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A reconsideration of the central pattern generator concept for locust flight

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Summary. Although it is generally agreed that locusts can generate flight similar rhythmic motor activity in the absence of sensory feedback from the wings, recent studies indicate that functional deafferentation produces significant changes in the flight motor pattern (Hedwig and Pearson 1984). These findings have raised doubts on the adequacy of the central pattern generator concept for the locust flight system (Pearson 1985). In this paper, we re-investigate the effects of deafferentation on the capacity of adult migratory locusts to generate the flight motor pattern. For this purpose, the experimental animals were dissected to various degrees, ranging from head-ventral nerve cord, to isolated pterothoracic nerve cord, and finally single isolated ganglion preparations. Flight motor activity was released by either wind stimulation, the more traditional method, or by applying octopamine (Sombati and Hoyle 1984; Stevenson and Kutsch 1986). In all cases the released motor activity was analysed, giving details of latency, and phase relationships between specific synergistic and antagonistic motor units, and then compared with the flight motor pattern generated by intact tethered locusts.

This analysis shows that deafferentation, although reducing the frequency, does not necessarily disrupt the basic flight motor pattern. By using octopamine we could show that even isolated thoracic nerve cord preparations can generate activity, which in all major aspects corresponds to this motor program. This could also be shown for the fully isolated metathoracic ganglion and we provide some evidence that the mesothoracic ganglion may be capable of a similar performance. In addition to releasing flight activity, octopamine was also found to enhance the responsiveness of deafferentated locusts to wind stimulation. This resulted in a considerable elevation of the frequency and prolongation of the flight motor activity to values comparable to the performance of intact tethered locusts.

Introduction

For many types of repetitive behaviours, it is apparent that the central nervous system (CNS) can generate properly timed rhythmic output in the absence of feedback from sense organs (Delcomyn 1980). The first convincing evidence for this came from the experiments of von Holst (1935), who showed that the coordinated movements of fish fins during swimming can be generated by the spinal cord without the need of sensory feedback. However, the concept of central pattern generators (CPG) was not firmly established until Wilson (1961) had presented evidence for 'an innate central pattern for the production of flight movements in the locust' (Selverston 1980). Sensory inputs were thought to exert only a slow control over the average flight performance rather than fast control over single wing-beat cycles (Waldron 1967a). This idea was supported by the finding that the destruction of wing sensory structures did not disrupt the normal phasing of antagonistic flight muscles, even though the frequency of the rhythm was reduced by about one half (Kutsch 1974). It would appear therefore that peripheral feedback from the moving wings is not essential for structuring the basic flight motor pattern.

It became evident, however, that peripheral sense organs can exert a phasic, cycle for cycle, influence on the flight pattern (Wendler 1972) and should therefore be considered as integral parts of the oscillating network (Altman 1983; Wendler 1983; Pearson et al. 1983). Although these studies bestow peripheral loops a more significant role than previously envisaged, they do not force us to reconsider the CPG concept. The latter hypothe-

sis merely requires that the CNS be able to generate properly timed rhythmic output in the absence of such feedback (Delcomyn 1980). However, more serious contention came from studies which indicated that the rhythmic activity generated by functionally deafferentated locusts (Robertson and Pearson 1982), i.e. removed from all conceivable sources of peripheral feedback, is not simply a slowed down version of the normal flight pattern (Hedwig and Pearson 1984; Pearson 1985). Apparently, the temporal activation pattern of synergistic and antagonistic flight muscles was altered to such an extent that it was suggested that the CPG concept is no longer applicable to the locust flight system (Pearson 1985; see also Note added in proof).

In this paper we attempt, by studying many preparations, to determine to what extent deafferentation leads to the disruption of the normal flight motor pattern, and whether this can be solely attributed to the lack of peripheral timing cues. This investigation was considerably assisted by employing the newly reported method of releasing flight activity with octopamine (Sombati and Hoyle 1984; Kinnamon et al. 1984; Stevenson and Kutsch 1986). This enabled us to evoke a flight motor response from the fully isolated CNS and also investigate the capacity of single ganglia to generate this motor program.

Methods

Preparation of experimental animals. Mature adult male locusts (*Locusta migratoria* L.) were taken from a crowded colony at Konstanz University. Some animals were left intact, and a copper harness waxed to the pronotal shield by which they could be tethered in a wind stream.

To study the effects of functional deafferentation on the details of the flight pattern, other animals were prepared in a way similar to that described by Robertson and Pearson (1982). In short, the legs and wings were first amputated at their base and the abdomen and pronotal shield cut away. Following a longitudinal, mid-line incision through the dorsal cuticle, the animal was pinned, taking care not to open the animal too broadly, ventral side down to a wax platform. The gut was then pulled forward and pinned to one side. Subsequent removal of tracheal sacs and fat body enabled the thoracic musculature to be visualized (Fig. 2A). At this stage the preparation was perfused with warmed (24–26 °C) aerated locust Ringer solution (Clements and May 1974, standard saline without sucrose). The suboesophageal, pro-, meso-, and metathoracic ganglia were then laid free and the connectives to the abdominal ganglia severed. With the exception of the hypopharyngeal and mandibulary nerves of the suboesophageal ganglion, and the meso- and metathoracic N4, (numbers of nerve roots after Campbell 1961) all nerve branches were cut close (=0.5 mm) to these ganglia. Particular care was taken to severe each branch of the very fine, sensory N2. Of the remaining N4, all its branches with the exception of N4D were ablated. The latter contains only efferent axons which innervate muscles M85, 90, 91 and 99 in the meso- and M114, 119, 120 and 129 in the metathoracic segment (muscles numbered after Snodgrass 1929). Finally, all major tracheal branches entering the thoracic ganglia were cut through. This preparation took about 30 min to complete and is referred to hereafter as 'deafferentated' (Fig. 2B).

Various experiments were also performed on totally isolated thoracic ganglia. For this purpose, the animals were prepared as above, with the exception that nerve branch N1D1 was cut close to its entry into the dorsal longitudinal wing depressor muscle (M81, resp. M112). All other branches of N1 were severed, including N6 which arises from the next anterior ganglion and fuses with N1. After cutting the anterior connectives of the mesothoracic ganglion and the four N4D before entering the target muscles, the meso-metathoracic ganglion complex (Fig. 7B) was removed to a small sylgard chamber and perfused as above with warmed aerated locust saline. Experiments on single isolated ganglia were also carried out after severing the remaining connectives (Fig. 11).

Release of flight motor activity. For tethered locusts, flight activity was evoked by suspending the animals in a warmed wind stream ($v=3.5$ m/s, ambient temp. 28 °C). For deafferentated locusts, this was achieved by blowing air ($v=2$–3 m/s) via a glass tube (internal diameter = 0.5 cm) placed approximately 2 cm in front of the head. The method of releasing flight with octopamine followed that of Sombati and Hoyle (1984). Glass micropipettes were filled with a solution of d-l octopamine hydrochloride (Fluka, 0.5 *M* in aq. dest., pH 6.8) and impaled in a site in the metathoracic ganglion which roughly corresponded to the 'position 2' described by Sombati and Hoyle (1984). A constant current pump served to eject octopamine from the micropipette. The effect of topically applying solutions of octopamine to the thoracic ganglia with a micro-syringe (2 µl) was also investigated (Kinnamon et al. 1984; Sombati and Hoyle 1984; Stevenson and Kutsch 1986).

Recordings of flight motor activity. For recording the flight motor activity of tethered locusts, stainless steel wire electrodes (40 µm), insulated to the tip, were inserted via holes punctured in the cuticle into various flight muscles. An earthed reference electrode was implanted in the abdomen. The location of electrodes was checked post-mortem by the Prussian blue method (Kutsch 1969).

In deafferentated preparations, muscle activity was recorded using similar, but bipolar electrodes. A silver chloride coated silver wire placed in the bathing medium served as an earthing device. In some experiments animals were prepared for intracellular recordings. For this the thoracic ganglia were supported by an underlying, wax coated, spoon and pinned through small trachea and fat tissue for stability. Glass microelectrodes (45–60 MΩ) were filled with potassium acetate (2 *M*). To enable better entry of electrodes through the sheath it was occasionally necessary to treat the ganglion with pronase (Sigma, 0.1% *w/v*). Impaled motoneurones were identified by antidromic stimulation of their target muscles and by the observation of 1:1 correlation of action potentials in the motoneurone and target muscle.

To record motor activity from isolated ganglion preparations, the distal ends of the N1D1 and N4D nerve branches were laid over bipolar steel wire hook electrodes, lifted from the perfusing medium and covered with vaseline to prevent drying. An earthed electrode was placed in the bath.

