THE POSTURE OF THE ABDOMEN DURING LOCUST FLIGHT: REGULATION BY STEERING AND VENTILATORY INTERNEURONES

BY ANDREAS BAADER*

Zoologisches Institut der Universität Basel, Rheinsprung 9, CH-4051 Basel, Switzerland

Summary

1. Tethered flying locusts (*Locusta migratoria*) make correctional steering movements with the abdomen when stimulated with a moving artificial horizon and integrated wind jet, simulating deviation from a straight course.

2. Neurones in the metathoracic and first abdominal neuromeres of the metathoracic ganglion have been characterized morphologically and physiologically. The selective stimulation of these cells causes movements of the abdomen.

3. One group of neurones responds directionally to visually perceived horizon movements and is excited by wind on the head. Some of these neurones are rhythmically activated at the flight frequency while others receive tonic drive from the flight oscillator. Electrical depolarization results in bending of the abdomen; the direction of this movement is always compatible with compensatory steering.

4. Interneurones which are active during the expiration phase of ventilation also contribute to the posture of the abdomen in flight. They are not visually responsive but their selective inhibition at the onset of flight activity helps to bring the abdomen into flight posture. Some of these interneurones are modulated at the flight frequency.

5. The efficiency of cooperation between different sets of interneurones in producing behavioural patterns and the signficance of single neurone stimulation are discussed.

Introduction

Directed locomotion requires the filtering and processing of a great deal of sensory information in order to produce coordinated behaviour. The flight system of the locust has been intensively studied in this respect and both sensory input and motor output have been investigated (for a review, see Rowell, 1988). Flying locusts detect deviations from a straight course both visually, with the compound eyes and the ocelli (Rowell and Reichert 1986), and mechanically, with the antennae (Gewecke, 1970; Arbas, 1986) and with directionally tuned, wind-sensitive hairs located on the head (Weis-Fogh, 1949; Camhi, 1970*a*) and the cerci

* Present address and address for offprint requests: Department of Biology, B-002, University of California at San Diego, La Jolla, CA 92093, USA.

(Altman, 1983). This sensory information is further processed by ascending (Pflüger, 1984; Boyan *et al.* 1986) and descending interneurones (Bacon and Tyrer, 1978; Griss and Rowell, 1986; Hensler, 1988). Thoracic premotor interneurones receive both sensory input and rhythmic drive from the central flight oscillator (Reichert and Rowell, 1985), thus providing an adequate input to flight motor neurones which results in steering reactions of the wings (Baker, 1979). Other components of steering include lateral bending of the abdomen (Dugard, 1967; Camhi, 1970a) and the hindlegs (Arbas, 1986) and compensatory head movements (Camhi, 1970b; Robert, 1988). Several studies have investigated the neuromuscular apparatus of anterior abdominal segments (Tyrer, 1971; Yang and Burrows, 1983; Baader, 1988) but nothing is known about its central nervous control. The efficacy of abdominal movements during steering behaviour has been proved, since Gewecke and Philippen (1978) found that ample torque was produced by the aerodynamic drag of the deflected abdomen. This is likely to be assisted by a shift of the centre of gravity, as the abdomen of young adult locusts forms $26.7 \pm 1.5 \%$ of the whole animal's mass ($N=7$; A. Baader, personal observation). Abdominal movements of flying locusts are complex. The abdomen is not used exclusively for steering but is also affected by two other superimposed motor components: the vibrations of the abdomen in time with the flight rhythm (Camhi and Hinkle, 1972) and predominantly vertical movements due to ventilatory activity (Miller, 1960). This study investigates how centrally generated rhythmic information (ventilation, flight) and peripheral sensory inputs are processed at the level of abdominal interneurones to produce coordinated behaviour of the abdomen in flying locusts.

Materials and methods

Preparation

The locusts, *Locusta migratoria*, were reared in a laboratory culture or were obtained from a commercial supplier. After removal of wings and legs, the animal was fixed with wax, dorsal side up, to a balsa-wood base. Pro- and pterothorax were cut open along the dorsal midline and the gut was removed to expose the metathoracic ganglion. Special care was taken to avoid damage to the abdominal segments. The ganglion was supported by a metal spoon and treated for 10 min with a small drop of Protease (type XIV, Sigma, 1 % in distilled water). In some preparations metathoracic nerve 1 or 3, containing motor axons of flight muscles, was cut to prevent excessive vibration during flight sequences from interfering with intracellular recordings. For the same reason the dorsal surface of the ganglion was stabilized with an additional small piece of metal. The ganglion was covered with locust saline which was periodically renewed.

Stimulation and recording

A hemisphere was constructed from 380 inwardly directed green light-emitting diodes (LED, Fig. 1) which could be activated by a constant current source to
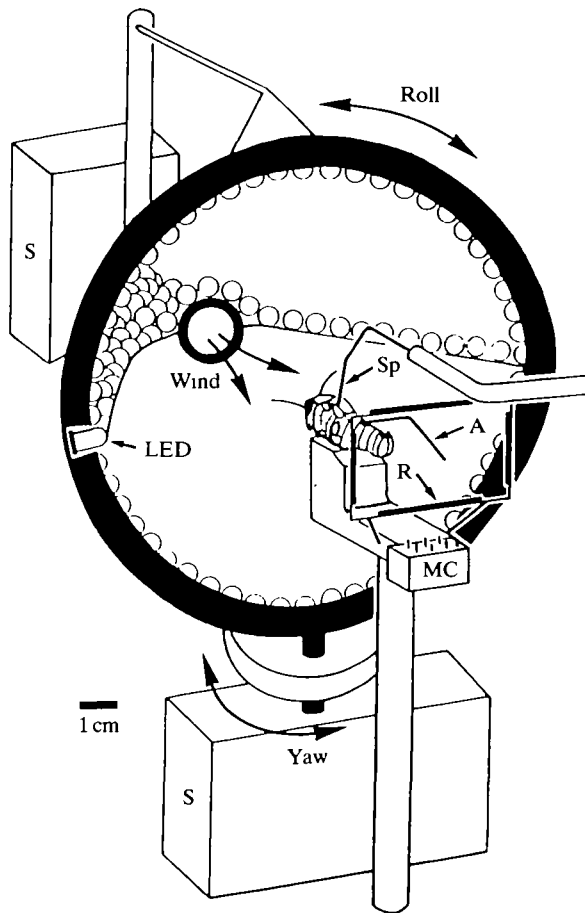
Fig. 1. Apparatus providing simulated course deviation during fictive flight. The hemisphere consists of 380 light-emitting diodes (LED). Only the upper half was illuminated, to provide an artificial horizon. It can be rotated up to ±30° around the roll and yaw axes by two servo motors (S). Constant non-laminar wind is blown through a tube directed towards the animal's head, which is positioned in the centre of rotation of the horizon. The nervous system is exposed and supported by a metal spoon (Sp) to improve the stability of intracellular recordings during fictive flight and abdominal movements. The transmitting aerial (A) and receiving antennae (R) allow the abdomen to bend ±30° horizontally and ±20° vertically while monitoring its position. The flight electromyogram and the output of the transducer are connected to the recording apparatus *via* a multiple connector (MC).

produce various patterns of illumination. For the experiments presented here the diodes of only the upper half of the hemisphere were illuminated to represent an artificial horizon, Servo motors rotated the horizon ±30° around two spatial axes, simulating roll and yaw deviations. Stimuli like these were shown to elicit pronounced steering responses in flying locusts both at the behavioural level

(Goodman, 1965) and in descending interneurones (Rowell and Reichert, 1986). The contrast ($\Delta I/I$) between the illuminated upper half and the dark lower half of the horizon was 0.85. The Faradic cage containing the animal was covered with black cotton material to eliminate extraneous light. Wind at variable speeds (up to 5 m s^{-1}) was provided through a central tube in the hemisphere and was usually set at 3 m s^{-1}, the average flight speed of locusts (Gewecke, 1977).

Two capacitative position transducers (Sandeman, 1968) registered independently movements of the abdomen in the horizontal and vertical directions. The transmitting aerial was fixed to the fourth abdominal segment, thus monitoring activity of the abdominal segments where the most pronounced bending occurs during steering (Camhi, 1970a). Maximum crosstalk between the two channels was 8%. Metal wire electrodes inserted through the cuticle into the mesothoracic right and/or left first basalar muscles (m97, depressors) recorded fictive flight activity. Usually the electrodes were positioned deep enough to monitor the activity of the first tergosternal muscle (m83, elevator) as well. For intracellular recordings the tips of thin-walled glass microelectrodes were filled with 5% Lucifer Yellow (Stewart, 1978) and then further backfilled with 0.1 mol l^{-1} lithium acetate. They had final resistances of 60–200 MΩ. Ganglia with successfully stained neurones were fixed in 4% paraformaldehyde, dehydrated, and cleared in methylsalicylate. Neurones were viewed under an epifluorescence microscope and either drawn directly or by tracing from colour slides. Some specimens were embedded in paraffin and sectioned frontally at 25 μm. Electrophysiological data were stored on FM magnetic tape.

