

## MOTONEURON RESPONSES TO VISUAL STIMULI IN *ONCOPELTUS FASCIATUS* DALLAS

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### INTRODUCTION

The optomotor response has been a subject of investigation for many years, both for its own sake and as a convenient response by which measurements of visual acuity and threshold may be obtained. Its presence in both vertebrates and invertebrates may be due either to underlying similarities of mechanism or to functional convergence of fundamentally dissimilar ones. Insects have proved particularly valuable in studying optomotor responses because of their hardiness and the advantages to the experimenter of an eye which can be blocked without surgical procedures. They have provided an experimental basis upon which sophisticated mathematical models of movement perception have been constructed (Hassenstein & Reichardt, 1956; Bliss, 1964).

While formal models of the optomotor response have been derived from behavioural experiments, physiological information has contributed less to our understanding of the components which interact to produce the final response. The discovery of units in insects and crustaceans (Burt & Catton, 1960; Blest & Collett, 1965; Waterman, Wiersma & Bush, 1964) that respond to complex visual stimuli indicate a possible source for the commands from the supraoesophageal ganglion which are presumed to regulate leg motoneurons in thoracic ganglia. These units derive information about a variety of visual events, and it is important to distinguish the units responsible for signalling turning to the legs from those that signal other commands. To do this, it is necessary to work from the known behavioural responses and to examine the motoneurons for evidences of visual control of their discharge. In this paper some experiments are reported on the effects of simple visual stimuli upon the motoneurons innervating the metathoracic legs of the milkweed bug *Oncopeltus fasciatus* Dallas.

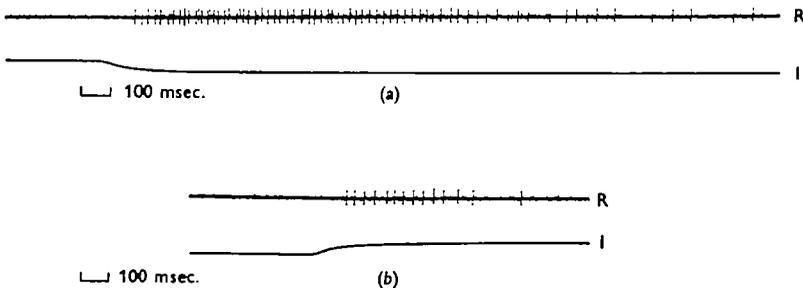
### METHODS

*Oncopeltus fasciatus* was reared on milkweed seeds and water in glass containers, from a strain kindly presented to us by Mr Elmer Cummings of the Stauffer Chemical Company. The anatomy of the central nervous system of *Oncopeltus* has been described by Rutschky & Stryjaker (1955) and by Johansson (1957). Physiological solution made up according to Burt & Catton (1954) was used for most experiments. Animals were anaesthetized with nitrogen; the pronotum and the abdominal and thoracic terga were removed by lateral cuts and the digestive tract and muscles of the

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thorax were also removed so that the nervous system was exposed. The metathoracic nerves were cut peripherally and drawn into suction electrodes, and the signals were amplified on an oscilloscope. In later experiments the signals were also recorded on an instrumentation tape recorder, to be later processed by playing them through a window circuit into a CDC 160 computer. The computer recorded the successive intervals between action potentials and calculated the instantaneous frequency against time (for plotting on an  $X$ - $Y$  plotter), the average interval, its variance and an interval histogram.

The light source for all experiments was a tungsten microscope lamp (with an intensity of  $1.42 \times 10^3$  lumens/m.<sup>2</sup>); a Compur shutter was employed to turn the light on or off, and lesser changes in light intensity were produced with Wratten neutral density filters. The animal was mounted on a platform suspended within a 4 in. diameter Lucite drum in those experiments involving moving stripes. Single stripes (subtending an angle of  $55^\circ$ ) were constructed with black electrical tape, while the regularly spaced stripes (subtending  $10^\circ$ ) were similar to those used previously by Bliss (1964). The light was directed toward the head (and parallel to the long axis of the animal), and the movement of the stripes was monitored with a photocell which displayed on the oscilloscope a d.c. voltage proportional to the light intensity. In some experiments a 3.0 N.D. filter was mounted upon a motor-driven wheel so as to interrupt the lamp beam for 0.3 sec. every second, thereby giving periodic decreases in light intensity.



