J. exp. Biol. 142, 401–424 (1989)
Printed in Great Britain © *The Company of Biologists Limited 1989*

ALTERATION OF THE RESPIRATORY SYSTEM AT THE ONSET OF LOCUST FLIGHT

I. ABDOMINAL PUMPING

BY J. M. RAMIREZ AND K. G. PEARSON
Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7

Accepted 17 November 1988

Summary

The respiratory behaviour of *Locusta migratoria* is altered at the onset of flight. The neuronal processes and some of the mechanisms underlying these alterations were studied by using intracellular recording and staining techniques.

It has previously been reported that abdominal pumping ceases for the first seconds of flight. Our data indicate that this phenomenon is not due to inhibition of the respiratory system, since most interneurones and some motoneurones maintain a respiratory rhythm during the onset of flight activity. Likely explanations for the cessation of the abdominal pumping are: (1) increased stiffness of the abdomen due to maintained activation of abdominal muscles and (2) decreased rhythmic modulation in abdominal motor units due to tonic excitatory input.

Two major changes occur in the respiratory system at the onset of flight: (1) the rhythm is reset by an activation of inspiratory and inactivation of expiratory neurones, and (2) the respiratory rate is increased. The increase in the respiratory rate at the onset of flight is in part due to an activation of inspiratory interneurones which are capable of accelerating the respiratory rhythm.

The changes in the respiratory system coinciding with the initiation of flight suggest a feedforward mechanism linking both behaviours. Tonic interneurones, involved in the initiation of flight and influencing respiration, might be involved in linking respiration and flight. At flight onset, one group of these simultaneously disinhibited respiration and flight and thus contributed both to an increase in the respiratory rate and to an activation of the flight system. Another group evoked flight and had variable effects on respiration. One tonic interneurone had a depressing effect on the respiratory rate.

We conclude that respiration is centrally linked to flight in part by the same interneurones controlling the initiation of flight. The existence of such a feedforward mechanism in the locust resembles the situation found in vertebrates, where locomotory and respiratory behaviour can be driven from the same brainstem region.

Introduction

In many animals respiration strongly depends on the animals' activity. It is

Key words: insect, locust, respiration, flight, interneurone.

generally found that the onset of movement is accompanied by an immediate increase in the respiratory rate (Di Marco *et al.* 1983; Feldman, 1986; Miller, 1960*c*). The mechanisms underlying this change in the respiratory system have been discussed for a long time for both vertebrates and invertebrates (Volkman, 1841; Miller, 1960*c*). Two major hypotheses have emerged. First, it has been hypothesized that the increased respiratory rate is caused by sensory *feedback*. Chemical stimuli, such as carbon dioxide, are unlikely to be involved, since they would be too slow to explain the immediate alteration of the respiratory system (Hoyle, 1959; Krogh & Lindhard, 1913). However, sensory feedback from mechanical or chemical receptors within the muscles could provide a rapid signal for exciting the respiratory system. The second hypothesis is that *feedforward* signals from the system driving movements to the respiratory system increase the respiratory rate (Krogh & Lindhard, 1913; Miller, 1960*c*). Evidence to support both hypotheses has been presented in vertebrates: feedback control by McCloskey & Mitchell (1972), Tibes (1977), Kao *et al.* (1979) and feedforward control by Eldridge *et al.* (1981, 1985). However, in invertebrates the neuronal mechanisms underlying alterations of the respiratory system related to locomotory movements are still unknown.

Previous studies have shown that two major alterations occur in the respiratory behaviour when a locust starts flying: (1) pumping movements of the abdomen cease at the onset of flight, but reappear after a few seconds at a higher rate than in the resting animal (Miller, 1960*c*), and (2) the thorax is moved rhythmically in phase with the flight rhythm instead of in phase with abdominal movements (Weis-Fogh, 1964). In this study we have concentrated on determining the neuronal mechanisms responsible for changes in the pumping rate of the abdomen at flight onset. A necessary prerequisite for an understanding of these mechanisms is knowledge of the events occurring in the respiratory system at the onset of flight. The cessation of pumping movements in the abdomen could be due to either: (1) an inhibition of the respiratory system, or (2) to the fact that respiratory activity cannot be expressed because of maintained activity in abdominal muscles. The latter possibility is likely since the abdomen is raised after flight onset into a typical flight position (Camhi & Hinkle, 1972), and many motor units on both sides of the abdomen are recruited, most of them tonically (Baader, 1988). Owing to the coactivation of many abdominal muscles the stiffness of the abdomen is presumably increased, which would tend to obscure ventilatory pumping movements.

To examine the issue of what the respiratory system does at flight onset we recorded intracellularly from respiratory motoneurones and interneurones. Our recordings revealed that the respiratory system remains rhythmically active at flight onset, that the respiratory rate is immediately increased and that it is reset at flight onset by an activation of inspiratory and an inactivation of expiratory neurones. These data demonstrate that the respiratory and flight systems are coupled by a feedforward mechanism. Interneurones that may be involved in the feedforward pathway were identified in the suboesophageal ganglion, a ganglion known to be important for the control of ventilation (Huber, 1960*a*).

Materials and methods

Animals

Adult male or female *Locusta migratoria* from colonies kept at the University of Alberta were used. All experiments were performed at room temperature.

Preparation and dissection

For most intracellular recordings the animals were mounted dorsal side up on a corkboard with legs and wings removed. The suboesophageal ganglion was exposed as described in detail by Kien & Altman (1984). The head capsule was opened leaving the frontal wind-sensitive hairs intact. The mandibular and dorsal neck muscles as well as the tentorium were removed. The tritocerebral commissure was left intact. The meso- and metathoracic ganglia were exposed, as described by Robertson & Pearson (1982). The thorax was opened with a dorsal incision and the gut and small muscles over the ganglia were removed. In some cases intracellular recordings were obtained in intact tethered flying locusts. This preparation was described by Wolf & Pearson (1987). The legs, but not the wings, were removed and the locust was fixed ventral side up on a steel holder. Recordings were obtained from a small window cut into the sternal cuticle above the ganglion. The tracheae were left intact. The ganglion from which intracellular recordings were obtained was supported by a stainless-steel platform and kept moist by a saline described by Robertson & Pearson (1982). In all preparations flight was induced by a frontal wind stimulus of 3–4 $m\,s^{-1}$. The onset of the wind stimulus was measured by a wind anemometer mounted at a distance of 1–2 mm from the frontal wind-sensitive hairs.

Recording and staining

Rhythmic activity in the flight system was monitored by electromyograph (EMG) recordings of either the tergosternal muscle 83, a forewing elevator, or the subalar muscle 129, a hindwing depressor. Respiratory activity was usually monitored by EMG recordings from either the inspiratory muscle 177 or the expiratory muscle 179. Recordings from these abdominal muscles were obtained by using low-resistance glass electrodes filled with potassium acetate (1 $mol\,l^{-1}$) inserted into the muscles. Sometimes respiratory activity was monitored by a hook electrode placed on either a median nerve or one of nerves 8, 9 or 10 of the metathoracic ganglion. The activity patterns in these nerves have been described by Lewis *et al.* (1973).

All intracellular recordings from neurones were obtained from their neuropile processes. The d.c. records were stored on an FM tape recorder and examined later. Recording electrodes were filled with a 5 % solution of the fluorescent dye Lucifer Yellow in distilled water. The electrode resistances varied between 60 and 200 MΩ. To allow the identification of the recorded neurone, dye was injected by passing negative current (4–7 nA) for up to 30 min. The ganglia were processed as described by Robertson & Pearson (1982) and the neurones were identified and

numbered according to the three-digit nomenclature of Robertson & Pearson (1982, 1983).

Results

Activity in abdominal muscles at the onset of flight

Miller (1960*c*) has demonstrated that abdominal pumping ceases for the first few seconds of flight, but he did not establish the mechanisms for this cessation. The cessation of abdominal pumping could be due to a strong activation of additional

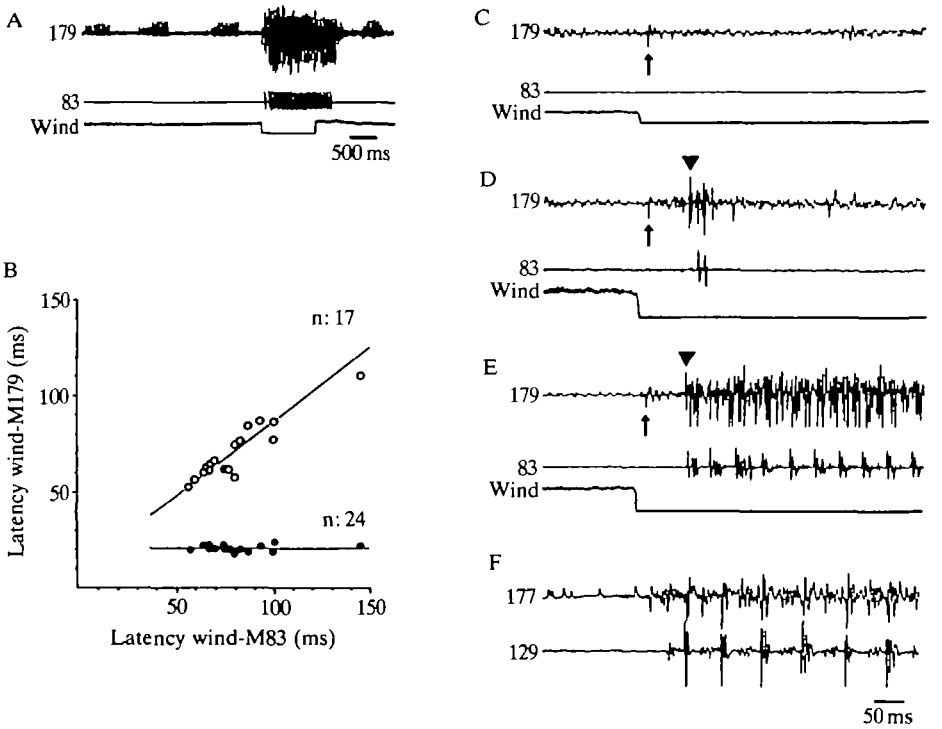

Fig. 1. Activity of abdominal muscles at the onset of flight. The recordings were obtained in intact tethered flying locusts. (A) Electromyograph (EMG) recording from expiratory muscle 179 (upper trace), recorded simultaneously with the EMG from wing elevator muscle 83 (middle trace) and the registration of the wind anemometer (lower trace). Note: during flight, respiratory rhythmic modulation is not visible. (B) Relationship between the onset of activity in the expiratory (ordinate) and the elevator muscle (abscissa). Closed circles, latency from the onset of wind to the onset of activity in a small abdominal motor unit (arrows in C–E). Open circles, latency from the onset of wind to the onset of activity in larger abdominal motor units (arrowheads in D,E). (C–E) Upper traces, activity in M179; middle traces, activity in M83; lower traces, wind stimulus. (C) Wind evoked no flight. (D) Wind evoked only one wing beat. (E) Long flight sequence evoked by wind. (F) Wind-evoked flight activates inspiratory muscle 177. Upper trace, activity in M177; lower trace, activity in the wing depressor muscle M129.