Nomenclature and definitions

Nerves and muscles were named according to Snodgrass (1929) and tracts and neuromeres according to Tyrer and Gregory (1982). The numbering of thoracic interneurones in the Acrididae is rather confusing in the literature, since almost every author uses a different system. Of these, the three-digit labelling originated by Robertson and Pearson (1982) was preferred in this study. The 500-series was extended to a fourth digit, since almost all numbers in this group are taken. New numbers start at 5100. The relationships and numbering of neurones within the fused metathoracic ganglion are especially critical. The median tracheae have been regarded as defining the metathoracic and first three abdominal neuromeres (Tyrer and Gregory, 1982). This, however, is not a reliable criterion since, for example, motor neurones of a given segment are frequently located in the anterior neuromere (Yang and Burrows, 1983; Baader, 1988). Other internal landmarks are provided by the locations of somata, for example, those of leg motor neurones which make up the posterior border of the metathoracic neuromere. For simplicity, the different neuromeres have been ignored in naming the interneurones described here. The terms ipsilateral and contralateral are used with respect to the location of the cell body. The directions of the horizon deviations indicated in the figures should be interpreted as follows: movements of the horizon to the left side are perceived by the animal as turns to the right side and *vice versa*.

Results

The examples shown in Fig. 2 illustrate the normal steering responses of the abdomen seen in flying and nonflying animals. The horizon simulated combined yaw and roll movements, first 25° to the left, then 50° to the right and back to the horizontal position (=0°). In Fig. 2A the abdomen of the flying animal vibrated slightly in time with the flight rhythm and weak vertical movement components are visible during horizon movements. In this example there was no strong lateral response to the initial left movement of the horizon but a stronger one to the subsequent right and final left turn in the four sequences shown. In many experiments abdominal steering reactions improved with longer flight sequences (see also Fig. 8B). Fig. 2B illustrates, in another preparation, the response while the animal is not flying but the wind jet is turned on. Vertical movement components are now mainly due to ventilatory activity. They occur as a result of the contraction of dorsoventral abdominal muscles. The posterior muscles of each segment are activated in the expiratory phase of ventilation, whereas the anterior
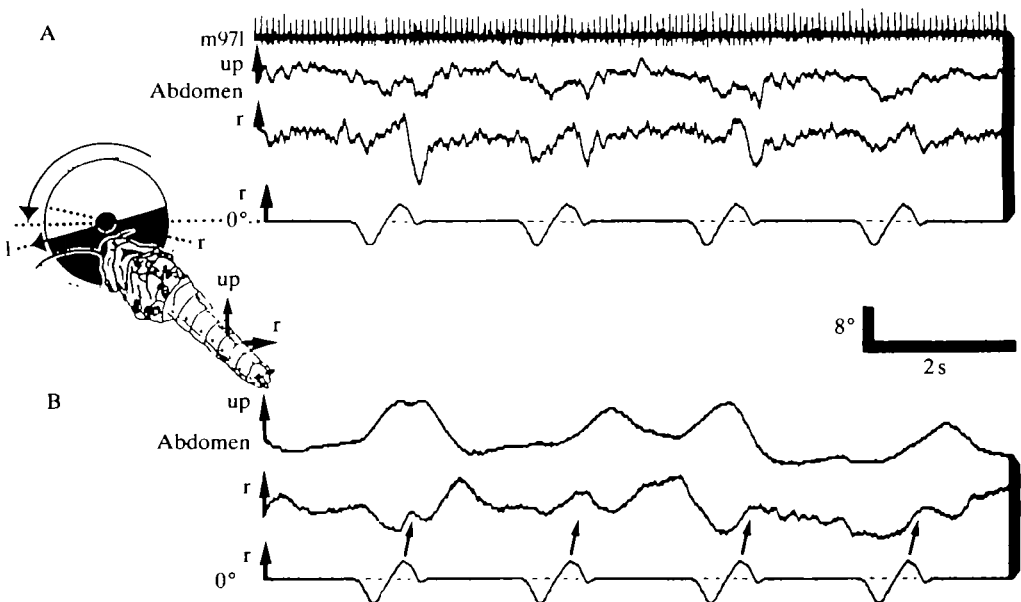
Fig. 2. Abdominal steering reactions in a restrained and tethered locust. (A) Fictive flight activity is induced by continuous wind at $3\,\mathrm{m\,s}^{-1}$ and monitored in a depressor muscle (m97left, first trace). Abdominal steering components are registered in the vertical (second trace) and horizontal (third trace) planes. The abdomen follows the moving horizon (fourth trace) which is rotated 25° around both the roll and yaw axes, beginning from 0°. The movements would serve to compensate for the perceived deviation in flight. The corrective steering response includes relatively strong horizontal and weaker vertical movement components. r, right, l, left. (B) When the animal is not flying, ventilation dominates abdominal movements in the vertical direction (first trace) but visual responses are still visible as small lateral deflections (arrows, second trace). The same visual stimulus was given as in A.

ones fire during inspiration (Lewis *et al.* 1973). Additional support for the abdomen is provided by the contraction of dorso-longitudinal muscles and the activity of all these muscles combined causes an upward bending of the abdomen during inspiration. Lateral bending shown in Fig. 2B is caused by the summation of ventilatory movements and the visual steering response, which is now weaker (see arrows) than during flight (Taylor, 1981).

Recordings from 21 individual neurones in the metathoracic and abdominal neuromeres fulfilled two criteria: (1) the neurones responded to at least one of the given stimuli, such as wind or visual stimuli, or they were activated in time with the respiratory rhythm and (2) moderate electrical stimulation of the neurones resulted in directional deflections of the abdomen. Owing to their generally small size, not all of them could be investigated sufficiently. The following section shows representative data from five of these neurones. According to their strongest responses, the cells have been subdivided into 'visual' and 'respiratory' populations. These terms will be used in the text without prejudice to other functions which the neurones may have. Each cell was recorded in 1–3 different preparations.

Visual steering interneurones

Steering interneurone 141

The morphology of 141 is shown in Fig. 3A. The soma is located in the ventral part of the first abdominal neuromere, close to the midline. All dendrites on the contralateral side are dorsally sited and have heavily beaded endings.

141 was recorded in two preparations. It was spontaneously active and flight activity resulted in an additional excitation of the neurone (Fig. 3B,C). 141 encoded visual deviations in both directions: it was excited by contralateral turns and inhibited by ipsilateral ones (Fig. 3B,C). Abdominal steering responses are the result of two stimulus modalities, the visual input and the simultaneously veering wind jet. Stimulation in the dark by veering wind alone resulted in a weaker but still directional response (not shown). In the histogram of Fig. 3D the averaged responses are counted within 100-ms intervals during consecutive left and right turns. Excitatory flight input to the neurone resulted in a reinforced

