J. exp. Biol. (1976), 65, 65–84
With 11 *figures*
Printed in Great Britain

ADULT MOTOR PATTERNS PRODUCED BY MOTH PUPAE DURING DEVELOPMENT

BY ANN E. KAMMER
Division of Biology, Kansas State University, Manhattan, Kansas 66506
AND MARY B. RHEUBEN*
Department of Biology, Yale University, New Haven, Connecticut

(*Received* 8 *January* 1976)

SUMMARY

Muscle potentials were recorded extracellularly from developing pupae and adults of the saturniid moths *Antheraea polyphemus* and *A. pernyi* and the sphingid moth *Manduca sexta*. During the week prior to the terminal ecdysis, developing moths still enclosed within the pupal cuticle produced motor patterns similar to those recorded from adults during flight and shivering. The pupal patterns had a longer cycle time and were more variable than the adult motor patterns. Characteristic inter-family differences in adult motor patterns were apparent in pupal motor patterns.

Development of motor patterns was followed over several days by observing individuals with chronically implanted leads. Early in the pupal period potentials were small and infrequent. The amount of activity gradually increased and became more patterned. As development proceeded adult patterns were produced for increasing lengths of time, although the patterns changed quickly and spontaneously.

Restricting the wing movements of *A. polyphemus* adults increased the cycle time, increased the number of spikes per burst in muscles opposing the restraint, and did not alter the interspike interval within a burst. The flight patterns produced by pharate moths, in which the wings are also immobile, also have a longer cycle time than that of adult flight, but the number of spikes per burst is the same and the interspike interval is longer than in adult flight. These observations suggest that the differences between pupal and adult patterns are not necessarily due to the confinement of the wings by the pupal cuticle.

INTRODUCTION

During the past decade neuromuscular patterns of activity involving relatively large, fast, thoracic muscles have been described for many insects. Particular attention has been given to motor patterns underlying stereotyped, rhythmically repeated movements, such as occur in flying and singing (Wilson, 1968; Elsner & Huber, 1974; Miller, 1974). Recently attention has turned to the development of these motor patterns. It has been shown that immature grasshoppers and crickets can be induced to produce motor patterns resembling in some respects those of adult flight and singing,

---

* Present address: Dr Mary B. Rheuben, Bldg. 36, Rm. 3B–24, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

although the nymphs possess only immobile wing-buds (Bentley & Hoy, 197d Kutsch, 1971, 1974*a*; Weber, 1972; Bentley, 1973; Altman & Tyrer, 1974; Altman, 1975). These Orthoptera undergo a gradual metamorphosis, and some of the muscles involved in the adult flight and singing are also used in walking by both nymphs and adults (Wilson, 1962; Bentley, 1973). Lepidoptera, in contrast, undergo a complete metamorphosis: larval, pupal, and adult stages differ markedly in form and behaviour. The pupal stage is devoted primarily to the transformation of larval structures into those of the adult, and its only overt behaviour is movement of the abdomen – flexion, telescoping, or rotation. Development of an adult moth involves construction of massive thoracic muscles (Snodgrass, 1954; Bienz-Isler, 1968), some of which are used only in producing wing movement, e.g. during flying, and in producing heat during warm-up. The motor patterns of flight and warm-up in adult moths are known (Kammer, 1967, 1968, 1970; Hanegan & Heath, 1970). We now report that moth pupae produce motor patterns similar to those of adults. Furthermore, the patterns are produced spontaneously, in pupae which look quiescent, as part of the normal developmental sequence.

A preliminary account of these results has been presented (Kammer & Rheuben, 1974).

MATERIALS AND METHODS

Diapausing pupae of *Antheraea polyphemus* and *A. pernyi* (Saturniidae) were purchased from a supplier and stored at about 5 °C until needed. Development was initiated by placing pupae at room temperature (21–26 °C) on a long-day light cycle. The developmental age of pharate saturniid moths was estimated from the number of days at room temperature and subsequently established either by dissection, using the morphological criteria of Nüesch (1965), or by back-calculation from the date of eclosion. *Manduca sexta* (Sphingidae) were raised on an artificial diet (modified from Yamamoto, 1969) and kept at room temperature or in an incubator at 26 °C. Ages of the *Manduca* pupae were estimated from the dates of pupation and adult emergence.

Muscle potentials were recorded by means of 30 μm insulated copper wires inserted into the muscles through a small hole in the exoskeleton. Some recordings were differential, with one electrode in the dorsal longitudinal muscle and the other in an elevator muscle or a nearby region of the hemocoel. A larger copper wire placed in the abdomen served as ground. Other recordings were made with a single active electrode relative to ground.

For chronic recording, pupae were fastened ventrally to a plastic support in such a way that the abdomen could rotate freely. Prior to implanting recording wires, tools and wires were disinfected by immersion in a Zephiran solution (0·13 % aqueous benzalkonium chloride, Winthrop Laboratories) and then rinsed with boiled water. The cuticle of the pupa was swabbed with the Zephiran solution and allowed to dry. Three recording wires were inserted about 2 mm through the mesonotum and fastened in place with a hot beeswax–resin mixture, and a ground was similarly placed in an anterior, immobile segment of the abdomen. Using these implanted wires, recordings (usually differential) were made from each individual almost every day until it emerged or neared emergence, or until the experiment was terminated. The time of day (between 08.00 and 01.00 h) at which observations were made and the length of the recording period varied irregularly. Recordings were made at room temperature, which ranged from 21 to 26 °C. When pupae were not being observed they were kept at 26° in an incubator on a long light-cycle; dark was midnight to 08.00 h.

For both acute and chronic preparations, electrode position was established by post-mortem dissection of animals preserved with the wires in place. The muscles studied were mesothoracic indirect flight muscles. Each of these muscles, except the tergo-trochanteral, which can move the leg, can move only the portion of the thorax to which it is attached and thereby move the wings. Muscle function, our nomenclature, and its correspondence with that of Nüesch (1953) are summarized by Kammer (1967). With the chronic recording method, we cannot be sure that the potentials observed early during pupal development came from the muscles which were around or adjacent to the electrode at the time of dissection, after subsequent pupal development. Furthermore, the temporal sequence of development of the different thoracic muscles is not known, and it is possible that the muscles do not develop simultaneously. During the middle and late stages of development, however, identifications can be made with some confidence. For example, the activity shown in Figs. 6 and 7, days 9–15, is most likely from the dorsal longitudinal muscle, since the electrode was deep and medial in the dorsal longitudinal muscle, and a new electrode inserted into a dorso-ventral muscle detected the activity of an antagonist (Fig. 7, day 16).

Selected records taken at high chart speeds (125 mm/s on a high-speed pen recorder (Brush 260)) were analysed to determine cycle time and phase. Cycle time was defined as the time from the beginning of one burst of impulses to the beginning of the next burst from the same motor unit (*a* in Fig. 3A). Phase was calculated as the ratio *b* over *a*, where *b* is the interval between the beginning of a depressor burst and beginning of the next elevator burst, and *a* is the concurrent cycle-time of the depressor unit (Fig. 3A).

RESULTS

Comparison of adult and pupal patterns in Antheraea polyphemus

Two readily distinguishable motor patterns have been described previously from saturniid moths including *A. polyphemus* (Kammer, 1967; Hanegan & Heath, 1970). One pattern (Fig. 1A) occurs during flight, and results in alternate contraction of antagonistic muscles and movement of wings up and down through a wide but variable arc. The other (Fig. 1B) occurs during warm-up, and results in synchronous excitation of antagonistic muscles, movement of the wings through a small arc, and elevation of thoracic temperature. Both of these motor patterns were observed in recordings from pupae during the last few days prior to ecdysis (Fig. 1C, D).

