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Neural Organization of the Median Ocellus of the Dragonfly

I. Intracellular electrical activity

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ABSTRACT Intracellular responses from receptors and postsynaptic units have been recorded in the median ocellus of the dragonfly. The receptors respond to light with a graded, depolarizing potential and a single, tetrodotoxin-sensitive impulse at "on." The postsynaptic units (ocellar nerve dendrites) hyperpolarize during illumination and show a transient, depolarizing response at "off." The light-evoked slow potential responses of the postsynaptic units are not altered by the application of tetrodotoxin to the ocellus. It appears, therefore, that the graded receptor potential, which survives the application of tetrodotoxin, is responsible for mediating synaptic transmission in the ocellus. Comparison of pre- and postsynaptic slow potential activity shows (*a*) longer latencies in postsynaptic units by 5–20 msec, (*b*) enhanced photosensitivity in postsynaptic units by 1–2 log units, and (*c*) more transient responses in postsynaptic units. It is suggested that enhanced photosensitivity of postsynaptic activity is a result of summation of many receptors onto the postsynaptic elements, and that transients in the postsynaptic responses are related to the complex synaptic arrangements in the ocellar plexus to be described in the following paper.

INTRODUCTION

The dorsal ocelli of many insects are relatively simple photoreceptor systems that consist of a layer of photoreceptor cells, a synaptic zone in which the axons of the photoreceptor cells come into contact with dendrites of the ocellar nerve fibers, and an ocellar nerve that leads from the eye to the brain (Cajal, 1918; Ruck and Edwards, 1964). Such an elementary system is obviously advantageous for study of receptor and synaptic mechanisms. A decade ago Ruck (1961) provided a first analysis of the electrical activity of the dragonfly dorsal ocelli by recording from them extracellularly. He concluded that light stimulation induces a slow depolarizing potential in the

photoreceptor cells (component 1) which results in secondary depolarizing activity in the receptor cell axons (component 2). This, in turn, leads to a hyperpolarizing postsynaptic potential in the dendrites of the ocellar nerve (component 3) which inhibits the spontaneous activity of the ocellar nerve fibers (component 4). The electrical activity of ocellar nerves has now been recorded in a variety of insects; all cases resemble the dragonfly in that spontaneous dark activity of the nerve is inhibited by light stimulation (Ruck, 1954; Hoyle, 1955; Ruck, 1957; Autrum and Metschl, 1961; Mimura et al., 1969).

One of the important questions left open by Ruck's analysis was whether the slow receptor potential contributes to synaptic transmission in the ocellus. The extracellular recordings from the photoreceptors showed an initial spike-like potential (component 2) superimposed on a sustained potential (component 1), but repetitive firing of impulse activity was not observed in the ocellar response evoked with long flashes. Such activity, if present, could have been masked to extracellular recording techniques by asynchronous firing of the receptor axons, and this was the interpretation favored by Ruck (1961). Indeed, Ruck (1961) argued that the sustained potential recorded from the dragonfly ocellus is incapable of initiating synaptic transmission in the eye.

In many invertebrate visual systems, only slow potential activity has been recorded from the receptors. Thus, it has been proposed that slow potentials are entirely responsible for mediating synaptic transmission at such photoreceptors and elsewhere (see, for example, Parry, 1947; Brooks and Eccles, 1947). Substantiation of such proposals has been difficult to obtain, however. Often subsequent studies have shown spikes in preparations where they were thought not to exist previously (Brock et al., 1962; Ruck, 1954; Hoyle, 1955; MacNichol and Love, 1960).

The present experiments were undertaken to record intracellular activity on both sides of the photoreceptor synapse in the dragonfly median ocellus, so as to examine the role of slow potentials at a synapse that has both spike and slow potential components presynaptically. We were encouraged to try such recordings by the anatomy of the median ocellus of the dragonfly which shows that there are two to three extraordinarily large nerve fibers (15–20 μ in diameter) among the 25–30 ocellar nerve fibers that innervate the 1500 or so photoreceptor cells (Cajal, 1918). The photoreceptor cells are themselves about 15 μ in diameter and have a length of 300–400 μ .

A second problem with which the present experiments became concerned is the relation between pre- and postsynaptic activity. How closely does postsynaptic activity follow presynaptic potential changes across an apparently simple synapse that involves just two elements? The extracellular recordings indicate that changes in membrane potential are of opposite sign in pre- and postsynaptic units during illumination, but beyond this, what alterations in the signal occur as it passes across the synapse?

This paper will describe the preparation, the intracellular activity of pre- and postsynaptic elements, and the effect of tetrodotoxin on pre- and postsynaptic activity. The following paper will describe the fine structure and organization of the synapses in the dragonfly ocellus and speculate on their function.

METHODS

Preparation During the summer, dragonflies were obtained by netting at local ponds and streams. To provide a supply of adult dragonflies during the winter, nymphs (Connecticut Valley Biological Supply Co., Southampton, Mass.) were raised to maturity in the laboratory (Chappell, 1970).

Receptor responses were recorded from nine dragonfly species: *Tramea carolina*, *Tramea lacerata*, *Libellula luctuosa*, *Plathemis lydia*, *Boyeria vinosa*, *Erythemis simplicicollis*, *Pantala flavescens*, *Anax junius*, and *Aeschna tuberculifera*. No differences were noted in the responses. Dissections were more easily accomplished on the larger species and this aided recording success. Of the recordings presented here, all extracellular records were obtained from *Plathemis lydia*. The record of receptor responses in the presence of tetrodotoxin shown in this paper was obtained from *Aeschna tuberculifera*. The stained cell shown in the receptor marking experiment is from *Tramea carolina*. All other records are from *Anax junius*.

Dissection The dragonfly median ocellus is located on the ventral side of the vertex which lies beneath the junction of the compound eyes (Fig. 1). It has a transparent convex crystalline lens the base of which is shaped roughly as an ellipse having a major axis of 1 mm and a minor axis of 0.5 mm. The photoreceptors are located just beneath the lens. For recording, the lens was left intact and a portion of the exoskeleton was removed from the front of the head for an approach perpendicular to the major axis of the receptors (Fig. 2). Ringer solution, isotonic with grasshopper embryonic cells (Carlson, 1946), bathed the ocellus. For the pharmacological experiments, tetrodotoxin at a concentration of 2×10^{-7} g/ml Ringer solution was applied to the exposed ocellus using a syringe driven by a micrometer.

Intracellular Recording Micropipettes for intracellular recording were made from Pyrex capillary tubing (Corning type 7740, Corning Glass Works, Corning, N.Y.) having a 1 mm outside diameter and 0.5 mm inside diameter, using a Livingston-type spring puller, modified by addition of a mechanical release. Tension, heat, and timing of release were adjusted to obtain electrodes having tip resistances of 70–140 M Ω when filled with 2 M KCl and measured in Ringer. Electrodes were filled either by heating to 85°C in 2 M KCl, boiling under vacuum, and repeating this process once; or by inserting a few fibers of glass wool into the capillary glass before pulling and injecting 2 M KCl into the pipettes with a syringe.

The microelectrode holder (Bioelectric Instruments Div., General Microwave Corp., Farmingdale, N.Y.) contained a chlorided silver wire in contact with the KCl solution of the pipette. The lead from this wire went to the probe of an ELSA-4 Bak wide-band electrometer amplifier (Electronics for Life Science, Inc., Rockville, Md.). A platinum-iridium wire immersed in the Ringer solution bathing the ocellus served as

reference electrode for most experiments. Identical recordings were obtained when a chlorided-silver wire was used for a reference electrode.

The amplifier fed directly into one channel of a Tektronix Type 502 Dual-Beam Oscilloscope (Tektronix, Inc., Beaverton, Ore.). The cathode follower stage of the vertical deflection plate of this channel of the scope was tapped and connected to an FM recording adaptor (Model 2D, A.R. Vetter Co., Rebersburg, Pa.) with a band-pass from DC to 1000 cps so that impulse activity as well as slow potential changes could be recorded on magnetic tape.

Extracellular Recording Extracellular records were made from the intact, breathing dragonfly in the manner commonly used for ocellar electroretinogram (ERG) recordings (Ruck, 1961). Uninsulated platinum-iridium electrodes were connected to a low level differential amplifier having a gain of 1000. The amplifier was AC coupled to the Tektronix oscilloscope. For the recordings reported here, the lens was impaled by the active electrode, while the reference electrode was located on the vertex.

Photostimulator A Sylvania tungsten halogen lamp (6.6 amp, 45 w, No. 58818-0, code No. FB-TH-1 quartz-iodide; General Telephone and Electronics Corp., Sylvania Electronic Systems, Mountain View, Calif.) was used as a light source. Two beams of equal intensity were brought to focus inside an electrostatic shield onto one end of a fiber optic bundle. The luminance at the other end of the 10 mm diameter fiber optic bundle, positioned 8 mm from the ocellar lens during an experiment, was measured using a direct reading photometer matched to the visual luminosity curve (Spectra Brightness Spot Meter, Photo Research Div., Kollmorgen Corp., Burbank, Calif.). This gave a corrected reading of 15.8 L for each beam at maximum intensity. This intensity is designated as $\log I = 0$ throughout the paper. The spot meter had been calibrated using a Macbeth Illuminometer (Leeds and Northrup Co., North Wales, Pa.). Intensity of the light beams was controlled using neutral density filters and a neutral density wedge.