Fig. 3. Steering interneurone 141. (A) A reconstruction of a Lucifer-Yellow-filled neurone. In all figures, numbers represent depths (in μm) measured from the dorsal surface of the ganglion. Main tracheae (T) are drawn to help define the borders between the metathoracic and abdominal neuromeres. (B) Activity of 141 in the resting animal (left part) and during wind-stimulated flight. The neurone is excited by the rotating horizon when this simulates a course deviation to the right-hand side. The abdominal steering response in this preparation is weak shortly after flight onset. (C) As in B, but after a longer flight sequence. The wind-induced tonic excitation is lower now and the visual response more distinct. (D) Average spike frequency calculated for each 0.1 s during visual stimulation, as shown in B. The neurone gets directional input during both rest and flight, but in the latter case this is superimposed on a higher background activity and produces a more distinct response.
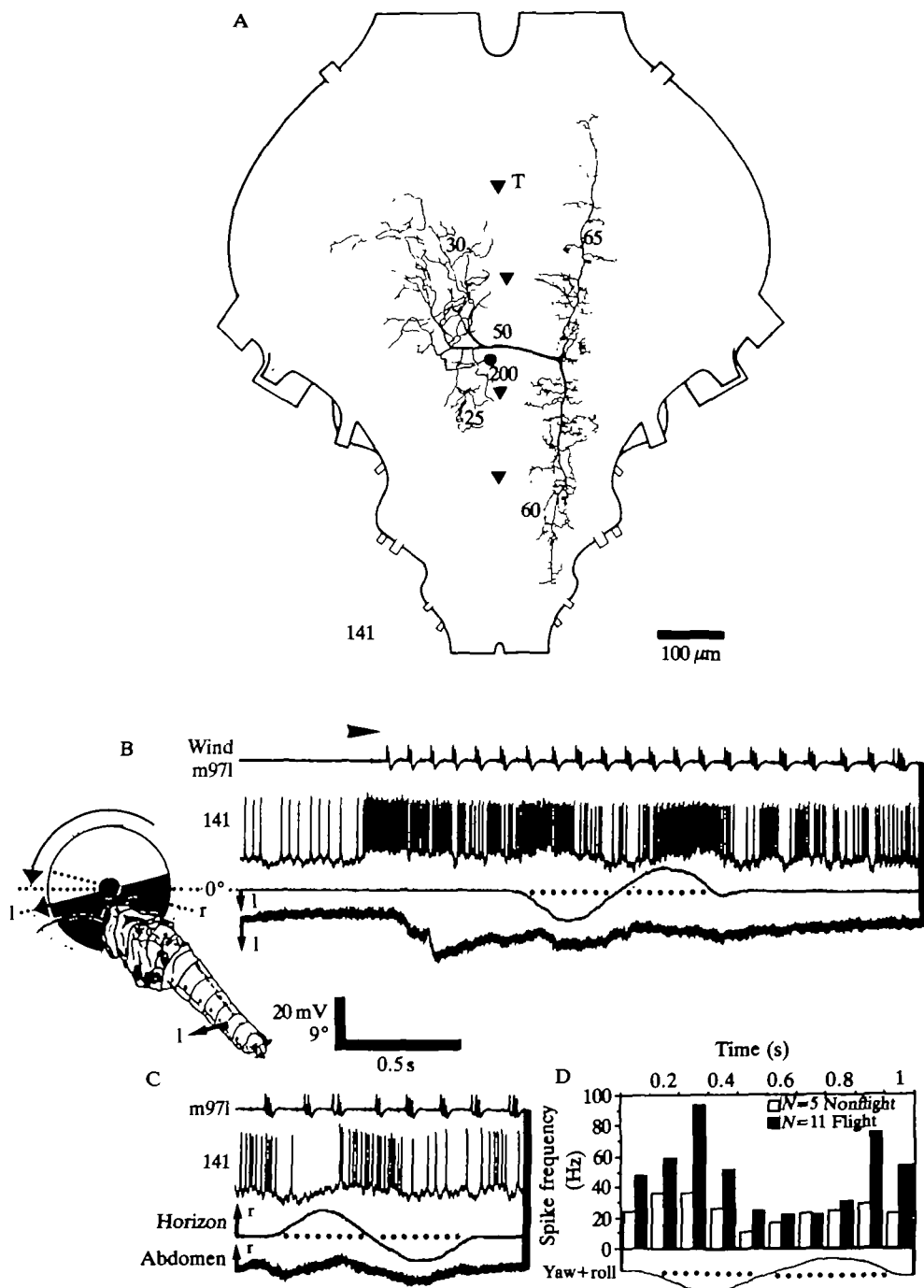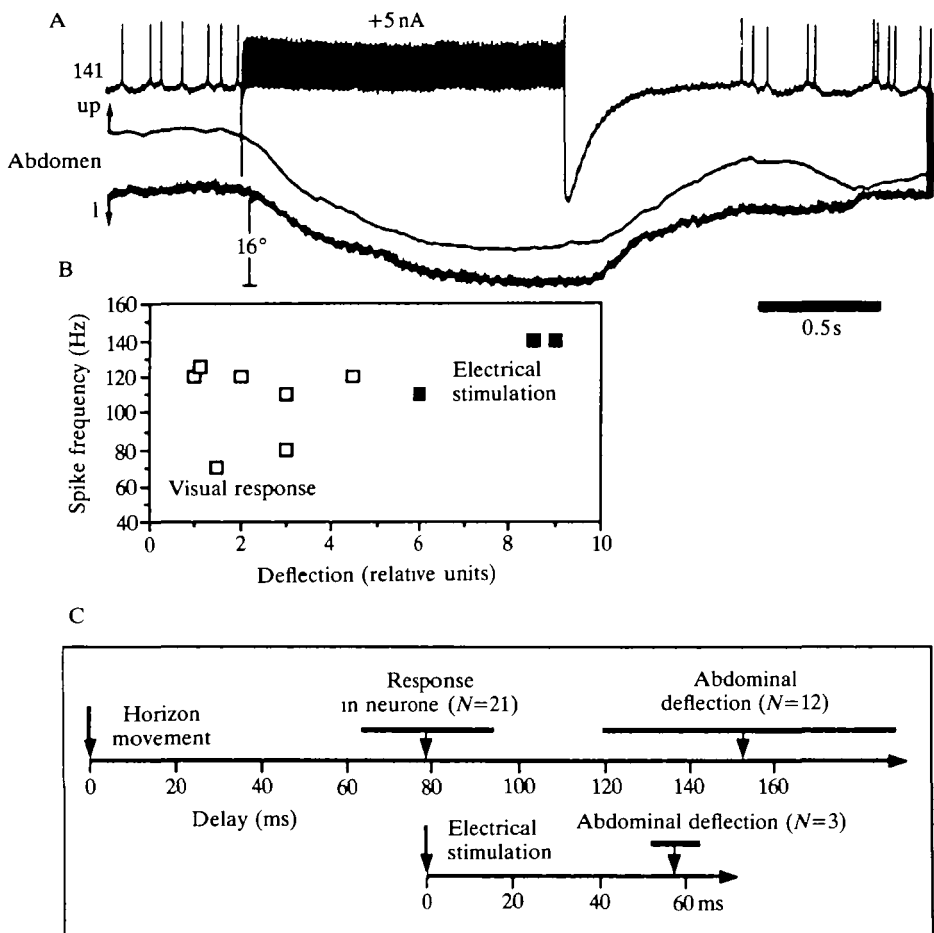
A

B

C

D

Fig. 3

A. BAADER

Fig. 4. (A) Electrical stimulation of 141 induces a train of action potentials with a frequency of about 130 Hz (a.c. recording) and reveals the output function of this cell: the abdomen is bent to the left (i.e. ipsilateral to the soma of the neurone) and downwards. (B) The amplitude of abdomen bending is smaller during horizon deviations (open squares) than during stimulation of 141 alone (black squares). Both produce a similar spike rate in 141 but the duration of stimulation is much longer during current injection. Further explanation in the text. (C) Temporal relationship between the response of 141 to a visual stimulus (with a mean delay of 78 ms, horizontal bars indicate standard deviation of the mean) and corrective lateral abdominal deflection (153 ms) during flight. Electrical stimulation produces a response after 58 ms.

response during turns in flight (solid bars); this is compared with the response of the animal stimulated with wind but not flying (open bars).

Positive current was injected into 141 to reveal its possible output function (Fig. 4A). The current produced a spike train at 100–140 Hz and bending of the abdomen up to 20° to the ipsilateral side and downwards. The direction of the artificially induced response reflects the activity of 141 during corrective flight

steering: course deviations of the animal to the right excite 141 and lead to compensatory abdomen ruddering to the left. In this example the spike train induced by the current lasted much longer than that induced by visual stimulation. In consequence, a continuously increasing abdomen response was produced which ultimately reached a larger amplitude than that elicited by visual stimulation, although the instantaneous spike frequency in 141 was the same in both cases (120–140 Hz, Fig. 4B). Fig. 4C illustrates the temporal relationship between the natural and artificially induced abdomen responses. The delay of the neurone response was 78 ± 15 ms (mean$\pm$s.D., $N=21$) and that of the abdomen was 153 ± 33 ms (mean$\pm$s.D., $N=12$), a difference of 75 ms. The latency of abdomen deflection elicited by current injection was 57 ± 5 ms (mean$\pm$s.D., $N=3$), not significantly different, which is compatible with the participation of 141 in the natural steering behaviour. 141 is modulated neither at flight frequency nor in time with the respiratory rhythm and is insensitive to acoustic stimuli.

Steering interneurone 5100

  The neurone has widespread arborizations in the dorsomedial plane of the metathoracic and abdominal neuromeres (Fig. 5A). The dorsal soma is located at the lateral posterior edge of the first abdominal neuromere (AG1). Fine, beaded branches in the contralateral hemiganglion are closely associated with the flight motor neurone neuropile. The ipsilateral branches run in the ventral and dorsal parts of the metathoracic neuromere (T3) and AG1. Activity of 5100 was modulated at flight frequency (Fig. 5B). Bursts were in phase with elevator activity, coinciding approximately with the muscle potential of m89, which is visible as crosstalk on the record of m97. Ipsilateral turns excited this neurone during fictive flight (Fig. 6) and elicited optomotor responses of the abdomen, which were expressed together with ventilatory movements. The histogram in Fig. 6B illustrates the average activity of 5100 during eight consecutive horizon movements.

