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# THE EYE MUSCLE OF *CALLIPHORA VOMITORIA* L.

## I. SPONTANEOUS ACTIVITY AND THE EFFECTS OF LIGHT AND DARK ADAPTATION

BY JOHN PATTERSON

*The Department of Zoology, the University of Newcastle upon Tyne, Newcastle upon Tyne, England**

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### INTRODUCTION

This paper and that which follows (Patterson, 1973) form a fuller account of the muscle associated with the compound eye of the blowfly, *Calliphora vomitoria*, which has been briefly described by Burtt & Patterson (1970).

Lowne (1892) contains a text-figure which shows the distal portion of a muscle inserted onto the medial edge of the compound eye in *Calliphora erythrocephala*. Lowne (1892) speculates that the muscle promotes changes in the arrangement of the visual apparatus, which in his own words, 'render it extremely probable that the ommatidea of the compound eye are capable of being adjusted for distinct vision'. To underline this supposed analogy with the process of accommodation found in the vertebrate eye, Lowne (1892) gave to the structure the name 'ciliary muscle'. Although this term has historical precedence it will be shown that this name is misleading, and Hengstenberg (1971) has proposed the technically more accurate term *Musculus orbitotentorialis*. This account will in general refer to the structure as simply the eye muscle.

Schiemenz (1957) describes a similar muscle in *Eristalis* (Diptera, fam. Syrphidae) which runs from the anterior arm of the tentorium, the internal supporting structure of the head capsule, to insert on the medial edge of the compound eye. Burtt & Patterson (1970) find comparable muscles in representatives of the Tipulidae and Culicidae (Diptera) as well as in the Syrphidae and also observe a similar structure in *Notonecta* (Hemiptera: Heteroptera).

In the Schizophora, that division of the Diptera of which *C. vomitoria* is a member, the anterior arms of the tentorium are not well developed as in other Diptera. Burtt & Patterson (1970) show that the origin of the eye muscle in this species is found on the tentorial structure which occupies the posterior part of the head capsule.

The best anatomical account of an eye muscle of this type is that given by Hengstenberg (1971) for the muscle found in *Musca domestica*. This shows that the muscle is composed of 14–20 fibres each of 7–10 $\mu$m diameter and that the muscle receives multiterminal innervation from a single motor neurone. The presence of a similar muscle in *Drosophila melanogaster* (Diptera; fam. Drosophilidae) is also noted. Hengstenberg (1971) also presents good electrophysiological evidence for that

* Present address: Department of Zoology and Comparative Physiology, Queen Mary College, Mile End Road, London, E1.

association to which Burtt & Patterson (1970) alluded, namely that the electrophysiological activity produced by the eye muscle is the same as the 'clock-spikes' which are recorded from, in particular, the ventral region of the optic lobes of the brain in *C. erythrocephala* (Kuiper & Leutscher-Hazelhoff 1965).

The Ph.D. thesis of van Barneveld (1971) is concerned primarily with the statistical analysis of 'clock-spike' activity, but this account also contains brief chapters on the effect of visual stimuli on, and the possible function of, 'clock-spikes' in *C. erythrocephala*. Van Barneveld (1971) has also recorded 'clock-spike' activity from the optic lobes of the bumblebee, *Bombus*, although no mention is made of any muscle associated with the compound eye.

This paper will be concerned with the spontaneous activity of the eye muscle in *C. vomitoria*, and with the effects of light and dark adaptation on eye-muscle activity and compound eye structure. Consideration will be given to the functional significance of the eye-muscle/compound eye system.

## MATERIALS AND METHODS

Blowflies were obtained as larvae from a local fishing tackle retailer. The larvae were reared in an insectary at constant temperature (21·5 °C) and constant relative humidity (70%). Adults were kept in the same insectary and fed on a diet of sugar and water.

The cultures were not pure and consisted primarily of *C. vomitoria* and *Phormia* spp. with occasional *C. erythrocephala* and *Lucilia* sp. Animals for experiment were checked routinely with Day's (1948) key and only individuals of *C. vomitoria* were used. The compound eyes of *C. vomitoria* are sexually dimorphic, and in the females the eyes are smaller and more separated on the dorsal surface of the head. Unless otherwise stated females alone were used in experiments and were not removed from the culture until a minimum of 3 days had elapsed after emergence from the puparium.

Prior to experiment animals were light- or dark-adapted for a period of 1 h. Groups of five animals were confined to glass specimen tubes either of clear glass (for light adaptation) or of glass coated with black acrylic polymer paint (for dark adaptation). Both types of tubes were then placed in the beam of white light produced by a Vickers high-intensity tungsten-filament lamp. The colour temperature of the tungsten filament was arbitrary but constant for all experiments, and intensities were varied by means of an iris diaphragm and Ilford neutral-density filters. Glass heat-reducing filters were placed between the light sources and the animals. Light sources, both adapting and stimulating, were placed 35 cm away from the animals or from the experimental preparation. Light intensities were measured in lux using a Holophane lumeter.

Dissection or fixation of material was performed either under the adapting intensity of illumination or, in the case of dark-adapted animals, under the weakest possible red light. Animals were prepared without using anaesthetic because Leutscher-Hazelhoff & Kuiper (1966) point out that carbon dioxide abolishes 'clock-spike' activity.

All experiments were performed in a darkened laboratory and at constant temperatures. Although no special apparatus was employed to ensure constant-temperature conditions the ambient temperature rarely varied by more than $\pm 0{\cdot}5$ °C in the course of an experiment.