Histology and Staining The methods used for the preparation of the light and electron micrographs are described in the following paper (Dowling and Chappell, 1972).

Intracellular staining was accomplished using microelectrodes filled by boiling with a saturated solution of both Niagara sky blue and methyl blue. Stain was ejected by pulsing the applied voltage of 45 v for 400 msec at 400-msec intervals (Miller and Dowling, 1970). Using this technique, sufficient amounts of dye could be injected without breaking the tip of the micropipette electrode. Thus, it was possible to record responses from the cells after completion of staining.

Upon completion of staining the preparation was allowed to sit for 1 hr. Before dissection, a few drops of fixative were applied to the preparation. After 2 or 3 min, the ocellar nerve was grasped near the brain with a pair of tweezers and the ocellus was gently worked away from the lens by pulling on the nerve. The nerve was then severed at the brain. The ocellus, with part of the ocellar nerve attached, was dropped into fixative and allowed to sit for 1 hr in an ice bath. The fixative used was a 2% OsO_4 solution in Ringer buffered to pH 4. After fixing, the tissue was dehydrated in

ethanol-water mixtures and embedded in soft plastic suitable for thick ($10\ \mu$) sections (Dowling and Werblin, 1969).

RESULTS

Receptor Structure

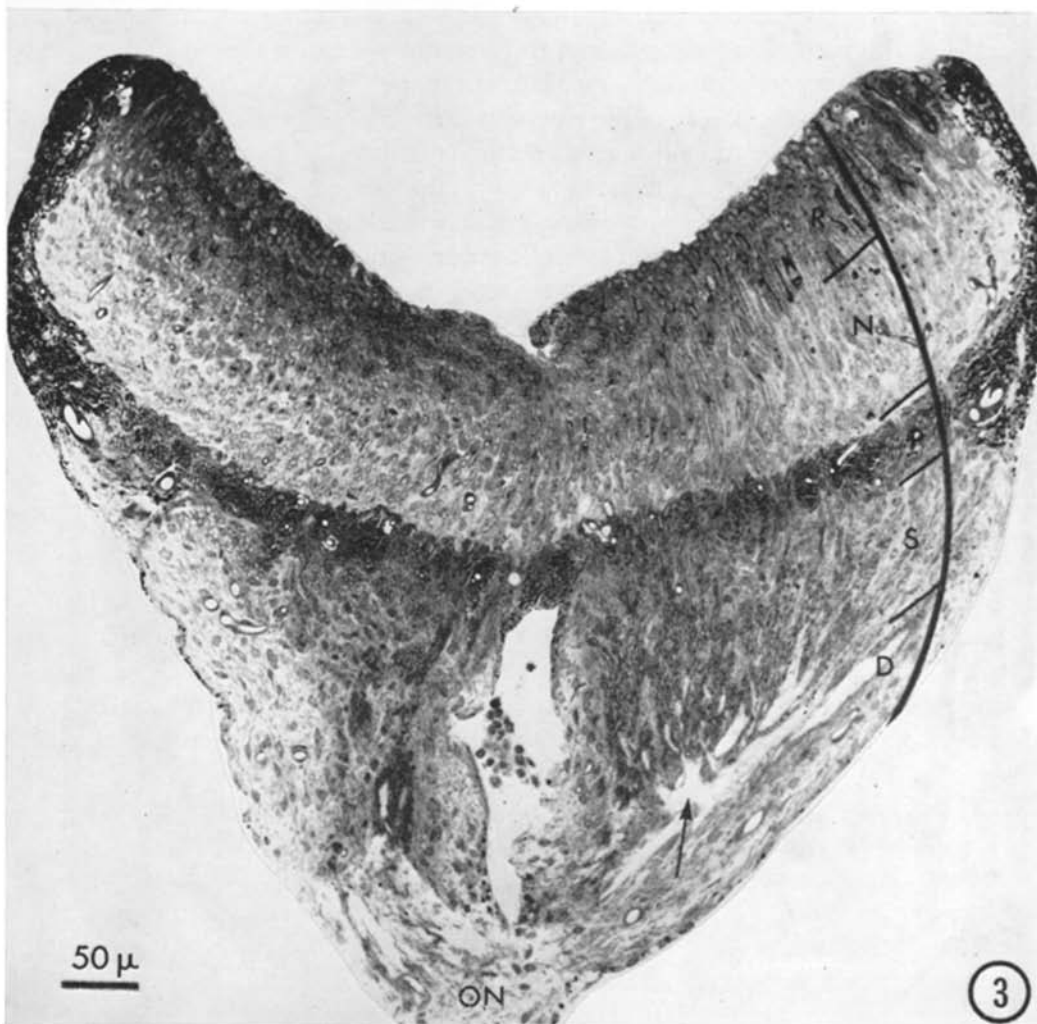
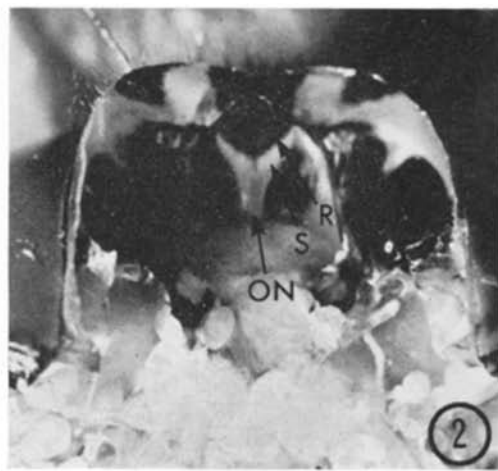
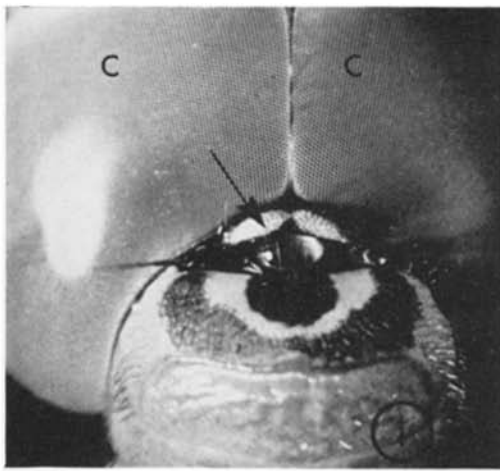
The over-all anatomy of the dragonfly medial ocellus is shown in Fig. 3 which is a light micrograph of a $10\ \mu$ section cut in the plane of Fig. 2. The rhabdomere-bearing regions of the receptors (*R*) are located distally in the ocellus just below the lens, which has been removed. The receptor cell nuclei (*N*) are located above a loosely pigmented region (*P*) through which receptor axons pass to reach the synaptic region (*S*). The larger dendrites (*D*) of the post-synaptic elements from the ocellar nerve (*ON*) can be seen branching as they traverse the base of the synaptic region (arrow).

A light micrograph of a section of the distal portion of the ocellus cut at right angles to the long axis of the receptors is shown in Fig. 4. The rhabdoms are densely staining, three-limbed structures and consist of the rhabdomeres of three retinular cells. An occasional atypical rhabdom is observed such as the one in the lower left corner which has six limbs (arrow).

Fig. 5 is an electron micrograph of three retinular cells (*R*₁, *R*₂, and *R*₃) which contribute rhabdomeres to one rhabdom complex. Each retinular cell contributes rhabdomeres to half of two limbs of the rhabdom complex. The rhabdomeres consist of tightly packed, parallel microvilli 500–600 Å in diameter. One limb of the rhabdom illustrated in the figure is cut so that the microvilli are seen in approximate cross-section (insert, Fig. 5); the microvilli of the other limbs are cut more in longitudinal section. At the inner junction of the three rhabdom limbs there is a small region where microvilli are absent.

The electron micrograph of Fig. 6 provides a high magnification view of the microvilli cut parallel to their axes. It is clear that while the microvilli from the two adjacent cells form an intricate, almost interdigitating structure, membrane integrity and separation are maintained at the tips of the microvilli where the two cells meet. Just beyond the distal-most microvilli, a desmosomal-like junction (*J*) is observed between the adjacent retinular cells (insert, Fig. 6). These junctions may serve to hold the retinular cells in close register.

Continuity of the cytoplasm in the microvilli with cell cytoplasm can occasionally be seen (thin arrows). At the base of many microvilli, however, there is a saclike dilation of the extracellular space (thick arrows). These sacs appear to pinch off partially the microvilli, and thus they often obscure the continuity of microvillus cytoplasm with cell cytoplasm. Such sacs at the base of microvilli are also seen in the rhabdom of the honey-bee drone (Perrelet and Bauman, 1969). Their role is not known, but they could serve



to isolate partially the microvilli from the rest of the cell or to provide a reservoir of extracellular fluid at the base of the microvillus.

The Retinular Cell Response

Retinular cells could be penetrated selectively by inserting microelectrodes into the most distal regions of the ocellus. Resting potentials of receptor cells were -35 to -45 mv. From receptors in a fresh preparation, intracellular responses could often be recorded for over half an hour.

Typical responses of a receptor cell to short (0.2 sec) light flashes of different intensities are shown in Fig. 7. Threshold for the response is at an intensity between 4 and 5 log units below the maximum intensity of the stimulator. At low intensities, a sustained depolarization is the basic feature of the response. Above $\log I = -3$, a spike is observed, but only at "on." The spike has a duration of 2 msec and does not overshoot zero membrane potential. With stimuli 3-4 log units above threshold a transient depolarizing wave is also seen between the "on" spike and the sustained depolarization. An unusual feature of the receptor response in the dragonfly ocellus is an oscillatory "off" wave, which is particularly prominent at low intensities.