Text-fig. 1. (a) Response of motoneurons in the right metathoracic nerve to a 2.0 log unit decrease in light intensity. Large, intermediate and small amplitude fibres present. I indicates time course of light intensity change monitored by a photocell. (b) Response of motoneurons in the right metathoracic nerve to a 2.0 log unit increase in light intensity. Intermediate and small amplitude fibres present.

## RESULTS

### *Responses to changes in light intensity*

In response to increases or decreases in light intensity a prolonged motoneuron discharge was observed in either of the metathoracic nerves cut peripherally and drawn into the electrode (Text-fig. 1). At threshold for this response a small-amplitude, spontaneously active unit was observed to speed up, and another normally silent fibre with an extracellular potential from two to three times as great fired 50–80 times a second. Greater changes in light intensity recruited a slightly larger fibre which fired at 50–60 times a second; this fibre adapted before the intermediate amplitude unit. In response to light-off the latency of the intermediate unit ranged from 115 to 130 msec.; the corresponding units of the paired nerves had initial latencies within

2–3 msec. of each other. Abnormally long latencies were observed in some preparations but could be clearly distinguished from a normal response by a more gradual increase in frequency after the units were first activated. Often the intermediate amplitude fibre fired alone for 4 or 5 sec. showing periodic variations in frequency; the larger fibre, in contrast, fired for a much shorter period of time. In many records it was possible to distinguish the intermediate and large amplitude units from one another by their relative sizes; the following description applies to the intermediate fibre except when otherwise noted.

An increase in light intensity produced a response with a different frequency envelope from that observed with decrease in light intensity, but showed similar relationships between different units. The 'on' threshold was higher than the 'off' one, as judged by the increase in light intensity needed to evoke the discharge and by its duration. A more gradual increase to a maximum frequency characterized the 'on' discharge compared to the sharp burst characteristic of the 'off' discharge (Text-fig. 1*b*).

Periodic fluctuations were observed in the frequencies of presumably homologous units in both right and left metathoracic roots (Pl. 1*a*). The normal response showed an initially high frequency which waxed and waned. In the initial part of the response the two sides fired together but then progressively moved out of phase. The period of these regular variations in frequency was about 1 sec. from peak to peak, thus being of the same order of magnitude as the frequency of leg movements during walking in many insects (Hughes, 1965).

In order to determine whether this motoneuron discharge was indeed correlated with movements of the legs, the left metathoracic nerve was drawn into the electrode after severing it distally, while the right metathoracic leg was left intact with a small piece of aluminium foil fixed around its tibia. The animal was mounted so that the left metathoracic leg extended over the side of the dish; light reflected from the flat surface of the foil to a photocell providing a means of monitoring gross movements of the leg. When the light was turned off (Pl. 1*b*), there was a discharge on the left side and extension of the tibia on the right, followed by alternate flexion and extension of the left metathoracic leg. The periodic fluctuations in the frequency of the unit in the left nerve was clearly related to extension of the other leg; the minimum frequency of the unit was correlated with extension of the opposite leg, after correction for phase lag due to conduction velocity and neuromuscular delay. The latency for leg extension varied in four preparations from 180 to 250 msec. In addition to extension of the tibia retraction of the femur was also observed (a much smaller movement).