Analysis of motor activity. All recordings were amplified by conventional techniques, viewed on an oscilloscope and stored on magnetic tape (Racal Store 7DS). Taped recordings were

later filmed (Recordine oscilloscope camera) at either 250 mm/s when analysing the activity of antagonistic motor units or at 500 mm/s when analysing activity of synergistic motor units. The term cycle length was defined as the interval between the first firing in the burst of impulses of a single motor unit to the first firing in the next burst of the same unit. Following the conventional terminology of Waldron (1967b), the term latency was applied to the interval between the first firing in the burst of impulses in one motor unit, and the first firing in the next burst of another motor unit, and phase was the depressor-elevator latency divided by the depressor-depressor cycle length. Intervals were measured with use of a digitizer (Hewlett-Packard 9774A) and the values stored for a subsequent computer assisted (HP 9825A calculator) statistical analysis (significance level $P=0.01$). Students t-test was applied to test for differences between the means of samples, and linear regression for correlation. Serial correlation coefficients for cross- and autocorrelograms were calculated to test for trends in sequential data (Wyman 1965).

Results

Tethered flight as reference of the normal flight pattern in intact locusts

Although an analysis of unrestrained flying locusts would be optimal, details of the flight motor pattern can still only be recorded when the animals are tethered in a wind stream. In this situation continuous flight performances generally last several minutes. Recordings from the pterothoracic flight muscles reveal that they are rhythmically activated in phase with the wing movements. The following features are characteristic of the underlying motor pattern (see Wilson and Weis-Fogh 1962): 1. All motor units are activated at the same overall frequency (for *Locusta*, ca. 20–25 Hz), once or twice, but rarely more than 3 times per wingbeat cycle. 2. Motor units of hindwing depressor muscles are activated several ms in advance of serial homologous motor units of the forewing segment of the same body side. 3. Contralateral homologous muscles are activated in near synchrony. 4. Wing elevator and depressor muscles are activated in alternation, whereby the latency depressor-elevator is longer than the reverse interval. 5. The elevator-depressor and depressor-elevator latencies both increase proportionally with increasing cycle length. As a result, the phase of elevator motor units within the depressor activity cycle remains relatively constant (Waldron 1967b; Kutsch and Stevenson 1984).

In each winged segment there are several elevator and depressor muscles. Based on the response of various flight motoneurones to sensory stimulation, the elevators appear to constitute an homogeneous group, whereas the depressors can be divided into two broad groups (Hedwig and Pearson 1984). This does not, however, appear to bear any consequence for their order of recruitment during straight tethered flight, since the actual sequence seems to be subject to considerable variation (Möhl 1985). We thus consider that an analysis of the above listed features for a single antagonistic muscle pair in each winged segment is adequate to describe the basic flight motor pattern. We chose the first remotor coxae (Remotor, M90 and M119), wing elevator, and subalar (Subalar, M99 and M129), wing depressor muscles. They are innervated by nerve branch N4D, which contains no afferent fibres (Kutsch and Schneider 1987); an attribute of major significance in the later analysis of the flight pattern in deafferentated preparations.

The activity of this muscle pair conformed to the above description of the flight motor pattern (Fig. 1). Electromyograms revealed 3 motor units for the remotor and 2 for the subalar. Due to a time lag of up to 6 ms between the activity of motor units which serve the same muscle (see also Möhl 1985), we strove to record from homologous units when examining latency relationships between serial and contralateral homologous muscles. This was easily achieved for the subalar, which is divided into a rostral and caudal part, each innervated by a single motor axon (Kutsch and Usherwood 1970; Kutsch and Schneider 1987). Contralateral homologous units of this muscle are activated in near synchrony, however, units of one body side might consistently lead the other by up to 4 ms (Wilson 1968; Möhl 1985). Hindwing subalar motor units are activated several ms in advance of the serial homologues of the forewings (Figs. 1 B, 8). Our analysis of elevator activity (14 animals) indicates that the hindwing remotor is activated either in synchrony, or at the most 1–2 ms in advance of the forewing remotor (Fig. 1 A). Due to the complex motor unit distribution of this muscle (Kutsch 1970), however, we could not be certain that our analysis was based on a comparison of homologous units. This may be a source of error, masking a possible time lag between serial homologous units.

The latency relationships between the examined antagonistic muscles was found to be surprisingly consistent in the 7 tethered locusts examined. The depressor-elevator latency was usually longer than the reverse interval. Both the elevator-depressor and depressor-elevator latencies increased proportionally with increasing cycle length (Figs. 3, 4 A), giving a mean intercept of nearly zero for both plots and slopes of 0.46 and 0.55, respectively (Table 1, Fig. 12). Thus, the phase of the elevator in

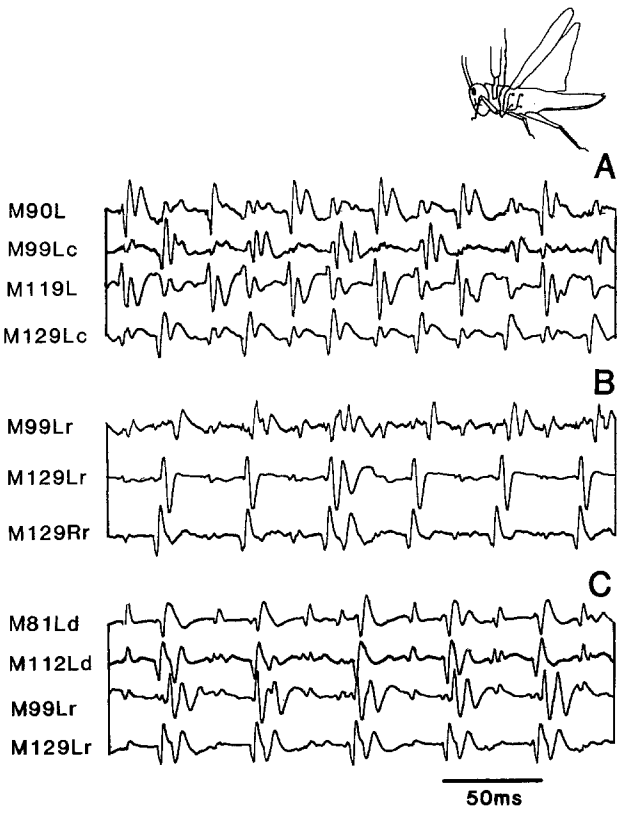

Fig. 1A–C. Electromyograms from an intact, tethered flying locust illustrating details of the flight motor pattern. Alternating activation of elevator (M90, M119) and depressor muscles (M99, M129) in both winged segments (**A**). Contralateral homologous motor units are activated in near synchrony, but one side might lead by up to 4 ms (**B** M129Lr-M129Rr). Hindwing depressor motor units are activated several ms in advance of serial homologues of the forewings (**B**, **C** M129Lr-M99Lr, **C** M112Ld-M81Ld). Lack of clear intersegmental time lag of elevator muscles (**A** M119L-M90L). Muscles numbered according to Snodgrass (1929). M90 fore-, M119 hindwing remotor; M99 fore-, M129 hindwing subalar; M81 fore-, M112 hindwing dorsal longitudinal; *L* left; *R* right body side; *r* rostral; *c* caudal unit of subalar; *d* most dorsal unit of dorsal longitudinal muscle

the depressor activity cycle remained relatively constant (mean 0.55, s.d. 0.02), irrespective of cycle length.

Flight motor activity recorded from deafferentated locust preparations

Wind stimulation evoked patterned motor activity in about 75% of all deafferentated locust preparations (Fig. 2C) up to 2 h after start of the experiment. Although the frequency was reduced and there was a tendency for the motor units to discharge more often per cycle, resulting in somewhat longer burst lengths, the activity clearly resembles the flight pattern. Since the preparation consisted basically of only the head and ventral nerve cord, having only the motor N4D intact (Fig. 2B), the only conceivable source of rhythmical peripheral feedback, which might be required for structuring the flight motor pattern, would be from remote receptors in the head, responding phasically to muscle contractions in the thorax. Although this is rather unlikely, we checked for possible effects by recording from motoneurones which innervate the remotor and subalar before and after having severed the four N4D. This abolished all rhythmical body movements, but had no detectable effect on details of the pattern evoked by a subsequent wind stimulus (Fig. 2D). Since this argues against remote receptors integrating in the system investigated, we left the N4D intact and included data obtained from myograms in the following analysis.