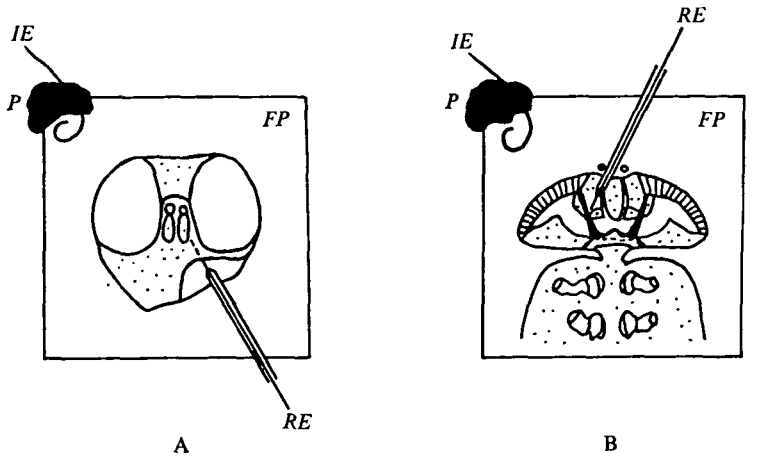


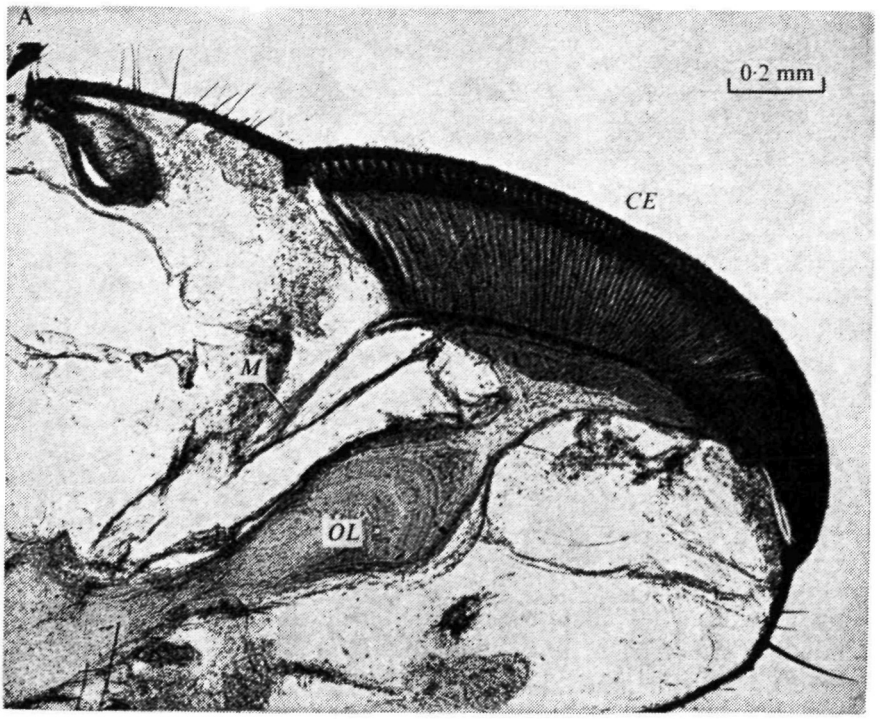

Fig. 1. Preparations used in electrophysiological experiments. (A) Isolated head with anterior surface uppermost and lateroventral aspect of head capsule removed. (B) Intact animal with mouthparts and ventral aspect of the head capsule removed. *IE* Indifferent electrode; *FP*, filter paper; *P*, Plasticene; *RE*, recording electrode.

Animals were fixed for microscopical examination by injecting aqueous Bouins' fixative into the head capsule. A hypodermic needle was pushed through the arthrodial membranes which surround the mouthparts and the animal appeared lifeless within 1 or 2 sec of the fixative entering the head. The mouthparts were dissected away and the isolated heads were washed with running tap water. In quantitative experiments the heads were then frozen on a Pelcool freezing microtome and horizontal sections of the head were cut away until the insertion of the eye muscle into the compound eye was clearly visible when the remaining block of material was viewed with a binocular microscope. Where post-morten shrinkage was of less importance the material was embedded in a 15% aqueous solution of gelatine, hardened with 10% formaldehyde and thick (100 $\mu$m) horizontal sections were cut with the freezing microtome.

For electrophysiological experiments the animals were light- or dark-adapted, decapitated and the isolated head was placed anterior surface uppermost on a piece of filter-paper moistened with insect saline (Hoyle, 1953). The latero-ventral quadrat of the head capsule was dissected away on one side enabling a 30 $\mu$m diameter tungsten wire electrode to be manipulated into the region of the eye muscle. The arrangement of the electrodes is shown in Fig. 1(*a*), electrical continuity being maintained by the severed neck of the isolated head. In a few experiments animals were pinned ventral side uppermost and the mouthparts and ventral region of the head capsule were dissected away to expose the eye muscle and surrounding tissue (Fig. 1*b*). The recording electrode in this case consisted of a glass micropipette filled with insect saline with a tip diameter $> 10$ $\mu$m.

Signals from the preparation were led into a Textronix type 122 preamplifier, and permanent records were made on 35 mm negative or paper film using a Cossor oscillograph camera. More frequently, however, electrophysiological data were quantified by employing a 'mean' interspike interval as a measure of eye muscle activity. These measurements were made in the following way.

The rising (+ve) phase of a muscle potential was used to trigger the beam of



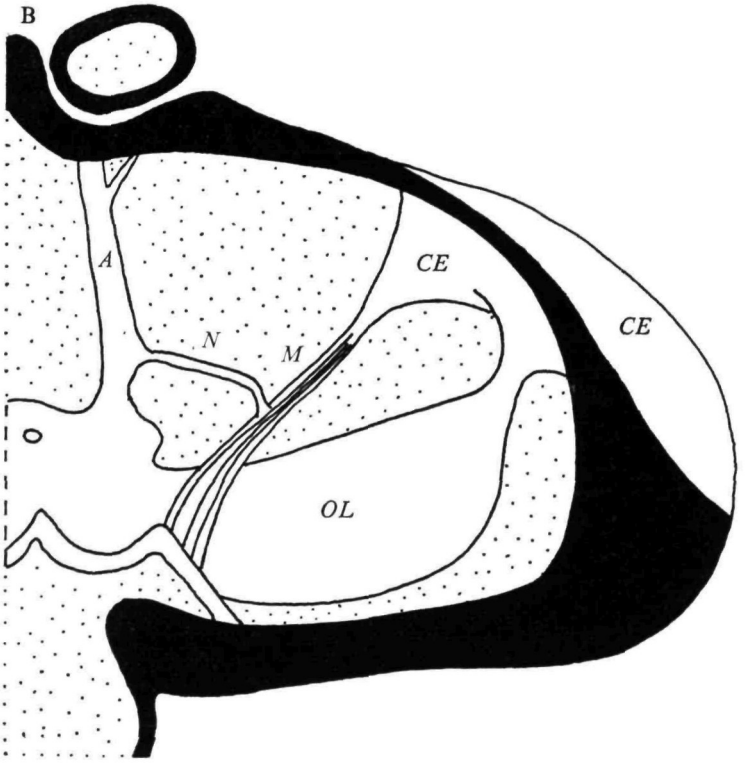




Fig. 2. For legend see facing page.

the oscilloscope (Telequipment D 43), and the trace was arranged so that the next potential in the train appeared at the centre of the oscilloscope screen. The next potential again triggered the beam. It can be seen that alternate muscle potentials are displayed as a standing wave and occur at a sufficiently high frequency for an observer to make a subjective estimate of the 'mean' interspike interval in, say, 1 or 2 sec of observation. Since the potentials are fairly constant in their temporal separation, and are especially so in resting preparations, this method was used extensively in the absence of automatic methods for the analysis of long trains of potentials. To give accuracy to the observations, experimental samples of ten animals were used and an analysis of variance was performed on the data. All the statistical methods employed are taken from Bailey (1968).