The frequency of firing and the duration of discharge of the unit depend upon the magnitude of the change in light intensity (and its sign, i.e. 'off' or 'on'), the time after the previous stimulus, and a variation in the threshold of the response which is more difficult to specify. In one experiment the number of spikes in the initial second of the discharge was plotted against the decrease in light intensity (in log units). When computer data-processing became available, the integral of the instantaneous frequency against time of the response was calculated for another experiment, by measuring the area under the frequency against time curves with a planimeter. The two curves (Text-fig. 2*a*) are similar except that points of the second experiment are shifted along the X-axis, indicating a lower threshold for this preparation. With greater decreases in light intensity the larger fibre is activated, making experiments involving large changes

in light intensity difficult to interpret. When a shutter was used (producing the greatest decrease in light intensity possible under these conditions), other units fired as well. There appeared to be several different thresholds for the response; initially the medium amplitude unit fired, at a higher stimulus intensity the larger unit joined it, and at a still higher stimulus intensity several other units appeared. The intermediate unit reached a maximum frequency level beyond which there was no further increase in frequency, but only in duration of discharge; this frequency level was just below threshold for the larger unit.

The second factor, that of the intensity of, and time elapsed since the previous stimulus, was very important in determining the intensity and duration of the response. In one set of experiments neutral density filters were interposed at regular intervals between light source and animal, and the number of stimuli required to adapt the response so that it could no longer be evoked was recorded (Text-fig. 2*b*). The number of stimuli required to adapt the response increased as a function of intensity. In a second set of experiments the response was similarly adapted, and various intervals of time were then allowed to elapse before the filter was again repositioned and the response again adapted. A plot of the number of spikes in each response after the repositioning of the filter is shown in Text-fig. 2*c*. Qualitatively, the intensity of the response and the amount of time necessary to re-adapt it increased the more time was allowed for recovery, but the recovery process seemed strikingly non-linear.

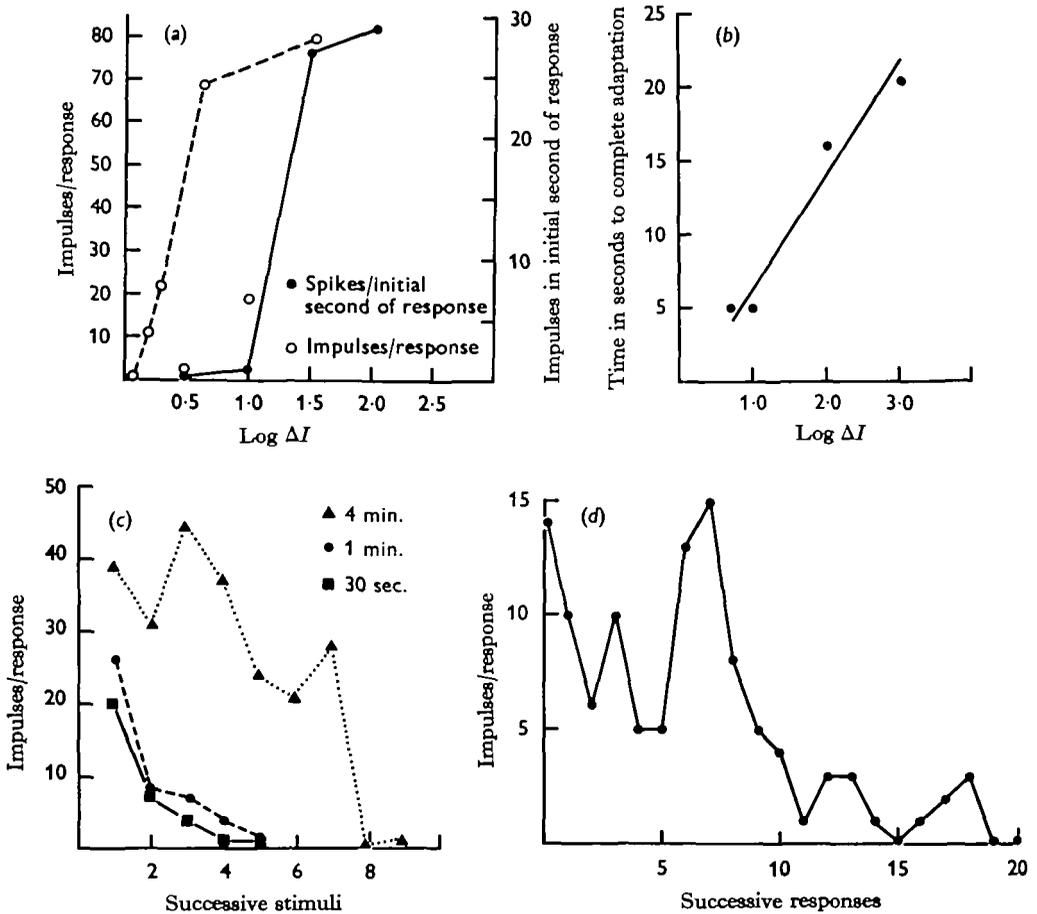
The third factor, long-term variation in the response to the same stimulus, is shown in Text-fig 2*d*. The response does not become steadily smaller, but after diminishing suddenly increases again; it is thus similar to the 4 min recovery curve in Text-fig. 2*c*. This variation in response to the same stimulus was always present. On some occasions a decrease of light intensity well above 'threshold' would produce no response at all; this did not appear to be due to injury.