Frequency and duration of flight motor activity. The flight motor sequences of deafferentated animals were usually only several seconds long. There was also a considerable reduction in frequency of the rhythm. Tethered locusts start a flight sequence at about 24 Hz, levelling off after a few seconds at about 22 Hz (Kutsch and Stevenson 1984). In deafferentated locusts, the highest values were also measured at the start of a sequence, however, even the first cycle (mean 17.1 Hz, s.d. 1.8 Hz; $n=11$) had a lower instantaneous frequency than that recorded for intact animals. The frequency then decayed within the next few seconds and remained relatively stable (mean 12.5 Hz, s.d. 1.7 Hz) until the end of a sequence, which was usually marked by a further drop to about 9 Hz.

Latency relationships between homologous motor units. The activity of the examined homologous muscles (Fig. 5A) was similar to that observed during tethered flight (Fig. 1). In all examined cases, identified units of the hindwing subalar led the serial homologues by several ms (Fig. 5B). Contralateral homologous units of this muscle were usually activated in near synchrony, although in some preparations one body side would lead consistently by up to 4 ms. The latency relationships between homologous elevator muscles was more difficult to evaluate, since our analysis of the remotor could still not be based on a comparison of homologous units. Nevertheless, as also found for tethered flight, the examined motor units of all remotors were usually activated at about the same time. In some preparations, however, the hindwing remotor clearly led that of the forewings, whereas contralateral remotors were activated in near synchrony (Fig. 5C).

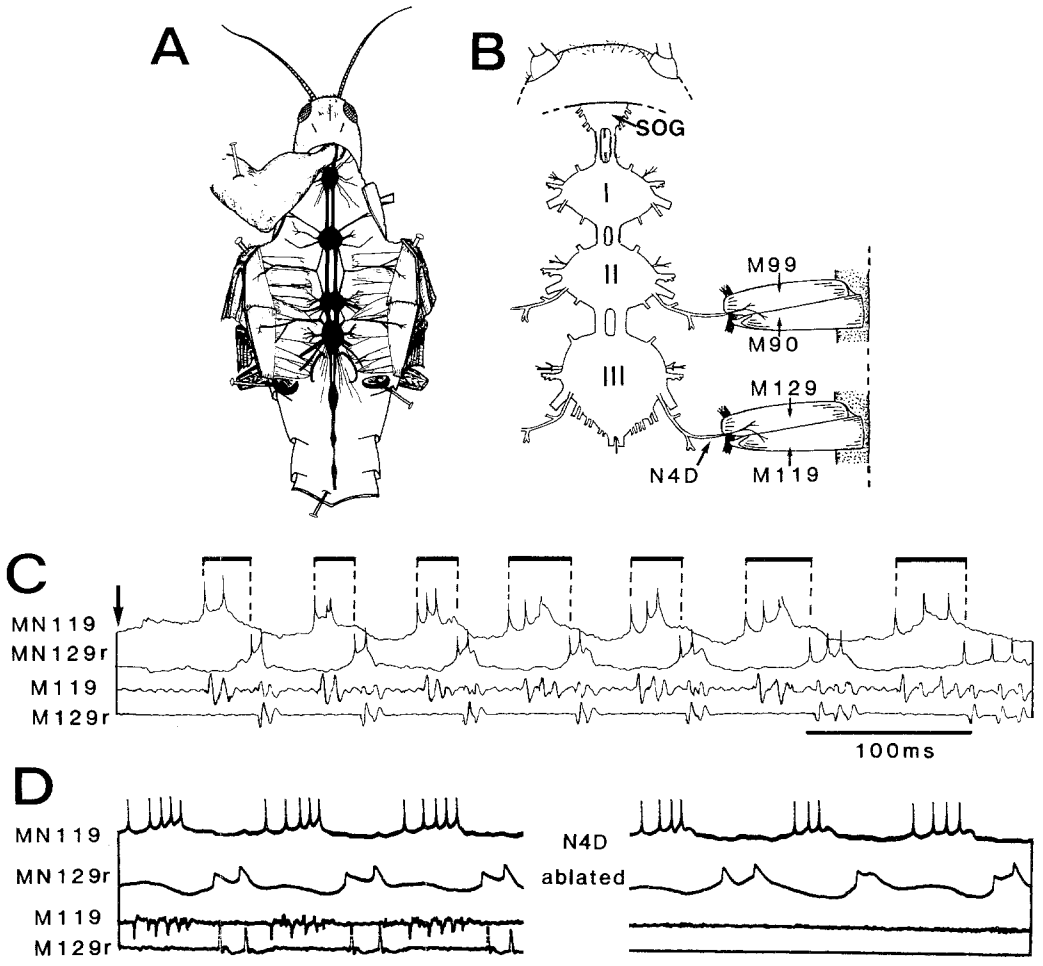

Fig. 2. Schematic representation of the locust preparation before (**A**) and after (**B**) functional deafferentation. In the latter preparation, all nerves arising from the thoracic nerve cord, with the exception of N4D, were severed. This nerve branch contains only efferent fibres which innervate a group of thoracic muscles (M85, 90, 91, 99 in the meso- and M114, 119, 120, 129 in the metathorax) of which only the remotor, wing elevator (M90, 119), and subalar, wing depressor (M99, 129), muscles are indicated. **C** Simultaneous intracellular recordings from the neuropilar segment of motoneurones which innervate the remotor (MN119) and rostral part of the subalar (MN129r). Electromyograms from these muscles are also shown (M119, M129r-rostral). Activity was initiated by a wind stimulus (arrow) directed at the head of a deafferentated locust. Black bars indicate the increase of elevator-depressor latency with increasing cycle length. Note, the muscle spikes of the recorded MN119 are not clearly visible in the myogram, which predominantly shows activity of another unit of this muscle. **D** Simultaneous intracellular recordings from the neuropilar segment of MN119 and soma of MN129r during flight motor activity before (note electromyograms) and after severing the remaining N4D of a deafferentated locust preparation

Phase relationships between antagonistic muscles. The alternating activation of the examined wing elevator and depressor muscles can only be observed during flight behaviour and is therefore one of the most characteristic features of the normal flight pattern. This aspect was analysed by evaluating each cycle of the first wind released sequence for 16 consecutive deafferentated preparations which had only the right metathoracic N4D intact. For the majority of animals (13), regression analysis revealed that both the remotor-subalar and subalar-remotor latencies increased proportionally with increases in total cycle length (Figs. 3, 4B) and the examined phase remained relatively constant (mean 0.6, s.d. 0.09). For these examples the mean phase and means of the intercepts and slopes calculated from linear regression analysis of elevator-depressor and depressor-elevator latencies against cycle length were not statistically different to the mean values calculated for intact locusts (Table 1, Fig. 12). Thus, the temporal activation pattern of antagonistic flight muscles is equivalent to the tethered flight situation, even though the cycle lengths are longer.

In 2 preparations, however, the remotor-subalar latency had rather constant values. This is best

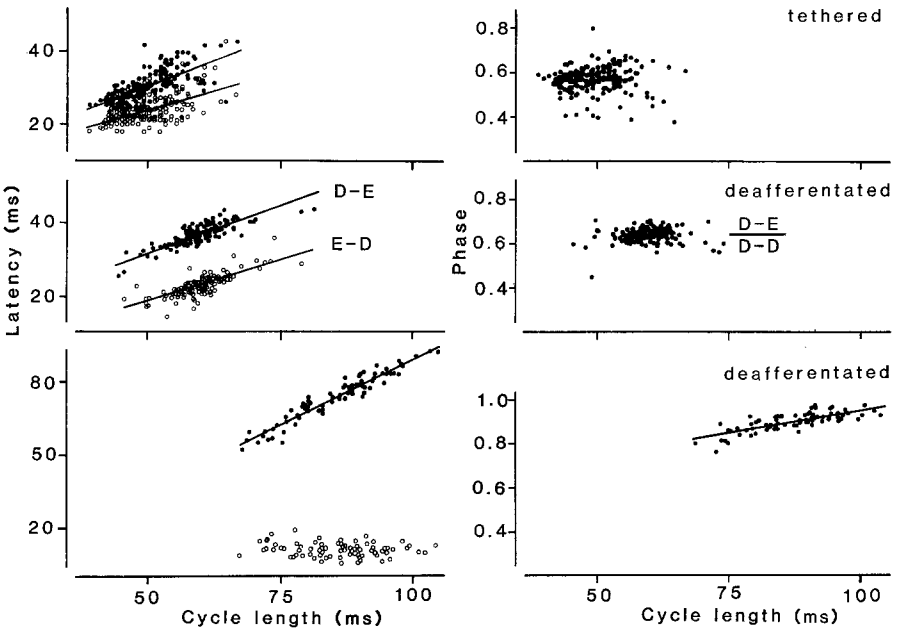

Fig. 3. Linear regression analysis of latency relationships between antagonistic motor units. Top: data from a tethered flying locust (169 intervals) showing that both the depressor-elevator (D-E, filled symbols) and elevator-depressor (E-D, open symbols) latencies increase with cycle length (left graph) resulting in a near constant phase of the elevator in the activity cycle of the depressor (right graph). Middle: data from a deafferentated locust (122 intervals) showing the same relationship as in tethered flight (slopes and intercepts are not statistically different), the phase is not related to cycle length, i.e., it remains relatively constant. Bottom: example of a deafferentated preparation for which the E-D latency was not related to cycle length (73 intervals). Values for the phase are positively correlated to cycle length and are considerably larger than in the above examples

