The Journal of Experimental Biology 201, 1785–1798 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1397

THE ROLE OF THE FRONTAL GANGLION IN THE FEEDING AND ECLOSION BEHAVIOR OF THE MOTH *MANDUCA SEXTA*

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Accepted 10 March; published on WWW 12 May 1998

Summary

We have examined the musculature and motor patterns of the foregut and the role of the frontal ganglion in the adult moth *Manduca sexta*. During adult development, the structure of the foregut changes from a simple straight tube to a pump consisting of a flexible-roofed chamber or cibarium, with dilator muscles that raise the roof to draw in fluids and a compressor to push it down and force the fluid down the thin-walled esophagus. The frontal ganglion drives the activity of this cibarial pump during feeding, which is triggered by the application of sucrose solution or water to the proboscis. The feeding motor pattern consists of coupled bursts of the pump dilators and shorter-duration, high-frequency bursts of spikes from the pump compressor.

The pump is also activated at the adult molt. At this time, it is used both before the moth emerges from the pupal case for swallowing molting fluid and again after emergence for swallowing air. These behaviors are important for eclosion and are necessary for the expansion of the wings after eclosion. Their motor patterns are similar to the feeding program. Up to 24 h before adult ecdysis, this motor pattern can be triggered by the peptide eclosion hormone. The other eclosion-related peptide, *Manduca sexta* eclosion-triggering hormone, does not appear to trigger activity of the cibarial pump.

Key words: ecdysis, stomatogastric, digestion, insect, tobacco hawkmoth, *Manduca sexta*, frontal ganglion, feeding.

Introduction

As it develops, an animal may show different behaviors at different stages of its life. Some of the most dramatic examples of behavioral ontogeny can be found among the holometabolous insects. These animals undergo metamorphosis, during which the crawling, feeding larval stage is replaced by a relatively quiescent pupal stage and ultimately by the flying, reproductive adult. The change from a larva to an adult involves enormous changes in body morphology as well as the development of a new set of behaviors specific to the adult. For example, while feeding behavior is exhibited throughout the larval and adult stages of the moth *Manduca sexta*, feeding style is dramatically altered as the leaf-eating larva develops into the nectar-feeding adult. This change in feeding style is accompanied by a massive restructuring of the feeding apparatus and foregut from chewing mandibles with a simple, straight foregut to a proboscis with a cibarial pump.

The changes in feeding style and foregut structure that take place during metamorphosis raise the question of the mechanisms that are used by the nervous system to accommodate them. Movements of the foregut are driven by neurons of the frontal ganglion (Miles and Booker, 1994). This small ganglion lies on the dorsal surface of the gut anterior to the brain, to which it is connected by a pair of frontal connectives. In an earlier study (Miles and Booker, 1994), we described the foregut musculature, the motor patterns and the role of the frontal ganglion in driving those patterns in the larval stage. As the next step in the analysis of how these characters change during development, we have examined the frontal ganglion and foregut of the adult. Very little previous work has addressed the role of the frontal ganglion in adult Lepidoptera. Removal of the frontal ganglion from adult *Heliothis zea* was shown to produce deficits in crop-emptying (Bushman and Nelson, 1990). The structure of the cibarial pump and its associated musculature has been described for *Pieris brassicae* and *P. rapae* (Eastham and Eassa, 1955; Daniel *et al.* 1989) and a generalized sphinx moth (Snodgrass, 1935), and a biomechanical analysis of feeding in adult *Pieris rapae* has been carried out (Daniel *et al.* 1989; Kingsolver and Daniel, 1995). However, there have been no neurophysiological studies of the motor patterns associated with feeding or the neurons that produce them in adult Lepidoptera. The present paper examines the musculature, motor patterns and role of the frontal ganglion in feeding in the adult moth *Manduca sexta*. We demonstrate that the frontal ganglion is essential for the activity of the cibarial pump during feeding. This activity is triggered by the application of sucrose solution or water to the proboscis. In addition, we have found that the frontal ganglion plays an

important role during the process of shedding the old cuticle, or ecdysis, from the pupal to the adult stage.

Earlier work by Bell (1986) suggested that, in *Manduca sexta*, the frontal ganglion may play a role in the ecdysis to the adult stage, or eclosion. His study described defects in eclosion and in the expansion of the wings of freshly eclosed adults from which the frontal ganglion had been removed. Bell (1986) suggested that the frontal ganglion was involved in swallowing air at eclosion, which could be important for the expansion of the wings in the newly emerged adult. We have re-examined the role of the frontal ganglion and pump musculature around the time of adult eclosion. At any of its molts, it is of critical importance to an insect that ecdysis behaviors are only carried out at the appropriate time, as the events that lead to a successful molt must take place in the correct sequence or the insect will die. Thus, the timing of many ecdysis-related behaviors has been found to be precisely regulated by hormones (Truman, 1971; Copenhaver and Truman, 1982; Miles and Weeks, 1991), particularly the peptides eclosion hormone (EH; Truman *et al.* 1981*a*) and *Manduca sexta* eclosion-triggering hormone (ETH; Zitnan *et al.* 1996). We therefore also examined the effects of these hormones on the activity of the cibarial pump. We have found that the pump is activated at the adult molt and that its activity is essential for the proper expansion of the wings. In addition, we demonstrate that the activation of the pump at eclosion is triggered by EH.

Materials and methods

Animals

Manduca sexta L. were obtained from our colony, where they were raised under a 16 h:8 h L:D schedule at 27 °C. Larvae were fed an artificial diet (Bell and Joachim, 1976) until the wandering stage, when they were placed into individual wooden chambers to pupate. The pupae were removed from the chambers after 1 week and placed in an open tray so that they could be easily examined for staging their development. Stages were determined using the morphological markers of Schwartz and Truman (1983), adapted for the rearing conditions of our colony. Animals that were to be used after adult eclosion were transferred to individual cages when they were within 2 days of eclosion.

Anatomy

For anatomical studies of the cibarial pump musculature and frontal ganglion nerve projections, heads were removed from adults that ranged in age from 1 to 4 days after eclosion. They were dissected either by making a frontal opening over the pump surface or by cutting the whole head sagittally with a double-edged razor blade. Preparations were examined under saline (Trimmer and Weeks, 1989) or Methylene Blue (Weeks and Truman, 1985). To determine whether fluid or air was swallowed around the time of eclosion, the gut was examined in staged animals. Five individuals from each of the following stages were used: 24–30 h before eclosion, 6–8 h before eclosion, 1–2 h before eclosion and 30 min after eclosion, when the wings were fully expanded but not yet folded. The animals were cold-anesthetized, the pupal cuticle was removed if necessary, and the abdomen was opened dorsally. An additional seven animals that were approximately 30 h from eclosion received injections of 50–100 µl of physiological saline saturated with carmine into the exuvial spaces of their heads. Their guts were examined 6, 24 or 30 h later for the presence of carmine particles.

To examine the morphologies of individual frontal ganglion neurons, the neurons were filled with cobalt chloride or Neurobiotin (Vector Laboratories) by backfilling from their nerve projections. Cobalt backfills were subsequently processed as described in Miles and Booker (1994), and Neurobiotin preparations were treated with a monoclonal antibody against biotin and conjugated to Cy3 (Jackson Immunoresearch Inc.), according to the methods described by Mesce *et al.* (1993), and viewed with fluorescence microscopy. Neurons were drawn using a *camera lucida* attachment to a microscope.

Neurophysiological techniques

Extracellular recordings

For recording the motor patterns of the pump musculature during feeding, animals were anesthetized with CO_2 gas, their legs and wings were cut off, and their bodies were secured to a wax platform with a pair of pins through the thorax. The scales on the surface of the head were rubbed off, and a pin was used to make small holes in the cuticle directly above the muscles to be recorded. Electrodes composed of 75 µm diameter silver or stainless-steel wire were inserted through the holes and into the muscle. Their positions were confirmed after the recording session by cutting the wires at the head surface and examining their position directly by dissecting the head. To initiate feeding, the proboscis was gently uncoiled and placed in a small (500 µl) dish containing a 20 % sucrose solution colored with food dye (Blue 1). After the recording session, the animal was again anesthetized with CO_2, and the abdomen was opened dorsally to examine the crop for the presence of the sucrose solution.

Isolated heads also ingested the sucrose solution, with a motor program that was indistinguishible from that of the intact animal. This preparation was also used for recording the feeding pattern. Up to four muscles were recorded at a time, using a four-channel extracellular amplifier (A-M Systems). Data were recorded onto a four-channel video recorder (Vetter Instruments) and analyzed after being played back in real time onto a high-speed chart recorder (Astro-Med Inc.). The mean and 95 % confidence intervals about the mean (CI) for the period and spike frequency for 10 consecutive bursts were averaged for each muscle recorded in an animal. To determine the mean ±95 % CI for a given muscle type, data were pooled for 10 consecutive bursts from each of the animals in which that muscle was recorded. Values are presented as the mean ±95 % CI , with *N* being the total number of bursts from all the animals that were used for the calculation. Means are considered significantly different when the 95 % CIs do not

overlap. The numbers of animals from which data were obtained for a given muscle were as follows: anterior dilators, *N*=8 animals; posterior dilators, *N*=7 animals; labral compressor, *N*=3 animals; pump compressor, *N*=8 animals.

To record pump activity immediately after eclosion, animals that had just emerged from the pupal case (*N*=9) were anesthetized briefly with CO_2 and quickly implanted with a single silver wire electrode into the pump, with a ground electrode placed in the thorax. Each wire was held in place with a drop of cyanoacrylate glue at the point where it passed through the cuticle. The animals were allowed to crawl on a sheet of paperboard, tethered by their electrode wires. Some individuals (*N*=5) were implanted with the electrodes as pharate adults (fully developed but still within the pupal cuticle) 2–3 days before eclosion. The activities of their pump muscles were recorded periodically over the course of the 2–3 days before eclosion.