The response of the retinular cells to sustained illumination (3-sec flashes) is shown in Fig. 8. The records illustrate more clearly some of the response features observed with the shorter flashes. For example, with long dim light flashes, the "off" oscillation is often the most prominent feature of the response. Also this figure illustrates that the sustained depolarization is maintained in the receptor response for the duration of the light flash. Finally, it should be noted that only a single "on" spike is seen in any of the records. We have found no evidence of repetitive firing in retinular cell recordings, re-

FIGURE 1. Dorsal view of the dragonfly head. The clear lens of the medial ocellus (arrow) is located between the antennae and in front of the vertex, a protruding region of the exoskeleton which is situated at the junction between the large compound eyes (*C*). The semicircular rings in front of the ocellus are the result of pigmentation in the exoskeleton of this species of dragonfly (*Anax junius*). $\times 10$.

FIGURE 2. Frontal view of the dissected dragonfly head ready for intracellular recording. The mandibles and frons have been removed to minimize movement of the musculature and provide access to the receptor (*R*) and synaptic (*S*) regions of the ocellus. The ocellar nerve (*ON*) runs from the synaptic region to the brain. Ringer solution was added to the natural cup around the ocellus provided by the dissection. $\times 10$.

FIGURE 3. Light micrograph of a $10\ \mu$ section of the ocellus cut in the plane of Fig. 2. The rhabdom region (*R*) of the receptors lies just beneath the lens which has been removed. The receptor cell nuclei are found in the region (*N*) just above a band of pigment cells (*P*) through which the receptor cell axons penetrate. In the synaptic region (*S*) the receptor axons synapse with dendrites (*D*) which have branched out from the ocellar nerve (*ON*). The arrow indicates one of the large ocellar nerve dendrites. $\times 200$.

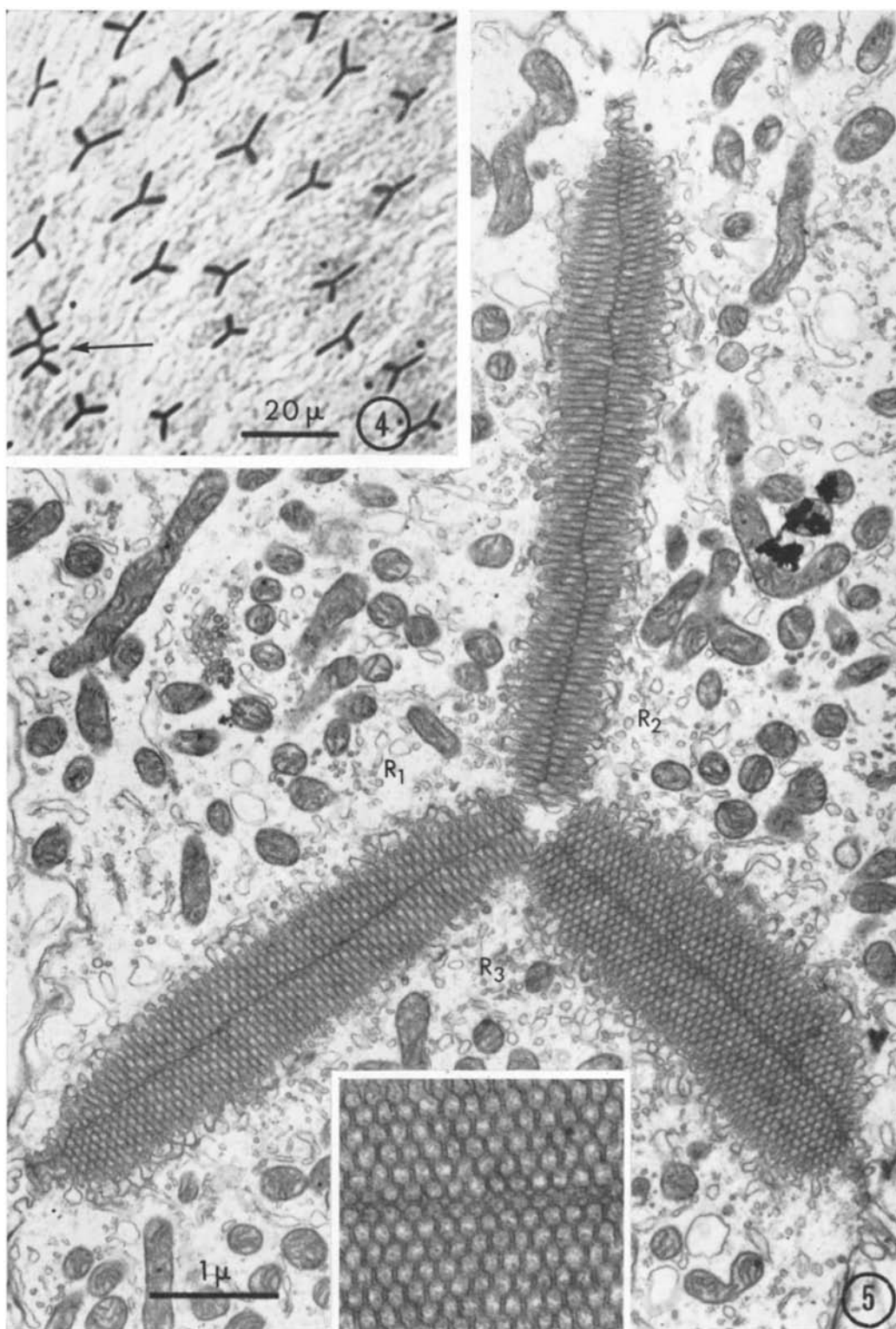


FIGURE 4. Light micrograph of the reticular cell region of the ocellus cut at right angles to the longitudinal axis of the receptors. The arrow indicates an atypical rhabdom having six limbs. $\times 1500$.

FIGURE 5. Electron micrograph of a rhabdom complex made up of rhabdomeres from three reticular cells (R_1 , R_2 , and R_3). A portion of one limb of the rhabdom, cut so that the microvilli are seen approximately in cross-section, is enlarged in the *insert*. $\times 20,000$; *insert*, $\times 50,000$.

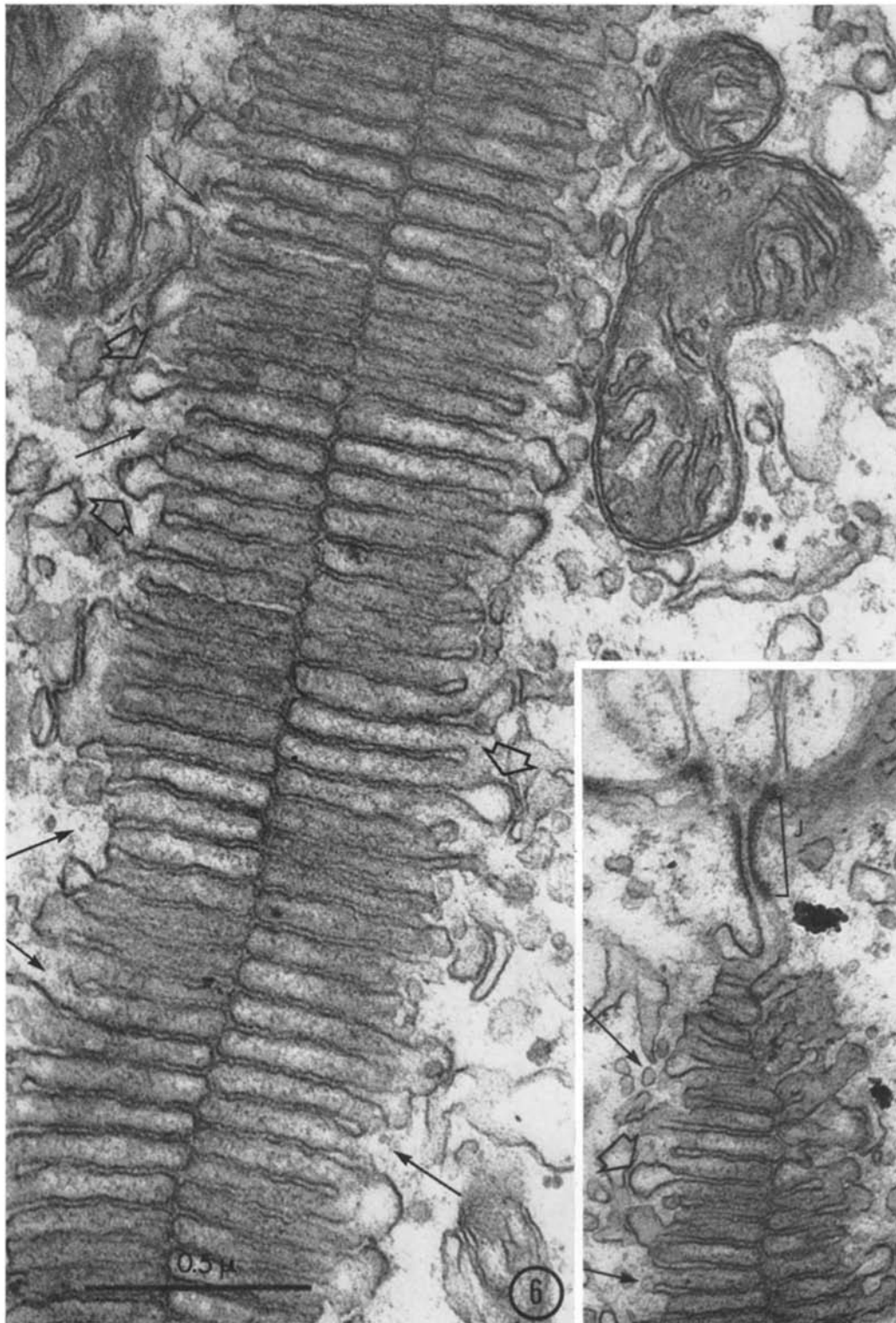


FIGURE 6. Microvilli cut parallel to their axes. Thin arrows indicate continuity between cell and microvillus cytoplasm; thick arrows point to the saclike dilatations observed at the base of many microvilli. The *insert* shows the desmosomal-like junctional complex (*J*) seen between adjacent reticular cells just beyond the distal-most microvilli. $\times 71,000$; *insert*, $\times 40,000$.