## RESULTS

### *Anatomy*

The eye muscle lies ventral to the brain and optic lobes and has an origin on the tentorium on the posterior region of the head capsule. Tapering as it does so, the muscle passes dorsally and anteriorly to insert on to the basement membrane of the medial edge of the compound eye. The proximal region of the muscle and its insertion onto the compound eye are shown in Fig. 2(*a*). The muscle is about 0·5 mm in length, is well supplied with tracheae and bears marked cross-striations which are visible in unstained sections and which become particularly noticeable when viewed through crossed polarizing filters. The striations are of about 5 $\mu$m separation and may be seen along most of the length of the muscle except towards the compound eye, where they are reduced and the muscle appears more tendon-like. Exact cross-sections of muscle have not been obtained, but in oblique cross-sections some seven muscle fibres have been counted. This is probably an underestimate, for Hengstenberg (1971) finds 14–20 fibres in the eye muscle of *Musca*. At its closest point the muscle passes within 100 $\mu$m of the optic lobe of the brain. Fig. 2(*b*) shows the eye muscle and its associated structures in diagrammatic form. The muscle is innervated by a fine branch of the antennal nerve and is surrounded by air sacs which have facilitated extracellular electrophysiological recording from the muscle, presumably because of the high-resistance pathway presented to local short-circuiting of the recorded signals.

### *Electrophysiology*

An electrode placed on or near the eye muscle records continuous potentials with a frequency of 40–100 Hz and of up to 1 mV in amplitude (Burtt & Patterson, 1970). The waveform of a single potential from this continuous train is shown in Fig. 3(A). The complex waveform is strikingly similar to that figured for 'clock-spikes' recorded from the optic lobe of *C. erythrocephala* (Kuiper & Leutscher-Hazelhoff, 1965) with the exception that the polarity is reversed. The waveform shown in Fig. 3(A) can be divided into two separate diphasic components. The first of these is of 2 msec duration and is separated by about 1 msec from a second component of much larger amplitude

Fig. 2. (A) Thick (100 $\mu$m) horizontal section of the head of a female *Calliphora vomitoria*; gelatine embedded. (B) Diagrammatic representation of the arrangement of the eye muscle and associated structures in the head capsule of *Calliphora vomitoria*. *A*, Antennal nerve; *CE*, compound eye; *M*, eye muscle; *N*, nerve supplying eye muscle; *OL*, optic lobe of the brain.

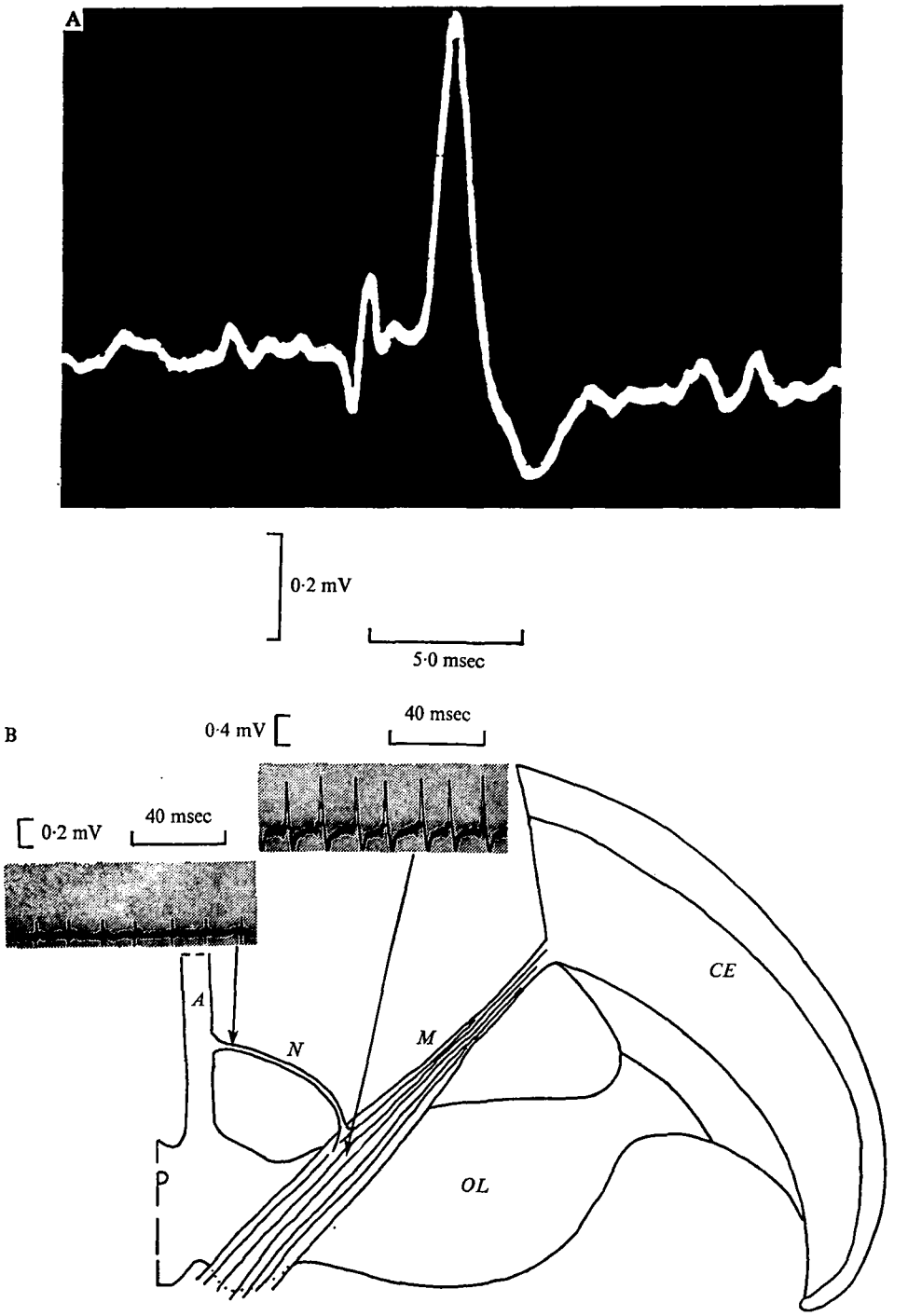



Fig. 3. (A) Single potential of a train recorded from an electrode placed on the eye muscle; temperature 15 °C. (B) Diagrammatic representation of the eye muscle and associated structures, showing the differences in the waveform of the recorded potentials with the recording electrode situated as indicated by the arrows. Labelling as for Fig. 2.

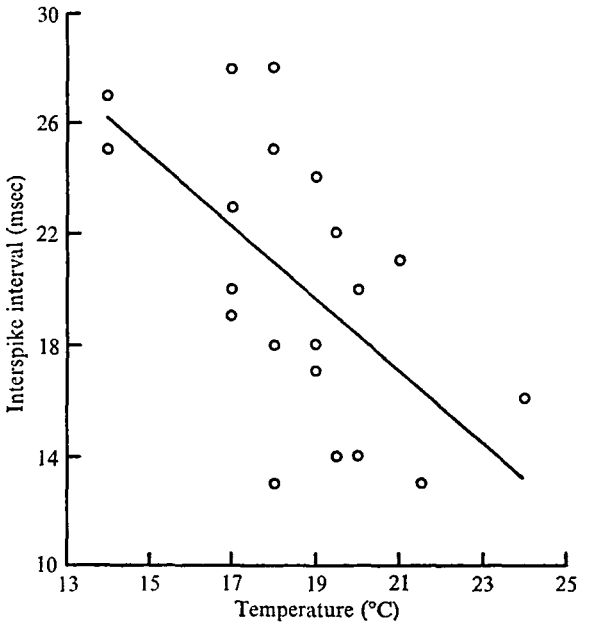



Fig. 4. 'Mean' interspike interval of eye muscle potentials in milliseconds plotted as a function of ambient temperature in degrees Centigrade.

which has a duration of about 6 msec. The observation that the second component becomes fatigued and disappears as preparations age suggests that it is produced by the eye muscle, while the first more rapid component is produced by the neurone supplying the muscle. This idea is strengthened by the results shown in Fig. 3(B) in which a Ringer-filled micropipette was used first to record activity directly over the muscle, and second to record activity at the antennal nerve end of the small nerve which runs to the eye muscle. In the first case, the second (large amplitude) component dominates the waveform and is preceded by a small first component. In the second case, with the recording electrode at the antennal nerve end, the small rapid component alone is present but is of about the same amplitude as was the first component when recorded from directly over the muscle.