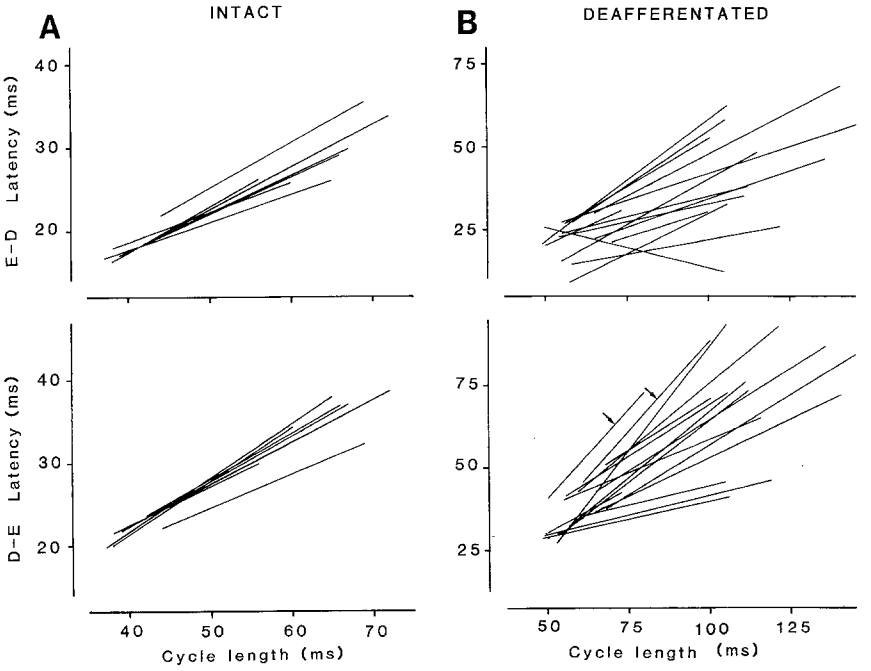

Fig. 4. Regression lines of data from 7 intact locusts (**A**) and 16 deafferentated preparations (**B**). In all intact locusts, and for the majority of deafferentated locusts, both the elevator-depressor (E-D) and depressor-elevator (D-E) latencies are positively correlated to cycle length, resulting in near constant phase relationships. Lines marked by arrows in **B** have a slope not statistically different to 1. In these cases, the E-D latency was not related to cycle length (hence regression lines were not drawn in top graph in **B**)

demonstrated in plots of subalar-remotor latency against cycle length; such data can then be fitted by a regression line with a slope not statistically different to 1 (Figs. 3, 4B). In one example, the remotor-subalar latency even shortened with increases in cycle length. In these 3 cases, phase was related to cycle length (Fig. 3) and had relatively large values (0.8–0.9). Even though all animals were deafferentated to the same degree, we initially attributed these occasional differences in phase relationships to some unknown manipulations performed by the experimenter. However, this possi-

Table 1. Table giving the means and s.d. of the slopes and intercepts calculated from values obtained by regression analysis of elevator-depressor (E-D) and depressor-elevator (D-E) latencies against cycle length during flight motor sequences of intact and deafferentated locusts and also for octopamine released activity (includes deafferentated and isolated ganglion preparations). *N* number of experimental animals in each test group

Test group	*N*	E-D Latency vs cycle length				D-E Latency vs cycle length			
		Slope		Intercept		Slope		Intercept	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Intact	7	0.46	0.01	−0.4	3.9	0.55	0.10	0.1	3.8
Deafferentated	13	0.44	0.19	−4.2	10.7	0.54	0.21	5.3	12.6
Octopamine released	5	0.45	0.18	0.4	14.7	0.58	0.23	−2.3	17.1

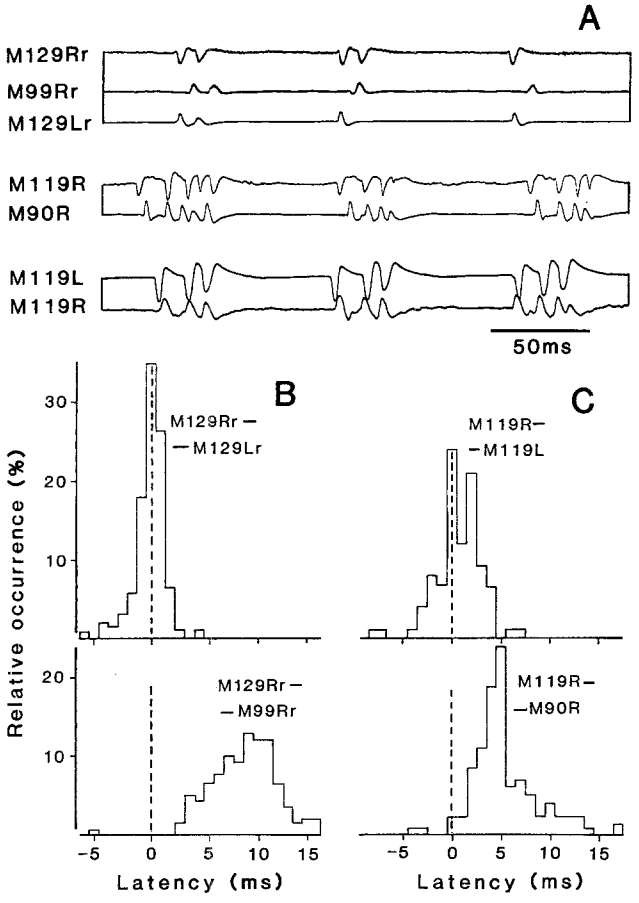

Fig. 5A–C. Latency relationships between synergistic motor units during flight activity of deafferentated preparations. **A** Electromyograms. Upper traces: homologous units (rostral) of the subalar of the left (M129Lr) and right hindwing (M129Rr) and the right forewing (M99Rr). Middle traces: hind- and forewing remotor (M119R, M90R). Lower traces: left and right hindwing remotor (M119L, M119R; same animal as in middle traces). **B** Interval histograms for subalar motor units ($n=138$). Note near synchrony of contralateral homologues (latency M129Rr-M129Lr) and delayed activation of the forewing subalar relative to the serial homologous unit of the hindwing (latency M129Rr-M99Rr). **C** Interval histograms for remotor muscles ($n=140$). The contralateral hindwing remotors (latency M119R-M119L) are activated in near synchrony and in this example they clearly lead the forewing remotor (latency M119R-M90R)

bility could be ruled out for at least 2 animals, for which we found that the phase alternated regularly, about every 8th cycle (ca. 2 Hz), between values encountered for constant phase, and constant latency relationships (Fig. 6A). Serial correlograms showed these fluctuations to be statistically significant (Fig. 6B).

Release of flight motor activity with octopamine

The strongest evidence for central control of a motor pattern comes from studies in which all or parts of the CNS are completely isolated from the rest of the animal. This has not yet been demonstrated for locust flight. The main difficulty in this system is that wind stimulation of head receptors is usually required to initiate a flight motor response. There are, however, reports that flight similar motor activity can be evoked by ionophoretically releasing octopamine into discrete regions of the metathoracic ganglion (Sombati and Hoyle 1984). Unfortunately, details of the pattern were not reported. It is therefore uncertain to what extent the octopamine released activity corresponds to the basic flight motor pattern. Furthermore, these previous experiments were performed on minimally dissected locusts, which raises the possibility that peripheral feedback loops are required to facilitate the response.