Intracellular recordings

Isolated heads were used for intracellular recordings. The heads were secured to a recording dish using two pins placed through the antennal bases. The dorsal surface of the head was scraped clean of scales, and an opening was made in the cuticle extending from the posterior dilators to the anterior dilators and laterally between the antennal bases. Silver wire extracellular electrodes were placed on up to three different pump muscles. The frontal ganglion was exposed and lifted onto a wax-coated stainless-steel platform, to which it was secured using cactus spines. The ganglion was desheathed, and recordings were made using standard neurophysiological techniques as described in Miles and Booker (1994). Once the intracellular recording was stable, the proboscis was uncoiled and placed in a 20 % sucrose solution to initiate feeding. Motoneurons were identified by the 1:1 correlation between spikes in the recorded neuron and the extracellular recording of spikes in the muscle. After the recording session, the neuron was ionophoretically filled with hexamminecobalt (III) chloride and processed and visualized using techniques described in Miles and Booker (1994). Motoneurons were identified on the basis of their morphology (from intracellular fills and backfills) and the muscle they innervated. Filled neurons were drawn using a *camera lucida* attachment to a compound microscope. Each of the neurons described in this paper was recorded and morphologically identified at least twice.

Surgical manipulations

To assess the effects of eliminating inputs to or from the frontal ganglion on adult behaviors, the nerves from the frontal ganglion were cut or the ganglion was removed entirely. Sham operations consisted of dissection procedures identical to those described below, except that the frontal ganglion and foregut were simply touched with the dissecting tools. Because of the accessibility of the frontal ganglion at the onset of the wandering stage (approximately 3 days before ecdysis to the pupa), surgery was carried out at this time for the following ablations: removal of the frontal ganglion, cutting the recurrent nerve and cutting the frontal connectives. The wandering animal was anesthetized with CO_2 gas, then placed in a chamber which kept a steady supply of CO_2 to the tracheae, but left the head capsule exposed for surgery. All dissecting tools were soaked in 95 % ethanol before use and between animals. The head capsule was cleaned with ethanol, then cut along the lateral margins of the clypeus, and the cuticle of the clypeus was folded down to reveal the frontal ganglion. The ablation or sham operation was carried out, a crystal of phenylthiocarbamide was placed in the wound to prevent oxidation of the hemolymph, and the cuticle was closed and sealed with dermatological glue (New-Skin, MEDTECH Labs, Inc.). The animals were then returned to the CO_2 gas until the glue had dried. Because we found that frontal nerves cut at the wandering stage regenerated in every case, this manipulation was carried out in pharate adults 2–3 days before eclosion. For this surgery, the animal was anesthetized with CO_2 gas and the pupal cuticle above the head was removed. The scales on the dorsal surface of the head were scraped away, and a window was cut in the head cuticle between the antennal bases and anteriorly over the pump compressor. The cuticle was folded back, and the frontal ganglion was located beneath the air sacs of the dorsal head. The ablation or sham operation was carried out, and the wound was treated as described above. After eclosion, operated and intact animals were tested for the quantity of sucrose solution they would consume in a feeding bout. The animals were starved for 24 h, then placed before a small dish containing colored 20 % sucrose solution. The proboscis was uncoiled and held gently in the solution for 20 min. The amount of sucrose solution consumed was determined by weighing the dish before and after the test period. After the data had been collected on the animals' physical conditions and their consumption of sucrose solution, they were killed and dissected to verify the type of ablation and to examine the crop for the presence of sucrose solution.

Hormonal manipulations

Eclosion hormone (EH) was applied directly to the isolated heads of pharate adults 24–30 h before eclosion to determine whether this peptide could trigger activity in the cibarial pump around the time of ecdysis. Animals were dissected as described above for intracellular recordings, and the activity of the cibarial pump was monitored with an extracellular electrode on the pump compressor. The corpus cardiacum–corpus allatum (CC-CA) complex has been shown to be a rich source of EH in the pharate adult (Truman, 1973). Therefore, to eliminate it as a possible source of endogenous EH in these preparations, the CC-CA complex was removed from 15 individuals. No significant differences were found in the results obtained from individuals with or without the CC-CA complex. The EH was either a purified form kindly supplied by Dr David B. Morton (Morton and Giunta, 1992) or an extract obtained from the CC-CA complexes of pharate adults (Weeks and Truman, 1984). For purified EH, 1 µl of 300 nmol l^{-1} EH was diluted in 50 µl of physiological saline and applied directly to the frontal ganglion and foregut. For the

preparations treated with CC-CA extract, the equivalent of the contents of half a CC-CA complex in 50 µl of physiological saline was used, applied in the same way.

Eclosion-triggering hormone (ETH) was obtained from ground Inka cells dissected from pre-pupal animals, according to the methods in Zitnan *et al.* (1996). Extract (50 µl) was injected through the dorsal thoraces of pharate adults 28–30 h before eclosion. Inka cells (10–16) from one pre-pupal animal were used for each injection.

Results

Anatomy of the adult foregut

The adult foregut consists of the large cibarial pump and the esophagus. The cibarial pump is a chamber with a flexible roof. Compressor muscles and dilator muscles serve to lower and raise the roof of the cibarium when the animal feeds. These act in conjunction with muscular valves at its entrance and exit to alternately bring nectar into the chamber and to eject it down the esophagus. We have examined the musculature of the adult *Manduca sexta* foregut (Fig. 1A,B). Many of these muscles are similar to those described for *Pieris brassicae* in an earlier study (Eastham and Eassa, 1955). There are three sets of dorso-ventral pump dilators, all of which originate on the roof of the cibarium. A pair of anterior dilators inserts on the clypeus, a lateral pair inserts on the frons near the antennal sockets, and four paired posterior dilators insert on the epicranium. The pump compressor, which depresses the roof of the cibarium, is a large muscle that covers the cibarial roof. It is composed of two layers of fibers, an outer layer running laterally between the eyes and an inner layer oriented rostro-caudally. At the point where the proboscis opens into the cibarial chamber, a dilator, the labral compressor, attaches to the dorsal surface of the pharynx and inserts on the clypeus. A constrictor muscle, the transverse sphincter, encircles the foregut just caudal to the labral compressor. At the pump's outlet to the esophagus, a constrictor muscle encircles the esophagus.

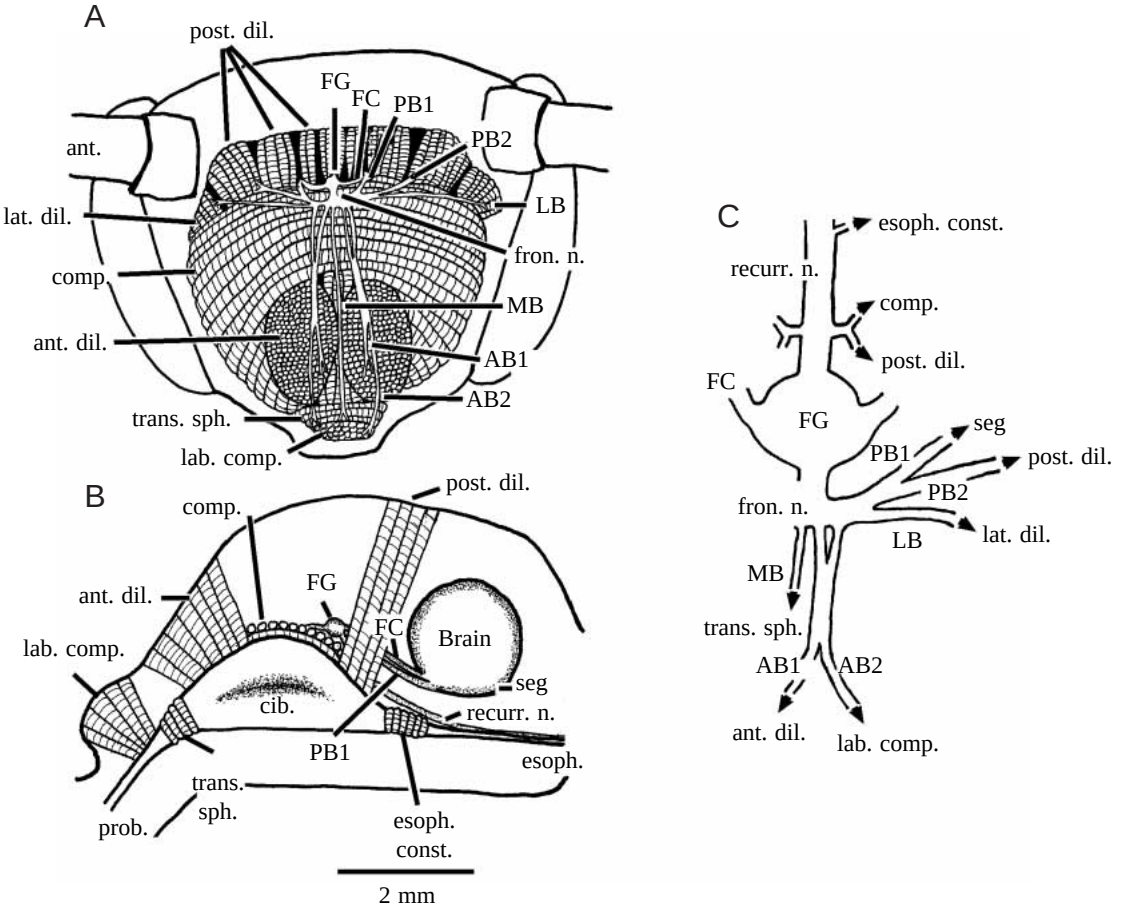

Fig. 1. Musculature and innervation of the cibarial pump in the adult *Manduca sexta*. (A) Frontal view, dorsal is uppermost. Muscle labels appear along the left side of the figure, nerves are labeled along the right side. (B) Sagittal section through the adult head. Dorsal is uppermost, rostral on the left. (C) Diagram (not to scale) of the frontal ganglion, its major nerve branches and the muscles they innervate (arrowheads). ant., antenna; ant. dil., anterior dilator; cib., cibarium; comp., pump compressor; esoph., esophagus; esoph. const., esophageal constrictor; fron. n., frontal nerve; lab. comp., labral compressor; lat. dil., lateral dilator; post. dil., posterior dilator; prob., proboscis; recurr. n., recurrent nerve; seg, subesophageal ganglion; trans. sph., transverse sphincter. AB1, anterior branch 1; AB2, anterior branch 2; FC, frontal connectives; FG, frontal ganglion; LB, lateral branch; MB, medial branch; PB1, posterior branch 1; PB2, posterior branch 2.