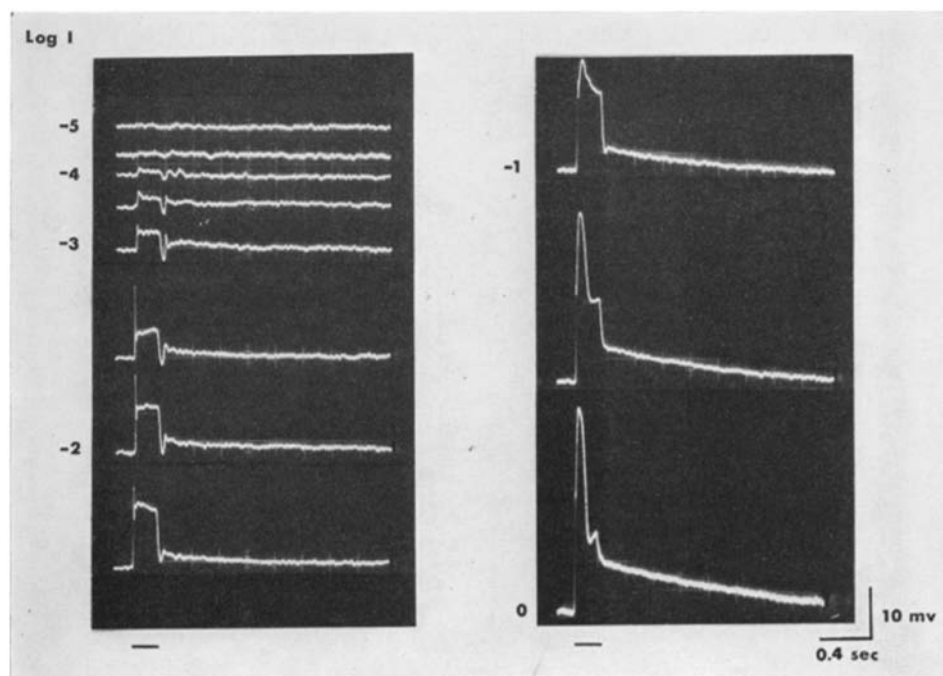


FIGURE 7. Receptor cell intracellular responses to short (0.2 sec) flashes. Intensity was increased in half log unit steps between consecutive traces. In this and subsequent figures an upward deflection indicates positivity of the active electrode, the log I values refer to the densities of neutral filter interposed in the test beam, and duration of illumination is indicated by horizontal bars beneath each column of responses.

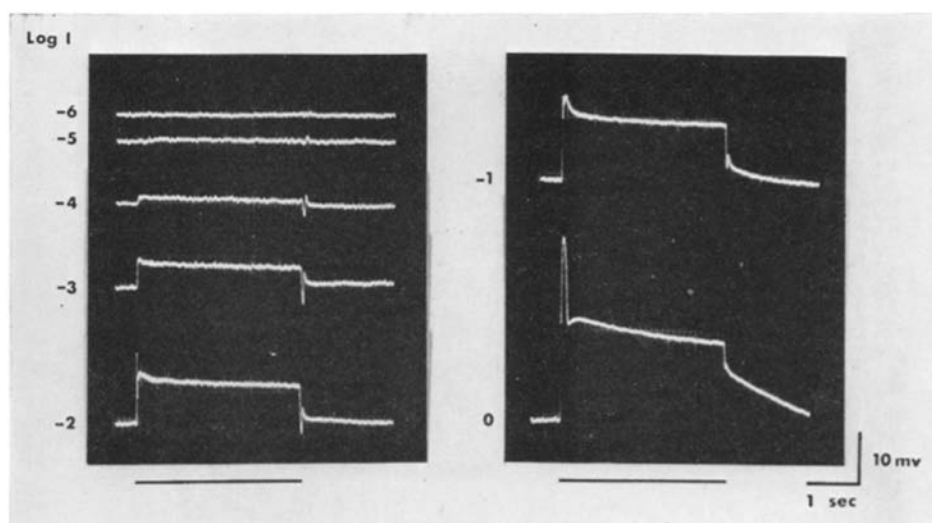


FIGURE 8. Receptor cell intracellular responses to sustained illumination (3-sec flashes).

ardless of stimulus or preparation conditions, with one exception. In that case, the normal response was observed as soon as the cell was penetrated and persisted as the electrode was advanced in $1\text{-}\mu$ steps through the cell for approximately $10\text{ }\mu$. After a further small advance of the pipette, however, repetitive firing was suddenly seen on top of the sustained depolarization. An additional $1\text{ }\mu$ step resulted in a sudden depolarization and loss of the

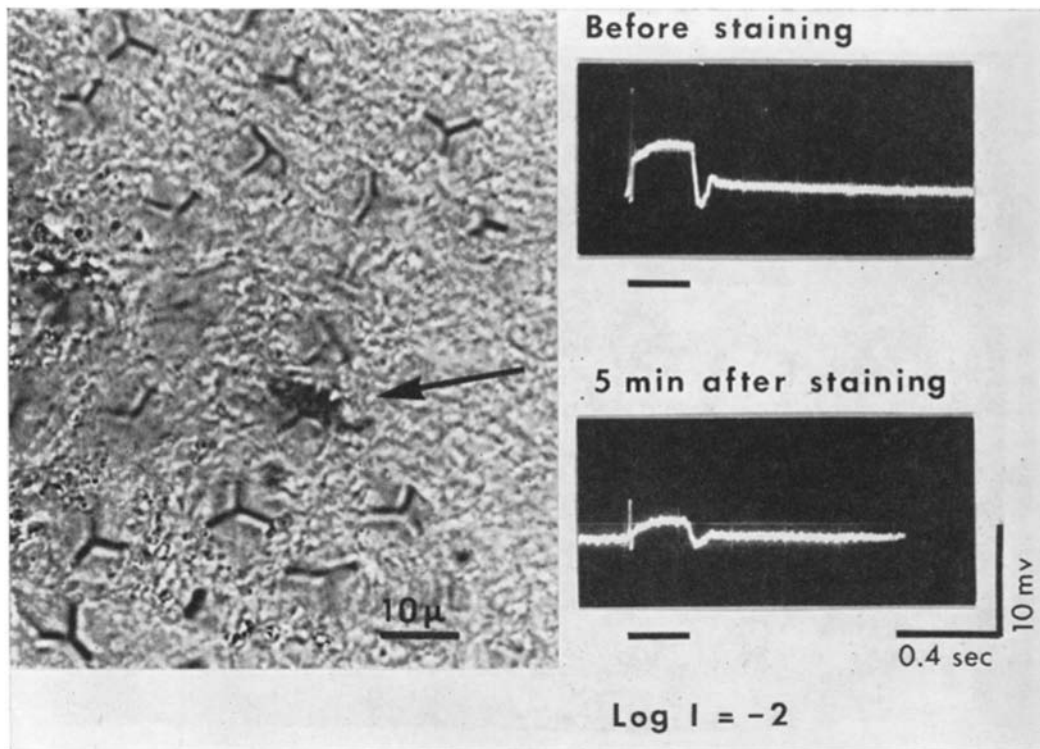


FIGURE 9. Light micrograph of a $10\text{ }\mu$ thick section showing a reticular cell stained by electrophoretic dye injection (arrow). The intracellular responses shown at the right were recorded before and 5 min after electrophoretic dye injection.

light response, indicating that the electrode had left the cell. It is probable, therefore, that the repetitive firing in this case was the result of injury to the receptor cell membrane. A similar suggestion was made by Bauman (1968) to explain repetitive firing of impulses in the reticular cell of the honeybee drone during illumination. This will be discussed further below.

Evidence that these depolarizing responses recorded from the distal regions of the ocellus originate in the reticular cells is given in Fig. 9, which shows typical photoreceptor responses before and 5 min after electrophoretic ejection of stain into a cell, along with a histological section of the stained cell.

Although the response of the cell after staining did not reach its original magnitude, probably due to the trauma of the staining process, its basic characteristics are similar to the original response. This provides evidence that the electrode remained inside the cell throughout the period of electrophoretic dye injection. The stain (arrow) is confined to one of three reticular cells making up a typical ocellar unit.

