible for the typically pure off-effects in the cockroach.

In the corneal lead, ocellar nerve impulses in both cockroaches and dragonflies appear as positive spikes. This is understandable if, in both cases, the impulses originate outside the synaptic region as suggested in the model of Fig. 1. In an earlier paper (Ruck, 1957) the polarity of the off-impulses of the cockroach ocellus, and the failure to record them following section of the ocellar nerve, were used to support an argument that they are efferent impulses. The argument is no longer attractive because their positivity in the corneal lead can now be explained as well on the assumption that these impulses are afferent. Failure to record off-impulses in the roach after sectioning the very short ocellar nerve probably signifies only that physiological deterioration of the dendritic terminals proceeds very rapidly.

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