### *The effect of temperature*

A number of animals of both sexes were adapted to light of 6500 lux and prepared as shown in Fig. 1(A). The 'mean' interspike interval was plotted as a function of ambient temperature and the results are shown in Fig. 4 as a scatter graph. Statistical analysis of the data showed a highly significant negative correlation between interspike interval and temperature. The correlation coefficient was found to have a value $r = -0{\cdot}595$, and the probability of no correlation a value $P = 0{\cdot}01$–$0{\cdot}002$. Linear regression analysis gave the regression line which has been fitted to the data of Fig. 4.

The results show that in the range 14–24 °C the interspike interval of the eye muscle potentials in *C. vomitoria* decreases by about 50% – that is, the frequency of the potentials increases from 38·4 Hz at 14 °C to 75 Hz at 24 °C. This amounts to an increase in frequency with temperature of 3·5 spikes/sec/°C. This value compares

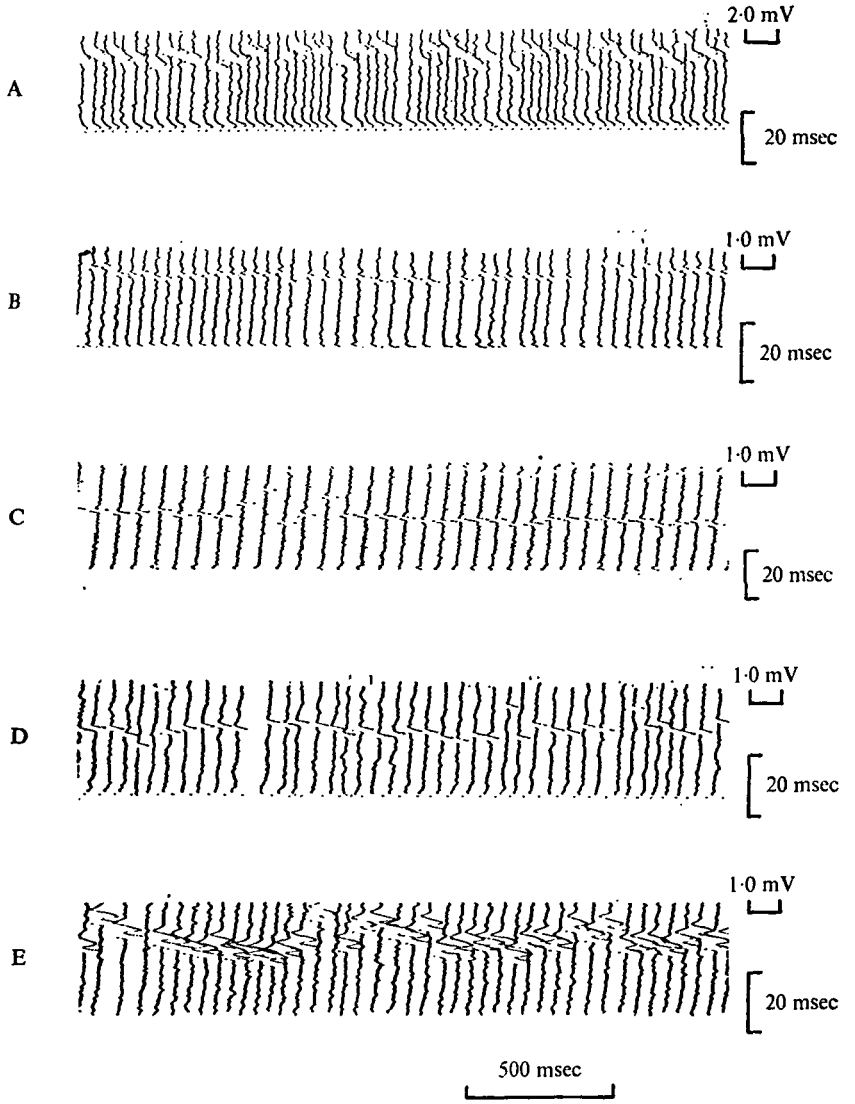



Fig. 5. (A–E) Successive eye-muscle potentials recorded from five dark-adapted female animals. For explanation see text.

favourably with the value found for the increase in 'clock-spike' frequency between 7 °C and 35 °C of 5·5 spikes/sec/°C in *C. erythrocephala* (Leutscher-Hazelhoff & Kuiper, 1966). Hengstenberg (1971) finds that values for the impulse frequency recorded from the eye muscle of *Musca* are about 70% of those obtained by Leutscher-Hazelhoff & Kuiper (1966) in *C. erythrocephala* at comparable temperatures. Values obtained from *Musca* are 30 impulses/sec at 15 °C, 62 impulses/sec at 24·4 °C and 100 impulses/sec at 40 °C (Hengstenberg, 1971).

The minimum interspike interval in *C. vomitoria* was observed in several cases to be about 6 msec, corresponding to a frequency of 167 Hz. This high-frequency discharge was usually found in dying animals. If a value of 6 msec is substituted in

