

CHANGES IN NEURONAL CIRCUITS DURING INSECT METAMORPHOSIS

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SUMMARY

During metamorphosis insects undergo dramatic changes in both form and behaviour. Cell birth and death, as well as neurone respecification all contribute to the overall reorganization of the nervous system. Within the visual and chemosensory processing areas of the insect brain large numbers of newly-generated adult neurones are incorporated into the larval nervous system. In the abdominal ganglia, however, identified larval neurones are retained to assume a new adult role. This respecification of motor neurone function involves not only the acquisition of a new target muscle, but also the reorganization of dendritic morphology, and alterations in the interconnections between neurones. For example, an identified abdominal motor neurone in the hawkmoth, *Manduca sexta*, grows new dendritic processes and changes its synaptic relationship with an abdominal stretch receptor such that an interaction that was inhibitory during larval life, becomes excitatory in the adult. In another example, identified sensory neurones that evoke a larval flexion behaviour, later participate in the defensive gin trap reflex that is characteristic of the pupa. In both instances the formation of new pathways is a two-step process in that the new circuits do not become behaviourally relevant as they are formed, but instead are activated abruptly at the appropriate time. For the gin trap reflex an identified peptide hormone is responsible for activating the circuit.

INTRODUCTION

Insects undergo a period of postembryonic development that can entail significant changes in both form and behaviour. In the holometabolous insects, larval and adult stages are bridged by a transitory pupal stage, and the changes associated with this metamorphosis are particularly dramatic. In some insects the two early stages are quite limited in their behaviour, but in others, such as the lepidopterans (moths and butterflies), each of the three stages is distinct and has its own unique set of activities. These behavioural changes require an extensive reorganization of the nervous system. Adult behaviour such as walking, flight, mating and egg-laying is markedly different from that of the previous stages and requires new, or more sophisticated, sensory systems, and motor control over newly developed appendages.

The options available for achieving the necessary neural reorganization are identical to those which are available to other tissues. The tissues of an adult holometabolous insect are derived from two sources. First, from imaginal discs,

Key words: Metamorphosis, insect, neurones.

which are nests of embryonic cells that remain undifferentiated in the newly hatched larva, and then differentiate into specific body parts such as eyes, antennae, legs and wings, during metamorphosis. Secondly, from polymorphic cells that persist throughout life, serving the same or slightly altered roles at different stages. In these ways, larval neurones can either be discarded to make way for a new set of adult elements, or remodelled to form new connections that are appropriate for pupal and adult behaviour. Both tactics are employed, with the choice depending upon the species of insect and the region of the nervous system. Clearly, metamorphosis shares many aspects of embryonic development including the necessity for neurones to grow and form specific synapses, but also requires some neurones to exchange one differentiated state for another.

One goal of recent work has been to describe the fate of individual neurones and their interactions with other neurones as metamorphosis proceeds. A second goal, and one of broader significance, has been to understand the means by which metamorphic changes are induced and regulated within the nervous system. Progress is being made on both fronts, and several issues will be explored following a brief overview of the process of insect metamorphosis.

BEHAVIOURAL CHANGES DURING METAMORPHOSIS

Lepidopteran larvae spend much of their time feeding, or crawling to a new food source. As it grows, the larva must periodically form a new cuticular coat underneath the old one. This moulting process culminates in a series of patterned muscular movements termed ecdysis, that allow the animal to escape from the old cuticle. After the last larval stage the animal must either crawl to a burrowing site or construct some sort of pupation cell. Most larval behaviour is no longer required after entry into the pupal stage, although this period is not necessarily quiescent (Bate, 1973b). Even though adult development occurs within the pupal case, there is behaviour unique to this transitory stage, and this places obvious constraints upon the developing adult nervous system.

HORMONAL CONTROL OF METAMORPHOSIS

Insect metamorphosis is controlled by two hormones, the steroid 20-hydroxyecdysone (20-HE), and the sesquiterpenoid juvenile hormone (JH). The most detailed investigations of their actions have been carried out on the epidermal cells, which are responsible for synthesizing and secreting the proteins comprising the cuticular exoskeleton (see Riddiford, 1980 for review). Although each group of insects is highly specialized, there are basic similarities which can best be illustrated by a specific example. In the moth *Manduca sexta* there are five larval stages (instars) during which the feeding caterpillar grows to a length of over 13 cm. Prior to each larval instar the epidermal cells secrete a new larval cuticle, but at the end of the fifth a more rigid pupal cuticle is secreted, and the adult forms inside within approximately 18 days.

Epidermal cells are induced to synthesize and secrete new cuticular proteins by the direct action of 20-HE. The type of proteins secreted varies for larval, pupal and adult

particles and is regulated by JH (Riddiford, 1981). In the presence of JH larval cuticle is secreted; in its absence pupal, and later adult cuticle forms. Thus, as demonstrated *in vitro*, epidermal cells are polymorphic, with JH and 20-HE acting on each cell to influence its pattern of mRNA and protein synthesis (Riddiford, 1981, 1982). During the normal postembryonic development of *Manduca*, JH is released from the corpora allata, and titres are high until midway through the last larval instar. 20-HE is released from the prothoracic glands under the influence of brain neurosecretory cells, which secrete prothoracicotropic hormone (PTTH) prior to each moult. At the end of the fifth larval instar there are two peaks of 20-HE in the blood; the first is important for epidermal cell commitment to a pupal pattern of mRNA synthesis, while the second induces the secretion of new cuticular proteins (Bollenbacher, Smith, Goodman & Gilbert, 1981; Riddiford, 1980). Following pupation 20-HE titres again rise, and in the absence of JH stimulate adult development.

METAMORPHOSIS OF THE VISUAL AND OLFACTORY SYSTEMS

The adult eyes and antennae are derived from imaginal discs and send thousands of afferent axons into the brain. Within the optic and antennal lobes new adult interneurons are generated from retained neuroblasts, and either differentiate alongside larval interneurons or replace them (Edwards, 1969). The incorporation of tritiated thymidine into the DNA of dividing cells has been used to establish the birthdays of neurons in the Monarch butterfly, *Danaus* (Nordlander & Edwards, 1969*a,b*, 1970), and in *Drosophila* (White & Kankel, 1978). In both, neuroblasts begin dividing and generating interneurons in the early larval instars, and continue through the early pupal stage.

