J. Exp. Biol. (1974), 60, 135–160
With 1 plate and 16 text-figures
Printed in Great Britain

A STUDY OF THE AFFERENT PATHWAYS OF THE DRAGONFLY LATERAL OCELLUS FROM EXTRACELLULARLY RECORDED SPIKE DISCHARGES

BY B. L. ROSSER*

Department of Zoology, University of Bristol, Woodland Road, Bristol, England

(*Received 20 June* 1973)

INTRODUCTION

A number of extracellular electrophysiological studies of the dorsal ocelli of several insect species have been published (Parry, 1947; Hoyle, 1955; Burtt & Catton, 1956; Ruck, 1957; 1958*a*, *b*; 1961*a*, *b*, *c*; 1966; Goldsmith & Ruck, 1958; Metschl, 1963) and are reviewed by Goodman (1970). Recently, an intracellular study has appeared (Chappell & Dowling, 1972). There is therefore a considerable body of evidence on ocellar physiology in addition to the anatomical data from electron-microscopy (Ruck & Edwards, 1964; Goodman, 1970; Toh, Tominga & Kuwabara, 1971; Dowling & Chappell, 1972). Ruck (1961*a*) has attempted to organize some of this data into a model of ocellar functioning, taking into account his recordings of ERGs from various parts of the ocellus and the main features of ocellar anatomy. Some doubts about this model have already been expressed by Autrum & Metschl (1963).

In the present account electrophysiological results based on recordings of spike discharges from several units in the dragonfly lateral ocellus are presented. They show that Ruck's model cannot in itself be an adequate account of ocellar functioning since the ocellar nerve units are not all the same, as Ruck and other authors have assumed, but show marked differences in their activity which must reflect differences in their physiology. These ocellar nerve units are of at least three kinds; two kinds of unit conduct action potentials centripetally and are referred to here as the single 'giant afferent unit' and the several 'small afferent units' respectively. Units of the third group conduct spikes centrifugally, and will be treated in a later publication.

Of the two kinds of afferent unit the small units, as far as the evidence shows, come the closer to following the essential features of Ruck's model. For the giant unit, a more complex and perhaps less conventional model is required, whose precise formulation, however, must await further evidence. For the present an interpretation of the anatomical relationship between the giant fibre and the receptor axons will be contrasted with that for the small fibres. The electrophysiological data will then be discussed in terms of some factors that must be considered and examined experimentally if a functional model is to be devised.

* Present address: M-P-I für biologische Kybernetik, 74 Tübingen, Spemannstrasse 38, West Germany.

MATERIALS AND METHODS

The animals used in this study were adults of both sexes of the dragonfly *Aeschna cyanea* Müll. They were usually reared in the laboratory from larvae caught in local ponds at the last or penultimate instar stages, and were used within 5 days after metamorphosis.

Intact animals were fastened to a block with plasticine, and their heads were firmly fixed by embedding the mouth parts in blackened tacky wax. An indifferent electrode of tungsten or chlorided silver wire was placed in the frontal cavity. The frontal cuticle had first been dissected away and the two large air sacs within had been removed. This allowed the frontal cavity to fill with haemolymph, which was topped up when necessary with saline (Fielden & Hughes, 1962).

Further dissection exposed the ocellar nerves. Most of the experiments described here were carried out on lateral ocelli *in situ*, but with their nerves cut near the brain. For some experiments the entire ocellus, except for the cornea, was transferred to a separate saline bath.

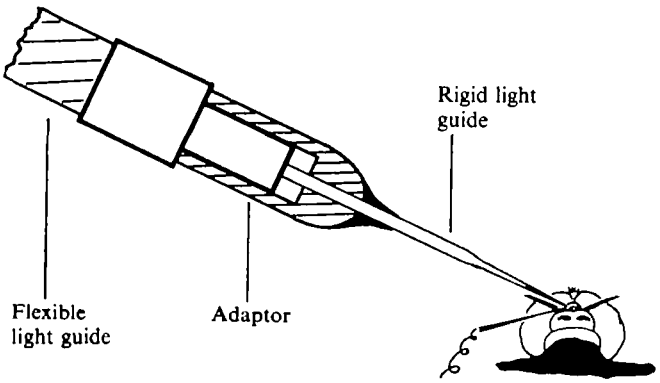
Metal recording electrodes were unvarnished, electrolytically sharpened tungsten wires and were placed in the ocellar cornea in *in situ* preparations. Recording from the nerve was effected with suction electrodes with internal tip-diameters between 70 and 120 μm. These were drawn by hand from thin-walled glass capillaries, and their tips were rounded-off in the heat of a flame. Care was taken to avoid suction artefacts – transient increases in spike rate – by using low suction pressures and allowing the preparation to settle down for some minutes after attaching the electrodes. Details of electrode application are given in the text.

Experiments were conducted in a darkened room at 20–25 °C. Standard recording equipment was used (usually with capacity coupling) in conjunction with a direct recording tape recorder for data storage.

Stimulation. Electrical stimuli in the form of D.C. pulses of variable duration were delivered to the ocellar nerve through a suction electrode, with an indifferent electrode in the head. The suction electrode was part of a mains-isolated stimulating circuit with variable output voltage. No attempt was made to measure current strengths.

For light stimulation the source was a tungsten lamp (Mazda, 48 watt) with a square filament, powered by two 6 V car batteries and a 6 V battery charger, all connected in parallel to provide a ripple-free D.C. current which was stable over the periods required. The light passed through a condenser of large aperture, heat filters, and a manual shutter before being focused on to one end of a flexible fibre-optic bundle (5 mm diameter). To the other end of the bundle was attached a small, rigid light guide, tapered to a tip diameter of the order of the ocellar corneal diameter. The tip of this guide was positioned almost in contact with the ocellar cornea (Text-fig. 1), and could be screened with blackened silicone grease if desired.

A stimulus-marker signal was provided by a phototransistor. The maximum intensity used (measured at the light-guide tip) was approximately $4{\cdot}3 \times 10^5$ lux; lower intensities were obtained with neutral density filters.



Text-fig. 1. Arrangement for presenting localized stimuli to the insect. The adaptor is light-proofed with black paint and plasticine; the rigid light-guide is coated with black paint to the tip. The end of the flexible guide is held in a micromanipulator. A corneal electrode is shown in position.

RESULTS

(1) *The structure and neuronal components of the lateral ocellus*

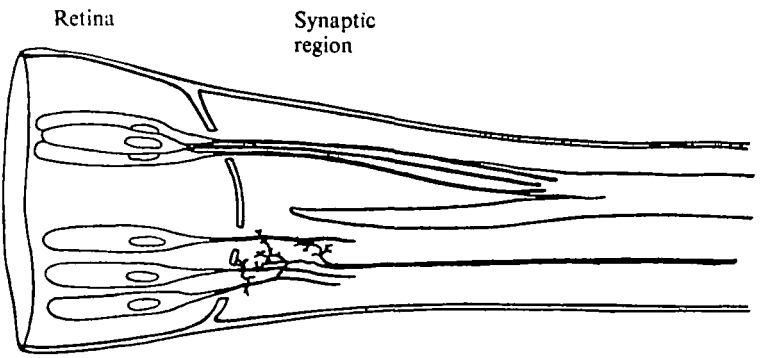
The basic structure of dragonfly ocelli is well described in the literature (Cajal, 1918; Ruck & Edwards, 1964; Dowling & Chappel, 1972). In this section the basic structure will be briefly summarized as a background to the electrophysiological data. Also, some data from the literature and original light-microscopical observations will be used to point out some structural differences between two kinds of ocellar nerve fibres. These structural differences are of interest in relation to electrophysiological differences to be described.

Distally in the ocellus a cuticular lens, the cornea, is underlain by a deep, cup-shaped retina composed of a single palisade of elongate receptor cells. Beneath the retina is a transverse, pigmented 'basement membrane', described by Ruck and Edwards as a layer of shielding pigment cells. Receptor axons pass through the basement membrane to enter the distal region of the ocellar nerve (the synaptic region) where they meet the terminal arborizations of the several ocellar nerve fibres.

In the dragonfly the synaptic region is a swollen structure which has no distinct proximal limit but rather tapers off into the ocellar nerve. Its swollen nature may be attributed to the large number of receptor axons and the thick branches of the larger ocellar nerve fibres that it contains, as well as to a plentiful supply of glial material (Cajal, 1918). Following the synaptic region the ocellar nerve runs to the brain, continuing to decrease in diameter.

Plate 1 A is a transverse section of the lateral ocellar nerve, cut just within the brain sheath, and gives an indication of the number and size range of the fibres present at this level.

The diagram of Text-fig. 2 gives the general anatomy of the ocellus and synaptic region, and shows the proposed relationships of two ocellar nerve fibres with the receptor axons. The thick fibre represents the giant fibre seen in the transverse section (Plate 1 A) and the smaller fibre represents one of those in the 5–12 μm range of diameters. Evidence is given in the next section that these correspond to the giant afferent unit and one of the small afferent units that feature in the electrophysiological records.



Text-fig. 2. Schematic representation of the lateral ocellus and synaptic region. The proposed branching configurations of the giant afferent fibre (upper) and a small afferent fibre (lower), and their relationships with the receptor axons, are shown. Further explanation in text.

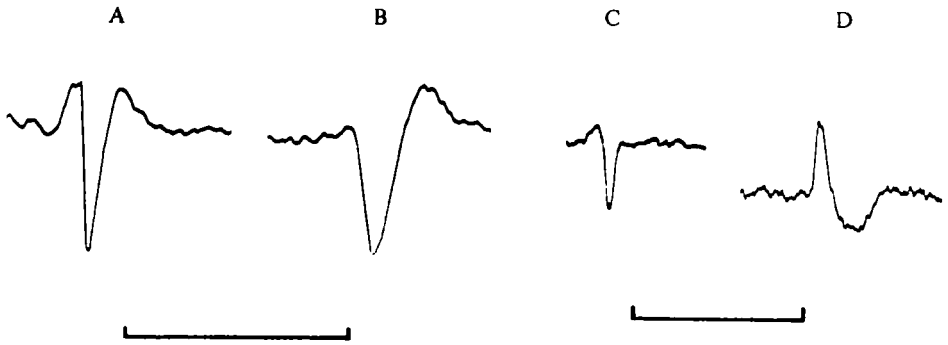
The only evidence for the arrangement of the terminal branches of the small fibre is Cajal's light-microscopical account. Although his description was primarily of such fibres in the median ocellus, he states that they also occur in the lateral ocellus. The fibre has a terminal arborization whose branches run more or less *transversely* to the direction of the receptor axons.

