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THE MECHANISM OF THE PUPAL GIN TRAP

II. THE CLOSURE MOVEMENT

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INTRODUCTION

Little attention has been given to the behaviour of pupae, despite its developmental interest and its obvious simplicity. From time to time it has been suggested that pupae are totally quiescent (e.g. Chapman, 1969) or that the movements which they make are elicited in some novel, non-neuronal way (Ashhurst & Richards, 1964). In part, this view is based on the assumption that the delicacy of the metamorphosing nervous system and the large-scale rearrangements which it undergoes prevent it from functioning. However, the interesting point is not that the pupal nervous system ceases to function but that it continues to do so throughout the period of reconstruction, until the onset of the adult moult.

The pupal stage is perhaps a highly modified last larval instar (Hinton, 1963). Certainly among the lepidoptera all of the muscles and most of the neurones functioning in pupal behaviour are the remnant of those which were once the organized basis of larval behaviour. In the privet hawk moth, *Sphinx ligustri*, a process of degeneration which begins at the end of the fifth instar and continues into the early hours of pupal life leaves persistent muscles in abdominal segments 3, 4, 5 and 6 (Fig. 1). With the exception of segment 3 these segments are jointed, and in segment 3 the muscles are reduced. In the diapausing pupa the reorganization of the larval nervous system has also begun, but the reconstruction of the neural lamella and the migration of ganglia are arrested at a particular and consistent point by the onset of diapause. The behaviour of the pupa at this stage therefore depends on a persistent fragment of the larval musculature which is driven by a partially reconstructed nervous system (Fig. 1).

The behaviour patterns of the pupa are defensive and hygienic. They enable the developing insect to repel predators and parasites and to clear debris which obstructs its cell and deforms its integument. The movements which sphingid pupae perform are of three kinds: the peristaltic contractions of the moult, sideways flexions of the abdomen, and twirling movements of the abdomen during which the cylindrically arranged muscles contract in a sequence which causes the tip of the abdomen to describe an ellipse about the longitudinal axis. In addition, those pupae like *Sphinx ligustri* which have gin traps perform a movement which closes the trap when the sensilla inside it are disturbed. The intention of this and a subsequent paper is to describe how the closure reflex is incorporated into the pupa's limited neuronal machinery.

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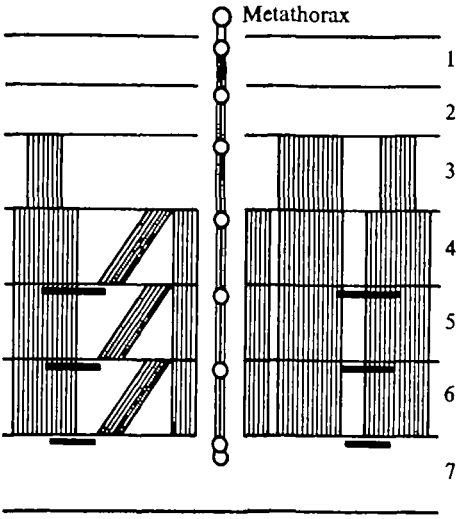

Fig. 1. A diagram of the persistent muscles and the abdominal nervous system in a diapausing pupa of *Sphinx ligustri*. On one side the ventral longitudinal muscles are partially removed to reveal diagonal muscles. At this stage the neuropile of the second abdominal ganglion is visible in the connective, arrested during its anterior migration to the thoracic ganglion of the adult. Abdominal segments numbered 1 to 7. Horizontal bars: gin traps.

METHODS

Pupae were prepared for experiment as before (Bate, 1973*a*). The muscles and fat body over the undersurface of the traps of interest were dissected away to leave the afferent bundles exposed for electrical stimulation. The remaining muscles and their innervation were left intact.

Nerve bundles selected for stimulation were sucked into fine glass pipette electrodes to which 500 μsec square pulses (amplitude 1·2–4 volts) were delivered at varying frequencies from the output of a pulse-generating system. The input stage of the pulse generator was modified to allow two trains of pulses of variable length to be delivered with a variable delay between the two.