Pupae which on the basis of the electrical activity of their flight muscles were 'flying' typically appeared to be motionless. However, at least in late stages of development the adult mesothoracic notum (the attachment site for many of the flight-muscles) could be seen moving underneath the pupal cuticle. The only movements made by an intact pupa were abdominal rotation and shortening, which occurred either spontaneously or in response to a touch on the abdomen. Activity in the flight muscles was not correlated with these abdominal movements, nor were we able to induce motor patterns in the thorax by light tactile stimulation anywhere on the pupal

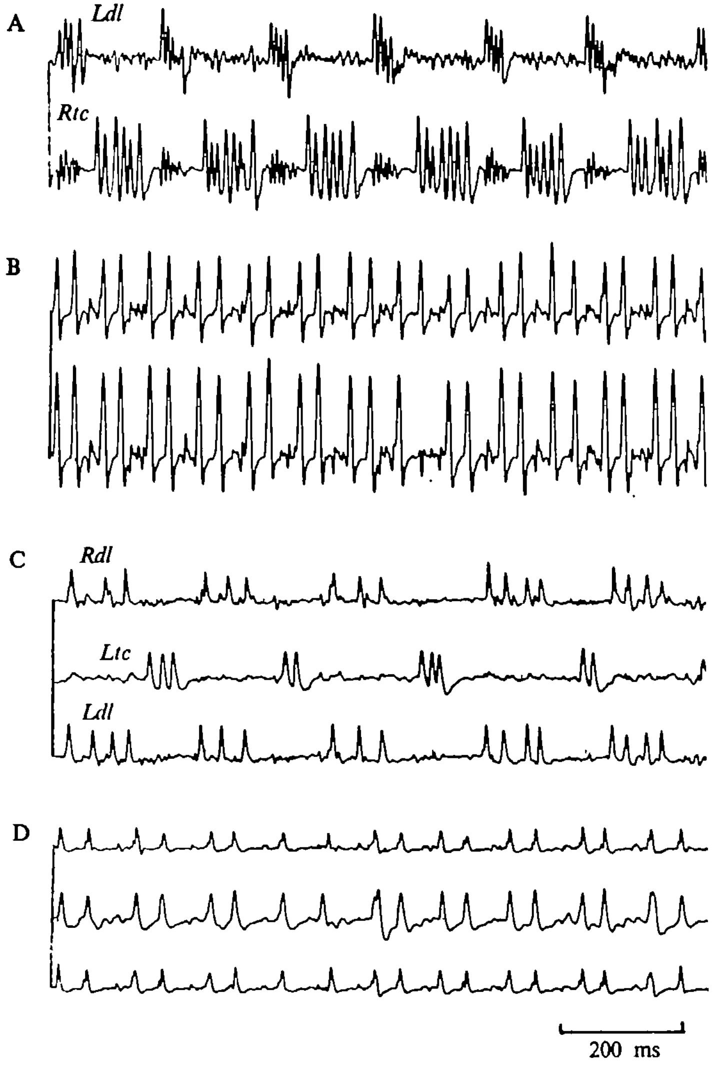

Fig. 1. Muscle potentials recorded extracellularly from *Antheraea polyphemus*. (A) Flight in adult. (B) Warm-up in adult, motor units as in (A). (C) Flight motor pattern from pupa 3 days prior to ecdysis. (D) Warm-up from pupa, motor units as in (C). Muscles: *L*, left; *R*, right; *dl*, dorsal longitudinal; *tc*, posterior tergocoxal (= dv_5 of Nüesch, 1953).

surface. The flight pattern was produced intermittantly and apparently spontaneously. Pupae were observed at various times of day – morning, afternoon, and evenings. Motor activity occurred at times other than those at which adult *A. polyphemus* moths are normally active, namely 23.00 to 24.00 h and 1 h before dawn (Rau & Rau, 1929).

The adult motor patterns of saturniid moths are distinctive. The flight pattern differs from those typical of several other insect groups in that during each wingbeat cycle a motor unit is excited by a small burst of impulses, rather than by one or two impulses (Kammer, 1967; Fig. 1 A). Furthermore, there is a positive correlation between cycle time and number of impulses per burst (Kammer, 1967), whereas in locusts the correlation is negative (Wilson & Weis-Fogh, 1962; Waldron, 1967). The

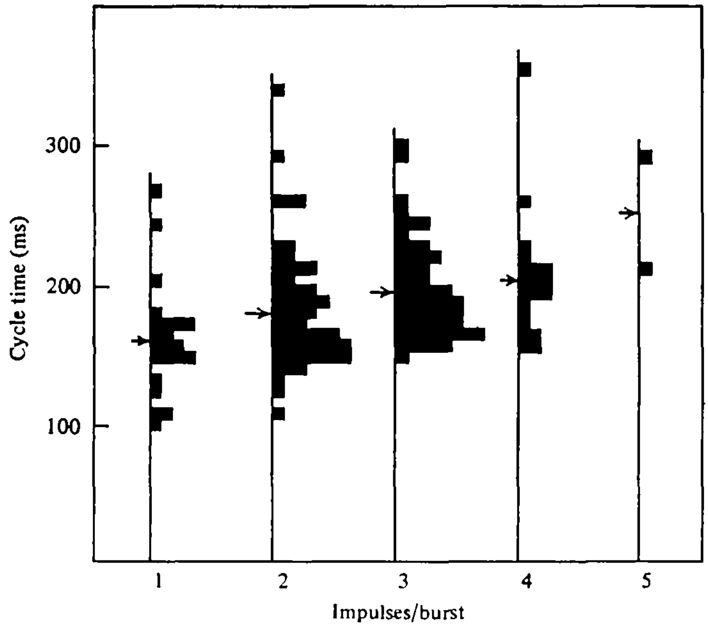

Fig. 2. Relationship between the number of impulses per burst (n) and cycle time in a pupa of *A. polyphemus*. For each n the distribution of cycle times observed is shown, and the mean cycle time is indicated by an arrow. The means for $n = 2$ and $n = 3$ are significantly different at the 0·05 level (t test) as are the means for $n = 3$ and $n = 4$.

flight motor pattern recorded from indirect flight muscles of pupae during the last few days of their development also possessed these distinctive features. In the pupal flight pattern, muscles which in adults are elevators and depressors of the wings during flight were excited alternately and with bursts of impulses (Fig. 1 C) and as in adults, there was a positive correlation between impulses per burst and cycle time (Fig. 2).

Although the major features of the pupal motor patterns were sufficiently similar to those of adult *A. polyphemus* that they could be readily classified as flight, there were some differences between the pupal and adult flight patterns. In pupae the cycle-time was usually greater than in adults, although in some cases pupal cycle-time approached that of adults (Figs. 3 B, 4). Pupal cycle-time was also more variable; that is, successive cycles varied markedly in length (Fig. 3 B), and a wider range of values occurred (Figs. 3 B, 4). Phase relationships in pupae were also more variable than those of adults, in terms of both the range of values which occurred during a bout of activity (Figs. 3 C, 4) and the values from cycle to cycle (Fig. 3 C). In addition to this greater variability of the pupal flight pattern, in pupae the phase of the elevator burst measured with respect to the depressor burst was usually greater than that of adults and sometimes greater than 0·5 (Fig. 4).

The warm-up motor pattern of adult saturniid moths differs from that of flight in several respects: the cycle time is shorter, there are only 1 or 2 impulses per burst, and muscles which are antagonists in flight are activated simultaneously (Kammer, 1967; Hanegan & Heath, 1970; Fig. 1 B). Such a motor pattern was observed in *A. polyphemus* pupae (Fig. 1 D). Like the pupal flight pattern, the pupal warm-up pattern had a longer cycle time and was more variable than that of the adult.