  As for 141, electrical stimulation of 5100 resulted in an immediate lateral bending of the abdomen in the compensatory direction (Fig. 7A). For comparison, the artificial horizon, deviation of which evoked both a weaker neurone activity and less bending of the abdomen, was moved shortly before the current pulse. The same visual stimulus given directly after the electrical stimulation produced no activity in the cell, owing to postexcitatory effects, but still elicited some abdominal responses (Fig. 7A, asterisks), which must be caused by parallel abdominal inputs. Fig. 7B analyses the abdominal movements quantitatively. Low spike activity, similar to that recorded during horizon deviations, correlates with a moderate deflection (open squares), which increases when the spike frequency is enhanced during depolarization (open circles). During concomitant fictive flight an equally high spike rate elicited an even stronger abdominal response (black squares). This again indicates the necessity for adequate parallel inputs (e.g. the flight motor) to produce optimal behavioural responses. Neuronal activity in 5100 occurs 53 ± 9 ms (mean$\pm$s.D., $N=14$) after the onset of horizon deviation and
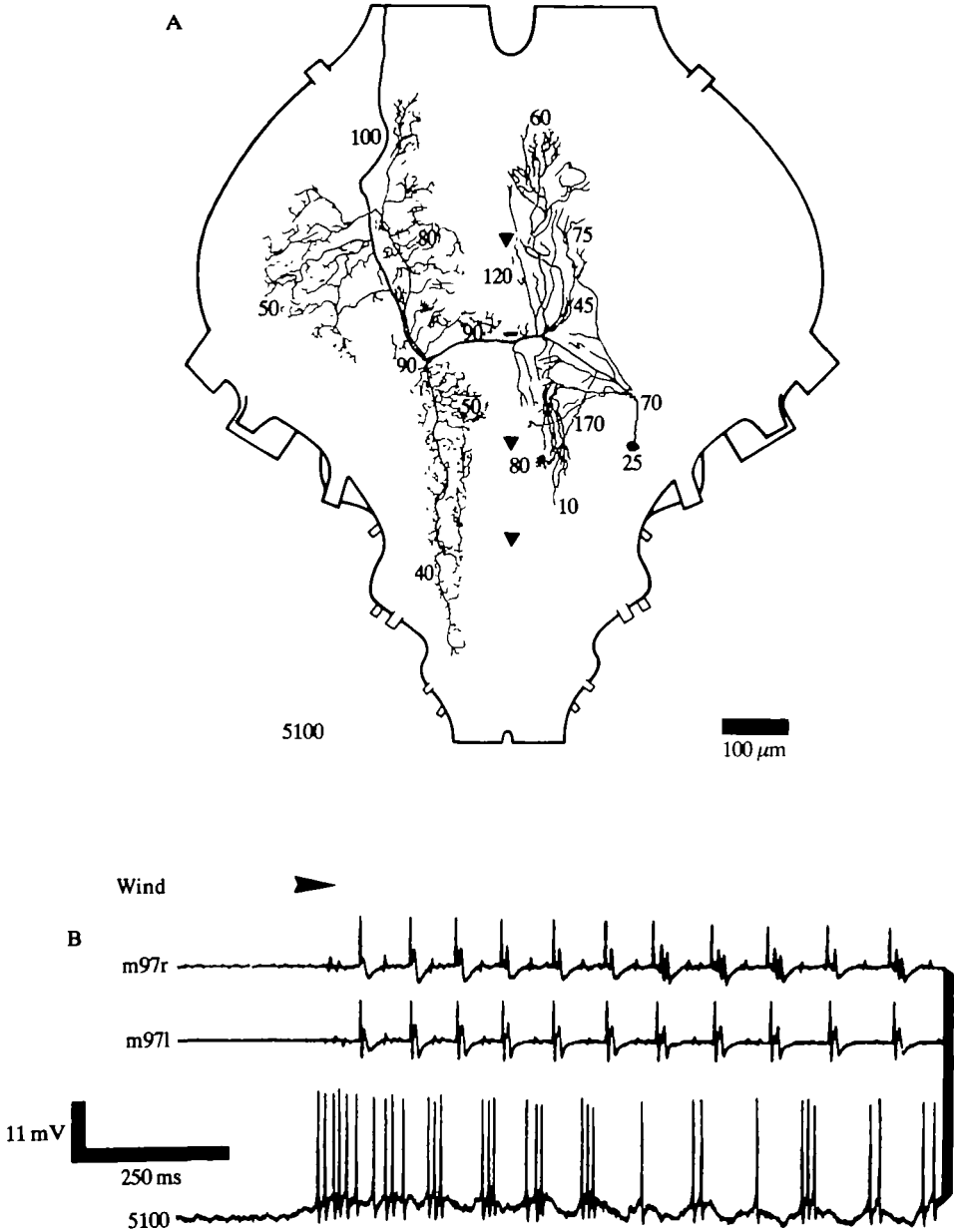
Fig. 5. Ascending steering interneurone 5100. (A) Morphology. See Fig. 3 for an explanation of the numbers. (B) Flight sequence monitored by the activity of two depressors on both sides (m97r,l), showing that 5100 is rhythmically activated in elevator phase.
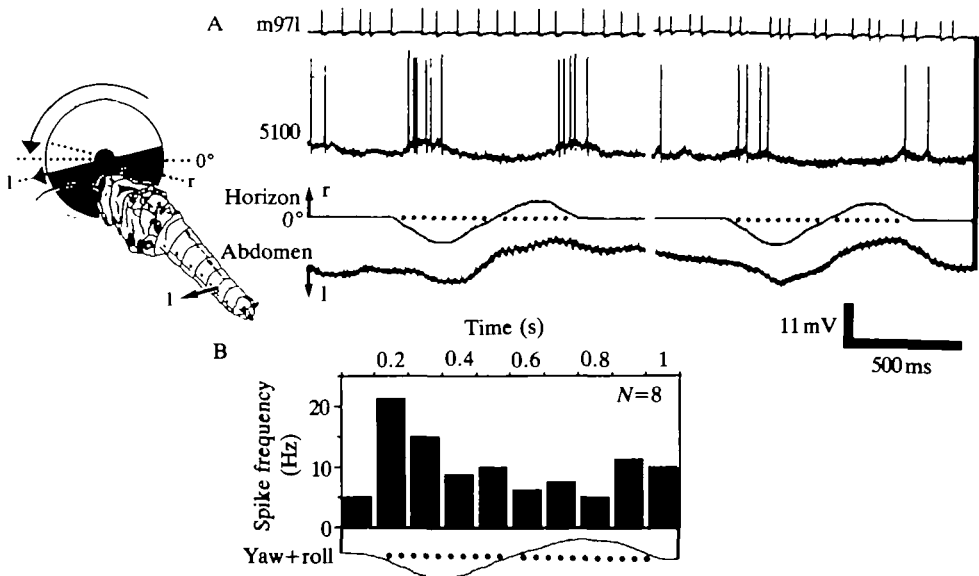
Fig. 6. (A) Recording during fictive flight. A simulated turn to the right (movement of the horizon to the left) causes an increase in the spike rate and correctional steering movements of the abdomen (lower trace). (B) Averaged response to eight movements of the artificial horizon as shown in A.

abdominal movement occurs after 156 ± 34 ms ($N=10$) (Fig. 7C). This means that the neurone responds 103 ms before the abdomen. Electrical stimulation of 5100 affects the muscles responsible for abdominal movement with a somewhat shorter delay (77 ± 19 ms, $N=17$).

200-series steering neurone

Though recorded in two preparations, the contralateral arborizations of this cell could not be obtained (Fig. 8A). The staining indicates, however, that it belongs to the 200-series. Axon and dendrites are in general very thin and arborize dorsally, running in AG1 and AG2. The soma is located at the posterior lateral edge of AG1. The axon leaves the ganglion through the ipsilateral posterior connective, in the direction of AG4. To determine whether this cell is a motor neurone, possibly innervating muscles of AG4, or an interneurone, the dorsal and ventral motor nerves of AG4 were backfilled with cobalt chloride using conventional methods (Tyrer and Altman, 1974). In four preparations neurones were stained in AG2 and AG3 (as well as in AG4 itself) but never in AG1. This suggests that the neurone is not a motor neurone. The possibility remains, however, that a neurone with such a thin axon could simply not be stained sufficiently by cobalt chloride.

The neurone has a low spontaneous activity but stimulation with wind alone results in a tonic excitation (Fig. 8B, upper panel), which is unaltered by the veering wind jet. Weak abdominal correctional steering is produced by this
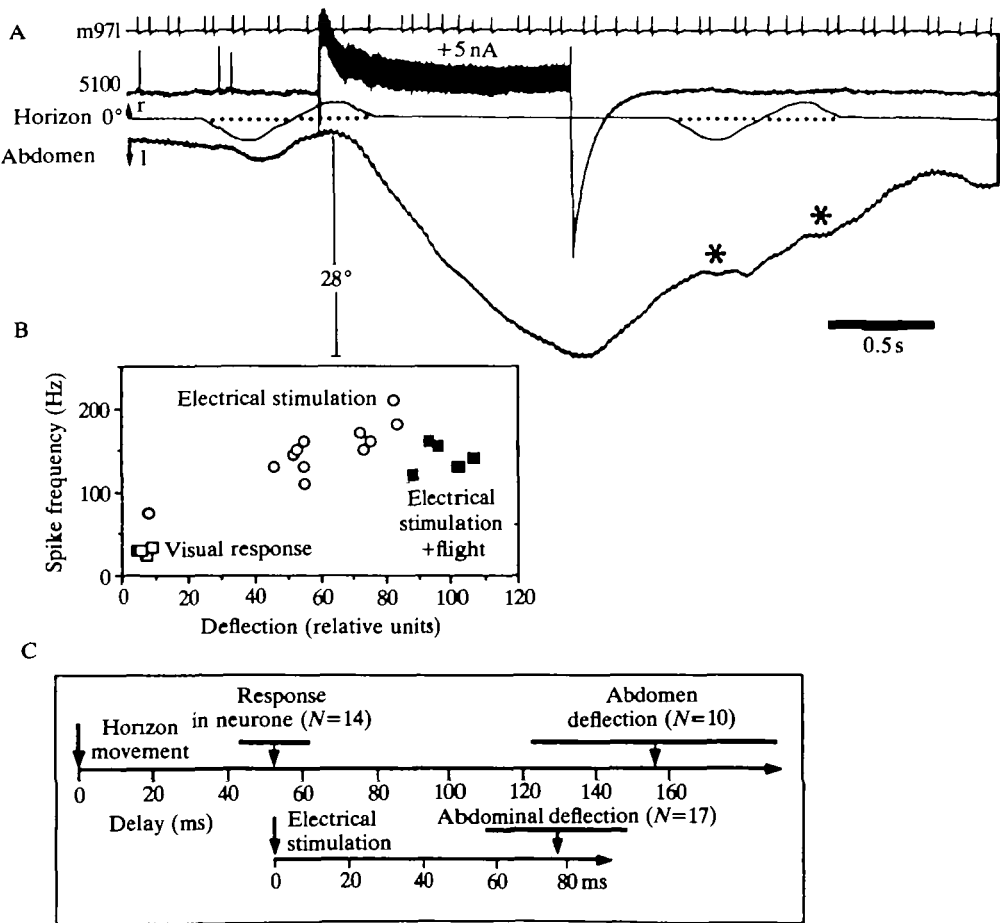
Fig. 7. (A) Depolarization of 5100 during fictive flight (a.c. recording) produces a train of action potentials resulting in prominent lateral abdominal bending. The bending is in the same direction as that produced by visual stimulation in the preferred direction. (B) Abdominal response *versus* spike rate in 5100. A given activity in the cell results in stronger bending during flight, presumably because more motor units are brought to or above threshold by the flight motor (black squares) than in the case of electrical stimulation of the non-flying animal (open circles). For comparison, see the 'natural' visual response of 5100 (open squares). (C) Mean latencies of the visual responses in 5100 (53 ms, $N=14$) and in the abdomen (156 ms, $N=10$, horizontal bars indicate standard deviation of the mean). Electrical stimulation of 5100 induces abdominal movement after 77 ms ($N=17$).