Dorsal longitudinal, ventral longitudinal and ventral diagonal muscle groups persist in the pupa (Fig. 1). The motor innervation in each segment is divided into dorsal and ventral branches. Extracellular recordings show that there are 4 dorsal longitudinal units, 4 ventral longitudinal units and a single diagonal unit (Bate, 1972). Recordings were readily made from the dorsal and ventral trunks, and after further dissection from the individual motor units. Nerve bundles and single motor units were lifted onto bipolar tungsten hook electrodes. Impulses were fed via a differential amplifier to an oscilloscope for display and an a.c. tape recorder for storage.

RESULTS

The closure movement

The trap is closed by a very simple movement. Abdominal segments 4, 5 and 6 end posteriorly in flexible articular membranes. A transient increase in the tension of the muscles in one half of any of these segments causes the extended articular membrane

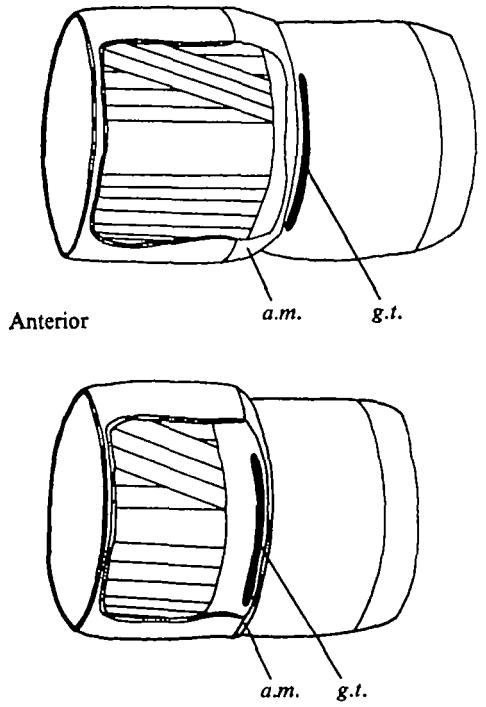

Fig. 2

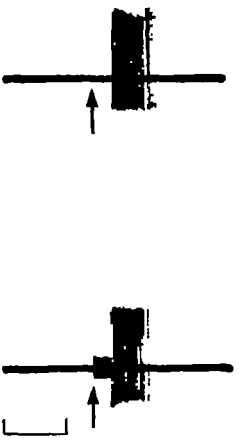

Fig. 3

Fig. 2. The closure of the gin trap. Upper: open; lower: closed. *a.m.*: articular membrane; *g.t.*: gin trap.

Fig. 3. A comparison of the response of a single efferent unit to (*a*) mechanical stimulation of the receptors in the adjacent trap and (*b*) an electrical stimulus (200 Hz) to nerve *B* leaving the trap. Time bar: 200 msec. Arrows: onset of stimulus.

to fold inwards, while the segment behind pivots about a dorso-ventral axis, pulling the trap under the folded membrane and crushing between it and the jaws objects which extend out of the pit (Fig. 2). The segments are stable in either the open or closed position, so that the trap once closed, remains shut.

The contraction which closes the trap is driven by a volley of impulses in the efferent nerves supplying the longitudinal and diagonal muscles of the segment anterior to the stimulated trap. Each unit in the ipsilateral half-segment fires at a similar frequency when the sensilla in the adjacent trap are disturbed.

The triggering stimulus may be mechanical or electrical. When the hairs in the trap are disturbed, single motor units respond with a train of impulses at a high frequency which abruptly declines to zero (Fig. 3). When nerve *B*, which carries axons from the receptors in the trap (Bate, 1973*a*), is stimulated electrically at frequencies from 100 to 200 Hz the response is identical. When a similar stimulus is delivered to nerve *A* (axons from receptors outside the trap) the response is characteristically different (see below).

