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VISUAL AND MULTIMODAL INTERNEURONES IN THE VENTRAL NERVE CORD OF THE COCKROACH, *PERIPLANETA AMERICANA*

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INTRODUCTION

Many studies have been made on the activity of visual units recorded within the central nervous system of insects and these have been shown to vary according to the source of input and to the nature of the applied stimulus (e.g. see Horridge *et al.* 1965; Dingle & Fox, 1966; Swihart, 1968; Northrop & Guignon, 1970).

A knowledge of the properties of visual interneurones in the ventral nerve cord is fundamental to any understanding of stimulus-response relationships since the traffic in these fibres must ultimately be concerned in regulation of motor output from the various ganglia of the cord.

In this paper an attempt has been made to characterize the ventral nerve cord units of the cockroach, *Periplaneta americana*, with regard to their responsiveness to stimulation of the compound eyes and ocelli.

MATERIALS AND METHODS

Cockroaches were maintained in a colony under a 12 h light: 12 h dark regimen in such a way as to ensure that experimental animals were in an active part of their diurnal cycle (i.e. 'dusk') immediately prior to an experiment. Male animals were used throughout.

Recording

Recordings were made from the ventral nerve cords using glass microelectrodes whilst stimulating the compound eyes and ocelli with light. The animal was mounted ventral surface up inside a light-tight box, which also acted as a Faraday's cage during recording, on a Perspex animal holder using plasticine and entomological pins.

A small region of the thoracic connectives, either between the suboesophageal ganglion and the first thoracic ganglion (S–T_1) or between the first and second thoracic ganglia (T_1–T_2), was exposed taking care not to damage the tracheal system. Occasionally recordings were made from the abdominal connectives between the third thoracic and first abdominal ganglia but in no cases were visual units recorded from this site.

The sheath covering the cord makes penetration with a glass microelectrode held at right-angles to the surface rather difficult. However, because in the thoracic region the cord is relatively taut between the ganglia, penetration is achieved with very little damage to the cord or to the fine glass tip of the electrode if the electrode approaches

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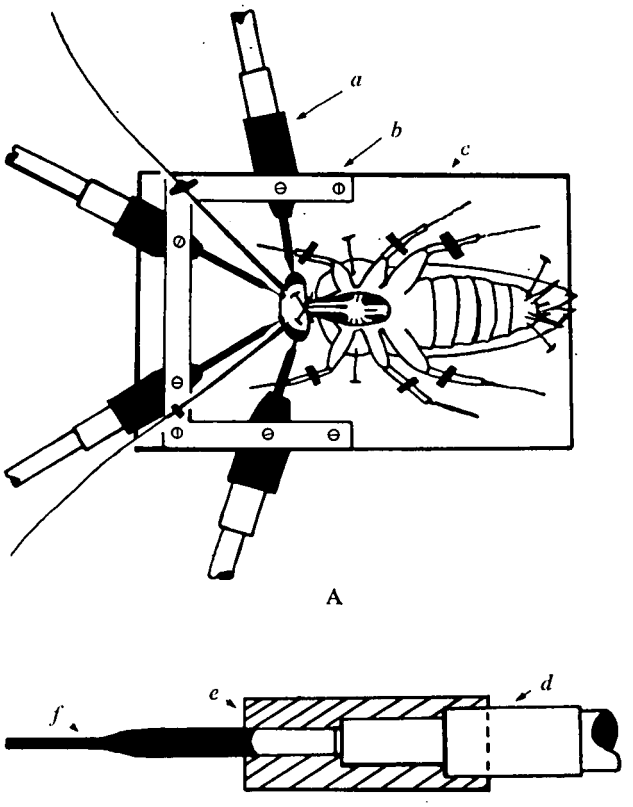

Fig. 1. (A) Diagram showing the positions of the four black Perspex light-guide terminations (*a*) when mounted on the animal holder (*c*) by means of clamps (*b*). (B) Sectional view of a light-guide termination (*e*), showing the arrangement of the light guide (*d*) and the light rod (*f*).

the sheath at a low angle with its shank parallel to the longitudinal axis of the connective. Glass micropipette electrodes of tip diameter less than 1 μm diameter were employed.

Conventional electrophysiological techniques were used to record electrical activity. Information was amplified using Tektronix 122 pre-amplifiers and displayed on a 502A oscilloscope. A Sony tape recorder was used to store records for future analysis by recording on to film and measuring directly or using a special purpose computer (Biomac) 1010 to plot histograms of spike intervals and latencies or to generate averaged responses.

Occasionally a single unit was immediately apparent; at other times a slight movement of the electrode tip either forwards or downwards was necessary to isolate a suitably large unit. Searching was facilitated by monitoring the recorded impulse traffic via an audio-amplifier. When a single unit was encountered a number of tests were performed on the animal and if responses were noted and considered to be of interest a more elaborate and controlled series of experiments was devised and executed. This was only suitable for identifying actively firing units so during pene-

tration of the connective sheath the animal was stimulated continuously in a variety of ways to avoid missing units which would otherwise have been silent. Experiments were never extended for longer than 40 min after the initial dissection to avoid possible slow changes due to anoxia. No anaesthetic was used.

Stimuli

Light stimuli were presented directly on to individual ocelli or compound eyes via light guides, each of which was controlled by a solenoid shutter mechanism. The light guides (Barr & Stroud: flexible) were terminated in tapered light rods coated throughout their lengths with black paint (Fig. 1 B) and clamped onto the animal holder by black Perspex blocks (Fig. 1 A). In this way the receptors could be illuminated separately or in any combination, with light spread kept to a minimum. The solenoid shutters produced fast transient spark artifacts which were picked up by the recording electrodes and thus indicated the exact point of stimulus change. When investigating flicker fusion frequency a strobe lamp (Grass Photostimulator) was used to deliver accurately repeatable flashes at rates in excess of 1 Hz; these were monitored by a photocell, whose output was recorded on the second beam of the oscilloscope.

Initial recordings had shown that certain multimodal units were sensitive to both visual and mechanical stimulation of the head receptors. Air was therefore piped from a compressed air line through an on-off valve on to either or both antennae via small pipettes; puffs or maintained flows of air could thus be delivered. Other forms of mechanical stimulation consisted of stroking the animal with a small paint brush or bristle.

Unit Types

The units reported here may be characterized according to their degree of coupling to the test stimulus and to the range of their responsiveness to various stimuli. The terms used are to a large extent those of Catton & Chakraborty (1969). Two broad groups were identified: tightly coupled units which respond or recover abruptly to a change in stimulus with a latency of less than 200 msec, and loosely coupled units which respond or recover less rapidly usually with a latency in excess of 200 msec. Some units, however, show one type of coupling to one stimulus (e.g. light OFF) and the other type to another stimulus (e.g. light ON) or *vice versa*. Furthermore, in some multimodal units coupling to different stimulus modalities was found to vary but coupling in any given unit to a particular stimulus modality was constant with time under the same experimental conditions. Both unimodal and multimodal units were recorded, and a distinction has been made between those units which respond to stimulation of receptors situated on both sides of the body (bilateral units) and those which respond to stimulation of receptors on one side (unilateral units). Adaptation and fatigue were impossible to define in all but the simplest cases as the reproducibility of stimuli was often difficult to ensure, especially as mechanical stimulation was sometimes followed by gross muscular activity. Whenever possible, however, visual units were tested for adaptation and results are reported where appropriate.