We were, nevertheless, able to establish that ionophoretic ejection of octopamine into the metathoracic ganglion of deafferentated locusts can release motor activity which illustrates major features of the basic flight motor pattern (Fig. 7C). The position of the micropipette within the ganglion was a crucial factor in determining whether or not flight motor activity was evoked. We only had success when the pipette was impaled in a region approximately corresponding to the 'position 2' described by Sombati and Hoyle (1984). The initial response was invariably recorded in elevator motor units. Occasionally, this was only a single discharge

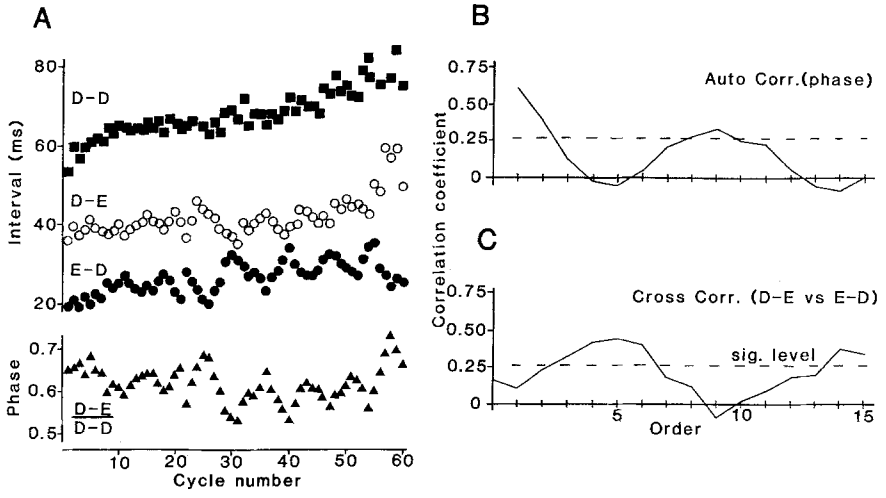

Fig. 6. A Sequential plot of a complete flight motor sequence of a deafferentated locust which illustrated the usual increase in cycle length (D-D) but fluctuations in depressor-elevator (D-E), and elevator-depressor (E-D) latencies and phase. **B** Serial auto-correlogram up to the 15th order for phase showing that the fluctuations occur regularly, about every 8th cycle. **C** Serial cross-correlogram of D-E latency vs E-D latency indicating that the opposing fluctuations are statistically significant. Significance level for the serial correlation coefficient ($P=0.01$) from Anderson (1942)

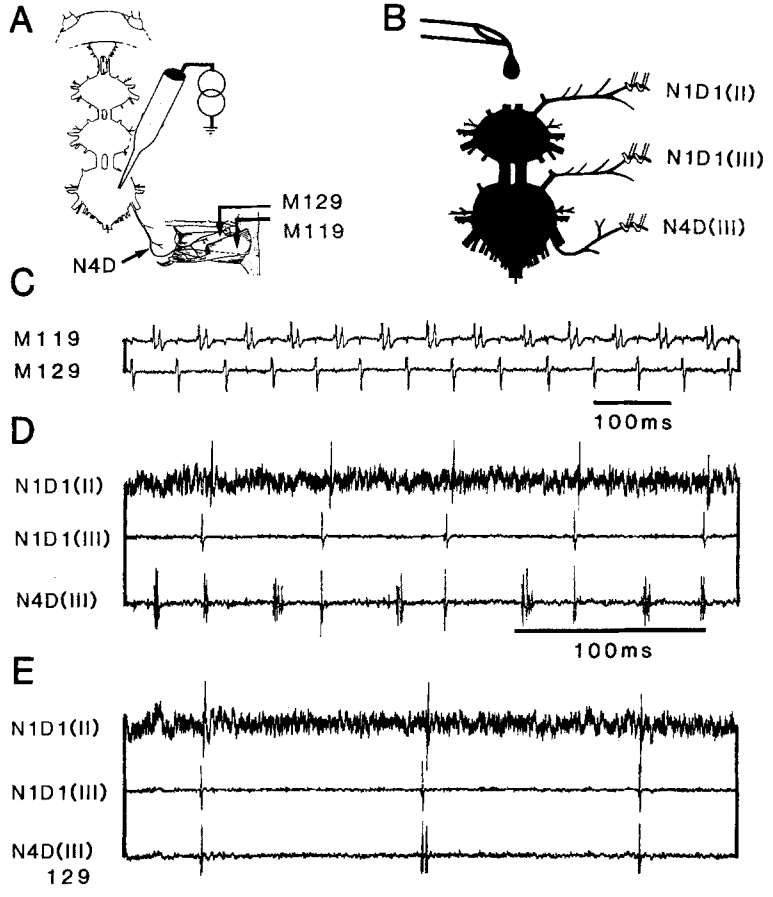

Fig. 7A–E. Release of flight motor activity by octopamine. Ionophoretic application to a deafferentated preparation (**A**) released patterned motor activity (**C**) of elevator (M119) and depressor (M129) muscles. Topical application to the isolated meso-metathoracic ganglion complex (**B**) released similarly patterned motor activity which was recorded from the stumps of severed motor nerves (**D**, **E**). Note the single unit of the dorsal longitudinal depressor muscle of the hindwing segment, visible in N1D1(III) leads the serial homologous unit of the forewing, N1D1(II). With reference to the activity of this depressor axon, and also by recording from the nerve branch which innervates the subalar alone (**E**, N4D(III), 129), an alternating activation pattern of elevator and depressor motor axons is clearly exhibited in N4D recordings (**D**)

followed after approximately 20 ms by a single discharge in depressor units. More often, we observed a continuous alternating rhythmic sequence which could continue for some minutes even with the current to the pipette turned off (Fig. 10A). The remotor and subalar motor units were activated only once or twice per cycle, which is more typical of the flight performances of tethered locusts. The frequency varied in different preparations from 10–17 Hz, but was surprisingly stable throughout any one sequence. A frequency decay after start, as observed for wind released flight activity was not apparent. The interval subalar-remotor was consistently longer than the reverse latency and

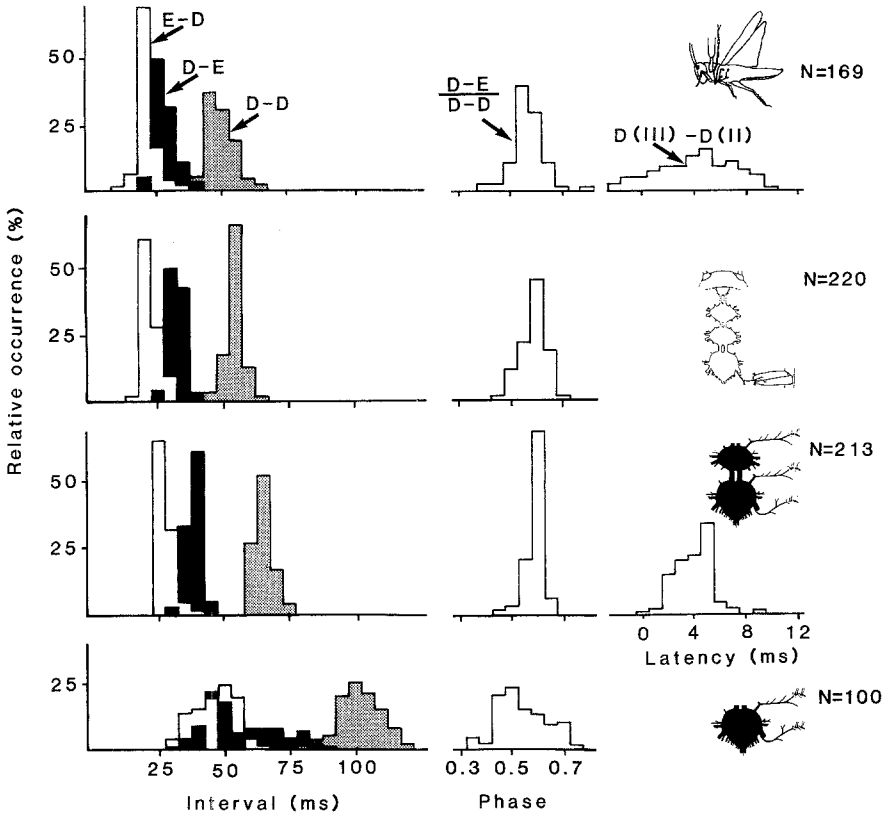

Fig. 8. Histograms of intervals and phase from recordings of wind released flight motor activity of a tethered locust (top) compared to the octopamine released pattern of a deafferentated, an isolated meso-metathoracic ganglion, and an isolated metathoracic ganglion preparation (see insets). E-D elevator-depressor latency (white), D-E depressor-elevator latency (black), D-D cycle length (grey). D-E/D-D phase of elevator in depressor activity cycle. D(III)-D(II) latency from a hindwing depressor motor unit to the activity of the serial homologous unit of the forewing. Neither the differences between the means of the phase, nor means of the D(III)-D(II) latency in the various preparations are statistically significant. Data for upper two animals from myograms, for lower two from nerve recordings

in most cases both latencies became proportionally longer with increases in cycle length, and the phase remained relatively constant (Figs. 8, 9). In some examples however, the subalar followed the remotor with a constant latency. Other types of motor activity were not observed.