Within the developing optic lobes newly generated cells surround the rudimentary larval centre. Massive death of larval cells was not observed in either *Danaus* (Nordlander & Edwards, 1969*a*) or *Drosophila* (White & Kankel, 1978), although Nordlander & Edwards (1969*b*) reported the degeneration of some differentiated neurons during the mid-pupal stage, and attributed it to the elimination of redundant adult neurons. Furthermore, in *Drosophila* unlabelled cells were present even in larvae exposed continuously to tritiated thymidine. Both studies concluded, therefore, that some larval neurons were incorporated into the adult optic lobes. The elaboration of the highly structured optic neuropiles has been detailed in several excellent reviews (Edwards, 1969; Meinertzhagen, 1973; Bate, 1978; Kankel *et al.* 1980). The precise spatial and temporal regularity of the developmental process makes the insect visual system ideal for the study of synaptogenesis (Frohlich & Meinertzhagen, 1982). In addition, the temporal relationship between the entry of reticular axons and the formation of the optic neuropiles suggests an important developmental interaction (Meinertzhagen, 1973), which has been confirmed by genetic as well as surgical perturbations (Meyerowitz & Kankel, 1978; Nassel & Geiger, 1983; Fischbach, 1983).

In contrast to the optic lobes, degenerating neurons were observed in the developing antennal lobes at the time of pupation, suggesting the elimination of at least some larval neurons (Nordlander & Edwards, 1970). The characteristic glomerular structure of the neuropil is lost at the end of the larval stage, only to reappear during the

pupal stage when adult interneurons extend their dendrites and antennal afferents enter (Nordlander & Edwards, 1970). The development of the adult antennal lobe has been examined in detail in *Manduca*, (see Hildebrand *et al.* 1982 for review). Antennal sensory neurones are born on days 1 and 2, and their axons enter the developing antennal lobe between days 3 and 10 of the pupal stage (roughly 10–50 % of adult development). Meanwhile, the interneurons begin to elaborate dendrites and continue through day 12. Ultrastructural as well as physiological evidence indicates that most synapses between antennal afferents and the interneurons mature between days 9 and 13 (Tolbert, Matsumoto & Hildebrand, 1983).

The imaginal discs that produce the adult antenna can be extirpated and transplanted before differentiation of the sensory neurones begins. While early removal of the retina leads to extensive death of central target neurones (Maxwell & Hildebrand, 1981), early removal of an antennal disc does not have such drastic consequences. Antennal lobe interneurons survive, and although the glomeruli are reduced to 'protoglomeruli', the neuropil is still segregated into synaptic and non-synaptic areas (Tolbert *et al.* 1983). An inductive relationship between the antennal afferents and the central interneurons is clearly demonstrated, however, when antennal discs are transplanted between males and females (Schneiderman, Matsumoto & Hildebrand, 1982). Normally the male is distinguished by its large macroglomerular complex (MGC), which receives the axons of pheromone receptors on the antenna. Genetic females which receive a male disc develop an MGC and interneurons which respond to the pheromone, while males receiving a female disc lack the MGC as adults.

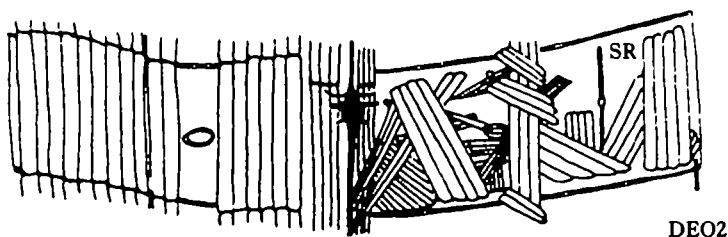
In summary, the sensory processing areas are enlarged significantly during metamorphosis by the differentiation of postembryonically-generated interneurons. The role of embryonically-generated neurones in the adult brain remains unclear, although the absence of massive cell death in many areas suggests that some are retained. In addition, since neuroblasts begin generating neurones early in larval life, the possibility remains that some of these new neurones participate in larval behaviour. Interneurons clearly do play both larval and adult roles in other parts of the brain. Kenyon cells in the *Drosophila* corpora pendunculata lose processes at the end of the larval stage, and extend new ones during metamorphosis (Technau & Heisenberg, 1982).

ABDOMINAL NERVOUS SYSTEM

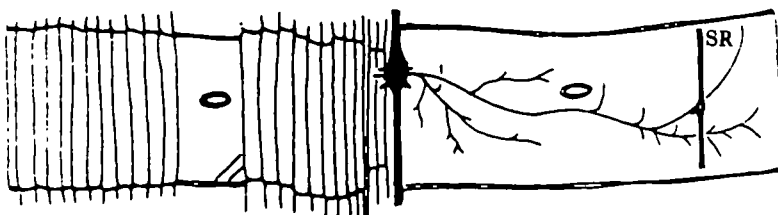
While the olfactory and visual areas of the insect brain provide dramatic examples of metamorphic change, the large number of neurones involved has made it difficult to analyse the process at the level of individual cells. Substantial progress in this regard has been achieved in recent analyses of the abdominal motor system of *Manduca*. Although the behaviour of the abdomen changes during metamorphosis, the number of neurones involved is sufficiently small that individuals may be identified and followed through the animal's life.

Movements of the abdomen are controlled by a network of body-wall muscles which is different in larva and adult (Fig. 1). Larval movements are dominated by the large intersegmental muscles (ISM). These persist through the pupal stage where they are important for a defensive reflex (Bate, 1973b), and participate in adult emergence only

Larva



Pupa



Adult

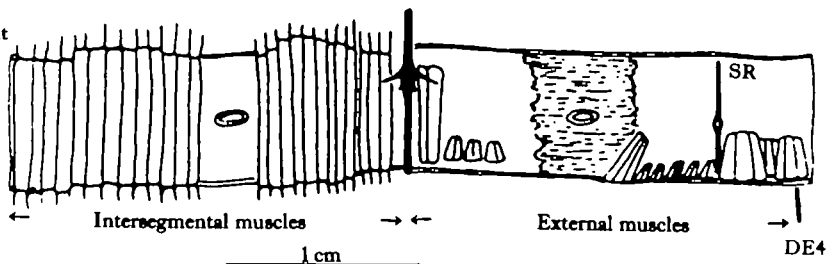


Fig. 1. Abdominal musculature in the larva, pupa and adult. In each case one abdominal segment is shown cut along the dorsal midline and pinned open, with the ventral nerve cord in the centre. The internal (intersegmental) muscles are shown on the left. Note that they persist unmodified into the adult stage (these muscles die shortly after adult emergence). On the right, the intersegmental muscles have been dissected away to reveal the external muscles. Essentially all of these die at the end of the larval stage and are replaced by newly-generated muscles in the adult. The motor neurone MN-1 innervates a dorsal external oblique muscle (DEO2) in the larva, and a dorsal external muscle (DE4) in the adult. The right stretch receptor (SR) is shown at each stage (modified from Truman & Levine, 1983).

to die soon thereafter (Truman & Schwartz, 1980). A second group of smaller external muscles lie immediately adjacent to the body wall, and in the larval stage control subtle movements of each abdominal segment. All of these muscles die at the end of the larval stage and are replaced during metamorphosis by newly-generated adult muscles. Due to the loss of the larger ISM, the external muscles of the adult have a greater behavioural role than that of their larval counterparts (Truman & Levine, 1983).

