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THE PHYSIOLOGY OF WANDERING BEHAVIOUR IN *MANDUCA SEXTA*

III. ORGANIZATION OF WANDERING BEHAVIOUR IN THE LARVAL NERVOUS SYSTEM

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SUMMARY

1. The locomotor patterns typical of wandering behaviour were studied electromyographically in abdominal segments of freely moving larvae of *Manduca sexta*. Crawling locomotion consisted of stereotyped, anteriorly-directed, peristaltic waves of intersegmental muscle contraction. During burrowing the intersegmental muscles of all abdominal segments contracted simultaneously for several consecutive cycles and then performed a single bout of the crawling pattern.

2. Sensory inputs determined which motor patterns were used and how they were modified. Local sensory inputs could modify patterns in the specific segments affected.

3. The neural circuitry that was required to generate the peristaltic and bracing patterns was repeated among the thoracic and abdominal ganglia, and normally was activated by the suboesophageal ganglion (SEG) and brain. In the absence of connections with the SEG and brain the segmental motor pattern generators could be activated by strong sensory stimuli. When the thoracic and abdominal segments lacked connections with the SEG, spontaneous movements were infrequent prior to wandering, but increased markedly at wandering or following 20-hydroxyecdysone (20-HE) infusion.

4. Prior to wandering the SEG drives spontaneous locomotion in debrained larvae, but this function disappears in wandering larvae, or following 20-HE infusion.

5. Prior to wandering the brain exerted a net inhibitory influence on locomotion. Removal of the medial region of the brain abolished this inhibition, resulting in strong, continuous locomotion which was driven by the lateral region of the brain. This lateral excitatory function of the brain was not altered by 20-HE infusion prior to wandering, nor did it change with the appearance of wandering behaviour.

6. We conclude that the locomotor patterns used during wandering are produced by pattern generators in the segmental ganglia and are modified by sensory information. The circuitry responsible for activating these motor pattern generators is associated with the SEG, and is under the control of the brain. The brain exerts a net inhibitory influence prior to wandering, which becomes excitatory during

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wandering. Ecdysteroids appear to alter locomotor function by acting at various levels including the segmental ganglia, the SEG and the brain. A model is advanced describing this effect.

INTRODUCTION

The ability of hormones to alter function in the nervous system underlies their role in modifying behaviour. Studies on various vertebrate systems indicate that the behavioural effects of steroids result from hormone action at several levels of neural organization, including peripheral as well as central targets (Arnold, 1981). For example, androgens are concentrated by neurones in each of the brain nuclei in the pathway regulating song in the zebra finch, as well as by the motoneurones and muscles of the syrinx (Arnold, 1979, 1981).

In larvae of the tobacco hornworm, *Manduca sexta,* the steroid 20-hydroxyecdysone (20-HE) induces a stereotyped behaviour termed wandering (Dominick & Truman, 1985) during which the caterpillar crawls from its feeding site and burrows underground, finally excavating an earthen chamber in which the pupa develops. Wandering behaviour involves the loss of specific larval behaviour, such as feeding, as well as the activation of new responses, such as a 20- to 30-h period of persistent locomotion and the appearance of burrowing movements (Dominick & Truman, 1984).

The endocrine events leading to wandering behaviour begin with the secretion of the peptide prothoracicotropic hormone (PTTH) which results in the appearance of ecdysteroids in the larva (Truman & Riddiford, 1974; Nijhout, 1976; Bollenbacher, Vedekis & Gilbert, 1975). The ecdysteroids then induce the behavioural changes (Dominick & Truman, 1985) as well as the associated changes in larval pigmentation (Nijhout, 1976).

By means of surgical lesions, previous studies of the neural organization of behaviour in insects have indicated that segmentally arranged motor pattern generators for locomotion, chirping and mating are activated or inhibited by the suboesophageal ganglion (SEG) and the brain (Roeder, Tozian & Weiant, 1960; Huber, 1960). In this paper we examine the organization of wandering locomotor patterns within the central nervous system (CNS) and the role played by 20-HE in modifying locomotor function at various levels within the CNS.

MATERIALS AND METHODS

Experimental animals

Manduca sexta larvae were reared under a short day (12L:12D) photoperiod as has been described (Dominick & Truman, 1984). Larvae were staged on the basis of weight (Dominick & Truman, 1984) which allowed predictive discrimination between larvae which would release prothoracicotropic hormone (PTTH) and ecdysone and subsequently wander during day 3 (Gate I) or day 4 (Gate II) of the last larval instar. This allowed the selection of larvae prior to PTTH and ecdysone release, or after ecdysone release but just prior to wandering. The onset and duration

of wandering behaviour of individual larvae were recorded with tilting dish actographs according to the methods outlined previously (Dominick & Truman, 1984).

Ecdysone infusion

20-Hydroxyecdysone (20-HE) (Rhoto, Osaka, Japan) was dissolved in *Manduca* saline (Cherbas, 1973) and was infused over 10 h at $0{\cdot}5\,\mu g\,h^{-1}$ as described by Dominick & Truman (1984). Infusion into neck-ligated larvae was accomplished by snipping off part of the larva's posterior horn and inserting a polyethylene cannula (PE-10; Clay Adams) which was fixed in place with a loop of thread and melted Tackiwax (Cenco).

Electromyograms (EMGs)

The locomotor patterns used by larvae during wandering were recorded using lacquered copper wire (100 μm diameter) exposed at the tip. These leads were inserted through the cuticle of CO_2-anaesthetized larvae into internal longitudinal muscle groups and cemented in place with fast-setting epoxy resin. The larvae were able to locomote freely with such myographic wires. EMG patterns were recorded during burrowing in a narrow (13 cm) Plexiglas chamber filled with soil, allowing visual correlation of EMG readings with behaviour.

Surgical procedures

Prior to surgery larvae were anaesthetized with CO_2 (Williams, 1946). Transections of the ventral nerve cord or deafferentation of segmental ganglia were performed under insect saline (Ephrussi & Beadle, 1936). A short (5 mm) incision was made in the ventral cuticle over the target region of the nervous system and the necessary manipulations were executed with micro-iridectomy scissors. The incision was closed with 3 to 4 sutures (6-0 needle, braided silk thread).