Another puzzling aspect of the response may be seen in the simultaneous recordings from left and right metathoracic nerves (Pl. 1*a*). One side of the same preparation produced a higher frequency response than the other; during cyclical variations of frequency, this side had a higher 'basal' level of frequency than the other. This asymmetry did not appear to be affected by the angle made by the light from the longitudinal axis; when the light was placed 45° to right or left of it there was no resulting change in the asymmetry. Neither did it appear to be a function of the angle of the head capsule with the thorax. This was tested by fixing the head at different angles to the longitudinal axis; again there was no variation in the asymmetry. These possibilities were not investigated quantitatively so that it only appears that the dramatic asymmetry observed is not radically altered by these factors.

#### *Responses to moving stripes*

*Oncopeltus fasciatus* has been shown to have an optomotor response similar to that of *Chlorophanus* (Bliss, Chapple, Crane & Seeley, 1964). It seemed reasonable to expect some alteration in the discharges of the motoneurons in response to moving stripes. Simultaneous recordings were made from both left and right metathoracic nerves when the animal was placed at the centre of a drum with regularly spaced stripes. Responses could be evoked when the speed of the drum was changed, but these responses occurred only in the small fibre, whose signal-to-noise ratio was too

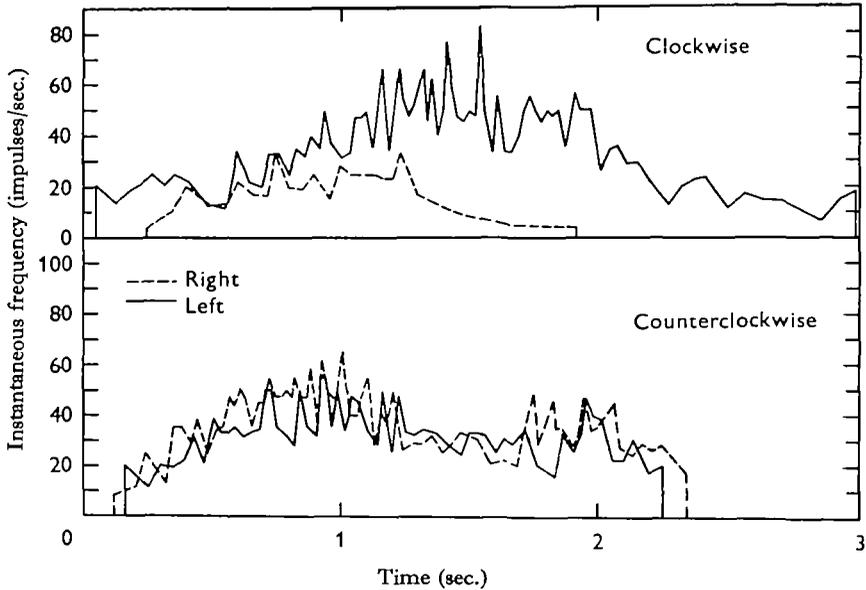
unfavourable for further study. On several occasions there was a transient burst of impulses in the intermediate amplitude fibre in response to changes in the speed or direction of the drum. This response, however, did not persist.



