J Comp Physiol A (1988) 163:287–299

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Metamorphosis of the ecdysis motor pattern in the hawkmoth, *Manduca sexta*

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Accepted February 9, 1988

Summary. The hawkmoth, *Manduca sexta,* undergoes periodic molts during its growth and metamorphosis. At the end of each molt, the old cuticle is shed by means of a hormonally-activated ecdysis behavior. The pharate adult, however, must not only shed its old cuticle but also dig itself out from its underground pupation chamber. To accomplish this, the adult performs a series of abdominal retractions and extensions; the extensions are coupled with movements of the wing bases. This ecdysis motor pattern is distinct from the slowly progressing, anteriorly-directed, abdominal peristalses expressed by ecdysing larvae and pupae.

We have found that the ability to produce the larval-like ecdysis pattern is retained in the adult. Although this behavior is not normally expressed by the adult, larval-like ecdysis could be unmasked when descending neuronal inputs, originating in the pterothoracic ganglion, were removed from the unfused abdominal ganglia. Transformation of the adult-specific ecdysis pattern to the larval-like pattern was accomplished by transecting the connectives between the pterothorax and the abdomen, or by reversibly blocking neuronal activity with a cold-block. A comparative analysis of the ecdysis motor patterns expressed by larvae and by isolated adult abdomens indicates that the two motor patterns are indistinguishable, suggesting that the larval ecdysis motor pattern is retained through metamorphosis. We speculate that its underlying neural circuitry is conserved through development and later modulated to produce the novel ecdysis pattern expressed in the adult stage.

Abbreviations: A(n) nth abdominal segment; *DL* dorsal longitudinal; *EH* eclosion hormone; *ISMs* intersegmental muscles; *MN* motoneuron; *SEG* subesophageal ganglion; *T1,T2,T3* prothoracic, mesothoracic, and metathoracic ganglion; *TSMs* tergosternal muscles; *TX* thorax

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Introduction

Sequential stages in the lives of insects that undergo a complete metamorphosis often show radically different behaviors. An important question to address is whether these new behaviors are related to the ones previously shown by the insect. In particular, what is the developmental fate of pattern-generating circuitry for a stage-specific behavior when that behavior has changed in form or is no longer expressed? Are old neural circuits lost and replaced with new ones or, alternatively, might they be retained and modulated to produce new and possibly more complex motor patterns?

A neural 'network' can be viewed as having the capacity to produce various patterned outputs depending on the synaptic inputs or chemical modulators it receives (Ayers et al. 1983; Getting 1983; Getting and Dekin 1985; Harris-Warrick and Cohen 1985; Selverston 1985, for review; Flamm and Harris-Warrick 1986). Such a strategy enables a nervous system to generate new and different behaviors economically and might also be used to construct adult behaviors during development (Bekoff 1981; Bekoff and Kauer 1982, 1984). We examined whether such a strategy is used in the construction of stage-specific behaviors in the developing hawkmoth, *Manduca sexta.* We chose to study this insect's periodic expression of ecdysis behavior (the shedding of the old cuticle), which occurs as it molts from one stage to the next.

Larval, pupal, and adult ecdyses are all 'triggered' by the same neuropeptide, eclosion hormone. However, adult ecdysis (also termed eclosion) differs strikingly from that of larvae and pupae. The pharate adult must dig itself out from its underground pupation chamber in addition to shedding its pupal cuticle. To accomplish this, the adult performs a series of rapid abdominal retractions and extensions; the extensions are coupled

with wing muscle activity or wing 'shrugging' (Truman and Endo 1974; Kammer and Kinnamon 1977). This eclosion motor pattern is distinct from the slowly progressing, anteriorly-directed, abdominal peristalses expressed by ecdysing larvae and pupae (Weeks and Truman 1984a, b).

It was initially assumed that the pattern-generating circuitry underlying larval and pupal ecdyses was dismantled during metamorphosis and replaced with adult-specific circuitry for the generation of the eclosion/digging behavior (Truman and Weeks 1983, 1985). We have found, however, that the larval/pupal ecdysis motor pattern is retained in the adult, although not normally expressed. Our data are consistent with the idea that the pattern generator for larval ecdysis persists through development to participate in the generation of the novel adult ecdysis behavior.

Materials and methods

Experimental animals. Larvae of the tobacco hornworm, *Manduca sexta*, were reared on an artificial diet (Bell and Joachim 1978) and both larvae and pupae were maintained at 26 °C in a 17 h light (L): 7 h dark (D) cycle. At the beginning of adult development, individuals were transferred to a 12 L:12 D cycle with a superimposed thermoperiod (day 27 °C: night 25 °C) which resulted in a greater synchrony of eclosion (Lockshin et al. 1975). Under these rearing conditions, adult development lasted about 18 days and eclosion occurred 1–4 h before lights off on day 18. Individuals were staged as being 1 day away from eclosion (Day −1) by their dark coloration and the advanced digestion of the overlying pupal cuticle (Schwartz and Truman 1983), and set aside for examination on the following day.

Electrophysiology. Extracellular muscle recordings were obtained from abdominal and thoracic muscles using teflon coated 0.003″ silver wire (A–M Systems, Inc.). The teflon was removed from the wire tips leaving a small area of bare wire. The electrodes were then inserted through the cuticle into the appropriate muscles involved in ecdysis movements. The major muscles that were recorded from were the intersegmental muscles (ISMs) and the tergosternal muscles (TSMs) in the 4th through 7th abdominal segments (A4–A7), and the dorsal longitudinal (DL) muscles in the thorax (TX). Recording leads were attached to the cuticle with cyanoacrylate glue (Duro Quick Gel, Loctite, Inc.). In all preparations recorded from, the movements of abdominal segments (both lateral constrictions and the partial inward telescoping of a given segment) were noted by an event marker. This ensured the correct identification of the TSM and ISM units. Electrical signals were amplified and displayed using conventional electrophysiological equipment. Electromyographic activity was taped by a Hewlett Packard 3960 Instrumentation Recorder and played out on a 6 channel Gould Brush 260 Chart Recorder.

Descending neuronal activity was blocked by either the surgical transection of the anterior connective or the use of a 'cold-block' apparatus. The original design of the cold-block device was provided by Dr. Hans-Georg Heinzel (University of California, San Diego). The thermoelectric cooling unit was manufactured by Melcor Inc. The surface area of the cooling platform measured 2 mm^2. A temperature drop from 26 °C to 3 °C, measured by a thermocouple, was accomplished at the connective site within approximately 15 s. At this time, neuronal activity was deemed blocked by the disappearance of electrical activity recorded by a suction electrode placed en passant on the connectives.

Sustained eclosion/digging behavior was achieved by placing the insect on its back with a wax harness around the thorax and portions of the abdomen. This prevented the moth from fully escaping from its old cuticle while also permitting easier access to nerves and muscles for experimental manipulation and electrical recording.

Results

The adult ecdysis motor pattern

A photographic sequence showing the abdominal movements of an intact adult during ecdysis is seen in Fig. 1. The basic motor pattern consists of a two-step process: (1) the abdominal segments are retracted anteriorly in a rapid metachronal manner (retraction component) and then, (2) pushed and extended out past the resting length of the abdomen (extension component). The extension of the abdomen is coincident with contractions of the dorsal longitudinal (DL) muscles in the thorax (TX), which cause movements of the wing bases. This basic motor pattern is expressed as the moth escapes from its pupal cuticle (eclosion), and also as it digs up through the ground towards the soil surface (digging). These repetitive abdominal movements can continue for hours while the insect is digging. Although the ecdysis movements shown by the adult during eclosion/digging consist primarily of a series of retraction-extension bouts, there are progressive changes in the form of the behavior (and pattern of muscle activity) from its initial expression to when robust digging is expressed. These will be described in sequence below.