Postsynaptic Activity

EXTRACELLULAR RESPONSES Records of impulse activity in the ocellar nerve are shown in Fig. 10. In the dark, the ocellar nerve is spontaneously

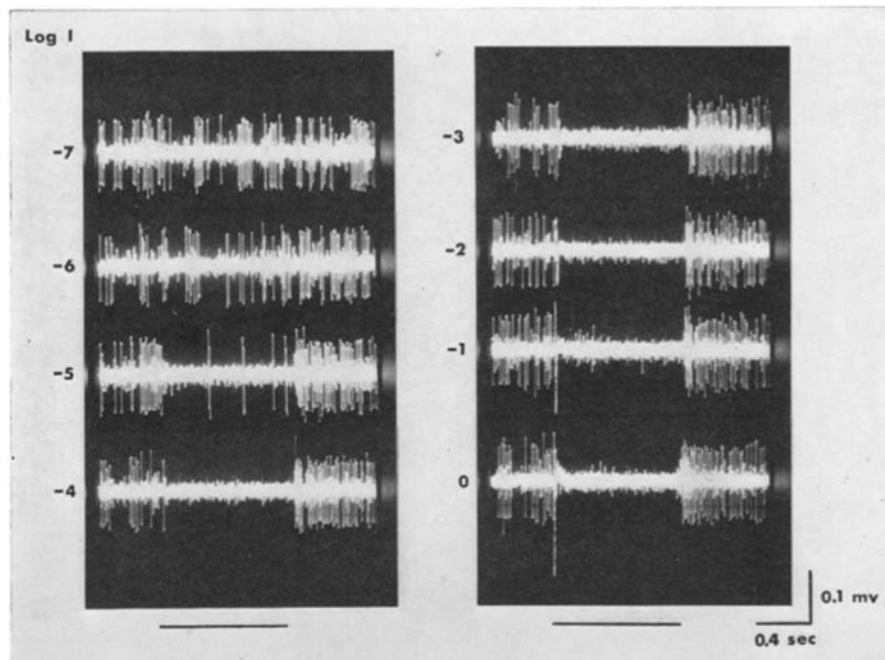


FIGURE 10. Ocellar nerve activity recorded extracellularly from the intact, breathing dragonfly. The large downward spikelike transient seen just after the light is turned on at $\log I = 1$ and 0 is an artefact, representing AC-coupled ERG activity.

active. Activity recorded in the dark appears the same as that shown in this figure for the low intensity flash of $\log I = -7$ and consists of a steady arrhythmic discharge. The variations in spike height seen in this record suggest that impulses were being recorded from three or four different ocellar nerve fibers. The smaller impulses which persist even during intense illumination represent background activity, possibly from neurons in the antennae which are located nearby.

In response to light, there is a sustained inhibition of spontaneous activity.

At the termination of the light stimulus, a vigorous burst of impulses is usually seen. Partial inhibition of the spontaneous activity is observed with light intensities as low as $\log I = -6$; complete inhibition is observed in response to stimuli 2 or more log units more intense.

INTRACELLULAR RESPONSES To record intracellularly from the post-synaptic elements, the micropipettes were directed into the proximal regions of the ocellus (layers marked *S* and *D* in Fig. 3). Usually only graded hyperpolarizing responses to light were recorded after penetration of a cell membrane in this region of the ocellus. Resting potentials of such units were -30 to -40 mv. On a few occasions, responses showing both spontaneous impulse activity and graded hyperpolarizing potentials during the period of illumination were obtained for a short period of time. An example is shown in Fig. 11. The light-evoked response consisted of an initial sharp hyperpolarization followed by a partial return toward the original membrane potential and

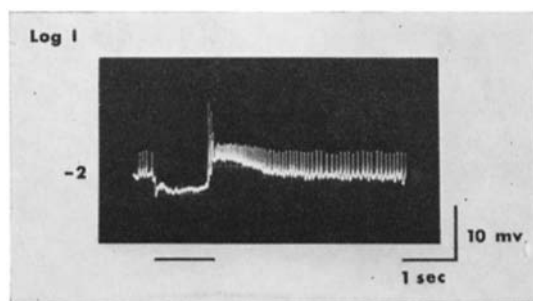


FIGURE 11. Intracellular response from postsynaptic unit showing impulse activity.

then a maintained hyperpolarization. For the duration of the light stimulus, spike firing was inhibited. At "off" the potential in the cell rebounded above the original dark membrane potential and the impulse firing rate increased above and then decreased toward the original base potential.

More often, however, recordings were made from units which hyperpolarized in response to light but did not show impulse activity. Although impulses were sometimes recorded initially, they would disappear without any noticeable change in resting membrane potential. However, the graded hyperpolarizing response could often be recorded for periods of 10–20 min and was stable over this period of time. A typical series of hyperpolarizing responses is shown in Fig. 12. 7 log units below the maximum stimulating intensity, discrete hyperpolarizing potentials are observed during the period of illumination. Such potentials are also seen occurring spontaneously in the dark (arrow, $\log I = -7$); dim lights simply increase their frequency. At slightly higher intensities, apparent summation of discrete events results in a sus-

tained hyperpolarization having noticeable fluctuations for the duration of the light period. At still higher intensities, there is observed a large transient hyperpolarization followed by a small sustained hyperpolarization having few fluctuations. At "off" at all intensities, the membrane potential shows a transient depolarization that overshoots and then returns relatively slowly to the original resting potential, taking several seconds at the highest intensities.

The discrete potentials observed in these units in the dark and in response

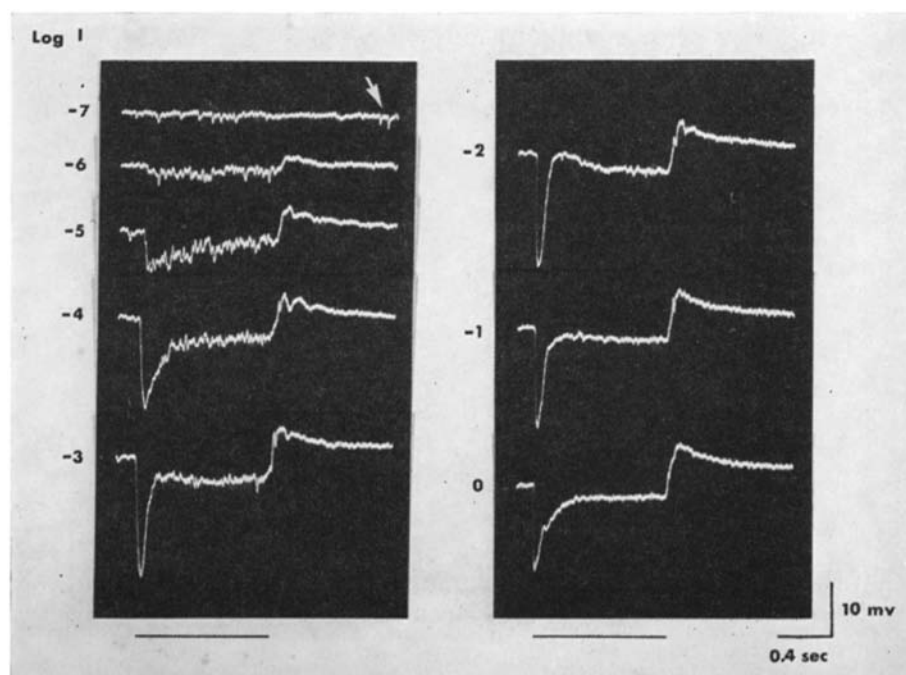


FIGURE 12. Intracellular slow potential responses from a postsynaptic unit in absence of impulse activity.

to dim lights appear similar to inhibitory postsynaptic potentials (IPSP's) seen at a variety of inhibitory synapses (Grundfest and Reuben, 1961; Eccles, 1964) and suggest that the postsynaptic potentials in the dragonfly ocellus result from the action of a hyperpolarizing neurotransmitter released by the receptors.

Comparison of Pre- and Postsynaptic Activity

LATENCY "On" latency measurements from the responses of four receptor cells are shown in the insert of Fig. 13. Lines connecting the data points for each receptor show that in every case latency of response decreases as the stimulus intensity increases, and that latency of each receptor maintains the

same relationship to the others at any given intensity. The variation in latency between receptors at a given incident light intensity is probably caused by differences in illumination of individual receptors. That is, some receptors by virtue of position and orientation receive more or less light than others at any given stimulus intensity.