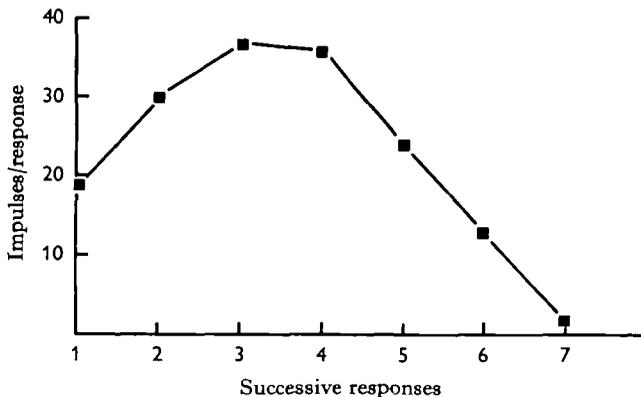
Text-fig. 2. (a) Relationship between strength of the response and change in light intensity measured by two different methods (see text). In each case the response was from the right metathoracic nerve. (b) Relationship between stimulus intensity and length of time required to fatigue the response of the right metathoracic nerve. Neutral density filters were repeatedly interposed between the preparation and light source to produce 0.6 sec. duration decreases in light with 0.6 sec. duration normal light intensity; the time was noted at which no further response could be elicited. (c) Similar to (b) except that the preparation was allowed to recover for various lengths of time and the response of the right metathoracic nerve was again fatigued to extinction. (d) Plot of the time course of the fatiguing process, showing the variation in intensity of successive responses. 3.0 N.D. filter interposed for 0.2 sec. and normal light intensity for 0.8 sec. Right metathoracic nerve.

By using a single-striped drum a discharge was evoked in the intermediate unit as the stripe passed across the visual field of the animal. When the stripe moved in a clockwise direction, the frequency of the intermediate unit in the left metathoracic nerve was greater than that of the homologous unit in the right metathoracic nerve (Text-fig. 3). If the drum was rotated in a counterclockwise direction, the response of the

unit on the left side diminished while that on the right increased. The duration of the response increased with the frequency of the unit, and at some speeds the responses to the successive appearances of the stripe merged into one another to produce a high-frequency discharge of a number of units which varied in intensity, increasing as the



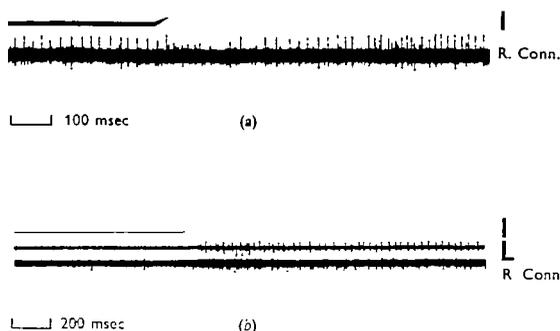
Text-fig. 3. Plot of the response of left and right metathoracic nerves to the movement of a  $55^\circ$  stripe around the animal at  $63^\circ/\text{sec}$ . Asymmetry related to movement superimposed upon normal asymmetry of the response.