The DL muscles in the thorax, the abdominal intersegmental muscles (ISMs), and the tergosternal muscles (TSMs) (Fig. 2), are the major muscles responsible for the movements expressed during adult ecdysis. ISM contractions in a given abdominal segment pull the adjacent posterior segment anteriorly, partially telescoping it into the next anterior segment. When ISM contractions in adjacent abdominal segments overlap, the abdomen is retracted (shortened). Contractions of the TSMs (dorso-ventrally oriented muscles) in a given segment cause that segment to constrict; co-activation of TSMs in all segments cause the entire abdomen to narrow greatly and to extend.

Figure 3 shows the electromyographic activity of the TSMs during the initiation of the adult ecdysis pattern. Typically, the initial cycles consist only of abdominal extensions brought about by co-con-

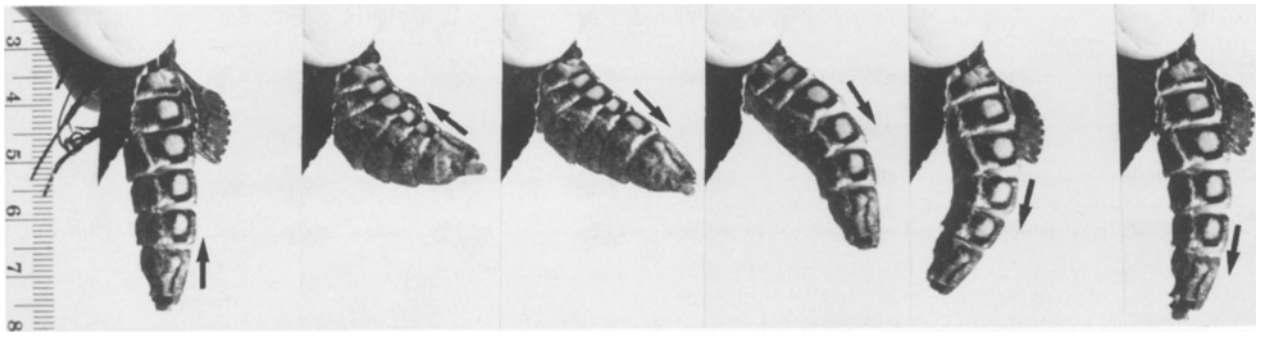

Fig. 1. A photographic sequence of the ecdysis movements expressed by the intact adult abdomen. Individual frames were taken at approximately one second intervals. A lateral view of the abdomen shows a retraction component where the entire abdomen is shortened anteriorly (upward arrows), this is then followed by an extension of the abdomen (downward arrows) past its resting length

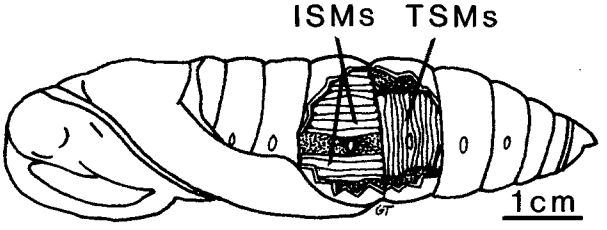

Fig. 2. Side view of a pharate adult, *Manduca sexta,* enclosed in its pupal cuticle. Parts of the pupal cuticle and the adult body wall have been cut away to expose the arrangement of the *ISM* and *TSM* muscle sets in A4 and A5, respectively. For illustration purposes only one muscle set is shown in each segment. The longitudinally oriented ISMs span the length of a given segment and insert along the anterior margin of the next posterior segment. The TSMs consist of a lateral and dorso-ventrally oriented mat of muscle fibers which lie just underneath the ISMs and flush with the body wall

tractions of the TSMs. The ISMs also contract along with the TSMs (data not shown). After 4 to 6 cycles the retraction component appears (Fig. 3). During these early retraction-extension cycles, both ISMs and TSMs are active during each retraction and extension phase (Fig. 4). The extension phase is always coincident with thoracic DL muscle activity.

The extension is produced by the co-contractions of the TSMs along the length of the abdomen, thereby constricting the segments and telescoping out the abdomen. During the early stages of the behavior, the TSM contractions are accompanied by contractions of the ISMs. These latter contractions prevent the abdomen from reaching its full extension. Although the extension of the abdomen appears as a peristaltic wave (Kammer and Kinnamon 1977), the elongation of the abdomen is actually accomplished by the synchronous firing of the TSMs. The flow of hemolymph forcing posterior segments out first is apparently responsible for the peristaltic appearance of the extension.

The patterned output of the ISMs and TSMs during the first few retraction cycles is often similar to the anteriorly-directed abdominal peristalses displayed during larval and pupal ecdyses (see next sect.). These initial retraction waves show a rather slow anterior progression with the firing of the TSMs in a given segment commencing as the activity of the TSMs in the next posterior segment is at or near completion (Fig. 4). Also, the TSMs of a given segment typically fire in synchrony with the ISMs in the next anterior segment (Fig. 4). Consequently, during these first few retraction cycles, each abdominal segment is constricted and pulled forwards as the peristaltic wave progresses up the abdomen.

With successive cycles there is a gradual but dramatic increase in the velocity with which the TSM (and ISM) contractions progress anteriorly along the abdomen (Figs. 5, 6A). Because the anterior-going wave moves along the abdomen so rapidly, without the relaxation of posterior segmental muscles, the entire abdomen becomes retracted towards the thorax. With the increase in velocity of the retraction wave, the delay between the onset of the retraction and the subsequent extension is shortened over time (Figs. 5, 6B). The cycle period between intrasegmental extension bursts, however, remains largely unaffected or increases slightly (Fig. 6B).

Figure 7 depicts the pattern of TSM and ISM activity mid-way through adult eclosion behavior and during the later expression of digging behavior. As the motor pattern continues, the ISM activity during the extension component gradually diminishes until it is no longer present. During these later cycles, robust extensions are observed that characterize the digging behavior.

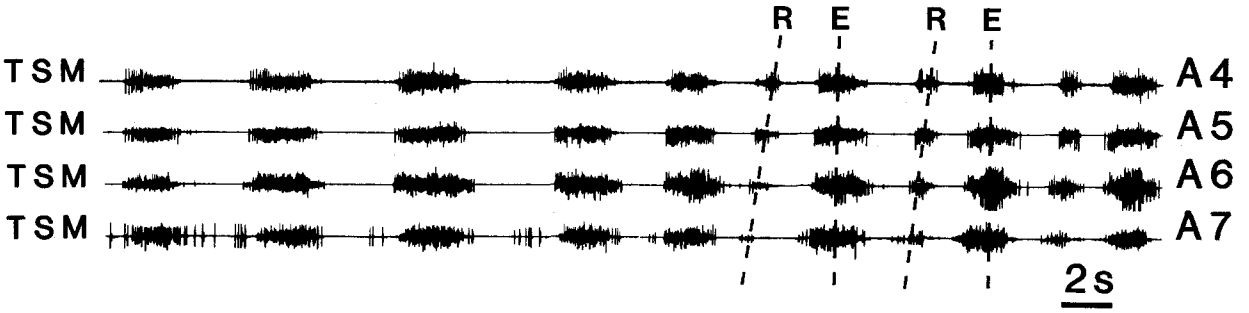

Fig. 3. Continuous electromyographic recordings from the *TSMs*, in A4–A7, at the onset of adult eclosion behavior. The first 5 cycles show only co-activity of all the TSMs, which results in an extension (*E*) of the abdomen. After cycle 5, the retraction (*R*) component becomes evident and alternates with the extension. During the retraction phase, the TSMs are activated in a metachronous wave starting from the most posterior segment. Dashed lines are fitted by eye through the midpoint of the bursts