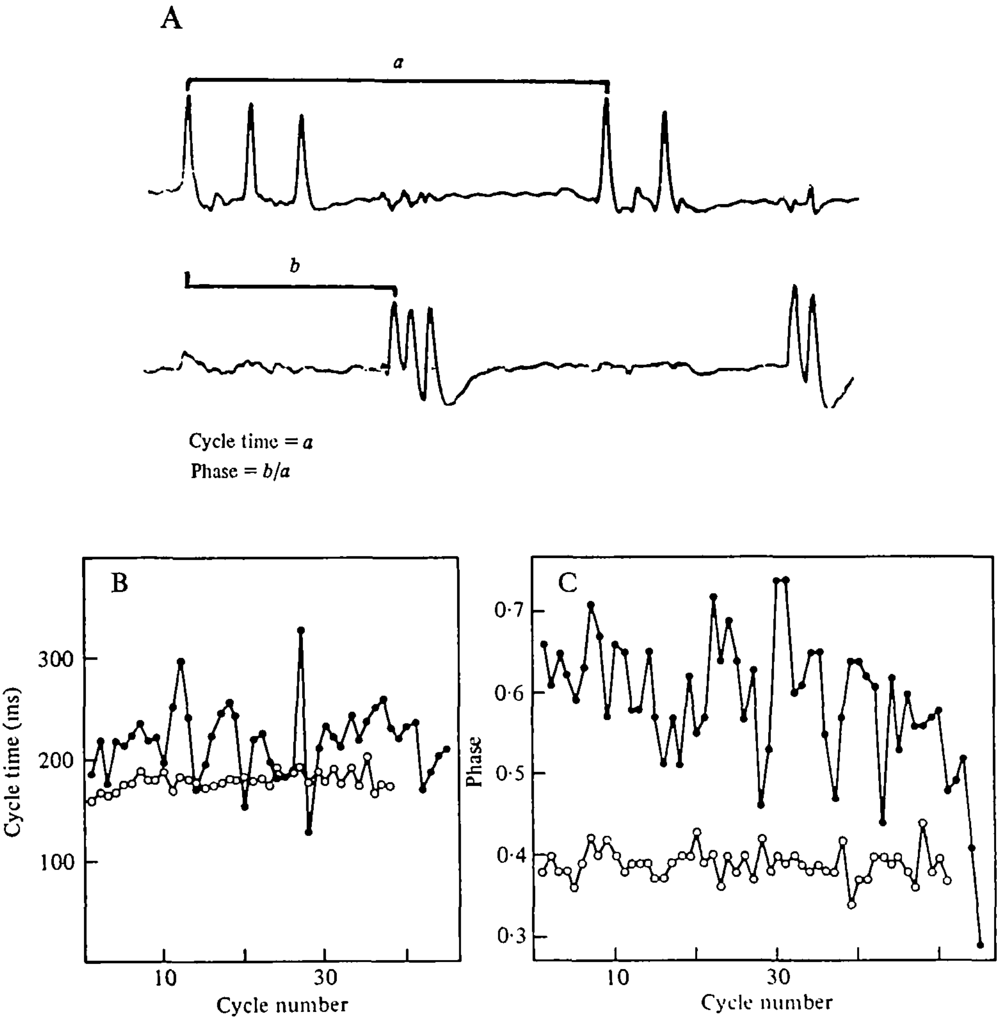

Fig. 3. Comparison of flight motor patterns from adult and pupal *A. polyphemus*. (A) Cycle time (*a*) and phase ($b.a^{-1}$) were measured as indicated. (B) Successive cycle times (abscissa is cycle number, starting from an arbitrary cycle in the middle of a bout of activity). Pupal cycle-times (●) are more variable and usually longer than those of the adult (○). (C) Sequence of phases in successive cycles of flight, showing that cycle-to-cycle variation is greater in the pupa (●) than in the adult (○).

Not all the activity observed fell neatly into one of these two categories of motor patterns. Sometimes motor units produced single impulses with no obvious relationship to the phase of other units (Fig. 5, *dl* and *tc*). Sometimes phase relationships were maintained but the pattern of interspike intervals did not resemble either warm-up or flight (Fig. 5, right and left *dl*). Animals spontaneously switched from patterned to unpatterned activity and from one pattern to another, or became quiescent. Since pupae which produced such apparently unpatterned activity also produced adult patterns, the activity cannot be attributed to incomplete development of the pattern-generating mechanism. It is possible that the activity corresponds to an adult behaviour

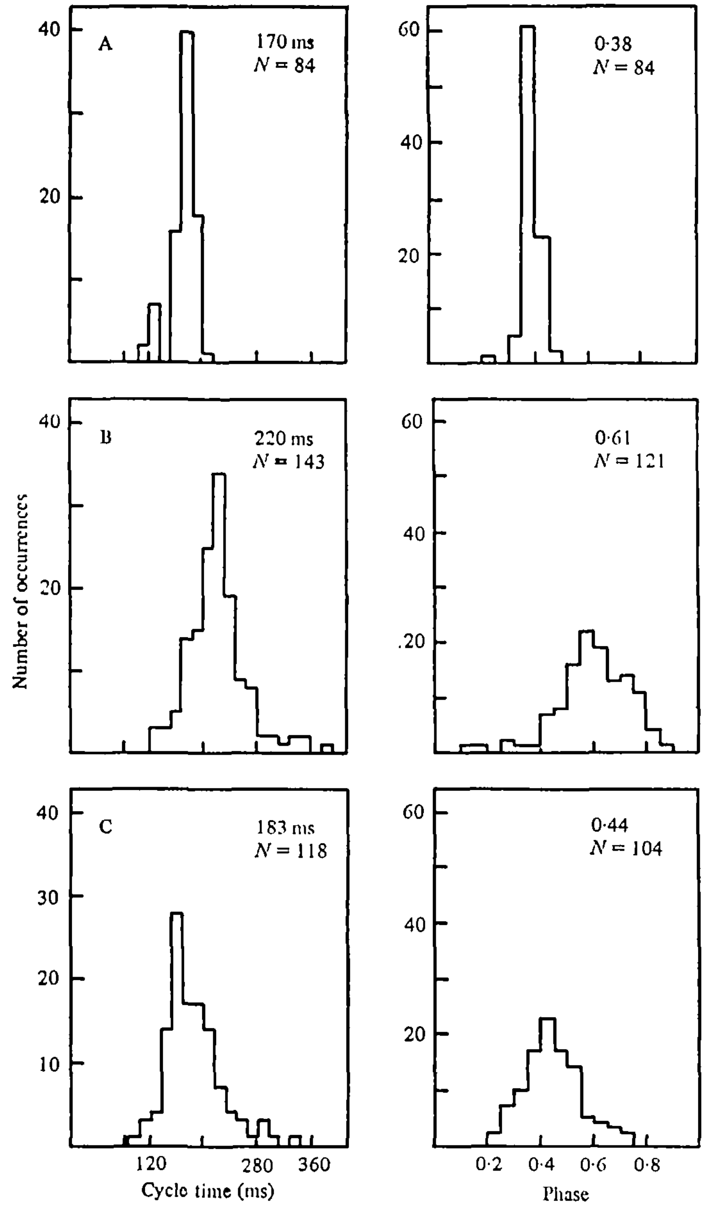

Fig 4. Histograms of cycle times (left) and phase relationships (right) in the flight motor patterns of *A. polyphemus*. (A) Adult. (B) Pupa 2 days before emergence (day 19). (C) Another more immature (day 16). Numbers in the upper right corners give the mean and the sample size.

which has not yet been analysed in neurophysiological terms, such as an eyespot display accompanied by wing vibrations (Blest, 1958), but since most of the muscles we have studied can move only the wings, there are few behavioural acts which might be considered.

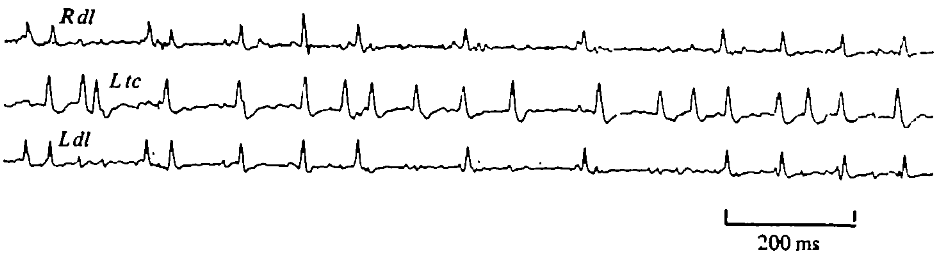

Fig. 5. Unpatterned activity from *A. polyphemus* pupa (same animal and abbreviations as in Fig. 1).

Development of motor patterns in A. polyphemus *pupae*

During the 21 days of pupal development, adult structures are gradually formed. In the dorsal longitudinal muscle, for example, cross-striations are visible on day 12 and the muscle is well developed by day 16, although fibre diameter continues to increase up to the time of eclosion (Bienz-Isler, 1968; Nüesch & Bienz-Isler, 1972).

To follow the development of motor patterns in the pupa, sterile wires were inserted dorsally into the thorax of six *A. polyphemus* pupae at different development stages, and recordings were made daily. Three of these pupae moulted the pupal cuticle in relatively normal fashion, suggesting that the experimental treatment did not seriously impair development. Post-mortem dissection of these adults plus the remaining pharate moths which were preserved prior to ecdysis showed that the muscles had developed around the wires, or, in a few cases, to one side of the wires, and the wires were coated with a layer of whitish tissue. In these chronic preparations, recording conditions were less favourable than in recently made preparations; the potentials recorded were small (less than 1 mV) and often reflected the activity of more than one unit. Because the signals were small, we could easily have failed to detect activity that was in fact present, particularly in the earlier days of development. The following description of the developmental sequence thus should be viewed as a minimal approximation of the actual sequence.