Of approximately 60 preparations in which a general survey of the unit types was attempted, compound-eye visual and multimodal units made up between 10% and 15% of the total number of units encountered. About 20% of the visual units were

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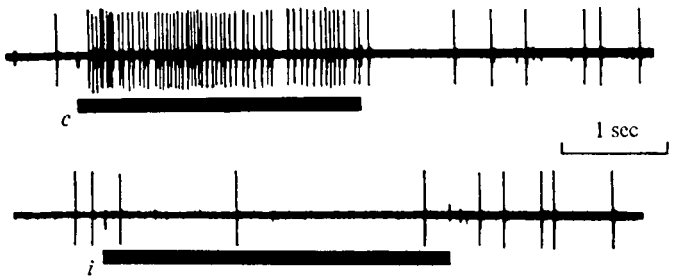


B

Fig. 2. (A) ON-unit A. Three consecutive stimuli applied to the ipsilateral (*i*), contralateral (*c*) and ipsilateral compound eyes respectively. Notice the weak discharge in the dark, lack of response to contralateral stimulation and the loosely coupled ipsilateral response to light ON. Black bar represents ON-period in both A and B. (B) ON-unit B. Stimulus marker and lettering as A above. Note here the tightly coupled contralateral response and the slight inhibition of the dark discharge by ipsilateral stimulation.

multimodal. This compares with a figure of 30% for multimodal units out of all the units encountered in the locust cord by Catton & Chakraborty (1969). A further 25 animals were used to study the effects of ocellar stimulation.

RESULTS

Detailed description of visual and visual multimodal units

For ease of description this section has been sub-divided into three parts: ON–, OFF– and ON–OFF-units. As all observed ocellar units in the cord showed only OFF– effects they are included in the sub-section devoted to OFF-units.

ON-units

The ON-units encountered were basically rather similar in general properties but could be sub-divided in terms of the degree of binocular interaction, modality and whether their response patterns were phasic or tonic.

A. *Compound-eye ipsilateral tonic ON-unit*

This unit is typically initiated from a previously silent unit, but if there is a dark discharge stimulation of the contralateral compound eye inhibits this firing (see Fig. 2A). Ipsilateral stimulation results in a loosely bound tonic ON-burst.

B. *Compound-eye and antennal multimodal, contralateral tonic ON-unit*

This unit (Fig. 2B) is very similar to ON-unit type A but was always shown to be multimodal, unlike type A.

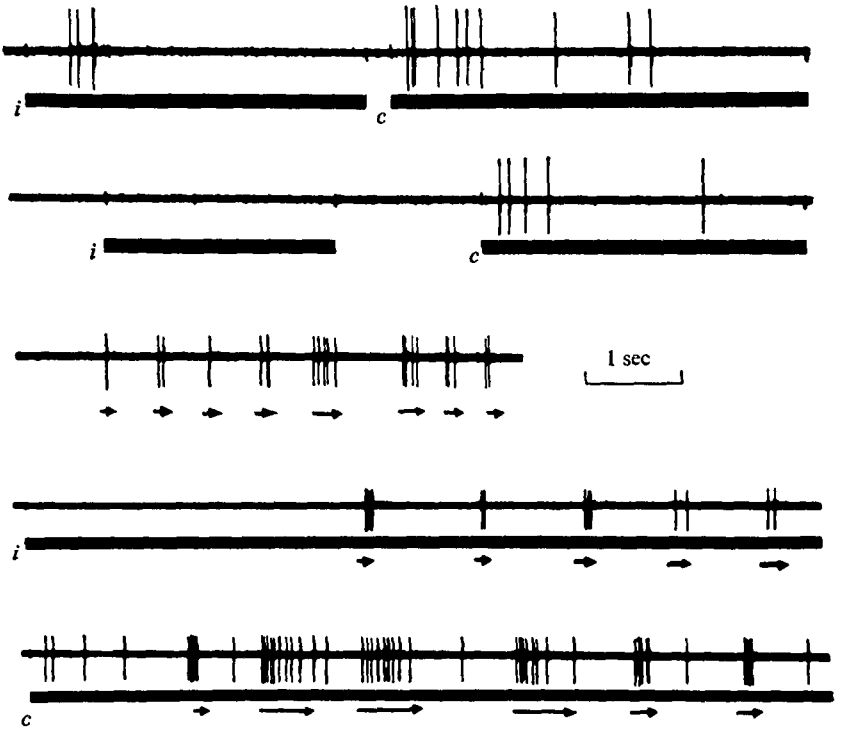
The tightly bound tonic ON-response shows an increased rate of firing to a steady level throughout the period of contralateral compound-eye stimulation. Like ON-unit type A, the dark discharge may be inhibited by compound-eye stimulation, in this case ipsilateral. Puffs of air on to either antenna evoke a short burst of spikes which often appeared to be less intense during contralateral compound-eye stimulation although this was not always apparent. Stimulation of the antennae during ipsilateral compound-eye stimulation results in a burst of firing which adds to the visual response.

It was found that these units were never silent in the dark for periods longer than one to 3 sec and that the 'dark discharge' consisted of short bursts of spikes not unlike those evoked by air puffs onto the antennae. Indeed if the antennae were unrestricted and free to undergo the 'searching'-type sweeps that occur naturally in the free animal the 'dark discharge' was more noticeable. It is therefore postulated that in this unit the firing that occurs without visual stimulation is due to activation of sensory hairs on the antennae which are stimulated whenever they come into contact with obstacles near the head. The sensory hairs on the antennae rather than any 'position sense' mediated by muscles would appear to operate in this case as the reaction was abolished if the antennae were held down firmly so that 'struggling' and presumably muscle activity occurred. In such a condition touching the hairs with a bristle evoked spikes in the cord unit.

C. *Compound-eye and antennal, multimodal, bilateral phasic ON-unit*

This unit was similar in its response to visual stimulation to A above, but ON-unit type C was always evoked from a previously silent unit. Light onto the contralateral compound eye evokes a rapidly declining burst of spikes which is generally longer in duration than the response to light on to the ipsilateral compound eye. Repeated ipsilateral presentation rapidly leads to adaptation after three or more repeated presentations. The refractory period lasts for approximately 5 sec and is shortened by contralateral stimulation. Adaptation to contralateral stimulation is less well marked. Simultaneous stimulation of both compound eyes produces a response which is essentially the same as that evoked by contralateral stimulation alone.

Puffs of air onto either antenna result in a phasic burst of spikes. This response is of the same intensity with or without compound-eye stimulation. If an air puff is presented at the same time as uni- or bilateral light stimulation the response to the air puff is superimposed on the visual response. The non-visual response is unaffected by the state of adaptation to a previous series of visual stimuli, but adaptation to air puffs was observed; it was never complete, as in the case of ipsilateral compound-eye stimulation, one or two spikes always remaining (see Fig. 3A).