Surgery on the brain required immobilizing the larva's head (frons upward) on the surface of a plastic Petri dish with Plasticene or tape while the rest of the animal was suspended through a hole in the Petri dish into a CO_2-filled chamber. To remove the brain or to sever the circumoesophageal connectives, a rectangular piece of cuticle was removed from one side of the head along the margin of the two principle sutures, exposing one side of the brain to view with minimal damage to muscles necessary for feeding (B. Cymborowski, personal communication). Medial transections or specific excisions of brain tissue were done through a window cut through the top of the frons. With both of these methods for exposing the brain, the removed cuticle was replaced into its former site and the wound sealed with Tackiwax.

RESULTS

Electromyograms of locomotor patterns during wandering behaviour

Crawling

Previous work has described the crawling of lepidopteran caterpillars as an anteriorly directed peristaltic wave. Within each segment contractions occur

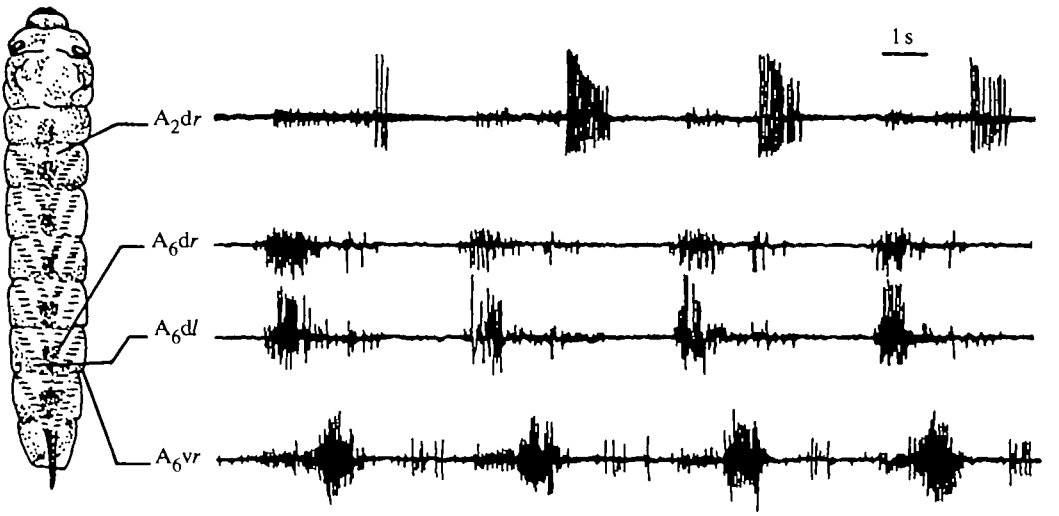

Fig. 1. Muscle activity patterns during normal crawling. EMG recordings are taken from the right dorsal longitudinal muscles of the second abdominal segment (A_2dr), the right and left dorsal longitudinals in A_6 (A_6dr and A_6dl) and from the ventral longitudinal muscles of A_6 (A_6vr). Each 'step' consisted of a bilaterally symmetrical, anteriorly directed wave of contraction. During each step contraction of the dorsal muscles of a given segment preceded ventral muscle contraction.

sequentially, starting in the dorsal longitudinal muscles (von Holst, 1934; Barth, 1937; Weevers, 1965). This pattern begins in the terminal segment, producing a wave of segmental lifting and promotion which travels toward the head.

In this investigation, myographic records of normal crawling showed a segmentally stereotyped and bilaterally symmetrical pattern which moved from posterior to anterior in the larvae (Fig. 1) and which was consistent with the sequence described above. The low level of activity in the dorsal and ventral muscles seen during the interburst periods when the segment was stationary may relate to hydrostatic adjustments of body wall tension (Weevers, 1965).

Burrowing

Wandering larvae which were placed on soil began burrowing into that substrate from the outset of wandering (Reinecke, Buckner & Grugel, 1980; Dominick & Truman, 1984). While burrowing, the larva made repeated thrusts and strong dorsal flexions with its head and thoracic segments while stiffening its entire abdomen as an anchor during each thoracic digging movement. This abdominal bracing was accomplished by simultaneous contraction (lasting 1 s) of the dorsal and ventral longitudinal muscles throughout the abdomen (Fig. 2). Typically a series of these braced digging flexions resulted in the excavation of a small space in front of the larva, into which the animal moved with a single crawling step. This was then followed by a new cycle of digging movements. Although the motor pattern for normal stepping was used for the former movements, the start of the locomotor wave emerged from the bracing movement in the more posterior segments and only became distinct as the wave progressed anteriorly (Fig. 2).

Motor pattern plasticity

In addition to crawling and burrowing, which constitute the principal motor patterns used during wandering, the larva can adapt its movements in response to specific challenges. When larvae were induced to crawl forward into a tightly fitting Plexiglas tube, the unconfined posterior segments exhibited peristaltic steps typical of normal crawling, in which dorsal muscle contraction preceded that of the ventrals (Fig. 3). When this wave arrived at the more anterior segments which were confined in the tube, the dorsal and ventral muscles contracted in virtual synchrony, thereby pulling the anterior segments through the tube. These results indicate that the motor patterns used during wandering are very flexible, and can be modified in specific segments in response to local sensory inputs independently of the pattern used in other segments.