Unlike the interneurons that comprise the sensory processing areas of the insect brain, all abdominal motor neurones of the adult are functional larval motor neurones (Taylor & Truman, 1974; R. B. Levine & J. W. Truman, in preparation). Cobalt backfills of the major peripheral nerves reveal motor neurone somata which, because of their characteristic locations in the ganglia, can be identified from stage to stage (Taylor & Truman, 1974; Truman & Reiss, 1976; Levine & Truman, 1982). In

addition, the motor neurones of the 'dorsal' segmental nerve have been individually identified on the basis of intracellular recording and staining (Levine & Truman, 1982, and in preparation).

The intersegmental muscle groups of the larva are each innervated by one excitatory motor neurone. During the pupal stage the muscles retain their innervation from these motor neurones, but following adult emergence both neurone and target die (Truman & Schwartz, 1980; Truman, 1983). The dendritic structure of the ISM motor neurones changes little during metamorphosis. There is an increase in the overall extent of fine processes, but the dendrites do not enter new areas of the neuropil (Fig. 2). Similarly, there are no changes in the extent of their peripheral field of innervation.

The remaining abdominal motor neurones lose their targets at the close of the larval stage. While some of these motor neurones die, the majority survive and acquire a new target during adult development. These 'respecified' motor neurones undergo both peripheral and central reorganization of their processes. In the periphery the motor neurone terminals retract from the dying larval muscle and expand over the developing adult target (Stocker & Nuesch, 1975). Meanwhile, some of the distal dendrites are lost, before the entire dendritic field, including adult-specific areas, begins to expand. The new adult growth is unique for each respecified motor neurone. In one, dendrites which are restricted to the posterior region of the larval neuropile, expand into anterior regions during adult development (Fig. 3). Other larval motor neurones with dendritic fields restricted to the neuropil ipsilateral to the target muscle, acquire bilateral dendritic fields (Figs 3, 4). Most dendritic growth is complete by day 14 of adult development (Levine & Truman, 1982), although there is a continued slow expansion even after adult emergence (R. B. Levine & J. W. Truman, in preparation).

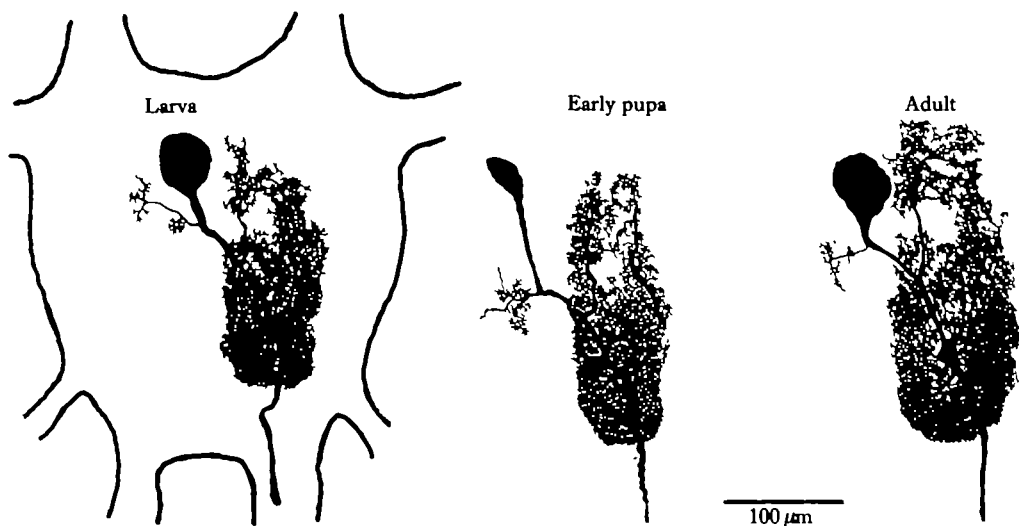


Fig. 2. Morphology of an intersegmental muscle motor neurone in the larva, early pupa and adult stages. *Camera lucida* drawings of neurones injected with Co^{2+} . Neurones were stained by passing positive current through an intracellular micropipette filled with 10 % cobalt nitrate, and the stain was enhanced in whole mount with a modification of the Timm's silver intensification procedure. The dendritic structure of the cell is largely conserved, but there is some growth of existing processes.

It has been suggested that such post-emergence growth could contribute to the continued improvement of adult behaviour (Kammer & Kinnamon, 1977).

One motor neurone, MN-1, has been subjected to a particularly detailed study. During the larval stage it innervates the DEO2 muscle, and its dendrites are restricted to the neuropile ipsilateral to the target (Fig. 4). Following the loss of this target at the onset of pupation, new dendrites begin to extend from a main neurite which had previously been devoid of fine processes (Fig. 4). At this point the dendrites either continue to grow as the animal enters the period of adult development, or the entire process enters a temporary hold as the animal begins a period of diapause. Following a surge of 20-HE, diapause is broken and adult development resumes. Dendritic growth continues as MN-1 innervates a newly-generated target, the adult DE4