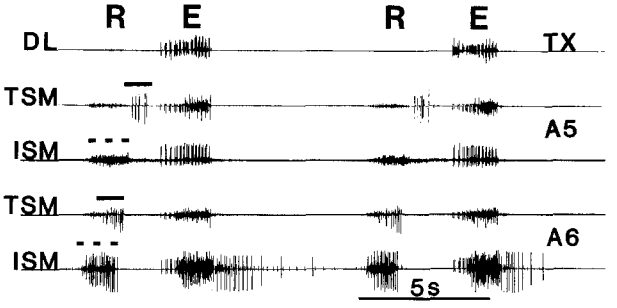

Fig. 4. Electromyographic recordings obtained from dorsal longitudinal (*DL*) muscles in the thorax (*TX*) and the ISMs and TSMs (in A5 and A6) during the early part of the adult-specific ecdysis motor pattern. The early retraction waves (*R*) consist of a slowly progressing, anterior-going, metachronal wave that is similar to the peristaltic wave expressed during larval/pupal ecdysis behavior. During retractions, TSM activity (solid bars) in adjacent segments is out of phase, whereas, the ISMs (dotted lines) are coactive with the TSMs in the next posterior segment. The small amplitude activity shown in the TSM trace (A5) represents electrical pick-up from the firing of the ISMs in that same segment. The phase lag between the ISMs in posterior segments, as compared to those in anterior segments (not shown), is much less. DL activity coincides with the coactivation of the TSMs and ISMs during the extension (*E*) phase of the cycle. Note that during these early extension bouts, the ISMs are active along with the TSMs

The initial presence and later loss of ISM activity during the extension phase is probably related to the changing role of the ecdysis motor program. During the early phases, the behavior is used for shedding the pupal cuticle. Too vigorous an extension of the abdomen would rupture the cuticle at the abdomen-thorax juncture, thereby preventing the insect from fully escaping from the cuticle over its head and thorax. The coactivation of the ISMs during the extension phase may therefore serve to moderate the force exerted on the cuticle during extension. Later in the ecdysis program, after the insect has had time to remove its old cuticle, the segmental ISM activity is lost (Fig. 7). The loss of the opposing forces generated by the ISMs enhances the power stroke during extension and, hence, the ability of the insect to dig itself out from its underground pupation site.

Eclosion motor pattern expressed by the isolated adult abdomen

At the start of adult ecdysis, an abdomen separated from the head and thorax continues to show rhythmic ecdysis movements. The behavior of such abdomens, however, is very different from that of the intact adult. The movements consist of a slow anteriorly-directed peristalsis (Fig. 8). During the progression of the wave, a given segment is constricted, due to TSM contractions in that segment, while it is pulled anteriorly by contractions of the ISMs in the next anterior segment, thereby partially telescoping the segment into the next anterior segment. As one muscle type in a given segment relaxes, the homologous muscle in the next anterior segment contracts. This anteriorly-directed peristaltic wave of activity is indistinguishable (see Discussion) from that expressed during larval and pupal ecdysis behavior (Weeks and Truman 1984a).

Figures 9 and 10 show the activity of the ISMs and TSMs during the ecdysis movements of the isolated adult abdomen. The larval-like pattern was produced when the connectives between the fused pterothoracic ganglion (T2–A2) and the abdomen were transected. Transections typically were made within seconds after the onset of adult eclosion. During the resulting pattern, ISM activity precedes TSM activity within a given segment, and the TSM activity in a given segment is in phase with the ISM activity in the next anterior segment. The period between successive peristaltic waves gradually increases (Figs. 9C, 11b) as the behavior

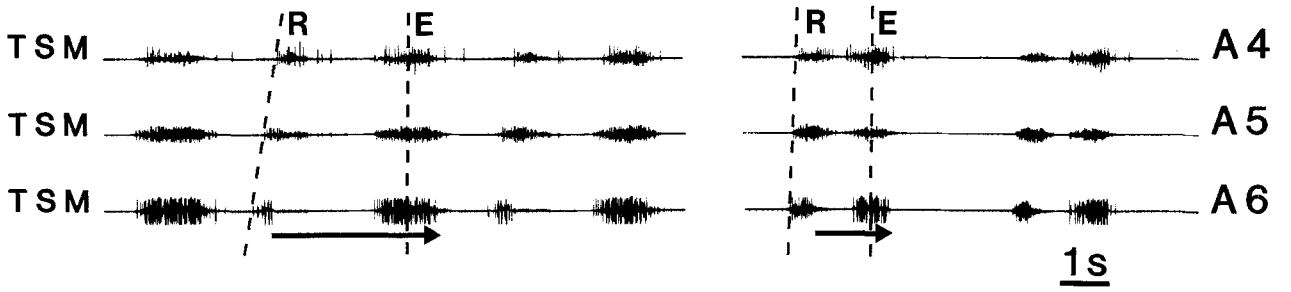

Fig. 5. TSM recordings from A4–A6 showing the increase in velocity of the retraction wave at early and later stages of eclosion. Left, retraction-extension cycles relatively early in the behavior; right, an example 11 cycles later. Note that the lag between the retraction (*R*) and extension (*E*) bursts in a given segment decreases over time but the duration of the period between successive extension bursts remains relatively stable, as measured by the time between the ends of the bursts under consideration

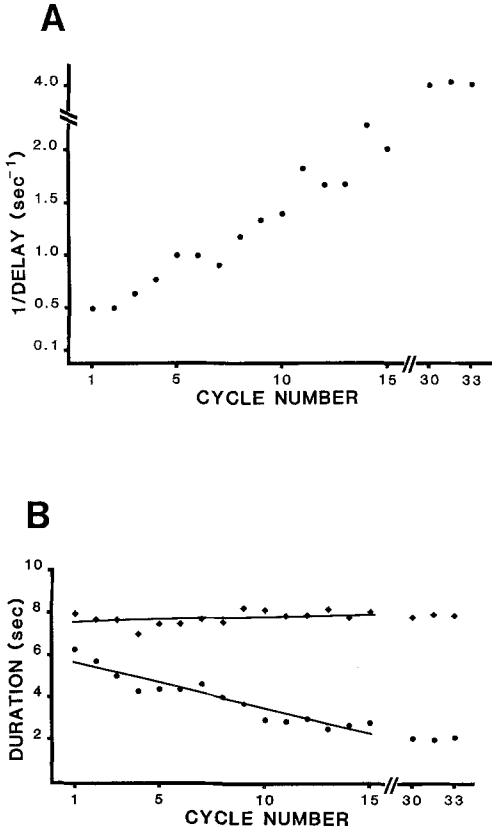

Fig. 6A, B. Progressive changes in the adult-specific ecdysis pattern are plotted over time. Data were obtained from the same preparation as in Fig. 5. **A** The inverse of the inersegmental delay, a measure of the velocity of the metachronal wave, is plotted versus cycle number. The intersegmental delay was measured as the time between the termination of a retraction burst in one segment and the termination of the retraction burst in the next anterior segment. Each point represents the inverse of the delay between one segmental pair. **B** The changes in the duration of the period between successive extension bursts (diamonds) and between retraction and extension bursts (circles) in one segment. Period duration was measured as the time between the ends of the bursts under consideration. While there is no significant change in the duration of the period between extension bursts over time, there is a decrease in the period duration between retraction and extension bursts

progresses. Figure 10 shows the transition from the adult-specific ecdysis pattern to the larval-like pattern when descending neuronal inputs to the abdomen were abolished by transection anterior to A3. Within the next retraction cycle after the transection, the pattern had switched. The extension component was immediately lost, and there was an abrupt decrease in period length between retraction (peristaltic) waves. The ISM activity in a given segment became coincident with the TSM activity in the next posterior segment. Removal of the descending inputs caused abrupt, stable shifts in the velocity of the peristaltic wave and shifts in cycle period (Fig. 11).