For the giant fibre the large branches – beginning deep in the synaptic region – run distally in a course more or less parallel to that of the receptor axons. Plate 1 B, an oblique section in the proximal part of the synaptic region, shows two large branches of the giant fibre which are each closely invested in a 'sleeve' of receptor axons running parallel to them. Thus, the giant fibre differs from the small ones in that the former lies enclosed in receptor axons while the latter branch freely amongst them.

Another observation may be added to this; namely, that the receptor axons pass through the basement membrane sometimes singly and sometimes in small bundles with a common glial sheath. This is described for the lateral ocellus by Ruck and Edwards (from electron-microscopy). In *Schistocerca* Goodman (1970) describes such bundles as sometimes merging into larger groups. It is therefore possible that the dendrites of the giant fibre are contained in a separate extraneuronal space – bounded by retinula axons – which is continuous with the retinal extraneuronal space via the bundles and distinct from the rest of the synaptic region. This is diagrammatically shown in Text-fig. 2. The implications of such an arrangement are discussed later.

(2) *Some features of recorded action potentials and conclusions about their cells of origin*

In this section information on the amplitudes, shapes and other features of the extracellularly recorded action potentials, and on the conditions under which they were recorded, is examined with the purpose of drawing some conclusions about their cells of origin. Since the precise electrical properties of the ocellar extracellular medium are not known, the examination is restricted mainly to qualitative differences between spikes of different units or from different recording sites. These differences between spikes, in conjunction with response characteristics, were also of great use in the identification of homologous units in different preparations.



Text-fig. 3. Examples of action potentials of the giant afferent unit recorded at four regions of the intact ocellus: *A*, the proximal end of the nerve; *B*, below the synaptic region; *C*, distal in the synaptic region; *D*, from the cornea. Time scales are each 4 msec. Upwards is positive.

The lateral ocellar giant unit. In intact preparations a simple, unvarnished tungsten electrode just piercing the surface of the cornea of the lateral ocellus records, relative to an indifferent electrode in the frons, action potentals of two distinct units (see also Ruck, 1961*b*). If the ocellar nerve is then cut near the brain, the smaller (in respect of spike amplitude) of these units disappears; it is one of four lateral ocellar nerve units which are discussed in a later paper (B. L. Rosser, in preparation). The remaining unit is the one studied by Ruck in similar cut-nerve preparations, and is the one referred to in this paper as the 'giant afferent unit'. It can also be recorded with a suction electrode on the nerve. Since, both in intact and cut nerves, its spikes are always considerably greater in amplitude than those of any other simultaneously recorded unit, and since positioning of the electrode is least critical in its recording, it seems reasonable to assign the giant afferent unit to the giant fibre seen in transverse sections of the nerve.

The giant afferent unit conducts action potentials by active propagation centripetally in the proximal part of the nerve and centrifugally in the distal part, with the spike generator lying at a point about one third of the distance from the retina. This is shown by the following three lines of evidence.

(i). The shape of the giant afferent spikes varies according to the position of the suction electrode on the nerve. At the proximal end of the nerve the spikes are triphasic (Text-fig. 3*A*), as expected for a fibre extending in both directions away from the recording site in a volume conductor (Lorente de Nó, 1947; Mauro, 1960). If the electrode is moved distally, the initial positive phase of the spike declines in relative amplitude until a point is reached where it is completely absent (Text-fig. 3*B*), indicating the site of the generator. More distally still, the initial phase reappears and increases in relative amplitude, and at the same time the final positive phase begins to decline. This, in its turn, disappears (Text-fig. 3*C*) when the electrode reaches the base of the retina, where the fibre is known to terminate. The corneally recorded spikes (Text-fig. 3*D*) also lack a final positive phase, though this may have a different cause.

(ii). Recording simultaneously with an electrode in the cornea and one on the nerve, the giant afferent spikes picked up by the corneal electrode either precede or follow their counterparts seen with the nerve electrode, according as the latter is respectively

proximal or distal to a point about half-way along the nerve. This would place the generator somewhat distal to this point.

(iii). Starting with the ocellar nerve intact it can be cut through at any point along its length. If the cut is made low on the nerve, giant afferent spikes appear only in the distal stump, and they respond strongly to light stimuli. With the cut made in the synaptic region, the spikes of this unit (identifiable by their great amplitude) can be recorded only from the proximal stump, but their discharges are now only very slightly modified by light.

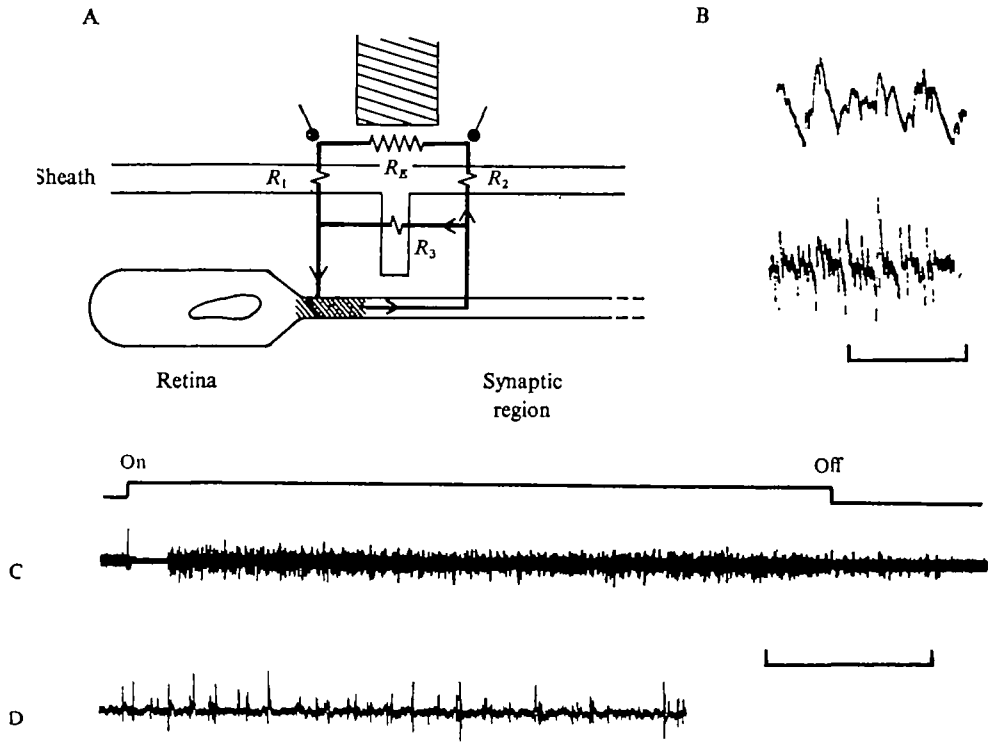
The smaller unit of the corneal record lent support to the above interpretation by its contrasting behaviour in respect of spike shape and the measurements of conduction time. There was no evidence that its spike generator lay anywhere within the ocellar nerve, and spike conduction was centrifugal along its whole length.

Small second-order units of the lateral ocellus. Normally when the nerve was cut below the giant afferent fibre generator site, only this unit was recorded in the distal stump of the nerve, and this too was silenced if the cut was made high enough. In a single preparation of this sort careful probing around the circumference of the nerve revealed a number of units with rapid spikes of small amplitude. Responsiveness to light, and its abolition with magnesium (see below), indicated them to be afferent and second-order.

The position of the electrode was highly critical for recording these units; maximum amplitude was achieved when the electrode mouth covered the antero-lateral quadrant of the nerve, near its cut end. Small deviations from this position resulted in a rapid decline in amplitude, and nothing could be recorded with the nerve sucked into the mouth of the electrode. It appears, therefore, that these units correspond to relatively small-diameter fibres situated near the sheath. Some of the fibres in the 5–12 μm size range (plate 1 A) seem likely to be the fibres of origin of these units; the other larger fibres are already accounted for by the centrifugal units.

Action potentials from retinula axons. Spike signals of a further category were recorded using the special 'excised' preparation of the lateral ocellus; that is, with the whole ocellus placed in a separate bath. The end of the nerve was sucked into the mouth of a suitably sized electrode, and an indifferent electrode of chlorided silver was placed in the bath. In most of these preparations the giant afferent unit continued to be active and to respond to light. The recording situation is that described, and termed *en passage*, by Easton (1965). Signals are recorded as current flows through the resistive channel between the nerve sheath and electrode glass at the electrode aperture.

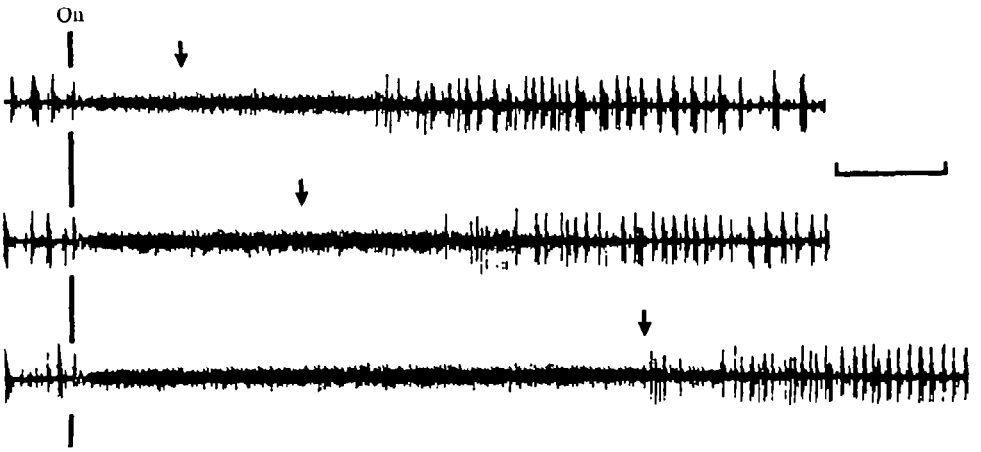
Until a certain stage in the steady progress of the nerve, under gentle suction, into the barrel of the electrode, the giant afferent was the only unit observed. When the base of the retina approaches the electrode aperture, however, the amplitude of the giant afferent spikes drops suddenly (from up to 2 mV to 200–300 μV), and simultaneously a high-frequency 'noise' makes its appearance. With slight further inward progress of the ocellus this 'noise' increases in amplitude and resolves itself into small, rapid spikes of various amplitudes (see Text-fig. 4B). Maximal amplitudes are obtained when the electrode mouth makes a close contact with the sheath surrounding one restricted region of the ocellus, the base of the retina: further movement of



Text-fig. 4. *A.* Interpretation of the conditions for recording receptor axon spikes. A portion of the ocellus in the region of the basement membrane is depicted with part of the mouth of a suction electrode (striated block) in the position for maximum recorded spike amplitude. The inside of the electrode is to the right. Signals are recorded between the two dots in the circuit diagram, i.e., across resistance R_E. The p.d. across R_E is large when R_E is large relative to R_1+R_2 (the sheath resistances) and when R_3 (the basement membrane resistance) is large relative to $R_E+R_1+R_2$. The shaded portion of the retinula cell axon represents an active region generating an action current in the circuit. *B.* Examples of receptor spikes recorded as in *A*. In the upper trace the band-pass limits were 20 Hz–1 KHz, and the predominantly negative spikes are superimposed on a slower oscillation of the baseline. The oscillation, a usual feature of such records, is filtered out in the lower trace (200 Hz–1KHz). Time scale, 0·1 sec: upwards is positive. C. Response to light of receptor spikes. The amplifier cuts out briefly at high-intensity illumination. The discharge does not cease abruptly at OFF in this record. Intensity, *ca.* 5×10^4 lux: time scale, 1 sec. *D.* Portion of receptor discharge adapted to illumination at approx. 50 lux. Time scale, 0·5 sec, spikes retouched.

the ocellus either into or out of the electrode results in rapid decline and disappearance of the spikes.