Neural organization of wandering

Abdominal and thoracic ganglia

We performed a series of surgical experiments to determine the locomotor roles played by different parts of the nervous system. Transection of the connectives between the first thoracic (T_1) and the suboesophageal ganglion (SEG) prevented persistent coordinated locomotion at all times during the fifth instar. Transection of

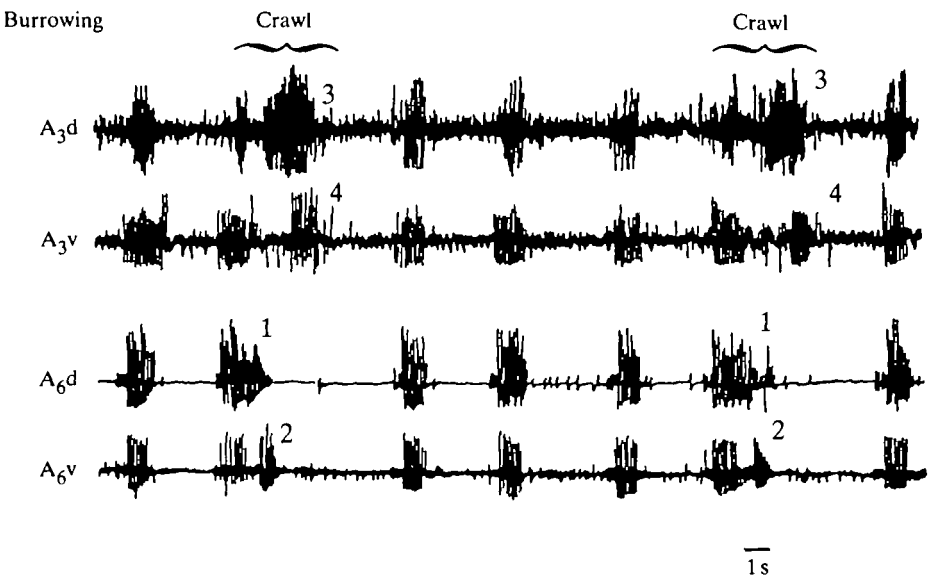

Fig. 2. Muscle activity patterns during burrowing in a wandering larva. Electrode placements in dorsal and ventral longitudinal muscles of the third (A_3d and A_3v) and sixth (A_6d and A_6v) abdominal segments. Synchronous bursts of activity in dorsal and ventral muscles in all abdominal segments occurred during bracing contractions, upon which a normal crawling step is periodically superimposed. See text for further description. Numbered motor bursts represent the order of muscle contractions during the crawling pattern.

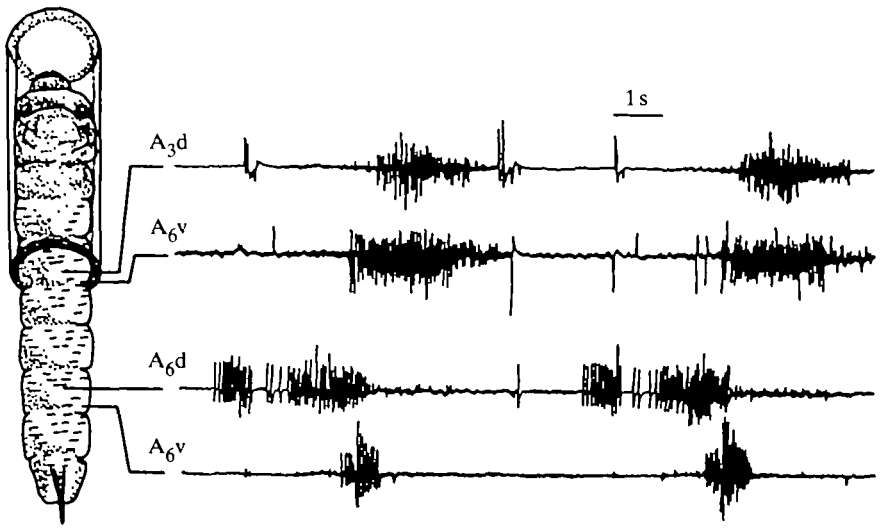

Fig. 3. Effect of sensory stimuli on the crawling pattern. Muscle activity patterns were recorded during entry into a tightly fitting Plexiglas tube. Electrode placements were as in Fig. 2. Posterior segments show the phase relationships characteristic of crawling whereas the more anterior segments (in tube) show synchronous firing.

connectives between the various abdominal or thoracic ganglia of wandering larvae caused the cessation of sustained spontaneous crawling in the segments posterior to the lesion while the anterior segments performed normal crawling patterns in which stepping frequency ($0{\cdot}5$–$0{\cdot}7\,s^{-1}$) was comparable to that of operated control larvae.

The segments posterior to the cut connectives occasionally produced crawling steps in response to stimulation, but such movements were slow, tremulous and quite exaggerated. This observation indicated that the neuronal circuitry required for crawling is present in the abdominal and thoracic portions of the nervous system, but that intact neural connections with the suboesophageal ganglion are necessary for the sustained and efficient activity of these pattern generators. Even when only the first thoracic segment had intact anterior connectives, that segment exhibited persistent movements similar to its behaviour during normal crawling. This result suggests that even a single segmental ganglion could generate crawling motor patterns if connections with the head ganglia were maintained.

The role of segments participating in the transmission of a crawling wave in *Manduca* was examined in an experiment originally performed by von Holst (1934). All the segmental nerves of the ganglia in abdominal segments 1–4 were severed, leaving only the connectives intact. This caused the denervated segments to become flaccid and distended, but it did not prevent the normal coordination of persistent crawling movements in the unoperated segments during wandering. Peristaltic waves were propagated as usual from the terminal segment and proceeded anteriorly until they 'disappeared' into the paralysed segments. The locomotor wave emerged from the anterior end of those segments after a delay which was approximately that expected for the propagation of the pattern through the four denervated segments. Thus, the failure of several segments to participate in the movement normally

generated by the wave did not interfere with the coordination of the pattern along the abdomen.

Response of decapitated larvae to ecdysteroids

Larvae were decapitated by neck-ligation to crush the ventral connection between the SEG and T_1 late during the day of ecdysone release. The abdomens of such larvae began to twitch and flex at the time of wandering, mashing the food and frass in their cups as happens when intact wandering larvae crawl and burrow (W. E. Bollenbacher, personal communication). To examine the possibility that ecdysteroids might elevate the spontaneous activity of the thoracic and abdominal segments independently of the brain, we ligatured larvae between SEG and T_1. Fifteen larvae before ecdysone release and eight larvae after ecdysone secretion but prior to wandering were used. After a 10- to 15-h recovery period the spontaneous flexions of the thorax and abdomen were counted for 1 h in both groups. The results (Fig. 4A) show that strong spontaneous movements occurred about seven times more frequently in larvae which had released PTTH and ecdysteroids, ($0{\cdot}7 \pm 0{\cdot}3$ movements min^{-1}; mean ± S.D.) than in larvae prior to ecdysone secretion ($0{\cdot}1 \pm 0{\cdot}1$ movements min^{-1}).