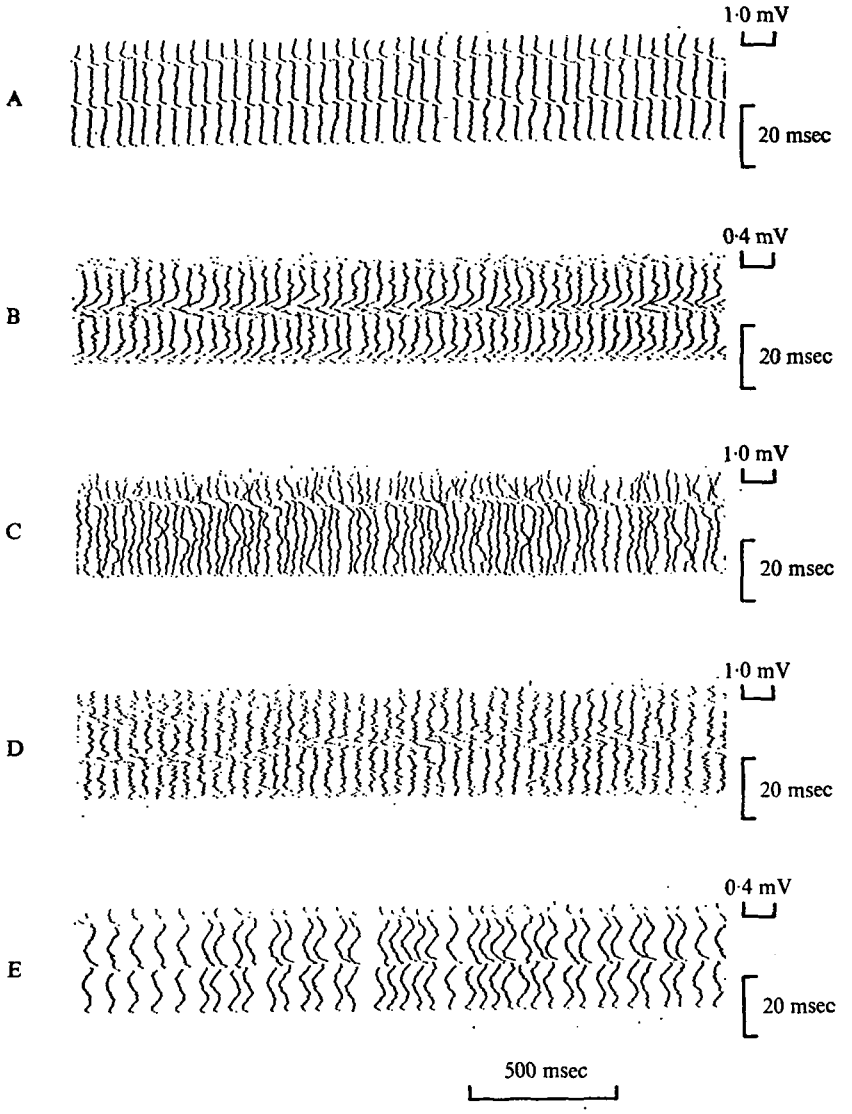



Fig. 6. (A–E) Successive eye-muscle potentials recorded from five light-adapted (5000 lux) female animals. For explanation see text.

in the formula used for regression analysis a corresponding temperature of 29·9 °C is the calculated result. This value falls in the middle of the range 25–36 °C in which 'clock-spike' frequency rises to around 200 Hz in *C. erythrocephala*, and animals show signs of dying (Leutscher-Hazelhoff & Kuiper, 1966).

### *Spontaneous activity*

Spontaneous eye-muscle activity in isolated heads of *C. vomitoria* was studied by making photographic records of the successive sweeps of the oscilloscope beam used to observe the 'mean' interspike interval. These records were obtained by rotating the oscillograph camera through 90° relative to its normal position with respect to

the oscilloscope screen. Each sweep of the beam then passed from the bottom to the top of the recording paper, rather than from left to right. Driving the recording paper slowly through the camera resulted in a record in which every second or every third interspike intervals could be compared. These experiments were performed at constant temperatures in the range 18–21 °C.

The results for five dark-adapted and five light-adapted (5000 lux) female animals are shown in Figs. 5 and 6 respectively. In the light-adapted animals the duration of the interspike intervals is fairly constant for each animal with the exception of Fig. 6(D). In the dark-adapted group, however, several animals (Fig. 5A, C, D, E) show considerable fluctuation in the interspike intervals. These fluctuations may be more or less periodic as in Fig. 5(D) and (E), or may consist of a slow lengthening of the intervals followed by a rapid shortening (Fig. 5C).

When both groups are compared there is a suggestion of a shorter interspike interval in the light-adapted as opposed to the dark-adapted animals.

*The effects of light- and dark-adaptation*

Changes in the illumination falling on the preparation produce two types of change in the interspike intervals of the eye-muscle potentials. The first type are the *transient* changes which occur within the first few seconds of a change in the level of illumination (Burtt & Patterson, 1970), and these changes will be discussed in more detail in a separate publication (Patterson, 1973). The second type are changes in the *resting* or spontaneous level of eye-muscle activity, changes which are maintained throughout periods of light or dark adaptation. It is this second category which will be described here.

After dark- or light- (6500 lux) adaptation the isolated heads of female *C. vomitoria* were prepared as shown in Fig. 1(B) and placed in the dark (dark-adapted group) or light of 6500 lux (light-adapted group). A period of 10 min was allowed for the preparations to recover from the initial effects of surgery and then the 'mean' interspike interval was measured at 1 min intervals for a period of 1 h. Ten light-adapted animals and ten dark-adapted animals were used, and the mean values for the 'mean' interspike intervals are plotted in Fig. 7.

Three features are striking about the data shown. First, the dark-adapted animals have a mean value for the interspike interval consistently longer than the light-adapted throughout the period of observation. Second, the dark-adapted animals show greater variance about the mean, as evidenced by the standard error of the mean, than do the light-adapted. Third, both groups show a progressive increase in the interspike interval during the first 20 min of observation after which a rough plateau is maintained until about 50 min, when the two groups become more variable.

The asymptotic shape of the functions indicated that linear regression analysis would not be the best method of assessing the statistical significance of the differences between dark-adapted and light-adapted animals. An indication of this significance was obtained by performing Student's *t*-tests, in a form modified for small samples, on pairs of mean interspike interval values at various times during the course of the experiment. The results of this analysis are shown in Table 1.

Taking a 5% level of probability ($P = 0{\cdot}05$) as indicative of a significant difference