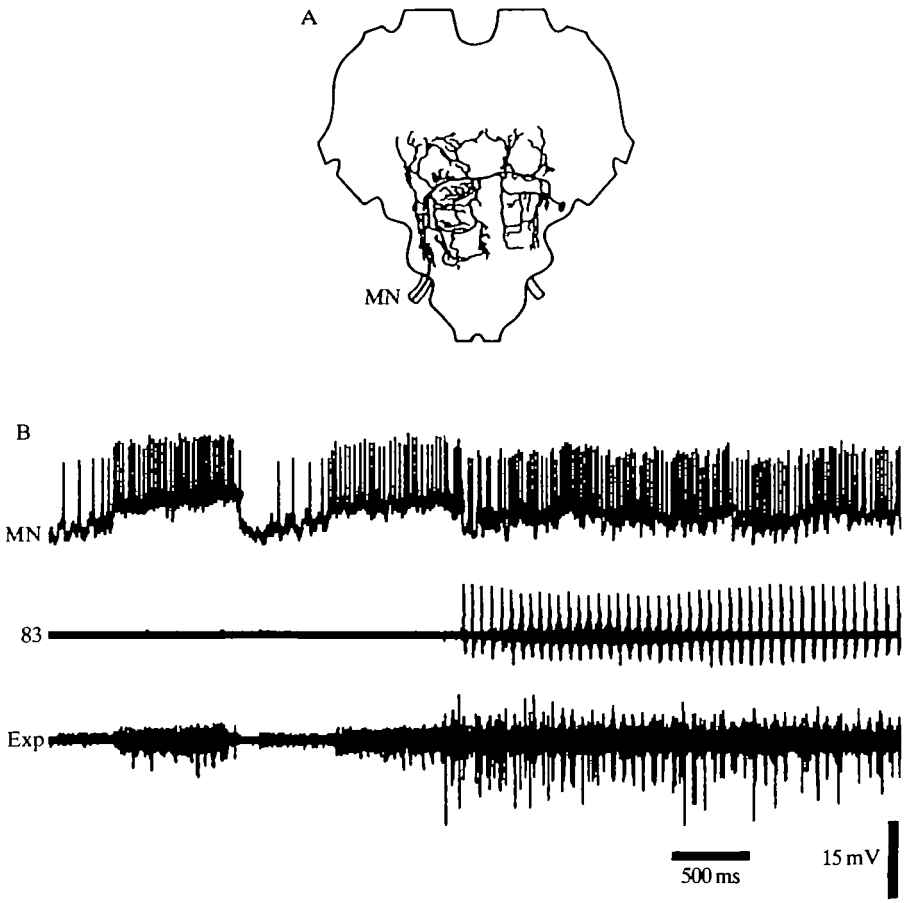

Fig. 2. Activity of an abdominal motoneurone (MN) during flight, recorded in an intact tethered flying locust. (A) Structure of the abdominal motoneurone which was located in the first abdominal ganglion. (B) Motoneurone recorded intracellularly (upper trace), together with an EMG from muscle 83 (middle trace) and a first abdominal nerve recording monitoring expiratory activity (lower trace). The expiratory rhythmic modulation in the motoneurone during flight was obscured by tonic excitation. In this and all following figures the vertical calibration bar reflects the amplitude of the intracellular recording (upper traces).

abdominal motor units which are inactive in the quiescent locust and which, during flight, obscure the activity of the respiratory system. We would therefore expect that this activation of abdominal muscles should be strictly correlated with the flight behaviour and should occur simultaneously with the onset of flight. Recordings from abdominal muscles have previously been obtained mainly with respect to steering behaviour (Camhi & Hinkle, 1972; Baader, 1988), but the exact time of activation of these muscles at the onset of flight was not established. Simultaneous recordings were obtained from the abdominal muscle 179, the wing elevator muscle 83 and the wind stimulus. In the quiescent locust, muscle 179 is

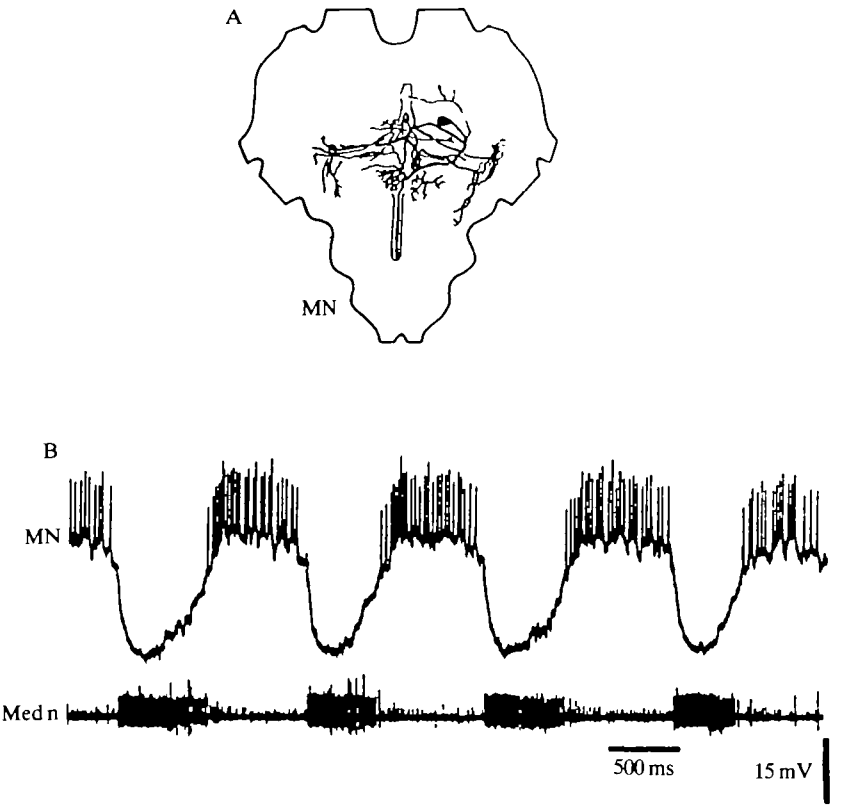

Fig. 3. Activity of the motoneurone innervating the closer muscle of spiracle 4, recorded in the quiescent locust. (A) Structure of the motoneurone in the first fused abdominal ganglion. (B) Upper trace, intracellular recording from the closer motoneurone. Lower trace, nerve recording from the first abdominal median nerve (Med n).

rhythmically active in phase with expiration. At flight onset, as well as during flight, additional motor units are recruited (Fig. 1A). The time of onset of activity in M179 was measured by taking the latency from the onset of wind blown on the head to the onset of activity in M179. The first muscle spike occurred with a relatively constant latency of 21 ± 2 ms (± S.D.) in M179 and was always visible in response to wind blown on the head, regardless of whether flight was initiated (Fig. 1D,E) or not (Fig. 1C). The latency of this response was not correlated with the latency of the onset of activity in elevator muscles (Fig. 1B, closed circles). We therefore conclude that this muscle spike is not a response to the onset of flight activity but a response to the sensory wind stimulus. Further muscle spikes occurred with a longer and more variable latency of 71 ± 9 ms. The latency of this M179 response was correlated with the latency of the onset of activity in the elevator muscle 83 (Fig. 1B, open circles). The muscle spikes in M179 were only visible if flight activity was elicited (Fig. 1D,E) and were not visible if the wind

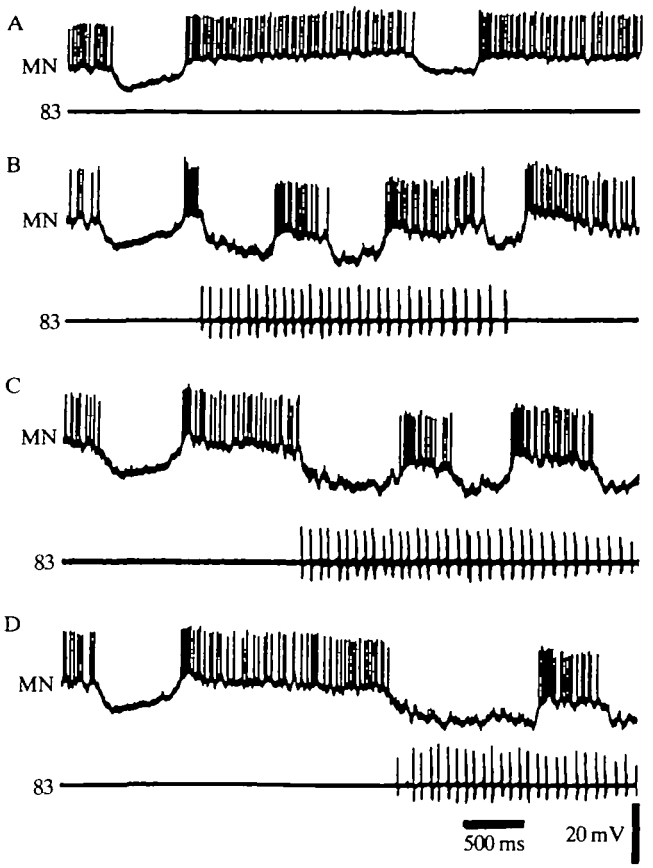

Fig. 4. Activity of the closer motoneurone innervating spiracle 4 at the onset of flight, recorded in an intact tethered flying locust. (A–D) Upper traces, intracellular recordings from the motoneurone. Lower traces, EMGs from wing elevator muscle 83. (A) Activity in the quiescent locust. (B–D) Activity at the onset of flight. The motoneurone was inhibited at the onset of flight, no matter in which phase of the respiratory cycle flight was initiated.

stimulus evoked no flight activity (Fig. 1C). The duration of activity in M179 was also correlated with the duration of activity in the elevator muscle M83. Although the wind stimulus was maintained for a long period, the activity in M179 ceased shortly after cessation of activity in M83 (Fig. 1D). However, the activity in M179 exceeded the duration of the wind stimulus if the flight episode was longer than the wind stimulus (Fig. 1A). Because of this coincidence of M179 activity with activity in the flight muscle 83, we conclude that this activity in M179 is a response due to the evoked flight activity and not due to a response to the wind stimulus. Similar results were also obtained for other expiratory muscles and for abdominal muscles which were active in phase with inspiration such as the muscle 177 (Fig. 1F). The high level of activity in abdominal muscles during flight made it impossible to determine whether the motor units which are rhythmically active in the quiescent

locust are still active, tonically active or inhibited during flight. Thus, it was impossible to determine what effect flight had on the respiratory system using this experimental arrangement. To examine this issue it was necessary to record intracellularly from single respiratory neurones.