Innervation of the foregut

The frontal ganglion is connected to the tritocerebral lobes of the brain by a pair of frontal connectives. Two additional nerves exit the frontal ganglion, an anteriorly directed frontal nerve and a posteriorly directed recurrent nerve. The frontal nerve, after leaving the ganglion, branches into several distinct, bilaterally paired branches. (Fig. 1A,C) The first of these, posterior branch 1, projects posteriorly and ventrally to join the subesophageal ganglion. Posterior branch 2 innervates the two most lateral posterior dilators, while the lateral branch of the frontal nerve innervates the lateral dilators. The anterior branches of the frontal nerve consist of two paired and one unpaired medial branch. The more medial of the paired anterior branches, anterior branch 1, projects over the compressor muscle mass to innervate the anterior dilator muscles. Anterior branch 2 lies just lateral to branch 1, traveling alongside it over the compressor mass to innervate the labral compressor. Anterior branches 1 and 2 are fused for much of the distance between their branchpoints from the frontal nerve and their muscle destinations. The medial branch of the frontal nerve is unpaired and projects to the transverse sphincter muscle.

Four of the pump muscles receive their innervation from the recurrent nerve (Fig. 1B,C). Approximately 75 μm after leaving the frontal ganglion, the recurrent nerve extends a pair of branches that innervate the two most posterior pairs of the posterior dilator muscles and the large pump compressor muscle. More posteriorly, approximately 720 μm from the frontal ganglion, another small branch of the recurrent nerve extends to the esophageal constrictor. The recurrent nerve then runs posteriorly for approximately 1.3 cm to the midgut, where it joins the large network of nerves of the enteric nervous system.

The foregut motor program

Unless the moth is feeding, the muscles of the cibarial pump are silent. A feeding bout can be initiated in an intact animal or an isolated head by placing the proboscis into a solution of sucrose or water. During feeding, fluid is brought into the foregut from the proboscis by expansion of the cibarium as the dilators raise its roof and the entrance to the cibarium is opened. The cibarial entrance is then closed, the esophagus is opened and the pump compressor forces fluid back into the esophagus (Kingsolver and Daniel, 1995; Eastham and Eassa, 1955). Peristaltic movements of the esophagus then move the fluid posteriorly to the crop. Although the recurrent nerve extends along the length of the esophagus, it is not required for peristalsis. Instead, the esophagus appears to be myogenically active since it exhibits regular peristalsis even when it is completely isolated from the animal.

Recordings from the dilator and compressor muscles during feeding revealed a rhythmic alternation of coupled dilator and pump compressor bursts (Fig. 2). The anterior and posterior dilator muscles typically exhibit bursts of spikes with durations of 0.87±0.1 s (*N*=80) and 0.81±0.08 s (*N*=70), respectively, and mean spike frequencies of 29.15±2.14 spikes s^{-1} (*N*=80) and 22.5±1.86 spikes s^{-1} (*N*=70), respectively, as the cibarial roof is raised. This is followed by a shorter-duration (0.086±0.009 s), high-frequency (83.8±5.76 spikes s^{-1}) burst from the pump compressor (*N*=80) as the cibarial roof is pushed rapidly down. The period of the feeding motor program is 1.37±0.12 s (*N*=80). These movements of the pump are easily observed in dissected feeding preparations.

The anterior and posterior dilators were not activated synchronously. In most cases (six out of eight), activity in the the anterior dilator preceded that in the posterior dilator by almost 300 ms. The anterior dilator bursts began 192±33.2 ms (*N*=60) after the end of the pump compressor burst, while the posterior dilator bursts began 480±84.5 ms (*N*=60) after the pump compressor burst. In each of these recordings, the anterior dilator burst ended before the posterior dilator burst. In two individuals, including the one shown in Fig. 2, the posterior dilator burst preceded the anterior dilator by approximately 250 ms. In these individuals, the posterior dilator burst began 153±45.3 ms (*N*=20) after the pump compressor burst stopped, while the anterior dilator burst began 408±113.7 ms (*N*=20) after the pump compressor burst. There was no significant difference in the times when the anterior and posterior bursts stopped.

Recordings from the labral compressor, which opens the junction of the proboscis and cibarium, revealed activity that was generally correlated with activity in the pump dilators, although with a longer duration of 1.24±0.23 s (*N*=30; Fig. 2). Mean onset time for the labral compressor was 56±17.23 ms (*N*=30) after the pump compressor burst ended and activity continued until 36±18.3 ms (*N*=30) before the onset of the next pump compressor burst. Spike frequency in the labral compressor was not significantly different from that of a typical dilator muscle.

A number of recordings of the activities of single frontal ganglion neurons during a feeding bout were made. Fig. 3 shows activity of an anterior dilator motoneuron designated anterior dilator 1 (AD1), while Fig. 4 illustrates activity in a posterior dilator motoneuron, designated posterior dilator 1 (PD1). The AD1 motoneuron had a cell body with a diameter of 25 μm in the dorsal anterior region of the ganglion. Intracellular recordings from this neuron showed a relatively gradual depolarization, leading to a prolonged (approximately 600 ms) depolarized period that terminated with a rapid hyperpolarization. PD1 also has its 24 μm diameter cell body in the dorsal anterior end of the frontal ganglion. Its primary arborization sends projections up both frontal connectives to the brain. Intracellular recordings during feeding reveal rapid de- and hyperpolarizations of this neuron, producing bursts of spikes.

The frontal ganglion is required for the feeding motor pattern, as animals without frontal ganglia or with cut frontal nerves fail to feed. Feedback from the crop or midgut *via* the recurrent nerve does not appear to play a role in the amount of food consumed during a feeding bout, as shown by the amount of 20 % sucrose solution they consumed in 20 min. This varied widely among animals with a cut recurrent nerve, sham-operated animals and unoperated control animals. Animals

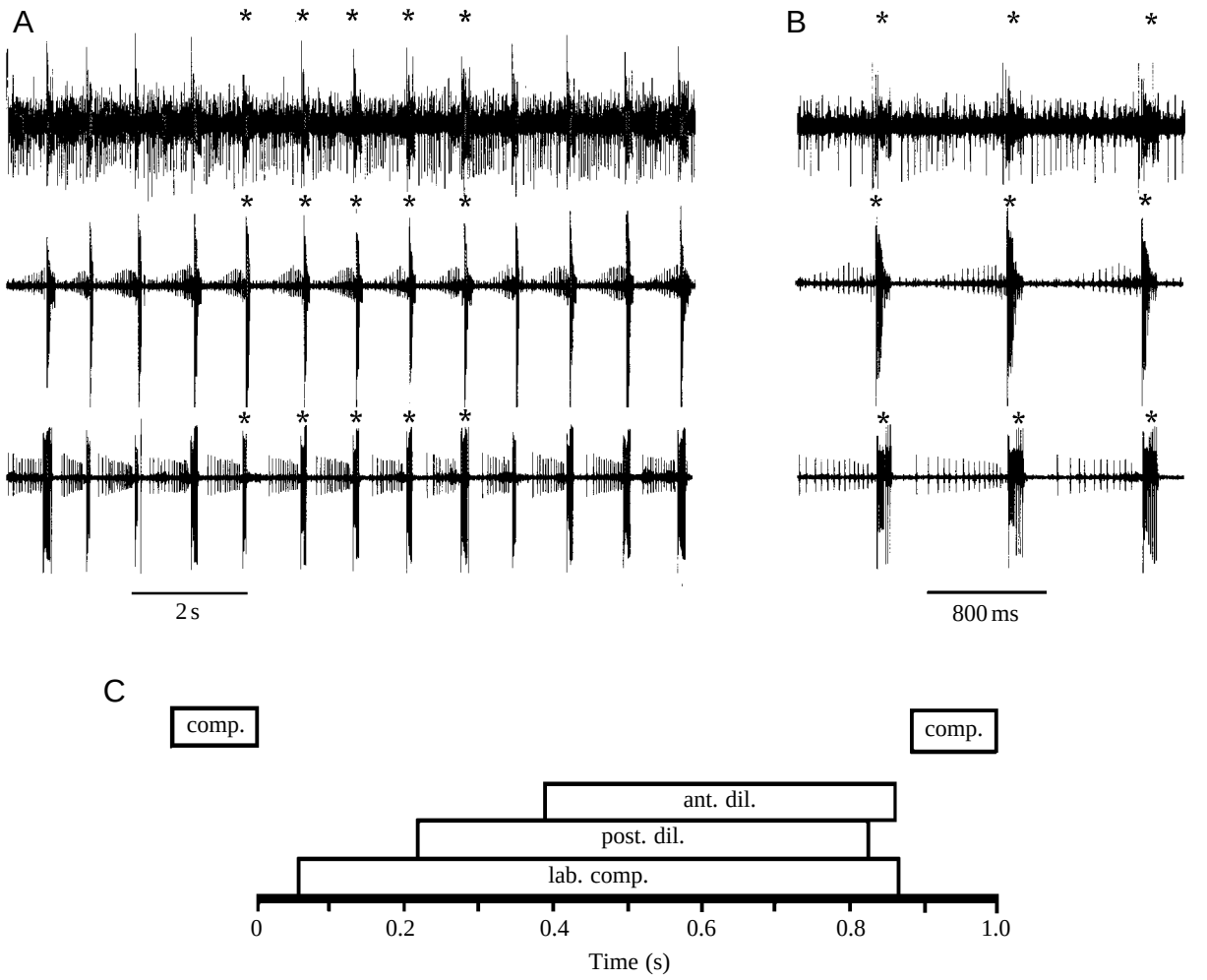

Fig. 2. The feeding motor program. (A) The motor pattern recorded in the labral compressor (top trace), the anterior dilator (middle trace) and the posterior dilator (bottom trace). The large-amplitude bursts in each trace, marked in several cases with an asterisk, are the activity of the pump compressor, which is often picked up by electrodes placed anywhere within the head. (B) As in A, same animal, but at a faster sweep speed. (C) Summary of the firing times for the muscles of this preparation, relative to the pump compressor bursts and averaged over 10 consecutive bursts for each muscle. Abbreviations are as in Fig. 1.

with cut recurrent nerves consumed an average of 286.29±282.4 µl of sucrose solution (*N*=7), while sham-operated animals consumed an average of 454.43±316.4 µl (*N*=7). Unoperated moths consumed 175±119.3 µl (*N*=4).