Modulation of the response to wind stimulation. In addition to releasing flight motor activity, octopamine ionophoresis was also found to enhance the responsiveness of deafferentated locusts to wind stimulation. To demonstrate this effect we ionophoretically injected octopamine for a length of time which was calculated to be just too short to actually evoke a flight response. Following this treatment, a wind stimulus then initiated a flight motor sequence (Fig. 10A) which, in some respects, differed to that observed prior to octopamine treatment. Typically, individual motor units were activated less often per individual cycle, the repetition frequency was significantly raised and the whole performance was much longer. Changes in phase relationships were not observed. In one example, we recorded flight motor activity which started at a frequency of 24 Hz, decayed to about 20 Hz and then remained stable for over 3 min (Fig. 10B). This performance approaches that of intact locusts and had never previously been observed for deafferentated preparations.

Flight activity released from isolated ganglion preparations. The main objective for the octopamine experiments was to find a reliable method which could be employed to release flight motor activity from isolated ganglion preparations. In deafferentated preparations, the ionophoretic release of octopamine in a specific site proved to be a powerful method. Essentially the same result could be achieved more simply, however, by topically applying an octopamine solution to the thoracic ganglia. The ganglion sheath would seem to be a formidable barrier to this substance since we did not observe any effect with concentrations less than 0.1 *M*. Although this prohibited us from performing satisfactory control experiments with other pharmaca, equimolar or equiosmotic concentrations of NaCl or sucrose were without effect.

Topically applied octopamine would even release patterned motor activity, which could persist for some minutes, from the fully isolated meso-metathoracic ganglion complex (Fig. 7D, E). This activity was monitored by recording from the stumps of severed motor nerves. Nerve branch 1D1 contains a total of 5 large motor axons which in-

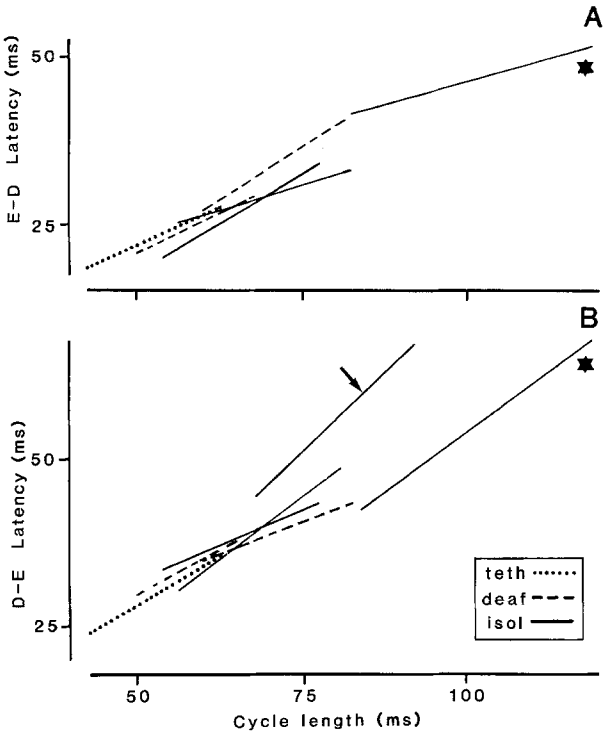

Fig. 9. Regression lines from an analysis of octopamine released flight motor activity showing that both the elevator-depressor (**A** E-D) and depressor-elevator (**B** D-E) latencies increase with cycle length indicating constant phase, rather than constant latency relationships. Dotted line: data from a tethered locust for comparison, broken lines: deafferentated, continuous lines: isolated meso-metathoracic, and one isolated metathoracic (*) ganglion preparations. Arrow in **B** marks a regression line of slope not statistically different to 1, indicating in this example constant values for E-D latency (hence no regression line in **A**)

nervate the dorsal longitudinal wing depressor muscle (Neville 1963). Since 4 of these initially run through the severed N6, only one large motor axon remained intact in our preparation. The recordings revealed this depressor motor unit to be cyclically activated once or twice per cycle at an overall frequency of 10–14 Hz. By comparing the serial homologues in the meso- and metathoracic N1D1, we found that the hindwing motor unit led the forewing homologue by several ms (Figs. 7D, E, 8, 11A) in all preparations examined (4). This time lag is similar to that recorded from serial homologous units of the dorsal longitudinal wing depressor muscle (M81 and M112) in intact animals (Fig. 1C).

The metathoracic N4D contains a total of 28 axons, all are efferent fibres but only 17 stem from motoneurones (Kutsch and Schneider 1987). Two of these motoneurones innervate the subalar, 3 the 1st remotor coxae and the remainder the 2nd remotor coxae and pleuroalar muscles. Recordings of this nerve branch reveal several active units. However, a clear pattern can be discerned by referring to the depressor unit in N1D1. One or two units are in phase with the N1D1 discharges, these probably reflect activity of subalar motor axons. Other units are activated in antiphase, and most likely represent activity of axons which serve the 1st and 2nd remotor coxae elevator muscles. This was confirmed by recording from the branch of N4D which only innervates the subalar (Fig. 7E). This revealed activity of only those axons which are in phase with the depressor unit in N1D1 recordings. Thus, in addition to the characteristic meta-mesothoracic time lag, a clear alternating activation of wing elevator and depressor motor units can be resolved (Figs. 7D, 11A). For the majority of preparations, regression analysis showed that both the elevator-depressor, and depressor-elevator latencies increased proportionally with cycle length (Figs. 9, 12), and the examined phase remained relatively constant (Fig. 8). Therefore, the recorded activity clearly corresponds to the basic flight motor pattern.

With this simple method in hand we were also able to test whether single isolated ganglia can autonomously generate the flight motor pattern. After having cut the connectives between a meso-metathoracic ganglion preparation, octopamine released activity from the metathoracic ganglion (Fig. 11C), which in all major respects corresponded to the basic flight motor pattern (Figs. 8, 9). However, no response could be evoked from the isolated mesothoracic ganglion in a total of 6 similar experiments. This would seem to indicate that the metathoracic ganglion contains an adequate complement of neuronal elements to generate basic features of the flight motor pattern, but that the mesothoracic ganglion requires additional information from the posterior ganglion or from the periphery for a similar performance. To test for the significance of the connectives between these ganglia, they were severed in otherwise intact animals which were then suspended in a wind stream. All of the 7 animals tested responded with low amplitude flutterings of the wings (cf. Wilson 1961). Myograms from 5 animals revealed that the mesothoracic antagonistic flight muscles were activated in a tonic, unpatterned fashion. In 2 animals, however, the same muscles were rhythmically activated in a flight similar fashion (Fig. 11D). Although these observations indicate that it is rather more difficult to release flight activity from the mesothorax in the absence of inputs from the metathoracic ganglion, they clearly demonstrate that such inputs are not entirely essential.

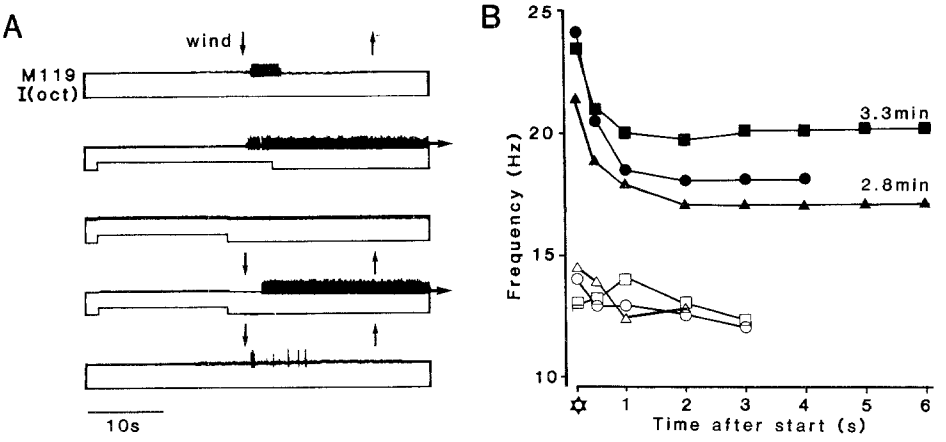

Fig. 10A, B. Enhanced flight response of deafferentated locusts to wind stimulation following octopamine ionophoresis. **A** Experimental procedure on a single animal indicating the various stimulus configurations (each separated by 5 min pauses). Arrows indicate wind stimulus. Top traces: electromyogram from the remotor (M119) indicating flight activity. Lower traces: deflections indicate current (50 nA) applied to the octopamine filled glass micropipette, I(oct). Note, in the 3rd trial octopamine application was too short to initiate a flight motor response, but if followed by wind stimulation (4th trial) an enhanced response was recorded (cf. 1st and last trial). **B** Graph showing frequency and duration (latter indicated in min when longer than 6 s) of the wind released flight motor activity, from start (✡ instantaneous frequency of the first depressor cycle) onwards for 3 deafferentated animals, before (open symbols) and after (filled symbols) octopamine ionophoresis