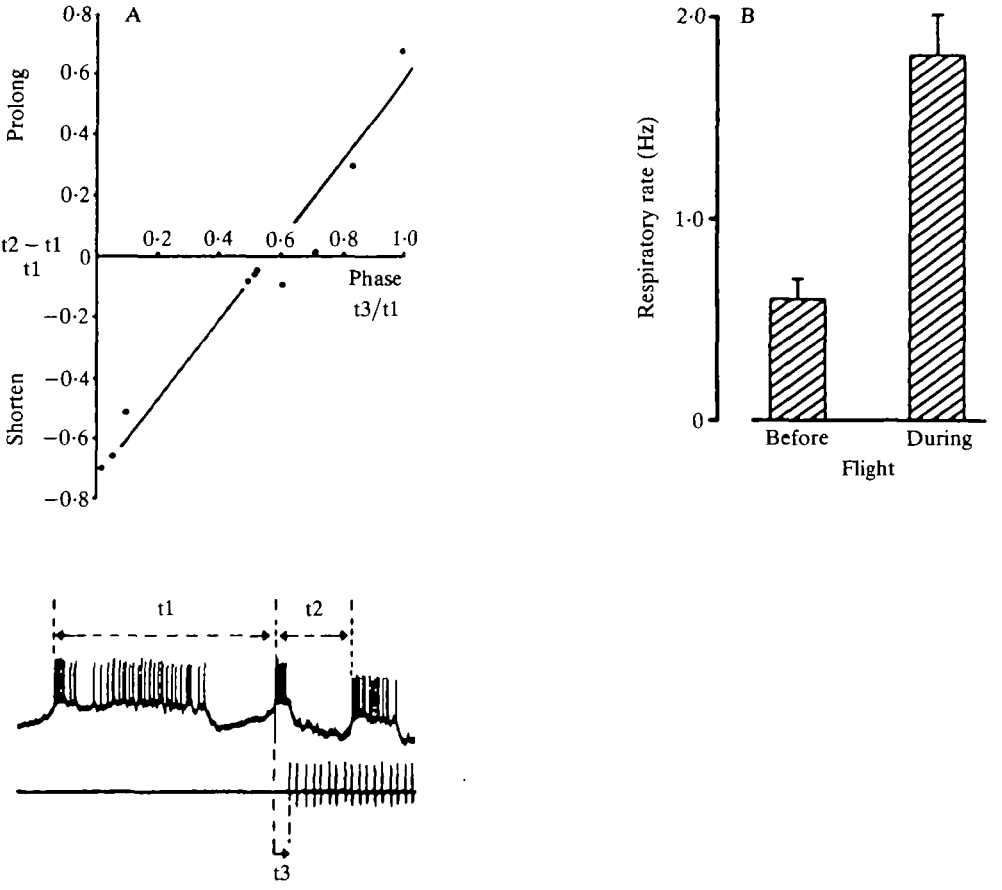

Fig. 5. Alteration of respiratory activity in the closer motoneurone to spiracle 4 at the onset of flight. (A) Reset of the respiratory rhythm at the onset of flight. Ordinate, effect of flight on the respiratory rhythm. Abscissa, phase at which flight was initiated. Inset: t1, duration of the respiratory cycle immediately preceding the cycle in which flight was initiated. One respiratory cycle was the time between two consecutive burst of activity in the closer motoneurone. t2, duration of the respiratory cycle in which flight was initiated. t3, duration from the onset of expiratory activity directly preceding the initiation of flight to the onset of flight monitored by the activity in muscle 83. (B) Respiratory rate increase immediately following the initiation of flight. The histogram represents the average respiratory rate before and immediately following the initiation of 10 succesive flight sequences in the same animal. Two consecutive respiratory cycles were measured to calculate the respiratory rate. Since flight always started with an inhibition of the closer motoneurone, one respiratory cycle was taken as the time between the onset of two consecutive inhibitions in the closer motoneurone.

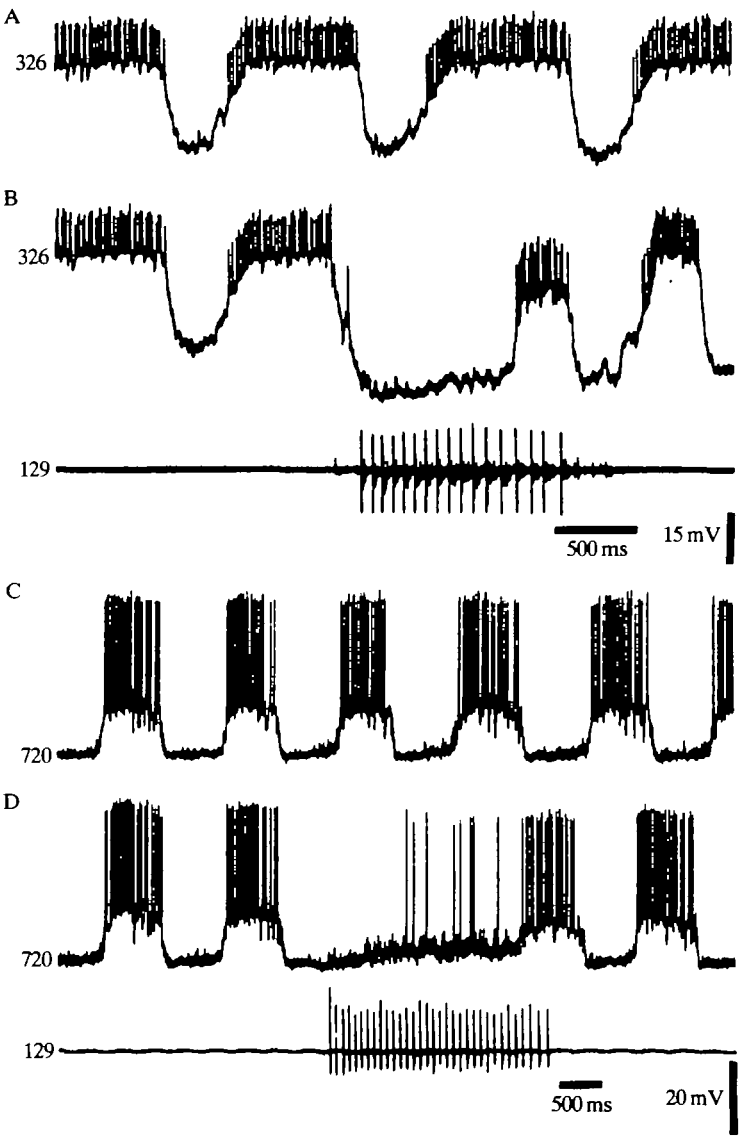

Fig. 6. Activity of the expiratory interneurones 326 and 720 during short flight sequences. The structures of these neurones have been previously described (Ramirez & Pearson, 1989). (A) Intracellular recording from 326 in the quiescent locust. (B) Intracellular recording from 326 (upper trace) at the onset of flight, as monitored by the EMG from depressor muscle 129 (lower trace). (C) Intracellular recording from 720 in the quiescent locust. (D) Intracellular recording from 720 (upper trace) at the onset of flight, as monitored by the EMG from muscle 129 (lower trace).

Activity of respiratory motoneurones at the onset of flight

Intracellular recordings were obtained from respiratory motoneurones in the first fused abdominal ganglion, a ganglion which seems to be important for the

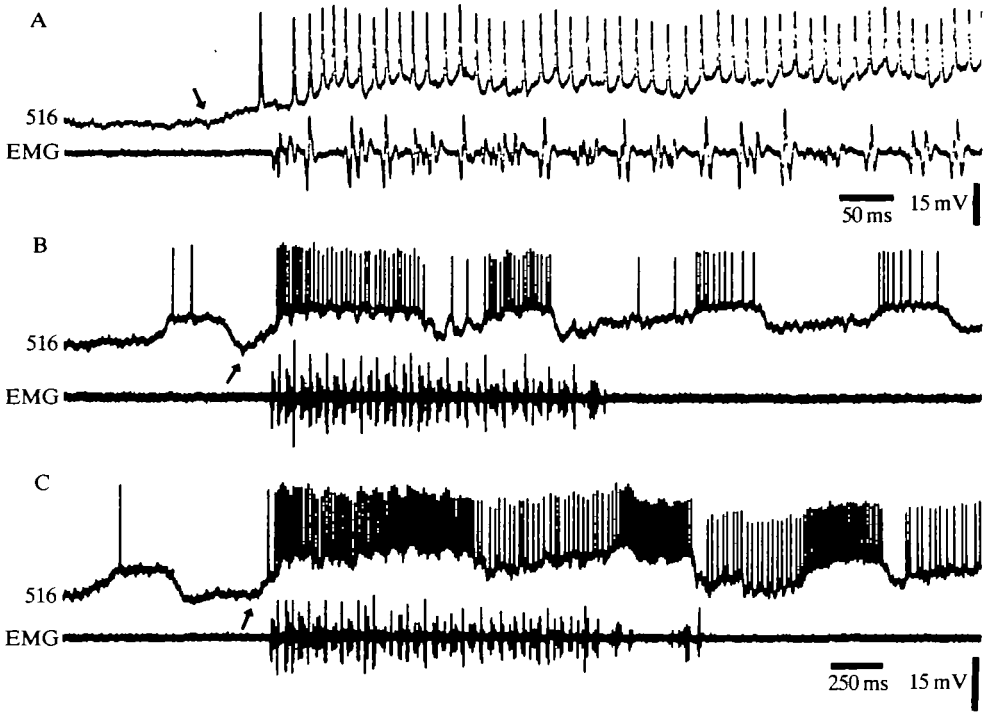

Fig. 7. Activity of the inspiratory interneurone 516 at the onset of flight. The structure of 516 has been previously described (Ramirez & Pearson, 1989). (A–C) Intracellular recordings from 516 (upper traces) at flight onset as monitored by the EMGs from a wing elevator muscle (lower traces). Note that the depolarization in 516 always started before the onset of activity in the wing muscle (arrows), indicating that the depolarizing input to 516 could not be caused by feedback from muscle activity. Spikes were sometimes produced at the same time or just after the onset of activity in the wing muscle. (B,C) The duration of the first inspiratory burst at flight onset depends on the respiratory phase in which flight was initiated.

