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ELECTRICAL RESPONSES TO VISUAL STIMULATION IN THE OPTIC LOBES OF THE LOCUST AND CERTAIN OTHER INSECTS

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Action potentials in the ventral nerve cord of the locust, elicited by visual stimulation, have been described previously (Burtt & Catton, 1954*a*). The present paper describes visual responses recorded from the optic lobe by the insertion of an insulated microelectrode through the corneal surface; a preliminary report has already appeared (Burtt & Catton, 1954*b*). Autrum (1950) has described the slow illumination potentials ('Belichtungspotentiale') in the eye of the blowfly *Calliphora* in response to light 'on' and 'off' stimulation, using a relatively coarse intraocular electrode. Other workers (e.g. Bernhard, 1942; Crescitelli & Jahn, 1939, 1942) have recorded potentials from superficial electrodes on the optic lobe of the grasshopper. Adrian (1937) made a study of the rhythmical discharges from the optic lobe of the waterbeetle *Dytiscus*, and noted also the presence of spike discharges.

Micro-anatomy of the insect optic lobe

The optic lobe of insects, which is interposed between the compound eye and protocerebral ganglion on each side, is a very complex organ. In general plan (see Fig. 1) it consists of three so-called 'ganglia', each made up of a central mass of fibres and synapses, surrounded by a layer of neurones. The first ganglion lies close under the 'retina' (the layer of retinula cells of the ommatidia) from which it receives numerous fibres. Between the first and second, and the second and third ganglia are zones of crossing fibres (chiasmata). Fibres then pass from the optic lobes into the protocerebral ganglia. The spatial distribution of the neurones and synaptic layers varies in different species, but all conform to this general pattern. The first, second and third 'ganglia' will be termed 'synaptic regions' in this paper. The spatial relations of these in *Locusta* are shown in Fig. 2. Measured along a direction axial to

the optic lobe, the depths of these regions from the corneal surface are 0·75, 1·25 and 1·75 mm respectively.

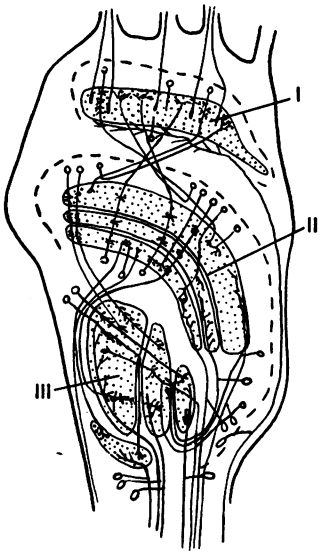

Fig. 1

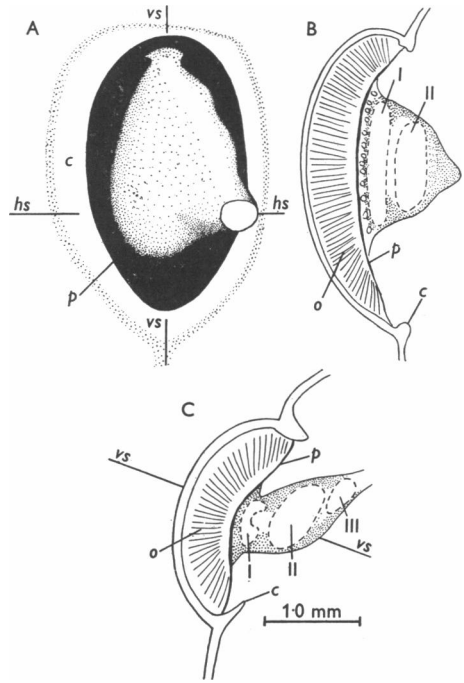

Fig. 2

Fig. 1. Diagram of neurone connexions in the optic lobe of *Aeshna* (redrawn from Zawarzin, 1914, with some connexions omitted). I, II, III, 1st, 2nd and 3rd synaptic regions. (The interrupted lines indicate the limits of distribution of the neurone somata.)

Fig. 2. *Locusta*. (A) Inner surface of compound eye and attached optic lobe severed from brain. (B) Median vertical section of eye and optic lobe, at plane *vs* in (A). (C) Horizontal section of eye and optic lobe at plane *hs* in (A). *c*, cuticular shelf supporting eye; *hs*, plane of horizontal section; *o*, ommatidial region; *p*, eye pigment; I, II and III, 1st, 2nd and 3rd synaptic regions of optic lobe; *vs*, plane of vertical section. In B and C synaptic and fibrillar regions of the optic lobe are shown plain and regions of neurone somata stippled.

METHODS

Adult specimens of the migratory locust (*Locusta migratoria migratorioides* L.), grasshopper (*Chortippus brunneus* Thunb.), blowfly (*Calliphora vomitoria* L.), and the dragonfly larva (*Aeshna* sp.) were used in different experiments, but most work was performed on the locust. In order to reduce voluntary movements the insects were lightly anaesthetized with urethane, injected into the body cavity; for *Locusta* 0·15–0·25 ml. of a 10% solution was used, and proportionate doses for the other species. The insect was placed in a groove cut in a cork platform and secured by pins and plasticene. A small hole was cut in the front of the head to receive the indifferent electrode (a silver wire, 0·2 mm diameter). The intraocular microelectrode consisted of a silver wire, electrolytically sharpened in dilute nitric acid, and thrust into a drawn-out glass capillary so that about 5–10μ of silver, about 10μ in diameter, protruded from the glass tip. By careful

heating in a nichrome coil the glass was caused to fuse round the silver, effectively sealing the electrode. In some later experiments indium-in-glass electrodes, as described by Malcolm (personal communication) were used. The resistance of the electrodes, measured in contact with Ringer's solution on a Kohlrausch bridge at 2000 c/s, was 50,000 to 100,000Ω. The microelectrode was mounted on a Zeiss mechanical stage (Fig. 3), having a vernier reading to 0·05 mm, and this was used to control the depth of penetration. Before insertion a small pilot hole was made by puncturing the cornea with a fine steel needle (Fig. 4); the microelectrode could then be inserted with no obvious resistance to movement. The extent to which the soft tissues were pushed in front of the electrode during its advance was uncertain. Sections prepared subsequently showed

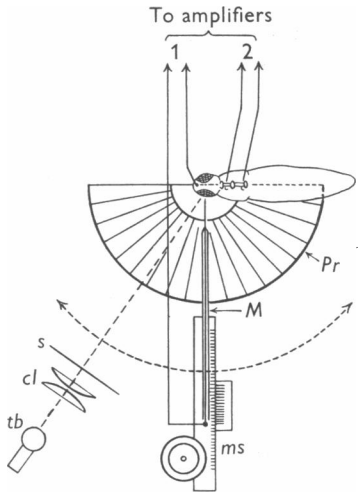

Fig. 3

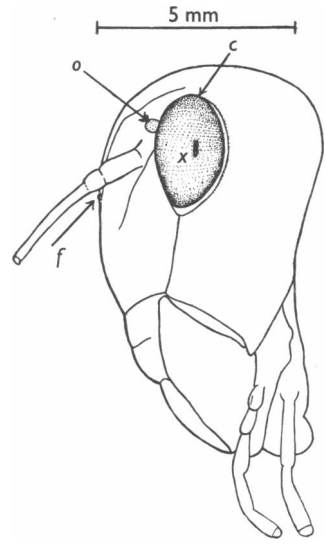

Fig. 4

Fig. 3. Diagram of experimental apparatus. *ms*, Zeiss mechanical stage with 0·05 mm vernier scale; *M*, microelectrode; *Pr*, protractor scale; *tb*, 6 V tungsten-filament bulb; *cl*, condenser lens; *s*, movable shutter. The electrode, mounted on the stage, could be swung through an arc as shown by the dotted line.