Within the ecdysis pattern shown by isolated abdomens, a new wave front can be initiated before the previous one is completed. Consequently, two wave fronts may occur simultaneously along the abdomen. Under these conditions, waves of anteriorly directed ISM or TSM activity were typically separated by an average of four segments. A measure of this intersegmental coordination is the intersegmental phase, a ratio of the intersegmental delay to cycle period. Intersegmental delay was measured as the time between the termination of the burst in a given segment and the end of the burst (same muscle set) in the next anterior segment. For example, the intersegmental phase value obtained from the recordings in Fig. 10 was 0.24 (S.D. ± 0.01; $N=5$; mean period length, 4.5 ± 0.1 s), such that muscles in A7 and A3 (a separation of four segments) are coactive. The period between successive peristaltic waves increases over time, whereas the velocity of the wave remains relatively constant or decreases slightly (Fig. 11). Thus, the intersegmental phase values show a progressive decrease. For example, for later bursts from the preparation in Fig. 10, the phase value dropped to 0.18 ± 0.04 ($N=5$) as the mean period increased from 4.5 ± 0.1 s to 7.4 ± 0.4 s.

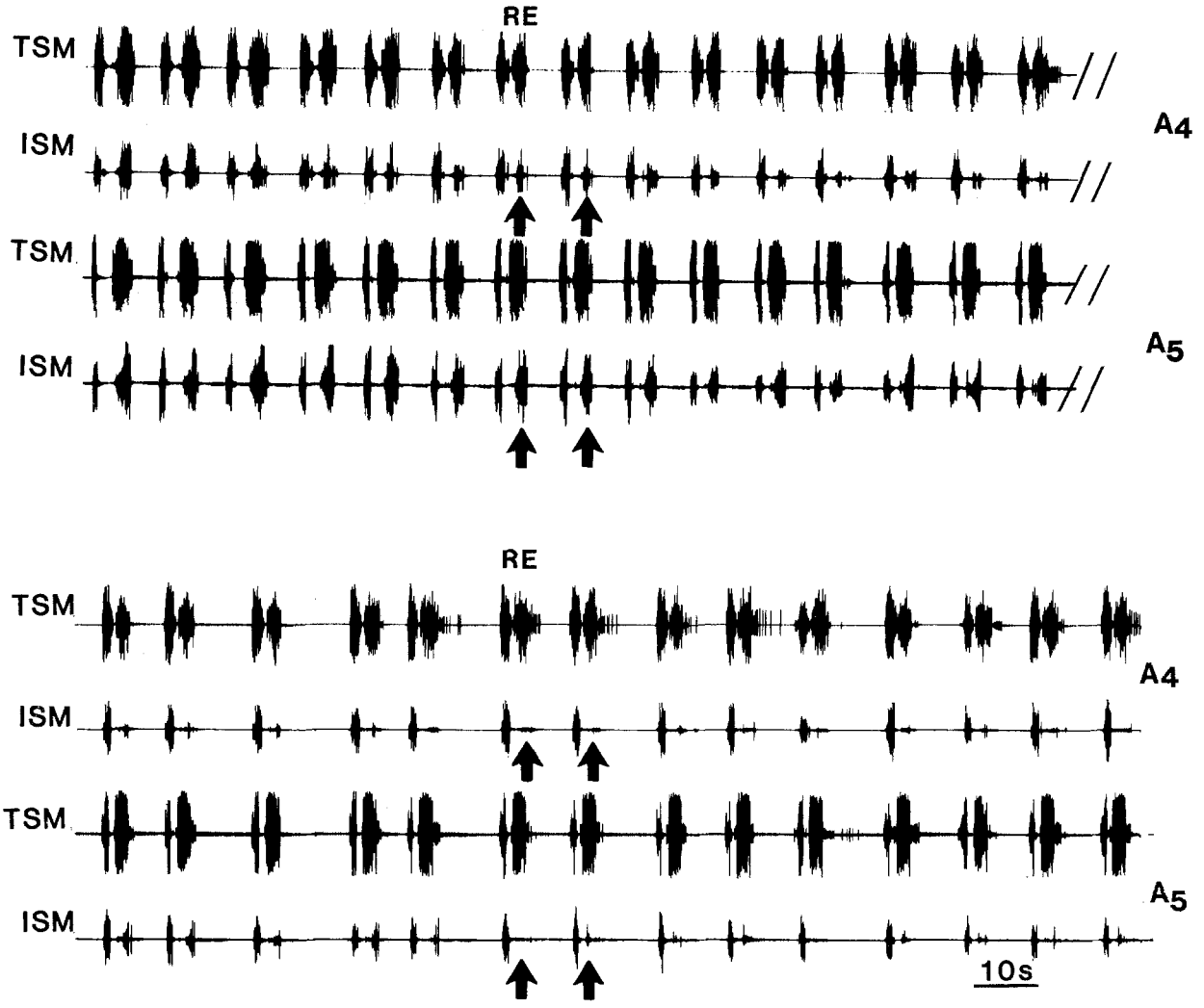

Fig. 7. Electromyographic activity of ISM and TSM muscle sets during the major movements of the adult eclosion/digging motor pattern. Recordings of ISM and TSM muscles show the progressive changes in the form of the eclosion motor pattern which produce a more robust digging behavior over time. Recordings were made from the TSMs and ISMs in the 4th (*A4*) and 5th (*A5*) abdominal segments. The record starts well into the ecdysis behavior when the adult was nearly out of its pupal cuticle. The upper and lower traces are continuous except for a gap of seven retraction-extension cycles (slashed lines). Note that during the earlier part of the motor pattern ISMs and TSMs are co-active during the extension phase. Later in the pattern, when robust digging behavior is observed, the ISMs no longer burst during the extensions; compare the ISM activity indicated in the upper and lower traces (arrows)

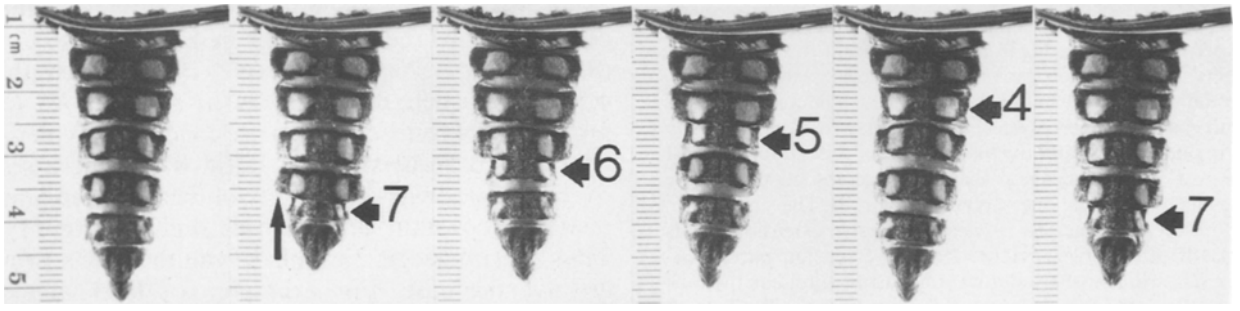

Fig. 8. A photographic sequence of the abdominal movements (dorsal view) expressed by the isolated adult abdomen. Individual frames were taken at approximately one-second intervals. Each abdominal segment, which is numbered sequentially, is constricted (horizontal arrows) as it is pulled up into the next anterior segment (vertical arrow)