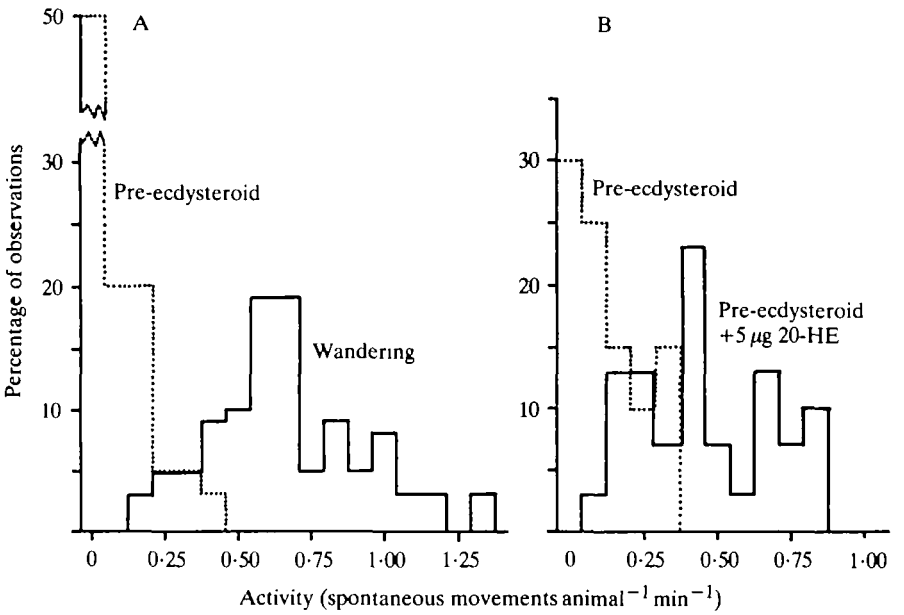

Fig. 4. The influence of 20-HE on spontaneous movements of thorax and abdomen of larvae decapitated by neck ligation. (A) Larvae ligated prior to ecdysone secretion ($N = 15$; dotted lines) or following ecdysteroid secretion and just a few hours before wandering ($N = 10$; solid lines). After 10–15 h for recovery, the number of spontaneous flexions performed was counted for both groups. The histogram presents the percentage of observation intervals (forty 2-min periods) in which animals exhibited a given activity level (or movement frequency). (B) Larvae (from A) ligated prior to ecdysone release were subsequently infused with 20-HE ($N = 10$; solid lines) or saline ($N = 5$; dotted lines) and then scored for movements as described above.

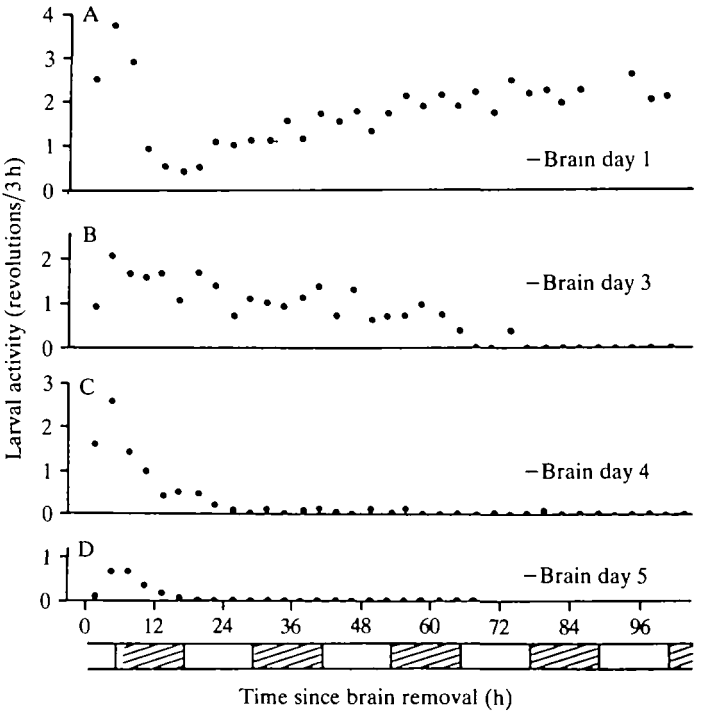

Fig. 5. Spontaneous locomotion of brainless larvae. Following removal of the brain at different ages, larvae were put in tilting actographs to monitor their locomotion as actograph revolutions/3 h. Each point represents the average locomotion of all larvae for that time. The 12L:12D photoperiod is represented by the open and cross-hatched bars at the bottom. (A) Larvae debrained on day 1 ($N = 15$). (B) Larvae debrained on day 3 (prior to PTTH release) ($N = 7$). (C) Larvae debrained after PTTH and ecdysone secretion, prior to wandering ($N = 20$). (D) Removal of the brain from wandering larva ($N = 13$).

Ten of the larvae ligated prior to ecdysone release were subsequently infused with 20-hydroxyecdysone (20-HE) ($0{\cdot}5\ \mu g\ h^{-1}$) for 10 h while the remaining five larvae received a saline infusion. Movements were scored 15 h after the start of the infusion as previously described. The 20-HE infused larvae were four times more active ($0{\cdot}45 \pm 0{\cdot}2$ movements min^{-1}) than they had been prior to 20-HE infusion and were four times as active as the saline-infused controls ($0{\cdot}1 \pm 0{\cdot}1$ movements min^{-1}). The responses of the decapitated larvae indicate that 20-HE can act on centres posterior to the SEG to induce increased activity which may facilitate wandering.