stimulus, indicating parallel wind-sensitive pathways. The cell showed distinct responses to the purely visual stimulus of the deviating horizon, being inhibited during ipsilateral yaw and roll stimulated by the movements of the horizon to the left and excited by movements in the opposite direction (centre panel). The powerful effect of wind given together with the same visual stimulus is shown in the lower panel. Additional motor units are brought to threshold, causing the
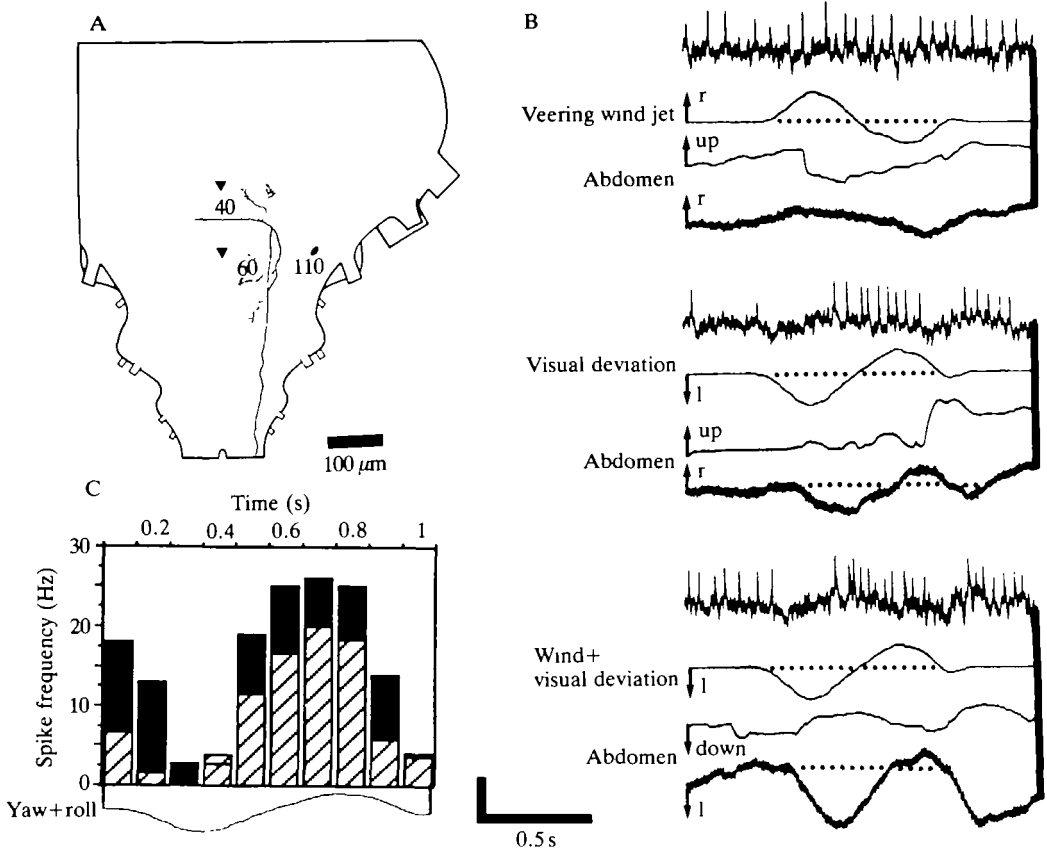
Fig. 8. Small steering neurone. (A) Reconstruction of the cell; the contralateral branches are incomplete. See Fig. 3 for an explanation of the numbers. (B) Upper record, response to the veering wind jet in the absence of visual inputs. The response is tonic and non-directional. Middle record, visual response to the horizon moving first to the left and then to the right side in a non-flying animal. The response is directional, being larger to contralateral turns. Bottom record, a combination of both stimuli causes stronger responses in the neurone and, even more pronounced, in the abdomen. (C) Histogram of the mean spike rate of the neurone in response to horizon deviations, with wind (black bars, $N=11$) and without wind (hatched bars, $N=12$). Vertical bar, $5°$ for B abdomen left, $10°$ for abdomen up, $4\,\text{mV}$ for neurone.

maximum response. The motor output responsible for vertical abdominal deflection is obviously not consistently influenced by the stimuli. The responses are summarized in Fig. 8C: purely visually induced activity (hatched bars) is greatly augmented by additional wind stimulation (black bars).

At the onset of fictive flight the neurone was slightly rhythmically inhibited in phase with activity in elevator muscles (see arrowheads in Fig. 9A) and the lowered abdomen was lifted (and, in this record, turned somewhat to the right side). The ability of the neurone to drive the abdomen is illustrated in Fig. 9B.
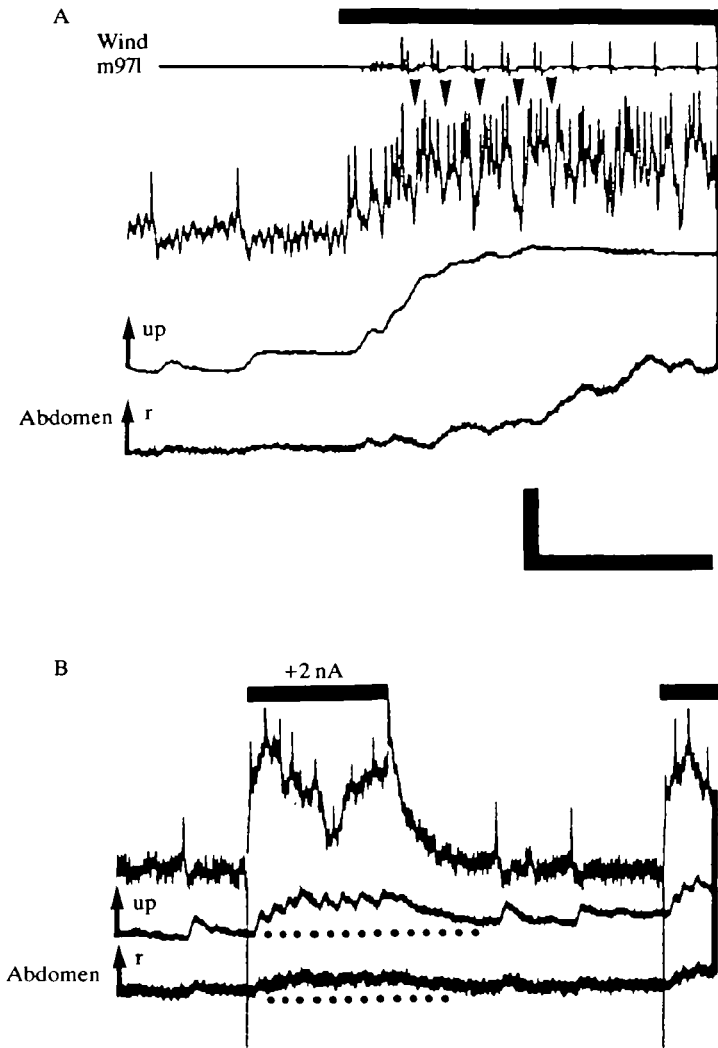
Fig. 9. (A) During flight the neurone shown in Fig. 8 is wind-activated and then slightly inhibited in time with the flight rhythm (elevator phase). Simultaneously, the abdomen is lifted into the horizontal flight position (and somewhat to the right). (B) Single spike activity in the neurone elicits behaviour. Each spike in the cell produces visible twitches in the abdomen which result in a constant bending of the abdomen upwards and to the right when the cell is driven by current injection. Time bar, 0.5 s for A, 1 s for B; vertical bar, 10° for A, 5° for B, 4 mV for neurone.

Single spikes caused visible twitches of the abdomen upwards and, less strongly, to the ipsilateral (right) side. The steering response was again compensatory. In this experiment the animal was completely at rest and presumably no other counteracting inputs influenced abdominal posture. Under these conditions the mean response latency of the abdomen was only 19.8±1.3 ms (mean±s.D., N=19) after each spike. This indicates strongly that the cell is directly coupled to fast

abdominal motor neurones and, thus, is suited to mediate rapid steering reactions. In contrast, the visually elicited steering reaction, which is the result of the whole motor output, has a much longer mean delay of 133 ± 21 ms (mean$\pm$s.D., $N=21$). Activity in this neurone starts on average 71 ± 21 ms (mean$\pm$s.D., $N=21$) after the beginning of the stimulus.

Participation of respiratory interneurones in abdominal flight posture

Seven different interneurones, which were rhythmically activated in phase with ventilation in resting and flying animals, were investigated during fictive flight. None of these was found to be involved in visually mediated compensatory steering. Two are described here.