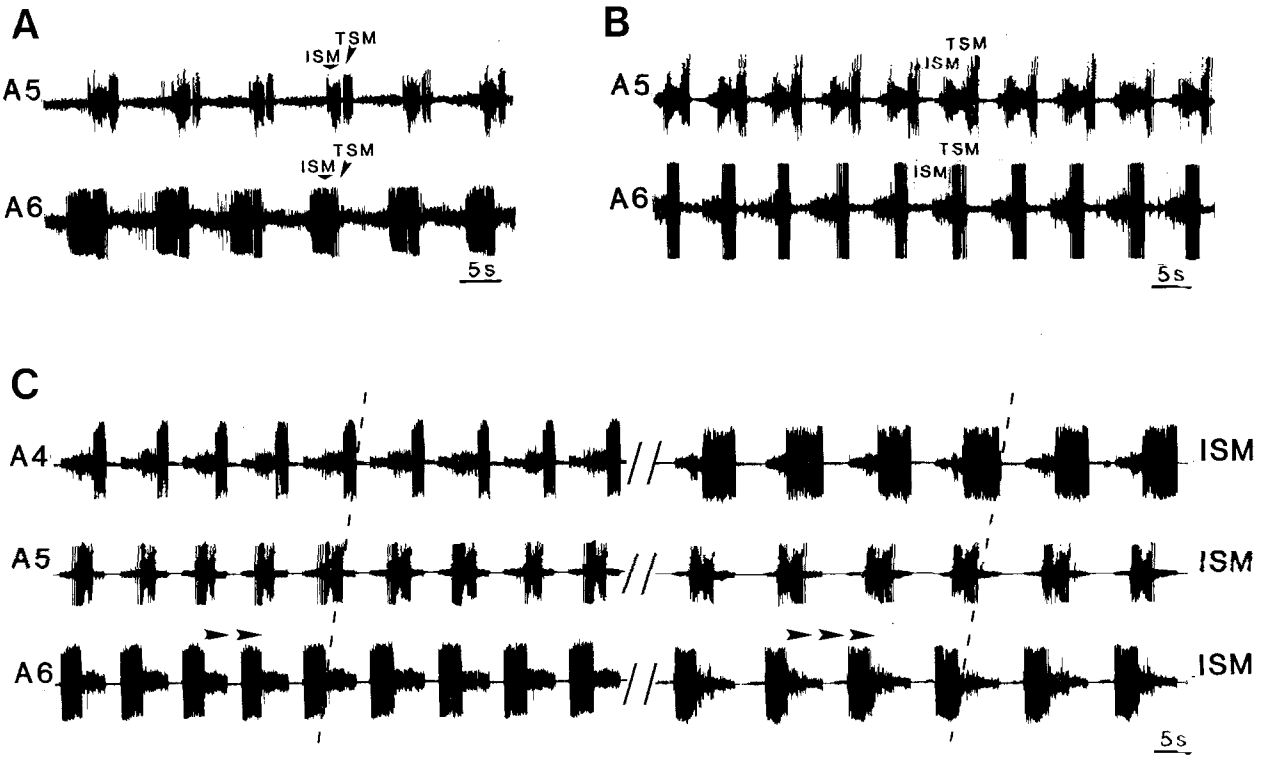

Fig. 9A–C. Recordings of muscles involved in the ecdysis behavior of the adult isolated abdomen. **A, B** Two different preparations are shown for comparison. In both preparations recording electrodes were placed in positions to record from both the ISMs and TSMs within a given segment. The motor pattern expressed shows that the firing of the TSMs coincides with the firing of the ISMs in the next anterior segment. **C** Muscle recordings from the ISMs in segments A4–A6, showing that the period between the bursts (arrows) in each segment increases over time but the velocity of the peristaltic wave (slope of dashed line) does not decrease. Only large amplitude units were visually correlated with ISM contractions; smaller units were due to electrical pick-up from adjacent muscles. The slash marks represent 6 cycles which were deleted

Use of a cold-block for temporary disruption of descending neuronal inputs

A cold-block device was employed to block descending neuronal activity to the abdomens of minimally dissected adults. The cooling platform was inserted through a small window in the cuticle and placed under the connectives between the fused pterothoracic ganglion and the first unfused abdominal ganglion (A3). Unlike the surgical transection of the connectives, the cold-block was reversible. Figure 12A, B demonstrates that the adult-specific eclosion pattern is transformed into the larval-like pattern during the cold-block and then back into the adult-specific pattern once the block is removed. During the block, the pattern of thoracic DL muscle activity becomes uncoupled from the abdominal activity (see Fig. 12B) and the cycle period of thoracic DL activity becomes more variable. As will be discussed below, it was not possible to go from the adult-specific pattern back into the larval pattern a second time.

Temporal constraints in expression of the larval-like ecdysis pattern

There is a narrow temporal window during which the removal of descending inputs to the abdomen results in the expression of the larval-like ecdysis pattern. When these inputs are interrupted before the first signs of rhythmic ecdysis activity, the isolated abdomen does not express any patterned motor output (Table 1). Only within the first 50 s after the insect has initiated ecdysis does abdominal isolation or application of a cold-block result in the abdomen sustaining rhythmic movements in the form of the larval-like ecdysis motor pattern. After about 60 s, these manipulations no longer result in the expression of rhythmic abdominal ecdysis movements despite the fact that, in the intact adult, ecdysis movements can last for hours if the insect is physically restrained (Kammer and Kinnamon 1977). At these later times, connective transection results in no abdominal activity even though thoracic activity continues (Fig. 13).

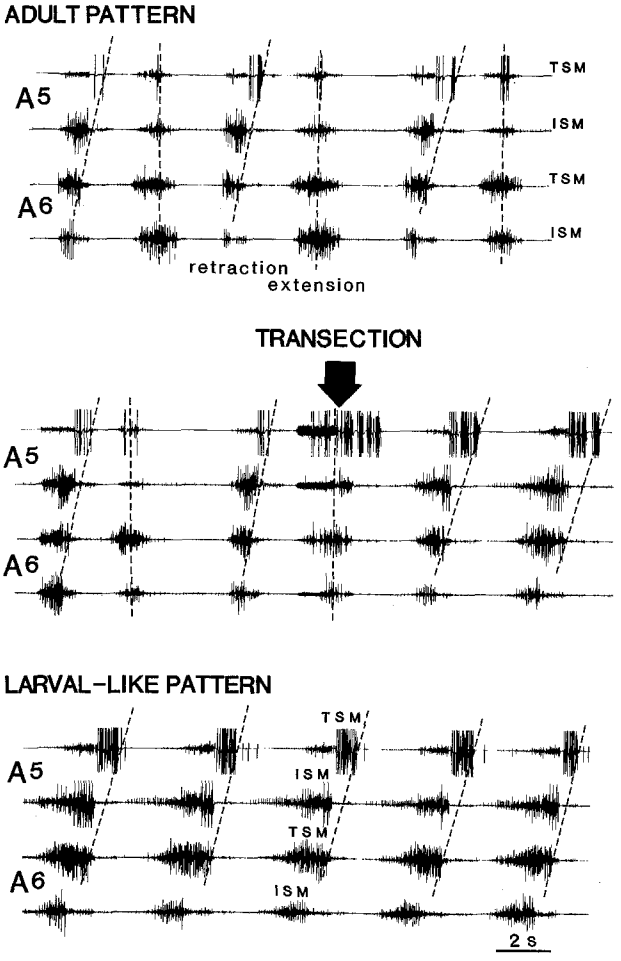

Fig. 10. Continuous record showing the transformation of the adult-specific ecdysis pattern into the larval-like pattern. Descending neuronal input was removed by transecting the connectives anterior to A3. Note the immediate and smooth transition from the adult-specific motor pattern to the larval-like pattern within the first cycle after transection. The slanted dashed lines, drawn through the ends of TSM bursts in adjacent segments, identify the retraction waves before and after transection; the vertical lines drawn through the midburst points denote the extension bouts