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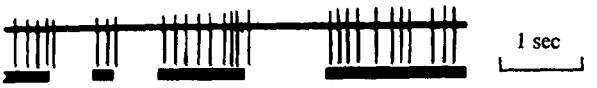

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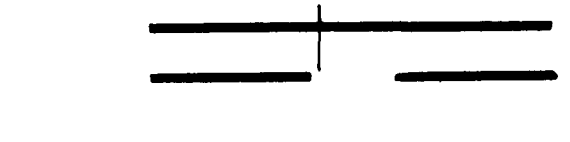
Fig. 3. (A) ON-unit C. *i*, ipsilateral stimulation, *c*, contralateral stimulation. Black bars indicate ON-period, arrows represent duration of air puffs on to the antennae. Top two traces; consecutive stimulations: note tightly coupled, phasic ON-burst to contralateral stimulation and rapid adaptation to repeated ipsilateral stimulation. Third trace; effect of air puffs onto the antennae, animal in the dark. Fourth and fifth traces; puffs superimposed on periods of light stimulation. (B) ON-unit D. Tightly coupled tonic responses during light-ON (black bars).

D. *Compound-eye tonic ON-unit*

Units of this type always involve the compound eyes and can be either contralateral or bilateral. Often the degree of inhibition is variable but usually there is a complete cessation of firing after light OFF (see Fig. 3B).



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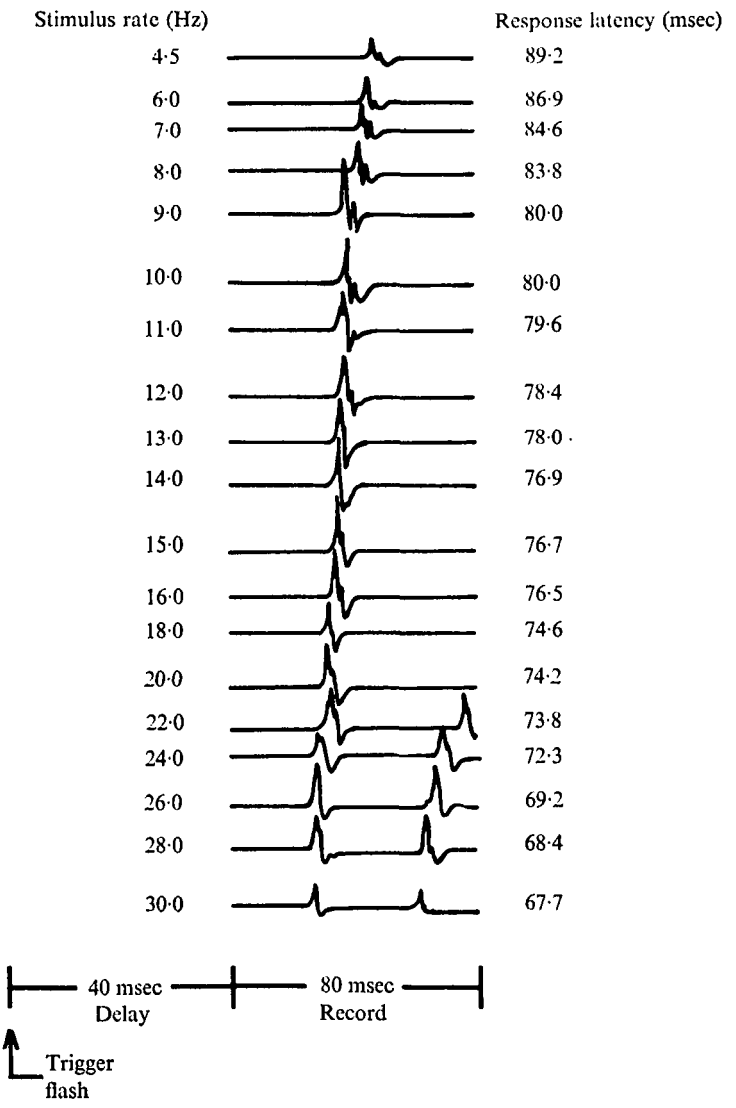

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Fig. 4. (A) A typical single-spike OFF-response exhibited by both ocellar and compound eye phasic OFF-units. (B) Ocellar phasic OFF-responses. A series of averaged responses to individual flashes in a train at rates indicated in the left-hand column. Note that the Biomac computer was programmed to start its cycle at a flash, wait for 40 msec before starting to digitize the waveform and to record for 80 msec before stopping and waiting for the next stimulus trigger. Thus at 22 Hz and above two responses are recorded during the 80 msec sweep period because a second flash has occurred within the 40 msec delay period without re-triggering the computation cycle.

OFF-units

A. *Phasic OFF-units*

Units of this type, firing with from one to four spikes at OFF, were the commonest visual units recorded from the cockroach cord at level S–T_1 and T_1–T_2 (see Fig. 4A).

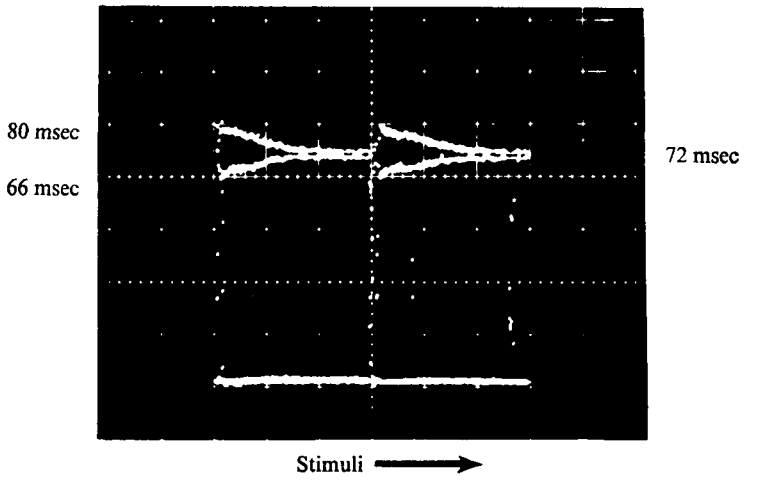
Both compound-eye and ocellar units are represented in this category. Generally ocellar units fire to stimulation of the contralateral ocellus although occasionally ipsilateral stimulation evoked a response in certain fibres. Ocellar units were never shown to be bilateral. Phasic OFF compound-eye units were less common than the ocellar types but ipsilateral and contralateral units were equally numerous and always unilateral. No ocellar/compound-eye interaction was noticed.

The compound-eye units are represented by more than one type. Some adapt very rapidly to repeated stimulation; others will only respond to a second light OFF stimulus if it follows the first after about 5 sec. Still others will apparently never adapt and will follow strobe-light flashes up to the reported flicker fusion frequency (FFF) of the compound eye or, in the case of the ocellar phasic OFF-unit, which also showed this lack of adaptation, up to the FFF of the ocellus.

A1. *Ocellar phasic OFF-unit*

This ocellar OFF-unit is characterized by a short burst of spikes at light OFF. The number of spikes in the burst depends upon the decay time of the light pulse; thus with strobe-light flashes (discharge-tube light-pulse duration of from 6–8 μsec) one or two spikes only occur whereas with a slowly dimmed filament bulb a short train of spikes can result, especially if the dimming is stepped. In this case spikes will be evoked at each step until a threshold level is reached below which no response occurs. The latency of this unit was found to be inversely proportional to the rate of flashing of a strobe-light up to about 55 Hz, when fusion typically occurs. The FFF recorded at the cord was found to vary between 45 and 55 Hz, for different individuals, with a mean value of about 51 Hz. This compares well with the figures reported by Ruck (1958) for the FFF of the cockroach ocellus. Typically the latency was 120 msec at less than one flash per second, shortened to 88 msec ($\pm$ 12 msec) at 5 Hz and progressively to 53 msec ($\pm$ 2 msec) at 50 Hz (see Fig. 4B). These values were constant for any particular unit after the first minute or so of stimulation. Variation from individual to individual was most marked at low frequencies, $\pm$ 12 msec at 5 Hz for nine individuals, and this declined progressively to a value of $\pm$ 2 msec, for four individuals at the highest frequencies.