Text-fig. 4. Fatigue of the response of the left metathoracic nerve to a  $55^\circ$  stripe rotating at  $130^\circ/\text{sec}$ .

stripe passed across the visual field. The asymmetry in the frequencies of the two units was superimposed upon the asymmetry natural to the system, as can be seen in Text-fig. 3. The difference in the frequencies of the units was not a transient phenomenon but persisted for the whole duration of the response and was present at all speeds. Attempts were made to determine whether the ratio of frequencies of the two units varied as a function of speed, but due to the presence of additional units at rotations

of 60–100 per sec, the response was too complex for analysis. It appeared that the frequencies of both units peaked in this range. Quantitative measurements were made but the variation was so great in the means of the units at each speed that this impression could not be substantiated.



Text-fig. 5. (a) Transient inhibition of unit in the right connective between prothoracic and central ganglia to light-off. I is light intensity. (b) Simultaneous recording from right prothoracic-central connectives (R. Conn.) and left metathoracic nerve (L) showing response to light-off. Phasic response in the connective precedes the response in the nerve by about 30 msec.

Responses to periodic movement of the stripe across the visual field were measured by obtaining the integral of the frequency for each response (Text-fig. 4). The response reaches a peak and then declines, showing little of the complex variations observed in the 'static' condition.

#### *Responses from the connectives*

In view of the large amount of work done on visual units in the insect connectives (Burt & Catton, 1954; Suga & Katsuki, 1962), it seemed important to see whether there were units in the inter-ganglionic connectives of *Oncopeltus* that responded to changes in light intensity with a latency appropriate for triggering the response of the motor fibres. The connectives between the prothoracic and central ganglia are about 1.5 mm long and enclosed in a tough connective sheath; single-fibre dissection, extracellular microelectrodes, or silver wire electrodes were not feasible; however, when the tip of a fine suction electrode was placed on the connectives, extracellular responses to light were observed. These were of two types: (a) a transient inhibition of a tonically firing unit to light-off (Text-fig. 5 a); and (b) the response to light-off by a phasic burst (Text-fig. 5 b). The latter record shows the concomitant discharge in the left metathoracic nerve. The firing frequencies of these fibres in the connectives and in the motor nerves were correlated, and the adaptation of such a connective unit was correlated with a similar adaptation in the motoneuron. The latency of the response in the connectives was usually about seventy milliseconds.

#### DISCUSSION

The responses of metathoracic motoneurons associated with tibia extension showed a number of features in common with some units observed at higher levels of the insect nervous system. Normally silent, they respond to changes in light intensity by a tonic discharge whose duration and frequency are proportional to the magnitude of the

change in light intensity. Complex adaptation of the tonic discharge is another property which the motoneurons share with visual units recorded from the optic lobes and medial protocerebrum. Indeed, the rhythmic fluctuations in frequency have been observed in units in the connectives and protocerebrum (Blest & Collett, 1965). But there are differences as well, particularly in the long tonic discharges (occasionally 20 sec. in duration) and generally lower frequencies of these units. Furthermore, the responses of the motoneurons are not precisely like any of the units described by Burt & Catton or by Blest & Collett, but seem to be produced by a combination of these units. There appeared to be no inhibition of spontaneously active motoneurons by either increases or decreases in light intensity.

The responses observed in the connectives, on the other hand, appear similar to those described by previous workers. The latency of these fibres is about 30 msec. less than that observed in the motor fibres, and presumably these interneurons trigger the motoneuron discharge. It thus seems likely that several units with different types of response to light sum in the central ganglion to produce the response of the motoneurons. Previous workers have established that walking and flight, although triggered from higher centres, owe much of their organization to events within the ganglion of the motoneurons which innervate the relevant musculature. It appears that the incoming fibres are summed by a centre with a long time constant which then acts as a source of excitation for the motoneurons. When the metathoracic ganglion is completely isolated the spontaneous activity in the leg nerves rapidly disappears, although it may be present in other nerves from the same ganglion.

The control of turning does not seem to require an elaborate mechanism to explain it. Homologous units passing down each connective might sum upon interneurons on either side of the ganglion; interaction between these cells might then produce the simultaneous response of motoneurons on each side. Such homologous units are known from the connectives (Suga & Katsuki, 1962; Burt & Catton, 1954; Goodman, 1965), and interactions between the 'motor units' of legs of the same ganglion have been known for many years.