generation of the respiratory rhythm (Ramirez & Pearson, 1989). As expected from the myogram studies, many motoneurones, regardless of whether they were rhythmically active in phase with inspiration or expiration, were tonically excited during flight. One example is shown in Fig. 2A. This motoneurone was rhythmically active in phase with expiration in the quiescent locust, but tonically active during flight (Fig. 2B). However, slight respiratory rhythmic activity was visible but this was not sufficiently clear to characterize the influence of flight on the respiratory system. A better insight was gained by studying motoneurones which, during flight, remained rhythmically active in phase with respiration. One example is the motoneurone innervating the closer muscle of spiracle 4. This motoneurone was identified by its anatomy (first characterized by Burrows, 1982) (Fig. 3A), as well as by its activity recorded extracellularly in the median nerve. In the quiescent locust, the spiracle closer motoneurone was rhythmically active in phase with

expiration and discharged reciprocally to the activity in the spiracle opener motor units, as indicated by the high-amplitude potentials in the median nerve recording (Fig. 3B). At the onset of flight two major alterations in the respiratory rhythm were found to be reflected in the motoneurone's activity.

The first major alteration was that the respiratory rhythm was reset (Figs 4, 5A). This reset was indicated by the inhibition of the closer motoneurone at the onset of

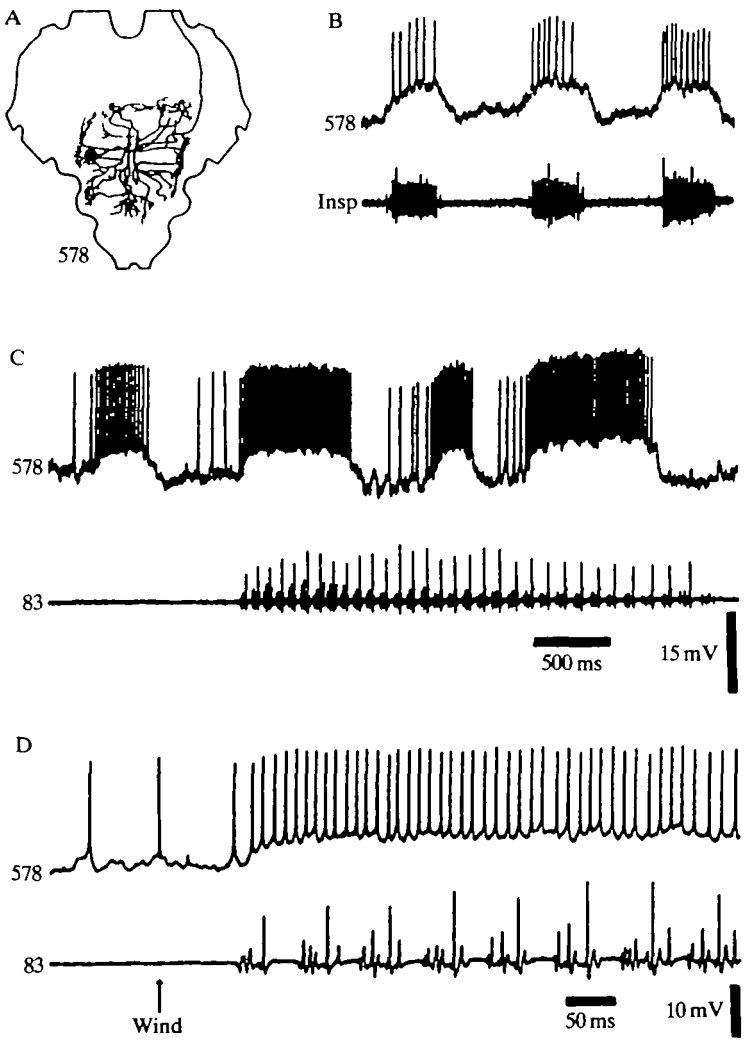

Fig. 8. Activity of the interneurone 578 in the quiescent locust and at the onset of flight. (A) Structure of the interneurone 578. The cell body of 578 is located in the first abdominal ganglion, the axon ascends contralaterally to the cell body at least into the prothoracic ganglion. (B) Activity of 578 in the quiescent locust. 578 (upper trace) discharged in phase with inspiration (Insp), as indicated by the EMG from muscle 177 (lower trace). (C,D) Activity of 578 at the onset of flight. Upper trace, intracellular recording from 578. Lower trace, EMG from muscle 83. Note that 578 was depolarized at the onset of flight.

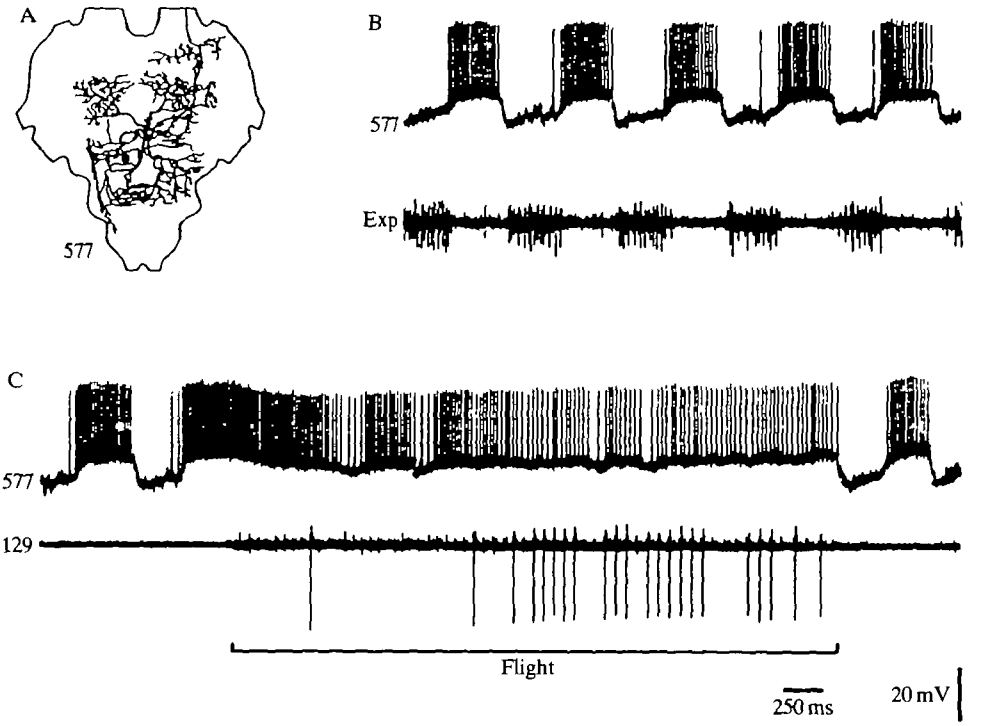

Fig. 9. Activity of the interneurone 577 (previously described by Burrows, 1982*b*) in the quiescent and tethered flying locust. (A) Structure of 577. The cell body is located ventromedially in the first abdominal ganglion. The axon ascends contralateral to the cell body at least into the prothoracic ganglion. (B) Activity of 577 in the quiescent locust. 577 (upper trace) was rhythmically active in phase with inspiration and discharged reciprocally to the activity in the expiratory muscle (Exp) as indicated by the EMG from muscle 179 (lower trace). (C) Activity of 577 during intact tethered flight. Upper trace, intracellular recording from interneurone 577. Lower trace, EMG from muscle 129. Note that 577 was tonically active during flight.

flight (Fig. 4B–D). The duration of this inhibition and the time of onset of expiratory activity were dependent on the respiratory phase in which flight was initiated. The earlier in the expiratory cycle that flight was initiated, the shorter was the inhibition and the sooner the next expiratory burst followed (Fig. 4B–D). Consequently, at phase values lower than 0·6 (Fig. 5A), the first expiratory burst during flight occurred earlier than would have been expected in the quiescent locust (Fig. 4A). Such a shortening of the respiratory cycle is reflected by negative values on the ordinate of the reset curve in Fig. 5A. The first expiratory burst during flight occurred later than would have been expected from the activity in the quiescent locust (positive values on the ordinate of the reset curve, Fig. 5), if flight had been initiated late in the expiratory (Fig. 4D) or during the inspiratory phase (phase values higher than 0·7).

The second major alteration of the respiratory system, as reflected in the

motoneurone's activity, was that the respiratory rate was increased immediately following the onset of flight. The duration of the first respiratory cycle during flight, measured from the onset of inhibition immediately following the beginning of flight to the onset of the next inhibition, was always shorter than in the quiescent locust. The respiratory rate increase is demonstrated by a histogram in Fig. 5B, in which the durations of two respiratory cycles before and after onset of flight were taken in account to assess the respiratory rate. The results described in this section indicate: (1) the respiratory system is reset at the onset of flight by an inhibition of expiratory neurones, and (2) the respiratory rate is increased immediately following the onset of flight. To confirm and extend these findings we recorded intracellularly from interneurones.