Interneurone 020

Closely confined to the dorsal midline of the ganglion, this neurone projects ventrally in the anterior T3 neuropile and in the posterior AG1–AG3 (Fig. 10A). Physiological data are from two recordings. 020 was inhibited at the onset of fictive flight (Fig. 10B), when there is a correlated lift of the abdomen into the flight position (dotted lines). There was otherwise no change in activity and no visual response during fictive flight. 020 was also weakly active in phase with ventilatory movements of the abdomen (arrowheads in Fig. 10C). The cell was most active shortly before and at the beginning of the expiration phase of ventilation, when the lifted abdomen starts to lower again (arrowheads). Depolarization of 020 resulted in a mainly downward bending of the abdomen and in activity of elevator flight muscles (compare Fig. 10C with flight recording in Fig. 10B, in which, on the same amplitude scale, activities of both elevator and depressor are visible).

Interneurone 321

This neurone is morphologically distinct from 020 but shares with it some physiological characteristics. It has been described previously as a respiratory interneurone with resetting properties (K. G. Pearson, unpublished results). It has dorsomedial branches in the anterior metathoracic neuromere, dorsal branches and a dorsal soma in AG1 and an axon descending contralaterally (Fig. 11A). Its activity correlated mainly with the downstroke of the abdomen during expiration (Fig. 11B). Depolarization of 321 produced a weak lateral response but a pronounced downward deflection of the abdomen (Fig. 11C). Like 020, it was inhibited at the onset of the flight sequence when the abdomen takes up its flight posture (Fig. 11D, dotted lines). In the left part of Fig. 11D two bursts are shown indicating respiratory activity. Wind then initiated fictive flight, there was prolonged hyperpolarization of the neurone's membrane potential, and it was rhythmically modulated at the flight frequency (depressor phase). Slight hyper-polarizations were seen at the beginning of 12 consecutive flight sequences in 321 and in all cases these prevented activity in the cell during the first 500 ms of flight. During fictive flight, its activity remained unchanged and it received no visual inputs. Both neurones share in the control of the posture of the abdomen during flight. They are both activated at the beginning of expiration when the abdomen

A

140
110
70
80
60
50
020

100 μm

B

Wind

m97l

020

Abdomen   up

+5nA

C   m97l
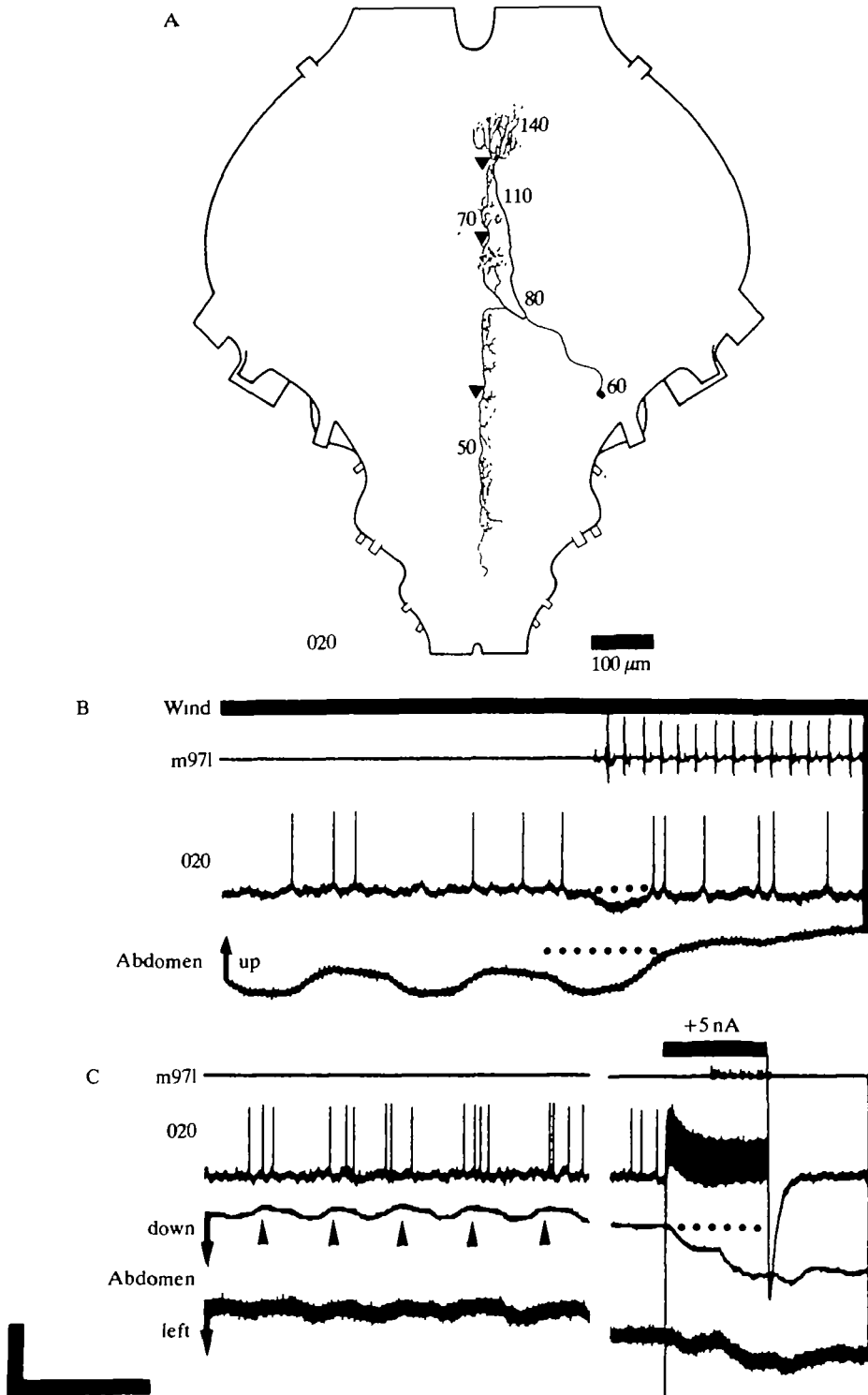
020

down

Abdomen

left

Fig. 10

Fig. 10. (A) Morphology of interneurone 020. See Fig. 3 for an explanation of the numbers. (B) Inhibition of 020 at flight onset occurs together with the raising of the abdomen into the flight position (compare dotted lines). (C) Modulation of 020 during respiration (arrowheads) and after selective stimulation with 5 nA current (a.c. recording), results in a downward (and somewhat contralateral) bending of the abdomen and activity in a flight muscle. Time bar, 1 s for B, 2 s for C; vertical bar, 4.5° for B and lateral abdomen movement in C, 9° for vertical movement in C, 22 mV for 020.

starts moving downwards. However, they are inhibited at the onset of flight which would tend to abolish the downward slant of the abdomen and so contribute to the horizontal posture seen in fictive flight.

Discussion

General considerations

This study describes interneurones that are involved in controlling the behaviour of the abdomen. The majority of the interneurones investigated did not produce visible abdominal twitches when electrically stimulated, though many were sensitive to the visual stimuli provided by the artificial horizon. This does not exclude the possibility that other visual interneurones could be functionally connected to abdominal motor neurones but produce only subthreshold excitation, as is the case for some flight interneurones and the flight motor neurones (Reichert and Rowell, 1986). The technique used in this study simply limits the number of possible candidates by identifying only those interneurones whose postsynaptic effects are powerful enough to produce overt behaviour. The few other interneurones in insects known to be directly involved in the expression of behaviour during flight include the wind-sensitive TCG neurone (Bicker and Pearson, 1983) and visual and acoustic interneurones of the locust which elicit directional abdominal and head movements (Hensler and Rowell, 1990; Baader, 1990). Another example is the acoustically sensitive IN1 of crickets, which produces abdominal ruddering when stimulated intracellularly during flight (Nolen and Hoy, 1984).

Abdominal steering behaviour as a result of visual stimulation

The latencies of neurone responses and abdominal movements relative to visual stimulation were 50–80 ms and 130–160 ms, respectively. This is quite long compared with responses of visual descending interneurones, which can be recorded in the thoracic ganglia 10–30 ms after stimulation of the ocelli, depending on the body temperature (Reichert and Rowell, 1986) and some 40 ms after stimulation of the compound eyes. What could account for these differences? Some delay is probably a mechanical artefact of the preparation used, in which tension and friction may hinder free abdominal movement. A much quicker steering reaction is possible, as shown by the results of electrical stimulation of 141 or the cell in Fig. 8, which can produce abdominal bending 58 or even 19 ms after