Importance of the frontal ganglion during adult eclosion

The frontal ganglion is important for expansion of the wings and abdomen at adult eclosion, as demonstrated in the series of surgical manipulations summarized in Table 1. Of 14 sham-operated controls (operation at wandering), one individual developed to adulthood but failed to emerge from the pupal case, while 13 developed, emerged and expanded their wings successfully (Fig. 5A). Dissections of these individuals or of unoperated control animals (*N*=10) shortly after the expanded and hardened wings were folded over the abdomen revealed that the crop was filled with air. In contrast, when the frontal ganglion was removed from 12 indviduals, eight of them developed to adults but failed to emerge successfully from the pupal cuticle. The remaining four animals emerged successfully but, unlike normally eclosing animals, they failed to expand their wings and their crops were empty and abdomens flat (Fig. 5B). If the proboscis of such an animal was later placed in a 20 % sucrose solution, it did not feed. Cutting the recurrent nerve (*N*=15) had no apparent effect on eclosion, wing expansion or feeding behavior in 12 of the animals, with the rest eclosing but failing to expand their wings. Frontal connective cuts, made 3 days before eclosion (*N*=8), resulted in seven animals successfully eclosing, none of which expanded its wings. The remaining individual failed to emerge. Sham-operated controls carried out at the same time (*N*=10) resulted in nine animals with normal eclosion and normal wings, and one individual that failed to emerge.

Cutting the frontal connectives produced animals with distended or burst abdomens 24 h after eclosion (Fig. 5C). Of 15 animals with cut frontal connectives, 11 emerged from the pupal case and expanded their wings normally. However, 24 h

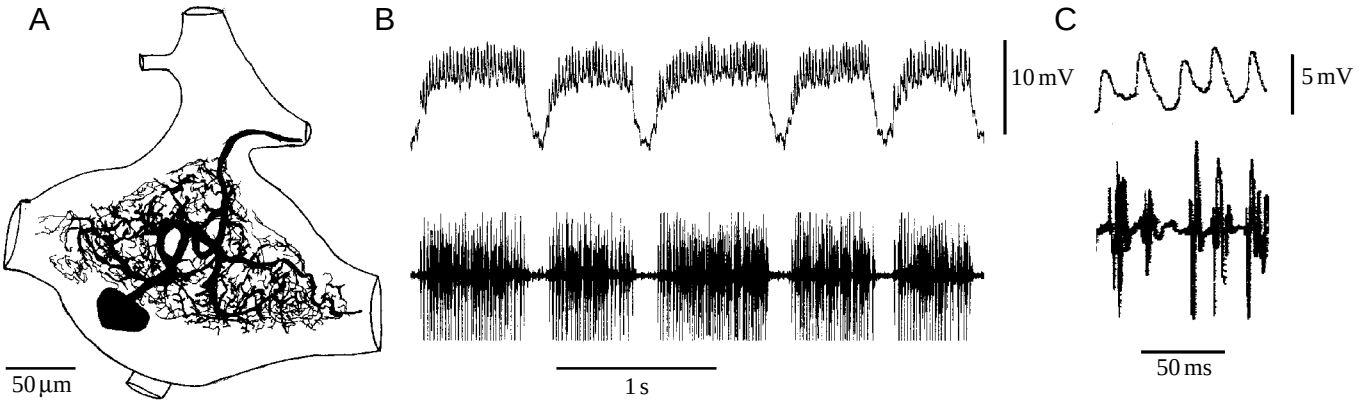

Fig. 3. Anterior dilator 1 (AD1) motoneuron. (A) *Camera lucida* drawing from an intracellular cobalt fill. The frontal nerve, extending anteriorly, is uppermost. (B) Activity of AD1 during feeding. The top trace is an intracellular recording from the motoneuron; the bottom trace is a simultaneous extracellular recording from the anterior dilator muscle. (C) Same as B, but at a faster sweep speed.

after eclosion, all of these animals had distended or burst abdomens. Two of the animals emerged sucessfully and no apparent effects were observed by 2 days after eclosion. The remaining two animals failed to emerge from the pupal case.

The foregut motor program at eclosion

The importance of an intact frontal ganglion for adult ecdysis and expansion of the wings indicated that the cibarial pump may be activated around the time of eclosion. This was found to be the case, with freshly emerged adults using the pump to swallow air. At eclosion, a stereotyped series of behaviors was displayed, as has been described in previous studies (Reynolds, 1980; Truman and Endo, 1974). After emerging from the pupal case, the animals (N=9) immediately began to crawl up the substratum provided. They crawled for variable lengths of time before stopping, always positioned with the head upwards on a vertical surface or on the underside of an inclined plane. The animals then stretched and coiled their proboscis. The wing bases were rotated so that the wings were positioned vertically over the thorax in the ‘butterfly’ position, and the air-swallowing motor program began 1.2–20 min (mean 4.7±4.7 min) after an animal had stopped crawling. During the quiescent period between the cessation of crawling and the initiation of pump activity, the wings began to stretch and expand, often reaching as much as two-thirds of their final size. The motor program for the pump at eclosion was generally similar to the feeding motor program, with alternating bursts of the pump dilators and compressor. However, unlike the very regular pattern during feeding, the pump activity during air swallowing began with irregular

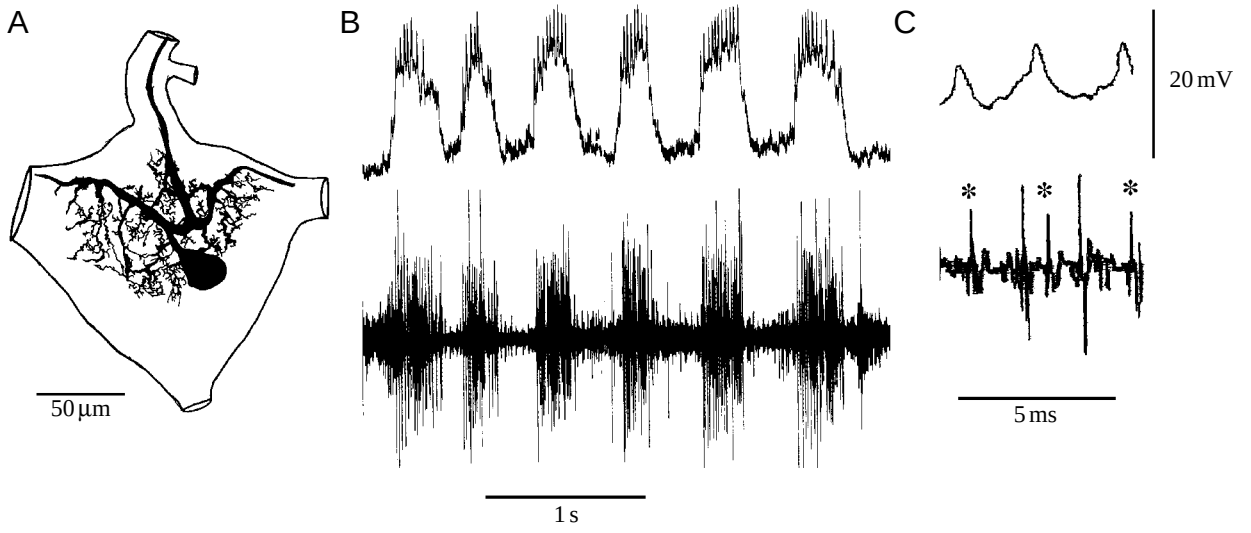

Fig. 4. Posterior dilator 1 (PD1) motoneuron. (A) *Camera lucida* drawing from an intracellular cobalt fill, The frontal nerve, extending anteriorly, is uppermost. (B) Activity of the same neuron as in A, during feeding. The top trace is an intracellular recording from the motoneuron; the bottom trace is a simultaneous extracellular recording from the posterior dilators. More than one muscle is represented in this recording. (C) Same as B, but at a faster sweep speed. Muscle spikes from the recorded neuron are marked with an asterisk in the lower trace.