To compare latency data from receptors with postsynaptic elements, it was assumed that every receptor responds equally when absorbing a similar

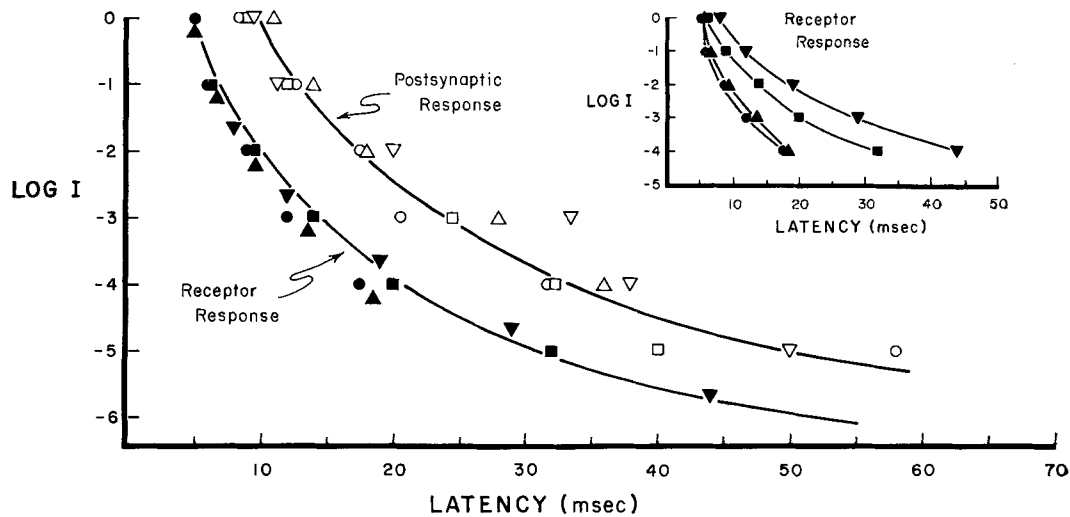


FIGURE 13. Latency of pre- and postsynaptic responses. "On" latency measurement data from the responses of four receptor cells are shown in the *insert*. Lines in the insert connect data points for individual receptors. The data for the three longer-latency receptors were shifted down the $\log I$ axis by increments of -0.2 , -1 , and -1.65 log units, respectively, until the shortest latency point of each curve fell on the curve of the receptor showing the shortest latency. The data was then replotted (filled symbols) to establish the lower curve of the figure. Latency data for the hyperpolarizing "on" response of four postsynaptic units are plotted (open symbols) along the upper curve.

number of quanta. Therefore the curves from the insert were shifted along the $\log I$ axis until the shortest latency point of each curve fell on the curve of the receptor showing the shortest latency (i.e., the receptor in the most favorable position to absorb light). This resulted in the excellent fit of receptor latency data shown in the lower curve of Fig. 13.

Latencies of the hyperpolarizing responses of four postsynaptic units at various stimulus intensities are also plotted in Fig. 13 (upper curve). Since the postsynaptic elements summate input from many receptors, probably widely spread in the ocellus, little difference in latency data was observed between units. A comparison of latencies between pre- and postsynaptic responses shows differences of 5–20 msec. This compares closely with latency

differences between pre- and postsynaptic responses in the locust compound eye (Shaw, 1968).

INTENSITY-RESPONSE RELATIONSHIPS Fig. 14 compares intensity-response relationships for the receptor and postsynaptic responses. Data for both the initial transient waves and sustained components of the responses are given.

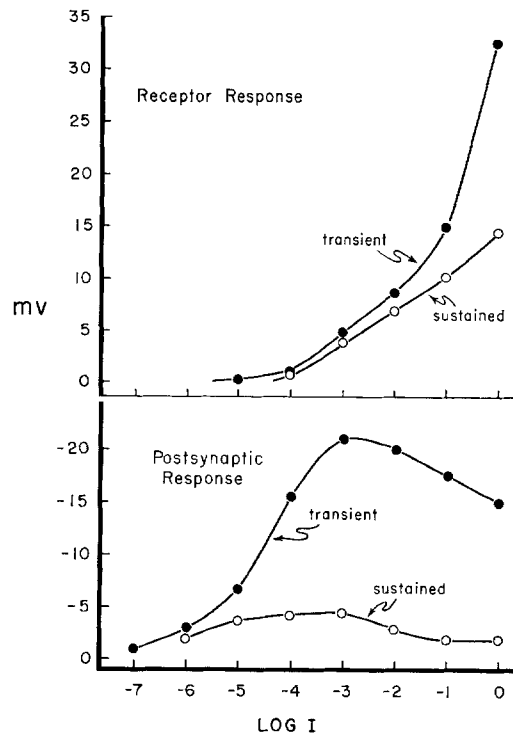


FIGURE 14. Intensity-response relationships for receptor and postsynaptic units. The amplitude of the peak of the transient wave and the sustained component of the receptor response (Fig. 8) and the postsynaptic response (Fig. 12) are plotted as function of intensity. The sustained component of the receptor response was measured 3 sec after the start of illumination.

The single impulse observed in the receptor response increases very little with intensity after it first appears and is not plotted here.

These data illustrate graphically two of the most significant differences between pre- and postsynaptic activity. First, the postsynaptic activity appears more sensitive to light by 1–2 log units. This probably reflects convergence of up to 100 or so receptors onto a single postsynaptic element. In addition to the enhancement of sensitivity, both transient and sustained phases of the postsynaptic response reach maximum amplitude at about $\log I = -3$, and decline in amplitude at higher intensities. For the receptors,

on the other hand, both transient and sustained phases of the response show no sign of saturation even at $\log I = 0$.

The second difference between pre- and postsynaptic activity which may be seen clearly in Fig. 14, as well as in Figs. 8 and 12, is that there is considerably less sustained potential relative to the initial transient wave in the postsynaptic response as compared with presynaptic activity. Especially at the higher stimulating intensities the sustained component of the postsynaptic

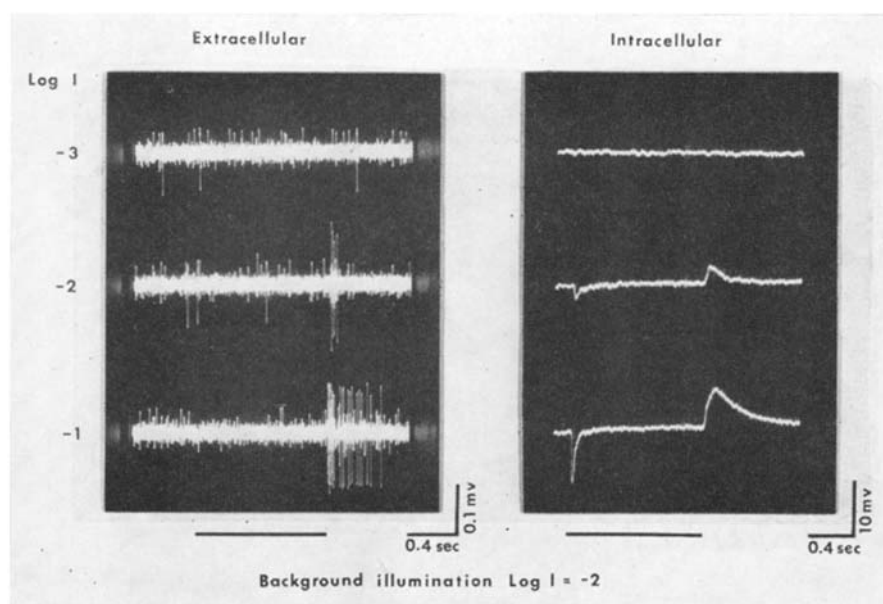


FIGURE 15. Postsynaptic activity in response to incremental illumination. The responses on the left were recorded extracellularly from an intact, breathing dragonfly. The responses shown on the right were recorded intracellularly from postsynaptic units in a different preparation under the same conditions of illumination. Flashes having intensities indicated at the left were superposed upon a steady illumination of $\log I = -2$.

response is very small compared to the initial hyperpolarization. Also, after the light is extinguished there is a prominent transient depolarizing potential in the postsynaptic response. Thus the striking features of the postsynaptic response, especially at the higher stimulating intensities, are the transient "on" and "off" responses.

With incremental stimuli superimposed on steady background illumination, postsynaptic activity is entirely transient in nature. An example of an extracellular response to one such condition of stimulation is shown on the left side of Fig. 15. The background illumination ($\log I = -2$) was turned on a few seconds before presenting increment light flashes, and it silenced most of the spontaneous activity. A flash of intensity 1 log unit below the

intensity of the background illumination elicited no response. However, a flash of the same intensity as the background illumination elicited a brief burst at "off" while a flash 1 log unit more intense elicited a more vigorous burst of impulses at "off." Similar increment responses were investigated against background illumination from the brightest intensity available ($\log I = 0$) down to an illumination of $\log I = -5$, which was just sufficient to provide total inhibition of the spontaneous impulses. In all cases an additional flash of the same intensity as the background illumination was just sufficient to elicit an "off" response in the ocellar nerve. Thus $\Delta I/I$ for the "off" threshold is nearly constant over five decades of background intensity.

Intracellular responses from hyperpolarizing units in the synaptic region were investigated under similar conditions of background illumination (Fig.

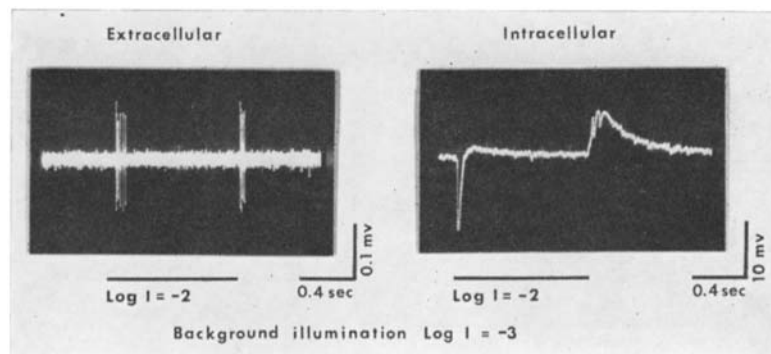


FIGURE 16. Postsynaptic response to incremental illumination showing "on" and "off" bursts of impulses. The experiment is similar to that of Fig. 15 and is described in greater detail in the text. The rebound after the "on" transient shown in the intracellular records rises to within 1 mv of the dark-adapted resting potential.