As adult development proceeded, there was a gradual increase in the amount of activity recorded and in the number of different units visible. The sequence observed in *A. polyphemus* (Figs. 6, 7) can be arbitrarily divided into stages: (1) isolated, small potentials, first seen on day 6; (2) small potentials in clusters, seen on day 10; (3) small bursts of similar potentials, initially repeated only a few times and then occurring rhythmically and for longer times, with antagonistic flight muscles in increasingly regular antiphase, characteristic of the flight pattern (days 12–13); (4) on day 15, clear warm-up and flight patterns were both apparent. During the last week a given pattern was produced for increasing lengths of time, but the pattern often changed relatively quickly from the flight to warm-up to unpatterned activity and back again. Also during the last week of development there occurred intermittently potentials which were 3 or 4 times larger than other units active concurrently (Fig. 7, day 14). These large potentials were not seen to participate in any patterns, and their origin remains unexplained. It is unlikely that they are electrical artifacts, since their time course resembled that of the smaller units, and they did not appear on other channels being recorded simultaneously. They may represent the occasional synchronous firing

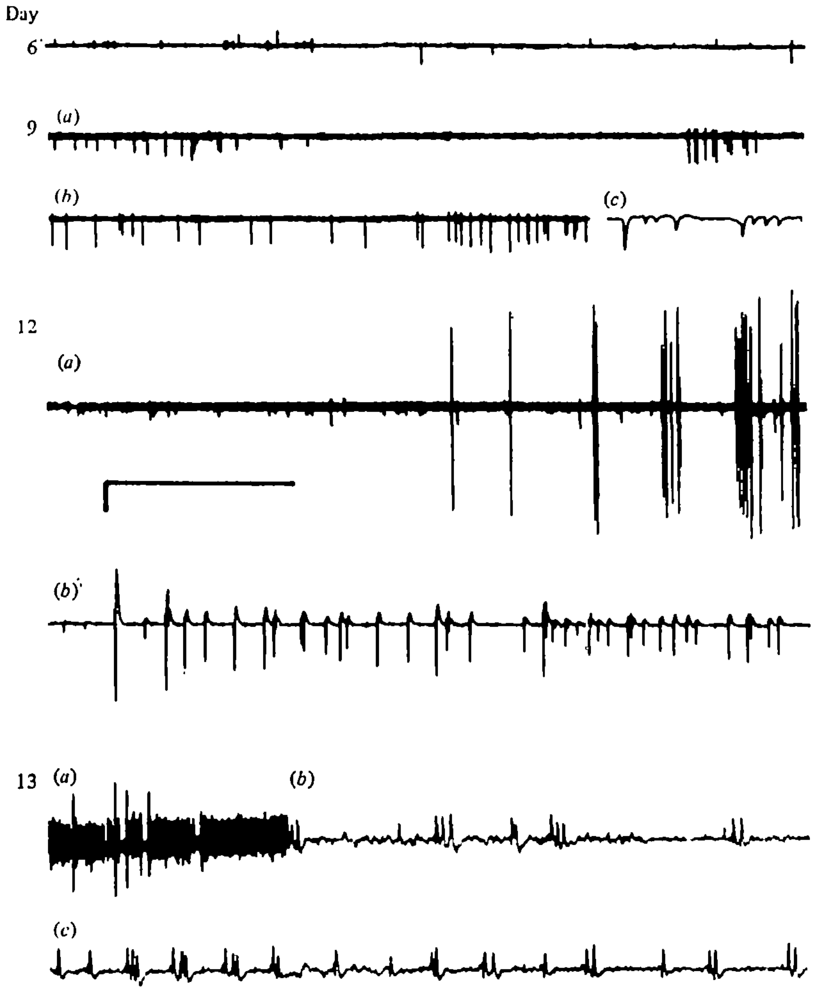

Fig. 6. Development of motor patterns in a pupa of *A. polyphemus*. The stage of development is given in terms of day number; full development of pupa to adult typically requires 21 days. In this animal rhythmic bursts were observed on day 13 but not on day 12, and there was also more activity in the older pupa. These traces were obtained with differential recording; some of the potentials probably were recorded from the dorsal longitudinal muscle (cf. Fig. 7). The time marked corresponds to 5 min in all records except 9(*c*), 12 (*b*), and 13 (*b*, *c*), in which it corresponds to 1 s. Voltage calibration is very approximately 0·5 mV for day 6, 1·0 mV for day 9, and 0·25 mV for the remainder.

of a number of units, or the activation of units which do not otherwise participate in the pupal patterns.

In summary, activity was recorded for 10–12 days in *A. polyphemus* pupae, showing that the developing muscles receive electrical signals from the central nervous system for at least half of the pupal development period. Bursts of potentials (i.e. the beginnings of the flight pattern) were observed 7 days prior to emergence, and full adult patterns, both flight and warm-up (although with a longer cycle-time) were seen during the 6 days before the day of adult eclosion.

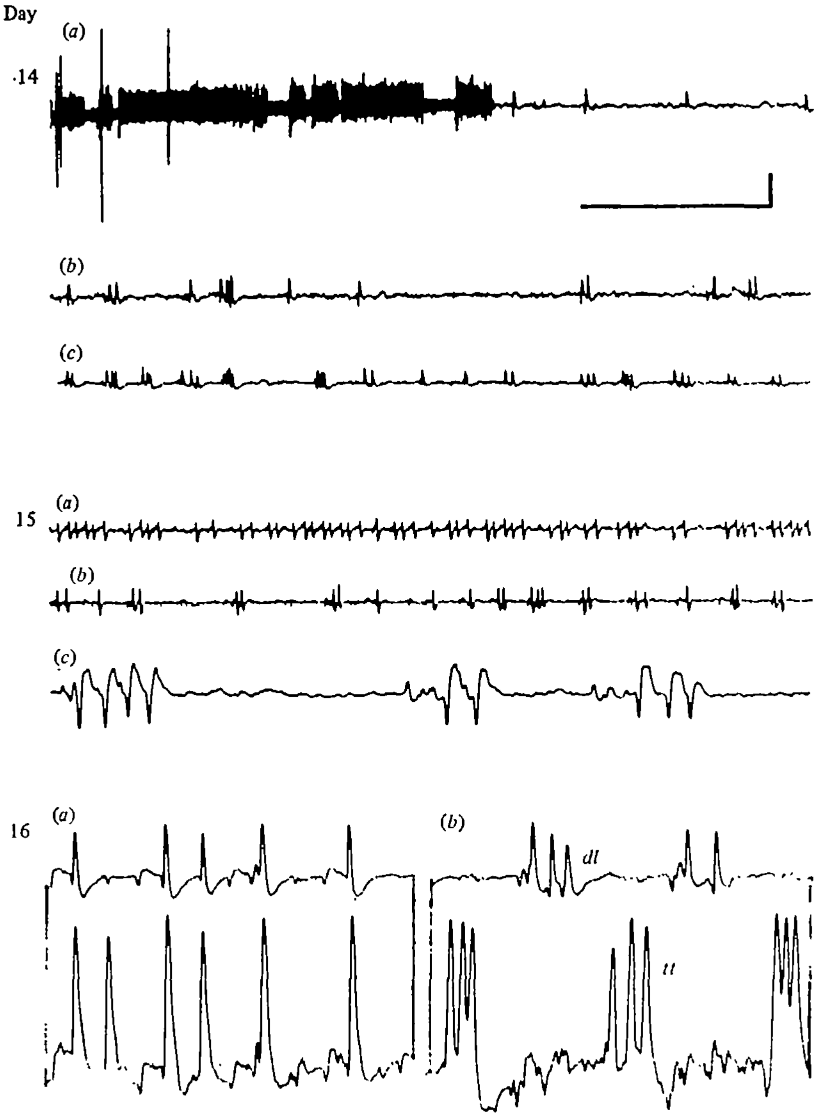

Fig. 7. Continuation of Fig. 6. Flight-like bursts (15*c*) were produced, mixed irregularly with single or double firings (14*c*, 15*b*). The warm-up motor pattern is apparent on day 15 (*a*). On day 16 a new electrode was inserted into an elevator muscle (*tt*, tergotrochanteral); the dorsal longitudinal muscle potentials (*dl*) were recorded with a chronically implanted wire. (This recording lead was probably detecting the activity of the dorsal longitudinal muscle in the other records shown in this and the preceding figure.) Patterns clearly resemble adult warm-up (16*a*) and flight (16*b*). Time mark: 5 min for day 14(*a*) (left half of record); 1 s for days 14(*a*) (right half of record) (*b*, *c*), 15(*a*, *b*); 200 ms for days 15(*c*), 16; approximate voltage: 0·1 mV for days 14(*a*, *b*), 15(*c*); 0·25 mV for days 14(*c*), 15(*a*, *b*); unknown for day 16.

Motor patterns from pupae of Antheraea pernyi

A. pernyi is closely related to *A. polyphemus*, and the dorsal longitudinal muscle develops in a similar sequence in both species (Bienz-Isler, 1968; Bienz-Isler & Nüesch, 1972). Electrical activity was recorded from three *A. pernyi* pupae which

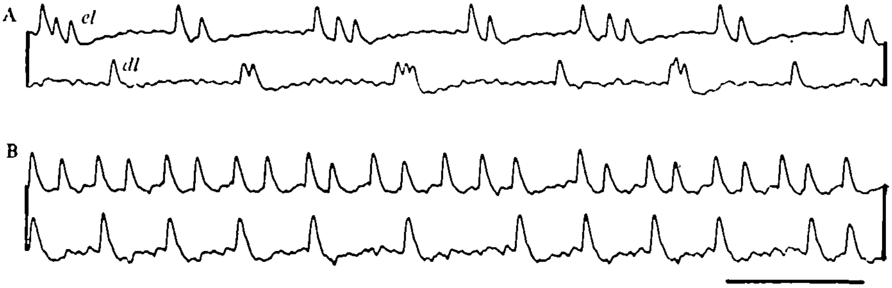

Fig. 8. Muscle potentials from a pupa of *Antheraea pernyi* a few days before eclosion. (A) Flight motor pattern. (B) Warm-up pattern from the same motor units; in this example there is less activity than normal in the dorsal longitudinal muscle (*dl*). *el*, Elevator muscle; time mark 200 ms.