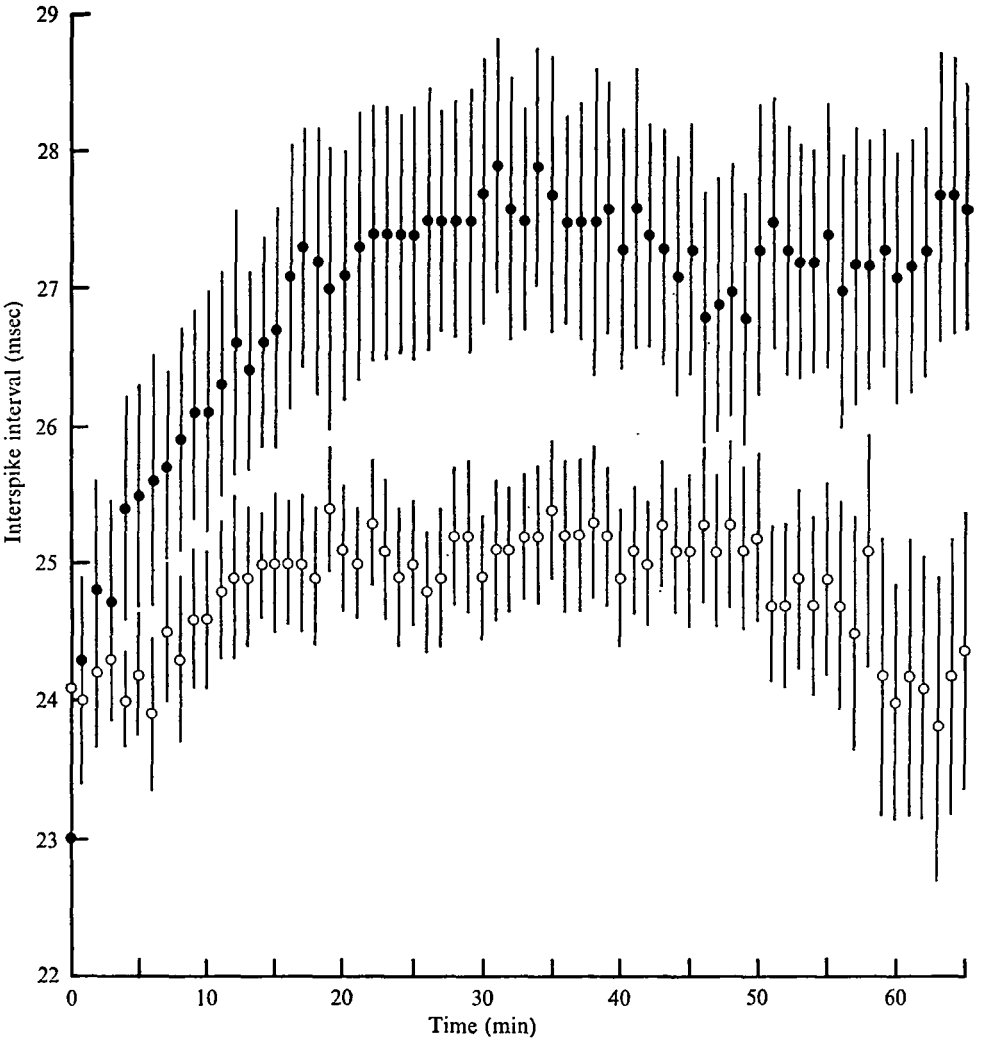



Fig. 7. 'Mean' interspike interval in milliseconds of eye-muscle potentials plotted as a function of time in minutes, for female animals. ○, light-adapted (6500 lux); ●, dark-adapted animals. Mean temperature for light-adapted group 17·5 °C, for dark-adapted 17·6 °C. All points are means of ten observations and bars indicate variance of the data expressed as ±1 standard error of the mean.

between the two sample means, the mean values for the interspike interval are significantly longer for dark-adapted animals at 30, 40 and 50 min after the beginning of the experiment, and are very nearly significantly different 20 min after the beginning of the experiment. The fact that these are only isolated points in whole curves which show the same trends must add to the significance of the data taken as a whole.

*Changes in compound eye depth with light- and dark-adaptation*

The proximal end of the eye muscle is attached to a rigid skeletal structure, the tentorium. The observed differences in the 'mean' interspike interval of the

Table 1. *Statistical analysis of resting mean interspike interval* (I.S.I.)

| | Time after start of expt. (min) | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | 60 |
| Dark-adapted mean I.S.I. (msec) $\bar{x}_1$ | 26·1 | 27·1 | 27·7 | 27·3 | 27·3 | 27·1 |
| Light adapted mean I.S.I. (msec) $\bar{x}_2$ | 24·6 | 25·1 | 24·9 | 24·9 | 25·2 | 24·0 |
| Standard deviation (based both on light and dark groups) (S) | 2·22 | 2·21 | 2·35 | 2·24 | 2·75 | 2·69 |
| *t*-value | 1·5 | 2·03 | 2·65 | 2·40 | 1·67 | 2·51 |
| Degrees of freedom ($n-2$) | 18 | 18 | 18 | 18 | 17 | 17 |
| *P* value | > 0·10 | 0·10–0·05 | 0·05–0·02 | 0·05–0·02 | > 0·10 | 0·05–0·02 |

Students' $t = \dfrac{\bar{x}_1 - \bar{x}_2}{S\sqrt{\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$.

$n$ = total no. of observations in both samples jointly.
$n_1$ = total no. of $x_1$ observations.
$n_2$ = total no. of $x_2$ observations.
$\bar{x}_1, \bar{x}_2$ = mean values.

eye-muscle potentials associated with light- and dark-adaptation should produce changes in the state of contraction of the muscle which should therefore be reflected in changes in the depth of the elastic compound eye at the insertion of the muscle.

To test this idea groups of five female (i.e. ten compound eyes) *C. vomitoria* were either dark or light (6500 lux) adapted at a constant ambient temperature. After fixation the heads were washed and frozen in water. The depth of the compound eye from the distal edge of the cornea to the basement membrane at the insertion of the eye muscle was measured with a micrometer eyepiece, as described under Materials and Methods. In Fig. 8 (circles) compound eye depth in $\mu$m is plotted for light-adapted and dark-adapted animals as a function of temperature.

The expected difference between dark-adapted and light-adapted eyes, with a longer interspike interval and therefore less contracted eye muscle for dark-adapted eyes, does in fact exist with dark-adapted eyes roughly 4–7% less deep than light-adapted. Table 2 gives a statistical analysis of the data and shows that mean eye depths are significantly different at the 5% level at 18·5, 19·0 and 21 °C.

Further analysis indicated that eye depth shows a slight, but statistically insignificant, negative correlation with ambient temperature. Correlation coefficient: $r = -0{\cdot}226$ dark-adapted, $r = -0{\cdot}281$ light-adapted, *P*, value, = > 0·10 dark-adapted, *P*, value, = > 0·10 light-adapted. These data indicate that, although the graphs of Fig. 8 are rather sawtooth-shaped, the observed marked temperature dependence of the 'mean' interspike interval for the eye-muscle potentials is not reflected in similar changes in compound eye depth: differences in muscle potential interval occurring with light- and dark-adaptation at a constant temperature do, however, produce expected changes in compound eye depth. These differences between light-adapted and dark-adapted eye depths were slight, and it was found difficult to obtain consistent measurements of the compound eye depth with the micrometer eyepiece. This, together with prior knowledge of the state of adaptation of the eyes and the absence of permanent material which could be subjected to arbitrary examination, meant that the existence of subjective bias on part of the observer could not be

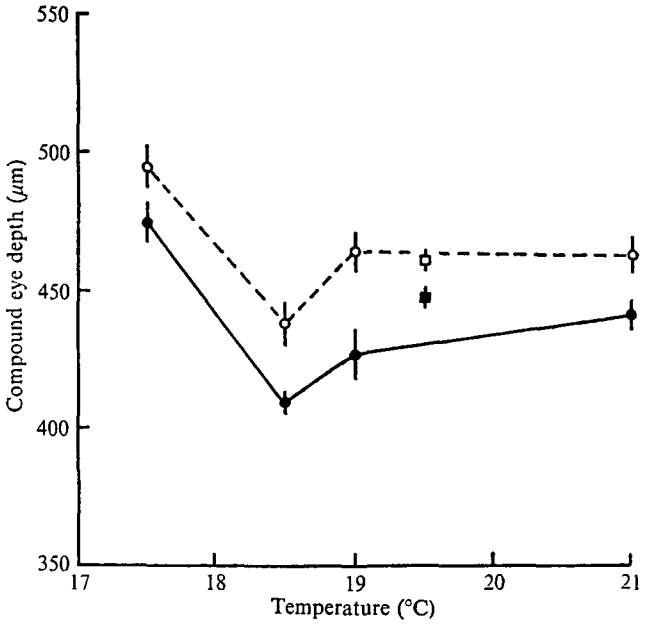



Fig. 8. Compound eye depth in $\mu$m at the insertion of the eye muscle, plotted as a function of temperature in degrees Centigrade, for female animals. ○, Light-adapted (6500 lux); ●, dark-adapted eyes. All points are means of up to ten observations and bars indicate the variance of the data expressed as $\pm 1$ standard error of the mean. Open squares are means of up to 30 light-adapted (6500 lux) and closed squares are means of up to 30 dark-adapted eyes. These latter data form the test for subjective error (see text).