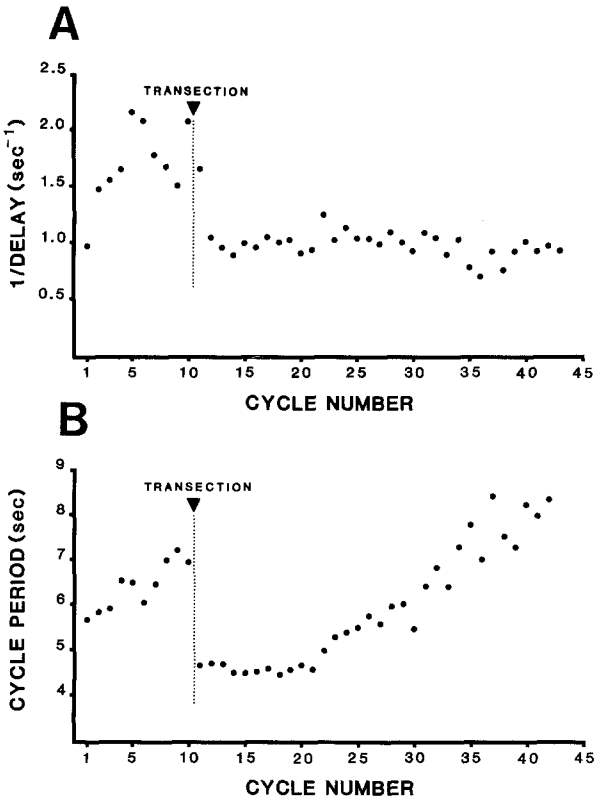

Fig. 11A, B. Changes in cycle period and wave velocity before transection of the connectives and throughout the larval-like pattern which was subsequently produced. Data were collected from the same preparation as shown in Fig. 10. **A** A plot of the inverse of the intersegmental delay (a measure of the velocity of the anterior-going wave). Intersegmental delay was measured between the end of the TSM burst in A6 and the end of the TSM burst in A5. Transection caused an abrupt decrease in wave velocity which then stayed stable for the remainder of the behavior. **B** Changes in the period of intrasegmental retraction cycles measured between the ends of successive bursts. After transection, the period abruptly shortens but then gradually lengthens as the behavior progresses

Ganglionic localization of the descending input

To determine the source of descending inputs, the connectives were transected at different levels anterior to the abdomen (Fig. 14). In all cases, these manipulations were made during the time when eclosion hormone is present in the blood (Reynolds et al. 1979; Truman 1985), and within the first minute after eclosion behavior had been initiated. Transection of the ventral nerve cord below the brain, subesophageal ganglion (SEG) and prothoracic ganglion (T1), had no effect on the expression of the adult-specific eclosion motor program in all animals tested ($N=30$). Transection posterior to the fused pterothoracic ganglion (T2–A2) resulted in the expression of the larval-like pattern by the remaining unfused abdominal ganglia in all animals tested ($N=50$). In adults ($N=8$) where T2 was surgically separated from T3, the adult-specific motor pattern remained intact although it was expressed more slowly. Thus, the locus of the descending input appears to reside somewhere in the T3–A2 region of the CNS.

Discussion

The larval ecdysis motor pattern: retention in the adult and its possible significance

Despite the dramatic changes in the ecdysis behavior, body morphology and neuronal organization

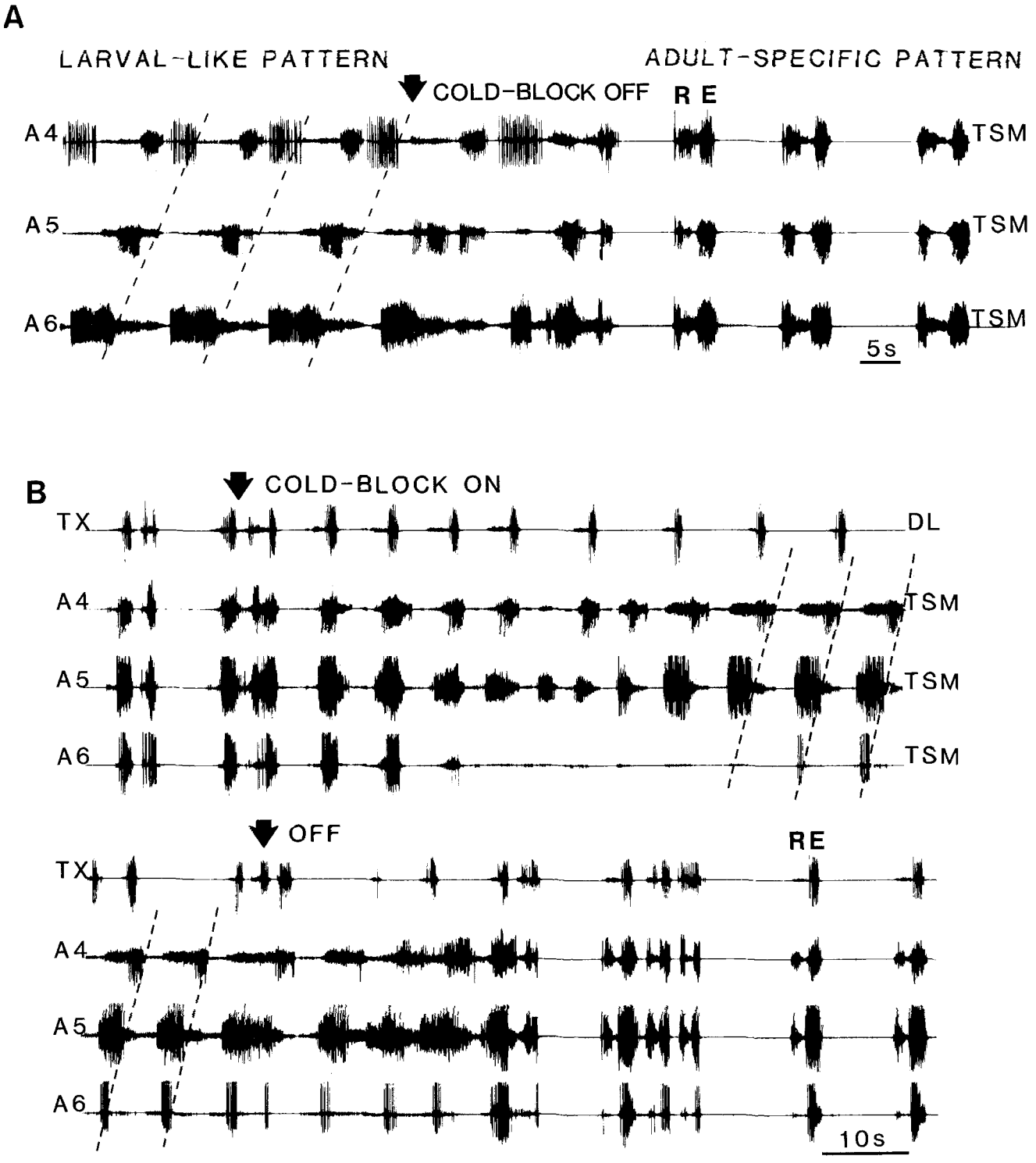

Fig. 12A, B. Use of a reversible cold-block, on the connectives between the thorax and abdomen, to alter the form of the ecdysis behavior. **A** The cold-block was applied at the onset of eclosion resulting in the production of the larval-like pattern, consisting of the slow anteriorly directed peristaltic waves. After removal of the cold-block, the pattern lapsed back into the adult pattern of cyclical retraction (*R*) and extension (*E*) bouts. The smaller amplitude units represent electrical pick-up from muscles other than the TSMs. **B** Example of recordings from the TSMs in A4–A6 and the thoracic DL muscles during application and removal of the cold-block. All records are continuous. The block was applied after the adult-specific pattern was expressed for several cycles. After a delay the larval-like pattern appears and the DL activity is no longer coordinated with activity in the abdomen. When the cold-block was removed, the adult-specific pattern was produced in full, with the thoracic DL muscle activity coincident with the extension component. Dashed lines indicate the series of larval-like peristalses