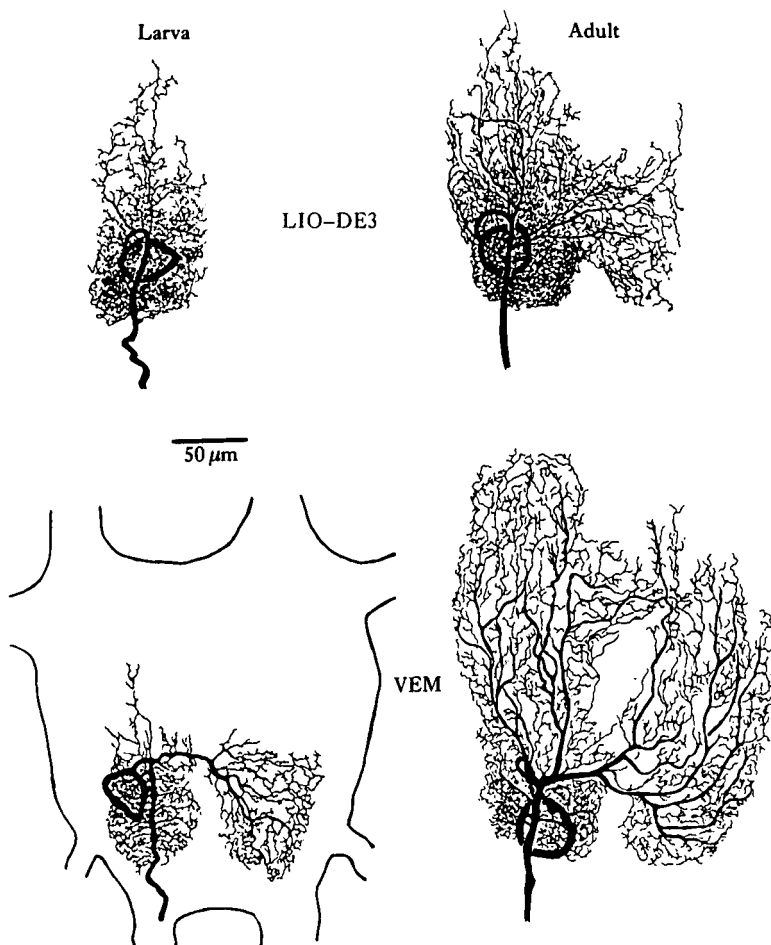


Fig. 3. Morphology of two respecified motor neurones in the larva and adult. Both neurones lose their larval targets at the onset of pupation, and innervate a newly-generated muscle during adult development. In the case of VEM (ventral external medial), both targets are given the same name, and are in a similar location (along the ventral midline). The two targets of LIO-DE3 (lateral internal oblique – dorsal external three) have been assigned different names because their orientation is slightly different, but both lie in the dorsal body wall. Note that both motor neurones undergo extensive growth during adult development.

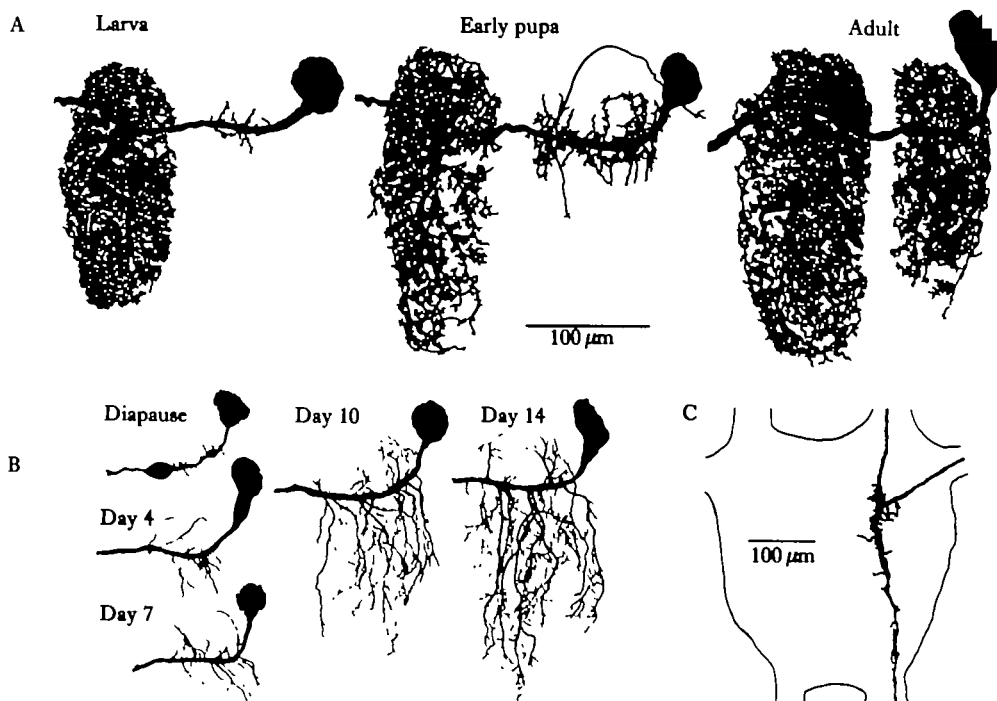


Fig. 4. Structural reorganization of MN-1. (A) *Camera lucida* drawings of MN-1 in the larval, early pupal and adult stages. In the larval and adult stages MN-1 was identified by correlating activity recorded intracellularly from it with that recorded extracellularly or intracellularly from the target muscle. In addition, action potentials in the MN-1 axon may be identified unambiguously in extracellular records from the third branch of the 'dorsal' segmental nerve, which allows MN-1 identification in the pupal stage. Note that during adult development MN-1 acquires a new dendritic field ipsilateral to its soma. (B) Growth of the dendritic field ipsilateral to the MN-1 cell body. Neurones were stained at various times during adult development by back diffusion of cobalt through the cut axon, followed by silver intensification of sectioned material. (C) Morphology of the stretch receptor (SR-3) sensory cell within the abdominal ganglion of an adult. The morphology of the cell is similar throughout the animal's life. Note that its processes do not cross the midline of the ganglion. Although not shown, the processes of the sensory cell extend into the adjacent abdominal ganglia (modified from Levine & Truman, 1982).

muscle, and at adult emergence it possesses a bilateral dendritic field. During the process of respecification MN-1 is not dormant. It continues, although unable to evoke any movement, to respond to synaptic inputs, and the axon maintains its ability to carry action potentials. As discussed below, however, there are marked changes in the identity of some of its synaptic inputs.

The abdominal motor neurones are not unique in their persistence through metamorphosis. Thoracic motor neurones are also respecified to innervate new adult targets. For example, the motor neurones controlling wing movements in adult *Man-duca* are retained from the larval stage, and extend new dendrites during metamorphosis (Casaday & Camhi, 1976).

CHANGES IN A STRETCH REFLEX DURING METAMORPHOSIS

The behavioural changes associated with metamorphosis require a reorganization of neural circuitry, and the reorganization of motor neurone dendrites is one correlate

Of this process. In MN-1 it has been possible to relate the acquisition of a bilateral dendritic field to the formation of a new synaptic input, and to a change in its behavioural role. Located bilaterally in each abdominal segment are a pair of stretch receptor organs. Within each is embedded a sensory neurone that responds to stretch of the segment with a train of impulses (Weevers, 1966). The sensory neurone projects into the CNS where it influences motor neurones in its own and adjacent segments (Fig. 4). In all three stages of the animal's life the same sensory neurone is present and its morphology within the CNS remains constant (Levine & Truman, 1982).