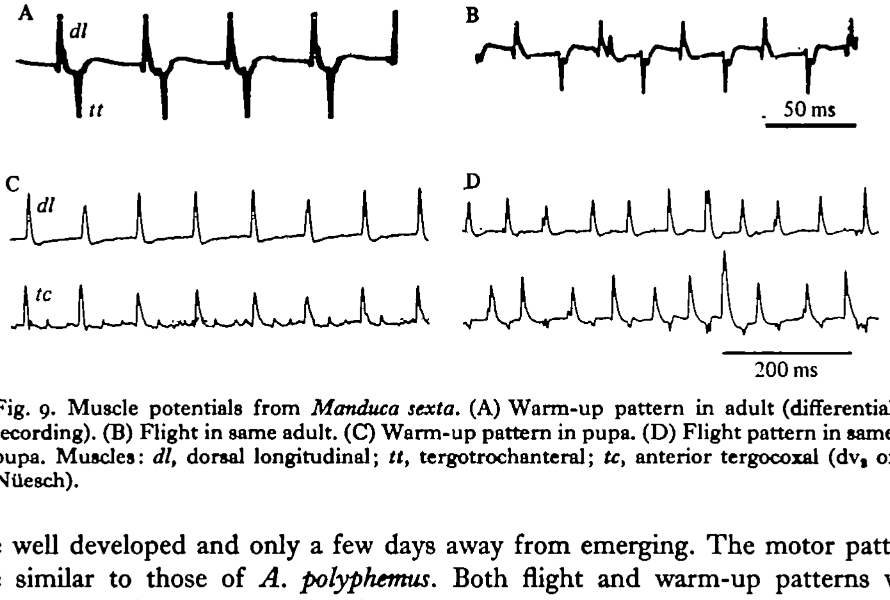

Fig. 9. Muscle potentials from *Manduca sexta*. (A) Warm-up pattern in adult (differential recording). (B) Flight in same adult. (C) Warm-up pattern in pupa. (D) Flight pattern in same pupa. Muscles: *dl*, dorsal longitudinal; *tt*, tergotrochanteral; *tc*, anterior tergocoxal (dv, of Nüesch).

were well developed and only a few days away from emerging. The motor patterns were similar to those of *A. polyphemus*. Both flight and warm-up patterns were observed (Fig. 8). As in *A. polyphemus*, *A. pernyi* produced motor patterns intermittently throughout the day and evening, not just during the normal activity period, which extends a few hours before and after midnight (Truman, 1974).

Motor patterns from pupae of Manduca sexta

Hawkmoth adults, like saturniids, exhibit both warm-up and flight motor patterns. During flight in hawkmoths, however, the power-producing muscles are excited with one or a pair of impulses per wingbeat rather than with a burst as in the saturniids (Kammer, 1968, 1971). The warm-up pattern of hawkmoths consists of a single impulse per cycle, with sets of antagonistic muscles firing simultaneously (Kammer, 1968, 1970) and at cycle-times similar to those of flight at the same body temperature (Heinrich & Bartholomew, 1971).

Both the warm-up and the flight patterns characteristic of adult hawkmoths were produced by *Manduca* pupae during the several days before ecdysis (Fig. 9). In this

Table 1. *Comparison of adult and pupal motor patterns in* Manduca sexta

(Each mean was derived from the performance of one individual; population values vary by approximately 10%.)

Motor pattern	Mean cycle-time (ms)	*N*	Coefficient of variation
Adult flight	51	123	2·5
Adult warm-up	63	31	2·5
Pupal flight	101	69	11.8
Pupal warm-up	89	25	3·8
	Mean phase		
Adult flight	0·4	123	4·9
Pupal flight	0·5	70	18·2

species the cycle-time of warm-up was similar to the cycle-time of flight in both adults and pupae, although pupal cycle-times were much longer than those of adults. Pupal patterns were also more variable than those of adults, as indicated by the greater coefficient of variation of the cycle-times (Table 1). Pupal phase relationships were also more variable than those of adults.

Preliminary information about the development of motor patterns in *Manduca* was obtained by chronic recording from three pupae. A sequence similar to that in *A. polyphemus* was observed: (1) single, small potentials occurring irregularly; (2) slightly larger potentials and more activity; (3) more regularity, i.e. potentials evenly spaced; and (4) appearance of antagonistic units (Fig. 10). Also as in *A. polyphemus* there were intermittent, large potentials of unexplained origin. Brief bouts of irregular activity were recorded on the first day of observation, which was 8–9 days before ecdysis. The flight pattern, with an antagonistic unit in antiphase, was first observed 4 days prior to ecdysis.

Effects of sensory input

To assess the possibility that pupal flight patterns differed from adult patterns because of the lack of appropriate phasic sensory input from the wings which in pupae are folded and restrained within the pupal cuticle, we recorded muscle potentials from adults with immobilized wings. Some animals were fastened to a dish with their wings pinned down horizontally, and others were fastened to a rod by wax on the coxae and the wings hand-held either fully depressed or fully elevated. With both methods bursts continued to be produced rhythmically (Fig. 11). With the latter method activity in depressor and elevator muscles was typically asymmetrical, as the animal attempted to counter the restraint (Fig. 11C, D). Particularly notable was the absence or reduction of activity in the dorsal longitudinal muscle relative to elevator muscles when the wings were held depressed (Fig. 11C, D), since this wing position was the closest approximation to the pupal wing position. In restrained moths cycle time was variable but greater than in normal flight and also greater than in the flight pattern of pupae. The number of impulses per burst was also greater than in normal flight, but inter-spike intervals within the burst were typically short. In the latter two respects the motor patterns elicited by wing-holding do not correspond with the pupal flight patterns, suggesting that sensory input from the wings of a pharate moth, or the lack thereof, influences the flight pattern-generator differently than does the input from

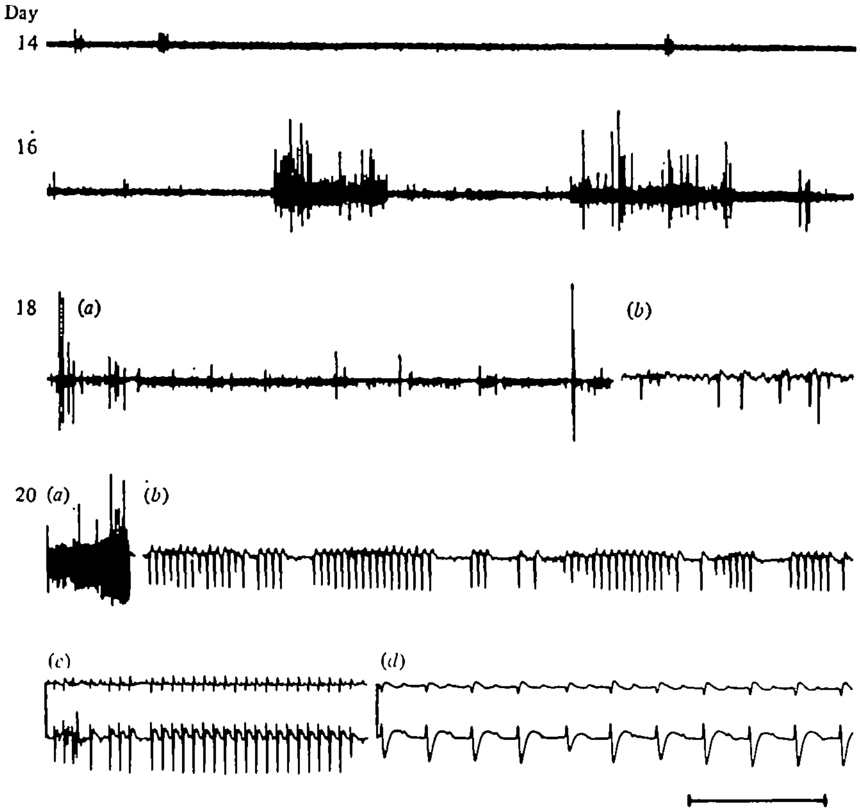

Fig. 10. Development of motor patterns in *Manduca sexta*. Recordings from the same pupa on the days indicated (pupation occurred on day 0 and emergence on day 21). In records (*c*) and (*d*) from day 20 the top trace was obtained by an additional electrode which detected the activity of the dorsal longitudinal and a more distant elevator muscle. The lower trace in day 20(*c*, *d*) also represents activity in the dorsal longitudinal muscle; since the same electrode was employed on previous days and for record 20(*a*, *b*), some of the activity probably came from this muscle. Time mark: 5 min for days 14, 16, 18(*a*) and 20(*a*); day 18(*b*), 1 s (gain is 5 times greater than in (*a*)); day 20(*b*) and (*c*), 1 s; day 20(*d*), 200 ms.

immobilized adult wings. However, since in both pupae and wing-held adults the cycle-time is longer than in freely flapping adults, it is possible that some wing receptors are exerting a similar control on cycle time in both cases.