Suboesophageal ganglion and brain

We examined the role of the brain and SEG in controlling locomotion and the effects of ecdysteroids on these neural centres by transecting the paired circumoesophageal connectives (CECs), which constitute the neural pathway between the SEG and the brain. The effects on locomotion varied considerably depending on the time of the surgery (Fig. 5A).

Cutting the CECs or removing the brain from larvae on the first day of the fifth instar resulted in tonic slow crawling lasting several weeks (Fig. 5A; $N = 15$). The

same operation performed on Gate II larvae just a few hours before the release of PTTH (Fig. 5B; $N = 7$) also resulted in the activation of tonic slow crawling, but it lasted only 4–5 days and then ceased. The cessation of crawling in these larvae was probably due to a low level of endogenous ecdysone secretion (Truman & Riddiford, 1974; Judy, 1972; Nijhout, 1976) for it coincided with the clearing of the epidermis over the heart, a phenomenon which depends on exposure to ecdysteroids (Nijhout, 1976) and which usually accompanies wandering behaviour. Interestingly, although these animals eventually showed the morphological manifestation of wandering they never showed the intense locomotion characteristics of this stage.

Brain removal in animals several hours prior to the onset of wandering, caused a brief post-surgical period of weak locomotion which rapidly declined during the expected time of wandering (Fig. 5C; $N = 20$). As with the above group, wandering locomotion did not occur. Importantly, larvae which had already begun to wander were unable to continue the behaviour following brain removal or transection of the CECs, and they showed an extremely low level of post-surgical locomotion (Fig. 5D; $N = 13$).

Although larvae debrained after the period of endogenous ecdysone secretion showed no spontaneous locomotion, they crawled persistently and at approximately normal rates for several minutes if provided with a tonic sensory stimulus, such as a slight elevation of the terminal segment above the substrate ($N = 8$). Thus peripheral inputs can evoke locomotion temporarily in brainless wandering stage larvae, suggesting that during wandering the SEG continues to be necessary for sustained locomotion, but that it can no longer by itself drive continuous spontaneous locomotion. Indeed, during wandering the brain becomes necessary to drive continuously the SEG-dependent locomotor system in the lower ganglia. By contrast, prior to wandering the net function of the brain appeared to be inhibitory to locomotion.

The changes in behaviour of brainless larvae coincided with the exposure of the heart, suggesting that these changes were consequences of ecdysteroid action. Therefore, the loss of sustained spontaneous locomotor activity in debrained larvae was examined as a function of 20-HE infusions. Eight larvae were debrained at 19.00 h on day 3 prior to PTTH release, and were then infused with 20-HE (5 μg/10 h) beginning at 21.00 h. Control larvae were debrained and infused with saline. Following infusion, locomotor activity of these larvae was monitored in tilting actographs.

Locomotion declined rapidly in the 20-HE-treated brainless larvae, beginning about 17 h after the start of the infusion (Fig. 6). This time course corresponded exactly with the timing of the heart exposure which is diagnostic for 20-HE action, and was the same as that previously described (Dominick & Truman, 1985) for the induction of wandering behaviour in intact larvae under these infusion conditions. In the control larvae, locomotion gradually declined over 96 h. Here too, the decline of locomotion was coincident with the exposure of the heart, which indicates autonomous secretion of ecdysone by the prothoracic glands (Nijhout, 1976; Truman & Riddiford, 1974). Therefore, even in the brainless saline-infused larvae,

the gradual loss of spontaneous crawling can be interpreted as a consequence of ecdysteroid action.

The brain

The above experiments suggest that in feeding larvae the brain serves to inhibit locomotion whereas after PTTH and ecdysteroid secretion, the brain is excitatory for persistent locomotion. Surgical manipulations on the brain were performed in fifth instar larvae at various stages in order to examine this transition (Fig. 7).

Larvae in which the brain was transected medially just prior to wandering ($N = 13$) showed no alteration of the normal onset time or duration of wandering, but the crawling movements were exaggerated and slow and the interval between steps was very long (18–35 s). When this operation was performed in larvae prior to PTTH release ($N = 9$), wandering and heart exposure were delayed by 1–2 days. Presumably this delay resulted from severing the axons of the brain PTTH neurones *en route* to their terminals in the contralateral corpora allata (Bollenbacher *et al.* 1980; Agui *et al.* 1979; Gibbs & Riddiford, 1977; Nijhout, 1975; Buys & Gibbs,

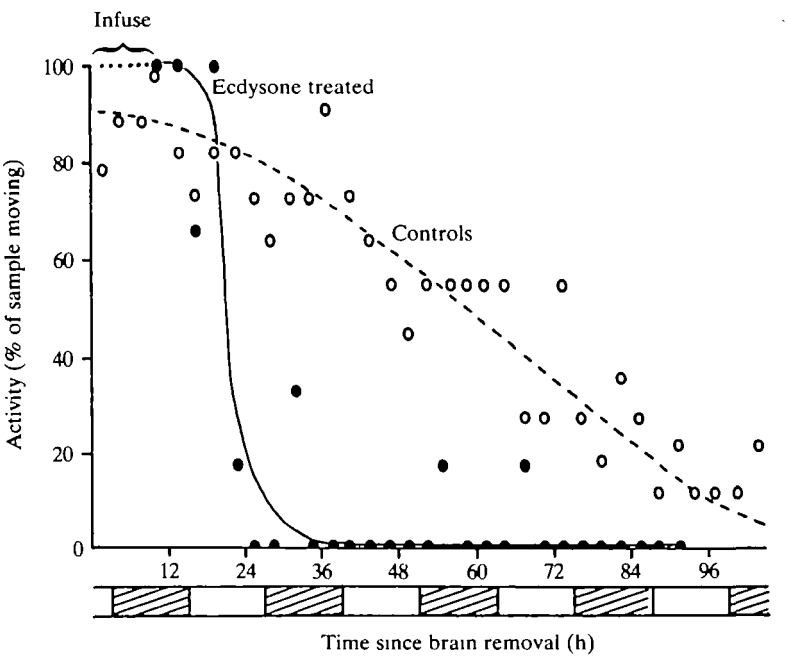

Fig. 6. The effect of 20-HE on spontaneous crawling in brainless larvae. Prior to PTTH and ecdysone release, Gate II larvae were debrained (19.00 h on day 3) and infused with 20-HE (5μg/10 h) beginning at 21.00 h (filled circles; $N = 6$). Locomotor activity was measured in terms of the percentage of the larvae making at least 1 actograph revolution per 3 h. Control larvae of the same weight were debrained at the same time but not infused (open circles; $N = 11$). The photoperiod and time since brain removal and start of 20-HE infusion are noted on the scale below the data. Lines represent eye-fitted curves through the data.