Two arguments favour the interpretation that these spikes originate in the retinula axons. Firstly, in contrast to the second-order units, their frequency is increased by illumination, as would be expected for receptor axons (Ruck, 1961*a*; Chappell & Dowling, 1972). (The possibility that the spikes are really light-sensitive polarization artefacts in the electrode is ruled out by the lack of response to light stimuli which are not localized on the retina.) Secondly, the fact that spikes are recorded at all is best understood by assuming the existence of a transverse, resistive barrier through which the fibres of origin must pass. The pigmented basement membrane may be



Text-fig. 5. Delayed off-responses. Three records showing retinula unit activity during and after illumination (4×10^5 lux) of varying, short duration. 10 min dark-adaptation between stimuli was allowed. The larger spikes belong to the giant afferent unit. Arrows indicate the light-off stimulus. Time scale, 1 sec.

just such a barrier, and the receptor fibres are the only ones known to cross it (Ruck & Edwards, 1964).

Signals from the fibres lying totally proximal to the barrier would be recorded at maximal amplitude when the electrode mouth is also proximal. This is the case for the giant unit. For fibres crossing the basement membrane, however, current flowing through it intracellularly must return across it extracellularly (Text-fig. 4A). If the basement membrane resistance is high enough, a signal will be recorded at maximal amplitude across the resistance of the electrode aperture when this is in parallel to it, i.e. when the aperture surrounds the basement membrane.

The number of units recorded is not known, but is certainly small in relation to the number of retinula axons (cf. Text-fig. 4D). Probably only those axons lying close to the outer sheath are represented.

(3) *Units of the afferent channels; responses to light*

Retinula units. Six preparations provided information on the behaviour of the reinula units in response to light stimuli. In four of these the units were almost silent in the dark-adapted state, giving a net discharge of less than 1 spike/sec. This rate may be tentatively considered to lie close to the normal dark-discharge since in the other two preparations (one of which was used for Text-fig. 5) there was some evidence that their higher rates were artefactual; the bursting discharge of the giant afferent unit in Text-fig. 5, for example, is abnormal.

Illumination results in a discharge of spikes whose frequency increases with intensity (compare Text-figs. 4C, D). Although frequencies were hard to estimate because of the number of units involved, a gradual and slight decline was sometimes observable during the first seconds (*ca.* 15 sec at 350 lux) following the onset of illumination. Thereafter, a relatively constant discharge remains for the duration of the stimulus (up to 10 min in these experiments). Unfortunately, the initial quarter second of responses to all but very low-intensity stimuli was not recorded, due t

overloading of the amplifier by the ocellar ERG. No values for latency can therefore be given, nor could the possible existence of an initial fast transient be established.

The response to light-off depends upon the intensity and duration of the stimulus. When intensity is low, or duration long, the light discharge ceases abruptly at 'off', generally with a brief silent period even in preparations with a relatively high dark-discharge. After brief, intense light flashes, however, the light-discharge continues in the dark for up to a few seconds more. Text-fig. 5 shows a few examples of this 'delay' phenomenon. Although the preparation in this case may have been abnormal (see above), the delays were not attributable to this. They were seen in better, but shorter-lived, preparations, and the associated delays of the giant afferent unit were studied in other experiments involving less drastic preparation.

The main characteristics of these delays are shown in Text-fig. 5. Delay shortens as stimulus duration lengthens (for constant intensity and constant interval of dark-adaptation between flashes). The retinula spike discharge declines gradually at the end of the delay with, for shorter flashes, no interruption to mark the 'off' stimulus. For longer flashes, there is a brief, partial break in the discharge at 'off' (better appreciated audibly), coinciding with a brief burst of the giant afferent unit.

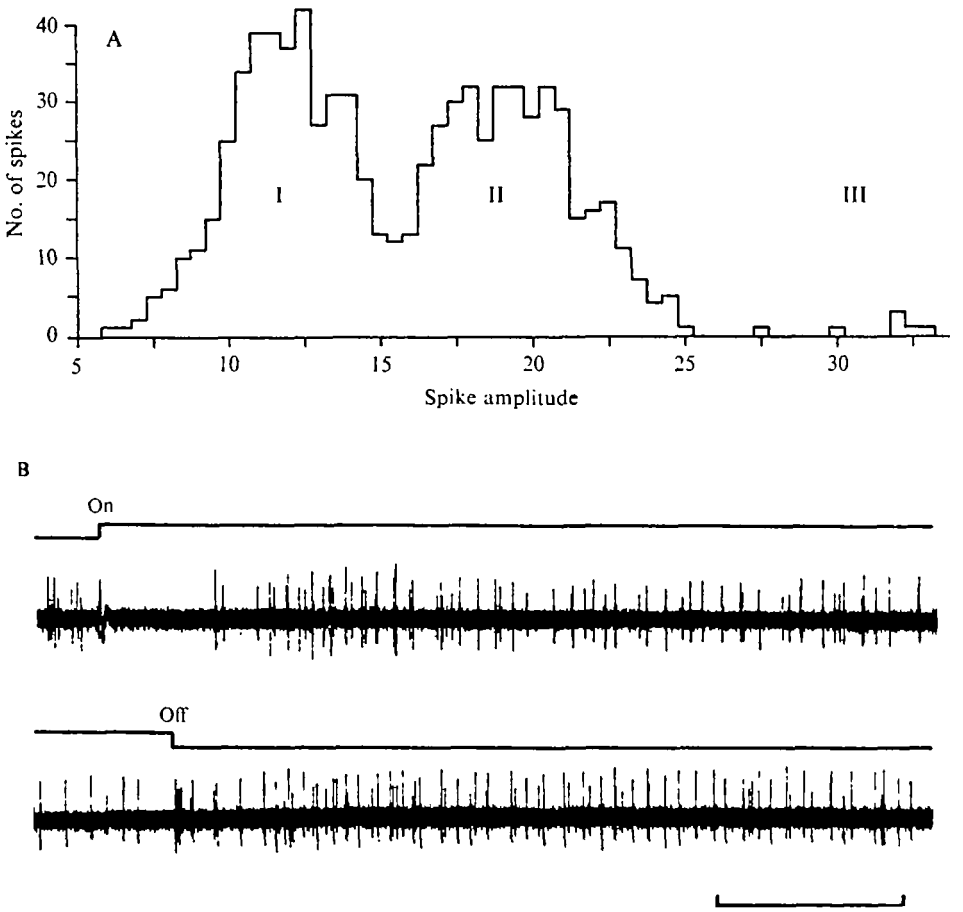
Small second-order units. In the single preparation in which small afferent units were recorded they were held long enough for their responses to simple light stimuli to be tested. It need not be emphasized that a single preparation is insufficient for confidence in the details of the responses; nor, since only the single light intensity of $3{\cdot}5 \times 10^5$ lux was used, can the responses be regarded as typical for all intensities. They will be briefly described, however, for comparison with the giant unit to be described below.

The number of units represented in the records is difficult to estimate. For those spikes clearly distinguishable from the noise level an amplitude histogram showed that the majority fell into two size categories (I, II in Text-fig. 6*A*). In addition, larger, infrequent spikes (III in Text-fig. 6*A*) almost certainly represent another size category. The description refers to categories I, II, taken as two 'units'.

The units fire in the dark at a fairly steady combined rate of about 16–20 spikes/sec. Text-fig. 6*B* shows the response to illumination after 10 min dark-adaptation. The response is a silent period lasting less than 1 sec, after which the renewed discharge quickly reaches the light-adapted level of about 10/sec, but with a slight overshoot in the first 2 sec. The whole of the difference between the light and the dark discharge rates appears to be accounted for by the smaller, category I spikes. That is, the category I unit has a phasic/tonic response to light, while that of the other unit (category II) is predominantly phasic. After 10 min of light-adaptation a weak response is produced at light-off. An initial, brief burst of spikes (Text-fig. 6*B*) precedes a rise in frequency over the first second to a rate of 30–35/sec, which then declines more gradually to the original dark-discharge.

The notable features of these responses are (i), the short duration of the transient components, (ii), the multiphasic nature of these transients, and (iii), the low maximal spike frequencies relative to the giant unit (see below). As a qualification to the second point the response to light after magnesium treatment loses the multiphasic character (Text-fig. 11*B*); it might, therefore, have been artefactual.