Table 2. *Statistical analysis of mean compound eye depth ($\mu$m)*

| Temperature (° C) | 17·5 | 18·5 | 19·0 | 21·0 |
|---|---|---|---|---|
| Dark-adapted depth ($\bar{x}_2$) ($\mu$m) | 474 | 409 | 427 | 440 |
| Light-adapted ($\bar{x}_1$) ($\mu$m) | 494 | 438 | 464 | 462 |
| Standard deviation based on both light and dark adapted groups (S) | 23·3 | 16·8 | 25·8 | 17·4 |
| *t* value | 1·92 | 3·63 | 3·28 | 2·64 |
| *P* value | 0·10–0·05 | 0·01–0·002 | 0·01–0·002 | 0·01–0·002 |

Student's *t* calculated as shown in Table 1.

overlooked. To eliminate some of these errors 30 dark-adapted and 30 light-adapted (6500 lux) eyes were prepared, and each head was placed in a numbered bottle by an assistant who noted whether the contents were light-adapted or dark-adapted. Eye depth was measured in the usual way, but knowledge of the state of adaptation was withheld until the end of the experiment. The results are plotted in Fig. 8 as squares, and although the separation of the means is less than in the other pairs of data, the difference between the means was highly significant, with a *P* value of 0·01–0·002. Mean eye depths were $447 \pm 3{\cdot}33$ $\mu$m (standard error of the mean) for the dark-adapted and $460 \pm 3{\cdot}56$ $\mu$m for the light-adapted eyes.

*Pseudopupil movements*

A pseudopupil is not normally found in the compound eye of *Calliphora* but one may be readily observed when the rhabdomeres are illuminated from inside the eye. This technique has been employed by Gemperlein & Jahrvileheto (1969) with *C. erythrocephala*, by Kirschfeld & Franceschini (1969) with *Musca* and by Franceschini & Kirschfeld (1971) with *Drosophila*.

In the present experiments with *C. vomitoria* animals were decapitated and a small area of the dorsal aspect of the compound eye was sliced off. The cut region of the compound eye was then placed on the end of a light-guide and the head was held in place by the surface tension created by a drop of saline applied to the end of the light-guide. The light-guide introduced bright white light into the compound eye in a plane at right angles to the plane of the rhabdomeres. Animals were neither light-adapted nor dark-adapted prior to the experiment.

The compound eye was viewed with a binocular microscope and attention was concentrated on the large image seen deep within the compound eye. This image corresponds to the 'pseudopupille profonde' of Franceschini & Kirschfeld (1971). The pseudopupil images which are formed by the illumination of the seven rhabdomere tips beneath each corneal lens were not studied in these experiments.

The deep image of the 'pseudopupille profonde' consists in *C. vomitoria* of seven bright points of light arranged in the arrowhead configuration common to many Diptera with the 'open' type of rhabdomere arrangement. This deep pseudopupil image was examined for the presence of movements which could be attributed to the activity of the eye myscle. Periodic movements of the deep pseudopupil image were found to occur spontaneously in many animals but in only 10–20% of the total number examined. The movements were for the most part slow, of quite small amplitude and of the order of one or two pseudopupil image diameters in each direction, and were predominantly towards and away from the midline. The frequency of the movements was variable and the movements were often irregular. In two cases where regular oscillation of the pseudopupil occurred the frequency was found to be 1 Hz and 1·7 Hz. These values compare favourably with oscillations in the spontaneous activity of the eye muscle as shown in Fig. 5(E).

The direction of pseudopupil movement was examined in different parts of the compound eye in nine animals. These observations were used to construct the composite diagram which forms Fig. 9. The individual images in this diagram are relatively larger than those found in the eye, and the arrows merely indicate the direction of movement and not the amplitude. It is possible that some optical deformation of the images has occurred; nevertheless the predominant directions of movement agree well with the contraction and relaxation of a muscle inserted on to the eye near the midline about half-way between the dorsal and ventral limits of the eye. Contraction of the muscle would tend to pull the back of the eye posteriorly, slightly ventrally and towards the midline.

Movements of the pseudopupil were produced when the antennae were moved by the animal and were especially large when the mouthparts were extended and retracted. Careful observation showed that pseudopupil movements occurred independently of either mouthpart or antennal movement. Electrophysiological

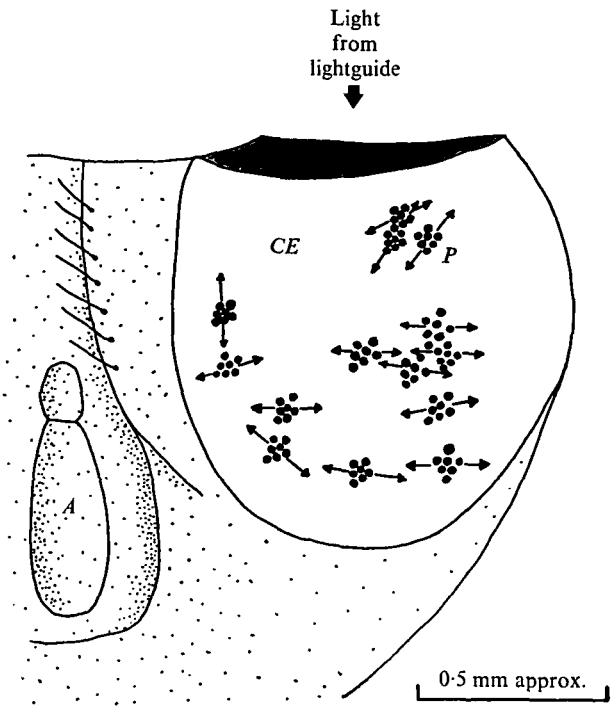



Fig. 9. Composite diagram based on observations of nine female animals to show the direction of the spontaneous deep pseudopupil movements as found in various regions of the compound eye. For clarity the deep pseudopupils have been shown with a diameter about twice that observed in the preparation.

experiments indicated that marked changes in eye-muscle activity often accompanied mouthpart or antennal movement so that pseudopupil movements associated with the motion of these organs may not be due simply to passive deformation of the compound eye.