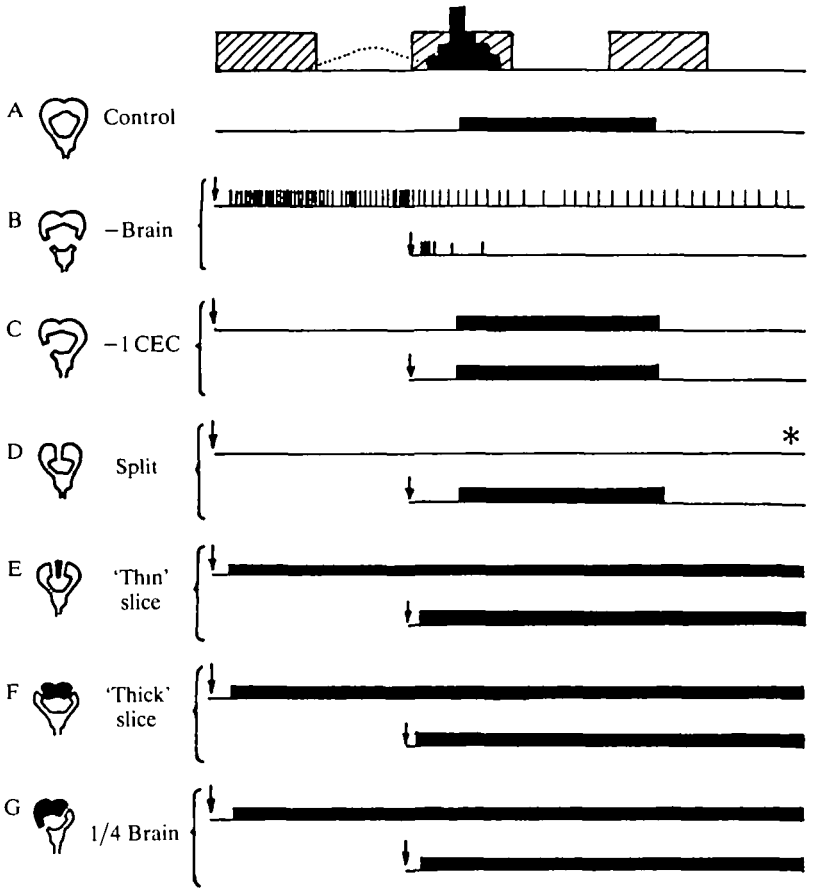

Fig. 7. Facsimile activity records of larvae following various surgical manipulations of the brain. Cross-hatched bars represent darkness. A diagrammatic ecdysone titre (dotted line) and a histogram (black bar) of wandering onset time for control larvae are shown at the top relative to the photoperiod. The specific surgical treatments of the brain and CEC are illustrated to the left of the figure. The arrows mark the time of surgery relative to the developmental regimen shown at the top. Representative activity records following each surgical treatment are schematically shown for one larva operated just prior to PTTH release (upper record) and for one larva operated just prior to wandering (lower record). Intense locomotion is shown as the fusion of individual actographs marks into a solid bar. (A) Exposure and handling the brain in control operated larvae (prior to PTTH secretion; $N = 20$; prior to wandering; $N = 20$). (B) Removal of the brain ($N = 22,20$). (C) Transecting a single CEC ($N = 9,4$). (D) Medial transection of the brain (split brain; $N = 9,13$). The asterisk indicates that splitting the brain prior to PTTH release delayed wandering by 1 day but the subsequent wandering was normal in duration and intensity (not shown). (E) Removal of a thin medial section from the brain ($N = 5,5$). (F) Removal of the large medial section of the brain ($N = 2,3$). (G) Removal of the entire brain except for one lateral quadrant ($N = 4,4$).

1981). Once it began, however, the wandering behaviour of these animals was normal in its duration.

When the medial region of the brain was removed as a slice of varying thickness, larvae became intensely active within 4 h of the surgery, crawling persistently (for

36–192 h) at a rate typical of wandering larvae, regardless of whether the surgery was performed on day 2 prior to PTTH release (6/8 became active) or just prior to the start of wandering (4/7 active). The results were the same for the thinnest slices removed (about 5–10% of the brain) and the thickest (about 80% of the brain). These larvae never constructed pupation chambers when presented with soil. 20-HE infusion (15 μg/10 h) prior to PTTH secretion into four larvae lacking the medial region of the brain had no effect on either the intensity or duration of the locomotion following the surgery.

When only the lateral region of one side of the brain was left ($N = 16$), intense and well coordinated locomotion started within a few hours of the surgery in larvae prior to ecdysone exposure and in those just prior to wandering. Crawling persisted in these larvae for 30–192 h.

DISCUSSION

Variability and neural organization of locomotor patterns

Crawling and burrowing are the most common motor patterns employed during wandering. Crawling consisted of rhythmic waves that began in the most posterior segment and progressed anteriorly through the abdomen and thorax. During the progression of a wave, activity in the dorsal longitudinal muscles preceded that of the ventral longitudinal muscles such that the ventral muscles in a given segment contracted synchronously with the dorsal muscles that were two segments anterior (Barth, 1937). During crawling, each wave travelled the length of the insect before the next wave started. The burrowing behaviour involved bracing movements of the abdomen caused by the simultaneous contraction of all the longitudinal muscles along the length of the abdomen. This motor pattern resulted in abdominal rigidity which anchored the abdomen while concurrent flexion of the head and thorax shovelled and compacted the soil. After several repetitions of this pattern, the larva employed a single crawling wave to move into the newly excavated space (Fig. 2). Previous observations of wandering larvae freely moving in soil have shown that virtually the entire period of wandering activity can occur underground (Dominick & Truman, 1984), suggesting that the pattern of synchronous activity seen during burrowing represents the most typical locomotor pattern of wandering larvae.