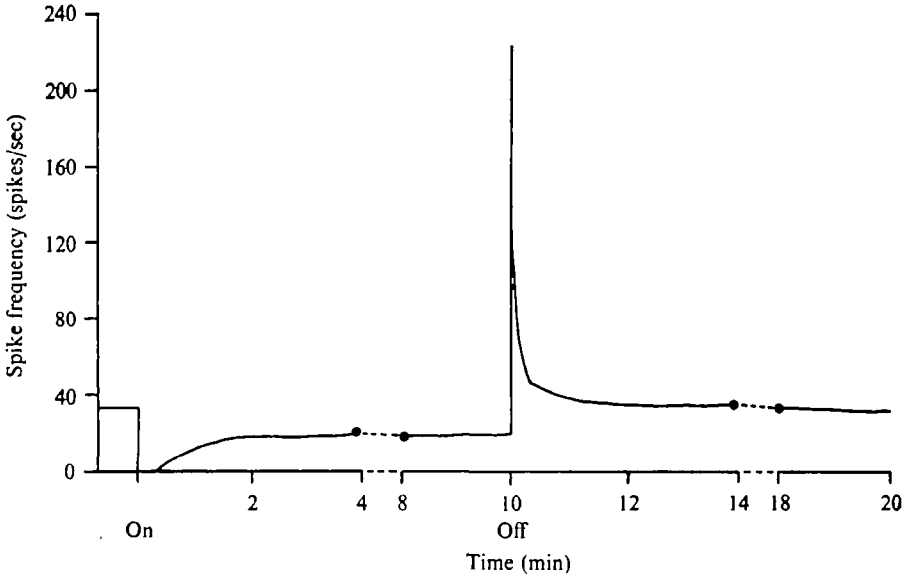
The giant afferent unit. The large second-order unit, the giant afferent unit, was



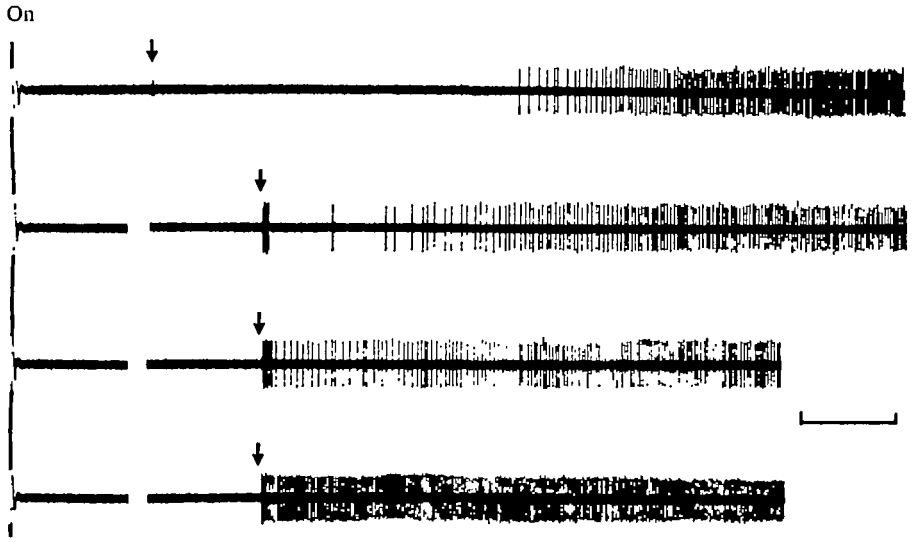
Text-fig. 6. *A*. Histogram of spike amplitude in a continuous record of the dark-discharge of small second-order units. Spikes fall into three size categories. (Amplitude in arbitrary units; total no. of spikes, 806). *B*. Responses of small second-order units. *Above*, on-response after 10 mins dark-adaptation. *Below*, off-response after 10 mins light-adaptation. Spikes are retouched: time scale, 1 sec.

studied in preparations in which the ocellar nerve had been cut near the brain. An effect of this operation was an increase in the dark discharge rate of the unit. At least a part of this increase is likely to be due to injury since it was found that the effect was greater the higher up the nerve the cut was made. No other systematic injury effects were observed as long as the cut was close to the brain.

The responses of this unit to step changes in light intensity are known from Ruck's work on other species of dragonflies (Ruck, 1961*b*, *c*), and are similar to those of afferent units in *Calliphora* ocellar nerve (Metschl, 1963). Text-fig. 7 is a typical frequency/time curve obtained during and after a light stimulus of long duration presented to a well dark-adapted preparation. Metschl has already drawn attention to the main qualitative features of the response: inhibition during illumination, with an initial silent period and a maintained, adapted discharge which is lower than the dark discharge; at off, a rapid rebound excitation subsiding to the original dark-discharge rate. At high intensities the transient responses to light-on and -off are



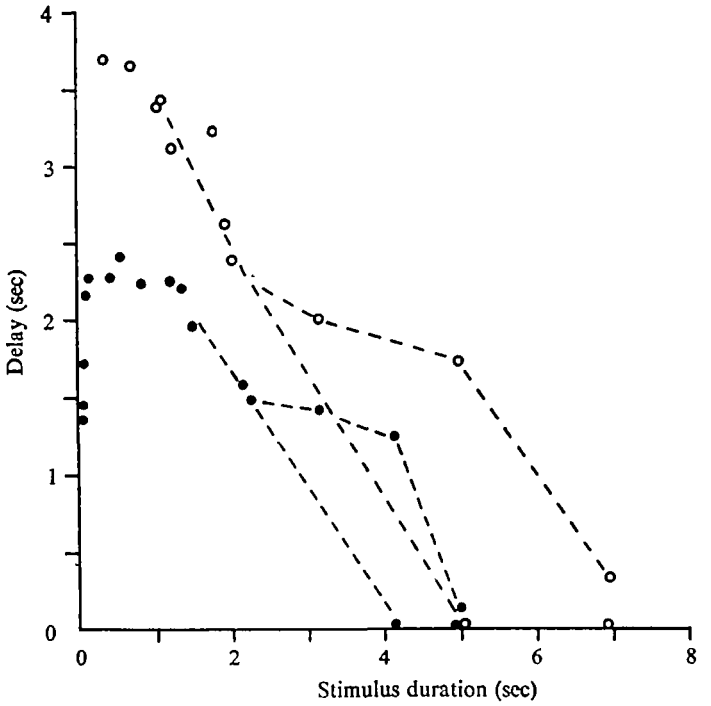
Text-fig. 7. On- and off-responses of the giant afferent unit. Plot of spike frequency against time for a typical preparation. The preparation was dark-adapted for 10 min prior to illumination. Stimulus intensity, $3{\cdot}5 \times 10^5$ lux.



Text-fig. 8. Four records showing delayed off-responses of the giant afferent unit and their transformation into the characteristic rebound off-excitation with increasing stimulus duration. Intensity was constant at $3{\cdot}5 \times 10^5$ lux, and dark-adaptation between stimuli was 15 min. Stimulus duration in the records, from above to below, was 1·5 sec; 7 sec; 10 sec and 15 sec. Spikes are retouched: time scale, 1 sec; OFF indicated by arrows.

remarkably long duration, up to 5 min, for complete adaptation of the off-transient at the maximum intensity of $4{\cdot}3 \times 10^5$ lux.

The phenomenon of the delayed off-response which follows short, bright light flashes has already been referred to. In Text-fig. 8 some examples from a more normal

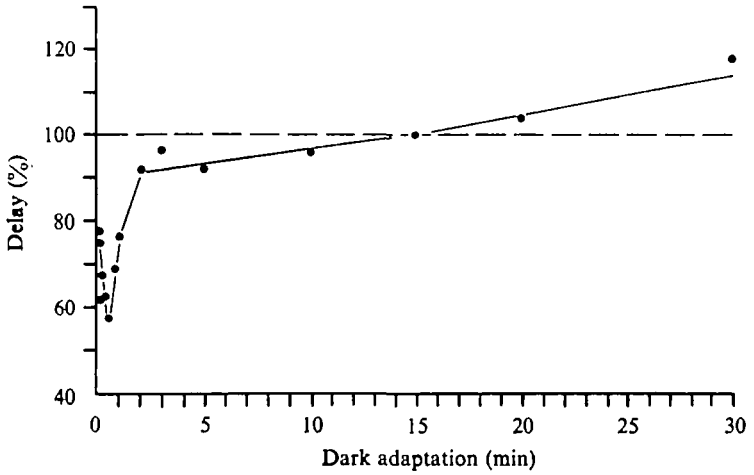


Text-fig. 9. Two curves of delay length against duration of illumination from two different preparations. The use of very brief stimuli in one preparation (closed circles) revealed a rising phase to the curve. The lowest point of this rising phase was produced by a 5-msec flash. The broken lines linking the points fork to include the short-latency phase (phase 1) of the response when this first appears with longer stimuli. Intensity, $3{\cdot}5 \times 10^5$ lux; dark-adaptation between stimuli, 15 min; stimuli were given in random order.

giant afferent unit are shown. For the figures, delays of only a few seconds duration were selected, but delays could be obtained up to 20 sec long in some preparations; in general they were longer when the ocellar nerve was intact. There are two phases to the response. Phase 1 is a short-latency burst of spikes which does not appear after very brief flashes; phase 2 occurs at longer, variable latency, and takes the form of a gradual reappearance of the dark-discharge with, usually, some overshoot. As flash duration lengthens the two phases merge and become the typical transient off-response.

Two curves showing the dependence of the delay duration on flash duration (constant intensity, constant dark-adaptation of 10 min) are shown in Text-fig. 9. As is expected, the delay of phase 2 has a maximum at a certain value of flash duration. Less expected is the 'hump' on the curve as phase 2 shortens in latency; this has implications when the origin of the delay is considered.

Another feature of the delayed response is its relationship to the period of dark-adaptation preceding the flash. Following about 10 min dark-adaptation a 1 sec flash is sufficient to produce a near-maximal delay (phase 2 latency). Subsequent 1-sec flashes at shorter intervals produce successively shorter delays until they are eliminated altogether. In experiments in which pairs of flashes of equal intensity and duration (*ca.* 1 sec) were presented with varying intervals between them (each pair being separated from the next by 15 min of dark-adaptation), a complex graph



Text-fig. 10. The delay produced by light stimuli of constant duration (1 sec) and constant intensity ($3{\cdot}5 \times 10^5$ lux) is plotted against the period of dark-adaptation between stimuli. Stimuli were given in pairs, each pair being preceded by 15 min dark-adaptation. To correct for instability the delays following the second stimulus of a pair are given as percentages of the delays of the nearest 'control' stimulus for which dark-adaptation was 15 min.

delay length (i.e. phase 2 latency; phase 1 never appeared under these conditions) of the second response against inter-flash interval is obtained (Text-fig. 10). Three phases of the curve are distinct: (i) a shortening in the delay duration with lengthening flash interval up to about ½ min; (ii) a rapid lengthening up to 2–2½ min; and (iii) a much slower lengthening which continues for up to at least 30 min flash-interval (the longest used).

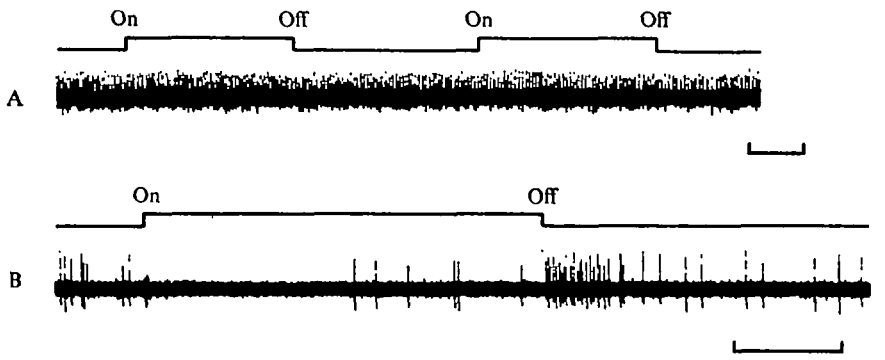
(4) *Electrical stimulation of ocellar nerve fibres and the effect of magnesium*

Two kinds of experiment for which data were obtained both from the giant and from the small afferent units of the lateral ocellar nerve, suggest that the two fibre types differ in some features of their organization. In the first group of experiments electrical (D.C.) pulses of variable, usually long, duration were delivered to the synaptic region of the ocellus by the method described above (see Materials and Methods).