During the larval stage the stretch receptor along with the right and left MN-1s are involved in a simple stretch reflex. Within each segment the stretch receptor excites the MN-1 innervating the ipsilateral DEO2, and inhibits the contralateral MN-1 (Levine & Truman, 1982). Each impulse in the stretch receptor evokes an EPSP in the ipsilateral MN-1 with a short and constant latency. Since processes of the sensory neurone overlap with the dendrites of the ipsilateral MN-1 (seen at the light microscope level), the excitatory connection is probably monosynaptic. Stretch of the receptor evokes a burst of stretch receptor impulses and causes a depolarization and spikes in the ipsilateral MN-1, and a hyperpolarization and a decrease in the spike frequency of the contralateral MN-1. Single impulses in the sensory neurone evoke an IPSP in the motor neurone that occurs with a relatively long and variable latency (Fig. 5). The processes of the two neurones do not overlap, suggesting that the inhibitory pathway is polysynaptic.

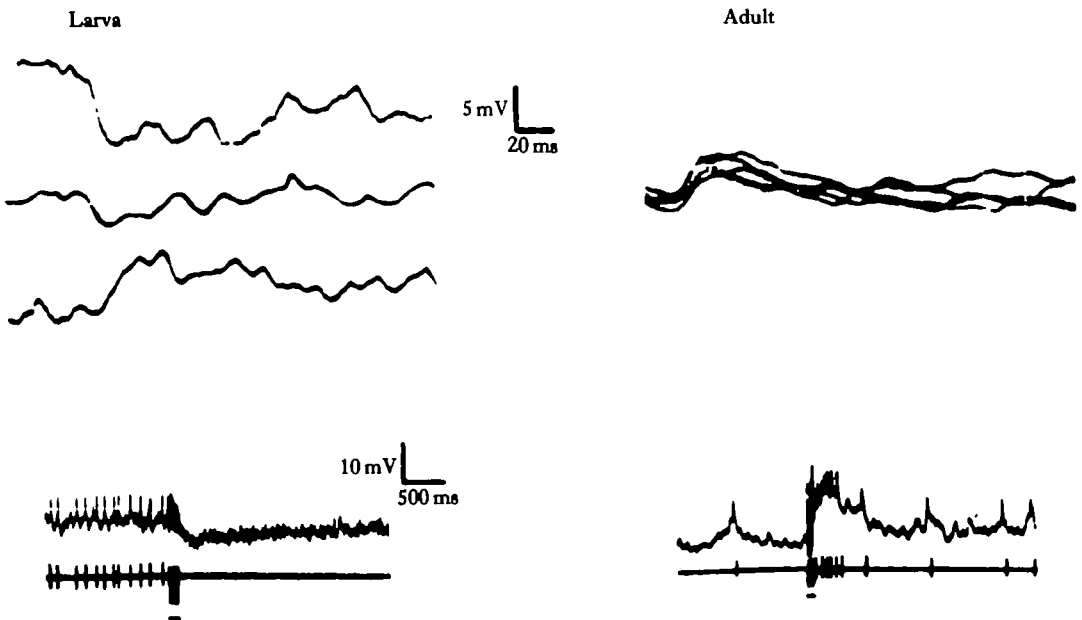


Fig. 5. Influence of the contralateral stretch receptor upon MN-1 in the larval and adult stage. Individual or bursts of electrical stimuli were delivered to the stretch receptor sensory neurone by a suction electrode placed on the sensory organ. In the larva, single stimuli evoke an IPSP which is reversed by hyperpolarization and enhanced by depolarization. Bursts of stimuli (stimulus marker and artifact in extracellular motor neurone record) of a frequency similar to that at which the sensory neurone normally fires (100 Hz), inhibit MN-1 activity. In the adult (30 h after emergence) single stimuli evoke a short latency EPSP and bursts of stimuli excite, rather than inhibit the motor neurone (modified from Levine & Truman, 1982).

The situation is quite different in the adult. Although the ipsilateral motor neurone is still excited through a short-latency pathway, the contralateral MN-1 is no longer inhibited (Fig. 5). Now each impulse in the sensory neurone evokes an EPSP in the contralateral MN-1 that has a short, constant latency. Furthermore, stretch of the receptor causes depolarization and spikes in the motor neurone. This change in the interaction between the two neurones is correlated with the dendritic growth of MN-1, which places dendrites near terminals of the contralateral stretch receptor.

This simple change in circuitry is related to changes in abdominal behaviour. In the larval stage the right and left DEO2 muscles, which are near the dorsal midline, often function as antagonists during lateral flexion of the abdomen, and the asymmetrical stretch receptor input to MN-1 would be important in this context. The adult abdomen, however, is dorso-ventrally flattened and is incapable of lateral flexion. Due to the rigidity of the cuticle, movements are confined to the dorsal-ventral plane in which the two DE4 muscles would function as synergists, and the symmetric stretch receptor input would support synchronous spiking of right and left motor neurones. In fact, during the adult stage the two MN-1s usually spike in synchrony, and, in addition to those from the two stretch receptors, receive many other common synaptic inputs.

In an effort to follow the development of the new excitatory pathway between MN-1 and the contralateral stretch receptor, the connection was tested during the latter part of adult development (Levine & Truman, 1982). Before adult emergence on the final day of the pupal stage, trains of stretch receptor impulses still inhibited the contralateral MN-1. Single stimuli delivered to the sensory neurones, however, evoked a biphasic response in the motor neurone, with a short latency EPSP preceding an IPSP (Fig. 6). The EPSP sometimes gave rise to an action potential, but during trains of stimuli the inhibitory component dominated. This situation persisted until the time of adult emergence, but by 30 min after emergence the inhibitory component had vanished, and trains of sensory neurone impulses excited the motor neurone.

The results suggest that the adult excitatory connection develops during the pupal stage, but due to the persistence of the inhibitory pathway is not allowed to influence behaviour (Fig. 6). The anatomical relationships between the two neurones suggest that the new excitatory synapse is formed upon the new dendritic field of MN-1. At adult emergence the inhibitory pathway is somehow deactivated, and the normal adult circuit becomes functional. Several interneurons die following adult emergence (Truman, 1983), and an inhibitory interneurone involved in this pathway may be among them. Since the deactivation is so rapid, however, cell death is unlikely to be the sole mechanism. As discussed below, a peptide hormone may also be involved.