DISCUSSION

Neural activity and behaviour

Several years ago the question of whether pupae exhibited any spontaneous neural activity or were entirely quiescent was debated (Van der Kloot, 1955; Schoonhoven, 1963; Tyshchenko, 1964; Tyshtchenko & Mandelstam, 1965). From our results it is now clear that the nervous system of pupae is indeed active and, in fact, is producing patterned neural output in the absence of overt behaviour. Such activity is surprising even when pupae are considered to be pharate ('cloaked') moths (Hinton, 1964).

Previous studies showed that pharate moths prematurely removed from the pupal cuticle could be induced to exhibit adult behaviour, such as leg movements, shivering,

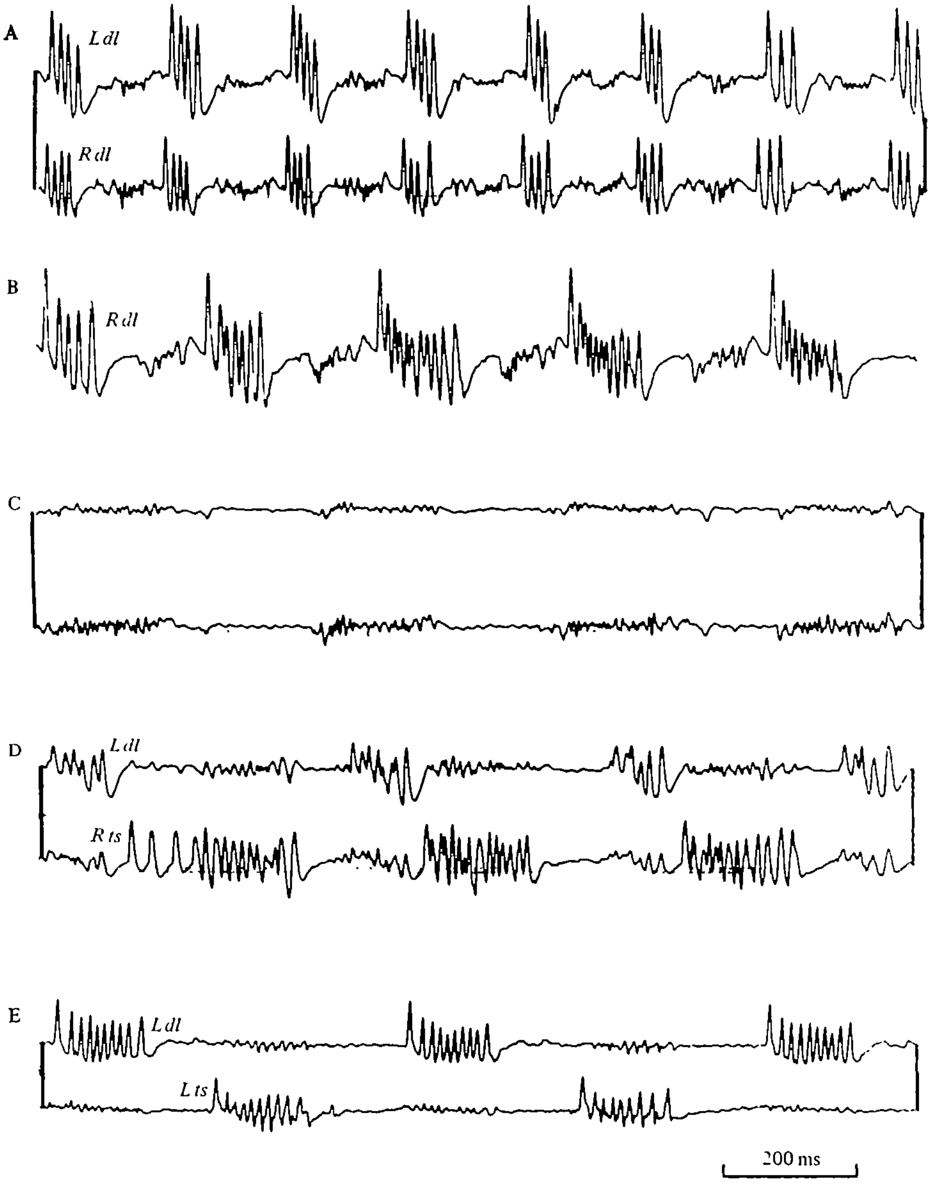

Fig. 11. Muscle potentials from adult *A. polyphemus*, showing that preventing wing movements results in long cycle times but not in pupal patterns. (A) Fixed flight, wings free to move (*Ldl*, *Rdl*, left and right dorsal longitudinal muscle). (B) Wings held up, more activity in wing depressor muscle (same *Rdl* as in (A)). (C) Wings held down, no activity in left or right depressor units; small potentials represent activity in distant elevator muscles (same preparation as in (A)). (D) Wings held down, some activity in depressor muscle (left *dl*), more activity in an elevator muscle (*Rts*, right tergosternal [= dv_1 of Nüesch]). (E) Wings pinned horizontally, symmetrical activity in depressor (left *dl*) and elevator (*Lts*, left tergosternal) muscles.

and wing flapping (Blest, 1960; Truman, 1971). Adult behaviour could be elicited only on certain days of the pupal instar and not on the day before the time of the normal terminal ecdysis. These behavioural observations are in accord with our conclusion based on electrical recordings that adult motor patterns develop gradually and are produced prematurely.

Gradual development of adult motor patterns has also been observed in several species of Orthoptera (Bentley & Hoy, 1970; Kutsch, 1971, 1974*a*, *b*; Weber, 1972; Bentley, 1973; Altman & Tyrer, 1974; Altman, 1975). Motor activity recorded from nymphs in the flight posture only partially resembles that of adult flight; for example, in both crickets and locusts correct phase relationships are established relatively late. The flight motor patterns in these orthopterans have been observed by suspending the nymphs in an airstream, and it is not known if the flight patterns are produced under natural circumstances (e.g. following a jump). In moth pupae, on the other hand, it is clear that the motor patterns are produced spontaneously, as part of the normal developmental sequence.

The pupal motor patterns are remarkably similar to those of adults. Sphingid moths such as *Manduca* have narrow wings and relatively high wingbeat frequencies (for Lepidoptera). Saturniid moths have broad wings, low wingbeat frequencies and a distinctive motor pattern. Both species of saturniid pupae produced the saturniid flight pattern, not just alternating activity of antagonistic muscles. In addition, bursts of several spikes, characteristic of saturniid flight, could be seen early in development, before much steady, rhythmic activity occurred. On the other hand, *Manduca* pupae produced the hawkmoth flight pattern, and no clusters of bursts were seen during their development. In addition to flight motor patterns, each of the three species produced the warm-up motor pattern specific to its family.

Role of sensory input

Important questions for which only partial answers can be given concern the role of sensory feedback in the production of adult motor patterns and in the development of the central nervous system. In mature locusts sensory input from mechanoreceptors in the wings and a stretch receptor at the base of each wing influences the centrally generated motor pattern in several respects, including cycle-time, number of spikes per burst, and phase relationships within a single cycle (Wilson & Gettrup, 1963; Waldron, 1967; Wendler, 1974; Kutsch, 1974*b*; Burrows, 1975). Development of the basic pattern-generating mechanism, however, appears to be independent of this sensory feedback, since elements of the pattern are produced while the nymphal wings are immobile rudiments (Bentley & Hoy, 1970; Kutsch, 1971; Weber, 1972; Altman, 1975), and development proceeds normally in young adults prevented from spreading their wings and practising (Kutsch, 1971; Altman, 1975). On the other hand the nymphal patterns do differ from those of the adult. In immature crickets induced to assume flight posture, the cycle time is longer than in adults, and in early instars the phase relationships among different units are incorrect (Bentley & Hoy, 1970; Weber, 1972; Bentley, 1973). In nymphs of a locust *Chortoicetes terminifera* there is rhythmic activity in flight motor units at about the adult flight frequency (unlike moths), but correct phase relationships are established only gradually and relatively late (Altman

& Tyrer, 1974; Altman, 1975). According to Altman (1975), some of the differences between mature and immature patterns reflect absent or non-adult sensory input.