In intact larvae, sensory inputs can dramatically modify a motor pattern or they can evoke an overt switch from one motor pattern to another (e.g. crawling to burrowing). Indeed, sensory inputs unique to just one portion of the larva can act locally to evoke modified motor output exclusively in the affected segments (Fig. 3).

Transection at various levels of the ventral nerve cord indicated that the locomotor patterns are organized by interactions of segmental neural pattern generators and are modified on the basis of sensory input, as has been described for other repetitive acts like locust flight (Wilson, 1961), cockroach walking (Pearson & Iles, 1973), silkmoth eclosion (Truman, 1978) and many others. Since crawling-like movements occur in the first thoracic segment of larvae in which the connectives posterior to this ganglion have been severed, it is likely that each segmental ganglion can independently

produce the various motor patterns as has been found for the leech swimming pattern (Weeks, 1981) and ecdysial movements in pupal *Manduca* (Truman & Weeks, 1985).

Although spontaneous crawling did not occur in segments posterior to a nerve cord transection, sensory stimuli could evoke one or two crawling steps from these regions. Even so, the movements were slow and tremulous. Therefore, it appears that in wandering animals the thoracic and abdominal motor pattern generators receive both activating and coordinating input from the higher neural centres (brain and SEG), the roles of which are discussed below.

20-Hydroxyecdysone and control of locomotor patterns

Thoracic and abdominal ganglia

The transections and other surgical manipulations of the CNS showed that the brain, SEG and the segmental ganglia underwent changes in their participation in locomotor control between the feeding and wandering stages. Moreover, these changes are attributable to the action of ecdysteroids.

In the absence of connections with the SEG, coordinated locomotor movements did not appear in the thorax and abdomen during the expected wandering period. However, an increase in the frequency of spontaneous movements of the thorax and abdomen at that time (Fig. 4) indicated that functional changes were occurring in the ventral nerve cord. Since a similar effect was also induced by 20-HE infusion into decapitated larvae, the increased activity is presumably due to ecdysteroid action. The significance of this change is not known, although it may result in enhanced responsiveness of the locomotor centres to input from the head ganglia (brain and SEG), thereby facilitating the increased level of locomotion.

Suboesophageal ganglion

A locomotor activating function is associated with the SEG in many insects as demonstrated by the induction of spontaneous locomotor activity that occurs after the circumoesophageal connectives are severed (e.g. Roeder *et al.* 1960). The similar results reported in this study confirm in *Manduca* a comparable locomotor activating role for the SEG under the influence of the brain. Prior to ecdysone secretion, ligations anterior to the SEG produce tonic crawling whereas ligations posterior to the SEG cause quiescence. This suggests that at this stage the SEG may have a descending excitatory influence on locomotor circuitry which is expressed in the absence of the brain. Interestingly, at wandering or after exposure to 20-HE infusion the SEG loses this ability to sustain spontaneous locomotion. Thus, rather than enhancing the activity of the proposed excitatory centre in the SEG, 20-HE appears to disable this mechanism, making it dependent on stimulatory inputs from the brain.

Brain

Because removal of the brain typically caused tonic locomotion in the praying mantis, Roeder (1937) proposed that the excitatory centres in the SEG were

inhibited by the brain, leading to the generalized view of the brain as an inhibitory regulator of many types of behaviour (Miller, 1976). The persistent SEG-dependent locomotion which followed either brain removal or transection of the circumoesophageal connectives during the feeding stage in *Manduca* indicates a similar inhibitory role for the brain relative to locomotion.

In contrast to the removal of the brain in feeding larvae, this operation in wandering animals eliminated all spontaneous locomotion. Thus, the exposure to ecdysteroids at wandering shifts the role of the brain to being necessary for locomotion, perhaps by exciting the otherwise inactive locomotor centres in the SEG. Similarly, in *Hyalophora cecropia,* the brain is required for cocoon spinning behaviour which is analogous to wandering behaviour (van der Kloot, 1954).

More insight into possible roles of the brain was provided by the lesion experiments. Prior to wandering, a medial bisection of the cerebral lobes did not reduce locomotor behaviour, but removal of the medial portion of the brain, even as a very thin slice (about 100 μm), resulted in strong, sustained locomotion which lasted up to 3 days (Fig. 7). Therefore, inhibition of locomotion appears to be associated with a small region adjoining each side of the cerebral midline, rather than with purely midline structures (such as the central body) or with cross-connections between the two brain hemispheres.

It is significant that these medial slices would damage the bilaterally paired corpora pedunculata (mushroom bodies), the β-lobes of which project nearly to the brain midline in *Manduca* larvae (personal observation), while a midline transection of the brain would leave them intact. The corpora pedunculata have been implicated in selective disinhibition and sequential organization of specific behavioural patterns in a variety of insects (Howse, 1976; Roeder, 1937; Huber, 1960; Otto, 1971; Kutsch & Otto, 1972). Interestingly, the brain lesions which release locomotion in *Manduca* shortly before wandering starts also appear to destroy the ability of the wandering caterpillar to show coherent cell building behaviour, whereas simply transecting the brain medially does not interfere with this behaviour (unpublished observations). Similarly, in *H. cecropia* larvae relatively localized lesions in the corpora pedunculata prior to cocoon spinning resulted in the spinning of a formless pad of silk rather than a cocoon (van der Kloot & Williams, 1954).