Fig. 4. *Locusta*. Lateral view of head. *c*, compound eye; *f*, position of frontal ocellus; *o*, lateral ocellus; *x*, usual point of entry of electrode.

no evidence of distortion, and it was often difficult to identify the electrode track. A check was always made on withdrawal to see that the depth reading was the same at contact as on insertion, so as to detect any movement of the head during the experiment. Agreement was usually found to within 0·05 mm. Although in most experiments the phenomenon occurring at any given depth during insertion was recorded at the same depth during withdrawal, it was still possible that some displacement of the optic lobe was occurring. The scatter of the results, at the greater depths particularly, may be explained in this way. In later experiments, in which parts of the optic lobe were excised, a physical check on the position of the electrode tip was possible, as this could be seen to emerge from the cut end of the optic lobe. The direction of insertion was in the earlier experiments commonly perpendicular to the corneal surface, the pilot hole being made a little below the geometrical centre of the eye. It was decided later that, in view of the spatial orientation of the optic lobes, other angles of entry might lead to a more axial penetration of

the optic lobe by the electrode, and so a protractor scale was fixed on the platform (Fig. 3), below the insect, to allow the electrode to be directed inwards at various angles in the horizontal plane. In prolonged experiments the head was irrigated from time to time with mammalian Ringer's solution. In some experiments action potentials in the ventral nerve cord were recorded simultaneously on a second amplifier channel. The amplifiers were a.c. coupled, with an overall time-constant of about 0·5 sec, so that some distortion of the relatively slow illumination potentials was to be expected. For recording action potentials only, the time-constant was reduced to 0·1 sec, in order to attenuate low frequency base-line disturbances. The input resistance of each amplifier was 1 MΩ. After each experiment the head was removed and fixed in either formol-saline or aqueous Bouin, and then embedded in gelatine by the methods given by Pantin (1946). Thick sections (100μ) were cut by the freezing method and stained with picro-carmine. The whole eye was thus obtained as a set of sections, and the relationship of the point of entry of the electrode to the underlying nervous structures could be worked out.

In order to obtain an estimate of the degree of shrinkage which occurred with fixation, frozen sections were cut from fresh unfixed material, and the depth of a readily observable layer (the 2nd synaptic region) from the corneal surface was measured and compared with the corresponding depth measurement in fixed material. It was found that agreement was within 5% when compared with Bouin-fixed material, but a greater and more variable shrinkage occurred with formol-saline. The sections shown in Fig. 9 are all taken from Bouin-fixed heads, and in all the later experiments the Bouin fixative was used.

Another variable is the size of the insects themselves. Since these were from cultures kept under identical conditions, little variation in size was to be expected. Measurements on adult female locusts of the same species as used in our work have shown a mean head width of $6{\cdot}43 \pm 0{\cdot}034$ mm (Duarte, 1939). This is a deviation either way of about 0·5%, which is much less than the probable errors inherent in our method of estimating the position of the electrode tip.

The types of stimulation used were by interruption of the light beam falling on the eye (light 'on' and 'off'), and by movement of small white cards in the field of view of the insect's eye (movement stimuli). The movements were performed rapidly but intermittently, to avoid fatiguing the responses.

RESULTS

Electrical responses were obtained from the eyes and optic lobes of *Locusta*, *Chortippus*, *Calliphora* and the *Aeshna* larva. It will be convenient henceforward to use the term 'eye' to include both eye and optic lobe. Two types of response were regularly obtained from the four species, all showing similar characteristics. These were (1) illumination potentials (possibly comparable with the electroretinogram of the vertebrate eye), and (2) action potential spikes. Other types of electrical activity, only seen occasionally and usually developing spontaneously, were (3) regular sinusoidal waves at a frequency of 8–20/sec, in *Locusta* (Fig. 5*a*), (4) large diphasic sharp waves, in *Locusta*, (5) small blunt waves, in *Locusta*, and (6) constant regular spike discharges at a frequency of about 75/sec, in *Calliphora* only (Fig. 5*c*). Since only types (1) and (2) appeared to be directly related to visual stimulation and were of regular occurrence attention was given to these, and the others, when they occurred, were usually neglected.

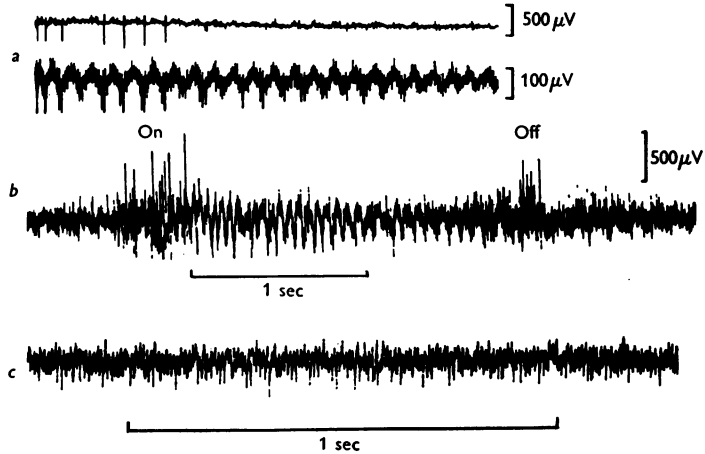

Fig. 5. Rhythms in eyes of *Locusta* and *Calliphora*. (*a*) *Locusta*. Upper trace, ventral nerve cord; lower trace, intraocular record. A spontaneous rhythm in the optic lobe is accompanied by spike discharges, which are particularly evident in the wave troughs. In the early part of the record single spikes in the nerve cord accompany the waves in six cases. (*b*) *Locusta*. Intraocular record, responses to light on and off. There is a low-amplitude resting rhythm of the same frequency as in (*a*). During illumination the frequency and amplitude are increased, but return to normal after light off. Note on and off spike discharges. Electrode tip 1·40 mm deep to surface of cornea. Illumination potentials do not appear. (*c*) *Calliphora*. Intraocular record, showing rapid spontaneous rhythm at about 75 waves per sec. (*a*) and (*b*) retouched.