Table 1. Ability of isolated adult abdomens to express spontaneously the larval-like ecdysis motor program as a function of the time of abdomen isolation

Time of nerve cord transection (s)[a]	# of animals	% showing larval program	Duration of larval program (min $\bar{x} \pm SD$)
pre-ecdysis	25	0	–
1–30	9	100	8.4±1.2
40–49	11	72	6.5±2.9
50–65	6	0	–
>65	8	0	–

[a] Relative to the start of eclosion behavior

of *Manduca* during development (Truman et al. 1986), the ability to express the larval ecdysis motor pattern is retained through metamorphosis. The ecdysis motor pattern that is unmasked in the adult when descending influences from the pterothoracic ganglion are removed (Figs. 8–10, 12) strongly resembles the ecdysis motor pattern expressed at larval and pupal ecdysis (Weeks and Truman 1984a). The duration of larval and pupal ecdysis behavior typically lasts 10–20 min and involves between 50–100 cycles (Truman et al. 1980; Weeks and Truman 1984a). The adult, larval-like ecdysis pattern typically lasted 8–10 min with 60–80 cycles expressed. Other comparisons between the larval and adult, larval-like ecdysis pattern reveal that parameters such as the duration of individual bursts of motor activity (ISM and TSM bursts lasted between 1–3 s), and the intra- and intersegmental coordination of burst activity are indistinguishable.

A distinctive feature of the larval/pupal ecdysis pattern is the anterior progression of two simultaneous peristaltic waves (Weeks and Truman 1984a). The presence of two waves is due to the first wave of peristalsis not being completed before the next one begins. The isolated adult abdomen showed a similar pattern of peristaltic wave activity where pairs of anterior and posterior segments (e.g. A7 and A3) contract together. However, because removal of the fused thoracic ganglion (which contains the first and second abdominal ganglia) was necessary for the expression of the larval-like ecdysis pattern in the adult, this loss precluded the progression of the waves up to segments A1 and A2.

The adult larval-like pattern showed no proportional decrease in the velocity of the peristaltic wave along the abdomen as the period between waves increased (Figs. 9c, 11). In two different adult preparations (not shown) where the period between waves had doubled, the intersegmental delays between A4, A5 and A6 showed only slight increases of 8% and 9%, respectively. As a result, the number of segments which separates the successive anterior-going waves varies over time. In these examples, the intersegmental phase values (ratio of intersegmental burst delay to cycle period) systematically changed from about 0.25 to less than 0.18; two peristaltic waves initially separated by 4 abdominal segments were later separated by 5 segments. Towards the termination of the behavior even longer separations were apparent. Thus, segmental coordination is not due to rigid neural coupling between successive segments. A similar conclusion was also reached for larval and pupal ecdyses (Weeks and Truman 1984a).

The ecdysis behavior shown by intact adults is a two phase behavior consisting of a retraction and extension of the abdomen during each cycle. During the retraction phase, the velocity of the metachronal wave dramatically increases over time (Fig. 6). During the initial expression of the retraction wave, however, the form and progression of the wave is similar to that seen in larvae and isolated adult abdomens (Figs. 4, 10). With successive

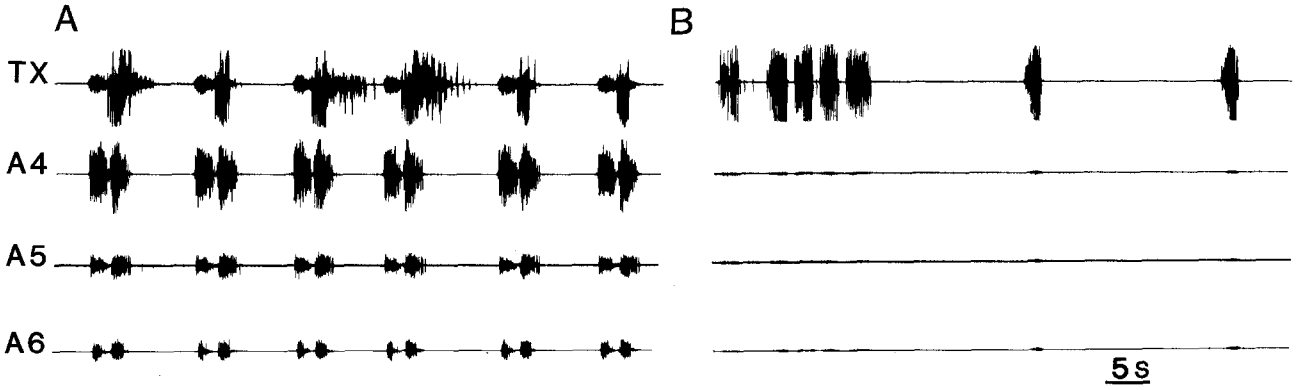

Fig. 13. TSM and thoracic DL muscle recordings during the adult-specific ecdysis pattern before (**A**) and after transection (**B**) of the connectives. The transection was made after the adult-specific pattern had been expressed for over 60 s. Note in **B** the absence of patterned muscle activity in the abdomen and the decrease in rythmic thoracic DL muscle activity after the transection

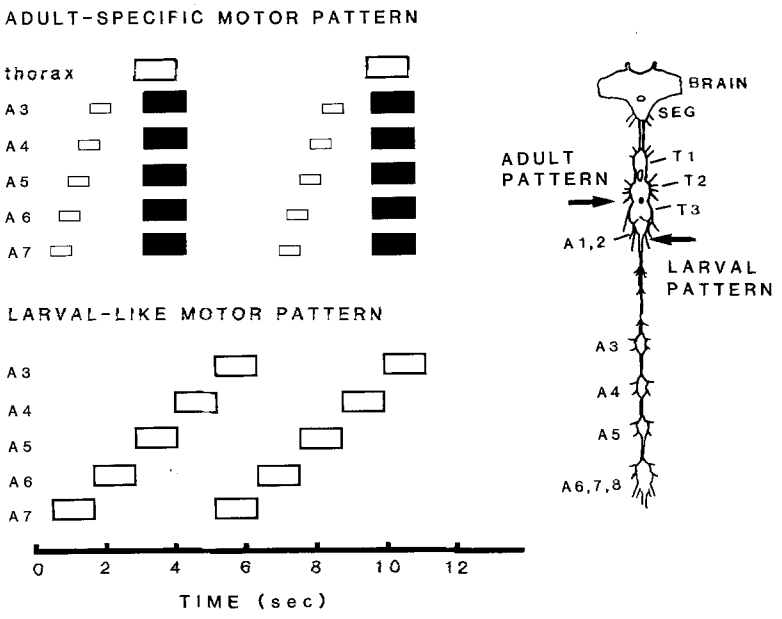

Fig. 14. Schematic diagram of the two types of ecdysis motor patterns expressed by the intact adult (adult-specific motor pattern) and the isolated adult abdomen (larval-like motor pattern). The intersegmental phase relationships of one muscle set (ISM or TSM) are illustrated from data averaged from several different preparations. The same time scale is used for both pattern types. Open blocks indicate segmental activity progressing anteriorly along the abdomen. In the intact adult, after wing muscle activity is initiated, the closed blocks represent the co-activity of the muscle sets resulting in the extension of the abdomen. This extension component is never expressed by the abdomen when descending inputs have been removed. The diagram on the right illustrates the various sites of transection where the adult-specific ecdysis pattern is preserved and where it is transformed into the larval-like pattern

cycles, the retraction wave gradually loses its resemblance to the larval peristalsis as the velocity of the retraction wave increases. Descending neuronal activity from the pterothoracic ganglion may provide modulatory inputs that alter the velocity of the anterior-going wave, as well as coordinating the co-contraction of the TSMs (and initially the ISMs) during extension.