In the single preparation in which they were studied the small afferent units were inhibited during cathodal stimulation of the synaptic region and excited during anodal stimulation. The responses were comparable to the responses to light, though not multiphasic; in each case there was an off-effect of reversed sign. These are the responses expected if the spike generators of these units do not lie under the electrode but, as for the giant fibre, lie some distance proximal to it (see Sandeman, 1969).

The giant unit responds to cathodal stimulation of the synaptic region in the same way as the small units, i.e. it is inhibited. The magnitude of the response is much greater. The threshold is lower, and at high voltages the duration of the silent period and the size of the off-transient compare with those for high-intensity light stimuli.

The response to anodal stimulation, however, is not the expected excitation but an inhibition, which resembles that to cathodal stimulation in magnitude and in the



Text-fig. 11. *A*. Discharge of the small second-order units in the first minute after adding $MgCl_2$ to the head haemolymph. Two light stimuli fail to produce recognizable responses. Time scale, 1 sec. *B*. The same during the first minute after washing with fresh saline. The response to light is restored, but has been somewhat altered by the magnesium treatment (cf. Text-fig. 6*B*). Time scale, 1 sec.

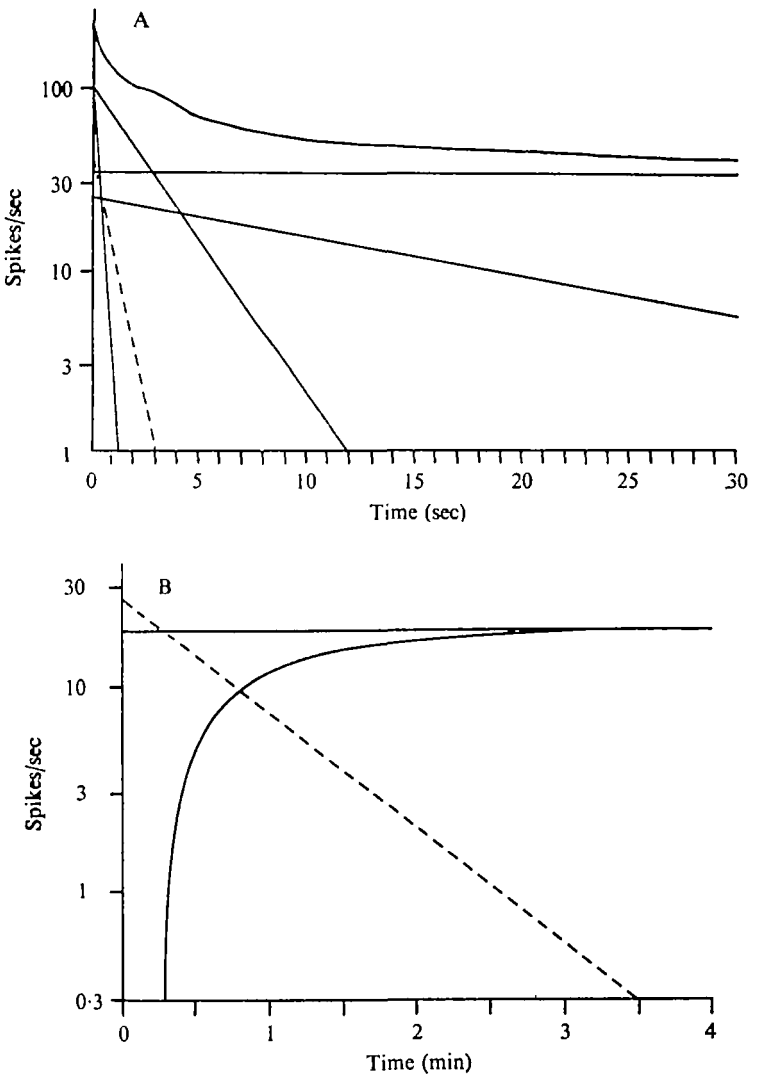
occurrence of an off-rebound excitation. Only at near-threshold voltages is a weak, short-lasting on-excitation seen preceding the inhibition. This contrasts not only with the small units, but also with the giant unit when stimulated at the face of the retina rather than at the synaptic region. In this case, cathodal stimuli, delivered to the excised preparation with an electrode just touching the face of the retina, produce inhibition, and anodal stimuli produce excitation over the whole range of voltages.

The second series of experiments involved the application of magnesium, an inhibitor of transmitter release at synapses (Katz, 1966). Here, too, the large and small afferent units are affected in quite different ways.

For the small units it was not possible to perform a carefully controlled experiment; a tiny crystal of $MgCl_2$ was simply allowed to dissolve in the head haemolymph. Responses to light were abolished within 15 sec of adding the crystal; as soon, in fact, as they could be tested (Text-fig. 11*A*). Similarly, responsiveness could be restored, again within 15 sec, when the head cavity was washed out with fresh, Mg-free saline (Text-fig. 11*B*).

The giant afferent unit was tested using both this crude method of adding Mg and, with the excised preparation, by completely refilling the bath with saline in which $MgCl_2$ had been substituted for $CaCl_2$. The results in each case were qualitatively the same, but experiments were carried on for longer using the second method.

After the addition of magnesium by either method the first noticeable effect was an increase in activity (also observed for the small units). This gradually subsided over the first few minutes to the original level or below. Light stimuli given at frequent intervals produced responses which were little different from normal. (This is an indication that the abolition of responsiveness of the small units was not due to damage at the first-order level). A change in the responses to light occurred at about 10 min from the start of the experiment. Illumination now produced a much longer silent period than normal, lasting 5 min before an adapted light-discharge was reached, and light-off produced a silent period of even longer duration. That is, both illumination and darkening now inhibit the giant unit. This condition lasted for at least an hour in the continued presence of Mg-saline.

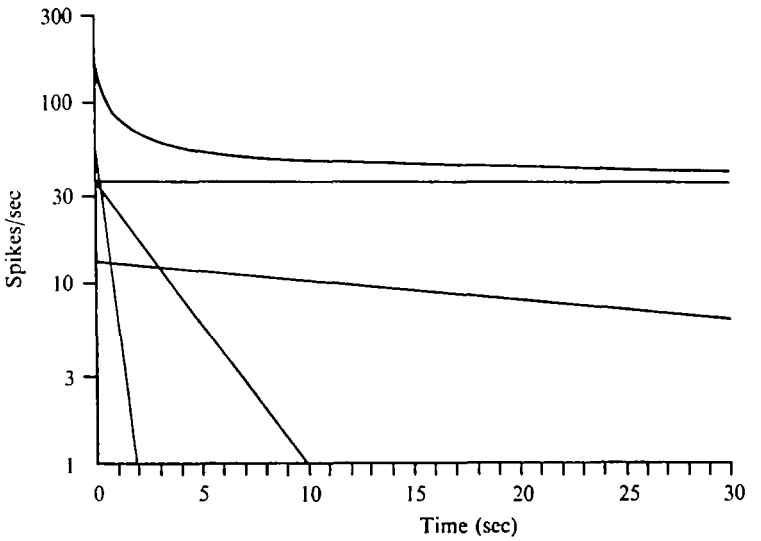


Text-fig. 12. *A*. Components of the off-response to light. The graph shows the breakdown of the transient response to light-off following 10 min of illumination at 5×10^4 lux. Frequency is plotted on logarithmic ordinate against a linear time scale ($t = 0$ is taken as the first spike of the response). Exponential components are represented as straight lines (solid lines, positive components, broken line, a negative component). The original curve (curved line) is described by the sum of the components; i.e.,

$$F_{(t)} = 35e^{-t/81\cdot90} + 25e^{-t/22\cdot8} + 100e^{-t/8\cdot45} - 40e^{-t/0\cdot83} + 93e^{-t/0\cdot19},$$

where the time constants are in seconds and the characteristics in spikes/sec. *B*. An analysed on-response of the same preparation. Dark-adaptation of 10 min duration preceded the stimulus. The time scale is measured from ON. Possible fast components would be lost in the silent period.

Eventually, about 2 h from the start of the experiment, a very regular discharge of about 30 spikes/sec ensued which was totally uninfluenced by light stimulation. When the saline was then (or at any time previously) replaced by normal saline, a period of *ca.* 10 min saw the return of responses of the original type.



Text-fig. 13. An analysed off-response to cathodal stimulation (D.C., 10 min duration). The negative component of Text-fig. 12*A* is consistently lacking in such responses.

It must be further noted that, for the small units at least, responses to electrical stimulation are not abolished by treatment with magnesium; this point was not tested for the giant unit.

(5) *Analysis of the responses of the giant unit*

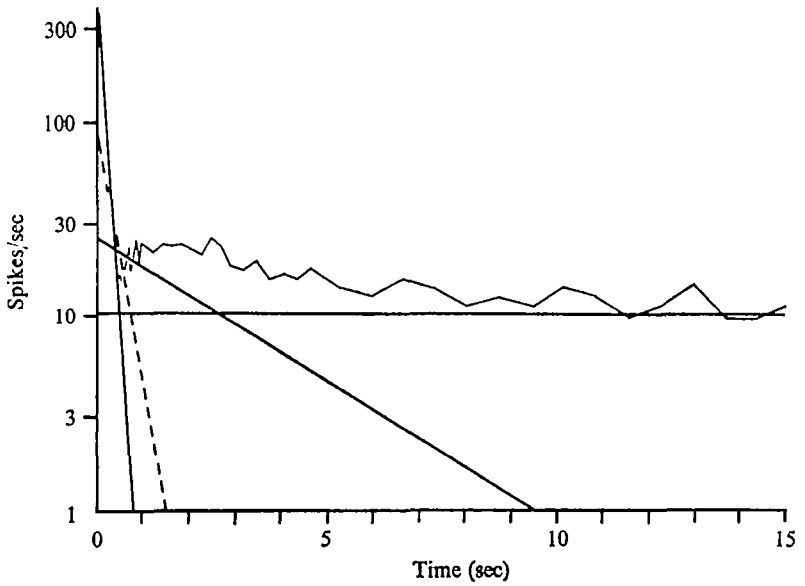
Ruck was concerned, throughout his investigation of ocelli, to identify distinct components in the net ERG responses and to trace these to their physico-chemical origins. An analogous procedure may be attempted with the spike discharges of the giant afferent unit.

Some responses of the giant afferent unit were analysed by the method of graphical extraction of exponential components which is described in detail by Mathews (1957) and Milsum (1966). Histograms of frequency against time were plotted with logarithmic ordinate for the 10 or 15 min following a stimulus, and a straight line was drawn through the tail of the graph. The straight line, an exponential component, was then subtracted from the graph, and the process was repeated until the last subtraction left only a straight line.