Changes in the intensity of the flash cause similar changes in latency, while at very low frequencies, one OFF-period every few seconds, the latency depends partly on the length of the ON-period, being longer with short ON-periods and shorter with long ON-periods. Sudden changes in flash rate cause rapid increases in the latency of the response (exceptionally up to 100%) but this returns gradually over a period of 1–2 min to a new steady value corresponding to the latency appropriate to the new flash rate. The state of light or dark adaptation of the ocellus prior to stimulation with light flashes affects the latency of the response, there often being an increase in latency with increase in background illumination. If the dark-adapted ocellus is suddenly exposed to a train of high-intensity flashes the latency of the response shows two preferred



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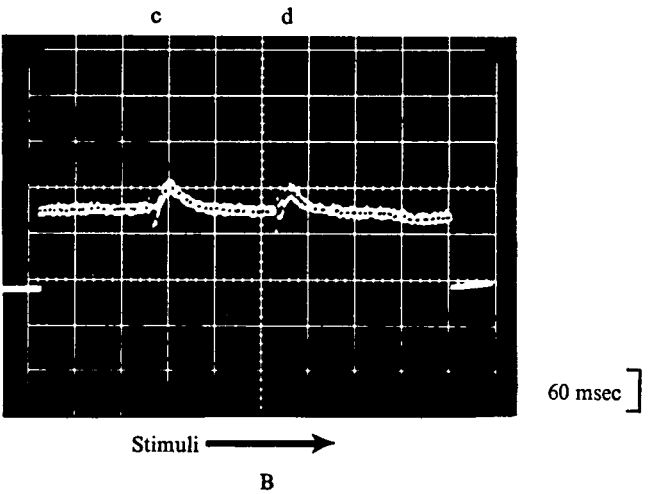

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Fig. 5. Latency histograms. In both these records a stimulus (flash) causes counts to be added to a store location at a pre-set rate until the response terminates the counting. The computer then waits for the next stimulus before adding counts into the next store location to the right (X-axis). In both cases responses to 1000 stimuli are recorded sequentially. Thus the X-axis represents the stimulus number and the Y-axis the latent period. (A) A dark-adapted animal was exposed at the start of the experiment to a 15 Hz flash train and responses were recorded for 500 flashes; the animal was then returned to the dark for 5 min and re-exposed to the flash train (midway along X-axis). It can be seen that initially the responses show two preferred latent periods which reduce to a value of 72 msec within about 250 stimulus/response cycles. (B) This shows the effect of exposing a light-adapted ocellus to a similar flash train. The ocellus and the photocell trigger are shielded from the flashes at points *c* and *d* for approximately 30 sec and then re-exposed. Latency shortens initially, lengthens and then returns to a steady value.

values which centre on a mean value appropriate for the particular flash rate and alters over the next 40 sec of stimulation in such a way as to approach the mean value (see Fig. 5A). If stimulation is stopped and the ocellus is kept in the dark for about 2 min, similar oscillations occur on re-exposure to the strobe lamp. Covering or simultaneously exposing the compound eyes has no noticeable effect on this response, which occurs at all frequencies below FFF. The results of such an experiment are shown in Fig. 5A. A latency histogram for 500 responses to flashes delivered at a rate of 15 Hz from an animal with a previously dark-adapted ocellus is shown. It can be seen that initially the responses occur alternately at two different instances in time after each stimulus corresponding to latencies of 80 msec and 66 msec and gradually shift to reach a steady value of approximately 72 msec in about 40 sec. Dark adaptation and subsequent re-exposure results in a repetition of the effect. If, however, a light-adapted ocellus is shielded from the strobe lamp for a short period, but not allowed to dark-adapt, on re-exposure the latency shortens initially, rapidly lengthens and then slowly returns to its original steady-state latency appropriate for the particular flash frequency (see Fig. 5B).

Intracellular recordings from ocellar OFF-units typically show a single spike at OFF but on occasion two or three spikes may occur especially at low flash rates (1 Hz), separated by between 2 and 6 msec. With a slow OFF or with dimming, more spikes may result, but generally these are separated by longer than 8 msec. Extracellular records sometimes reveal spikes of different amplitude. It is assumed that in these cases two or more fibres lying close together are carrying similar information (see Fig. 6A). Movement of the electrode will often increase the amplitude of one at the expense of the others.

Two types of double spiking were identified. One occurred at frequencies just below the FFF and consists of small numbers of spikes separated by time periods in the order of tens of milliseconds (see Fig. 6B). At and beyond the FFF responses were still recordable but appeared to be random in time. However, when such signals were averaged by triggering the Biomac 1010 from the light pulses they were found to be responses to discrete flashes in the flash train. For example, at 60 Hz (stimulus separation 16·67 msec) the spikes occurred every 33·8 msec $\pm$ 1·1 msec or very nearly one response every other flash. Response latencies were found not to be constant with time and if the latency was such that the second flash fell within the refractory period following the first response the unit would fire to every third flash, for example. Because these variations in latency were superimposed on a steady flash train, responses appeared as shown in Fig. 6B with occasional spikes appearing close together (responses to the first and third flashes in a consecutive sequence) and with longer spaces between such groups (more than one flash falling within the refractory period following a previous response).

The second type of 'double' spike response can be identified after signal averaging at flash frequencies below 15 Hz. At this frequency one of a pair of spikes may fail and that which remains alternates between two temporal positions originally occupied by the two spikes individually (see Fig. 6C). Here two single spikes firing to adjacent flashes at 15 Hz are shown to sum at two points in time (X-axis). This same effect is apparent in the latency histogram shown in Fig. 5B where a double row of dots represents the two latencies.