The components of the reflex

Axons from the sensilla in the trap enter the CNS via the second nerve trunk of the ganglion in their segment (Fig. 4). Their path within the CNS was followed by

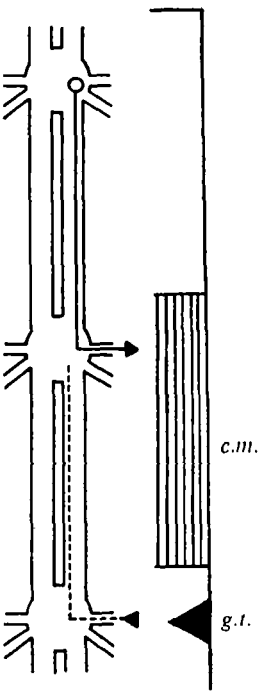

Fig. 4. The pathways of afferent (dashed line) and efferent neurones (continuous line) involved in the closure reflex, and the segments with which they are associated. Three ganglia are required for the performance of the reflex and the arrangement is repeated for each of the three pairs of gin traps. *g.t.*: gin trap; *c.m.*: muscles which close the trap.

stimulating the connectives and recording the antidromic pulse in the afferent bundle beneath the trap. The axons ascend the ipsilateral connective and terminate in the ganglion of the segment next anterior. The conduction velocity of the antidromic spike was 0·6 m sec^{-1}.

Similarly, the motor neurones driving the muscles which close the trap lie in the segment anterior to the one they innervate. Their axons descend the ipsilateral connective and emerge in the adjacent segment as a discrete bundle without synapsing in the more posterior ganglion. The arrangement of the motor neurones is identical to that previously described by Weevers (1966) in the larval nervous system of *Antherea pernyi*. The conduction velocity of the efferent spikes was 1·8 m sec^{-1}.

The arrangement of the reflex circuit can be confirmed by cutting the connectives; three ganglia are required for the performance of the closure response and the connectives of only one side need be intact (Fig. 4). The system is repeated for each of the three pairs of gin traps.

A comparison of the response elicited by stimulation of receptors inside and outside the trap

The high-frequency volley which closes the trap is confined to the motor units in the ipsilateral half of the next anterior segment. The efferent neurones which are outside this group either fail to fire (innervating muscles in contralateral half segments) or fire at a lower frequency (innervating ipsilateral muscles outside the closure segment).

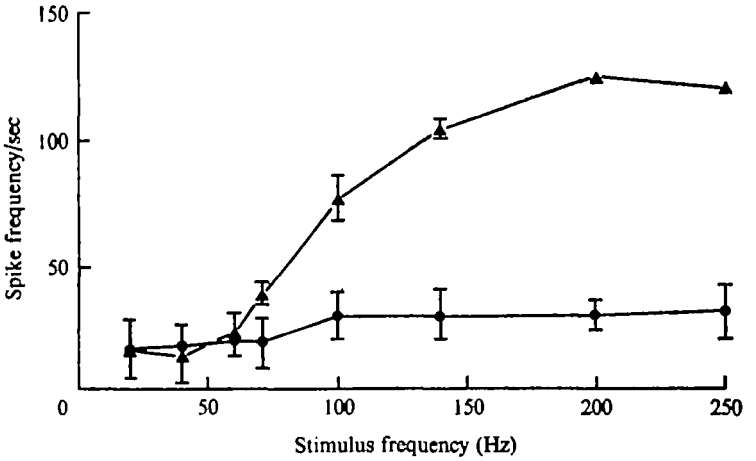

Fig. 5. The average frequency of the first ten spikes recorded in corresponding ventral motor units in segments 4 (triangles) and 5 (circles) in response to an electrical stimulus to nerve *B* in segment 5 at the indicated frequencies. The average frequency of the unit in the anterior segment rises with the stimulus frequency.