Text-fig. 12*A* shows the result of such an analysis of the off-response to prolonged (10 min) illumination. Five components are seen, the number found in analyses of four similar responses from four different preparations. Solid straight lines are 'positive' components of declining 'excitatory' influence; the broken line represents a 'negative' component of declining *inhibitory* influence, whose effect is seen as a depression in the original curve. The whole time course of the off-response is given by the equation

$$F_{(t)} = Ae^{-t/\tau_1} + Be^{-t/\tau_2} + Ce^{-t/\tau_3} - De^{-t/\tau_4} + Ee^{-t/\tau_5}.$$

It must be pointed out that the latency, the rise times of the components, and a short break in the discharge (see also Ruck, 1961*b*), all occurring during the firs

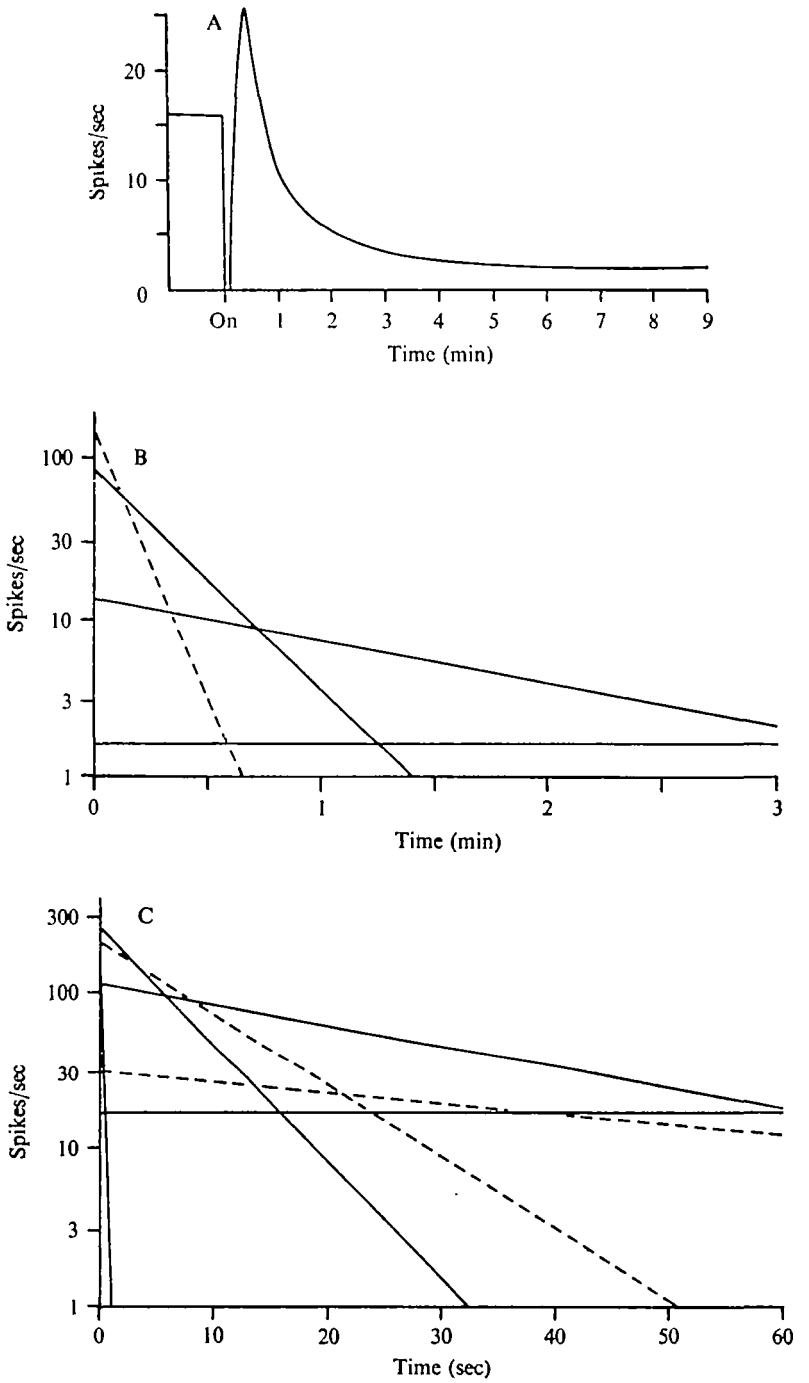


Text-fig. 14. An off-response to illumination of 10 min duration in a preparation which had been treated with magnesium (see text). Previous to treatment the off-response had resembled that of Text-fig. 12*A*.

20 msec following 'off', are not accounted for in this form of equation. Also, a continuous and irregular high-frequency oscillation, usually of small amplitude (also observed by Ruck) is averaged out by the method of calculation.

It is tempting to attribute to these five components the status of distinct *physiological* components. As an hypothesis, this receives some support from comparisons of variously produced 'off'-discharges in which the number of components is altered experimentally. Thus: (i) the off-response to cathodal inhibition lacks the negative component (Text-fig. 13); (ii) prolonged treatment with magnesium, by which a preparation initially showing all five components in light-off responses is subjected to alternating (long) periods in ordinary and Mg-saline over several hours, results, after some transitional stages, in the elimination of the second-slowest positive component from the recovered off-response (Text-fig. 14); (iii) the continuous presence of Mg leaves only the steady dark-discharge component, responsiveness to light being abolished.

Other experimental procedures described in the text have not been evaluated in terms of the component content of responses because the rather stringent requirements for analysis of a spike train are not always met. A further interesting observation, however, was provided by a preparation whose unusual responses allowed a more detailed comparison of the on- and off-responses than was normally possible. An on-response from this preparation is shown in Text-fig. 15*A* and analysed in Text-fig. 15*B*. Two strong and relatively slow 'positive' components are present which are absent from normal on-responses (cf. Text-fig. 12*B*). The off-response shows two slow 'negative' components (compare Text-fig. 15*C* with Text-fig. 12*A*). Although the time constants are different in the on- and off-responses, a correspondence of the pairs is suggested.



Text-fig. 15. *A*. The time course of an on-response (following 10 min dark-adaptation) in an unusual preparation. Intensity, $3{\cdot}9 \times 10^4$ lux. *B*. Analysis of the on-response shown in *A*. *C*. Analysis of an off-response of the same preparation.

Further consideration of exponential components is left to the next section and to the general discussion.

(6) *Exponential components and delayed responses*

The idea that delayed responses may arise as the result of the summation of excitatory and inhibitory components which outlast the stimulus has already been suggested for ganglion cells in the frog retina (Pickering & Varjú, 1971). A similar idea will be employed here to derive a qualitative model for the delayed responses of the giant afferent unit of the ocellus. In frog ganglion cells delayed responses have basically the same characteristics as do those of the giant afferent unit; notably, a brief burst at short latency (phase 1) and a train of spikes at varying latency (phase 2). Pickering and Varjú exclude phase 1 from their model, ascribing to it a separate origin. Here, however, in considering components of the delayed response, the components already described for the off-discharge following long light stimuli were taken into account, and it will be seen that phase 1 fits nicely into the resulting model.

This model is not claimed to be a complete and accurate model for the phenomena it attempts to describe. The purpose was simply to compare with the data an hypothesis, namely, that delayed responses do ***not*** imply the delayed onset of the previously described off-components. This hypothesis figures in the later discussion, and it is the central assumption of those listed below.

(i). The exponential components of the off-response represent the effects on the giant fibre of 'physiological components' which are, as an approximation, simply additive. Some evidence supporting this has already been given.

(ii). The same components are also at work, but with reversed sign, during the response to light-on. The unusual responses analysed in Text-fig. 15 support this, at least for some of the components. To simplify calculation, it was assumed that the time constants are the same in light and dark; this is not borne out by the evidence, but the difference is quantitative only.

(iii). The components behave somewhat as condenser discharges with light as the applied 'current'. That is, the starting value taken by an off-component at the instant of light-off is determined by the duration of the light stimulus. The effect of electrical stimulation suggests the analogy, and some data (not presented here) on the variation of the peak frequency at 'off' with the duration of applied cathodal stimulation support the assumption.

(iv). A further component (inhibitory) which behaves differently from the others must be assumed. This component is additive. It rises, during continual light, to a given maximal value after about the first second, and decays at a slower rate. Light-off curtails the rise of this component, which then decays with the same rate (as a convenient approximation) as in light. This component may be called the 'delay component'.

Using typical values for the off-response components and suitable values for the magnitude and the rise and fall rates of the assumed delay component, 'responses' in very good agreement with those shown in Text-fig. 8 were obtained, and the graphs of Text-fig. 9 could be matched in their main features. The delay component was designed, of course, to account for three things: the simple fact of response delays, the rising phase of the delay/stimulus-duration curves, and the falling phase of

the same curves. In addition to these the model accounts for the occurrence of phase above a given value of stimulus duration, and the 'hump' on the falling phase of the curves of Text-fig. 9. These two features are, therefore, tests of the model.

The curve of Text-fig. 10 is not accounted for by the model as presented above. To account for it the model would have to be extended by the addition of assumptions concerning the behaviour of the components, especially the delay component, during dark-adaptation. It is no part of the present purpose to attempt this.

The model has two implications which are important for the later discussion. First, if, as was assumed, the exponential components of the off-discharge begin always at short latency, and occur alongside the delay component, then there are at least two separate input channels from the retina to the second-order unit. Second, the receptor spikes follow the time course of the delay component (Text-fig. 5), and therefore do not release, by their cessation, the off-components.

DISCUSSION

Spikes are generated at one particular region of the cell, the spike generator region. Discharges of spikes reflect, therefore, electrical events at this region, i.e. current fluxes across the membrane, superimposed, of course, upon the electrical properties of the membrane itself. It follows from this that spike discharges will not necessarily behave in the same way as graded potentials recorded, either from inside or outside the cell, relative to an indifferent electrode at an electrically distant point; that they do not always do so in the ocellus has been remarked by Autrum & Metschl (1963). This limits the degree to which the present findings can be compared with those of Ruck and of Chappell and Dowling. In particular, the components of the giant afferent spike discharges are not to be regarded as equivalent to Ruck's ERG components.

Contrasting the small and giant afferent units

Ruck's model (1961*a*) seems to be quite adequate to account for the observed behaviour of the small afferent units. In particular, transmission from the receptor axons to these units must be synaptic, as proposed by Ruck, since it is blocked by magnesium. As a possible minor modification of the model the multiphasic transient responses of the units may indicate the sort of feed-back synaptic arrangement described by Dowling & Chappell (1972) for the median ocellus. Also, the observed dark-discharge may be artefactual, since the ocellar nerve was cut relatively far from the brain, a condition seen to enhance the dark-discharge of the giant unit. Interestingly, the absence of a dark-discharge would render these units off-receptors or on-off-receptors.