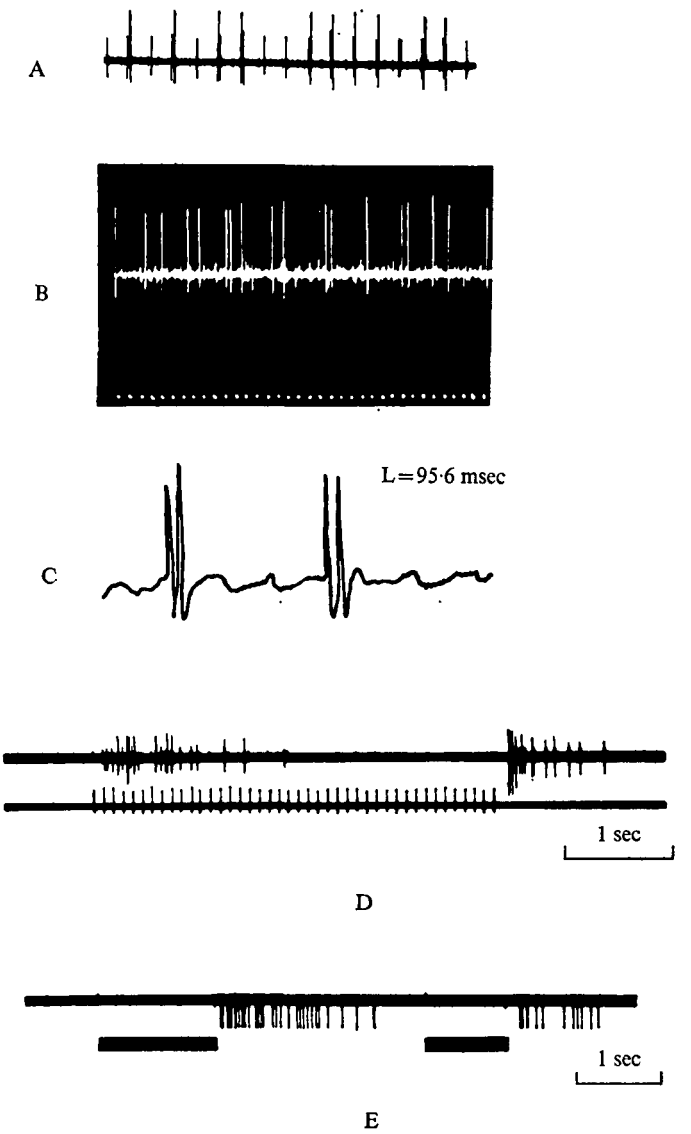

Fig. 6. (A) A series of multiple responses to a train of flashes; a difference in spike height indicates that responses from more than one fibre are recorded. (B) Double spikes recorded at a frequency close to FFF. White dots indicate time of flashes. (C) A Biomac average of two responses in a flash train (15 Hz). Note that single spikes have here summed into two bins in such a way that the averaged response appears to show two sets of double spikes. Close correspondence of 'spike' height indicates that the single spike fell equally in the two different time bins. (D) Compound-eye phasic OFF-unit A 2. Note that the large spikes follow certain of the stimuli (lower trace; photocell responses) whereas the small spikes appear to 'see' the flash train as an ON-period–ON-burst. An intermediate-sized spike bursts at the end of the flash train as does the large spike. (E) Compound-eye phasic, bursting OFF-unit. Black bars beneath trace indicate ON-periods.

It must be mentioned here that certain of the above effects have been noted to occur in the ocellar electroretinogram where the OFF-spikes appear to exhibit similar properties. These are described in a later paper (in preparation).

A2. *Compound-eye phasic OFF-units*

These are less frequently encountered in the thoracic cord than the ocellar phasic OFF-units described above and as a result their properties have not been studied in such detail. However, it is thought that those compound-eye OFF-units which follow a strobe light without adapting show a longer latency than the ocellar phasic OFF-units with similar properties, by as much as 20 msec throughout the flicker response range. It would seem, therefore, that ocellar OFF information reaches the thoracic ganglion before similar information from the compound eyes even at high rates of flicker, but generally the FFF of 50–60 Hz for the compound-eye phasic OFF-response is slightly higher than the FFF for the ocellar phasic OFF-unit.

Although some of the compound-eye phasic OFF-units will follow a strobe light others have been shown to respond only to flash rates below one every 1–5 sec. This type of response is shown in Fig. 6D where a number of units are responding to a short burst of strobe flashes delivered at a rate of 7/sec. It will be seen that whereas some follow most of the flashes others respond to individuals in the train; still others 'see' the whole flash burst as an ON-period and fire only at the apparent OFF.

It is doubtful whether these phasic OFF-units are involved in movement detection although on certain occasions OFF-spikes were evoked by sharp shadows moving across the eye. OFF-units of type C below are thought to be of greater importance to the animal in this respect.

A3. *Compound eye phasic, bursting OFF-unit*

These units fire with more than three spikes to a rapid OFF-stimulus depending upon some unknown condition (see Fig. 6E). They are not to be confused with those units which fire similarly to a dimming light stimulus (see C below). They are not sensitive to shadows moving across the eye. They are compound-eye units and have been recorded only occasionally from S–T_1 and from T_1–T_2, with or without the ocelli covered. It is believed that ocelli can give this type of OFF-response but no conclusive evidence is available. The length of the burst at OFF is proportional to the length of the previous ON-period up to approximately 2 min, when longer ON-periods tend to evoke the same number of spikes, usually up to 25.

B. *Ocellar unit with phasic OFF-component and sustained dark-firing*

This ipsilateral ocellar OFF-unit has both phasic and tonic components and has been found on only six occasions, always at S–T_1 separated by many weeks of experimentation.

There is always a tightly bound OFF-response to a sharp OFF which, like A1 above, will follow a strobe flash up to between 45 and 55 flashes/sec. For this reason it can easily be confused with phasic OFF-unit type A1, but after some time in the dark, greater than 7 sec, spikes reappear at a frequency ranging from 3/sec to one every 2–3 sec in irregular bursts for as long as the dark period is maintained. It has been recorded for periods in excess of 30 min without any apparent adaptation and has

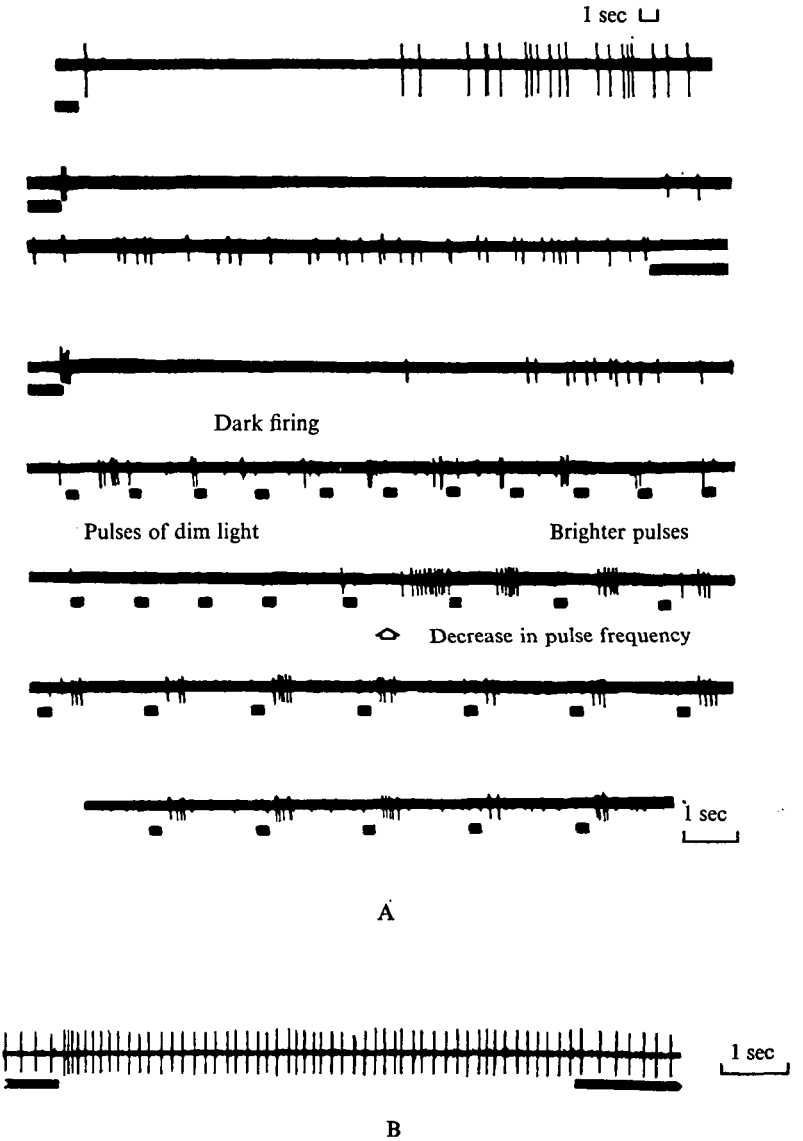

Fig. 7. (A) Ocellar phasic OFF-unit with tonic dark firing. Top trace; typical response characteristics of the unit. Second and third traces; consecutive traces showing a longer period of dark-firing and cessation at light ON. Black bars beneath traces represent ON-period. Fourth to eighth traces; fourth as second, showing dark-firing; subsequent traces show the effect of pulses of light on the phasic and tonic components. Time marker at end of eighth trace refers to all traces except first. (B) Compound-eye OFF-unit D. Black bars indicate ON-period.