A

B

321

up

Abdomen

r

+2 nA

C

321

down

0.2 s

l

D

Wind
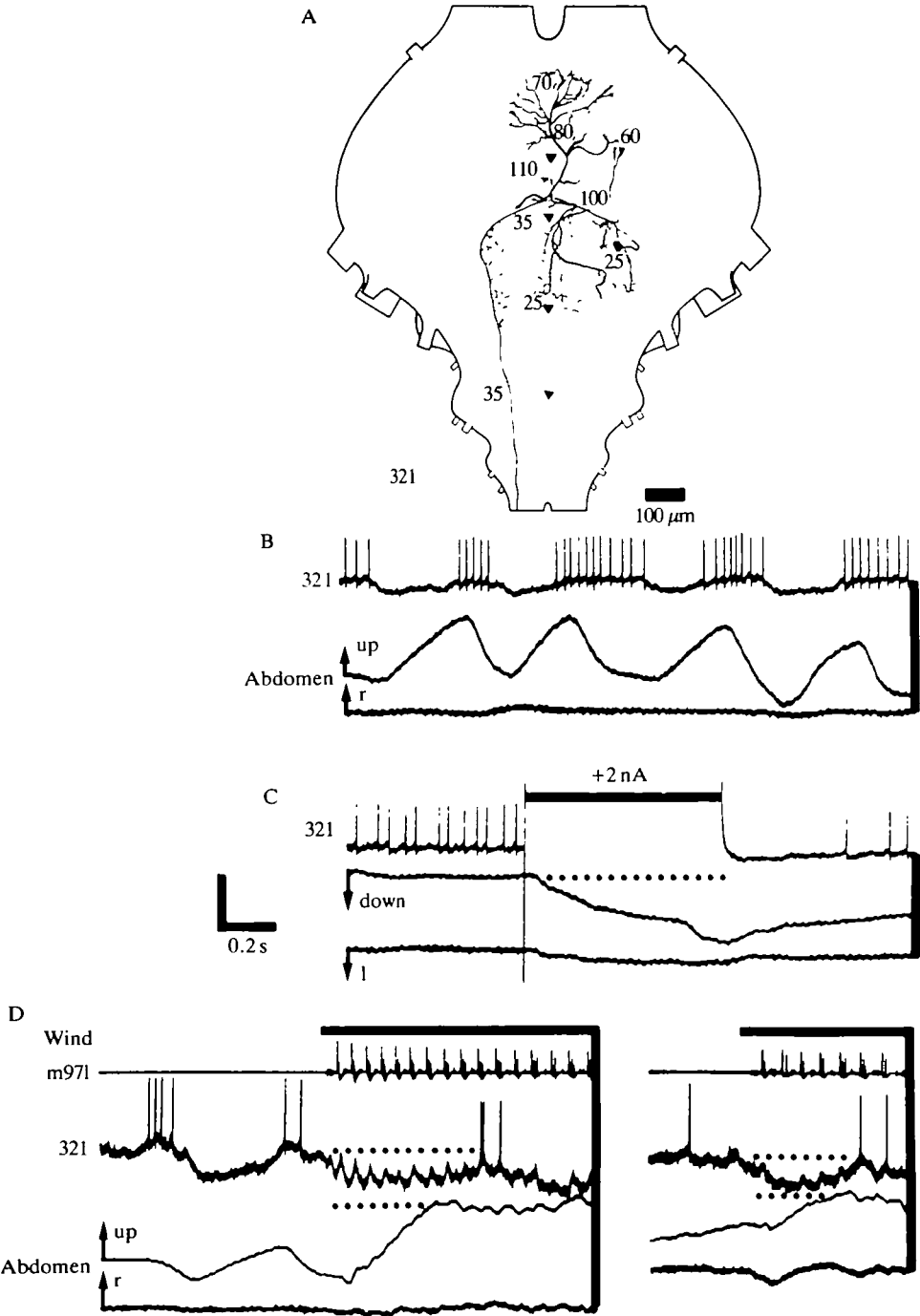
m97l

321

up

Abdomen

r

Fig. 11

Fig. 11. Morphology of 321 (A) and its activity during ventilation (B). See Fig. 3 for an explanation of the numbers. Action potentials in 321 are correlated with the downstroke of the abdomen during expiration. (C) Positive current injection clarifies its motor effect: the abdomen moves mainly downwards and slightly to the left. The cell is slightly inhibited at the beginning of fictive flight (D, dotted line) reliably preventing spikes. The abdomen is lifted simultaneously. The membrane potential of 321 oscillates at flight frequency (depolarized in depressor phase). Note that spikes are clipped in D. Vertical bar, 4.5° for B,C,D, abdomen right, 9° for D abdomen up, 20 mV for 321 in B,C, 10 mV for 321 in D.

activity in the cell. By adding the latter value to the 40 ms needed for visual information about course deviation to arrive to the thorax, one obtains latencies which fit well those of abdominal steering reactions in unrestrained flying locusts, that is 59 ± 15 ms ($N=80$, D. Robert, in preparation).

In contrast, the directional dependence of the interneurone's responses is invariable. During electrical stimulation the abdomen is ruddered in all cases towards the side which would be correctional for the perceived deviation. Further, this is always the preferred direction of the visual response of the neurone. The wind response can also be directional, as in 141, or generally excitatory, as in the unidentified neurone of Fig. 8 or in the respiratory interneurones (A. Baader, unpublished observation). In any case, wind input is sufficient to produce the optimum response of the abdomen. Finally, flight oscillator information is carried by some of these neurones, such as 5100, causing the abdominal vibrations seen in the behavioural experiments (Fig. 2A). An additional reafferent input reporting the actual flight frequency in unrestrained animals was disabled in this preparation: it is known that rhythmic wind turbulence produced by the beating wings elicits phasic deflections of the head wind hairs (Horsmann *et al.* 1983) and related activity in descending wind-sensitive interneurones (Bacon and Möhl, 1983). These signals would presumably modulate these steering interneurones on both sides of the animal and thus contribute to the observed vertical abdominal oscillations during flight. In conclusion, the visually responsive interneurones described here can contribute to the abdominal behaviour exhibited by flying locusts during steering.

Necessity for respiratory interneurones

Pumping movements of the abdomen continue and are accelerated during flight, except during the first seconds after flight onset, when they usually cease (Miller, 1960). Some respiratory interneurones have been described as tonically excited in flight (Ramirez and Pearson, 1989*b*). The interneurones from which these authors recorded also showed distinct differences at flight onset: inspiratory neurones were activated and expiratory ones were inhibited. The present study confirms this, by showing that expiratory interneurones 020 and 321 are inhibited at the onset of fictive flight. How can these effects be reconciled? Activity in 020 or 321 would normally result in a downward deflection of the abdomen (as revealed by current injection); inhibition, however, will allow the abdomen to be lifted into the

horizontal position. Parallel excitation caused by what are presumed to be other respiratory interneurones will also increase the stiffness of the abdomen, as suggested by Ramirez and Pearson (1989*b*). None of the respiratory neurones appears to be actively involved in lateral steering. This is supported by the observation that electrical stimulation produced only vertical abdominal deflections; lateral ruddering was weak or absent. The question of whether these cells play a role in the respiratory oscillator remains to be investigated. Some of the oscillator cells have been characterized recently (Ramirez and Pearson, 1989*a*), but unfortunately without respect to steering movements.

The summed activity of abdominal interneurones

Fig. 12 shows how the different input modalities are integrated by abdominal interneurones. It seems that all the sensory and centrally generated parameters contained in the signal flow of multimodal descending interneurones (Hensler, 1988) are still present at this level of neuronal processing. Other thoracic interneurones sharing similar features have recently been described (Elson, 1987): they receive exteroceptive (ocellar, weak acoustic) and proprioceptive (neck,
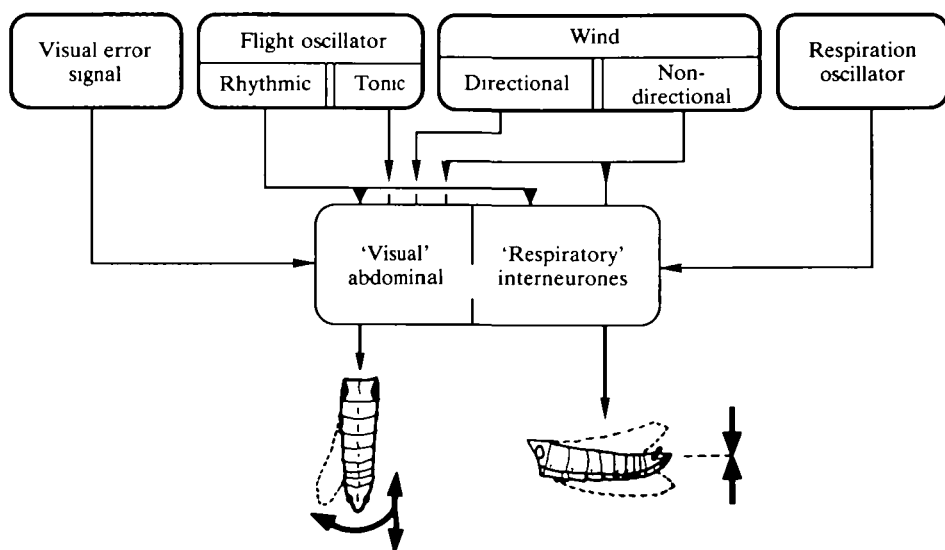
Fig. 12. Diagram illustrating the different inputs which determine activity in abdominal interneurones and their output functions. 'Visual' steering interneurones activate abdominal muscles, invariably in such a way as to produce correctional ruddering of the abdomen with respect to the perceived visual deviation. Some receive rhythmic or tonic drive from the flight oscillator and directional or generally excitatory wind input. 'Respiratory' interneurones can be excited by wind and excited or inhibited by the flight oscillator (A. Baader, unpublished observation) but receive no directional visual input during fictive flight. Synergistic activity in these cells would be sufficient to lift the abdomen into the horizontal flight position.

wing) input. However, in the neurones of this study, some compartmentalization does take place, since those receiving signals about course deviation do not receive input from the respiratory oscillator. Visual interneurones can be modulated by the flight oscillator and receive powerful wind input, as do interneurones that are activated during ventilation (A. Baader, unpublished observation). The membrane potential of cell 321 (and of two other respiratory interneurones not illustrated) is modulated at the flight frequency, an observation that is particularly interesting, since two different rhythms can be recorded in the same cell (see also Burrows, 1975) and the pumping rhythm is suppressed by the flight motor at the onset of flight (Fig. 11D). The efficacy of tonic wind stimulation explains the behavioural context in which the recorded units are preferentially sensitive: they function optimally during flight. The way in which the cells affect the posture of the abdomen can account fully for the abdominal steering. Visually sensitive units cause lateral correctional movements and some observations suggest that respiratory neurones help to bring the abdomen into the flight position and probably increase its stiffness. The role of the ventilatory interneurones in flight steering requires further investigation. The same is true for the unidentified 200-series neurone. Although all morphological data gathered so far characterize it as an interneurone, the physiological features, in particularly the one-to-one-drive onto the abdominal motor output, indicate a motor neurone. This is supported by the observation that so far no fast premotor interneurone has been shown in locusts. Some of the neurones described here, and other abdominal interneurones, are additionally sensitive to acoustic stimuli. This will be discussed in a subsequent paper (A. Baader, in preparation).