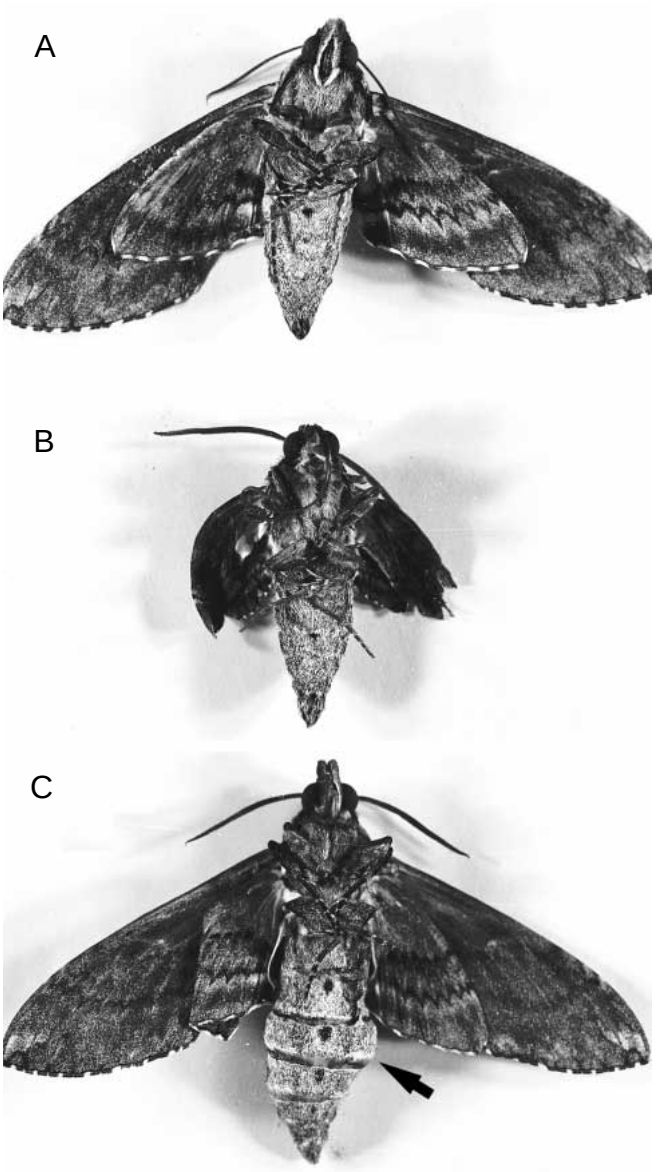

Fig. 5. Photographs of animals in which the frontal ganglion was altered. (A) Sham-operated control. (B) The frontal ganglion was removed at the onset of the wandering stage. The wings are not expanded and the abdomen is flattened. (C) Animal with bilaterally cut frontal connectives, 2 days after eclosion. Note the distorted abdomen, especially apparent at the arrow.

bursts in the dilator and compressor muscles that became regular and increased in frequency before slowing and stopping (Fig. 6A). The peak period for air swallowing was significantly shorter than the period for feeding, at 0.87±0.12 s (*N*=90). Pump activity lasted an average of 1.45±0.34 min and during this time, the wings could be observed expanding to approximately their full size.

The air-swallowing motor program was activated for only a brief period shortly after eclosion. Once the wings had reached their full size, hardened and were folded over the animal's back, this motor pattern was never observed again. In contrast, animals with cut frontal connectives showed pump activity immediately after eclosion, in some cases even while crawling. In all of these animals, the air-swallowing motor program continued after the wings were fully expanded and folded (Fig. 7). Indeed, this pattern could be recorded as late as 2 days after eclosion although, by this time, the animals' abdomens had burst. The period of the pump musculature in animals with the frontal connective cut on the first day after emergence was not significantly different from that of normal animals, at 1.03±0.15 s (*N*=50 from five animals). After 2 days, it had generally slowed substantially.

Air swallowing requires less activity of the pump dilator muscles than feeding. This was demonstrated by experiments in which animals displaying continuous air swallowing following transection of the frontal connectives were given sucrose solution (*N*=4). Immediately upon placing the proboscis in sucrose, the motor pattern showed a significant increase in the number of spikes in the dilator burst in three of these animals (Fig. 8). The remaining animal also showed an increase in the number of spikes per burst, although the increase was not significant. Three of these four animals also showed a significant increase in the period of the pump motor pattern after the proboscis had been placed in sucrose solution. It was not possible in these experiments to determine whether one set of dilator muscles was more likely to increase its activity than another.

Recordings of the swallowing motor program prior to eclosion

A motor program very similar to air swallowing was recorded before eclosion. Bouts of what appeared to be swallowing activity could be recorded in the pump musculature 6 h before eclosion in normal animals (*N*=5, Fig. 9). The timing of this swallowing motor pattern suggested that these animals could be swallowing molting fluid. To test this, the frontal ganglia were removed from seven pharate adults approximately 3 days before eclosion. They were then observed carefully at the expected time of eclosion to determine whether the molting fluid had been reabsorbed the moment they emerged from the pupal case. In normal animals, the scales of a freshly eclosed moth are a light gray color and dry to the touch. In contrast, the animals without frontal ganglia emerged at the expected time, but they were wet to the touch and a dark gray, almost black, color, indicating that the molting fluid had not been reabsorbed. These animals did dry to their normal light gray color within a few minutes of being exposed to the air. Examination of the crop contents of normal animals before ecdysis revealed that, approximately 24 h before eclosion, there was fluid within the crop. At 6 h before eclosion, the crop contained both fluid and some bubbles of air, and by the time the animal was within an hour or two of eclosion, the crop contained only air. To verify that the moths were ingesting their molting fluid, carmine particles were injected into the space between the developing adult cuticle and the overlying pupal case. Animals that had received carmine injections into the exuvial space 30 h before the expected time of ecdysis had carmine particles throughout the

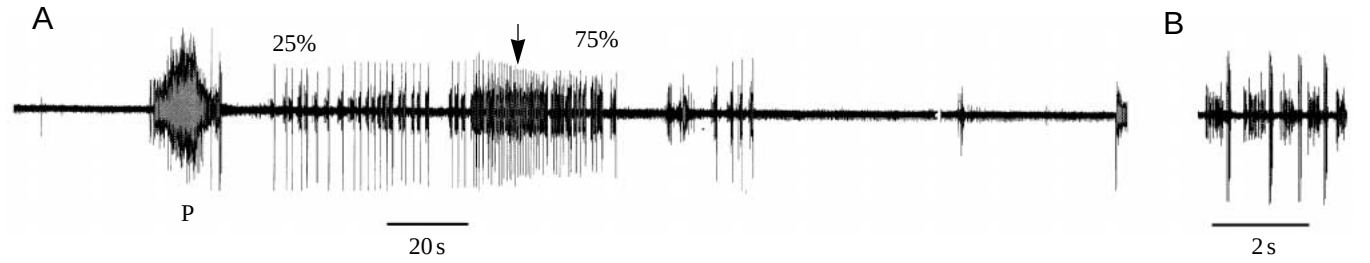

Fig. 6. The air-swallowing motor program in an intact freely moving animal. (A) The recording begins at the time when the animal stopped crawling, coming to rest in a vertical position. P indicates muscle activity associated with proboscis coiling. The degree of wing expansion during the recording is given as an approximate percentage of final wing size. (B) Part of A at a faster sweep speed (taken at the arrow). The large-amplitude bursts are the compressor muscle, the lower-amplitude bursts are from several dilators.

gut, including the crop, within 24 h before the expected time of ecdysis.

Triggering of the swallowing motor program by eclosion hormone

The timing of air-swallowing behavior at eclosion suggested that it could be triggered by the peptide hormones EH or ETH. The role of EH in air swallowing was tested by applying the peptide to isolated heads of animals 24–30 h from eclosing (*N*=22). To test whether the hormone was effective without the input from the brain to the frontal ganglion by way of the frontal connectives, these were severed in all animals. In some individuals, the small posterior branch 1 from the frontal nerve, which extends to the subesophageal ganglion, was also cut. No significant differences were found among individuals with intact or cut posterior branch 1. In most animals, some irregular spiking within the pump musculature could be recorded initially, which ceased after approximately 10 min. The pump musculature then remained silent for a period ranging from 7 to 105 min among the animals recorded (mean 43.16±9.16 min). At this time, irregular spiking began in one or more of the pump muscles, which typically developed into a rhythmic bursting pattern 72.75±16.55 min after the initial application of EH (Fig. 10). For 13 animals, the rhythmic pump activity could be correlated with easily observed alternating contractions of the pump dilators and compressor that looked like normal pump activity; for seven individuals, rhythmic activity could be recorded, but obvious pumping was not observed; one individual only showed a regular twitch of the pump compressor muscle, and one individual showed no pump activity. For the 20 animals that developed regular activity of the cibarial pump, the period of bursting was longer than that for the air-swallowing motor program recorded at eclosion, with a mean of 4.36±0.78 s (*N*=167).

In contrast, saline-treated controls either remained silent (*N*=9) or exhibited only occasional random spiking in the pump muscles (*N*=2) over the same period.