Illumination potentials

The illumination potentials were relatively slow waves elicited in response to light on and off, each wave lasting about 0·1 sec (Fig. 6). Wave activity of a similar nature was also detected when large movement stimuli were given. In each of the four species studied the illumination potentials underwent a reversal of sign at a critical depth, characteristic of the species. Thus with the electrode tip at, or near, the surface of the eye, the on-wave was negative, the off-wave positive (micro-electrode potential with respect to indifferent electrode: Fig. 6*a* (i)). At the reversal point little or no potential change was recorded (Fig. 6*a* (ii)); deeper than this the waves appeared again, the on-wave now positive, the off-wave negative (Fig. 6*a* (iii)). With progressively deeper penetration, both waves became gradually attenuated (Fig. 6*a* (iv)), after passing through a rather flat maximum. Between the on- and off-waves was a plateau region, the base-line showing a positive deflexion in the superficial recordings, and a negative deflexion at depths below the reversal zone. The exposure given in the experiment from which Fig. 6 is taken was 0·5 sec, so that this plateau may be distorted by the a.c. amplifier. Autrum's records (Autrum, 1950), using a d.c. amplifier, are, however, substantially the same, but he did not observe the reversal of sign which

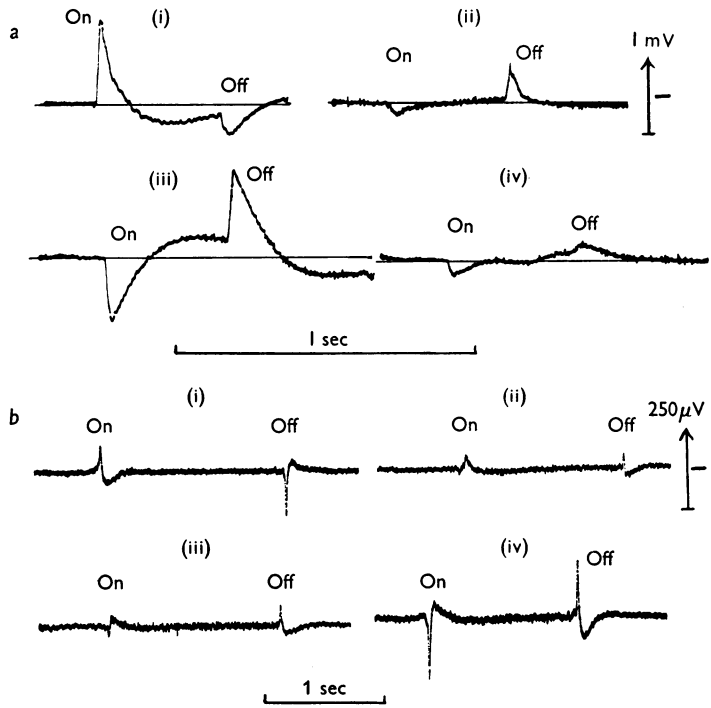

Fig. 6. Illumination potentials recorded by intraocular electrode, at various depths. Stimulation—light on and off. (*a*) *Locusta* compound eye. Electrode tip at depths: (i) 0·5 mm, (ii) 1·0 mm, (iii) 1·5 mm, (iv) 2·0 mm. 180μ electrode. (*b*) *Calliphora* compound eye. Electrode tip at depths: (i) 0·15 mm, (ii) 0·45 mm, (iii) 0·50 mm, (iv) 0·60 mm. 10μ electrode. (*a*) (i) and (*a*) (iii) retouched.

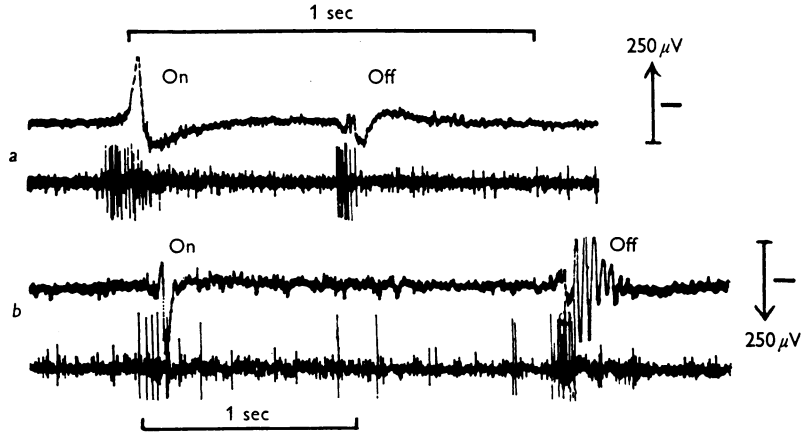

Fig. 7. (*a*) and (*b*). Simultaneous records of on and off responses in *Locusta*. Upper trace, intraocular; lower trace, ventral nerve cord responses. The nerve cord spikes slightly precede the intraocular potential waves. In (*b*) the off response is oscillatory.

arises during penetration. The plateau was not seen in all records, but only in those showing the largest wave activity; the example in Fig. 6*a* is particularly favourable. Whilst the on-wave was usually single, and relatively constant in form, the off-wave was more variable, and occasionally markedly oscillatory (Fig. 7*b*). In most experiments the reversal was so criticallly localized in depth that it was difficult, with the manipulator used, to set the electrode tip exactly to the minimum position. This depth in *Locusta* ranged from 0·75 to 0·9 mm, in different experiments. In some experiments with the grasshopper a second reversal zone was found, when the electrode tip was in the second synaptic region.

The on- and off-waves were accompanied by action potential discharges in the ventral nerve cord (Fig. 7), the onset of which in each case appeared to precede the onset of the intraocular wave. These nerve cord on- and off-responses have been reported previously (Burtt & Catton, 1954*a*). Ventral nerve cord spikes were also observed to accompany the sinusoidal wave activity (type (3) above), single spikes tending to synchronize with each wave (Fig. 5*a*). In one experiment (*Locusta*) the resting background sinusoidal wave was increased in amplitude and frequency during illumination of the eye (Fig. 5*b*). The illumination potential of the *Calliphora* eye, shown in Fig. 6*b*, behaved in the same way as in *Locusta*.

Intraocular spike potentials

During slow progressive insertion of the microelectrode, movement and on-off stimuli were repeatedly given. Faint spike responses were audible at all depths, but rapidly gained strength when a certain depth was reached. In many experiments, but not invariably, the onset was abrupt, occurring within one vernier division (0·05 mm). In *Locusta*, on which most experiments were performed, the depth of onset of loud spike activity was 1·0–1·25 mm, and was reproducible in the same specimen. With increasing penetration the spikes increased in number and amplitude, and then as a rule progressively diminished, the fade-away being much less abrupt than the onset. This zone of maximal spike activity (first spike maximum) had a mean depth of 1·27 mm ($\pm$0·13 S.D.). There was usually a region of strong spontaneous activity, a little deeper than that of the onset (spontaneous maximum, 1·20–1·40 mm). This can be seen in Fig. 8, which also shows simultaneous discharges in the ventral nerve cord (movement stimuli). This figure shows that the intraocular response goes through a maximum at a depth of about 1·15 mm in this experiment, and that the preparation is not damaged by the electrode, as indicated by constancy of the ventral nerve cord response throughout the penetration. The intraocular on and off spikes reached a maximum in the same region as the movement spikes, i.e. usually between 1·0 and 1·5 mm; they are clearly seen in Fig. 5*b*.