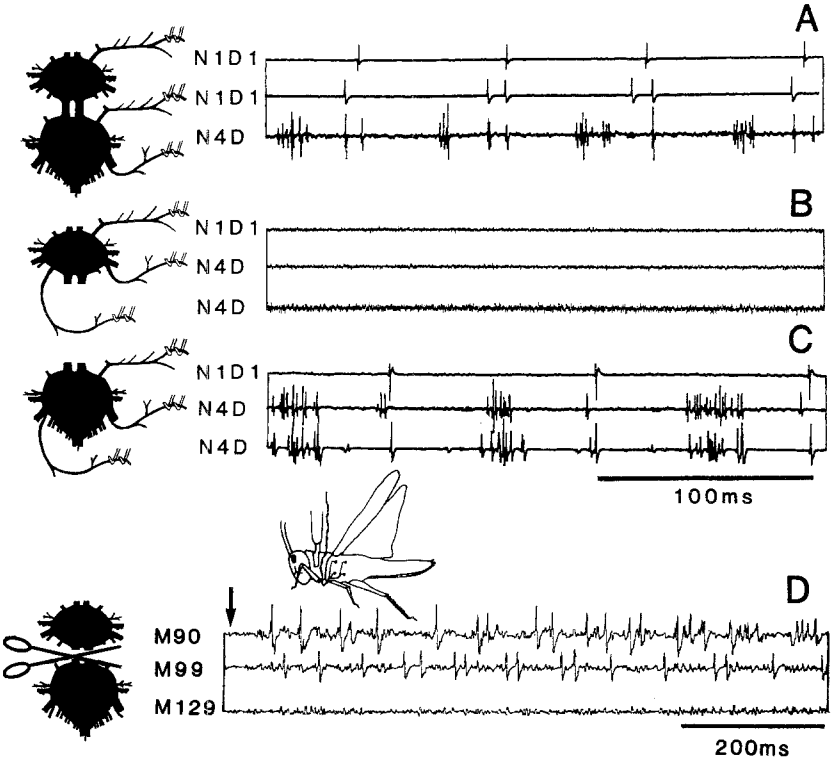

Fig. 11. Nerve recordings of octopamine released flight motor activity from an isolated meso-metathoracic ganglion complex (**A**). After cutting the connectives, similar activity could still be released from the metathoracic ganglion (**C**), but not from the mesothoracic ganglion (**B**). **D** Electromyograms from a tethered locust (arrow indicates wind on) after having severed the meso-metathoracic connectives. Note alternating activation of the mesothoracic wing elevator (M90) and depressor (M99) muscles and silence of metathoracic muscles (M129)

Discussion

The object of this paper was to determine whether the central pattern generator (CPG) concept can still be strictly applied to the locust flight system. A CPG is generally regarded as a central nervous network capable of generating a motor output, similar to that occurring in intact animals, in the absence of peripheral feedback (Delcomyn 1980; Selverston 1980; Pearson 1985). Sensory timing cues are then considered to serve only to modulate the overall activity and frequency, but not to be essential for establishing the basic motor pattern (Delcomyn 1980; Pearson 1985). Support for this general concept has been obtained for nearly 50 species of animals distributed among 4 phyla, in-

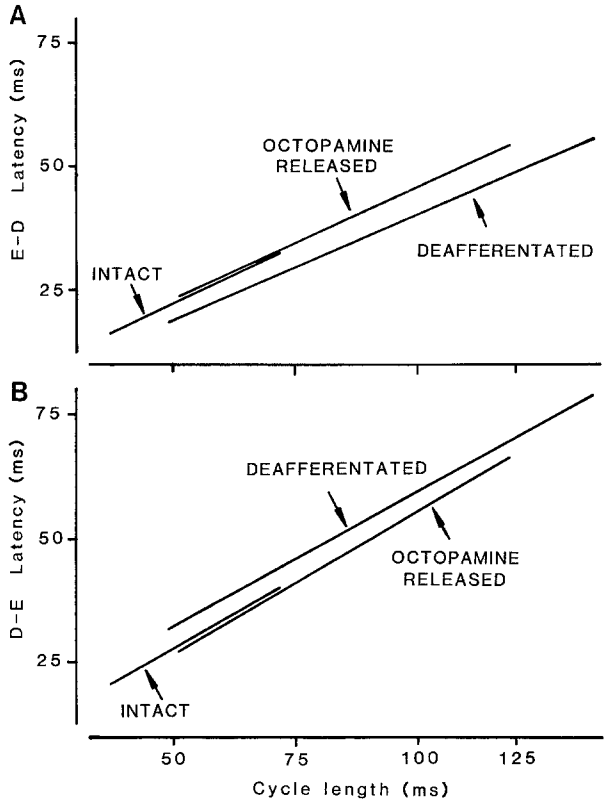

Fig. 12. Graphs showing the relationship of elevator-depressor (**A** E-D) and depressor-elevator (**B** D-E) latencies to cycle length during wind released flight activity of intact (7 animals) and deafferentated locusts (13 animals) and octopamine released flight motor activity (5 animals, includes deafferentated and isolated ganglion preparations). Each line represents the mean slope and intercept obtained from values calculated from regression analysis (Table 1) and is plotted from the minimum to maximum recorded cycle length for each test group. The differences between the mean slopes and intercepts of the data from the 3 groups are not statistically significant

cluding vertebrates (for summary see Delcomyn 1980). Evidence for a CPG in the locust flight system first arose from the work of Wilson (1961), who showed that 'an output in the flight motor axons, which resembles the basic flight pattern, but at lower frequency' can be generated in the absence of sensory input from the wing receptors.

More recently, however, it has been claimed that the rhythmic activity generated by functionally deafferentated locusts is not simply a slowed down version of the normal flight pattern (Pearson 1985). Deafferentation apparently led to the loss of the normal time shift of forewing motor activity relative to hindwing activity and of the usual order of recruitment of different depressor muscles serving the same wing, and a change from the characteristic constant phase relationship of wing elevator and depressor motor units (Waldron 1967b) to a pattern where depressor bursts follow elevator bursts with a constant latency (Hedwig and Pearson 1984; Pearson 1985). Although it was not disputed that some form of central nervous oscillating network exists, the main conclusion was that the 'idea of a CPG being primarily responsible for the patterning of motor activity in the flight system cannot be accepted' (Pearson 1985). Many, no doubt, regard this opinion as semantic conjecture. It is important to realize, however, that these experiments lead us to acknowledge that phasic peripheral feedback loops (Wendler 1972; summary Altman 1982) are essential in order to establish the basic latency and phase relationships which are characteristic of the normal flight motor pattern.

The results presented in the first half of this paper, however, show that deafferentated locusts are in fact capable of generating a motor pattern which is not essentially different to the flight motor pattern of intact locusts. Firstly, identified motor units of hindwing depressor muscles were consistently found to lead serial homologous units of the forewing segment by several ms. This strict lag in forewing motor activity has been reported for practically all depressor muscles (Wilson and Weis-Fogh 1962) of intact animals and is considered as a fundamental feature of the normal flight pattern. The latency relationships between serial homologous elevator muscles has not been well documented. Our studies of the remotors during flight of intact locusts indicate that there may be no strict intersegmental time lag between elevator muscles. However, this still needs verification, for example by comparing serial homologous units of elevator muscles. In any event, the situation for deafferentated locusts was again not different to that of intact animals. Even the timing of contralateral homologous units was found to be similar to the tethered flight situation; they were usually activated in near synchrony, but as is also commonly seen in intact locusts (Wilson 1968, Möhl 1985), one body side might consistently lead the other by up to 4 ms.

The most characteristic feature of the normal flight pattern is that the latencies of elevator to depressor, and depressor to elevator discharges both increase proportionally with increases in total cycle length. As a result, the phase of elevator discharges in the depressor activity cycle remains relatively constant, regardless of cycle length (Waldron 1976b; Kutsch and Stevenson 1984). This basic relationship was surprisingly consistent in all examined intact animals and was not dramatically altered in the majority of experiments by deafferentation, even though the cycle durations were lengthened. Thus, sensory feedback cannot be essential

for generating the normal phasing of the alternating pattern. This is in accordance with the finding that ablating wing sensory nerves of otherwise intact animals does not upset the normal phase relationship, even though the frequency is similarly reduced (Kutsch 1974).