15). Flashes were superposed on background levels of illumination ranging from $\log I = -4$ to $\log I = 0$. In all cases, a flash at an intensity 1 log unit below that of the background illumination evoked no response. A flash of the same intensity as the background illumination evoked an initial transient hyperpolarization and a depolarizing "off" response, but no sustained potential was observed during the increment flash. A flash having an intensity 1 log unit above that of the sustained illumination evoked larger transient responses at "on" and "off," as shown in the bottom trace on the right of Fig. 15, but again no sustained component was observed. Under these conditions of illumination, therefore, both the intracellular responses from hyperpolarizing units in the synaptic region and responses recorded extracellularly from the ocellar nerve fibers are only transient events. On the other hand, under similar conditions of illumination, the receptor intracellular responses always show a sustained depolarization for the duration of incremental illumination.

Under certain conditions of illumination, it is possible to obtain both "on" and "off" bursts of impulses from the ocellar nerve. An example is shown in Fig. 16. With sustained illumination of $\log I = -3$, a flash of $\log I = -2$ evoked bursts of impulses at both "on" and "off." Intracellular records made under the same conditions of illumination are shown on the right. After the initial transient hyperpolarization, the membrane potential rebounded transiently *above* the level maintained during background illumination and approached the dark-adapted membrane potential. This rebound to the dark-adapted membrane potential following the initial transient hyperpolarization probably accounts for the transient discharge of impulses at "on" of the light.

Effects of Tetrodotoxin

When recording intracellularly from the ocellar receptors, only a single impulse at "on" is usually observed. It is possible, however, that the failure to record additional spikes is caused by damage to the reticular cell during micropipette penetration. Whether impulse activity in the receptor axons is necessary for the transfer of information across the photoreceptor synapse can be tested by applying tetrodotoxin to the eye which blocks impulse activity in the receptor while leaving the slow potential intact. Tetrodotoxin, which has been used in a variety of experimental preparations to block nerve impulses without appearing to affect either slow potential generation or synaptic transmission (see, for example, Lowenstein et al., 1963), rapidly eliminates impulse activity in the ocellar nerve of dragonfly (Gallin and Chappell, unpublished observations).

Fig. 17 shows the results of an experiment in which tetrodotoxin at a concentration of 2×10^{-7} g/ml Ringer solution was applied to the preparation. The records on the left are typical intracellular receptor responses and served as controls. 3 min after application of the tetrodotoxin to the eye, the dramatic change observed in the receptor response is the severe reduction in amplitude of the initial spike. With further time in most preparations the initial spike entirely disappears (see Fig. 13 of Dowling and Chappell, 1972), except at the brightest intensity ($\log I = 0$), where a slowed, small, spikelike potential can still be seen on the leading edge of the receptor potential. In the experiment illustrated in Fig. 17, the "off" oscillation appeared to decrease somewhat as a result of tetrodotoxin application to the eye. In four other experiments, however, the "off" oscillation completely survived tetrodotoxin while the initial impulses were blocked (see Fig. 13 of Dowling and Chappell, 1972).

Similar experiments were repeated while recording from hyperpolarizing units of the synaptic region. No significant alterations of the responses were seen even 15 min after application of tetrodotoxin (Fig. 18). The observed potentials exhibited the characteristics of typical postsynaptic responses at all stimulus intensities, including the initial, large transient hyperpolarizing

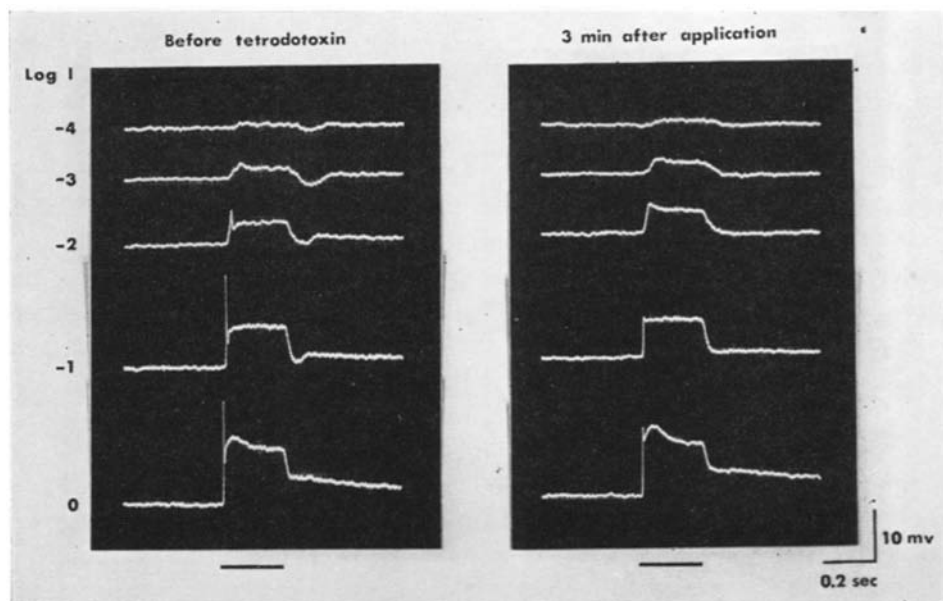


FIGURE 17. Receptor responses before and after application of tetrodotoxin.

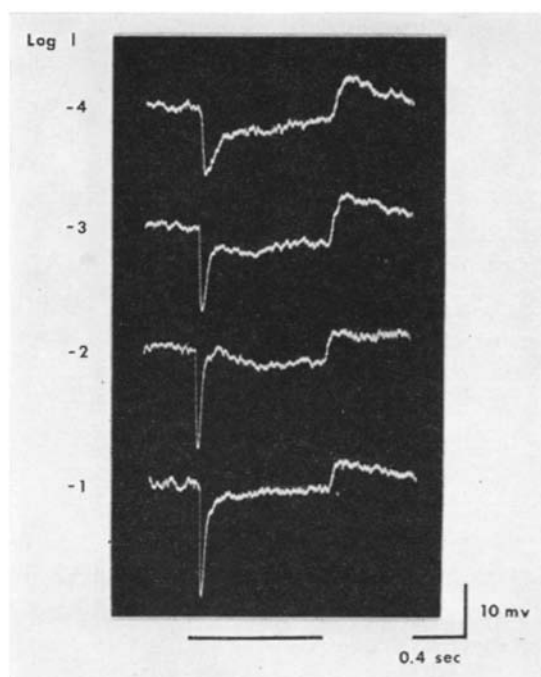


FIGURE 18. Responses of a postsynaptic unit 15 min after tetrodotoxin application. No alterations in slow potential postsynaptic activity were noted after tetrodotoxin at a concentration of 2×10^{-7} g/ml Ringer solution was applied.

wave, the small sustained component, and the depolarizing "off" rebound. These experiments indicate, therefore, that presynaptic spike activity is not required, or even at all responsible, for synaptic transmission in the dragonfly median ocellus. They leave open, however, the question of what the function of the presynaptic spike might be and why it does not have any apparent effect on synaptic transmission in the eye.

DISCUSSION

Receptor Responses

The activity recorded intracellularly from the photoreceptors in the dragonfly ocellus can be discussed in terms of the components of the extracellularly recorded receptor response described by Ruck (1961). Ruck's component 1 corresponds to the sustained depolarizing potential which first appears at the lowest intensities at which a response can be detected. At high stimulating intensities, an additional transient wave occurs shortly after light is turned on, preceding the sustained depolarization. These components of the response, referred to here as the slow potential, are typical of potentials recorded intracellularly from photoreceptors of many invertebrates. For example, similar responses have been recorded in the lateral eye of the horseshoe crab *Limulus* (MacNichol, 1956); in the compound eye of the blowfly (Burkhardt and Autrum, 1960); the housefly (Scholes, 1969), the damselfly (Naka, 1961), the dragonfly (Fuortes, 1963), the worker honeybee (Naka and Eguchi, 1962), and the locust (Scholes, 1964, 1965); as well as from ocelli of the barnacle (Gwilliam, 1963) and the wolf spider (DeVoe, 1972). In the housefly *Musca* similar responses have been recorded extracellularly from compound eyes abnormally developed in the abdomen and lacking second-order neurons (Eichenbaum and Goldsmith, 1968).

Component 2 of the extracellular response (Ruck, 1961) corresponds to the single spike which is evident at "on" in the intracellular recordings at intensities above $\log I = -3$. Such "on" impulses have not been observed commonly in intracellular receptor responses from other preparations. A similar spike is seen in recordings from reticular cells in the compound eye of the drone honeybee (Naka and Eguchi, 1962; Bauman, 1968), but in this eye there may also be a train of spikes fired during the sustained component of the photoreceptor response. Bauman (1968) reports, however, that trains of spikes were recorded in only five out of over 1000 honeybee drone reticular cells examined and then only under unusual experimental conditions such as toward the end of a long experiment, during impalement, when the preparation was drying out, or at the beginning of a response in strongly light-adapted preparations. Otherwise, only a single spike at "on" is observed, which agrees closely with our observations.

The unique feature of the intracellular receptor response from the dragon-

fly medial ocellus is the "off" oscillation. Such oscillatory activity at the cessation of light stimulation has not been described in receptor responses of other invertebrates, although records showing hints of such responses have been published (Alawi and Pak, 1971). Speculation concerning the origin of this phenomenon will be presented in the second paper of this series (Dowling and Chappell, 1972).