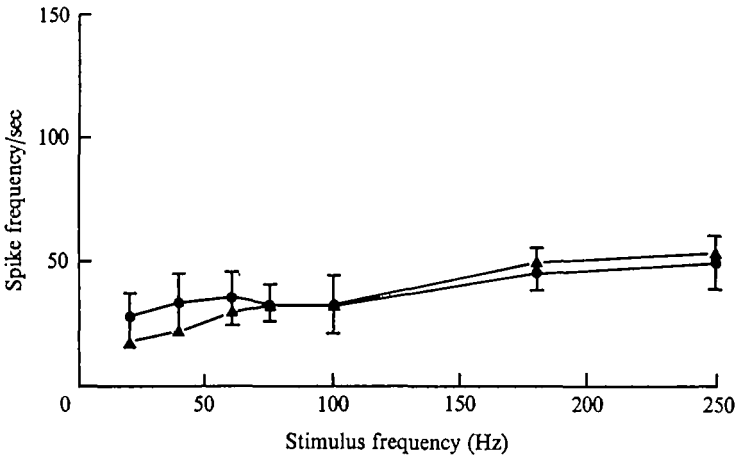

Fig. 6. As in Fig. 5 but with the stimulating electrode transferred to nerve *A*. Notice that the rise in the frequency of the anterior unit, which is characteristic of closure, fails to occur.

A comparison of the response of a unit in the closure segment with its homologue in the segment posterior (Fig. 5) shows that at low input frequencies the response of the two units is similar, but as the input frequency is raised there is an accelerated discharge which is confined to the unit in the segment anterior to the stimulated trap.

If the experiment is repeated with the stimulating electrode transferred from nerve *B* to nerve *A*, the response of the two units remains the same over the whole range of input frequencies (Fig. 6) and the accelerated discharge characteristic of closure fails to occur. A similar response can be recorded from units in each half-segment on the stimulated side of the abdomen, and the output is characteristic of movements in which the muscles of one side contract together, flexing the abdomen about a dorso-ventral axis.

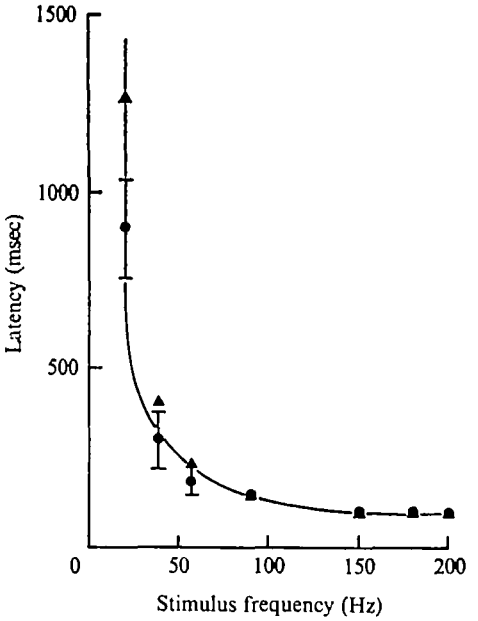

Fig. 7. The latency of the response recorded in single dorsal (circles) and ventral motor units (triangles) to stimuli to nerve *B* from the adjacent trap at the indicated frequencies. Each point is the average of five responses.

The high-frequency closure volley occurs only when the input is from the axons of trap receptors and only when this input is at the frequency at which these receptors transiently discharge when they are disturbed (Bate, 1973*a*). At lower input frequencies the response to receptors inside and outside the trap is the same.

Latency of the closure response

As the frequency of an electrical stimulus to nerve *B* is raised, the latency of the response in the anterior segment rapidly declines. The relation (Fig. 7) is a common one in the insect nervous system (e.g. Fielden, 1963) but previous experiments have been criticized by Rowell (1963) who showed that the effective amplitude of an electrical stimulus may increase at high frequencies so that an apparent temporal summation is the consequence of a misleading spatial summation. These objections were avoided in the present work by using stimuli which were well over threshold and by verifying that at each frequency increases in stimulus amplitude were not accompanied by a decline in the latency.