THE DEVELOPMENT OF ADULT BEHAVIOUR

More complicated adult behaviour develops gradually during the pupal stage. By simply removing the pupal cuticle from developing moths it is possible to observe their behavioural capabilities at different stages (Blest, 1960; Truman, 1976; Kammer & Kinnamon, 1977). Coordinated emergence (eclosion) movements, for example, appear gradually in the silkmooths after about 75 % of adult development, but are repressed during the final 5 % (Truman, 1976). Similarly, the eclosion

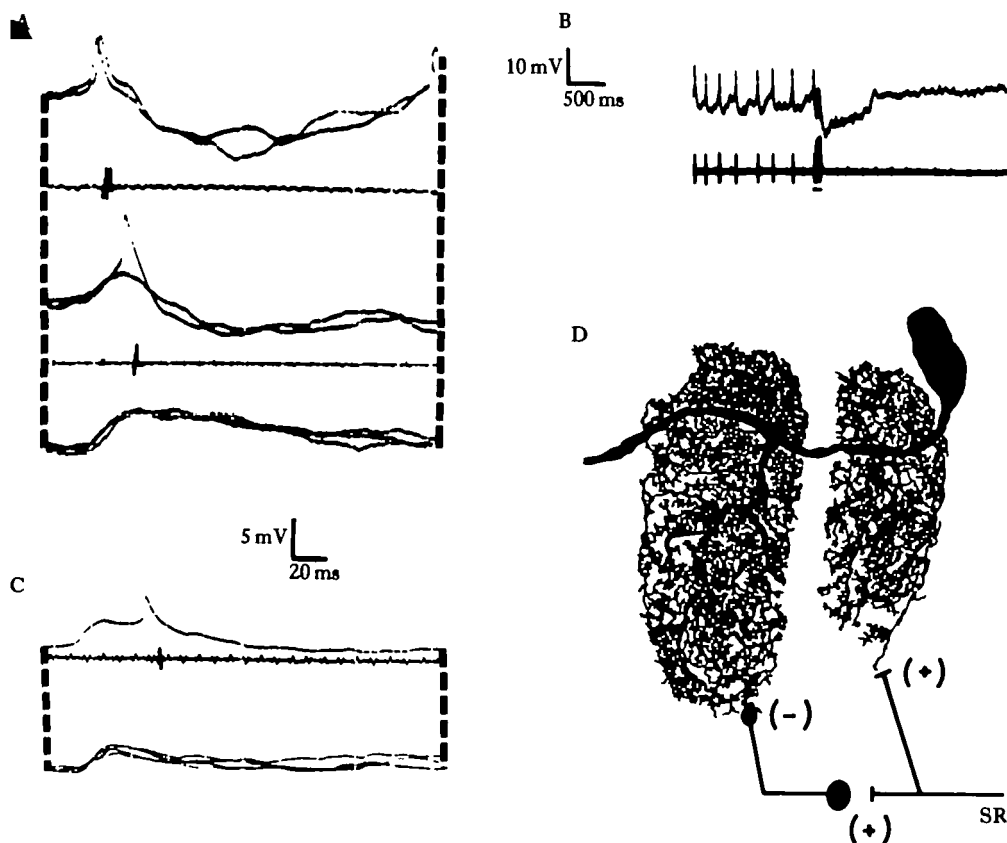


Fig. 6. Influence of the contralateral stretch receptor upon MN-1 immediately before and after adult emergence. (A) About 5 h prior to emergence single stimuli to the sensory neurone evoke a biphasic response in the contralateral MN-1. The later, inhibitory component is enhanced by depolarization of the motor neurone (top), and reversed by hyperpolarization (bottom). The early component may lead to an action potential (also visible in the extracellular records). (B) Same preparation as (A). Bursts of stimuli still inhibit MN-1 activity as in the larva. (C) Thirty minutes after emergence, single stimuli to the stretch receptor evoke only the short-latency depolarizing response in the contralateral MN-1 (bottom). Depolarization of the motor neurone does not reveal an inhibitory component (top). Note that action potentials were not blocked during the period formerly associated with the IPSP. (D) A model to explain the biphasic response evoked just prior to adult emergence. After emergence the indirect, inhibitory component is deactivated. SR, stretch receptor (modified from Levine & Truman, 1982).

behaviour is also repressed during the final hours of adult development in *Manduca*, but not as strongly as it is in the silkmoths (Kammer & Kinnamon, 1977). Appropriate sensory stimuli, such as evoked by manipulation of a leg, can induce eclosion movements in peeled *Manduca* pupae as early as 36 h prior to the normal time of emergence, but become less effective shortly before eclosion.

The developing flight motor pattern has been subjected to a detailed myographic analysis in several species of moths (Kammer & Rheuben, 1976). Although flight motor neurones display sporadic activity starting at 45 % of adult development, proper phase relationships between elevators and depressors is not observed until about 85 %. Thereafter the frequency of movements gradually increases towards its normal value in flying adults. During this later period the flight muscles are not fully

responsive to motor input (Kammer & Kinnamon, 1979). As the muscle membrane matures to the point where it responds in a manner similar to that of adult muscle, central pattern generation of the flight motor programme is repressed.

DEVELOPMENT OF THE GIN TRAP CIRCUIT

Rather than being quiescent during adult development, *Manduca* pupae display a stage-specific defensive reflex termed the gin trap behaviour. Along with the changes in general body shape that accompany the larval-pupal transition there must be certain changes within the nervous system which allow this behaviour to be displayed. The behaviour is initiated by the tactile stimulation of small sensory hairs that lie within sharp-edged cuticular pits (the gin traps) located at the anterior margins of abdominal segments 5, 6 and 7. Appropriate stimuli evoke a contraction of the ISM in the ipsilateral half of the next anterior segment (Bate, 1973*a,b*). Motor neurones innervating other segments or contralateral muscles are inhibited during the behaviour (Bate, 1973*c*; R. B. Levine, unpublished observations). Contraction of the ISM brings two segments together rapidly, crushing the agent of stimulation.

The pathway between the sensory neurones and the ISM motor neurones is not direct. The sensory neurones terminate within the ganglion one segment anterior to their segment of origin, and one segment posterior to the motor neurone dendrites (Bate, 1973*b*; R. B. Levine, unpublished observations; Fig. 7). At least one interneurone is therefore interposed, and such an interneurone has been identified in extracellular records of the interganglionic connectives (Bate, 1973*c*). Thus three segmental ganglia are required for each reflex circuit.