In some specimens of locust and dragonfly larva the sequence of observations differed from the above in that the spike responses to movement, after passing through the first maximum, declined somewhat (in the region of strong spontaneous activity) and then increased again to a second maximum before finally dying away. The separation in depth between these two maxima of movement response was about 0·2 mm in the locust, and 0·5 mm in the dragonfly larva. The presence or absence of the second spike maximum could possibly be associated with the precise angle of entry of the electrode, either encountering or missing the deeper-active zone.

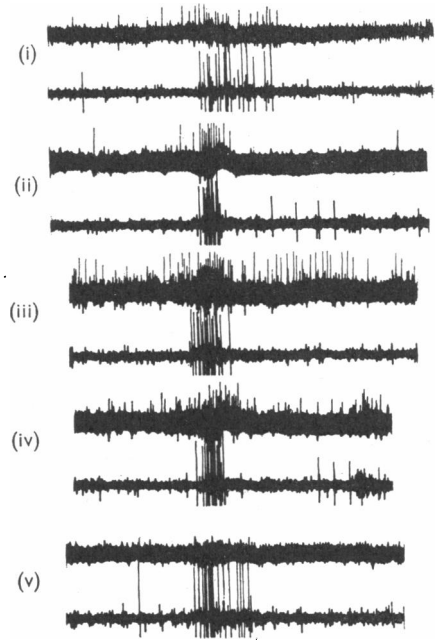

Fig. 8. Simultaneous records of movement responses in *Locusta*, upper trace intraocular electrode, lower trace ventral nerve cord electrodes, taken at various depths of the intraocular electrode: (i) 1·0 mm, (ii) 1·15 mm, (iii) 1·35 mm, (iv) 1·45 mm, (v) 1·65 mm. The nerve cord responses remain practically constant, but the intraocular response rises to a maximum in (ii), and vanishes in (v). Note marked spontaneous activity in (iii), which is characteristic of this depth, and that there are no corresponding impulses in the ventral nerve cord.

The intraocular spikes were more numerous, of lesser recorded amplitude, and more scattered than those in the ventral nerve cord. There was no one-to-one correspondence, and the first intraocular spike commonly preceded the first spike in the nerve cord. The spontaneous intraocular spikes were not always accompanied by nerve cord spikes (Fig. 8 (iii)). Since it is intended to investigate the neurological connexions involved in the visual pathway by more precise methods, the approximate time relations between intraocular and ventral nerve cord events observed so far will not be quoted here.

Comparison of the acuity of movement perception using the ventral nerve cord and the optic lobe responses

Since we now had in the intraocular spike phenomenon a neurological response to visual stimulation closer to the source than that afforded by the ventral nerve cord on which we have previously reported, it was of interest to attempt to measure the threshold sensitivity to movement stimulation, using the intraocular spikes as indicators of a visual movement response. It was expected that the sensitivity should be better than for the ventral nerve cord, in which some degree of convergence and integration of the visual impulses might be expected to have occurred.

A comparison was made of the visual sensitivity in *Locusta*, using (*a*) the nerve impulses in the ventral nerve cord led off between the 2nd and 3rd thoracic ganglia, and (*b*) the impulses detected in the optic lobe with the intraocular electrode. The stimulus used was the movement of a small tungsten-filament bulb on a pivoted arm, and sets of ten trials with 10 sec intervals between each trial were made (Burtt & Catton, 1954*a*). The intraocular electrode was inserted until the point of maximum loudness of movement spike response was reached. A test carried out with the electrode at different depths confirmed the expectation that the point where the movement spikes were loudest was also that where the highest number of responses was obtained in ten trials, at a threshold movement of the bulb. Movements of angular extent ranging from 0·85 to 0·1° were used, scaling down by stages of 0·1°. In order to guard against the effects of possible deterioration of the sensitivity of the preparation, readings were taken as quickly as possible, and the tests were made at the three values of 0·8°, 0·5° and 0·2°. If, as commonly happened, the nerve cord gave 10/10 responses for 0·8°, 0/10 for 0·2° and an intermediate figure for 0·5°, then further subdivisions of the extent of the movement were not used, but we passed at once to establish threshold responses from the intraocular electrode. Two sets of ten movements were made on all occasions where values other than 10/10 or 0/10 had been obtained. The comparison was made in nine locusts and gave the following figures for the smallest angular movement giving five or more responses out of ten (Table1).

TABLE 1. Threshold angles of movement stimulation for responses in the nerve cord and in the optic lobe of locusts

Thoracic nerve cord (°)	0·5	0·5	0·5	0·5	0·5	0·4	0·3	0·2	0·2
Optic lobe (°)	0·2	0·2	0·2	0·2	0·2	0·4	0·3	0·2	0·1

Thus, in six cases the optic ganglion was more sensitive, and in three cases it was as sensitive as the ventral nerve cord, but in none of the nine insects was it less so. Further, the greatest sensitivity of all was recorded via the optic lobe, with a response of 5/10 for a movement of 0·1°. The sensitivity of

the two sites of recording can also be compared by finding the total number of responses for movements of the same extent in each. Thus, it is seen (Table 2) that for the smaller movements, the response of the optic lobe is better maintained than that of the nerve cord. The value for the optic lobe response at 0·2° (57/90) is comparable to that of the ventral nerve cord at 0·5° (61/90).

TABLE 2. Frequency of occurrence of movement responses in nerve cord and optic lobe, for different angular movements, in the locust

Angular movement (°) ...	0·8	0·5	0·2
Responses:			
Ventral nerve cord (90 trials)	83	61	22
Optic lobe (90 trials)	89	85	57

A few measurements of sensitivity to movement, using the same method, were made on *Chortippus*, but the values were obtained in different specimens in these experiments. The lowest intraocular threshold was 0·2° and the lowest for ventral nerve cord 0·8°. In *Aeshna* only a weak response was obtained from the nerve cord, while a significant response was obtained to a movement of 0·2°, using the deeper active spike layer.

Results for species other than locust. These are discussed in more detail in the next section, but in general it may be said that regions of reversal of sign of the illumination potential and of localized spike responses were found in the grasshopper, dragonfly larva, and blowfly.