The intense locomotion which followed removal of the medial region of the brain in both feeding and wandering larvae continued for many days and was much stronger than that seen in the larvae debrained prior to ecdysone secretion, suggesting that the lateral brain regions actively promoted locomotion. This idea was supported by the finding that even a small lateral portion of a single cerebral hemisphere was sufficient to stimulate locomotion. In the present experiments this excitation cannot be proved to be identical to that which underlies wandering. However, the diminished stepping frequency of larvae with a single lateral brain fragment is comparable to that of wandering larvae with a medially transected brain and these latter larvae show the other types of behaviour characteristic of wandering, i.e. burrowing and cell construction. The ability of the lateral regions to promote locomotion is seen in larvae both prior to ecdysone secretion and during wandering

and in larvae infused with 20-HE prior to endogenous ecdysone secretion. Consequently, it appears that ecdysteroids do not induce wandering by directly altering the function of cells in this lateral area of the brain. Rather, the inhibitory influence of the medial region of the brain seems to cease at wandering, since its removal during wandering does not further enhance locomotion. The elimination of this inhibition by ecdysteroids could then permit the excitatory lateral regions of the brain to activate locomotor centres in the SEG and lower ganglia. These interactions are summarized in a working model illustrated in Fig. 8. The ventral ganglia contain the motor pattern generators for the crawling and burrowing behaviour while the brain and SEG play a regulatory and perhaps coordinating role. Target sites for the ecdysteroids appear to include the ventral ganglia, the SEG and the brain. Steroid action on the ventral ganglia is not well understood but it may raise the excitability

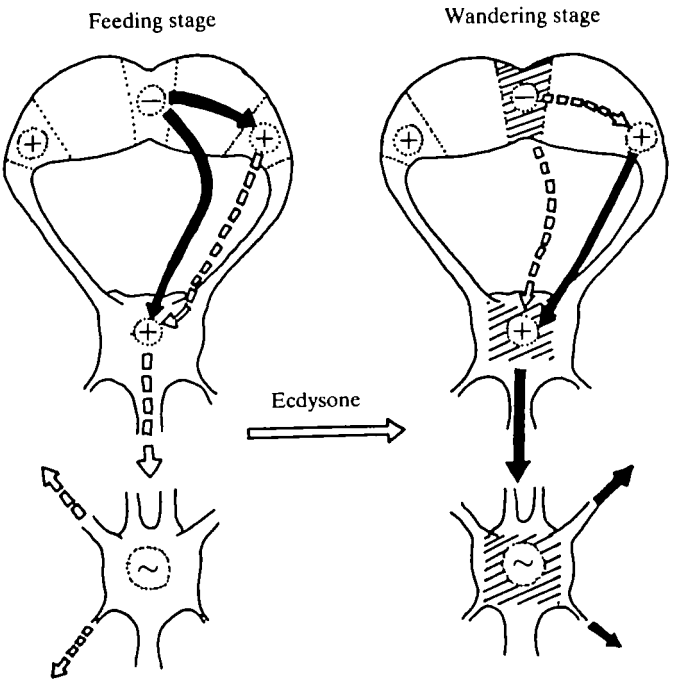

Fig. 8. A working model of the interaction of various regions in the CNS in regulating locomotion and the likely sites of ecdysteroid action related to wandering behaviour. Areas with apparent locomotor inhibitory effects are shown as (−); excitatory regions are designated (+). Active neural pathways are represented with solid arrows; suppressed pathways as dotted arrows. Regions thought to be targets of ecdysteroid action producing wandering are shaded. Prior to ecdysone release ('feeding stage' larva) the medial region of the brain exerts inhibitory control both over the powerful excitatory locomotor influence in the lateral area of both sides of the brain and over the moderately active locomotor initiating function in the SEG. Ecdysteroids suppress the spontaneous locomotor initiating activity of the SEG and temporarily repress the medial inhibitory function of the brain. Consequently the lateral excitatory region of the brain can stimulate intense locomotion from the SEG. Ecdysteroids also act on lower centres in the thoracic and abdominal ganglia and may increase their level of excitability.

level of the ganglia so that it is easier to drive the pattern generating circuitry. Hormone action in the head appears to alter the respective roles of the brain and SEG. Ecdysteroid exposure suppresses the excitatory role of the SEG, but the major target for the hormone may be the proposed inhibitory centre in the medial region of the brain. Suppression of this centre would then allow the lateral excitatory centre to drive the locomotor behaviour. We have illustrated this influence as acting through the SEG centre but it is also possible that control fibres from the brain may bypass this centre and extend directly to the pattern generating elements in the thorax and abdomen.

Cessation of spontaneous wandering could be due either to the decline of descending excitation from the lateral centres or to a reappearance of the prior inhibition. Termination of wandering behaviour is not based on a loss of motor ability, since activity can be temporarily re-evoked by disturbing the larvae (Dominick & Truman, 1984). It is significant that removal of the medial region of the brain from wandering larvae results in continuation of locomotor activity for several days, rather than in cessation of movement at the normal time. This suggests that wandering may normally cease in response to a reappearance of inhibition from the medial region of the brain. Thus we suggest that the medial inhibitory function is temporarily inactivated by 20-HE, leading to wandering locomotion, and that its subsequent reappearance causes cessation of further spontaneous wandering. This temporary change in brain inhibitory function contrasts with the apparently irreversible change in role played by the SEG following ecdysteroid exposure. The relationship observed between wandering duration and the length of exposure to 20-HE (Dominick & Truman, 1985) suggests that the time course of such a temporary disinhibition varies with differing lengths of 20-HE exposure.

In summary, the induction of wandering behaviour by 20-HE appears to result in operational changes in several distinct regions of the nervous system. In this respect it is similar to many examples of steroid-regulated behaviour in vertebrates in which the hormone appears to act at various levels in the behavioural circuitry (Arnold, 1981). In addition, although the evidence presented here supporting the idea that wandering locomotion appears as a result of the hormonally defined period of disinhibition is as yet preliminary, the principle of selective disinhibition as a means for activating specific behaviour has been established in insects (Howse, 1976) and in vertebrates (Ewart, 1967; Delgado, 1967).

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