been shown to have repeatable properties for periods of up to 3 h. The dark-firing is immediately inhibited by light above a threshold intensity, the phasic OFF response being evoked only if the intensity drops rapidly to OFF or to a level below that threshold.

The number of spikes in the original phasic OFF-burst varies from one for a sharp OFF (near square pulse from a shutter) to 5 or 6 spikes at a high frequency following

a slow intensity reduction (dimming) to OFF. The single spike response never adapts to single high-intensity flashes, but the initial OFF-burst can be lost if dimming is sufficiently slow, although even in this case the dark-firing remains. The length of time between the phasic and tonic components may be related to the 'amount' of light (intensity or exposure time) during the preceding ON-period, i.e. if the preceding ON-period was bright and long the silent phase is also long (up to 30 sec) but if the ON-period is shorter this silent phase is much reduced. If short pulses of light are presented at regular short intervals the OFF-burst is followed, fairly rapidly, by the dark-firing which gives a bursting response close to the ON-phase (see Fig. 7A). There appears to be an ON-component in addition, but this may well be due to interaction between the phasic and tonic OFF-components.

Maintaining light onto the compound eyes or contralateral ocellus during ipsilateral ocellar stimulation does not appear to alter the properties of this unit, either in the time to the dark-firing or in the rate of firing in the dark. However, the non-firing period between the initial OFF-burst and the dark-firing appears to be linked to the 'amount' of light which falls onto the ipsilateral ocellus prior to light OFF.

C. *Compound-eye dimming and movement-sensitive unit*

This unit responded with a short train of spikes to dimming and to movement of a small target in any part of the contralateral visual field. It was encountered in about 10% of the preparations. Only rarely did sudden OFF-stimuli evoke a response in this unit and no evidence of directional sensitivity was noted.

Rapid or jerky movements gave sharp bursts of two or more spikes to each displacement of the target; slow steady movements tended to evoke low-frequency trains but very slow movements failed to elicit spikes.

Similar units have been described from the ventral cord of *Schistocerca* and *Acheta domesticus* by Palka (1967 and 1969 respectively). Type C units may be similar to those studied by Rowell & Horn (Rowell & Horn, 1967, 1968; Horn & Rowell, 1968) in the tritocerebrum of locusts and in the locust cord (Rowell, 1971*a*, *b*, *c*).

A detailed examination of these movement-sensitive units was not attempted.

D. *Compound-eye stimulatory tonic OFF-unit*

These units were recorded from about 15% of preparations at S–T_1 and T_1–T_2 and were equally responsive to stimulation of either eye. They fired continuously during light-ON, but at light-OFF there was a burst of firing which slowly returned to the ON level. This rate of return could be increased by shortening the light period with respect to the dark period (see Fig. 7B).

ON–OFF-units

Units in this group are unimodal but show both phasic and tonic components.

A1. *Compound-eye phasic ON–OFF-unit with tonic OFF-firing*

Units of this type have been recorded in about 5% of preparations from T_1–T_2. They are thought to fire under ipsilateral compound-eye stimulation. The short burst at ON soon adapts, usually in under 2 sec, to give single random spikes. The small OFF-burst declines over approximately 7 sec to give a tonic low-frequency discharge throughout the OFF-period (see Fig. 8A).

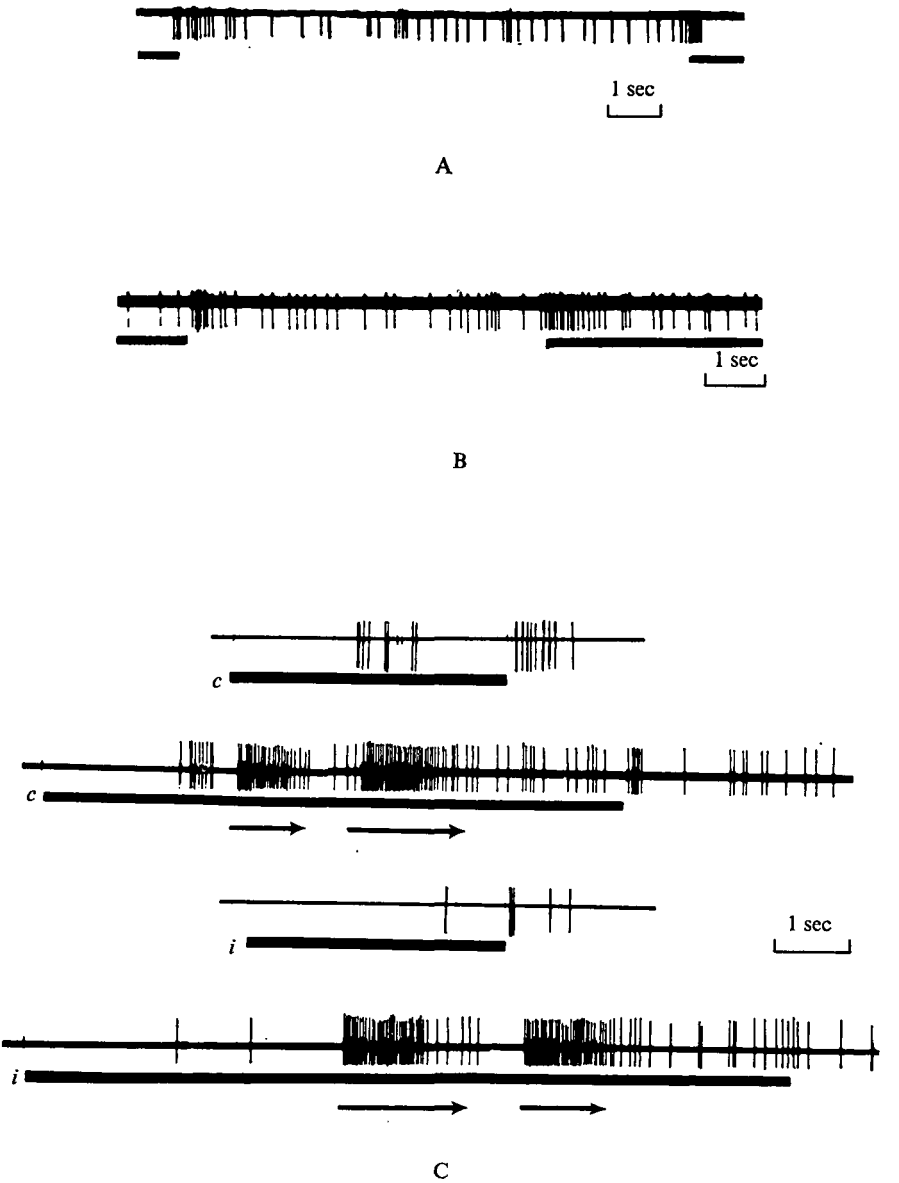

Fig. 8. ON–OFF-units. (A) ON–OFF-unit A 1. (B) ON–OFF-unit A 2. (C) ON–OFF-unit B. Note that the first two traces show the effects of exposing the contralateral (c) compound eye to light and the lower two traces show ipsilateral (i) responses. In the latter case the ON-component is very weak and loosely coupled to the stimulus. The bottom trace in each pair shows the effect of air puffs (arrows) on to the antennae during visual stimulation. Time marker refers to all four traces.