Correlation of the electrical responses with the anatomical structure. In the sections of the compound eye and optic lobe prepared subsequent to the experiment it was usually not possible to identify the electrode track. A few attempts were made to produce localized silver deposits at the electrode tip at selected sites, using electrochemical methods, but without success. Thus the position of the tip was estimated by dead reckoning, knowing the point of insertion, the angle of insertion (protractor scale) and the depth of penetration. Thick sections (100 μ) were cut in the horizontal plane. Measurements were made in that section in which the pilot hole in the chitinous cornea could be seen, thus determining the horizontal plane of the electrode. The outline of the eye and the positions of the neuronal masses in the optic lobe were sketched by camera lucida on squared paper, to a scale of 2 in. to 1 mm. From the experimental data four points were plotted for each experiment, one for the position of reversal of sign of the illumination potential, one for the position of the first maximum of action potential spikes, one for maximum spontaneous activity and one for the deeper spike maximum when this was observed. In a few experiments pilot holes in the cornea were made at points other than central, and similar responses were generally obtained, excepting in the most edgewise insertions, when the deep synaptic layers would be 'missed' entirely, and spike activity was then not recorded. Similarly, for

widely divergent angles of entry the deep synaptic layers might be missed by the electrode tip. The two factors, position of insertion on the cornea and angle of insertion had to be carefully controlled, more especially in *Aeshna* larva and *Calliphora* in which the optic lobe (Fig. 9C, D) is relatively long and narrow and not well anchored by the surrounding tissues, as compared with *Locusta* and *Chortippus*, in which the optic lobe is more compact and less mobile.

Results for individual species

Locusta. From Fig. 9A we put forward tentatively the following correlations. The region of reversal of the illumination potentials seems to coincide with the more superficial part of the first synaptic layer (the retina inter-

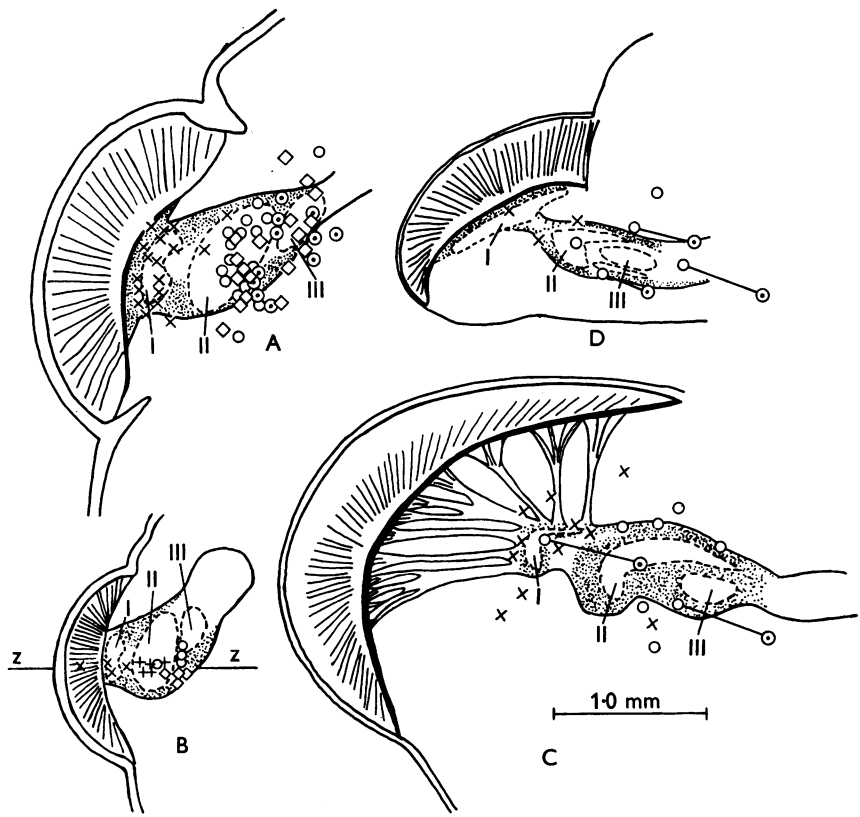

Fig. 9. Horizontal sections of compound eyes and optic lobes at comparable levels with the positions of the responses shown. A, *Locusta*; B, *Chortippus*; C, *Aeshna*; D, *Calliphora*. ○, first spike maximum; ⊙, second spike maximum; ×, first reversal; +, second reversal found in *Chortippus*; ◇, spontaneous maximum; I, II, III, 1st, 2nd and 3rd synaptic regions. Synaptic and fibrillar regions of optic lobe plain, regions of neurone somata stippled. In *Chortippus* the positions of the responses are all referred to the one line of entry shown by the line (*Z*). In the other cases the points are plotted from various lines of entry.

mediaria of Cajal). More tentatively, we suggest that the first spike maximum corresponds to the deeper part of the second synaptic region, the spontaneous maximum with the neuronal mass lying between the second and third synaptic regions, and the second spike maximum with the third synaptic region.

Chortippus. Fig. 9B shows that the eye and optic lobe are very similar to *Locusta* in form and in the disposition of the three synaptic regions. This similarity in general bodily structure and function is expressed by their both being placed in the family Acridiidae of the order Orthoptera. The positions for the first point of reversal (×) the first spike maximum (○), and spontaneous maximum occur in a comparable situation to those in *Locusta*, provided always that allowance is made for *Chortippus* being half the size. The insect is, however, rather too small for accurate correlations to be made between the structure and the responses. One phenomenon not found in *Locusta* was observed in four of the five specimens of *Chortippus* examined, namely a second reversal at a deeper level than the first. The first reversal showed a rough indication of a relationship to the first synaptic region, and the second with the second synaptic region.

Aeshna larva. This insect (Fig. 9C) was particularly interesting because not only is the scale different from that of *Locusta*, being larger in the ratio 5 : 3, but the form and disposition of the synaptic layers show marked differences. The chief are that the whole optic lobe is more elongated as seen in horizontal section, and the distance between the eye itself and the optic lobe is much greater, the post-retinal fibres being elongated. Nevertheless, there is a clear indication that the first reversal occurs in the first synaptic layer. The points for spike maxima lay deeper, embracing the region of the 2nd and 3rd synaptic layers. A double spike maximum separated by a region of attenuation was found in two of the four insects examined. It is not possible, on the evidence available, to relate these maxima to any definite structure. It should be said that the more elongated loosely attached optic lobe of *Aeshna* is more flexible than that of *Locusta*, and this is probably the explanation of the greater scatter of the points in Fig. 9C.

Calliphora. Fig. 9D shows an eye and optic lobe of yet another pattern, different from any of those so far considered. The eye and optic lobe of this insect is even softer and more flexible than in *Aeshna*, and this doubtless explains an even greater scatter of points. One can say with certainty that reversal is succeeded by spike maxima in all cases, and that in three sets of observations a double spike maximum was found. With reservation, it can further be said that the reversals centre around the first, and the spike maxima around the 2nd and 3rd synaptic regions, but the fact that a reading for a spike maximum is recorded quite beyond the optic lobe itself suggests so much stretching and distortion by the electrode, that it is impossible to relate structure and response more closely.