Activity of respiratory interneurones at the onset of flight

The most direct approach to obtain insight into the neuronal mechanisms underlying the alterations of the respiratory rhythm was to record, at the onset of flight, the activity of interneurones which are involved in the generation of the respiratory rhythm. Such interneurones have been described previously (Ramirez & Pearson, 1989). The expiratory interneurone 326, located in the first fused abdominal ganglion, could reset and accelerate the respiratory rhythm and is therefore an element of the respiratory rhythm generator (Ramirez & Pearson, 1989). At the onset of flight, 326 was immediately hyperpolarized (Fig. 6B). The duration of the first burst during flight was considerably shorter (Fig. 6B) than those in the quiescent animal (Fig. 6A). This is also consistent with the findings in the closer motoneurone. A similar result was also obtained for other expiratory interneurones located in the first fused abdominal ganglion, such as 327, 328, 329 and 606 (Ramirez & Pearson, 1989). Another expiratory interneurone examined was the interneurone 720. It is located in the mesothoracic ganglion, and could reset, entrain and slow the respiratory rhythm (Ramirez & Pearson, 1989). Excitatory input to 720 was suppressed immediately after flight onset (Fig. 6C,D). Our data, obtained from moto- and interneurones, demonstrate, therefore, that expiratory neurones are inactive immediately following the onset of flight. This inactivity can be due either to a hyperpolarization (326, 327, 328, 329, 606) or to a suppression of excitatory input (720).

Because the respiratory system is reciprocally organized, one would expect that inspiratory neurones would be activated at flight onset. This was found to be true. The interneurone 516, which is located in the first fused abdominal ganglion, could reset and accelerate the respiratory rhythm (Ramirez & Pearson, 1989). As predicted, 516 was excited at the onset of flight (Fig. 7, arrows). The onset of spike activity in 516 coincided with the onset of flight and the depolarization in 516 started before the onset of activity in wing muscles (Fig. 7). The duration of the excitatory burst at the onset of flight was dependent on the phase in which flight was initiated. The excitatory burst was short if flight was initiated early in the expiratory phase (Fig. 7B) and longer if flight was initiated later in the expiratory phase (Fig. 7C). Thus, the excitatory burst of the inspiratory interneurone has

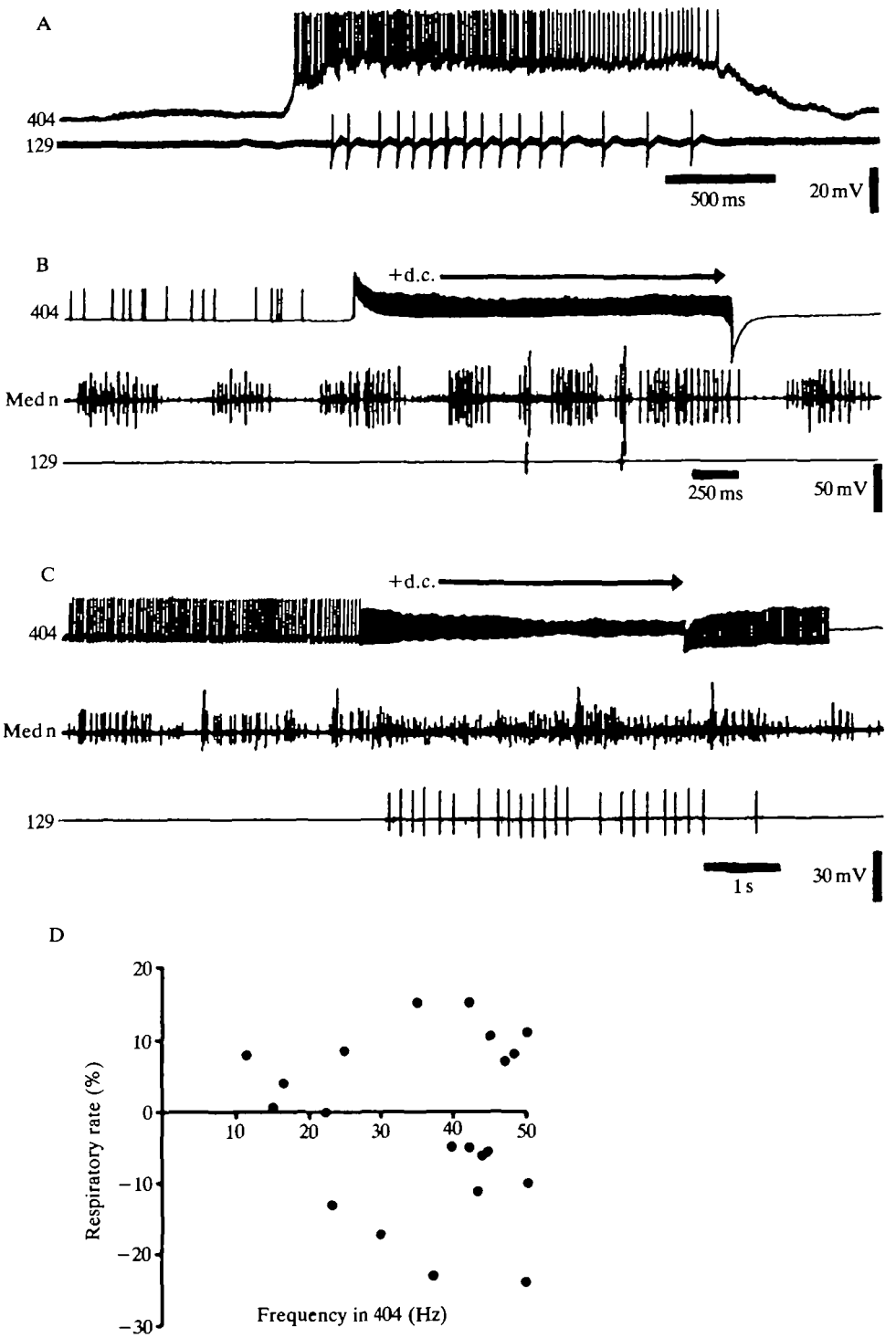
A
404
129
500 ms
20 mV
B
+d.c.
404
Med n
129
250 ms
50 mV
C
+d.c.
404
Med n
129
1 s
30 mV
D
Respiratory rate (%)
20
10
0
−10
−20
−30
10
20
30
40
50
Frequency in 404 (Hz)

Fig. 10

similar characteristics to the onset inhibition of the expiratory neurones. The strong excitation in 516 was due to a tonic depolarization. Therefore, spikes were produced not only during the burst at the onset of flight but also during the expiratory phase (Fig. 7C). Since intracellular injection of constant depolarizing current into 516 caused a considerable increase in the respiratory rate (Ramirez & Pearson, 1989), the strong excitation observed in 516 during flight presumably contributed to the acceleration of the respiratory rate at flight onset.

A similar result was obtained for one other inspiratory interneurone, 578 (Fig. 8). Interneurone 578 was strongly excited at the onset of flight (Fig. 8C), with the excitation coinciding exactly with the onset of flight (Fig. 8D). However, interneurone 578 had no effect on the respiratory rhythm and, therefore, probably did not contribute to the increase in the respiratory rate.

Another inspiratory interneurone excited at the onset of flight was the interneurone 577 (Fig. 9A), which has been described previously by Burrows, (1982*b*). In contrast to the interneurones described above, it remained tonically excited during flight (Fig. 9C).

Tonic interneurones influencing respiration and flight

The data obtained from respiratory interneurones and motoneurones revealed that the respiratory rhythm is reset at the onset of flight by an inhibition of expiratory and an excitation of inspiratory neurones. This suggests that the respiratory and the flight systems are coupled by a feedforward mechanism. Neurones that might be involved in such a feedforward mechanism are interneurones contributing to the initiation of flight.

One group of flight-initiating neurones are the neurones 404 in the mesothoracic ganglion (Fig. 10; Pearson *et al.* 1985). These neurones were tonically active during flight (Fig. 10A) and evoked flight activity when stimulated intracellularly (Fig. 10C). In all nine animals we examined, the activity in abdominal muscles was

Fig. 10. Influence of flight-initiating interneurones 404 on respiration. (A) Activity of 404 during flight. Upper trace, 404 recording, lower trace, EMG recording from muscle 129. (B) Upper trace, 404 recording (a.c.-filtered). Middle trace, recording from first abdominal median nerve. Lower trace, EMG recording from muscle 129. The stimulation of 404 in this animal was too weak to evoke prolonged flight, but sufficient to evoke two wing beats. Note that the respiratory activity was altered before these wing beats. During the wing beats, respiratory activity occurred in short bursts with a duration similar to the duration of the wing beats. During 404 stimulation, the intensity of activity was increased in the median nerve. (C) Upper trace, 404 recording. Middle trace, recording from third abdominal median nerve. Lower trace, EMG recording from muscle 129. Intracellular stimulation of 404 evoked flight activity and altered respiratory activity. (D) Effect of 404 stimulation on respiration at strengths insufficient to evoke flight activity. Each dot in the graph represents one 404 stimulation, data were obtained from seven animals. Two respiratory cycles before and during stimulation were measured and the respiratory rate calculated. The difference between the respiratory rate before and during stimulation is represented as a percentage (ordinate). The frequency of 404 was measured for each stimulation (abscissa).

influenced by 404 stimulation at strengths that initiated flight (Fig. 10B,C). This 404-evoked activity in abdominal muscles resembled the activity during wind-evoked flight. However, owing to the reasons mentioned in the first section (Fig. 1), it was impossible to determine whether the respiratory rate was also increased. Therefore, we examined the effect on respiration of stimulating 404 at intensities insufficient to evoke flight activity (spike activity in 404 interneurones during stimulation: 10–50 spikes s^{-1}). This type of stimulation did alter the respiratory rate but in a very inconsistent manner. Stimulation could lead, even in the same animal, to an increase (up to 15 %) or decrease (more than 20 %) in the respiratory rate, and in many cases the respiratory rate was unaffected. The graph shown in Fig. 10D was taken from 24 stimulations in seven animals. Fig. 10B also