A 2. *Compound-eye phasic ON–OFF-unit with tonic light- and dark-firing*

This unit differs from A 1, firing continuously in the light and dark at about the same frequency with burst of approximately equal intensity at ON and OFF, declining rapidly to the basal frequency in under 1 sec. It therefore monitors sudden increases or reductions in the background illumination without indicating the direction of the change (see Fig. 8B). Only three such units were recorded.

B. *Contralateral compound-eye ON–OFF: ipsilateral compound-eye OFF, multimodal unit*

Units of this type were found fairly frequently in about 25% of all preparations at S–T_1. Contralateral stimulation (see Fig. 8C) resulted in a loosely bound ON-response followed by widely spaced random spikes during the ON-period and a short, tightly bound, OFF-response. With ipsilateral stimulation only the OFF-response was evoked. Air puffs on to either antenna elicited a reaction similar to the OFF-response and light on to either compound eye during the puff had no effect on the intensity of the response to antennal stimulation.

DISCUSSION

ON-units

Pure ON-units have rarely been reported from insects and in all cases they relate to compound-eye stimulation.

A tonic ON-unit exhibiting exactly the same characteristics as ON-unit A is also represented in the locust cord (Catton & Chakraborty, 1969, see their Fig. 1*a*) and in the optic lobes (Burtt & Catton, 1960) of *Schistocerca*. Swihart (1968) recorded a pure tonic ON-response from the medulla interna of the optic lobes of *Heliconius erato* and a pure phasic ON-fibre from the protocerebrum of the same animal which fired with trains of spikes, the number of spikes in the burst being proportional to the light intensity up to about 20 spikes/On-response.

Northrop & Guignon (1970) have reported recording a number of units, which fired to light ON, from the optic lobes of *Romalea*. These *L*-units (light-units) were monocular and constituted 37% of all units they studied. Two types were distinguished, tonic *L*-units and ON-type *L*-units. They also recorded a limited number of units of the former type which exhibited an ON-gated mechano-response, there being another, smaller, burst of firing to mechanical stimulation following light ON. Although this type of response was not recorded from the cockroach cord a light-gated antennal unit was recorded on two occasions; mechanical stimulation (air puffs onto the antennae) only elicited a response if the animal was in the light. The ON-gated mechano-response recorded by Northrop & Guignon failed after several seconds of light ON (light steady state) and was not observed in the dark (dark steady state).

Phasic ON-unit B, which in the cockroach is binocular and multimodal, appears to be similar to the ON-type *L*-units of Northrop & Guignon (1970) recorded from the optic lobes of *Romalea*, the ON-units of Burtt & Catton (1960) from locust optic lobes, and from the locust cord by Catton & Chakraborty (1969).

OFF-units

Dingle & Caldwell (1967) comment that OFF-stimuli were the most effective visual stimuli when recording from the cockroach protocerebrum. The commonest visual units to be recorded from the thoracic ventral nerve cord connectives in the cockroach, using glass microelectrodes, were OFF-units.

Ventral cord OFF-units have been studied by Burtt & Catton (1954) who found both median ocellar and compound-eye phasic OFF-units in *Locusta* cord similar to those reported here. The survey of single-unit responses by Catton & Chakraborty (1969) in *Schistocerca* included units similar to the above but they comment that ocellar responses were rarely found.

Ocellar OFF-units

Hoyle (1955) mentions three types of ocellar response recorded at the level of the circum-oesophageal commissures of *Locusta*, a continuous and irregular 'dark discharge' which was reduced during illumination of the ocellus, a brief discharge to both increases and decreases in the intensity of ocellar stimulation (ON- and OFF-discharges) and thirdly an OFF-discharge which was distinguishable from, and closely followed by, the dark discharge. Burtt & Catton (1954) also recording from the whole cord, here in the thorax, report recording OFF-discharge only; there was no maintained dark discharge or response to movement in the ocellar visual field; however, the OFF-unit followed a flickering light up to 30 flashes/sec. No ON-responses were noted. Goodman (1971*a*, and personal communication) has found a phasic OFF-unit, ON–OFF-units which fire to dimming and brightening of the ocellar field and another which gives a sustained burst in response to illumination with a very slow rate of adaptation.

Only two ocellar units have been recorded here from the cockroach cord, the phasic OFF-unit and another which, not unlike that reported by Hoyle (1955) from the circumoesophageal connectives of *Locusta*, gave a rapid burst at OFF followed by a maintained dark discharge. The unit from the cockroach differs from that in *Locusta* in that firing is completely suppressed by illumination of the ocellus and not merely reduced. An ocellar unit with similar properties was recorded by Suga & Katsuki (1962) from the thoracic cord (at S–T_1) of the grasshopper, *Gampsocleis buergeri*. This would also respond to single light flashes or to a flash train with a short burst at OFF and showed spontaneous dark-firing after several seconds in the dark. They comment that the quiescent period could be increased by increasing the light intensity during the ON-period or by increasing the flash length or rate. Their units were recorded from the contralateral connective and also responded with an OFF-burst to stimulation of the median ocellus. Cauterisation of the median ocellus reduced the intensity (number of spikes) of the lateral ocellar OFF-response by about one third. Chalmers (1971) reports recording ocellar units from the thoracic cord of *Schistocerca gregaria* which fired phasically at light OFF and tonically after many seconds in the dark. No ocellar ON-units were recorded here from the cockroach cord, or from the locust cord by Catton & Chakraborty (1969), but they mention a unit which showed inhibition of a steady firing rate with illumination of the ocellus. Such a unit was not recorded from the cockroach.

Compound eye OFF-units

A common compound-eye OFF-unit was the phasic OFF-unit A2. Similar units have been recorded by Burtt & Catton (1954) and Catton & Chakraborty (1969) from the cord of *Locusta* and from *Schistocerca* by Goodman (personal communication) and by Cosens (1966).

Bursting OFF-units (OFF-unit A3) similar to those reported here have been recorded by Dingle & Fox (1966) from the brain of *Gryllus domesticus*, but they found a continuous discharge in the light and dark with phasic burst at light OFF superimposed upon this discharge. The duration of the OFF burst was linked to the length of the previous ON-period as in the cockroach cord.

Dimming and movement-sensitive units recorded from the cockroach appear to be similar to those recorded from the cord of *Schistocerca* (Palka, 1967; Catton & Chakraborty, 1969), responding to jerky movements within the visual field. All these units are probably similar to those studied by Rowell (1971 *a*, *b*, *c*).