It is noteworthy that Franceschini & Kirschfeld (1971) observed spontaneous movements of the deep pseudopupil in *Drosophila* and admitted to the temptation of ascribing these movements to the action of a muscle such as that described by Burtt & Patterson (1970) and Hengstenberg (1971).

## DISCUSSION

Lownes' (1892) suggestion that the compound eye is accommodated for different object-to-eye distances by the action of the eye muscle becomes unsatisfactory when consideration is given to the dioptric properties of the compound eye of *Calliphora*. The small physical dimensions of each of the corneal lenses means that both diffraction and refraction contribute to the formation of the image which lies at the distal tips of the rhabdomeres. A point source in the visual field of an ommatidium does not form a point image but rather a complex intensity pattern distributed along the visual axis of the ommatidium (Kuiper, 1966). This implies that the corneal lens-to-image distance remains relatively constant for a wide range of object distances so that accommodation of the visual apparatus to different image positions is an unlikely consequence of the activity of the eye muscle.

Two aspects of the visual process prove more profitable in the search for a hypothesis for the functions of the muscle. These are retinomotor phenomena and scanning of the visual environment.

Retinomotor phenomena (reviewed by Mazokhin-Porshnyakov, 1969) are relative movements of parts of the visual apparatus which are correlated with the process of light- and dark-adaptation. Such movements commonly affect the rhabdomeres, retinula cell nuclei and the distribution of screening pigments between the ommatidia. The rhabdomere movements described show with dark adaptation a consistent and often marked distal migration towards the crystalline cone. This has been observed in *Culex* and *Anopheles* (Diptera; Nematocera) by Sato (1957, 1959) and by Sato, Kato & Toriumi (1957); in certain chironomids (Diptera; Nematocera) by Tuurala (1963); in *Notonecta* (Hemiptera; Heteroptera) by Lüdkte (1953) and by Reis (1960) in *Lethrocerus* (Hemiptera; Heteroptera) and in *Dytiscus* (Coleoptera) by Walcott (1969). All of these animals with the exception of *Dytiscus* have eyes of the apposition type where the rhabdomeres extend for most of the length of the ommatidium. For these eyes Sotavalta, Tuurula & Oura (1962) suggest that with dark-adaptation increased sensitivity is achieved by widening the 'aperture' formed by the rhabdomeres, pigment, crystalline cone and crystalline thread, all the structures acting in concert to produce an 'iris-diaphragm' effect. The changes in the depth of the compound eye of *Calliphora* described above and the correlated changes in eye-muscle activity could act so as to bring the rhabdomeres closer to the corneal lens with dark-adaptation. Movements of the rhabdomeres must be slight, however, for light microscopy has failed to show gross movements of the rhabdomeres such as occur in other species. A further complication arises from the pseudocone construction of the compound eye in *Calliphora*. The cones in this case are not strictly crystalline and the corneal lens is the major refractive element of the system. In addition, the eye does not possess a crystalline thread so that it is unlikely that an 'iris-diaphragm' effect can operate in the usual way.

If it is assumed that each rhabdomere has a diameter of 1 $\mu$m and that the volume remains constant during the changes in eye depth which accompany light and dark adaptation, then a 5% decrease in eye depth with dark adaptation will produce an increase in rhabdomere diameter of 25 nm. It is difficult to see how such a dimensional change could significantly affect the light-trapping or guiding properties of the rhabdomere, although Snyder & Miller (1972) suggest that, in *Calliphora*, the dimensions of the rhabdomeres may determine which wavelengths of light are absorbed by the photoreceptors. Larger-diameter rhabdomeres would tend to absorb a greater proportion of longer wavelengths.

A clearer function for the eye muscle is found in the production of eye movements which result in scanning of the image produced by the corneal lens. The cornea is relatively rigid and forms the hinge about which the rest of the compound eye moves with contractions and relaxations of the eye muscle. The movements of the deep pseudopupil image described above seem to be the consequence of eye-muscle activity, and the movements suggest that the rhabdomeres change the aspect of the visual field at which they are 'looking'. The distal tips of the rhabdomeres are quite close to the cornea and therefore close to the fulcrum of the system, so that any movements of the tips produced by eye-muscle activity must be small. It is still

possible, however, that the complex intensity pattern, which is distributed along the visual axis and which, because it is predominantly produced by the corneal lens, is immobile, will allow the rhabdomere tips to move through contrast boundaries and so increase the flow of visual information into the central nervous system.

Apparatus which promotes the movement of contrast boundaries across photoreceptors is found in the visual system of many animals. The great importance to the quality of vision of the rapid eye movements produced by the extrinsic eye muscles in man has been demonstrated by Ditchburn & Ginsborg (1952) in experiments in which the visual image is stabilized on the retina. Similarities with the vertebrate system are found in the movements of the eyestalk which bears the compound eye in crabs (Burrows & Horridge, 1968). In these systems, movement of contrast boundaries across photoreceptors is achieved by moving both the visual organs relative to the visual environment, but independently of the body of the animal. In locust nymphs Wallace (1959) describes 'peering' movements, involving movement of both compound eyes simultaneously relative to the environment, which are produced by the action of the pro- and mesothoracic legs.

It is only in arthropods that the photoreceptors alone are moved relative to the static image produced by the corneal dioptrics. Land (1969*a*, *b*) has shown that the retina of the principal eyes of salticid spiders is moved by a system of muscles so as to scan the image produced by the corneal lens. The system responds to visual stimuli and also produces a tremor of frequency of 1·0–0·5 Hz. The copepod crustacean *Copilia* has been studied by several authors. The two photoreceptors each consist of five rhabdomeres of the 'open' type associated with a small lens (Wolken, 1969). An attached muscle causes the photoreceptors to scan the image produced by the corneal lens with a maximum frequency of 5 Hz (Gregory, Ross & Moray, 1964).

Not only does it appear that *Calliphora* has a system capable of producing a similar type of photoreceptor movement but also the observed frequencies of about 1 Hz for the movement of the deep pseudopupil image and for fluctuations in the interspike interval of the eye-muscle potentials are similar to those found in comparable arthropod systems.

## SUMMARY

1. A muscle attached to the medial edge of the compound eye is described for the blowfly *Calliphora vomitoria*.

2. Electrophysiological activity in the form of continuous tonically firing potentials can be recorded extracellularly from the muscle. These potentials are generated by the muscle and have the same origin as the 'clock-spikes' recorded previously from the optic lobe of *Calliphora erythrocephala*.

3. The interspike interval of the eye muscle potentials varies inversely with the ambient temperature.

4. Light-adaptation results in a decrease and dark-adaptation an increase in the *resting* interspike interval of the eye-muscle potentials.

5. Light-adaptation is correlated with increase and dark-adaptation with decrease in the depth of the compound eye as measured at the insertion of the muscle.

6. The pseudopupil produced by illumination of the compound eye from the

inside displays spontaneous movements which can be correlated with the anatomical arrangement and spontaneous activity of the eye muscle.

7. The probable function of spontaneous and transient changes in eye-muscle activity is to promote scanning of the visual images produced by the dioptrics of the compound eye.

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