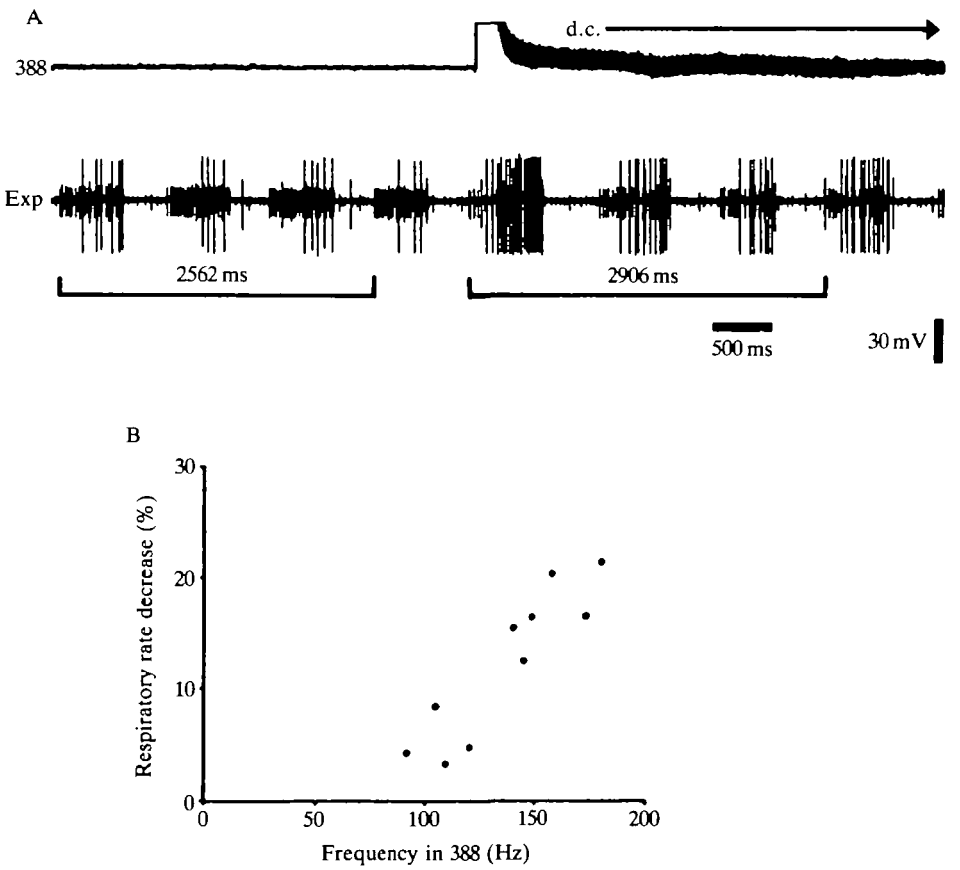

Fig. 11. Intracellular stimulation of 388 decreases the respiratory rate. (A) Upper trace, intracellular recording from 388 (a.c.-filtered). Lower trace, recording from an expiratory abdominal nerve. Note that the respiratory rate was decreased during 388 stimulation, but the intensity of activity in the nerve recording increased. (B) Each dot represents one 388 stimulation, obtained in the same animal. The procedure for calculating the effect of 388 on respiration was the same as described for Fig. 10.

gives an example of how the respiratory rhythm was altered if 404 stimulation evoked only a few wing beats.

In the suboesophageal ganglion further interneurones exist which are associated with the initiation of flight (Ramirez, 1988). The effect on respiration caused by three of these interneurones was examined in this study. The interneurone 388 received indirect excitatory input from 404 interneurones and, like these interneurones, it was also tonically active during flight. In all four animals examined, 388 stimulation caused a decrease in the respiratory rate (Fig. 11A,B). Thus, the tonic activity observed in 388 during flight (approximately, 140 spikes s^{-1}) could not contribute to the increase in the respiratory rate. Instead, it might act in opposition.

Two pairs of descending interneurones (398 and 399) originate in the suboesophageal ganglion and contributed to flight initiation by disinhibiting the flight system at flight onset. In the quiescent locust they were tonically active; 399 was inhibited by flight-initiating stimuli prior to the onset of flight (Fig. 12A) and 398 was inhibited 20–30 ms later than 399, coincident with the onset of flight. Intracellular stimulation of these neurones could inhibit flight activity (Ramirez, 1988). Both interneurones (398 and 399) had an inhibitory influence on the respiratory system (398 was examined in 12, 399 in five different animals). The strength of the inhibitory effect varied from animal to animal, ranging from a complete inhibition of respiration (Fig. 12B,C) to only a 10% decrease in the respiratory rate (the example in Fig. 12D represents a 28% respiratory rate decrease). Variability was also observed in the same animal. In 22 successive presentations of the same stimulus to 398 (average activity in 398 during stimulation was 162 spikes s^{-1}) there was an average decrease in the respiratory rate of $21{\cdot}4 \pm 9\%$. The intracellular injection of hyperpolarizing current into the spontaneously active interneurones (activity in 398 and 399 between 50 and 100 spikes s^{-1}) increased the respiratory rate (Fig. 13). The amount of the increase in respiratory rate caused by inhibiting the interneurones 398 and 399 was also variable, ranging from more than a 30% increase to no effect. Since all four interneurones of the bilaterally paired 398 and 399 were tonically active in the quiescent animal and inhibited at the onset of flight, they presumably contribute to an increase in the respiratory rate at flight onset. However, owing to the variability of the disinhibitory effect in a single neurone, it was not possible to estimate quantitatively the increase in respiratory rate caused by all four inhibitory interneurones.

Discussion

Alteration of abdominal pumping at the onset of flight

In the quiescent locust, the abdomen is moved rhythmically to ventilate the tracheal trunks (Hustert, 1975; Lewis *et al.* 1973; Miller, 1960*a*,*b*). When the locust starts flying, abdominal pumping cannot be expressed but reappears after a few seconds of flight at a considerably higher rate (Miller, 1960*c*). The cessation of

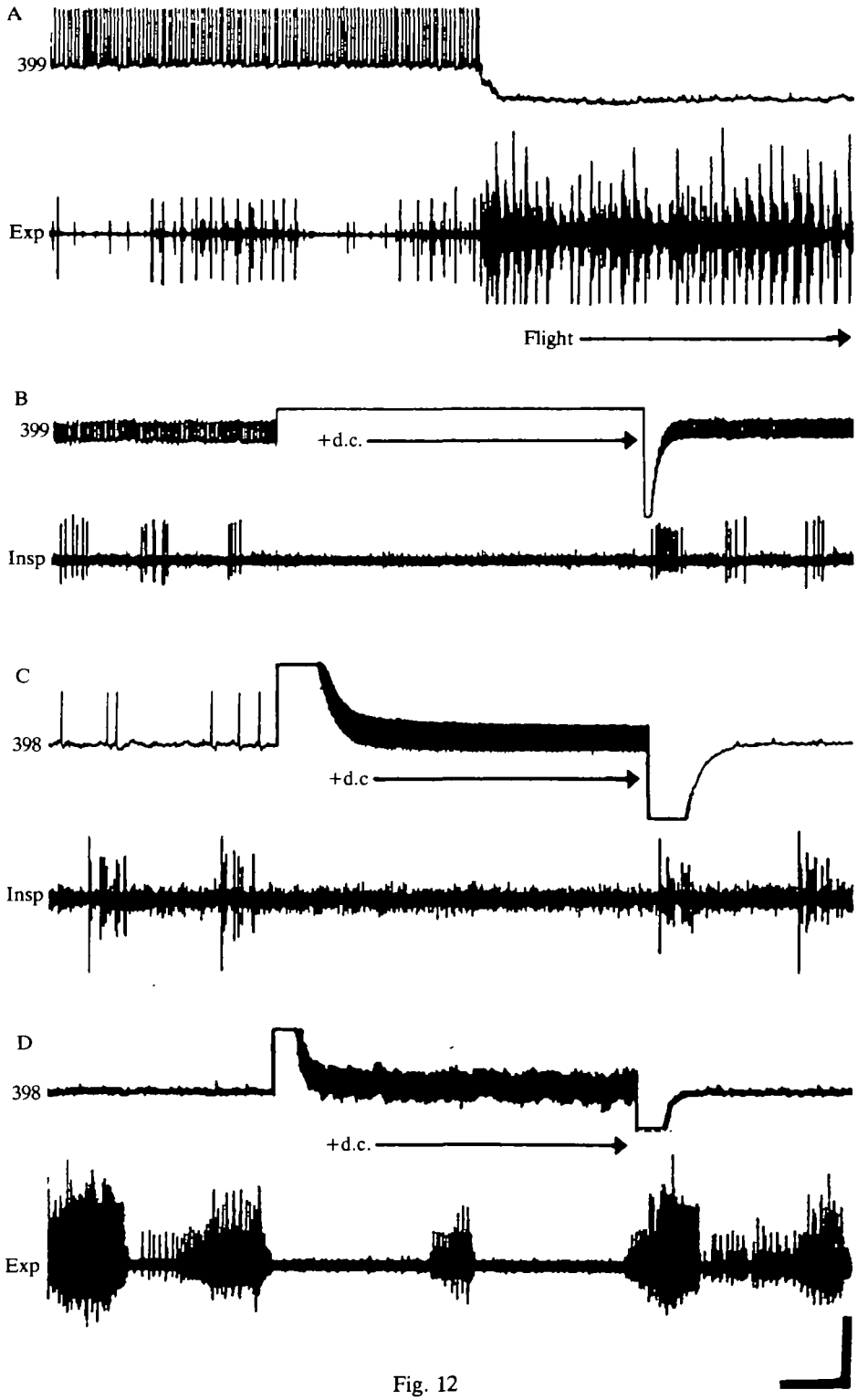

Fig. 12

abdominal pumping in the first seconds of flight is not due to an inhibition of the respiratory system, since interneurones which are involved in the generation of the respiratory rhythm (326, 327, 328, 329, 516, 606 and 720; Ramirez & Pearson, 1989) maintained respiratory rhythm at the onset of flight (Figs 6, 7). A probable explanation for the cessation of abdominal pumping is that the respiratory rhythm is obscured by maintained activity in abdominal muscles. In this study we have demonstrated that this possibility is likely since activity in abdominal muscles is considerably increased in strict correlation with flight and at the same time as flight is initiated (Fig. 1). Maintained abdominal muscle activity might contribute to obscure the respiratory rhythm in two different ways: first, by increasing the stiffness of the abdomen; second, by causing many abdominal motoneurones which are rhythmically active in the quiescent locust to become tonically active during flight (Fig. 2).