The stimulatory tonic units reported here (OFF-unit D) exhibit properties similar to those of the 'nett dimming units' recorded by Northrop & Guignon (1970) where light on to the ipsilateral eye inhibits firing. In the cockroach this inhibition was never shown to be complete although very few units of this type were encountered.

ON–OFF units

ON–OFF-units A1 and A2 were similar to sustaining units reported from crustaceans by Wiersma & Yamaguchi (1966) and by Dingle & Fox (1966) from the brain of *Gryllus domesticus*. A1 was probably more like the latter authors' dark unit (they only recorded from two such fibres) which had a higher spontaneous frequency in the dark than in the light (as here) with ON- and OFF-bursts, but A2 was more like the classical sustaining unit firing at a rate proportional to the intensity of stimulation and often with high-frequency bursts at ON and OFF followed by a decline to a new level appropriate for the steady-state light intensity. The properties of unit A2 were also similar to those of Burtt & Catton's (1960) D-units which gave both ON- and OFF-bursts and a dark-adapted discharge rate either equal to or greater than the light-adapated rate. The units from the cockroach cord were not shown to respond to movement stimuli as were the D-units of Burtt & Catton.

ON–OFF-units A1 and A2 may also be similar to the multimodal units recorded by Northrop & Guignon (1970) where ON- and OFF-bursts and erratic firing in dark and light steady states occurred as well as responses to touching the body with a fine paint brush. The monocular, cockroach ON–OFF-units of type A were never shown to be multimodal.

ON–OFF-unit type B was the most complicated, in the sense of diversity of input, that was found in the cockroach cord, and a similar unit has been recorded in the locust cord by Catton & Chakraborty (1969, see their Fig. 1*c*). In the cockroach these units were always multimodal.

Of the thousands of fibres known to exist within the ventral nerve cord of the cockroach (Guthrie & Tindall, 1968) only a few have been recorded from. For the purpose of this study they can be divided into two classes, viz: (a) giant fibres (> 15 μm and < 60 μm in diameter) and others of large diameter (> 5 μm), and (b) small-diameter

fibres ($< 5\ \mu$m). Giants, or their anterior projections (Dagan & Parnas, 1970), were frequently encountered and were sometimes penetrated so that intracellular recordings were possible from these fibres. The only other types of fibre which were definitely penetrated, as indicated by the size of the recorded action potential, were pure antennal units and very occasionally pure ocellar OFF-units. It can therefore be assumed that these fibres are of large diameter also. Other antennal and visual fibres were generally thought to be smaller in size although the use of microelectrodes enabled good records to be obtained from such fibres. There must be a large number of units passing between the thoracic ganglia in each connective which were not recorded from; one suspects that it is these fibres which control and mediate the behaviour of the motor neurones. It is possible that the giant fibres only serve to stop on-going activity prior to the arrival of the pre-motor information.

Goodman (1971*b*) has reported recording ocellar and compound-eye phasic OFF-units from the cord of *Schistocerca gregaria* and mentions that the ocellar OFF-unit is amongst the largest that can be recorded from the ventral connectives in that animal. She suggests that ocellar OFF-spikes are carried by one or more of the large fibres in the cord; and it would seem likely that also in the cockroach fibres which are easily penetrated and encountered most frequently during unit searching are of large diameter, and this would indicate that many of the units reported here were recorded from the larger members of the axon population. One can be more specific than this; the contralateral OFF-unit may possibly be one of the descending 'giant' fibres. This is not to imply that all visual units are large-diameter fibres, there must be many hundreds of smaller fibres from which recording is impossible using the present techniques and some of these are, no doubt, visual units.

The ventral nerve-cord units reported here have been found to show repeatable general properties in a number of individuals. Slight changes in the latency of the response or in the discharge frequency have been taken as acceptable, bearing in mind that comparisons were being made between different individuals.

In all cases single units were identified during recording either because of their large amplitude relative to other units picked up simultaneously or because they were the only spike events recorded at the time, i.e. the fibre concerned was so close to the electrode tip that it was the only fibre recorded. Two or more units showing similar properties were occasionally recorded at the same site; usually they were OFF-units, but there was no real indication of association of fibres into tracts within the nerve cord with similar functions as has been found in the brain of insects (Collett, 1970).

Ocellar OFF-units have been reported from both the contralateral and ipsilateral connectives in the locust cord, and Satija (1957, 1958), on the basis of anatomical studies, considers that ocellar fibres enter both thoracic connectives. Patterson (personal communication) has recently found electrophysiological evidence for this in *Schistocerca*. Chalmers (1971), recording from locusts, and Suga & Katsuki (1962) from another orthopteran, the grasshopper *Gampsocleis buergeri*, report finding only contralateral ocellar fibres, at least from the lateral ocelli. In the cockroach, contralateral ocellar units are by far the most frequently encountered ocellar units in the cord. Occasionally ipsilateral ocellar units are encountered but these may really be contralateral units fired and recorded as a result of light spread or poor isolation of the ocellus

under test. This does not exclude the possibility of there being ipsilateral ocellar fibres in the thoracic cord but it would suggest that if they exist they are small-diameter fibres and few in number. Ocellar OFF-unit B is exceptional.

In the cockroach pure ocellar units appear to be the rule. In *Schistocerca* Goodman (1971*b*) has reported interaction between ocellar and compound-eye units, the former affecting the latter, and Mimura *et al.* (1970) have recorded antennal units which are affected by ocellar stimulation in the brain of the fleshfly. The reported interactions would indicate that in Diptera ocellar fibres pass to regions of the brain neuropile concerned with antennal and compound-eye unit integration. Power (1943) has in fact reported that a branch of the ocellar tract on each side of the brain runs in the posterior optic tract of the compound eye in *Drosophila melanogaster*.

Multimodal units, both from the brain and ventral nerve cord, have been recorded by most workers who have studied arthropod nervous systems. However, along with these fibres there are large populations of units which only fire to stimulation of a single receptor modality. These units are of importance in monitoring activity in a specific receptor or the change in a single stimuls parameter, and it can be assumed that this information is utilized in a pure form. As multimodal units carry information from two or more sense organs, it must be assumed that all inputs are treated similarly by the effector neurones driven by these fibres. On the other hand it may be that these fibres are capable of 'filtering' or 'routing' information along different terminal branches to separate sites, and recently such a role has been tentatively suggested for giant axons where narrow regions of the fibre have been shown to block high-frequency signals in a particular direction (Parnas & Dagan, 1971).

No real differences which could be correlated with the nocturnal habits and weak flight of cockroaches have been found between the units reported here and by other workers on diurnal, fast flying insects.

SUMMARY

1. Visual and multimodal units were recorded from the thoracic nerve cord of the cockroach, *Periplaneta americana*, using glass microelectrodes.

2. Compound-eye units could be classified as ON-, OFF- or ON–OFF-units according to their response to visual stimulation. Some were multimodal, firing to both visual and tactile stimulation of the antennae.

3. Although some units were found to be either fired by ipsilateral or by contralateral stimulation only, others were fired by both types of stimulation, often in different ways.

4. Ocellar units were invariably OFF-units, mainly phasic, but one type showed tonic dark-firing in addition to the phasic OFF-burst.

5. The general properties of cockroach visual units are discussed and compared with those reported by other workers for different insects.

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