Effects of the flight oscillator

One interneurone (020) has a direct influence on the flight motor. The spikes in the tergosternal muscle 83 seen after stimulation of 020 could be repeatedly elicited and clearly represented flight activity, since, in contrast to some other flight muscles, which are used during both flight and walking (Wilson, 1962), m83 is a unifunctional flight muscle (Ramirez and Pearson, 1988). If 020 is triggering a central flight oscillator, this should subsequently affect both elevators *and* depressors; this is not observed. Wolf *et al.* (1988) have given evidence that the flight oscillator consists of subsets of oscillators distributed in the meso- and metathoracic ganglia. Although they did not suggest separate elevator and depressor oscillators, their hypothesis is supported here, in that single activation of such an oscillation subset by 020 might result in partial flight motor output.

Ruddering of the abdomen is an important factor in the flight steering of locusts (see Introduction). The investigation of such adaptive behaviour in reduced preparations is in general questionable. The present investigation, however, shows once again that it is possible to study behavioural patterns which are at least very similar to those seen in free-flying or tethered animals and to get information about their neuronal origin.

References

ALTMAN, J. (1983). Sensory inputs and the generation of the locust flight motor pattern: from the past to the future. In *Insect Flight* (ed. W. Nachtigall), *BIONA Report*, pp. 127-136. Stuttgart: G. Fischer.

ARBAS, E. A. (1986). Control of hindlimb posture by wind sensitive hairs and antennae during locust flight. *J. comp. Physiol.* A 159, 849–857.

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.

BAADER, A. (1990). An ascending visual neural pathway in locusts. *Naturwissenschaften* 77, (in press).

BACON, J. AND MÖHL, B. (1983). The tritocerebral commissure giant (TCG) wind-sensitive interneurone in the locust. I. Its activity in straight flight. *J. comp. Physiol.* 150, 439–452.

BACON, J. AND TYRER, M. (1978). The tritocerebral commissure giant (TCG): a bimodal interneurone in the locust, *Schistocerca gregaria*. *J. comp. Physiol.* 126, 317–325.

BAKER, P. S. (1979). The wing movements of flying locusts during steering behaviour. *J. comp. Physiol.* 131, 49–58.

BICKER, G. AND PEARSON, K. G. (1983). Initiation of flight by an identified wind-sensitive neurone (TCG) in the locust. *J. exp. Biol.* 104, 289–293.

BOYAN, G. S., ASHMAN, S. AND BALL, E. E. (1986). Initiation and modulation of flight by a single giant interneuron in the cercal system of the locust. *Naturwissenschaften* 73, 272–274.

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

CAMHI, J. M. (1970a). Yaw-correcting postural changes in locusts. *J. exp. Biol.* 52, 519–531.

CAMHI, J. M. (1970b). Sensory control of abdomen posture in flying locusts. *J. exp. Biol.* 52, 533–537.

CAMHI, M. J. AND HINKLE, M. (1972). Attentiveness to sensory stimuli: central control in locusts. *Science* 175, 550–552.

DUGARD, J. J. (1967). Directional change in flying locusts. *J. Insect. Physiol.* 13, 1055–1063.

ELSON, R. C. (1987). Integration of wing proprioceptive and descending exteroceptive sensory inputs by thoracic interneurones of the locust. *J. exp. Biol.* 128, 193–217.

GEWECKE, M. (1970). Antennae: another wind-sensitive receptor in locusts. *Nature* 225, 1263–1264.

GEWECKE, M. (1977). Control of flight in relation to the air in *Locusta migratoria* (Insecta, Orthoptera). *J. Physiol., Paris* 73, 581–592.

GEWECKE, M. AND PHILIPPEN, J. (1978). Control of horizontal flight-course by air-current sense organs in *Locusta migratoria*. *Physiol. Ent.* 3, 43–52.

GOODMAN, L. J. (1965). The role of certain optomotor reactions in regulating stability in the rolling plane during flight in the desert locust, *Schistocerca gregaria*. *J. exp. Biol.* 42, 385–407.

GRISS, C. AND ROWELL, C. H. F. (1986). Three descending interneurones reporting deviation from course in the locust. I. Anatomy. *J. comp. Physiol.* A 158, 765–774.

HENSLER, K. (1988). The pars intercerebralis neurone PI(2)5 of locusts: convergent processing of inputs reporting head movements and deviations from straight flight. *J. exp. Biol.* 140, 511–533.

HEUSLER, K. AND ROWELL, C. H. F. (1990). Control of optomotor responses by descending deviation detector neurones in intact flying locusts. *J. exp. Biol.* 149, 191–205.

HORSMANN, U., HEINZEL, H.-G. AND WENDLER, G. (1983). The phasic influence of self-generated air current modulations on the locust flight motor. *J. comp. Physiol.* 150, 427–438.

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

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

NOLEN, T. G. AND HOY, R. R. (1984). Initiation of behavior by single neurons: the role of behavioural context. *Science* **226**, 992–994.

PFLÜGER, H. J. (1984). The large fourth abdominal intersegmental interneuron: a new type of wind-sensitive ventral cord interneuron in locusts. *J. comp. Neurol.* **222**, 343–357.

RAMIREZ, J.-M. AND PEARSON, K. G. (1988). Generation of motor patterns for walking and flight in motoneurons supplying bifunctional muscles in the locust. *J. Neurobiol.* **19**, 257–282.

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

RAMIREZ, J.-M. AND PEARSON, K. G. (1989b). Alteration of the respiratory system at the onset of locust flight. I. Abdominal pumping. *J. exp. Biol.* **142**, 401–424.

REICHERT, H. AND ROWELL, C. H. F. (1985). Integration of nonphaselocked exteroceptive information in the control of rhythmic flight in the locust. *J. Neurophysiol.* **53**, 1201–1218.

ROBERT, D. (1988). Visual steering under closed-loop conditions by flying locusts: flexibility of optomotor response and mechanisms of correctional steering. *J. comp. Physiol.* **164**, 15–24,

ROBERTSON, R. M. AND PEARSON, K. G. (1982). Interneurons in the flight system of the locust: distribution, connections and resetting properties. *J. comp. Neurol.* **215**, 33–50.

ROWELL, C. H. F. (1988). Mechanisms of flight steering in locusts. *Experientia* **44**, 389–395.

ROWELL, C. H. F. AND REICHERT, H. (1986). Three descending interneurones reporting deviation from course in locust. II. Physiology. *J. comp. Physiol.* A **158**, 775–794.

SANDEMAN, D. C. (1968). A sensitive position measuring device for biological systems. *Comp. Biochem. Physiol.* **24**, 635–638.

SNODGRASS, R. E. (1929). The thoracic mechanism of a grasshopper and its antecedents. *Smithson. misc. Collns* **82**, 1–111.

STEWART, W. W. (1978). Functional connections between cells as revealed by dye-coupling with a highly fluorescent napthalimide tracer. *Cell* **14**, 741–759.

TAYLOR, C. P. (1981). Contribution of compound eyes and ocelli to steering of locusts in flight. I. Behavioural analysis. *J. exp. Biol.* **93**, 1–18.

TYRER, N. M. (1971). Innervation of the abdominal intersegmental muscles in the grasshopper. II. Physiological analysis. *J. exp. Biol.* **55**, 315–324.

TYRER, N. M. AND ALTMAN, J. S. (1974). Motor and sensory flight neurones in a locust demonstrated using cobalt chloride. *J. comp. Neurol.* **157**, 117–138.

TYRER, N. M. AND GREGORY, G. E. (1982). A guide to the neuroanatomy of locust suboesophageal and thoracic ganglia. *Phil. Trans. R. Soc. Ser.* B **297**, 91–123.

WEIS-FOGH, T. (1949). An aerodynamic sense organ stimulating and regulating flight in locusts. *Nature* **163**, 873–874.

WILSON, D. M. (1962). Bifunctional muscles in the thorax of grasshoppers. *J. exp. Biol.* **39**, 669–677.

WOLF, H., RONACHER, B. AND REICHERT, H. (1988). Patterned synaptic drive to locust flight motoneurons after hemisection of thoracic ganglia. *J. comp. Physiol.* **163**, 761–769.

YANG, Q.-Z. AND BURROWS, M. (1983). The identification of motoneurones innervating an abdominal ventilatory muscle in the locust. *J. exp. Biol.* **107**, 115–127.