How does abdominal pumping restart after a certain time in flight? The analysis presented was only for the first few seconds of flight because flight sequences lasted for only a short time. However, we know that rhythmicity comes back; therefore, we would predict that the rhythmicity would return in many motoneurones after some seconds of flight. This issue remains to be examined.

Alteration of activity in respiratory rhythmic interneurones at the onset of flight

This study has not only demonstrated that the respiratory system is still active at the onset of flight but also shows that the respiratory system is reset by an inhibition of expiratory and an excitation of inspiratory neurones and that the respiratory rate is increased immediately following the onset of flight. To understand the neuronal mechanisms involved in these alterations we analysed the changes of activity in elements of the respiratory rhythm generator. Our data suggest that the increase in the respiratory rate at flight onset is mainly caused by an activation of inspiratory interneurones, such as the interneurone 516 (Fig. 7).

Fig. 12. Inhibition of respiration by the interneurones 398 and 399. (A) Activity of 399 at the onset of flight. Upper trace, intracellular recording from 399. Lower trace, nerve recording from an expiratory abdominal nerve (Exp). Note that 399 was inhibited at flight onset before an increase in activity in the expiratory nerve. (B) Intracellular stimulation of 399 inhibits respiration. Upper trace, intracellular recording from 399. No spikes are visible during injection of depolarizing current (10 nA), because the amplifier was out of balance. Lower trace, EMG from opener muscle of spiracle 3 which is active in phase with inspiration (Insp). Note the activity increase in the opener muscle immediately after stimulation. (C) Intracellular stimulation of 398 inhibits respiration. Upper trace, intracellular recording from 398. The 398 recording was a.c.-filtered. Depolarizing current: 7 nA. Lower trace, EMG from opener muscle of spiracle 3. (D) Intracellular stimulation of 398 decreases the respiratory rate. Upper trace, intracellular recording (a.c.-filtered) from 398. Depolarizing current: 5 nA. Lower trace, nerve recording from an expiratory abdominal nerve. Note that not only the respiratory rate but also the intensity of nerve activity is considerably decreased during 398 stimulation. Calibration bar, A 500 ms, 20 mV; B 1000 ms, 40 mV; C 500 ms, 30 mV, D 320 ms, 30 mV.

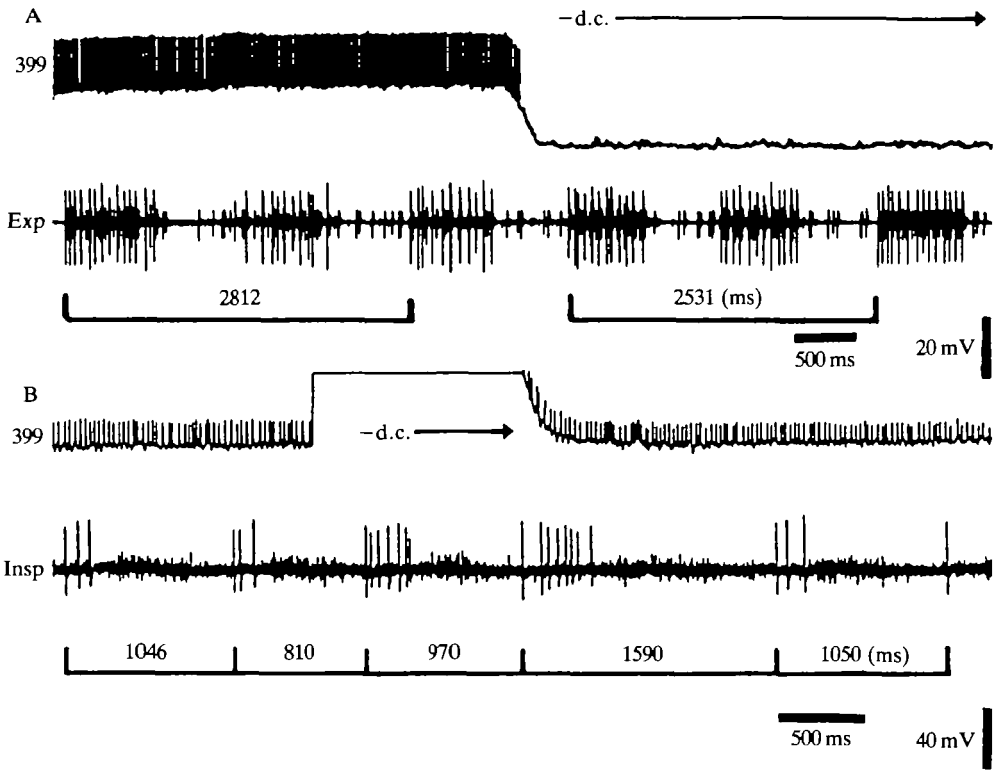

Fig. 13. Inhibition of 399 disinhibits respiration. (A) Upper trace, intracellular recording from 399. Hyperpolarizing current: −2 nA. Lower trace, nerve recording from an expiratory abdominal nerve. (B) Upper trace, intracellular recording from 399, a.c.-filtered. Negative current: −5 nA; the recording was out of balance during current injection. Lower trace, EMG from the opener muscle of spiracle 3. Note that in A and B not only the respiratory rate but also the intensity of activity in the nerve/muscle increased during injection of negative current into the spontaneously active 399.

516 was tonically depolarized at the onset of flight and remained so throughout the whole flight sequence. Consequently, the spike activity in 516 increased considerably during the inspiratory phase and this neurone was sometimes active during the expiratory phase. Intracellular injection of short current pulses into 516 had an acceleratory effect on the respiratory rhythm, even when stimulation was during the expiratory phase. Constant depolarizing currents, causing 516 to discharge at a spike frequency similar to the spike frequency during flight, could increase the respiratory rate by 2·6 times (Ramirez & Pearson, 1989). Thus, the activation of the bilateral pair of 516 could easily account for the threefold respiratory rate increase observed at the onset of flight (Fig. 5).

All expiratory interneurones, including the accelerating interneurones 327, 328 and 329 (Pearson, 1980; Ramirez & Pearson, 1989) were inactivated at the onset of flight (Fig. 6). The activity of these neurones during the expiratory phase was not

much greater than it was in the quiescent locust. Thus, although more expiratory than inspiratory interneurones are known in the respiratory rhythm generator (Ramirez & Pearson, 1989), the role of these interneurones in increasing the respiratory rate during flight appears to be small.

Alteration of spiracle activity at the onset of flight

Although it was not the major aim of this study to investigate the alteration of spiracle activity at flight onset, some of our data provide further insight into the control of spiracles during flight. We have demonstrated that the motoneurone innervating the closer muscle of spiracle 4 was rhythmically active at the onset of flight (Fig. 4). Its rhythmic activity and the rhythmic activity of motoneurones innervating the muscles of the abdominal spiracles 5–10 and the thoracic spiracle 1 are important to guarantee the airflow through the tracheae which primarily ventilates the central nervous system (Miller, 1960*b*). In contrast, spiracles 2 and 3 are opened at the onset of flight and remain open throughout the whole flight sequence to guarantee the ventilation of the rhythmically active flight wing muscles (Miller, 1960*c*, 1966; Weis-Fogh, 1964). Their opening is caused by a tonic inhibition of closer motoneurones (Miller, 1960*b*) which seems to be mediated by only a few interneurones (Burrows, 1985*a*,*b*, 1978, 1982*a*). An interneurone that might contribute to the tonic inhibition of the closer motoneurone is the interneurone 577. In the quiescent locust it discharged in phase with inspiration and therefore in antiphase to the activity in the closer motoneurone. At the onset of flight and throughout the whole flight sequence it was tonically active (Fig. 9C). Its morphology indicates that it has inhibitory outputs (Pearson & Robertson, 1987) and, indeed, inhibitory connections to the motoneurones innervating spiracle 2 were demonstrated for an anatomically and physiologically similar neurone (Burrows, 1982*b*).

Tonic interneurones influencing respiration and flight

The findings that the respiratory rhythm is reset and the respiratory rate increased at the same time as flight is initiated suggest that the respiratory system is altered by a feedforward mechanism. The advantage of such a feedforward control is that the respiratory rate is increased in anticipation of need; a strategy which was also described in vertebrate systems (Feldman, 1986). One possible mechanism to provide feedforward signals from flight to respiration, is through neurones which are involved in the initiation of flight. To examine this hypothesis we studied the influence on respiration of interneurones which are involved in the initiation of flight. Two pairs of suboesophageal ganglion interneurones, the descending interneurones 398 and 399, were found to have an inhibitory effect on both the flight (Ramirez, 1988) and the respiratory systems (Fig. 12). These interneurones were tonically active in the quiescent locust and were inhibited just before (399) or at the same time (398) as flight was initiated (Ramirez, 1988). The inhibition of these interneurones disinhibited both the flight and the respiratory systems, thus contributing to a synchronous activation of both systems and to an increase in the

respiratory rate. However, as already mentioned in the Results section, the variability of the disinhibitory effect in a single neurone made it impossible to estimate the contribution of all four interneurones to the increase in the respiratory rate. Also, the data obtained by inhibiting single interneurones could not explain how a cessation of activity in these neurones could activate inspiratory and inactivate expiratory interneurones. These issues might be resolved if more were known about the pathways by which these neurones disinhibit the respiratory system.

An influence on both flight and respiration was also found for the flight-initiating interneurones 404 (Fig. 10). Intracellular stimulation of these interneurones usually evoked flight and altered the activity recorded in abdominal muscles in a similar way to that observed during wind-evoked flight. Thus, the respiratory rhythm was obscured, as mentioned above, (Fig. 1) and it was not possible to determine how the respiratory system was influenced. The influences of 404 on the respiratory rhythm could be demonstrated by stimulation at strengths that were insufficient to evoke flight activity (Fig. 10B). However, a puzzling finding was that the respiratory system was influenced in an inconsistent manner. The respiratory rate was sometimes increased and at other times decreased. Often 404 stimulation had no effect on the respiratory rate. One possible explanation for this is that 404 stimulation indirectly excited 388 and indirectly inhibited 398 and 399 (Ramirez, 1988). These two groups of suboesophageal ganglion interneurones all influenced respiratory behaviour, but in opposing manners; 388 presumably decreased, and 398 and 399 presumably increased, the respiratory rate when 404 was stimulated. Thus, the effect that the 404 neurones had on the respiratory rate might depend on the relative effect they had on these two groups of neurones. One explanation of why 404 sometimes decreased the respiratory rate in the quiescent animal might be the relatively stronger excitatory connection to 388. Intracellular stimulation of neurone 388 in the quiescent locust decreased the respiratory rate (Fig. 11).