Recently, the peptide ETH was shown to trigger adult eclosion up to 24 h prior to the expected time of eclosion (Zitnan *et al.* 1996). Staged individuals (*N*=5) were injected with ground Inka cell extract 28–30 h before the expected time of eclosion. Control animals at the same stage received injections of saline. The animals injected with Inka cell extract eclosed that day at the normal eclosion gate for our colony (16:00–19:00 h). Saline-injected animals eclosed the following day. The animals injected with Inka cell extract displayed most of the normal eclosion-related behaviors upon emerging from the pupal case, including abdominal rotations followed by peristalsis, splitting of the pupal cuticle, crawling, climbing to a vertical surface, proboscis coiling, vertical rotation of the wing bases and, finally, folding of the hardened wings over the abdomen. These animals differed from normally eclosing

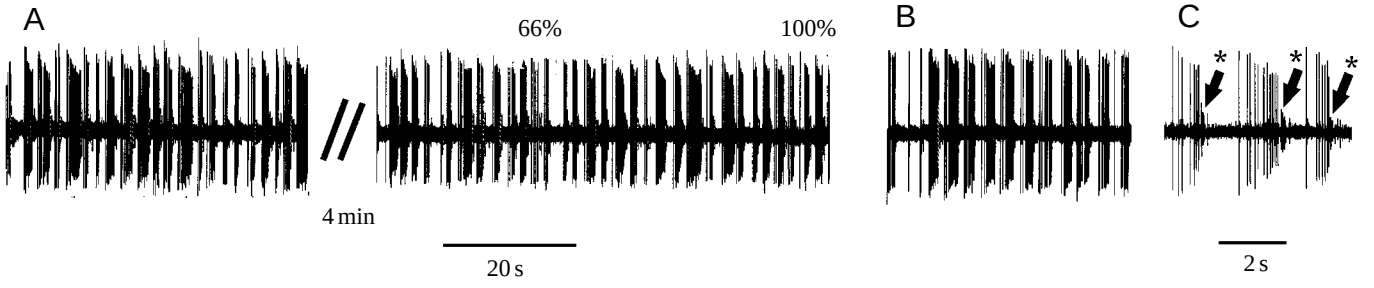

Fig. 7. Pump activity recorded in an animal with cut frontal connectives at eclosion. The electrode was placed in the posterior dilator muscle. (A) The recording begins at the time when the animal stopped crawling and came to rest in a vertical position. The degrees of wing expansion as an approximate percentage of the final size of the wings are given at two points along the recording. (B) Same animal 90 min after the start of A. The electrode was not moved between these recordings. (C) Same as A at a faster sweep speed. The pump compressor bursts (asterisks) are the small-amplitude bursts marked with arrows.

Table 1. *Success of eclosion following surgical manipulations*

Surgical manipulation	*N*	Eclosion success			
		Eclose, expand wings	Eclose, no wing expansion	No eclosion	Expand wings, abdomen bursts
Remove frontal ganglion	12	0	4	8	0
Cut frontal connectives	15	2	2	0	11
Cut recurrent nerve	15	12	3	0	0
Sham-operated	14	13	0	1	0
Frontal nerve cut*	8	0	7	1	0
Sham-operated*	10	9	0	1	0

*Operations performed 3 days before eclosion. All other operations were performed at the wandering stage.

animals in two respects, however: they were very wet when they emerged from the pupal case, and they did not expand their wings. Recordings from the cibarial pump from the time of eclosion until the wings were hardened and folded 1–2 h later revealed that the air-swallowing motor program was never initiated in the animals injected with Inka cell extract.

Discussion

Changes in foregut structure and the role of the frontal ganglion in the adult

In both larvae and adults, the frontal ganglion contains the neural circuitry that drives the foregut. In the larva, the foregut is continuously active whether or not the larva is feeding, except for a period preceding the larval–larval molts (Bestman *et al.* 1997). Two types of larval foregut movements have been described: a posteriorly directed peristalsis and a squeeze movement (Miles and Booker, 1994). As long as innervation from the frontal ganglion remains intact, these movements will continue in the absence of the brain and ventral nerve cord. They could be considered to be the swallowing phase of feeding behavior, with biting and other mandibular movements being controlled by the subesophageal ganglion (Griss, 1990; Rohrbacher, 1994).

In adults, the frontal ganglion is essential for two behaviors. One of these is feeding, which is exhibited many times in adult life, while the other behavior is displayed only at one specific time during the adult animal's life. This is the swallowing behavior that takes place around the time of eclosion and is essential for the proper expansion of the wings of the freshly emerged moth.

During adult development, the structure of the foregut changes dramatically from the simple straight tube of the larva. The buccal region of the larval foregut expands greatly to form the cibarial chamber and pump of the adult, and the esophagus lengthens and narrows. As may be expected from these massive changes in foregut structure, the mechanism of feeding is also changed in adults. Adult feeding behavior consists of the manipulation of the newly developed proboscis into a nectar source and pumping of the nectar into the foregut using the cibarial pump. Proboscis movements are probably controlled by neurons in the subesophageal ganglion (Eastham and Eassa, 1955), while the motor pattern of the cibarial pump is driven by the frontal ganglion. The rhythmic activity of the cibarial pump consists of synchronous contractions of the dilators and the labral compressor, followed by a much shorter-

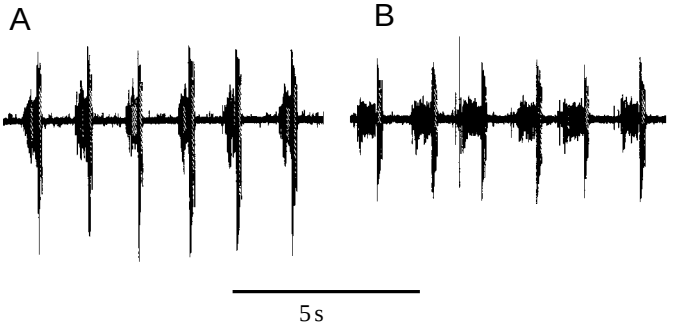

Fig. 8. A comparison of pump activity from an animal with a cut frontal connective with its proboscis in air or in 20 % sucrose solution. The bursts with the largest amplitude are from the pump compressor, which shows no significant change (the 95 % confidence intervals overlap) in the number of spikes per burst, the lower-amplitude bursts are from several dilator muscles. (A) Proboscis in air. For the dilators, the mean number of spikes per burst was 20.3±3.68 (*N*=10 bursts). (B) Proboscis in 20 % sucrose. For the dilators, the mean number of spikes per burst was 45.5±4.6 (*N*=10 bursts). The electrode was not moved between these recordings.

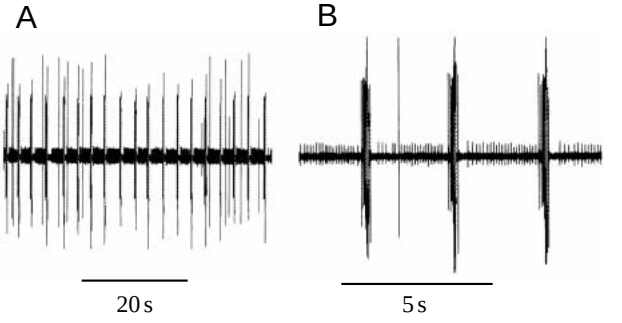

Fig. 9. Cibarial pump activity before eclosion. Recordings are from an animal that eclosed 6 h later. (A) Motor pattern showing large-amplitude pump compressor bursts and lower-amplitude dilator muscle bursts. (B) Same as A at a faster sweep speed.

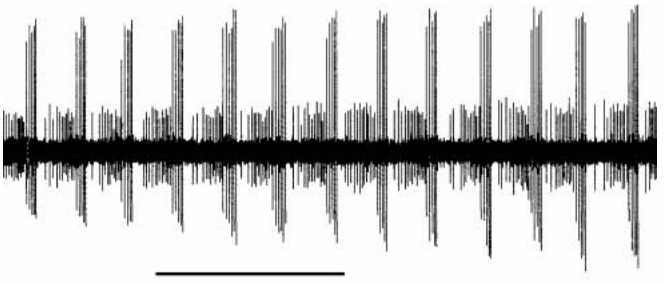

Fig. 10. Cibarial pump activity in a pre-eclosed animal resulting from treatment with eclosion hormone 50 min earlier. This animal was approximately 24 h before its expected time of eclosion. The spikes with the largest amplitude are the pump compressor, and the smaller-amplitude bursts are from several dilator muscles. In this preparation, the pump was observed to move as in a normal animal.

duration contraction of the pump compressor. Unlike the larval stage, the frontal ganglion is not required for the peristaltic movements of the adult esophagus, which appeares to be myogenic. Another change from the larval stage is that the activity of the adult foregut is apparently triggered by sensory input from the proboscis, as this motor pattern is initiated only when the proboscis is placed in water or a sucrose solution. How those inputs reach the frontal ganglion and initiate the feeding motor pattern is not known, however.

The activities of two motoneurons that have been identified so far in the adult frontal ganglion during feeding show regular oscillations of de- and hyperpolarization leading to bursts of spikes that correlate with the bursts in the muscles they innervate. For the anterior dilator motoneuron AD1, the depolarized phase is prolonged, lasting approximately 0.6 s. These activity patterns resemble the activities recorded in motoneurons of the larval frontal ganglion (Miles and Booker, 1994). During adult development in *Manduca sexta*, many adult neurons are derived from remodeled larval neurons (Levine and Truman, 1985). This is likely to be the case for neurons of the frontal ganglion, as we have found no evidence of neurons either being born or dying during adult development in this ganglion (C. I. Miles and R. Booker, personal observation). Which larval neurons may have developed into the adult PD1 and AD1 neurons remains to be determined.

The termination of a feeding bout in adult *Manduca sexta* is apparently not mediated by inputs from the recurrent nerve, as was shown to be the case in the blowfly *Phormia regina* (Gelperin, 1971, 1972), since intact moths or those with cut recurrent nerves did not exhibit significant differences in the amount of sucrose solution they consumed. It is possible that they use a system of stretch receptors on the crop similar to that described in the fly (Bernays, 1985). In larval *Manduca sexta*, however, volumetric feedback from the gut did not have an effect on the amount of food consumed (Timmins and Reynolds, 1992). Another possibility is that a feeding bout is terminated by feedback from abdominal stretch receptors within the body wall, as in the bug *Rhodnius prolixus* (Chaing and Davey, 1988). Stretch receptors on the crop or body wall have yet to be described in the moth, but it is likely that some form of feedback by way of the brain is used, as indicated by the over-expansion of the abdomen that occurs when the frontal ganglion is disconnected from the brain.