Role of Impulses in Synaptic Transmission

The results presented in Fig. 17 show that the initial spike of the receptor response in the dragonfly ocellus is blocked by tetrodotoxin, while the sustained generator potential is not affected. This implies that two separate processes are involved in generating these components of the receptor response. Similar results were reported by Bauman (1968) who applied tetrodotoxin to the honeybee drone compound eye while recording intracellularly from receptors. The initial spikelike component of the receptor response was abolished while the rest of the response remained intact. In the *Limulus* lateral eye attenuation of the transient component of the slow potential by tetrodotoxin has been reported in eccentric cell recordings (Benolken and Russell, 1965), but a more recent study found only the eccentric cell impulses to be affected (Dowling, 1968), in agreement with the results from receptors in the dragonfly ocellus.

Evidence for the survival of synaptic transmission in the presence of tetrodotoxin is already available. For example, there is no decrease in the response of frog sartorius muscle to external application of acetylcholine in the presence of tetrodotoxin (Furukawa et al., 1959). Also, in the frog sartorius muscle preparation, it has been shown that short pulses of depolarizing current applied to motor nerve terminals can elicit small end-plate potentials (Katz and Miledi, 1965). The presence of miniature end-plate potentials indicating spontaneous release of transmitter at rat motor nerve terminals in the presence of tetrodotoxin has likewise been demonstrated (Elmqvist and Feldman, 1965). And, finally, release of transmitter in response to depolarizing pre-synaptic current in the presence of sufficient tetrodotoxin to eliminate action potentials was shown at the squid giant synapse (Bloedel et al., 1966).

In postsynaptic units in the dragonfly ocellus it was found that, even 15 min after application of tetrodotoxin to the eye, no changes occur in the basic characteristics of the hyperpolarizing responses elicited by light. This observation confirms that synaptic transmission can occur in the presence of tetrodotoxin and indicates that regenerative spike activity in receptor axons is not the process by which synaptic transmission is mediated in the dragonfly ocellus. It appears rather that the receptor slow potential, which survives the application of tetrodotoxin, is directly responsible for transmission of information to the synapse and for the initiation of the synaptic activity which results in the postsynaptic hyperpolarizing response.

In other preparations, evidence for slow potentials mediating synaptic transmission has been presented. For example, a thorough investigation of the barnacle eye carried out to evaluate the question of impulse activity in its receptors was reported by Gwilliam (1963, 1965). Neither intra- nor extracellular recordings provided evidence of impulse activity in the receptors or their axons, although impulses associated with an "off" response were readily recorded from the supraesophageal ganglion. In addition, Gwilliam was able to block the postsynaptic impulse activity by application of procaine to the supraesophageal ganglion, while application to the receptors and their axons had no effect. Thus Gwilliam suggested that slow potentials may be the mechanism by which information travels up to 4 mm along the photoreceptor axons to the supraesophageal ganglion of the barnacle.

Slow potential activity from receptors has been recorded in the first synaptic layer of several insect visual systems. Bauman (1968), for example, reported recording a graded potential typical of the receptor response and its associated initial spike in the first synaptic region of compound eye of the honeybee drone. More recently, Jarvilehto and Zettler (1970) have unequivocally identified the recording sites of slow potential responses recorded at the base of the lamina in the blowfly compound eye to be retinula cell axons. After staining the axon from which a response had been recorded, they also visualized the location of the electrode tip in a freeze-dried preparation. These experiments show clearly that receptor slow potentials reach the receptor synapses.

In the vertebrate retina, the distal neurons (receptor, horizontal, and bipolar cells) have been found to generate only slow, graded potentials in response to retinal illumination (Werblin and Dowling, 1969; Kaneko, 1970). Thus, all the synaptic interactions of the outer plexiform layer of the vertebrate retina appear to be mediated by slow potentials. In support of this, Murakami and Shigematsu (1970) have reported that graded postsynaptic potentials can be recorded in ganglion cells of the frog retina in the presence of tetrodotoxin in a concentration sufficient to block impulse activity. Hence, in the vertebrate visual system, the slow, graded potentials appear to be capable of initiating postsynaptic responses as far centrally as the inner plexiform layer.

Postsynaptic Responses

The extracellular recordings described here confirm in an intact nerve preparation what Ruck (1961) reported for a preparation in which the ocellar nerve was severed near the brain and held in forceps electrodes. The two studies agree in that inhibition of impulse activity observed during the period of illumination is followed by a burst of impulses at a higher frequency than the original spontaneous activity when the light stimulus is terminated.

Intracellular penetrations in the postsynaptic region of the dragonfly

ocellus have shown graded hyperpolarizing responses for the duration of a light flash, followed by a transient depolarization of the unit at "off." Shaw (1968) reported similar responses while recording intracellularly in the first synaptic region (the lamina) of the locust compound eye.

In the dragonfly ocellus, it has not been possible to record impulse activity long enough in the intracellular recordings from postsynaptic units to investigate the relation between impulses and the slow potential changes under various conditions of illumination. Shaw (1968) experienced similar difficulties in the locust eye. The lability of impulses in such intracellular recordings is not an uncommon problem. For example, McReynolds and Gorman (1970) experienced difficulty in recording intracellular impulse activity in the distal receptor cells in the scallop eye, even though a slow, graded hyperpolarizing response could be recorded for a substantial period, as we have found in the dragonfly ocellus. The reason why impulses do not last after penetration is open to speculation, but presumably involves cell damage.

Recently, Autrum et al. (1970) reported intracellular responses from a monopolar neuron identified by stain in the lamina of the compound eye of the blowfly, *Calliphora*. Such monopolar neurons have been shown anatomically to be postsynaptic to receptor axons. Over the first 4 or 5 log units of intensity, the responses from the monopolar neuron of the blowfly eye are similar to the postsynaptic hyperpolarizing responses from the dragonfly ocellus or locust lamina (Shaw, 1968). At higher intensities, however, the response from the blowfly lamina during the period of illumination shows a depolarization above the original resting potential following the initial hyperpolarization. This depolarization was explained by Autrum et al. (1970) as being the result of electrotonic spread of the retinular cell response, and not an active part of the monopolar neuron response. They reported that spike activity was not observed in the monopolar neurons, although they suggest that such neurons produce a spontaneous spike activity which is suppressed by the hyperpolarizing postsynaptic potential. Spike potentials have been recorded extracellularly from fibers believed to be en route to the second optic ganglion of the fly *Phaenicia sericata* (Arnett, 1971). Two types of responses have been described. One shows an "on" center, "off" surround type of response; the other gives transient bursts of impulses at "on" and "off" of illumination.

Function of the Ocellar Synapse

A comparison of pre- and postsynaptic potentials in the dragonfly ocellus shows three striking alterations in the light-evoked responses which are, presumably, a result of transmission across the synapse. First, the postsynaptic potentials are of opposite polarity as compared with the presynaptic potentials. Second, the relative sensitivity to light appears to be enhanced in the post-

synaptic units when compared with the presynaptic cells. And third, the postsynaptic potentials are more phasic in nature than are the presynaptic potentials, demonstrating prominent "on" and "off" transients.

It is likely that the polarity reversal occurs across the ocellar synapse because the receptor synapses release a transmitter which hyperpolarizes the postsynaptic element. Indeed, the observation of discrete IPSP-like potentials in the postsynaptic element whose frequency is intensity dependent is consistent with this notion. The apparent increase in sensitivity of the postsynaptic units, on the other hand, may simply result from summation of inputs from many receptors onto the relatively few postsynaptic elements. The anatomy of the dragonfly ocellus which shows that some 1500 receptors are present in an eye having only 20–30 ocellar nerve fibers provides evidence that there is considerable convergence in the eye (Cajal, 1918).

Reasons why the postsynaptic activity in the ocellus is more transient in nature than presynaptic activity and how the transient depolarization at "off" is evoked are not so easily suggested. The failure of tetrodotoxin to alter the postsynaptic potentials in the eye eliminates the possibility that the large initial transient in the hyperpolarizing response is related to the "on" impulse in the receptor response, for the receptor spike is rapidly lost in the presence of tetrodotoxin. All of the present evidence suggests that the slow potential part of the receptor response is responsible for synaptic transmission in the ocellus. Also the presynaptic counterpart for the prominent, transient depolarization at "off" observed in the post-synaptic response has not been identified. The oscillatory potential which dips below the dark resting potential of the receptor momentarily at "off" is one obvious candidate, although it exhibits a much shorter time-course than the postsynaptic "off" response.

In many visual systems, transient responses at "off" of illumination are observed, and such responses are often believed to result from the complex interplay of excitatory and inhibitory influences upon pre- and postsynaptic elements. In the *Limulus* visual system, for example, Hartline and his colleagues have shown this to occur in the eccentric cell as a result of lateral and self-inhibitory circuits in that eye, and have further demonstrated that the "on" activity of the cell is considerably more transient as a result of these inhibitory interactions (Hartline et al., 1961; Ratliff, 1961). In the following paper, we provide anatomical evidence for the existence of both lateral and feedback synapses in the dragonfly ocellus, and we suggest there that the transient nature of the postsynaptic response and the generation of the "off" response may result from the interplay of such synaptic interactions in the ocellus.

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