The involvement of the suboesophageal ganglion interneurones 388, 398 and 399 in the control of respiration further emphasized the importance of the suboesophageal ganglion in the control of insect ventilation. In a previous study, we described a rhythmically active interneurone which seems to be an element of the respiratory rhythm generator (Ramirez & Pearson, 1989). In crickets, it has been demonstrated that the suboesophageal ganglion strongly influences ventilation (Huber, 1960*a*), and several respiratory rhythmic interneurones have been described in this ganglion (Otto & Campan, 1978; Otto & Weber, 1982), some of which also influenced the generation of the respiratory rhythm (D. Otto & J. Janiszewski, in preparation). That 398 and 399 not only influenced respiration but also flight is, furthermore, consistent with the role of the suboesophageal ganglion in the control and regulation of different behaviours, as has been suggested by various authors (Altman & Kien, 1979, 1987*a*,*b*; Hedwig, 1986; Huber, 1960*b*; Ramirez, 1986, 1988; Ronacher *et al.* 1986). A better understanding of the mechanisms by which suboesophageal ganglion interneurones influence

behaviour could therefore lead to a better understanding of how complex behavioural functions, such as the coordination and coupling of different behaviours, is controlled by the nervous system.

We thank Y. Tang for her technical assistance. This study was supported by a grant from the Medical Research Council of Canada.

References

ALTMAN, J. S. & KIEN, J. (1979). Suboesophageal neurons involved in head movements and feeding. *Proc. R. Soc.* B **205**, 209–227.

ALTMAN, J. S. & KIEN, J. (1987*a*). Functional organization of the suboesophageal ganglion in insects and other arthropods. In *Arthropod Brain: Its Evolution, Development, Structure and Functions* (ed. A. P. Gupta), pp. 265–301. New York: John Wiley & Son.

ALTMAN, J. S. & KIEN, J. (1987*b*). A model for decision making in the insect nervous system. In *Nervous Systems in Invertebrates. NATO-ASI Series A, Life Sciences* (ed. M. Ali), pp. 621–643. New York: Pergamon Press.

BAADER, A. (1988). Some motor neurones of the abdominal longitudinal muscles of grasshoppers and their role in steering behaviour. *J. exp. Biol.* **134**, 455–462.

BURROWS, M. (1975*a*). Co-ordinating interneurones of the locust which convey two patterns of motor commands: their connexions with flight motoneurones. *J. exp. Biol.* **63**, 713–733.

BURROWS, M. (1975*b*). Co-ordinating interneurones of the locust which convey two patterns of motor commands: their connexions with ventilatory motoneurones. *J. exp. Biol.* **63**, 735–753.

BURROWS, M. (1978). Sources of variation in the output of locust spiracular motoneurones receiving common synaptic driving. *J. exp. Biol.* **74**, 175–186.

BURROWS, M. (1982*a*). The physiology and morphology of median nerve motor neurones in the thoracic ganglia of the locust. *J. exp. Biol.* **96**, 325–341.

BURROWS, M. (1982*b*). Interneurones co-ordinating the ventilatory movements of the thoracic spiracles in the locust. *J. exp. Biol.* **97**, 385–400.

CAMHI, J. M. & HINKLE, M. (1972). Attentiveness to sensory stimuli: central control in locusts. *Science* **175**, 550–553.

DI MARCO, A. F., ROMANIUK, J. R., VON EULER, C. & YAMAMOTO, Y. (1983). Immediate changes in ventilation and respiratory pattern associated with onset and cessation of locomotion in the cat. *J. Physiol., Lond.* **343**, 1–16.

ELDRIDGE, F. L., MILLHORN, D. E., KILEY, J. P. & WALDROP, T. G. (1985). Stimulation by central command of locomotion, respiration and circulation during exercise. *Respir. Physiol.* **59**, 313–337.

ELDRIDGE, F. L., MILLHORN, D. E. & WALDROP, T. G. (1981). Exercise hyperpnea and locomotion: Parallel activation from the hypothalamus. *Science* **211**, 844–846.

FELDMAN, J. L. (1986). Neurophysiology of breathing in mammals. In *Handbook of Physiology*, vol. IV, *The Nervous System, Intrinsic Regulatory Systems of the Brain* (ed. F. E. Bloom), pp. 463–524. Bethesda, Maryland: American Physiological Society.

HEDWIG, B. (1986). On the role in stridulation of plurisegmental interneurons of the acridid grasshopper *Omocestus viridulus* L. I. Anatomy and physiology of descending cephalothoracic interneurons. *J. comp. Physiol. A* **158**, 413–427.

HOYLE, G. (1959). Action of carbon dioxide on an insect spiracular muscle. *J. Insect Physiol.* **3**, 378–394.

HUBER, F. (1960*a*). Experimentelle Untersuchungen zur nervösen Atmungsregulation der Orthopteren (Saltatoria: Gryllidae). *Z. vergl. Physiol.* **43**, 359–391.

HUBER, F. (1960*b*). Untersuchung über die Funktion des Zentralnervensystems und insbesondere des Gehirns bei der Fortbewegung und der Lauterzeugung der Grillen. *Z. vergl. Physiol.* **44**, 60–132.

HUSTERT, R. (1975). Neuromuscular co-ordination and proprioceptive control of rhythmical abdominal ventilation in intact *Locusta migratoria migratorioides*. *J. comp. Physiol.* **97**, 159–179.

KAO, F. F., MEI, S. S. & KALIA, M. (1979). Interaction between neurogenic exercise drive and chemical drive. In *Central Nervous Control Mechanisms in Breathing* (ed. C. Von Euler & H. Lagercrantz), pp. 75–89. Oxford: Pergamon Press.

KIEN, J. & ALTMAN, J. S. (1984). Descending interneurons from the brain and suboesophageal ganglion and their role in the control of locust behaviour. *J. Insect Physiol.* **30**, 54–72.

KROGH, A. & LINDHARD, J. (1913). The regulation of respiration and circulation during the initial stages of muscular work. *J. Physiol., Lond.* **47**, 112–136.

LEWIS, G. W., MILLER, P. L. & MILLS, P. S. (1973). Neuro-muscular mechanisms of abdominal pumping in the locust. *J. exp. Biol.* **59**, 149–168.

MCCLOSKEY, D. I. & MITCHELL, J. H. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *J. Physiol., Lond.* **224**, 173–186.

MILLER, P. L. (1960*a*). Respiration in the desert locust. I. The control of ventilation. *J. exp. Biol.* **37**, 224–236.

MILLER, P. L. (1960*b*). Respiration in the desert locust. II. The control of the spiracles. *J. exp. Biol.* **37**, 237–263.

MILLER, P. L. (1960*c*). Respiration in the desert locust. III. Ventilation and the spiracles during flight. *J. exp. Biol.* **37**, 264–278.

MILLER, P. L. (1966). The regulation of breathing in insects. *Adv. Insect Physiol.* **3**, 279–354.

OTTO, D. & CAMPAN, R. (1978). Descending interneurons from the cricket suboesophageal ganglion. *Naturwissenschaften* **65**, 491.

OTTO, D. & WEBER, T. (1982). Interneurons descending from the cricket cephalic ganglia that discharge in the pattern of two motor rhythms. *J. comp. Physiol.* **148**, 209–219.

PEARSON, K. G. (1980). Burst generation in coordinating interneurons of the ventilatory system of the locust. *J. comp. Physiol.* **137**, 305–313.

PEARSON, K. G., REYE, D. N., PARSONS, D. W. & BICKER, G. (1985). Flight initiating interneurons in the locust. *J. Neurophysiol.* **53**, 910–934.

PEARSON, K. G. & ROBERTSON, R. M. (1987). Structure predicts synaptic function of two classes of interneurones in the thoracic ganglia of *Locusta migratoria*. *Cell Tissue Res.* **250**, 105–114.

RAMIREZ, J. M. (1986). Interneuronal control of locust flight. PhD thesis, University of Regensburg, FRG.

RAMIREZ, J. M. (1988). Interneurons in the suboesophageal ganglion of the locust associated with flight initiation. *J. comp Physiol.* **162**, 669–685.

RAMIREZ, J. M. & PEARSON, K. G. (1989). Distribution of intersegmental interneurones that can reset the respiratory rhythm of the locust. *J. exp. Biol.* **141**, 151–176.

ROBERTSON, R. M. & PEARSON, K. G. (1982). A preparation for the intracellular analysis of neuronal activity during flight in the locust. *J. comp. Physiol.* **146**, 311–320.

ROBERTSON, R. M. & PEARSON, K. G. (1983). Interneurons in flight system of the locust: distribution, connections and resetting properties. *J. comp. Neurol.* **215**, 33–50.

RONACHER, B., VON HELVERSEN, D. & VON HELVERSON O. (1986). Routes and stations in the processing of auditory directional information in the CNS of a grasshopper, as revealed by surgical experiments. *J. comp. Physiol.* A **158**, 363–374.

TIBES, U. (1977). Reflex inputs to the cardiovascular and respiratory centers from dynamically working canine muscles. *Circulation Res.* **41**, 332–341.

VOLKMAN, A. W. (1841). Über die Bewegungen des Atmens und Schluckens. *Arch. Anat. Physiol.* 332–360.

WEIS-FOGH, T. (1964). Functional design of the tracheal system of flying insects as compared with the avian lung. *J. exp. Biol.* **41**, 207–227.

WOLF, H. & PEARSON, K. G. (1987). Comparison of motor patterns in the intact and deafferented flight system of the locust. II. Intracellular recordings from flight motoneurons. *J. comp. Physiol. A* **160**, 269–279.