From the results in these four insects we can draw the following conclusions: The successive phenomena which were found when a microelectrode penetrated the locust's eye occur in all the other three insects. Further they occur in the same order, and their distance apart is related to the size and anatomical arrangement of the optic lobe. In *Locusta* and *Aeshna*, the first reversal is related to the first synaptic layer, while the same is probably true of *Chortippus* and *Calliphora*. That the spikes are related to the 2nd and 3rd synaptic regions is definite in *Locusta*, *Aeshna* and *Chortippus*, and probably also the case in *Calliphora*.

It would be premature to generalize from the above to all other insects, but these four species represent three main types of winged insect; i.e. *Aeshna* is a survivor of the Palaeoptera, while *Locusta* and *Chortippus* represent the exopterygote branch, and *Calliphora* the endopterygote branch, of the Neoptera. This constancy of electrical response in the insect eye corresponds to its basic structural similarity throughout the class.

Effects of removal of the 2nd and 3rd synaptic regions

A number of operations were performed in which the aim was to remove a portion of the optic lobe. It was not possible to remove an exactly predetermined region, but varying amounts of the optic lobe were removed and the results were then checked anatomically. The procedure was:

(*a*) To cut a window in the front of the head and expose the protocerebral ganglia and optic lobe.

(*b*) To record from the intact insect's eye in the usual way with the electrode traversing it to different depths. This was done to ensure that the preparation gave the normal responses.

(*c*) To transect the lobe at various levels and remove the region adjacent to the protocerebrum. This left an intact eye joined to various amounts of the 1st, 2nd and 3rd synaptic regions. The electrode was again inserted and the behaviour of the preparation noted.

(*d*) The preparation was fixed, and frozen sections were cut and stained as above.

The synaptic regions are so placed that an approach from the front provides a fair chance of transecting between the 1st and 2nd, or 2nd and 3rd regions. We did not succeed in separating the 1st synaptic region from the ommatidia themselves (i.e. severing the short post-retinal fibres).

Table 3 summarizes the results of eleven operations. When the experiments are arranged in order of amount of lobe remaining, it is clear that injury to the brain, or connexion of the optic lobe to the brain, has no detectable effect, apart of course from eliminating the responses from the opposite eye (see below). Thus the optic lobe seems to respond as a functional whole as far as the effects dealt with in the present paper are concerned. The results of

removal of portions of the 2nd and 3rd regions suggest the following tentative conclusions:

(i) The phenomenon of reversal still remains as far as the transection was carried, i.e. up to the 1st synaptic region.

(ii) On and off spikes occur, provided there is a complete 2nd synaptic region, and even with a portion of one, but in the latter case the results were abnormal, i.e. there were off spikes only, or spikes appearing continuously on illumination of the eye.

(iii) The presence of movement spikes requires both the 2nd and a part of the 3rd region to be intact, and the same applies to spontaneous spikes.

TABLE 3. The effects of operative removal of parts of the optic lobe on the responses of the locust eye

	Results of operation: eye plus				
Phenomenon	1st synaptic region	1st and part of 2nd	1st and 2nd	1st, 2nd and part of 3rd	1st, 2nd and 3rd (no connexion to 'brain')
Reversal of illumination potential	+	+ − + +	+	+ + +	+ +
On-off spikes	−	− + − +	+	+ + +	+ +
Movement spikes (1st spike maximum)	−	− − − −	−	− − +	+ +
Spontaneous spikes	−	− − − −	−	− + −	+ +

Responses from the contralateral eye

It was observed in many experiments that spike responses were obtained from the electrode in one eye when stimuli were given to the opposite eye. This phenomenon was not examined in detail, but the following comments are relevant. In a concomitant microanatomical study of the locust brain and optic lobes, crossing tracts of fibres have been identified (Satija, unpublished). Also, when the short stalk connecting the optic lobe with the 'brain' is severed, these contralateral responses are abolished. The functional significance of these crossed responses is obscure, but it is interesting to recall the fibres in the vertebrate eye which run from one retina to the other, and for which the function is also unknown.

The rhythmic discharges

Rhythmic sinusoidal discharges were observed in a few locust preparations, and usually appeared spontaneously (Fig. 5*a*). Spike discharges in the optic lobe and ventral nerve cord were discharged at the negative crests. In one experiment (Fig. 5*b*) a high frequency rhythm ('bright rhythm' of Adrian?) developed at 'light on', abolishing the previous slow rhythm (dark rhythm?).

If these rhythms are to be regarded as due to pathological change (Adrian, 1937), their relatively rare occurrence in our experiments may be accounted for by the minimal trauma inflicted by the present technique as compared with the more extensive dissections used by earlier workers.

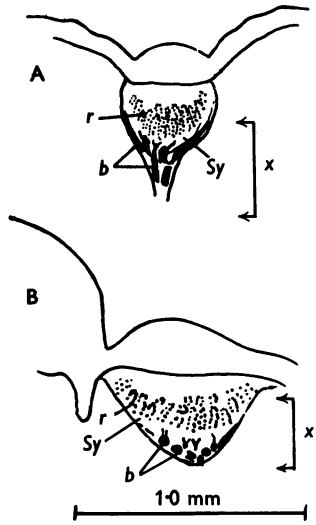

Fig. 10

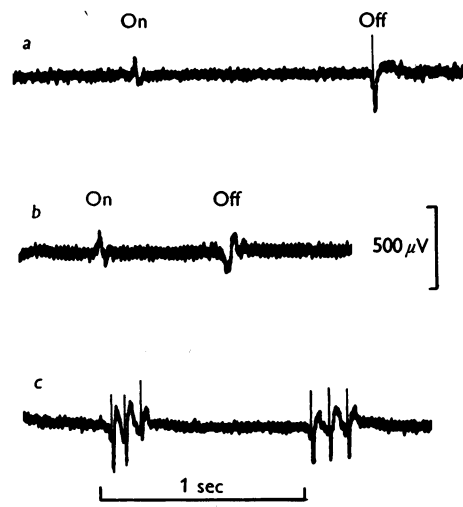

Fig. 11

Fig. 10. Horizontal sections of A, frontal; and B, lateral ocellus. *b*, branches of ocellar nerve fibres; *r*, retinula cells; *Sy*, synaptic region; *x*, line showing depth over which response was obtained.

Fig. 11. Ocellar potentials recorded by intraocellar electrode. (*a*) Frontal ocellus, on and off responses. (*b*) Left lateral ocellus, on and off responses. (*c*) Frontal ocellus, rapid repetitive stimulation, by passing three fingers of the hand through the light beam. Regular sinusoidal base-line due to 50 c/s pick-up.

Intraocellar potentials

In a few experiments the microelectrode was inserted into either the frontal ocellus or one of the lateral ocelli, in order to compare the responses in such a simple eye with those found in the much more complex compound eye. The maximum depth of the ocellus (Fig. 10) measured from the surface is about 0·5 mm. As soon as the electrode penetrated the corneal surface small on- and off-wave responses were recorded in response to strong illumination of the ocellus (Fig. 11*a*, *b*) and the off-wave was often accompanied by a single spike. On rapid repetitive stimulation, separate on- and off-waves could not be distinguished (Fig. 11*c*) and the discharges appeared to consist of off-waves and spikes only. No reversal effect with deeper penetration was observed. The on-waves were not accompanied by spikes in the intraocellar record, and neither were ocellar spike discharges for 'on' recorded in the ventral nerve cord, but 'off' responses were always found in the cord.