The motor neurone response to stimulation of the gin trap sensory neurones is frequency-dependent. Low frequency stimuli (50 s^{-1}) evoke only a weak motor response, but at slightly higher frequencies there is an abrupt increase in the response intensity. A large, rapid depolarization and a high frequency (80 s^{-1}) burst of action potentials are evoked in the ISM motor neurones, and abruptly terminated after about 50 ms (Bate, 1973*b*; R. B. Levine, unpublished data; Fig. 7).

The sensory neurones that innervate the gin trap sensilla are already present, and functional in the larval stage (Bate, 1972) as are the ISM motor neurones (Truman & Levine, 1983). Sensory neurone stimulation at this stage, however, evokes a much weaker motor neurone response. Even very high frequency stimulation evokes only low frequency, prolonged firing of the motor neurones. Furthermore, the larval response is not laterally or segmentally specific, depending instead upon the posture of the animal prior to stimulation (Bate, 1972; R. B. Levine, unpublished data). Behaviourally, the larval response consists of a small lateral bend caused by the weak contraction of all the ISM on one side of the body.

During the final days of the larval stage there must be an alteration in the CNS in preparation for the pupal gin trap behaviour. The full extent of the changes within the gin trap circuit has yet to be determined, but at least at the level of the sensory neurones there is a structural correlate. Cobalt backfills of the entire gin trap nerve, or fills of individual afferents reveal that the sensory neurones expand their terminal arborizations during the final days of the larval stage (R. B. Levine, unpublished). The relationship between this morphological change and alterations in the

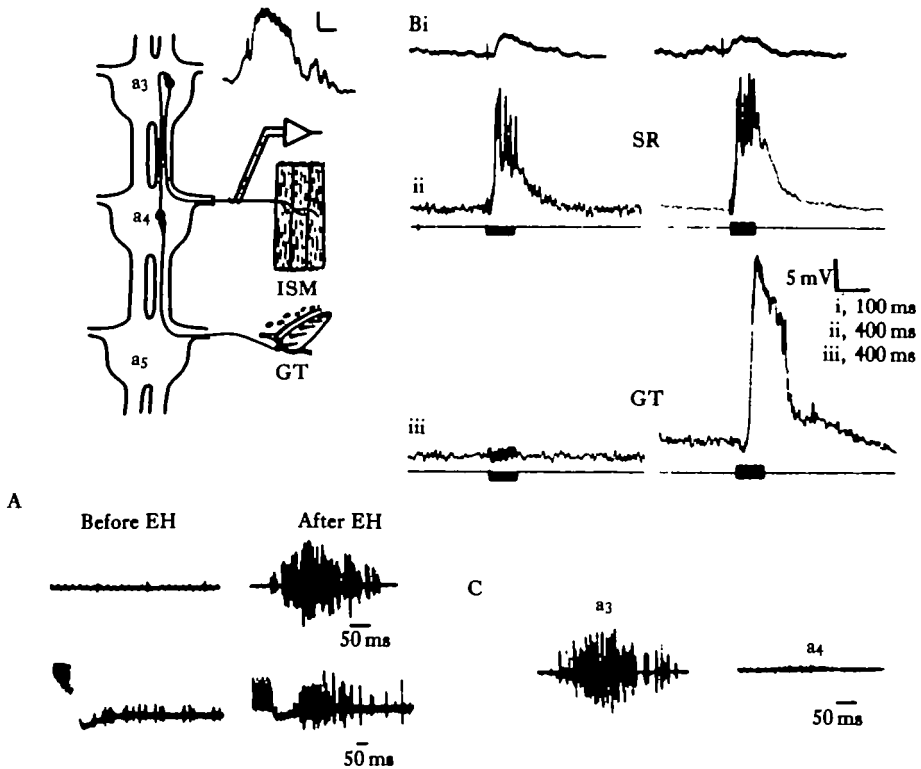


Fig. 7. Activation of the gin trap reflex by eclosion hormone (EH). Insert (upper left) shows a representation of the proposed circuitry underlying the reflex and the location of extracellular electrodes recording motor neurone activity. ISM, intersegmental muscles; GT, gin trap. On the upper right is an intracellular record from the cell body of an ISM motor neurone showing the response to tactile stimulation of the gin trap (calibration: 2.5 mV, 50 ms). (A) Extracellular records of the response of ISM motor neurones to tactile stimulation of the gin trap receptors (top) and electrical stimulation of the gin trap nerve (bottom) before and 1 h after exposure to EH. (B) Same type of preparation as in (A) except that recordings are made intracellularly from the cell body of the motor neurone. Records on the left are before EH addition while those on the right are after EH exposure and subsequent ecdysis. Before EH addition a single electrical stimulus to the ipsilateral stretch receptor evokes an excitatory potential in an ISM motor neurone (Bi), and a train of stimuli (stimulus marker) evokes a burst of action potentials (Bii). A train of stimuli to the gin trap nerve evokes no response (Biii). After ecdysis the motor neurone response to stretch receptor stimulation is unchanged (Bi, Bii), while stimuli to the gin trap nerve evoke a large depolarization and a high frequency burst of action potentials (Biii). (C) Selective exposure of individual ganglia to EH. Recordings from the lateral nerve of a_4 as in (A). Left, exposure of a_3 to EH activates the entire reflex pathway as shown by the motor neurone response to tactile stimulation of the receptors 1 h after EH exposure. Right, exposure of a_4 in another preparation to EH does not activate the pathway (modified from Levine & Truman, 1984).

behavioural influence of the sensory neurones has not yet been clearly defined.

Although the external gin trap structures are fully formed, and the sensory afferents have attained the pupal projection pattern several hours prior to the larval-pupal ecdysis, stimulation of the sensory neurones does not evoke the pupal response at this time. If the larval cuticle covering the newly-formed gin trap is peeled away 3–4 h prior to pupal ecdysis, sensory neurone responses can be evoked by tactile stimulation, but there is no behavioural response. Immediately after the brief ecdysis behaviour,

when the animal sheds the larval skin and officially becomes a pupa, the reflex is fully active (Bate, 1972; Levine & Truman, 1984).

BEHAVIOURAL ACTIVATION AT ECDYSIS

As described previously, each moult is culminated by a series of muscular contractions that enable the animal to shed its old cuticle. The ecdysis behaviour is not identical at each stage of life, but always achieves the same goal. Emergence (eclosion) of the adult moth is achieved through a stereotyped series of abdominal and thoracic movements that are induced following the release of the peptide eclosion hormone (EH) into the haemolymph (Truman, 1980). The peptide acts directly upon the nervous system to activate a centrally-programmed series of patterned motor neurone bursts (Truman, 1978). It is now clear that EH is also responsible for inducing the larval-larval (Copenhaver & Truman, 1982) and larval-pupal (Truman, Taghert & Reynolds, 1980) ecdysis behaviour as well.