At input frequencies greater than 80 Hz the latency in units supplying dorsal and ventral muscles is closely similar. They fire in a burst which suggests that they have been driven well over the threshold for impulse initiation and are firing repetitively when the sensilla in the adjacent trap are disturbed. At lower frequencies differences in the timing of the first spike appear in individual units, and the latencies diverge and become more variable. Junctions with individual efferents or fibres in series with them are therefore sensitive to the input frequency, and as this declines the latency of the response is increasingly affected by individual variations in threshold. Large differences in threshold may be engineered by eliciting abdominal twirling movements, and

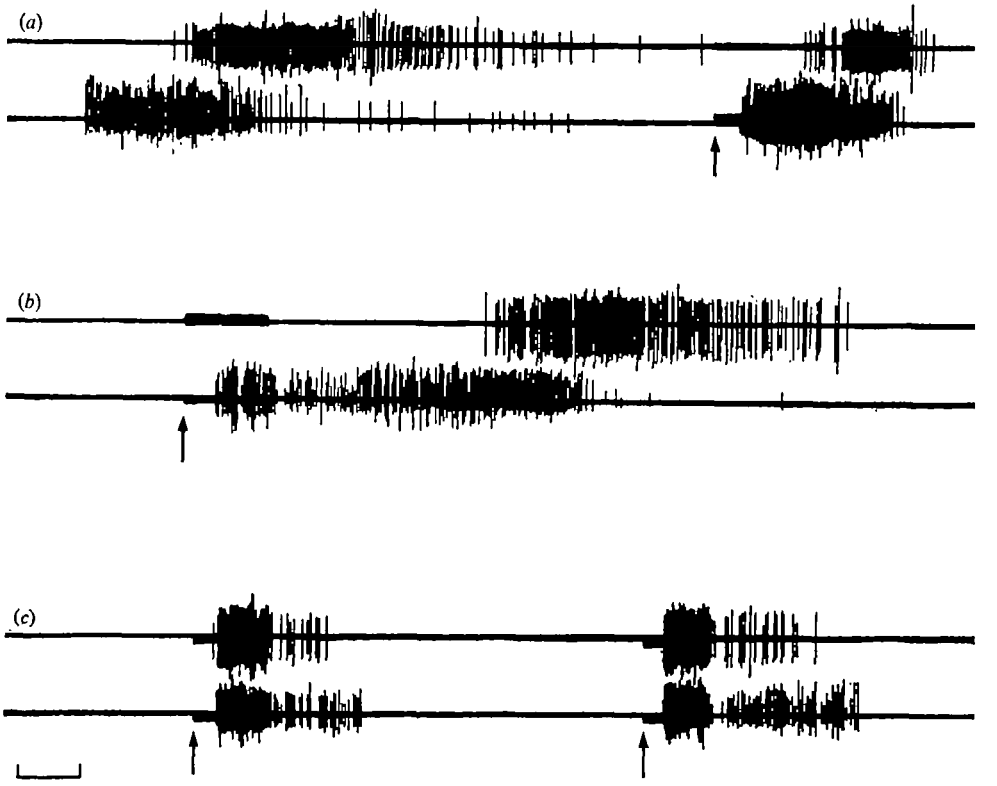

Fig. 8. The response of efferent neurones involved in closure to a high-frequency stimulus to nerve *B* during (*a*) and (*b*) and between (*c*) twirling sequences. All connexions between motor neurones and muscles were severed. Upper trace, ventral efferent neurones; lower trace, dorsal efferent neurones. Time bar: 500 msec. Stimuli (200 Hz) arrowed.

under these circumstances the response in dorsal and ventral units is uncoupled at even the highest input frequencies.

The neuronal basis of abdominal twirling movements is a burst of spikes generated in adjacent motor units in sequence about the cylinder of abdominal muscles. These sequences can be elicited in the absence of movement, and they indicate the existence of a central programme driving the motor neurones in clockwise or anticlockwise sequences (Bate, 1972).

Sequences of this kind can be elicited from an experimental preparation where connexions between motor neurones and muscles have been cut, by severing the connectives between the mesothoracic and metathoracic ganglia. Animals treated in this way produce repetitive sequences for several minutes. Between sequences the response to a trap stimulus is normal (Fig. 8), but during sequences the response in dorsal and ventral units is separated and occasionally the ventral units fail to fire at all. It seems possible that the central twirling programme consists of a sequence of inhibitory and excitatory inputs to individual units and that it is this which causes the divergence in threshold which the experiment reveals.