In adult Lepidoptera the effects of sensory input on flight have not been well studied. The wings are supplied with numerous sensilla (Vogel, 1911, 1912; Cartheuser, 1974). Muscle stretch receptor organs have not been observed in the adult thorax although they are present in association with dorsal muscles in the caterpillar (Finlayson & Lowenstein, 1955). In addition, it is possible, although not established, that moths possess a stretch receptor associated with the wing base, as in locusts.

Two observations indicate that sensory input can modify flight motor patterns in adult moths. Cutting the large sensory nerves from the wings increases the flight cycle-time (Kammer, 1967; Hanegan, 1972). Holding the wings immobile, thus producing abnormal sensory input, also modifies the flight pattern (Fig. 11). Conversely these experiments also show that in adult moths production of a recognizable flight motor pattern does not depend on phasic feedback from the wing receptors.

Most probably any sensory feedback received by the pupal nervous system differs markedly from that of the adult. The developing wings of pharate moths are soft and very flexible. They are folded ventrally and may be at least partially dissociated from movements of the notum. These anatomical considerations lead us to favour the hypothesis that the neuronal mechanisms for producing flight and warm-up patterns develop independently of normal sensory feedback.

The differences between pupal and adult patterns (i.e. longer cycle time and greater variability) remain unexplained. They could be due to lack of normal sensory input in the pupa, or they could reflect developmental processes within the central nervous system, or some combination of these factors could pertain. Since restraining the wings of adults increased the cycle-time, it is possible that the longer cycle-times in the pupa are due in part to sensory input from immature wings folded within the pupal cuticle. Since cutting the wing nerves in the adult specifically produced a longer cycle time (Kammer, 1967; Hanegan, 1972), the longer cycle-times seen in pupae may also be due to lack of sensory input from the wings. The longer cycle-times observed in pupae cannot be explained by a difference in temperature between 'flying' pupae and flying adults. In *Manduca sexta* adults the flight cycle-time decreases with increasing temperature; these moths typically fly with a thoracic temperature of 38–40 °C (Heinrich, 1971) and a cycle-time of 40–50 ms. In moths forced to flap their wings with a body temperature of 25 °C the cycle time is about 65 ms (Heinrich & Bartholomew, 1971). *Manduca* pupae at that temperature had a cycle time of 101 ms, about 1·6 times longer than would be predicted on the basis of body temperature.

In summary, the available data suggest that, although adult flight patterns are modulated by sensory feedback, the pattern-generating mechanism probably develops in the absence of normal sensory input. The data do not allow us to discern which of the differences between pupal and adult patterns, particularly the long cycle-time, may be attributed to immaturity of the basic pattern-generating mechanism and which are due to differences in sensory input. These questions await direct manipulation of the developing nervous system.

Development of muscles and neurones

The development of one of the muscles from which we have recorded, the dorsal longitudinal muscle, has been studied in *A. polyphemus* and *A. pernyi*. In diapausing pupae this muscle is present as a thin strand of cells probably retained from a larval muscle (Eigenmann, 1965; Bienz-Isler, 1968). In the first days of development myoblasts fuse with this anlage (Stocker, 1974). On the 9th day the muscle reacts to electrical stimulation with a weak local contraction (Basler, 1969), but it is not clearly cross-striated (Eigenmann, 1965; Basler, 1969) and the T-system is only beginning to form (Bienz-Isler, 1968; Nüesch & Bienz-Isler, 1972). On the 12th day, the muscle is clearly cross-striated, and a repetitive electrical stimulus evokes a tetanus (Basler, 1969). By day 16 the basic structure is finished, and on successive days there is additional growth in number of A-filaments and mitochondria (Bienz-Isler, 1968).

The motor neurones which supply the adult mesothoracic dorsal longitudinal muscle of *Manduca sexta* are retained from the larva (Casaday, 1975; Rheuben and Kammer, in prep.). Development of the motor neurones in the pupa involves a gradual increase in the size of the dendritic field from day 6 (when the first stained preparations from pupae were obtained) to the adult (Casaday, 1975).

The adult neuromuscular junctions of the dorsal longitudinal muscle in *A. polyphemus* develop from new sprouts of the motor axons. Stocker & Nüesch (1975) observed rather unspecialized nerve–muscle contacts in diapausing pupae. By day 4 structures with most of the elements of a synaptic junction were seen. By day 10 the synapses resembled those of the adult; subsequent changes reflected further differentiation and elaboration of the synaptic complex (Stocker & Nüesch, 1975).

In these developing muscle fibres, end-plate potentials could be recorded by day 13 (Kaufmann, 1971), but no successful recordings were made earlier. Contraction could be evoked by ganglion stimulation on day 10 (Nüesch, 1965), suggesting that the junctions may be functional at least by then (barring spread of stimulating current and direct stimulation of the muscle fibres). Intracellularly recorded end-plate potentials were seen to increase in size during development after day 13, but only on day 21, the last pupal day, were full-size end-plate potentials plus active membrane responses observed (Kaufmann, 1971).

Some tentative comparisons can be made between these findings and the results of the present study. The increase in amplitude of the end-plate potentials observed by Kaufmann (1971) is consistent with the increase in muscle potentials which we recorded extracellularly. However, because of the limitations of our recording technique, we cannot be sure of the source of the potentials recorded early in development. It is clear that the muscles are excited via their motor neurones several days before the complete complement of contractile proteins is present and before the neuromuscular junction is mature. It appears that the developing muscles are excited via their motor neurones shortly after the T-system begins to form. Similarly, the developing motor neurones are active before the growth of their dendritic trees is complete, and before they can elicit large depolarizations of the muscle fibres.

What functional significance the patterned neural activity may have, if any, is not clear. Possibly patterned activity is essential for the maturation of the muscles and/or the central nervous system. One set of results suggests that it is not important, at least in the gross morphological development of the muscles. Tetrodotoxin causes flacci paralysis of moth pupae, and moths so paralysed early in the pupal stage developed flight muscles which were normal in appearance (Williams, 1969; Lockshin, 1971). However, the mechanism of paralysis is not clear. Since tetrodotoxin blocks sodium spikes in insect axons (Narahashi, 1965), it is possible (but not yet demonstrated) that the muscles of the paralysed pupae developed in the absence of action potentials in the motor axons. It is known that innervation of the flight muscles is required for development of the complete muscle mass (Nüesch, 1952, 1968), but perhaps the neural influence does not depend on electrical activity. Whether or not patterned activity participates in the development of synaptic connexions in the moth central nervous system remains an unanswered question.

This work was done partly during the tenure by M.B.R. of a Research Fellowship of the Muscular Dystrophy Associations of America, and was supported in part by U.S.P.H.S. Grant 2R01 NS 08996 to M. J. Cohen. Laboratory facilities were provided by the Marine Biological Laboratory, Woods Hole, Massachusetts.

REFERENCES

ALTMAN, J. S. (1975). Changes in the flight motor pattern during the development of the Australian plague locust, *Chortoicetes terminifera*. *J. Comp. Physiol.* 97, 127–42.

ALTMAN, J. S. & TYRER, N. M. (1974). Insect flight as a system for the study of the development of neuronal connections. In *Experimental Analysis of Insect Behaviour* (ed. L. Barton Brown), pp. 159–79. New York, Heidelberg, Berlin: Springer-Verlag.

BASLER, W. (1969). Untersuchung der Nervenwirkung bei *Antheraea pernyi* Guer. (Lep.). *Rev. Suisse Zool.* 76, 297–362.

BENTLEY, D. R. (1973). Postembryonic development of insect motor systems. *Developmental Neurobiology of Arthropods* (ed. D. Young), pp. 147–77. Cambridge University Press.

BENTLEY, D. R. & HOY, R. R. (1970). Postembryonic development of adult motor patterns in crickets: a neural analysis. *Science, N.Y.* 170, 1409–11.

BIENZ-ISLER, G. (1968). Elektronenmikroskopische Untersuchungen über die Entwicklung der dorsolongitudinalen Flugmuskeln von *Antheraea pernyi* Guer. (Lepidoptera): II. *Acta Anat.* 70, 524–33.

BLEST, A. D. (1958). Some interactions between flight, protective display, and oviposition behaviour in *Callosamia* and *Rothschildia* spp. (Lepidoptera, Saturniidae). *Behaviour* 13, 297–318.

BLEST, A. D. (1960). The evolution, ontogeny and quantitative control of settling movements of some New World saturniid moths, with some comments on distance communication by honeybees. *Behaviour* 16, 188–253.