Based on these observations, we suggest that the underlying neural elements for the larval/pupal ecdysis pattern are conserved through metamorphosis to the adult. Most likely, these conserved elements are incorporated with others, most notably the descending inputs from the thorax, to produce a new, adult-specific motor pattern. With time, the descending inputs may modify the output from this conserved pattern generator, bringing about the rapid retraction waves characteristic of the adult. When these inputs are blocked, however, the larval form of the pattern is immediately re-expressed.

An alternative possibility is that the pattern generator responsible for the larval ecdysis pattern is normally inhibited in the adult and that separate circuitry (perhaps newly constructed) is responsible for the adult ecdysis motor pattern. Removal of descending inputs would then release the larval-like ecdysis program from inhibition. The observation, however, that the adult-specific pattern initially contains a larval-like peristalsis which is gradually transformed into a rapid retraction wave, argues against this hypothesis. More importantly, when descending inputs are blocked, the first cycle of larval-like peristalsis that is initiated occurs at nearly the identical time that the adult-specific retraction wave would ordinarily appear (Fig. 10). Such precise timing is not consistent with the idea that a separate pattern generator is activated after loss of the putative inhibition. In such a situation, a more variable delay and temporary alteration in segmental coordination might be expected as the new pattern generator becomes activated.

Stage-specific behaviors

In the holometabolous insect, *Manduca sexta,* the formation of the adult ventral nervous system is achieved by the death of some larval neurons (Truman 1983; Weeks and Truman 1985), the reorganization of other larval cells for new functions in the adult (Levine and Truman 1982, 1985), and the addition of adult-specific arrays of cells (Booker and Truman 1987). Associated with this remodeling of the CNS are dramatic changes in overt behavior. Such changes, however, may not necessarily reflect alterations in the underlying neural circuitry. For example, pupal ecdysis behavior in *Manduca* looks quite different from that shown by the larva because the pupa lacks the larval prolegs which show rhythmic retractions during larval ecdysis (Weeks and Truman 1984a, b). However, the proleg motoneurons, retained in the pupa, fire at their appropriate phase even though their peripheral targets have degenerated (Weeks and Truman 1984b). Thus, in this case the behavioral alteration arises from a peripheral change rather than a central one.

The transformation from the pupa to the adult results in even greater changes in neurons and mus-

cles, including those involved in ecdysis behavior. In the case of adult ecdysis, the novel aspects of the behavior appear to arise from central rather than peripheral changes. Our results are consistent with the idea that the adult ecdysis pattern generator does not arise by building a completely new central pattern generator, but rather by the addition of a new adult-specific component in the form of descending modulatory inputs to the pre-existing larval pattern generator. From a developmental standpoint, it will be of interest to determine whether the added descending neuronal activity, which is necessary for the generation of the adult-specific behavior, is produced by new neurons that appear during metamorphosis or by elements present at earlier developmental stages which are remodeled during metamorphosis for their new functions in the adult.

Curiously, sustained larval-like ecdysis movements can no longer be evoked by abdominal isolation after the adult ecdysis behavior has progressed for longer than 50 s (Fig. 13, Table 1). However, the abdomens isolated after this time are capable of showing a few bouts of larval-like ecdysis behavior in response to tactile stimulation. For example, tactile stimulation of the isolated abdomen, in a manner that mimics the shedding of the pupal cuticle along the abdomen, results in a reactivation of the larval-like ecdysis program for several cycles or longer. This was observed in insects whose abdomens were isolated after 20 min of digging behavior (data not shown). The nature of the short-term and perhaps longer-term control of expression of the larval-like motor program remains to be explored.

A simplified model for understanding the neural organization of the adult-specific ecdysis program is one based on the coupling of two pattern generators: an adult-specific thoracic one, and the conserved larval pattern generator distributed throughout the unfused abdominal ganglia. Neural elements localized in, and descending from, the thoracic pattern generator may coordinate the activity of abdominal segments with each other (during extensions) and also with the thoracic activity expressed during wing shrugging. These same elements might also serve to modulate the conserved larval peristalsis program to produce the rapid adult-specific metachronal wave. Since rhythmic expression of spontaneous wing shrugging is apparently dependent on ascending neuronal inputs (Figs. 12, 13), information regarding the completion of the retraction wave may then be communicated back to the thorax (by sensory and/or interneurons) to set the timing of the next cycle of wing shrugging and extension behavior. The two pattern generators may become coupled late in adult development. For example, in the giant silkmoth, *Antheraea pernyi,* the insect goes through a period, late in adult development, when it will perform eclosion movements if stimulated by the manual removal of the pupal cuticle. However, these movements involve only thoracic muscle contractions with abdominal extensions and lack the peristaltic retraction component (Truman 1976). A few days later, at the time of ecdysis, the moth then shows the complete pattern with both retraction and extension.

Pattern generators and their developmental fates

The appearance of stage-specific behaviors throughout development may depend on a variety of neural mechanisms. For example, in both invertebrate and vertebrate systems, the neural circuitry underlying a particular behavior may be constructed well before it is expressed later in development (Bentley and Hoy 1970; Kammer and Rheuben 1976; Bekoff 1981; Bekoff and Lau 1980). Subsequent activation of such 'stored' neural circuits may be dependent on the removal of inhibition and/or the presence of a neuronal or chemical (hormonal) stimulus present at the appropriate time (Bentley and Hoy 1970; Truman 1976). In the case of behaviors that are expressed sequentially throughout the life of an animal, a new behavioral program may come about through modification of an earlier behavior as we speculate occurs for adult *Manduca* ecdysis behavior.

Another example of the use of this strategy is the conservation and modification of the motor patterns underlying hatching in the chick. The leg motor pattern produced by a hatching chick is still present at 61 days post-hatching (Bekoff and Kauer 1984), even though this behavior is not normally expressed once the chick escapes the egg. It is suspected that this behavior is conserved because some elements of its underlying neural circuitry are used to produce locomotory behaviors, including walking, in the post-hatching animal. A striking parallel to the present study is that the chick-hatching motor program, expressed earlier in development, can be fully activated in the adult stage when it is no longer appropriate. These observations suggest that pattern-generating circuitry used earlier in development may persist in a relatively unmodified state into later stages. This circuitry could then participate in new behaviors generated by short-term influences, such as sensory cues in the case of the chick (Bekoff and Kauer

1982) or descending influences in the case of *Manduca*. The ability to identify and manipulate such short-term influences, or behavioral switches, may prove extremely useful in characterizing how new behaviors are formed during development. In addition, the organization of more complex behaviors may be better understood if they are analyzed in the context of the conservation and modification of simpler programs expressed earlier in development.

Acknowledgements. We thank Drs. R. Booker, C. Cypher, S.E. Fahrbach, D.B. Morton, and J.L. Witten for comments on the manuscript and helpful discussions during the course of this work. Special thanks to Ms. G.M. Terzi for preparing Fig. 2. This research was supported by an NIH postdoctoral fellowship (NS07639) to K.A.M. and an NIH grant (NS13079) to J.W.T.

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