One curious feature of the present experiments is the presence of a bias, as evinced by a higher firing frequency of one of a pair of (presumably) homologous units to the same stimulus. Behavioural asymmetry has been observed by Fraser Rowell (1963) during experiments on the scratch reflex in locusts, so the existence of a neurophysiological asymmetry is not too surprising. No symmetrical animals were observed in any of the experiments. It seems likely that the asymmetry is to some extent an artifact since insects do not normally run in circles. These experiments involved cutting the metathoracic nerves to record from them, thus depriving the motoneurons of the normal sensory feedback which presumably cancels out the asymmetry during walking. The absence of this normal sensory feedback may also explain the lack of any response to the regularly striped drum. Normal sensory feedback might maintain the 'motor unit' in an excited state, so that the repetitive stimuli of the regularly striped drum would not fall below threshold, but would sum to produce a turning reaction. In the absence of sensory feedback the regularly striped drum would not excite the 'motor unit'.

Several points need further work. Latency measurements of the response to movement assume a particular importance in view of the recent work of Blest & Collett

who classified units in the medial protocerebrum by latency, response characteristics, and visual field. Similar work is needed to establish the latency and visual fields of the motoneuron responses. In particular, latency measurements might make it possible to separate 'static' from 'moving' changes in light intensity. Such experiments were attempted but proved unsuccessful for technical reasons.

The question of the way in which the ratio of the frequencies of the units on the two sides varies with stripe speed is an important one. One possibility is that the ratio increases in a fairly continuous fashion as the stripe speed increases. Another possibility is that the frequency ratio remains constant but the discharge is maintained for a longer period of time, so that the duration of motoneuron discharge rather than its frequency is related to stripe speed in the manner described by Hassenstein & Reichardt (1956) for their behavioural experiments. This would make unnecessary the precise adjustment of the frequencies of the units on the two sides; a more leisurely adjustment of the duration of the response would require less elaborate mechanisms for its control. Although units have been observed that respond to increasing speeds with continuous increases in frequency, these might conceivably sum at some point to produce such an output.

#### SUMMARY

1. Tonic discharges of homologous units in the left and right metathoracic leg nerves in response to increases and decreases in light intensity were observed.
2. Periodic variations in the frequencies of these units was shown to be related to leg movement and at a frequency appropriate for walking.
3. The intensity and duration of discharge of these units was related to the change in light intensity and the frequency of stimulation.
4. The unit of one side normally fired at a higher frequency than that of the other.
5. This was separate from the asymmetry produced by placing the animal in a rotating drum with a single 55° stripe. In this case, clockwise movement of the stripe produced a higher frequency discharge on the left side, while a counterclockwise movement reversed the frequency ratio.
6. Units in the connectives appeared to trigger this discharge.

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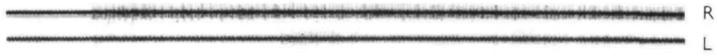
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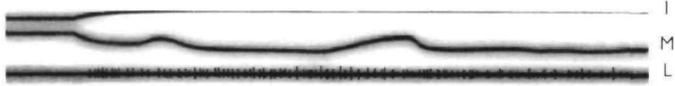
## EXPLANATION OF PLATE

(a) Simultaneous recording from left and right metathoracic nerves showing marked asymmetry in the firing frequency of units on the two sides and the periodic variations in frequency probably associated with walking. Note that in the later portions of the record, the units of the two sides are out of phase. (b) Simultaneous recording from the left metathoracic nerve and movement of the tibia of the right metathoracic leg. Photocell records peak of extension (M); initial deflexion is due to stimulus light source (I) being shut off. Note periodic frequency variations in nerve similar to (a).



—| 200 msec.

(a)



—| 200 msec

(b)