The frontal ganglion is important for wing expansion at adult eclosion

Air swallowing is an important component of molting behavior in a number of insects (reviewed in Reynolds, 1980). By filling the gut with air, larval locusts *Schistocerca gregaria* can generate enough internal pressure to expand their bodies to split open the old cuticle at ecdysis (Bernays, 1972). Increasing internal pressure through air swallowing is also used by the blowfly *Calliphora erythrocephala* after ecdysis to expand the new cuticle (Cottrell, 1962). Air swallowing is important following adult ecdysis in blowflies, locusts and crickets *Teleogryllus oceanicus*, where it is essential for expanding the wings (Cottrell, 1962; Hughes 1980*a*,*b*; Carlson, 1977). Among Lepidoptera, air swallowing is apparently not a critical component of wing expansion in *Pieris brassicae* (Cottrell, 1964), although the butterfly does exhibit this behavior.

In an earlier report by Truman and Endo, (1974), air swallowing was not considered to be involved in the expansion of the wings of *Manduca sexta*, as this could take place without the head. However, we found that eliminating input from the frontal ganglion disrupts wing expansion, as was reported in an earlier study (Bell, 1986). If the frontal ganglion was removed entirely, thereby eliminating all innervation of the cibarial pump, the few animals that emerged successfully from the pupal case were wet and failed to expand their wings. These animals also failed to feed. Animals with cut frontal nerves eclosed successfully, but none of them expanded their wings. This manipulation eliminated input to the anterior and lateral dilators and thus disrupted the animal's ability to expand the pump and swallow air. Cutting the recurrent nerve did not noticeably disrupt eclosion, although a few individuals (three out of 15) failed to expand their wings after eclosion. In animals with a cut recurrent nerve, innervation of the compressor and the posterior dilators would be disrupted. Apparently, such animals are able to take in enough air with the remaining dilator muscles for successful eclosion and, in most cases, expansion of their wings.

The air-swallowing motor pattern, like that for feeding, consists of prolonged bursts from the dilator muscles alternating with the brief, high-frequency pump compressor bursts. Feeding requires more dilator muscle activity than air swallowing, however, as might be expected since the more viscous sucrose solution would require a greater force generated by the pump to draw it into the foregut. The pattern of coupled dilator and compressor bursts also differs from feeding by its characteristic increase then decrease in burst frequency. A similar pattern of pump activity has been described in freshly eclosed blowflies (Cottrell, 1962).

The frontal ganglion is important before eclosion

The post-eclosion air swallowing cannot be the only

behavior driven by the frontal ganglion that is needed for expansion of the wings. We found that by the time that this motor program is initiated, the wings had already expanded to as much as two-thirds of their final size. This wing expansion is probably due to a combination of the elasticizing effects of the peptides EH and bursicon on the wing cuticle, as described by Reynolds (1977), and the pumping movements of the abdomen, which assist in forcing blood into the wing veins (Truman and Endo, 1974). However, animals without a frontal ganglion failed to expand their wings at all, indicating that the frontal ganglion is important at some time before the initiation of post-eclosion air swallowing. We have recorded a steady swallowing motor program in animals up to 6 h before eclosion. This is approximately the time at which the final phase of molting fluid resorption begins (Reynolds *et al.* 1979) and indicates that the pump may be used before eclosion to swallow molting fluid. Previous work suggested that this was the case at larval molts in the silkworm *Bombyx mori* (Wachter, 1930), and more recently for *Manduca sexta* larvae as well (J. Bestman, personal communication). Cornell and Pan (1983) provided evidence that, at the larval–pupal molt, molting fluid is swallowed. At the adult molt of *Manduca sexta*, Lensky *et al.* (1970) described specific sites in the integument where the molting fluid was reabsorbed, but also noted that, at the head, it appeared to be swallowed. Several observations we have made support this hypothesis. We found that animals incapable of swallowing because of the removal of their frontal ganglion emerge from the pupal case wet, unlike normal animals. In addition, our observations of the guts of animals near the time of eclosion showed that, 24 h before eclosion, the crop began filling with liquid. Approximately 6 h before eclosion, air began to appear in the crop as well. We have also found that carmine particles injected into the exuvial space of the head appear in the gut by 24 h before eclosion. The appearance of fluid and carmine particles in the gut at 24 h before eclosion is approximately 18 h earlier than we recorded a clear regular swallowing pattern in the pump musculature. The pump shows sporadic activity at this early time, however, and it is possible that this type of activity could lead to some fluid ingestion. Swallowing molting fluid would aid in its removal from the exuvial space and in the conservation of the molting fluid proteins and the breakdown products from the old cuticle. In addition, swallowing molting fluid could help to increase abdominal pressure by filling the gut to facilitate the moth's escape from the pupal cuticle. Evidence to support this function is provided by our observation, as well as that of Bell (1986), that only a fairly low proportion of the animals missing the frontal ganglion successfully emerged from the pupal cuticle. Expansion of the crop before eclosion may also provide the freshly eclosed moth with enough internal pressure to help in the first part of wing expansion, before the characteristic air-swallowing motor program is initiated.

In normal animals, swallowing behavior at eclosion ends after the wings have fully expanded. However, animals with cut frontal connectives appear to be unable to terminate air swallowing once it has started. We found that 11 of 15 animals in which the frontal connectives were cut displayed distended or burst abdomens 24 h after eclosion. These results are similar to those of Bell (1986), who reported that all of his 12 animals with a cut frontal ganglion showed this effect. Clearly, there must be some form of feedback from the crop or abdomen that reaches the frontal ganglion through the frontal connectives to terminate the air-swallowing motor program in normal animals. Whether this is by stretch receptors in the crop or abdominal body wall, as suggested above for feeding, is not known. While these results show that the frontal ganglion's connection to the brain is necessary for inactivating air swallowing, they also show that the activation of swallowing behavior does not require the brain or central nervous system (CNS), but is apparently by a mechanism that acts directly on the frontal ganglion. This result suggests that its activation at eclosion may be by a blood-borne factor, as we have found.

The swallowing motor program is activated by eclosion hormone

The application of eclosion hormone triggered activation of pump musculature in 21 out of 22 frontal ganglion–cibarial pump preparations from staged animals 24–30 h before the expected time of eclosion. In the majority of cases (13 animals), the pump displayed what appeared to be a normal pumping cycle of alternating expansions and compressions of the cibarial roof. Seven of the remaining animals showed activity that was typically rhythmic, although the pump's activity was not easily observed. Saline-treated controls never exhibited rhythmic pump activity.

With the recent discovery of the peptide hormone ETH, the precise role for EH in eclosion behavior has been re-examined (Zitnan *et al.* 1996; Ewer *et al.* 1997; Kingan *et al.* 1997; Gammie and Truman, 1997). The two peptides are believed to interact in a positive feedback loop to stimulate each other's further release. The eclosion motor program is then triggered by EH acting on specific neurons within the CNS (Ewer *et al.* 1994; Gammie and Truman, 1997).

Because air-swallowing is an integral and critical part of the series of behaviors associated with eclosion, it is perhaps not surprising that it is triggered by eclosion hormone. However, if the swallowing motor pattern we recorded approximately 6 h before eclosion is triggered by EH, it is several hours earlier than might be expected, as this peptide cannot be detected in the blood of *Manduca sexta* until 2.5–3 h before eclosion (Reynolds *et al.* 1979). An intriguing piece of evidence for EH triggering the uptake of molting fluid is the observation by Truman, cited in Reynolds (1980), that some brainless silkmoths responded to injections of EH while they were still wet and that these animals showed accelerated resorption of molting fluid. It is possible that EH may be present in the blood at earlier times, in quantities that are too low to be detected by the wing-stretch assay used in the earlier study (Reynolds *et al.* 1979) yet are capable of activating the frontal ganglion. The possibility of an early low-level release of EH has also been raised by Kingan *et al.* (1997), who proposed this could trigger the initial release of ETH by the Inka cells. Clearly, a detailed

analysis of the hemolymph for the presence of EH at earlier times in the molt would be of great value.

On the basis of two lines of evidence, we have found that ETH does not activate the swallowing motor progam. First, the isolated head preparations used in our study did not include a source for this peptide, as so far it has only been found to be released from the Inka cells, which are located in the thorax and abdomen (Zitnan *et al.* 1996). Second, animals given injections of ETH a day before the expected time of emergence eclosed prematurely and displayed all of the behaviors associated with eclosion, such as abdominal rotations, peristaltic waves of contraction, climbing, proboscis coiling and appropriate movements of the wing bases, but they were wet at eclosion, they did not swallow air and their wings did not expand properly. Similar observations were reported by Zitnan *et al.* (1996). These results suggest that early injections of ETH fail to trigger the activation of the frontal ganglion. Two possible explanations for these results are as follows. First, the early application of a high dose of ETH may trigger the central release of EH, thereby initiating eclosion behaviors, but does not trigger its release into the periphery, perhaps because the physiological mechanisms for this release are not yet competent. Second, it is also possible that, if the pump is used for swallowing molting fluid, it could be turned off near eclosion by high levels of ETH. After eclosion, the frontal ganglion would be activated again by sensory inputs that indicate that the moth is in the appropriate position to expand its wings. If ETH inhibits the swallowing motor program of the frontal ganglion, it would have to be by activating inhibitory inputs that reach the frontal ganglion through the frontal connectives, as severing these leads to a swallowing motor program that does not inactivate.

Activation of the frontal ganglion and cibarial pump prior to eclosion is only one of many molt-related behaviors that are displayed well before the much-studied pre-ecdysis and ecdysis behaviors are observed. It would be reasonable to expect that many of these behaviors will be found to be triggered by hormones that are known to be modulated at the molt.

We thank Dr D. Morton for the gift of purified eclosion hormone. We also thank Dr C. Gilbert and Ms J Bestman for critically reading the manuscript and Ms J. Bestman for carrying out carmine injections. Supported in part by USDA 96-35302-3287.