The giant unit differs significantly from the small units in respect of three features of its electrophysiology. These are (i) the transient components with long time constants in its responses to light, (ii) the difference in responses to anodal stimulation of the synaptic region, and (iii) the different effect of magnesium.

Probably the firmest conclusion that can be made concerning the giant unit is that it does not conform to Ruck's model. The nature of transmission from the first-order cells is especially brought into question. This forms the main theme of the following discussion.

Synapses and the effect of magnesium

Ruck postulated synaptic transmission in the ocellus because of the difficulty of explaining the hyperpolarizing response of the second-order cells in any other way. Electron-microscopical studies of various insect ocelli show that the synaptic region is indeed rich in synapses. On the other hand, the ocellar nerve fibres are not described individually in these accounts, and Ruck & Edwards (1964) give no details of synaptic arrangements for the dragonfly lateral ocellus.

In the present work magnesium was used to attempt to block synaptic transmission. While the responses to light of the small units were almost immediately abolished, those of the giant unit persisted for about 10 min, and then altered in a complex manner. The results must be considered in respect of two possible causal factors; (i) synaptic block, (ii) other effects due to ionic disturbance.

Synaptic block at the receptor axon/giant fibre interface would be expected, by comparison with the small units, to occur rapidly. It is possible that a careful examination, e.g. by component analysis, of the responses during the early stages of magnesium treatment would have revealed some change, but if this is so the role of synapses must be at most a minor one. It is also possible that the later changes in the responses, amounting to a lengthening of the light-on silent period and reversal of the off-rebound excitation, were due to synaptic block. It is perhaps conceivable, in view of the differing anatomical characteristics of the two neurone types, that the giant-fibre synapses could be so much less accessible to infiltration by magnesium than those of the small units; but this interpretation, too, indicates a secondary role for synapses in the elicitation of inhibition in the giant unit.

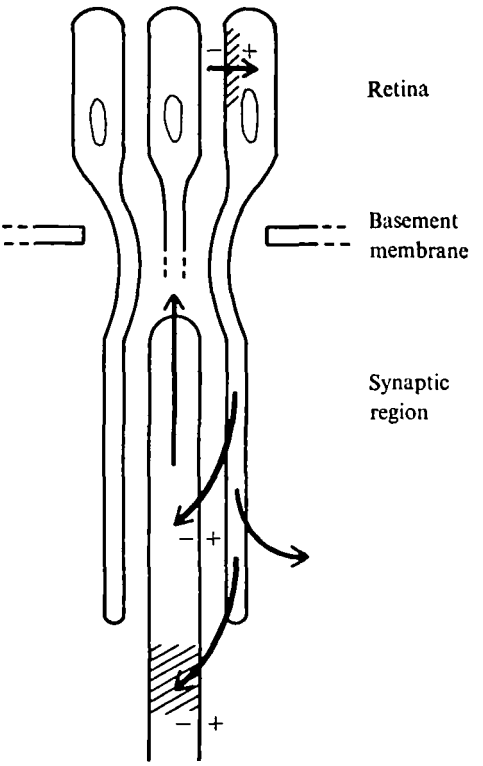
It is simpler, however, to regard the late effect as due, at least predominantly, to other causes. Photoreceptor cells in *Eupagurus* show a prolonged after-depolarization in an ionic medium containing Mg^{2+} but no Ca^{2+} (Stieve, 1965); and, furthermore, this occurs only after several minutes of treatment. A similar condition in the ocellus could account for the 'on' and 'off' inhibition observed; a single excitatory component, falling rapidly at 'off' would then be required to account for the eventual recovery of the light discharge. The final abolition of responsiveness to light may be due to similar causes (see Stieve, 1965).

The experiments with magnesium, therefore, suggest that inhibition in the giant unit is not synaptically mediated. If functioning synapses do occur there is no evidence that their contribution is inhibitory rather than excitatory.

Electrotonic inhibition

The alternative to inhibition mediated synaptically is inhibition mediated electrotonically, and some suggestion should be made as to how this could come about. Clearly, tight-junctions between the receptor axons and the giant-fibre dendrites will not serve, since the receptor cells become depolarized in response to light.

The literature affords two examples of electrically mediated inhibition of significant magnitude, *in vivo*: both involve the Mauthner cell of the goldfish. In the one case, non-propagated depolarizations occurring in the fine axon-cap endings surrounding the Mauthner cell axon hillock suppress spike generation in the axon hillock (Furukawa & Furschpan, 1963); in the other, action potentials in the Mauthner cell axon



Text-fig. 16. Scheme for partial electrotonic coupling between receptor cells and the giant fibre producing inhibition in the latter. Active depolarization in the receptor somata is shown imposing a passive hyperpolarization on the distal region of the giant fibre (spike-generator cross-hatched). Action potentials in the receptor axons could also hyperpolarize the giant-fibre generator, or could depolarize it if it lies within the receptor axon 'sleeve'.

itself induce passive hyperpolarizations in adjacent neurones (Faber & Korn, 1973). In the former case, a degree of structural specialization is seen in the tight meshwork of small fibres of the axon-cap; in the latter, the structural requirement seems to be that some part of the axon of the hyperpolarized cell lies along the extracellular current path of the Mauthner cell axon (Faber & Korn, 1973).

The anatomy of the synaptic region as summarized in Text-fig. 2 suggests that similar conditions for partial electrical coupling between receptor axons and the giant fibre are to some degree fulfilled. The giant-fibre dendrites are surrounded by receptor axons and lie parallel to them, i.e. along their extracellular current paths. Also, it was suggested that the dendrites lie in an extraneuronal space which has a direct link with the retinal space via the receptor axon bundles. Text-fig. 16 shows how an anatomical arrangement of this kind could make possible the passive hyperpolarization of the giant-fibre generator region during depolarization of the receptor cells. Depending on the precise electrical properties of the system and on the exact position of the giant afferent generator, the effect of an active response (receptor potential or action potential) in the receptor cells could be always inhibitory or, a possibility not excluded by the evidence, could be mixed, inhibitory and excitatory.

Receptor spikes

There is controversy in the literature as to whether or not insect photoreceptor axons conduct action potentials. In the drone bee (Naka & Eguchi, 1962; Baumann, 1968) and in the dragonfly median ocellus (Chappel & Dowling, 1972) a single spike-like depolarization at the start of the receptor potential is consistently seen. Trains of spikes during illumination are, however, rare in intracellular records; their absence (Naka & Eguchi, 1962; Kennedy, 1964) or presence (Baumann, 1968; Chappell & Dowling, 1972) is interpreted as artefactual, according to the standpoint of the various authors.

In some insect photoreceptors conduction by non-propagated graded potentials has been convincingly demonstrated (notably by Ioannides & Walcott, 1971). The question thus arises as to the respective roles of graded potentials and spike trains (if they occur) in determining the responses of second-order units. This question has been partly answered by Chappell and Dowling, who showed that the intracellularly recorded hyperpolarization of the median ocellar nerve dendrites occurs with little modification in the presence of tetraodotoxin.

Earlier in this paper trains of spikes occurring during illumination were described, which almost certainly originate in receptor axons. Although they were recorded extracellularly, these spikes are open to the charge of being artefactual, because of the particular recording conditions. It was suggested that these spikes, whether they are artefactual or not, do not drive the whole of the giant-unit response. They may, on the other hand, be responsible for part of it.

These conclusions rest on the validity of the model of delayed response production. The receptor spike discharge follows, at least roughly, the time course of the hypothesized 'delay component', so that, in terms of the model, the off-response components are released during the receptor spike discharge. A point against this model is that the receptor discharge falls briefly at 'off' for stimuli longer than a few seconds, so that a delay component with this characteristic would allow the short-latency burst (phase 1) of the second-order unit. It would then not be necessary to postulate the parallel occurrence of the off-components to explain this burst. The hump on the falling phase of the delay/stimulus duration curve (Text-fig. 9), on the other hand, is hard to explain unless at least the negative off-component occurs in parallel to the delay component and is, therefore, independent of the receptor spike discharge.

Electrical stimulation, and the origins of slow transients

Although the effects on the giant unit of cathodal and anodal stimulation of the synaptic region provide a strong indication of dissimilarity in the functioning of this and the small units, detailed interpretation of these effects is not possible within the limits of reasonable speculation. In general terms they may be regarded as supporting the proposition of electrotonic interaction between receptor cells and the giant, in view of the similarity of responses to light and to electrical stimulation. Also, the difference between anodal stimulation of the synaptic region and of the retina is suggestive of something like the anatomical arrangement described for the giant afferent dendrites, particularly since the small units do not show the anomalous behaviour of the giant. Further experiments with electrical stimulation may be very informative in the

future investigation of the giant afferent unit. It would be interesting, for example, to compare the component composition of 'off' or 'off'-like responses to the four electrical stimuli used (i.e. anodal and cathodal stimulation of the synaptic region and the retina) with off-responses to light; in the case of cathodal stimulation of the synaptic region all four positive components were present while the negative one was lacking (Text-fig. 13).

Another feature of the giant afferent unit which it is necessary to account for is the extremely long duration of its transient responses. In the analysed off-response (Text-fig. 12*A*) it is seen that this long duration is largely the effect of a single exponential component. Abolition of this component by poisoning with magnesium, reduces the off-response to a brief burst followed by a slower phase lasting only about 15 sec instead of 4–5 min (Text-fig. 14). As an explanation of this component the obvious candidates are either some ionic pool, drawn upon by the giant fibre, which becomes depleted during prolonged illumination and is only slowly replenished thereafter; or glial cells responding sluggishly to light or electrical changes by altering the ionic medium. Since the irreversible poisoning by prolonged magnesium treatment is selective for this component, the latter explanation seems the more likely.

CONCLUSION

The experimental findings of this study are sufficient to show that the lateral ocellus of the dragonfly is an inhomogeneous physiological system, at least a part of which, the part concerning the giant afferent unit, has certain unconventional properties. While some attempt has been made to indicate the possible nature of the mechanisms of the afferent system, it should be clear that further experiments using intracellular and extracellular recording techniques are required before a securely based functional model may be devised. Given this, the ocellus itself seems likely to prove a useful model for the study of several biophysical phenomena such as electrical inhibition, glial/neuronal interactions, etc. Some anatomical and electrophysiological similarities with the elements of the distal ganglion of insect optic lobes suggest, also, that some of the functional principles of the ocellar synaptic region may be general in arthropod visual systems.

SUMMARY

1. The lateral ocellar nerve of adult dragonflies contains at least two kinds of afferent nerve fibres: the 'giant' afferent and small afferents. Efferent fibres are also present, but are not described here.

2. The afferent fibres and receptor axons were studied by extracellular recording of their spike discharges. Experiments using light and electrical (D.C.) stimulation and the application of magnesium were performed.