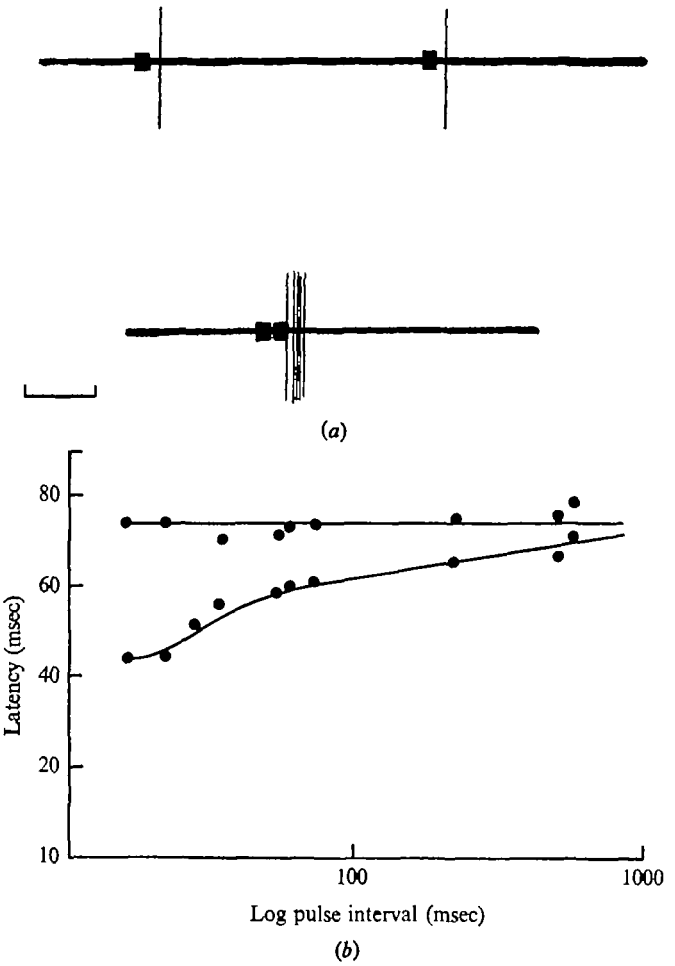

Fig. 9. (*a*) The latency of the response in a single ventral motor unit to two stimuli (200 Hz) to nerve *B* in the adjacent segment with a variable delay between the two. Time bar: 200 msec. (*b*) The decline in the latency as the interval between the two stimulus pulses is reduced. Upper line, latency to first pulse in the stimulus pair; lower line, latency to second pulse.

Conduction time between receptors and motor neurones

The minimum latency for the closure response varies from 80 to 150 msec. Assuming that the fibres in the connective anterior to the ganglion in which the sensory fibres terminate have a similar conduction velocity to the afferent nerves, the minimum conduction time for the system is 35–40 msec. Clearly the difference between this value and the actual latency allows for complicated circuitry between the afferent and efferent neurones. However, the failure of the latency to decline further at frequencies above 100 Hz (Fig. 7) may not be due to a limit which conduction time places on the system. If the system fails to follow input at frequencies greater than 100 Hz then the shortest latency is not necessarily the conduction time for the system; as at lower frequencies, a proportion of the latency may be taken up by the rise of central excitation to a point at which the efferent neurones fire.

This possibility was explored by determining the time taken by the next impulse to

travel round the system after the central excitation had risen to the threshold at which the motor neurones fire. Efferent connexions with muscles were cut and two successive bursts of stimulus pulses were delivered to nerve *B* while recording from a single ventral motor unit in the anterior segment. The length of the first burst in the stimulus pair was set so that the efferent neurone fired no more than once and the latency of the response to a second burst, delivered after a variable delay, was measured. As the interval between the bursts declines to zero, the situation approaches the ideal of the first impulse in the second burst travelling round the circuit to coincide with a central excitation just at threshold. Fig. 9 shows the typical results of such an experiment. In practice the interval between the two stimuli was never less than 15 msec and it was necessary to set the length of the first burst so that the neurone was driven some way over threshold to avoid the consequences of small variations in sensitivity. These two experimental concessions may have caused some decline in the central excitation before the arrival of the second pulse and an increase in the threshold of the motor neurone as a consequence of having fired (Wilson, 1964). Nevertheless, the curve (Fig. 9*b*) reaches a minimum before the interval between the two pulses has been reduced to its lowest point, and the latency which is observed here may be the limit which conduction time places on the system.