DISCUSSION

The experiments described appear to be the first in which insulated microelectrodes of the order of size of 10μ have been used in the insect eye and optic lobes, with a view to obtaining information about activity in localized regions. The results thus differ in certain respects from those obtained by previous workers seeking to analyse the neurological behaviour of these organs. Autrum (1950) and Autrum & Gallwitz (1951), have made a thorough study of the illumination potential in the eyes of a number of insects, including *Calliphora*. They used the isolated head supported on a fixed silver wire, acting also as the indifferent electrode, with a second electrode inserted superficially into the eye as the intraocular electrode. This electrode consisted of silver wire of original diameter 0·5 mm, with sharpened tip, and the depth of penetration was 0·1–0·2 mm. The potential changes were amplified by a d.c. amplifier and recorded on a cathode-ray oscillograph. The preparation was kept in a light-proof metal box, which served also as a moist chamber and an electrical shield. Their records from *Calliphora* show the on-wave as positive ('cornea with respect to the bases of the ommatidia'), which would correspond to a negative wave with our convention (i.e. potential of intraocular electrode with respect to indifferent electrode). They did not detect the reversal of sign with increasing depth and possibly with a thicker, non-insulated electrode this would not be clearly seen. In the same way they did not record any spike activity. Our results for the illumination potential are generally in agreement with the above, except that the recorded amplitude of the waves was somewhat less; this may be attributable to the use of a much finer electrode. In an early experiment on *Locusta*, using a relatively coarse electrode of about 180μ diameter, we obtained a large response (on-wave of about 1 mV amplitude, Fig. 6*a*) comparable to those obtained by Autrum. Using the standard 10μ electrode the on-wave amplitude was usually about 250 μV, and the plateau effect seen in the early locust experiment is not so well seen in experiments using the fine electrode (e.g. Figs. 6*b*, 7).

The origin of the reversal phenomenon we supposed might possibly be related to the existence of a layer of neurones with their axons or dendrites running parallel, and perpendicular to the surface of the cornea. Cragg & Hamlyn (1955), recording action potentials from the pyramidal neurones in the hippocampus of the rabbit by means of a search microelectrode, found a reversal of sign of slow-wave activity as the electrode was progressively inserted. This reversal occurred in the region of the parallel-running inwardly-directed axons of the neurones. They applied repetitive electrical stimulation via a needle electrode whose tip was implanted deeper than the recording site. The pyramidal neurones of the rabbit's hippocampus form a well-defined layer, with profusely branching dendrites directed towards the surface, and

axons running parallel and away from the surface. We have not identified such a layer of neurones in our own preparations, which were not cut in a suitable way for this purpose. On referring to the work of Cajal on the histology of the insect optic lobe, however, there is clear evidence presented of the existence of a layer of neurones, situated in the first synaptic region, which possess a comparable morphological character (Cajal & Sánchez, 1915). These neurones (the 'giant monopolar' cells of Cajal) form a very clearly defined layer; they have few dendrites, but prominent axons which run parallel for

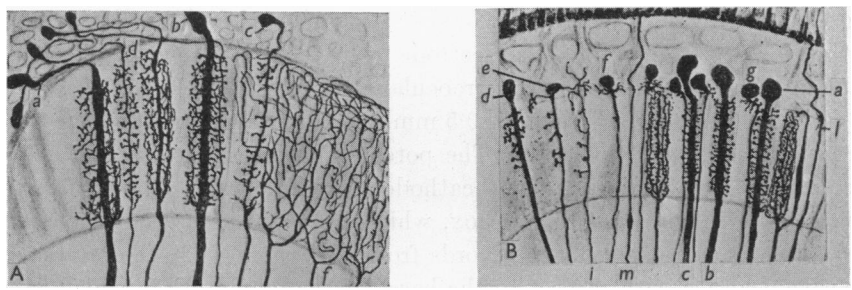

Fig. 12. (A) Retina intermediaria (first synaptic region) of an orthopteran. *a*, *b*, giant monopolar cells; *c*, *d*, small monopolars; *f*, centrifugal fibres. (B) Various types of large and small monopolar cells in the retina intermediaria (first synaptic region) of *Calliphora*. *a*, *b*, giant monopolars; *c*, the same associated with two small monopolars; *d*, small monopolar with few dendrites; *e*, *f*, monopolars with somatic dendrites; *i*, centrifugal fibres; *l*, *m*, long post-retinal fibres. (From Cajal.)

the first part of their courses and are directed perpendicularly inwards. These giant monopolar cells of the first synaptic layer appear to be a feature of the optic lobes of many, if not all insects; they are found in the order Orthoptera (which includes *Locusta* and *Chortippus*), and in *Calliphora*, as shown in Fig. 12A and B. It seems possible that, since this cell layer corresponds closely in situation to the region of reversal of sign of the illumination potential in our experiments, it is in fact the source or 'generator zone' of the greater part of the illumination potential. There is a considerable difference in morphological scale, the rabbit neurones being much larger in overall extent (500 μ) than those in the optic lobe of the insect (about 100 μ in extent). Correspondingly, our reversal occurred over a much narrower zone (about 50 μ) than in Cragg & Hamlyn's experiments (250 μ).

The hypothesis that the origin of the illumination potential is localized to the first synaptic region, and probably to the layer of giant monopolar cells, is supported by the evidence from our transection experiments, in which the illumination potential persisted after severance of the second and third synaptic regions. Further, the reversal of sign still occurred when only the first of these regions remained.

Autrum & Gallwitz (1951) investigated the effect of removal of parts of the optic lobe in *Calliphora*. After removal of all but the first synaptic layer, it was found in general that the illumination potential could still be recorded, although it now had a form different from the normal. Owing to uncertainties as to the amounts of tissue removed, the results showed a range of variation, but in general there was a tendency for a prolonged negative plateau to develop, on which the off-wave was superimposed. The more complete the extirpation the more marked was the negative plateau. In the few preparations in which the whole of the optic lobe, including the first synaptic region, was successfully severed from the 'retina', equivalent to a severance of the post-retinal fibres, the response was a pure negative plateau, with no on- or off-waves. Autrum & Gallwitz regarded this negative plateau as the pure response of the isolated retina. We have not observed such a phenomenon in *Locusta*, not having succeeded in isolating the retina in this way. The plateau region seen in our record (Fig. 6*a*) is positive in the superficial position of the electrode, which we presume to correspond with the site from which Autrum & Gallwitz recorded.