References

BELL, R. A. (1986). Role of the frontal ganglion in lepidopterous insects. In *Insect Neurochemistry and Neurophysiology* (ed. A. B. Borkovee and D. B. Gelman), pp. 321–324. Clifton, NJ: Humana Press.

BELL, R. A. AND JOACHIM, F. A. (1976). Technique for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann. ent. Soc. Am.* **69**, 365–373.

BERNAYS, E. A. (1972). The intermediate moult (first ecdysis) of *Schistocerca gregaria* (Forskal) (Insecta, Orthoptera). *Z. morph. Tiere* **71**, 160–179.

BERNAYS, E. A. (1985). Regulation of feeding behaviour. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 4 (ed. G. A. Kerkut and L. I. Gilbert), pp. 1–32. Oxford, New York: Plenum

BESTMAN, J. E., MILES, C. I. AND BOOKER, R. (1997). Neural and behavioral changes associated with larval molts in the moth *Manduca sexta. Soc. Neurosci. Abstr.* **23**, 768.

BUSHMAN, D. W. AND NELSON, J. O. (1990). The role of the frontal ganglion and corpora cardiaca/corpora allata complex in post-feeding weight loss in adult *Heliothus zea. Physiol. Ent.* **15**, 269–274.

CARLSON, J. R. (1977). The imaginal ecdysis of the cricket (*Teleogryllus oceanicus*). I. Organization of motor patterns and roles of central and sensory control. *J. comp. Physiol.* A **115**, 299–317.

CHAING, R. G. AND DAVEY, K. G. (1988). A novel receptor capable of monitoring applied pressure in the abdomen of an insect. *Science* **241**, 1665–1667.

COPENHAVER, P. F. AND TRUMAN, J. W. (1982). The role eclosion hormone in the larval ecdyses of *Manduca sexta. J. Insect Physiol.* **28**, 695–701.

CORNELL, J. C. AND PAN, M. L. (1983). The disappearance of moulting fluid in the tobacco hornworm, *Manduca sexta. J. exp. Biol.* **107**, 501–504.

COTTRELL, C. B. (1962). General observations on the imaginal ecdysis of blowflies. *Trans. R. ent. Soc. Lond* **114**, 317–333.

COTTRELL, C. B. (1964). Insect ecdysis with particular emphasis on cuticular hardening and darkening. *Adv. Insect Physiol.* **2**, 175–218.

DANIEL, T. L. KINGSOLVER, J. G. AND MEYHÖFER, E. (1989). Mechanical determinants of nectar-feeding energetics in butterflies: muscle mechanics, feeding geometry and functional equivalence. *Oecologia* **79**, 66–75.

EASTHAM, L. E. S. AND EASSA, Y. E. E. (1955). The feeding mechanism of the butterfly *Pieris brassicae. Phil. Trans. R. Soc. Lond. B* **239**, 1–43.

EWER, J., DEVENTE, J. AND TRUMAN, J. W. (1994). Neuropeptide induction of cyclic GMP increases in the insect CNS: Resolution at the level of single identified neurons. *J. Neurosci.* **14**, 7704–7714.

EWER, J., GAMMIE, S. C. AND TRUMAN, J. W. (1997). Control of insect ecdysis by a positive feedback endocrine system: roles of eclosion hormone and ecdysis triggering hormone. *J. exp. Biol.* **200**, 809–881.

GAMMIE, S. C. AND TRUMAN, J. W. (1997). Neuropeptide heirarchies and the activation of sequential motor behaviors in the hawkmoth, *Manduca sexta. J. Neurosci.* **17**, 4389–4397.

GELPERIN, A. (1971). Abdominal sensory neurons providing negative feedback to the feeding behaviour of the blowfly. *Z. vergl. Physiol.* **72**, 17–31.

GELPERIN, A. (1972). Neural control systems underlying insect feeding behaviour. *Am. Zool.* **12**, 489–496.

GRISS, C. (1990). Mandibular motor neurons of the caterpillar of the hawk moth *Manduca sexta. J. comp. Neurol.* **296**, 393–402.

HUGHES, T. D. (1980*a*). The imaginal ecdysis of the desert locust, *Schistocerca gregaria.* I. A description of the behaviour. *Physiol. Ent.* **5**, 47–54.

HUGHES, T. D. (1980*b*). The imaginal ecdysis of the desert locust *Schistocerca gregaria.* III. Motor activity underlying the

expansional and post-expansional behaviour. *Physiol. Ent.* **5**, 141–152.

KINGAN, T. G., GRAY, W., ZITNAN, D. AND ADAMS, M. E. (1997). Regulation of ecdysis-triggering hormone release by eclosion hormone. *J. exp. Biol.* **200**, 3245–3256.

KINGSOLVER, J. G. AND DANIEL, T. L. (1995). Mechanics of food handling by fluid feeding insects. In *Regulatory Mechanisms in Insect Feeding* (ed. R. F. Chapman and G. DeBoer), pp. 32–73. New York: Chapman & Hall.

LENSKY, Y., COHEN, C. AND SCHNEIDERMAN, H. A. (1970). The origin, distribution and fate of the molting fluid proteins of the cecropia silkworm. *Biol. Bull. mar. biol. Lab., Woods Hole* **139**, 277–295.

LEVINE, R. B. AND TRUMAN, J. W. (1985). Dendritic reorganization of abdominal motoneurons during metamorphosis of the moth, *Manduca sexta. J. Neurosci.* **5**, 2424–2431.

MESCE, K. A., KLUKAS, K. A. AND BRELJE, T. C. (1993). Improvements for the anatomical characterization of insect neurons in whole mount: the use of cyanine-derived fluorophores and laser scanning confocal microscopy. *Cell Tissue Res.* **271**, 381–397.

MILES, C. I. AND BOOKER, R. (1994). The role of the frontal ganglion in foregut movements of the moth *Manduca sexta*. *J. comp. Physiol.* A **174**, 755–767.

MILES, C. I. AND WEEKS, J. C. (1991). Developmental attenuation of the pre-ecdysis motor pattern in the tobacco hornworm, *Manduca sexta. J. comp. Physiol.* A **168**, 179–190.

MORTON, D. B. AND GIUNTA, M. A. (1992). Eclosion hormone stimulates cyclic GMP in the nervous system of the hawkmoth, *Manduca sexta. J. Neurochem.* **59**, 1522–1530.

REYNOLDS, S. E. (1977). Control of cuticle extensibility in the wings of adult *Manduca* at the time of eclosion: effects of eclosion hormone and bursicon. *J. exp. Biol.* **70**, 27–39.

REYNOLDS, S. E. (1980). Integration of behaviour and physiology in ecdysis. *Adv. Insect Physiol.* **15**, 475–595.

REYNOLDS, S. E., TAGHERT, P. H. AND TRUMAN, J. W. (1979). Eclosion hormone and bursicon titres and the onset of hormonal responsiveness during the last day of adult development in *Manduca sexta J. exp. Biol.* **78**, 77–86.

ROHRBACHER, J. (1994). Fictive chewing activity in motor neurons and interneurons of the subesophageal ganglion of *Manduca sexta* larvae. *J. comp. Physiol.* A **175**, 629–637.

SCHWARTZ, L. M. AND TRUMAN, J. W. (1983). Hormonal control of rates of metamorphic development in the tobacco hornworm *Manduca sexta. Devl Biol.* **99**, 103–114.

SNODGRASS, R. E. (1935). *Principles of Insect Morphology.* New York, London: McGraw-Hill.

TIMMINS, W. A. AND REYNOLDS, S. E. (1992). Physiological mechanisms underlying the control of meal size in *Manduca sexta* larvae. *Physiol. Ent.* **17**, 81–89.

TRIMMER, B. A. AND WEEKS, J. C. (1989). Effects of nicotinic and muscarinic agents on an identified motoneurone and its direct afferent inputs in larval *Manduca sexta*. *J. exp. Biol.* **144**, 303–337.

TRUMAN, J. W. (1971). Physiology of insect ecdysis. I. The eclosion behavior of saturniid moths and its hormonal release. *J. exp. Biol.* **54**, 805–814.

TRUMAN, J. W. (1973). Physiology of insect ecdysis. III. Relationship between the hormonal control of eclosion and of tanning in the tobacco hornworm, *Manduca sexta. J. exp. Biol.* **58**, 821–829.

TRUMAN, J. W. AND ENDO, P. T. (1974). Physiology of insect ecdysis: neural and hormonal factors involved in wing-spreading behaviour of moths. *J. exp. Biol.* **61**, 47–55.

TRUMAN, J. W., TAGHERT, P. H., COPENHAVER, P. F., TUBLITZ, N. J. AND SCHWARTZ, L. M. (1981*a*). Eclosion hormone may control all ecdyses in insects. *Nature* **291**, 70–71.

TRUMAN, J. W., TAGHERT, P. H. AND REYNOLDS, S. E. (1981*b*). Physiology of pupal ecdysis in the tobacco hornworm, *Manduca sexta*. I. Evidence for control by eclosion hormone. *J. exp. Biol.* **88**, 327–337.

WACHTER, S. (1930). The moulting of the silkworm and a histological study of the moulting gland. *Ann. ent. Soc. Am.* **23**, 381–393.

WEEKS, J. C. AND TRUMAN, J. W. (1984). Neural organization of peptide-activated ecdysis behaviors during the metamorphosis of *Manduca sexta*. I. Conservation of the peristalsis motor pattern at the larval–pupal transformation. *J. comp. Physiol.* **155**, 407–422.

WEEKS, J. C. AND TRUMAN, J. W. (1985). Independent steroid control of the fates of motoneurons and their muscles during insect metamorphosis. *J. Neurosci.* **5**, 2290–2300.

ZITNAN, D., KINGAN, T. G., HERMESMAN, J. L. AND ADAMS, M. E. (1996). Identification of ecdysis-triggering hormone from an epitracheal endocrine system. *Science* **271**, 88–91.