In addition to the rather obvious morphological alterations associated with the larval-pupal and pupal-adult ecdyses, these events represent times of dramatic behavioural change. Although new adult circuits develop gradually during the pupal stage, the behavioural and myographic studies discussed earlier revealed that the new circuits were not actively expressed on the last day of adult development. In the silkmoth, *Antheraea pernyi*, fully developed adults freed prematurely from the pupal cuticle displayed no adult behaviour until, at the normal time of eclosion, the animals began a pantomime emergence behaviour and other adult behaviour patterns were suddenly activated (Truman, 1976). This activation could be induced prematurely following the injection of EH (Truman, 1976).

Manduca flight, which is normally repressed just prior to eclosion, can be activated by octopamine (Kinnamon, Klaassen & Kammer, 1980). Octopamine and its agonists influence not only the central generation of the flight pattern, but also the peripheral response of the flight muscles to motor neurone activity (Klaassen & Kammer, 1980; Klaassen, 1982). Octopamine does not mimic all of the behavioural effects of EH, such as the induction of pupal and adult ecdysis, but at least for flight it appears to play an important role at the time of adult emergence.

The new excitatory connection between MN-1 and the contralateral abdominal stretch receptor is, as described previously, not expressed prior to adult emergence because an inhibitory pathway is co-activated. This example may not be completely analogous to the behavioural repression described above, since the inhibition is retained from an earlier stage rather than being imposed near the end of development. Nevertheless, the stretch receptor system could serve as a useful model for the release of inhibition at the time of adult emergence. The role of EH has not been established, but the rapid deactivation of the inhibitory path is coincident with the appearance of EH in the haemolymph.

ACTIVATION OF THE GIN TRAP BEHAVIOUR

The abrupt appearance of the gin trap behaviour at the time of pupal ecdysis, and the recent demonstration that EH is responsible for inducing this ecdysis, suggested

That the peptide might play a role in activating the behaviour. Animals staged 3–4 h prior to ecdysis were injected with physiological doses of EH. Such animals not only initiated premature ecdysis, but also displayed the gin trap behaviour in response to tactile stimulation of the sensilla (Levine & Truman, 1984).

In semi-intact, pre-pupal preparations motor neurone activity can be monitored, and the hormonal content of the bathing medium can be controlled. Prior to addition of EH to the bath, electrical stimulation of the gin trap sensory axons evoked either a weak, or no response in the ISM motor neurones (Fig. 7). Approximately 1 h after addition of EH to the bath, a series of motor neurone bursts began that were patterned appropriately for the ecdysis behaviour. Upon their conclusion, stimulation of the gin trap sensory neurones evoked the normal pupal motor neurone response (Fig. 7). Preparations maintained in the absence of EH remained healthy, but never became capable of displaying this response (Levine & Truman, 1984).

Within the neural circuit responsible for the gin trap behaviour there are several possible sites at which EH could act. The responses of sensory neurones to tactile stimuli are not affected by the peptide, nor are responses of the motor neurone to intracellular injection of current. Furthermore, other synaptic inputs to the ISM motor neurones, such as those from the stretch receptor, are not depressed prior to ecdysis and are not enhanced by EH (Fig. 7). This last observation argues against the possibility that the entire CNS is generally inhibited just prior to ecdysis.

By taking advantage of the distributed nature of the gin trap circuit, possible sites of hormone action can be restricted. In semi-intact, pre-pupal preparations, EH presented only to the ganglion containing the sensory neurone terminals is without effect (Fig. 7). In contrast, EH presented to the ganglion containing the motor neurone dendrites activates the entire reflex, suggesting that the peptide influences (directly or indirectly) the interneurone–motor neurone connection. In neither case is the ecdysis motor programme generated, suggesting that there are multiple sites of EH action within the CNS.

CONCLUSIONS

Although there is extensive neurogenesis during insect metamorphosis, the reorganization of the nervous system is not explained entirely by this process. Motor neurones, interneurones and even sensory neurones can be recycled to serve in more than one stage of life. These respecified neurones display a remarkable degree of plasticity of both structure and function. Not only are neuronal shapes altered in a predictable way, but the factors which specify neurone–target interactions during development must be altered to allow the formation of new connections. Similarly, newly-generated neurones must form synapses with new and existing targets. This may require modulation, at least to some degree, of cell-surface markers. In this light, the recent demonstration that the expression of certain neuropile-associated antigens is modulated during metamorphosis, is quite intriguing (White, Periera & Cannon, 1983).

Both delayed neurogenesis and neurone respecification must somehow be triggered at the appropriate time. Progress in the effort to identify the relevant signals is beginning. The crucial roles played by 20-HE and JH in directing the metamorphosis

of non-neuronal tissues suggests that these hormones are relevant in the nervous system as well. Variations in the haemolymph titres of 20-HE and JH are temporally related to several aspects of neurone metamorphosis (Hildebrand *et al.* 1982). The analysis of programmed muscle and neurone death in the *Manduca* abdomen has been particularly revealing in this regard. By experimentally manipulating the 20-HE titres it has been clearly established that cell death is under the direct control of this steroid hormone (Schwartz & Truman, 1982; Truman & Schwartz, 1982, 1984; Weeks & Truman, 1983). Rapid functional validation of new circuits is common to both larval-pupal and pupal-adult ecdysis. Circulating factors responsible for this activation, such as EH, have been identified, and their mode of action is under investigation (Truman & Levine, 1983).

A number of straightforward questions pertaining to the reorganization of neural circuits remain unanswered. Although individual neurones and some of their interconnections have been followed through metamorphosis, and alterations consistent with behavioural changes have been described, the ontogeny of important adult behaviour is not fully understood at the cellular level. Especially important in this regard will be the further characterization of both inter- and intra-ganglionic interneurons. These interneurons certainly play a crucial role in the generation of behaviour such as walking and flight, and a thorough understanding of their developmental history and function is central to the attempt to understand behavioural transitions during metamorphosis.

Much of the original work reviewed was performed while the author was a postdoctoral fellow in the laboratory of Professor J. W. Truman. The author was supported by an NIH postdoctoral fellowship, and grants from NIH and the McKnight Foundation to JWT. I thank Professor Truman and Mr B. Waldrop for a critical reading of the manuscript, and Dr M. Bate for access to his Ph.D. thesis.

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