3. Responses of the giant afferent were analysed into a number of exponential components. The number could be altered experimentally.

4. The giant afferent shows the phenomenon of 'delayed response', a delayed onset of the off-response to brief light stimuli. A qualitative model for delayed responses, incorporating the exponential components, is described.

5. It is concluded that the small afferents behave in accordance with Ruck's model

of ocellar functioning but that the giant afferent does not. It is proposed that inhibition in the giant afferent fibre is produced electrotonically, but there may be a synaptic contribution also.

This work was carried out at the University of Bristol and was supported by an SRC Research Studentship. I thank my former colleagues at Bristol, especially my supervisor Dr R. de G. Weevers and Dr R. M. Rose, for their help and encouragement. I also thank Drs L. J. Goodman, R. L. Chappell, and J. E. Dowling for useful discussions, and Dr T. Poggio for criticizing the manuscript.

REFERENCES

AUTRUM, H. & METSCHL, N. (1963). Die Arbeitsweise der Ocellen der Insekten. *Z. vergl. Physiol.* **47**, 256–73.

BAUMANN, F. (1968). Slow and spike potentials recorded from retinula cells of the honeybee drone in response to light. *J. gen. Physiol.* **52**, 855–75.

BURTT, E. T. & CATTON, W. T. (1956). Electrical responses to visual stimulation in the optic lobes of the locust and certain other insects. *J. Physiol. Lond.* **133**, 68–88.

CAJAL, S. R. (1918). Observaciones sobre la estructura de los ocelos y vias nerviosas ocelares de algunos insectos. *Trab. Lab. Invest. biol. Univ. Madr.* **16**, 109–39.

CHAPPELL, R. L. & DOWLING, J. E. (1972). Neural organization of the median ocellus of the dragonfly. I. Intracellular electrical activity. *J. gen. Physiol.* **60**, 121–47.

DOWLING, J. E. & CHAPPELL, R. L. (1972). Neural organization of the median ocellus of the dragonfly. II. Synaptic structure. *J. gen. Physiol.* **60**, 148–65.

EASTON, D. M. (1965). Impulses at the artefactual nerve end. *Cold Spring Harb. Symp. quant. Biol.* **30**, 15–28.

FABER, D. S. & KORN, H. (1973). A neuronal inhibition mediated electrically. *Science, N.Y.* **179**, 577–8.

FIELDEN, A. & HUGHES, G. M. (1962). Unit activity in the abdominal nerve cord of a dragonfly nymph. *J. exp. Biol.* **39**, 31–44.

FURUKAWA, T. & FURSCHPAN, E. J. (1963). Two inhibitory mechanisms in the Mauthner neurons of goldfish. *J. Neurophysiol.* **26**, 140–76.

GOLDSMITH, T. H. & RUCK, P. R. (1958). The spectral sensitivities of the dorsal ocelli of cockroaches and honeybees. *J. gen. Physiol.* **41**, 1171–85.

GOODMAN, L. J. (1970). The structure and function of the insect dorsal ocellus. *Adv. Insect Physiol.* **7**, 97–196.

HOYLE, G. (1955). Functioning of the insect ocellar nerve. *J. exp. Biol.* **32**, 397–407.

IOANNIDES, A. C. & WALCOTT, B. (1971). Graded illumination potentials from retinula cell axons in the bug *Lethocerus*. *Z. vergl. Physiol.* **71**, 315–25.

KATZ, B. (1966). *Nerve, Muscle and Synapse*. New York and London: McGraw-Hill.

KENNEDY, D. (1964). The photoreceptor process in lower animals. In *Photophysiology* (ed. A. C. Giese), pp. 79–121. New York: Academic Press.

LORENTE DE NÓ, R. (1947). A study of nerve physiology. *Stud. Rockefeller Inst. med. Res.* **132**.

MATHEWS, C. M. E. (1957). The theory of tracer experiments with ^{131}I-labelled plasma proteins. *Physics Med. Biol.* **2**, 36–53.

MAURO, A. (1960). Properties of thin generators pertaining to electrophysiological potentials in volume conductors. *J. Neurophysiol.* **23**, 132–43.

METSCHL, N. (1963). Elektrophysiologische Untersuchungen an den Ocellen von *Calliphora*. *Z. vergl. Physiol.* **47**, 230–55.

MILSUM, J. H. (1966). *Biological Control Systems Analysis*, pp. 249 *et seq.* New York and London: McGraw-Hill.

NAKA, K-I. & EGUCHI, E. (1962). Spike potentials recorded from the insect photoreceptor. *J. gen. Physiol.* **45**, 663–80.

PARRY, D. A. (1947). The function of the insect ocellus. *J. exp. Biol.* **24**, 211–19.

PICKERING, S. G. & VARJÚ, D. (1971). The retinal ON-OFF components giving rise to the delayed response. *Kybernetik* **8**, 145–50.

RUCK, P. (1957). The electrical responses of dorsal ocelli in cockroaches and grasshoppers. *J. Insect Physiol.* **1**, 109–23.

RUCK, P. (1958*a*). Dark adaptation of the ocellus in *Periplaneta americana*: a study of the electrical response to illumination. *J. Insect Physiol.* **2**, 189–98.

RUCK, P. (1958*b*). A comparison of the electrical responses of compound eyes and dorsal ocelli in fo insect species. *J. Insect Physiol.* **2**, 261–74.

RUCK, P. (1961*a*). Electrophysiology of the insect dorsal ocellus. I. Origin of the components of the electroretinogram. *J. gen. Physiol.* **44**, 605–27.

RUCK, P. (1961*b*). Electrophysiology of the insect dorsal ocellus. II. Mechanisms of generation and inhibition of impulses in the ocellar nerve of dragonflies. *J. gen. Physiol.* **44**, 629–39.

RUCK, P. (1961*c*). Electrophysiology of the insect dorsal ocellus. III. Responses to flickering light of the dragonfly ocellus. *J. gen. Physiol.* **44**, 641–57.

RUCK, P. (1966). Extracellular aspects of receptor excitation in the dorsal ocellus. In *The Functional Organization of the Compound Eye* (ed. C. G. Bernhard). *Wenner-Gren Center Int. Symp. Ser.* **7**, 195–205.

RUCK, P. & EDWARDS, G. A. (1964). The structure of the insect dorsal ocellus. I. General organization of the ocellus in dragonflies. *J. Morph.* **115**, 1–25.

SANDEMAN, D. C. (1969). The site of synaptic activity and impulse initiation in an identified motoneurone in the crab brain. *J. exp. Biol.* **50**, 771–84.

STIEVE, H. (1965). Interpretation of the generator potential in terms of ionic processes. *Cold Spring Harb. Symp. quant. Biol.* **30**, 451–56.

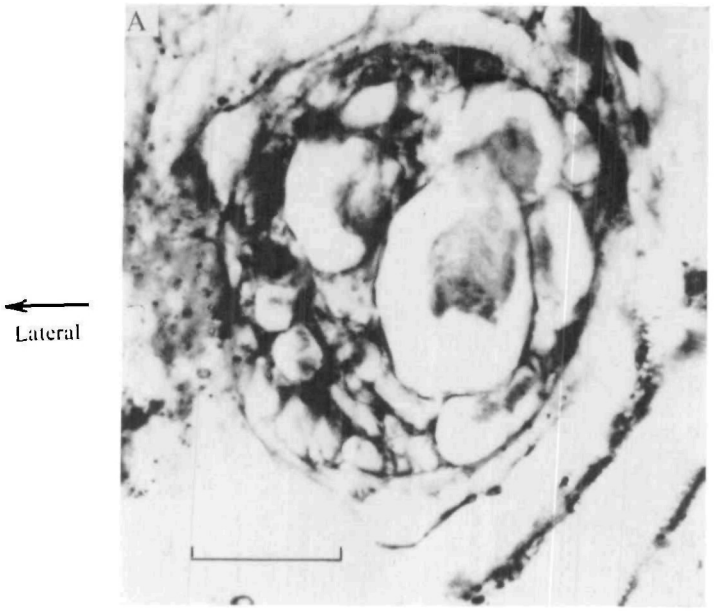
TOH, Y., TOMINGA, Y. & KUWABARA, M. (1971). The fine structure of the dorsal ocellus of the fleshfly. *J. Electron Microscopy* **20**, 56–66.

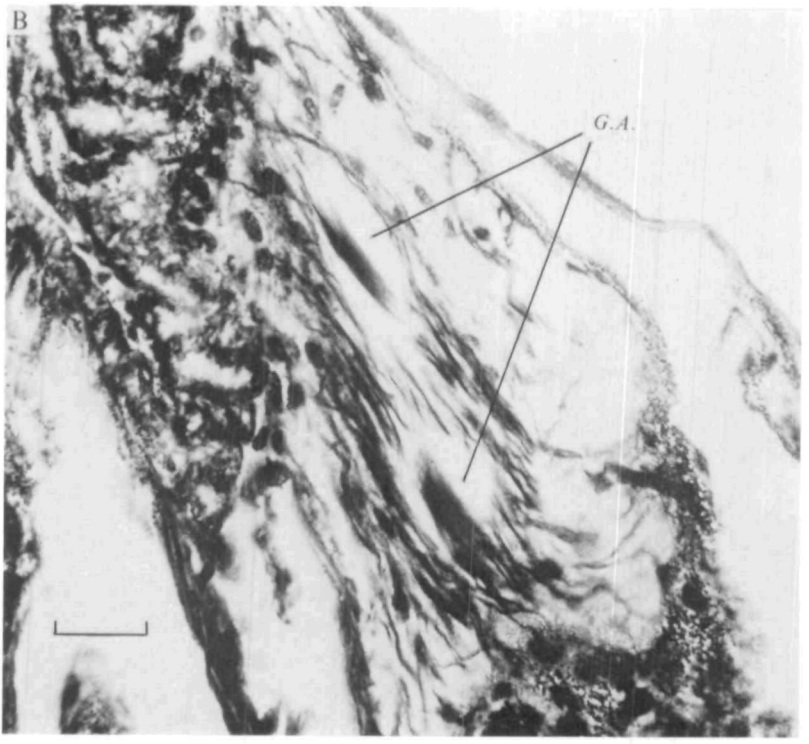
EXPLANATION OF PLATE

PLATE 1

A. Transverse section of a lateral ocellar nerve at the level of the brain, stained with osmium and zinc iodide. Five fibres of large diameter are seen. Many of the remaining fibres have diameters in the range of 5–12 μm. Scale, 20 μm.

B. Oblique section of a lateral ocellus taken deep in the synaptic region; stained with Holmes' silver. The section shows two large dendrites (*G.A.*) of the giant fibre, each tightly enclosed by receptor axons of which the neurofibrillar complement is stained. The two branches merge in more proximal sections. Scale, 20 μm.


A
Lateral


B
G.A.