In a few deafferentated preparations, however, this phase constant relationship was not apparent, depressors followed elevator activity with a rather short constant latency. This situation, which is in accordance with the results of Hedwig and Pearson (1984), cannot, however, be solely attributed to the lack of peripheral feedback since all other deafferentated locusts generated the phase constant pattern. In support of this notion was the observation that the phase in a few preparations regularly alternated between values typical for constant latency, and constant phase relationships. This could reflect activity of the ventilation rhythm generator, which sends input to flight motoneurones (Burrows 1975), and oscillates at a similar frequency during flight (Fraenkel 1932). It should also be stressed that deafferentated preparations are not only isolated from sensory feedback, but are also subjected to physical conditions which could equally affect the performance of the neural circuits. A depressed physiological state is also to be expected due to extensive dissection. The conditions of the animals need not have been the same in all experiments, which could account for occasional differences in phase relationships between antagonistic motor units.

In these experiments we did not investigate whether deafferentation leads to the loss of the usual order of recruitment of depressor motor units (see Pearson 1985) which serve the same wing, since in tethered flight there appears in fact to be no specific order (Möhl 1985). Our account of the activity of a single depressor and elevator muscle in each winged segment is therefore sufficient to provide an adequate description of the basic rhythmic flight pattern.

The only consistent differences between the pattern generated by deafferentated locusts and the normal flight pattern was that the duration of the performance was short, the overall frequency reduced and the motor units tended to be activated more often per cycle (cf. Hedwig and Pearson 1984). These parameters could be controlled by sensory input. For example, random stimulation of ablated wing sensory nerves has been shown to elevate the frequency of the rhythm (Wilson and Wyman 1965). More recently, it was shown that the frequency could only be elevated when stimulation was delivered at a precise time in each cycle, i.e. phasically (Pearson et al. 1983). This treatment was even shown to prolong the duration of flight activity. However, we could achieve a similar result which could not be attributed to phasic stimulation. Following octopamine treatment we often observed that the responsiveness of deafferentated locusts to wind stimulation was enhanced, resulting in flight motor performances which were even more similar to tethered flight. They lasted much longer, the frequency was significantly higher and the motor units were activated only once or twice per cycle. A similar modulatory action of octopamine has been shown in moths (Kinnamon et al. 1984) which, apparently, could not be explained by an effect on peripheral cells, or on axonal membranes in general. It was suggested that octopamine alters the general level of excitation or the effectiveness of synaptic transmission among central neurones, including those involved in producing the flight motor pattern. Thus, although phasic peripheral feedback may play some role in frequency control and flight maintenance (Pearson et al. 1983), our results indicate that this need not be the only mechanism.

The method of octopamine ionophoresis was not only found to modulate the response to wind stimulation, but also to actually release flight motor activity in deafferentated preparations. This confirmed Sombati and Hoyle's (1984) experiments on minimally dissected locusts. Furthermore, we could achieve essentially the same result by topically applying an octopamine solution to the thoracic ganglia, although this had previously been shown to have no effect on locusts (Sombati and Hoyle 1984). For both methods, the motor pattern recorded from deafferentated locusts corresponded to that observed following wind stimulation alone (Fig. 12), with the exception that the octopamine released activity lasted considerably longer and the motor units were activated less often per cycle. In these respects the released activity was more similar to the flight performances of tethered locusts.

It was not our intention in these experiments to address the question as to how octopamine brings about such dramatic effects, but rather to investigate a potential method which could be employed as an instrument to release flight activity from isolated ganglia. Isolation experiments have been successfully performed in other systems to provide conclusive evidence for central generation of various motor patterns (for references see Delcomyn 1980). This has not yet been convincingly demonstrated for the locust flight pattern. Although Wilson (1961; Wilson and Wyman 1965)

had shown that electrical stimulation could occasionally evoke a flight similar motor response from decapitated, wingless preparations, it is not absolutely clear in his description of the preparations whether all possible sources of peripheral feedback had been removed. For example, we have observed that receptors of the rather fine, diffuse N2, respond phasically to even the slightest movements of thoracic muscles (Stevenson and Kutsch, in prep.). We therefore favoured the physical removal of parts of the CNS from the animal's body, and employed the simpler method of topical application of octopamine. With this method we were finally able to evoke a flight motor reponse not only from the isolated meso-metathoracic ganglion complex, but also alone from the metathoracic ganglion. Recordings from severed motor nerves revealed a motor pattern which corresponded to the basic flight pattern of intact locusts. Although the frequency was reduced, motor units were activated once or twice per cycle, homologous motor units of the hindwing segment led those of the forewing by several ms, and elevator and depressor motor units were activated in alternation, in many cases at the correct phase relationship. The pattern was surprisingly stable, and could persist for minutes without pause.

The above experiments provide the strongest evidence yet presented that the basic locust flight motor pattern can be generated in complete isolation of peripheral timing cues. We were, however, able to go one step further and address the question as to whether separate oscillators exist in the fore- and hindwing segments. Although there is evidence that this may be the case (Wilson 1961), it was recently suggested that the flight pattern generator is a single entity, distributed among several segmental ganglia, and operates as a unit (Robertson and Pearson 1984). This was based on the finding that interneurones, which have so far been found to influence the timing of the flight pattern, do not appear to be organized into two homologous systems for the separate control of the fore- and hindwings. Our results, however, do not fully conform to this model. Firstly, basic features of the flight pattern could be recorded from the metathoracic ganglion in isolation. Secondly, in the absence of inputs from this ganglion flight activity could also be recorded from the mesothoracic segment. Therefore, each pterothoracic segment would appear to have some capacity to generate the basic features of the flight pattern.

We conclude that phasic peripheral feedback is not essentially required for structuring the basic flight motor pattern and see no reason to modify Wilson's (1961) original concept of 'an innate central pattern for the production of the basic flight movements in the locust'. There can of course be no doubt that phasic sensory feedback can influence this central pattern, for example in order to compensate for external perturbations. However, as important as such loops may be, 'they act on top of what is already determined by the central nervous structure and function' (Wilson 1961).

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Note added in proof. Some results presented in this paper differ to those recently published by Pearson and Wolf (1987, J Comp Physiol A 160:259–268). For intact flying *Locusta* they find a constant depressor-elevator (D-E) latency irrespective of wingbeat frequency. This contrasts not only our present findings, but also those of Waldron (1967b) and Kutsch and Stevenson (1984). In deafferentated preparations, however, they find a decrease in D-E latency with increasing frequency, which is comparable to our results for both intact and deafferentated locusts. They thus claim, in contrary to us, that deafferentation produces qualitative changes in the flight motor pattern.

Pearson and Wolf's data were won, however, from locusts flying upside-down rather than in the normal position. As this may be the basis of our conflicting results, we compared EMG recordings from 5 intact animals flying under these two situations (Fig. 13). Paired observations (200 intervals each animal and situation) revealed an increase in D-E latency with cycle length for locusts flying in the normal position (full linear regression lines) but a rather constant D-E latency when upside-down (stippled lines). This difference was found both for muscle pair 129 and 119 we investigated and also muscle pair 97 and 83 Pearson and Wolf examined (lines marked by arrow). The results is similar irrespective of whether latency is plotted against cycle length or frequency, although we prefer the former since units of time and frequency are not linearly related. We thus see no reason to modify any conclusion drawn in this paper.

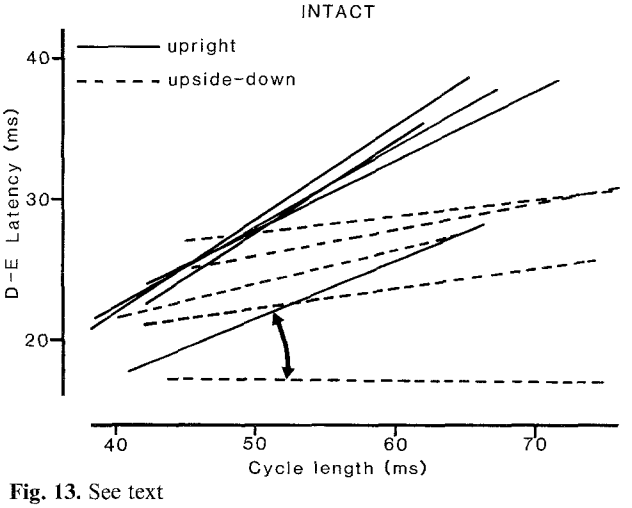

Fig. 13. See text