The essential feature of the experiment is a comparison of the length of a stimulus which just causes the neurone to fire with one which just does not. The central threshold varies, so that the more direct method is preferable, because with each pair of pulses it is possible to confirm that the threshold has been reduced to zero. Were the threshold constant, then a simple substraction of one from the other would give a consistent conduction time for the system.

The minimum value which can be obtained from such an experiment is about 44 msec. It suggests that the connexion between the afferent and efferent neurones may be rather simple, with two or at most three junctions involved.

DISCUSSION

A. The selective connection of the triggering sensilla

The discrimination between the triggering the non-triggering sensilla which is a feature of the mechanism of the gin trap becomes apparent only when the frequency of the input is similar to the frequency at which the abdominal hair sensilla briefly discharge when they are disturbed (Bate, 1973).

A comparison of the response to input from these receptors at high and low frequencies shows that the efferent neurones are driven by two parallel systems. The first, which links the efferent neurones on one side in a low-frequency discharge, is characteristic of abdominal flexion; the second, which drives a restricted group of motor neurones in a high-frequency volley, is characteristic of closure. The receptors outside the trap are connected only with the first system, whereas those inside the trap have access to both. It is the selective connexion of the receptors inside the trap with this second system, and its sensitivity to the frequency of the input, that ensure that the trap closes only when objects move within its margins. The closure system first appears at pupation, and its development is considered in a subsequent paper (Bate, 1973*b*).

B. The interaction between twirling movements and closure

The closure system is relatively insensitive to tonic postural inputs (Bate, 1973*b*), but differences between the thresholds of individual motor neurones which appear at low input frequencies are probably a consequence of peripheral biasing. Variations in threshold of a central origin also occur; the pupal motor neurones are multifunctional units, and their simultaneous response to input from the trap sensilla is incompatible with their sequential activation in an alternative, centrally determined pattern.

The inhibitory effects observed during twirling sequences were consistently asymmetrical, that is to say ventral neurones failed to fire when dorsal motor neurones fired, but never the reverse. This is surprising, for sequences in both senses are equally available in the intact animal. Possibly the removal of one half of the experimental preparation introduces a bias which permits sequences in one direction only. The asymmetry is reminiscent of that described by Pearson & Iles (1970) in the centrally patterned activation of the coxal levator and depressor muscles in the leg of the cockroach. In that preparation bursts in levator motor neurones may occur without impulses being generated in the antagonistic slow depressor neurone, but never vice versa. Pearson and Iles suggest that the absence of the alternative pattern is a consequence of an inhibitory input to the slow depressor neurone from a bursting interneurone which drives the levator motor neurones. The possibility that in the pupa the abdominal efferent neurones are driven in twirling sequences by parallel mirror image systems with asymmetrical excitatory and inhibitory connexions awaits investigation.

SUMMARY

1. Despite partial degeneration of the larval muscles and reorganization of the nervous system, pupae of the privet hawk moth, *Sphinx ligustri*, show simple behaviour patterns including abdominal flexion and twirling, and a movement which closes the gin trap.

2. The gin trap closes when the motor neurones driving muscles in the ipsilateral half of the adjacent anterior segment discharge together in a brief high-frequency volley.

3. The system which excites the volley is restricted to these motor neurones and is activated only by input at a high frequency from triggering receptors.

4. A second system excites efferent neurones in several segments and is driven by triggering and non-triggering receptors. Therefore at low input frequencies the discrimination between the two kinds of receptors disappears.

5. Motor patterns associated with twirling movements drive the efferent neurones in a sequence which interferes with their simultaneous response to triggering inputs.

6. The arrangement of the closure system shows that the afferent neurones are separated from the efferent neurones by at least one interneurone.

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