Autrum & Gallwitz, from their studies on *Calliphora* and *Aeshna*, reached quite a different conclusion as to the origin of the illumination potential. They regarded the potential as divisible into two components, a purely negative one derived from the retina, and a positive one derived from the optic lobe. The retinal component acted as the 'generator potential' for the positive potential from the lobe. The combination of the two gave rise to the illumination potential, as recorded superficially. Certainly the evidence from the vertebrate retina indicates that the electroretinogram (to which the illumination potential of the insect eye may possibly be compared) is of complex origin, there being probably three or more components (Granit, 1947). Nevertheless, the phenomenon of reversal of sign of the illumination potential, critically localized in our experiments to the first synaptic region, suggests a simpler interpretation in the case of the insect eye.

The occurrence of a second region of reversal in our experiments (seen only in a few specimens of grasshopper), we are not at present able to explain.

Wave and spike potentials have been recorded from the eye of the water-beetle (*Dytiscus*) by Bernhard (1942). He used an isolated head preparation with an exposure of the optic lobes and protocerebral ganglia, and moist thread extensions from Ag–AgCl electrodes were used to make contact with the tissue. The indifferent electrode was placed on the cornea and the search electrode on various parts of the optic lobe. With the search electrode on the optic lobe brief exposures to the light from a 100 W lamp were made. At 'light on' a brief negative wave occurred, followed by a negative plateau persisting for the duration of the exposure (d.c. amplification). Superimposed

on this plateau were small oscillations and spike potentials. At 'light off' a brief negative wave occurred, during which the spike potentials disappeared, and the mean recorded potential then fell sharply back to the base-line. When the optic lobe was transected at its connexion with the 'retina', and the search electrode was placed in contact with the back of the eye, a clean negative plateau was recorded during exposure to light. Bernhard concluded that the negative plateau recorded by the ganglion lead was simply electrical spread from a source in the 'retina'. When 4% cocaine was applied to the optic lobe in the intact preparation the oscillations and spikes both disappeared leaving a smooth negative plateau. The persistence of the negative plateau after transection of the optic lobe and after treatment with cocaine in these experiments corresponds with the findings of Autrum & Gallwitz on *Calliphora* eye, and certainly suggests that such a potential change may originate in the 'retina'. In our experiments using the microelectrode, however, the plateau region between the on- and off-waves underwent reversal of sign together with the waves, suggesting that both have a common origin, in the first synaptic layer.

Ruck & Jahn (1954) investigated the illumination potentials in the eye of *Ligia* (an isopod crustacean), the optic lobes of which have an anatomical pattern similar to those of *Locusta*. They found negative on-wave and positive off-wave responses to short-duration high-intensity light stimuli. Their preparation involved an extensive exposure of the ganglia within the head, and surface electrodes of small tip size were used. One electrode (the indifferent) was placed on the corneal surface of an eye which was occluded; the other, a search electrode, was placed on various regions of the inside surface of the other eye and on the optic lobe surface. They found that the illumination potentials became inverted when the search electrode was moved from the inner surface of the unoccluded eye on to the adjacent part of the optic lobe. This phenomenon at first sight seems closely comparable to the inversion found by us in the locust eye. On the other hand, the illumination potential remained unchanged when the complete optic lobe was removed, suggesting that in *Ligia* this potential is generated largely or entirely in the photoreceptor cells themselves. This conclusion is opposed to the findings of Autrum & Gallwitz (1951) and to those reported in this paper. In view of the technical difficulty of cleanly separating the first synaptic layer from the bases of the ommatidia, as encountered in our experiments, we feel that some doubt may yet exist on the question of the precise locus of origin of the illumination potential in *Ligia*. It certainly appears to originate close to the photoreceptor layer. It is interesting to find that recent work on the vertebrate retina, using insulated microelectrodes giving sharply localized responses (e.g. Tomita & Funaishi, 1952; Ottoson & Svaetichin, 1953) points to the conclusion that in certain vertebrate eyes the e.r.g. may indeed be largely generated in the

photoreceptor layer. This conclusion is in marked contradiction to earlier hypotheses as postulated for example by Granit (1947).

Previous workers have recorded rhythmical wave activity, of a nature similar to that reported above, in the optic lobes of insects. Adrian (1937) recorded dark and light rhythms from the optic lobe of *Dytiscus*. The dark rhythm became established after a period of keeping the eye in darkness; it was readily inhibited by faint light. When strong light was used the dark rhythm was abolished and replaced by a bright rhythm (cf. Fig. 5*b*). Adrian ascribed these rhythms to synchronized activity in large numbers of neurones. The dark rhythm could be elicited by physical injury to the ganglion. Adrian recorded spike discharges coincident with the negative crests of the waves, as shown in our Fig. 5*a*. Crescitelli & Jahn (1942) detected similar rhythms in the eye of the grasshopper. Our results agree in general with those of previous workers.

Hoyle (1955) investigated the potentials in the ocellar nerve of the locust in response to illumination. He found that impulses were discharged for light on, light off and during dark exposure. The on and off discharges were very brief, the on-response consisting of a single action potential. In contrast, we have never detected a spike response to 'on', but only a brief off-discharge, consisting of a single spike from the ocellus and a short train of spikes in the ventral nerve cord. Hoyle found both on and off ocellar discharges in the circum-oesophageal commissures.

SUMMARY

1. Fine insulated microelectrodes of exposed tip diameter 10 μ have been used to record potentials in response to visual stimulation in the compound eyes and optic lobes of four species of insect: a locust (*Locusta migratoria migratorioides* L.), a grasshopper (*Chortippus brunneus* Thunb.), a dragonfly larva (*Aeshna* sp.), and a blowfly (*Calliphora vomitoria* L.).

2. Three kinds of observed activity are reported: (*a*) slow wave activity when light is switched on or off (='illumination potentials'), (*b*) action potential spike discharges in response to movement and light on or off, and (*c*) rhythmical sinusoidal wave activity.

3. The on and off wave responses of the illumination potential are of opposite sign at all depths, and undergo a sharp reversal of sign at a particular depth, corresponding to the superficial part of the first synaptic region of the optic lobe. In this region Cajal described a zone of giant monopolar cells. Evidence is presented to support the view that this layer of cells is the source of a major part of the illumination potential.

4. The action potential spike discharges were also found to be localized in origin, arising in the deeper part of the second synaptic region, and in some

cases also from the third synaptic region of the optic lobe. The sensitivity to movement stimuli, measured as the smallest movement of a test object to elicit a response from the optic lobe, was found to be superior to that determined by similar measurements performed on the ventral nerve cord responses of the same insect. The spike discharges in the ventral nerve cord show no one-to-one correlation with the intraocular spikes, are fewer in number, and of larger amplitude.

5. Rhythmical sinusoidal waves, similar to the dark and bright rhythms reported by other workers, were observed in some preparations.

6. The ocelli produce simple illumination potentials and discharge single spikes in response to light-off stimulation.

7. The intraocular microelectrode provides a more critical method of analysis than do earlier techniques of investigation of the insect optic lobe.

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