BURROWS, M. (1975). Monosynaptic connections between wing stretch receptors and flight motoneurones of the locust. *J. exp. Biol.* 62, 189–219.

CARTHEUSER, C. F. (1974). Zur Funktion der Sinneskuppeln auf den Schmetterlingsflügeln. *Naturwissenschaften* 61, 132.

CASADAY, G. B. (1975). Neurodevelopment in a thoracic ganglion of a moth: identified motor neurons and microanatomical structure. Ph.D. thesis, Cornell University.

EIGENMANN, R. (1965). Untersuchungen über die Entwicklung der dorsolongitudinalen Flugmuskeln von *Antheraea pernyi* Guer. (Lepidoptera). *Rev. Suisse Zool.* 72, 789–840.

ELSNER, N. & HUBER, F. (1974). Neurale Grundlagen artspezifischer Kommunikation bei Orthopteren. *Fortschr. Zool.* 22, 1–48.

FINLAYSON, L. H. & LOWENSTEIN, O. (1955). A proprioceptor in the body musculature of Lepidoptera. *Nature, Lond.* 176, 1031.

HANEGAN, J. L. (1972). Pattern generators of the moth flight motor. *Comp. Biochem. Physiol.* 41A, 105–13.

HANEGAN, J. L. & HEATH, J. E. (1970). Temperature dependence of the neural control of the moth flight system. *J. exp. Biol.* 53, 629–39.

HEINRICH, B. (1971). Temperature regulation of the sphinx moth, *Manduca sexta*. I. Flight energetics and body temperature during free and tethered flight. *J. exp. Biol.* 54, 141–52.

HEINRICH, B. & BARTHOLOMEW, G. A. (1971). An analysis of pre-flight warm-up in the sphinx moth, *Manduca sexta*. *J. exp. Biol.* 55, 223–39.

Hinton, H. E. (1946). Concealed phases in the metamorphosis of insects. *Nature, Lond.* **157**, 552–3.

Kammer, A. E. (1967). Muscle activity during flight in some large Lepidoptera. *J. exp. Biol.* **47**, 277–95.

Kammer, A. E. (1968). Motor patterns during flight and warm-up in Lepidoptera. *J. exp. Biol.* **48**, 89–109.

Kammer, A. E. (1970). A comparative study of motor patterns during pre-flight warm-up in hawkmoths. *Z. vergl. Physiol.* **70**, 45–56.

Kammer, A. E. & Rheuben, M. B. (1974). Adult motor patterns recorded from the muscles of moth pupae. *Am. Zool.* **14**, 1272.

Kaufmann, L. (1971). Untersuchungen zur Funktion der Flugmuskulatur von *Antheraea polyphemus* (Lep.) während der Imaginalentwicklung. *Rev. suisse Zool.* **78**, 1007–36.

Kutsch, W. (1971). The development of the flight pattern in the desert locust, *Schistocerca gregaria*. *Z. vergl. Physiol.* **74**, 156–68.

Kutsch, W. (1973). The influence of age and culture temperature on the wingbeat frequency of the migratory locust, *Locusta migratoria*. *J. Insect Physiol.* **19**, 763–72.

Kutsch, W. (1974*a*). The development of the flight pattern in locusts. In *Experimental Analysis of Insect Behaviour* (ed. L. Barton Brown), pp. 149–58. New York, Heidelberg, Berlin: Springer-Verlag.

Kutsch, W. (1974*b*). The influence of the wing sense organs on the flight motor pattern in maturing adult locusts. *J. comp. Physiol.* **88**, 413–24.

Lockshin, R. A. (1971). Programmed cell death: nature of the nervous signal controlling breakdown of intersegmental muscles. *J. Insect Physiol.* **17**, 149–58.

Miller, P. L. (1974). Rhythmic activities and the insect nervous system. In *Experimental Analysis of Insect Behaviour* (ed. L. Barton Brown), pp. 114–38. New York, Heidelberg, Berlin: Springer-Verlag.

Narahashi, T. (1965). The physiology of insect axons. In *The Physiology of the Insect Central Nervous System* (ed. J. E. Treherne and J. W. L. Beamont), pp. 1–20. New York and London: Academic Press.

Nüesch, H. (1952). Über den Einflüsse der Nerven auf die Muskelentwicklung bei *Telea polyphemus* (Lepid.). *Rev. Suisse Zool.* **59**, 294–301.

Nüesch, H. (1953). The morphology of the thorax of *Telea polyphemus* (Lepidoptera). *J. Morph.* **93**, 589–609.

Nüesch, H. (1965). Die Imaginalentwicklung von *Antheraea polyphemus* Cr. (Lepidoptera). *Zool. Jb. Anat.* **82**, 393–418.

Nüesch, H. (1968). The role of the nervous system in insect morphogenesis and regeneration. *A. Rev. Entomol.* **13**, 27–44.

Nüesch, H. & Bienz-Isler, G. (1972). Die Entwicklung denervierter Imaginalmuskeln bei *Antheraea polyphemus* (Lep.) und die Frage der entwicklungsfordernden Nervenwirkung. *Zool. Jb. Anat.* **89**, 333–50.

Rau, H. P. & Rau, N. (1929). The sex attraction and rhythmic periodicity in giant saturniid moths. *Trans. Acad. Sci., St Louis* **26**, 83–221.

Schoonhoven, L. M. (1963). Spontaneous electrical activity in the brains of diapausing insects. *Science, N.Y.* **141**, 173–4.

Snodgrass, R. E. (1954). Insect metamorphosis. *Smithsonian Misc. Coll.* **122** (9), 1–124.

Stocker, R. F. (1974). Elektronenmikroskopische Beobachtungen über die Fusion myogener Zellen bei *Antheraea polyphemus* (Lepidoptera). *Experientia* **30**, 896–8.

Stocker, R. F. & Nüesch, H. (1975). Ultrastructural studies on neuromuscular contacts and the formation of junctions in the flight muscle of *Antheraea polyphemus* (Lep.). I. Normal adult development. *Cell Tiss. Res.* **159**, 245–66.

Truman, J. W. (1971). The physiology of insect ecdysis. I. The eclosion behaviour of saturniid moths and its hormonal release. *J. exp. Biol.* **54**, 804–14.

Truman, J. W. (1974). Physiology of insect rhythms. IV. Role of the brain in the regulation of the flight rhythm of the giant silkmoths. *J. comp. Physiol.* **95**, 281–96.

Tyshchenko, V. P. (1964). Bioelectric activity of the nervous system in the developing and diapausing pupae of Lepidoptera. *Ent. Rev.* **43**, 59–65.

Tyshtchenko, V. P. & Mandelstam, J. E. (1965). A study of spontaneous electrical activity and localization of cholinesterase in the nerve ganglia of *Antheraea pernyi* at different stages of metamorphosis and in pupal diapause. *J. Insect Physiol.* **11**, 1233–9.

Van der Kloot, W. G. (1955). The control of neurosecretion and diapause by physiological changes in the brain of the cecropia silkworm. *Biol. Bull., Woods Hole* **109**, 276–94.

Vogel, R. (1911). Über die Innervierung der Schmetterlingsflügel und über den Bau und die Verbreitung der Sinnesorgane auf denselben. *Z. wiss. Zool.* **98**, 68–134.

Vogel, R. (1912). Über die Chordotonalorgane in der Wurzel der Schmetterlingsflügel. *Z. wiss. Zool.* **100**, 210–44.

Waldron, I. (1967). Neural mechanism by which controlling inputs influence motor output in the flying locust. *J. exp. Biol.* **47**, 213–28.

Weber, T. (1972). Stabilisierung des Flugrhythmus durch 'Erfahrung' bei der Feldgrille. *Naturwissenschaften* **59**, 366.

WENDLER, G. (1974). The influence of proprioceptive feedback on locust flight co-ordination. *J. com*
Physiol. **88**, 173–200.

WILLIAMS, C. M. (1969). Photoperiodism and the endocrine aspects of insect diapause. *Symp. Soc. exp. Biol.* **23**, 285–300.

WILSON, D. M. (1962). Bifunctional muscles in the thorax of grasshoppers. *J. exp. Biol.* **39**, 669–77.

WILSON, D. M. (1968). The nervous control of insect flight and related behaviour. *Adv. Insect Physiol.* **5**, 289–338.

WILSON, D. M. & GETTRUP, E. (1963). A stretch reflex controlling wingbeat frequency in grasshoppers. *J. exp. Biol.* **40**, 171–85.

WILSON, D. M. & WEIS-FOGH, T. (1962). Patterned activity of co-ordinated motor units, studied in flying locusts. *J. exp. Biol.* **39**, 643–67.

YAMAMOTO, R. T. (1969). Mass rearing of the tobacco hornworm. II. Larval rearing and pupation. *J. econ. Ent.* **62**, 1427–31.