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MONOSYNAPTIC CONNEXIONS BETWEEN WING STRETCH RECEPTORS AND FLIGHT MOTONEURONES OF THE LOCUST

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SUMMARY

1. The connexions between stretch receptors of the wings and motoneurones innervating flight muscles have been studied anatomically and physiologically.

2. Filling with cobaltous chloride shows that the single neurone of a forewing stretch receptor has a complex pattern of branches within the mesothoracic ganglion and branches which extend into the pro- and metathoracic ganglia. The single neurone of a hindwing stretch receptor has extensive branches in the metathoracic ganglion and branches in the mesothoracic ganglion. The branches of both receptors are confined to the ipsilateral halves of the ganglia.

3. A stretch receptor gives information about the velocity and extent of elevation of a wing.

4. Each spike of a forewing stretch receptor causes an EPSP in ipsilateral mesothoracic depressor motoneurones and an IPSP in elevators. The connexions are thought to be monosynaptic for the following reasons. The EPSPs in the first basalar (depressor) motoneurone follow each spike of the stretch receptor at a frequency of 125 Hz and with a constant latency of about 1 msec. In a Ringer solution containing 20 mM-Mg^{2+} the amplitude EPSP declines gradually. The IPSPs upon elevators have similar properties but occur with a latency of 4–6 msec.

5. The connexions therefore comprise a monosynaptic negative feed-back loop; elevation of the wing excites the stretch receptor which then inhibits the elevator motoneurones and excites the depressors.

6. A hindwing stretch receptor synapses upon metathoracic flight motoneurones in the same way, causing EPSPs in depressor and IPSPs in elevator motoneurones.

7. No connexions of either fore- or hindwing stretch receptors have been found with contralateral flight motoneurones.

8. Interganglionic connexions are made by both receptors. For example, both fore- and hindwing stretch receptors cause EPSPs upon the meso- and metathoracic first basalar motoneurones.

9. Stimulation of the axon of a stretch receptor with groups of three stimuli repeated every 50–100 msec thus simulating the pattern which it shows during flight, causes subthreshold waves of depolarization in depressor motoneurones. When summed with an unpatterned input, the stretch receptor is able to influence the production of spikes in motoneurones on each

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cycle. During flight, it is expected that the stretch receptor will influence the time at which a motoneurone will spike and hence have an effect on the amplitude of the upstroke and upon the phase relationship between spikes of motoneurones.

INTRODUCTION

The flight of the locust has come to be regarded as a classic example of a behaviour whose basic pattern is controlled by the central nervous system. Undoubtedly within the central nervous system there resides a pattern generating mechanism capable of producing an alternation of the spikes in depressor and elevator motoneurones of the wings. If sensory nerves of the wings are cut, a locust can still produce a rhythmical motor output to its flight muscles but of a reduced frequency (Wilson, 1961). This finding does not preclude the existence of peripheral feedback loops but indicates that they must act in parallel to or sum with the central pattern generator. What influence do the profusion of sense organs of the wing and its hinge have upon the central neurones of the flight system? Of the many sense organs only the unicellular stretch receptor at the base of each wing hinge has been studied in any detail. It responds to elevation of the wing (Gettrup, 1962, 1963; Pabst, 1965) and during flight produces a few spikes towards the end of each upstroke (Wilson, 1961). It can thus provide information about the amplitude and velocity of each upstroke (Gettrup, 1963; Pabst, 1965) and possibly of the phasing between the two pairs of wings (Pabst & Schwartzkopff, 1962). The central nervous system apparently makes no use of the phasic information, the effect of which outlasts a single wing beat and is summed over many cycles (Wilson & Gettrup, 1963). This conclusion was based on three types of experiment. In the first, cautery of two, three or four stretch receptors reduced the frequency of flight by 10, 25 or 50% respectively. In the second, gluing the fore and hindwings together rendered the locust unable to lift its own weight, but the frequency of the wingbeats and the phasing of the muscles was unchanged. In the third, stimulation of the nerve innervating the wing hinge in a locust, bisected except at the head, which flaps its wings at only half the normal frequency, raised that frequency by 30%. The wingbeat frequency increased with a time constant of 2 sec or 25 wingbeats, but with no pattern of stimulation could the output be entrained (Wilson & Wyman, 1965). The conclusion was that such timing cues as the stretch receptor may provide are not used and that the information is summed over many cycles to raise the frequency of wingbeats. The interpretation of the experiments is, however, based on the untenable assumption that only one sensory neurone, the stretch receptor, is being activated. First, cautery of the wing hinge was believed to destroy selectively the stretch receptor, although no recordings were made after the operation to verify this assumption. Gettrup (1963) admits that the multicellular scolopidial organ close to the stretch receptor may be damaged and Kutsch (1974) shows by recording that similar surgery abolishes inputs from the stretch receptor, the scolopidial organ and, when applied to the forewing, some receptors of the hindwing as well. Secondly, gluing wings together should alter the phasing of inputs from all sense organs of the wing and thirdly, stimulation of the nerve from the wing hinge is unlikely to activate the stretch receptor selectively. I do not question that afferents influence the frequency of wingbeats but this effect cannot be attributed to the stretch receptor alone. Kutsch (1974)

produced a 50% reduction in the frequency of wingbeats by destroying the sense organs of three or four wings but their destruction in one or two wings was without effect. In addition the coefficient of variation of the intervals between the spikes of antagonistic muscles was increased when the frequency was reduced.

Afferents of the wing might be expected to perform two functions during flight which would seem to require that use be made of the information they provide at each wingbeat. First, they might serve to match the central motor score to the resonant frequency of the thorax and wings which will change as the locust matures and the cuticle hardens. Removal of sense organs of the wing in a young adult, however, does not prevent the gradual increase in the frequency of wingbeats that occurs in maturing adults (Kutsch, 1971, 1974). The development of the flight motor score, therefore, can proceed without afferents from the wing which, when present, can raise the frequency of wingbeats by the same amount in both young and old adults. Secondly, afferents might signal the rapid correctional changes needed as the locust flies through turbulent air. Experiments in which the locust was subjected to imposed roll or other manoeuvres demonstrated, however, that afferents only cause slow changes in the average level of excitation of the motoneurones (Waldron, 1967*b*). Rapid variations in the motor pattern were attributed to changes within individual motoneurones and were not thought to be caused directly by afferents of the wings.

All these types of experiment fail to demonstrate a use for the phasic information provided by the stretch receptor and other sense organs of the wing. If, however, a forewing of a flying locust is moved forcibly at a frequency which differs from the natural one by 10–15%, all wings can be induced to adopt the imposed frequency (Wendler, 1972, 1974). The entrainment occurs within one or two cycles of the wingbeat so that the central nervous system must be using the phasic information from sense organs of the wing to co-ordinate the motor output. Wendler makes no attempt to ascribe the effect to a particular sense organ and predicts that a variety act together.

Few attempts have been made to determine what connexions the sense organs of the wing make with the central components of the flight system. Stimulation of unknown numbers of unidentified sensory neurones in the nerve to the wing hinge produced depolarizing potentials with a delay of 1·5–3·0 msec in unidentified, but presumed second-order neurones of the mesothoracic ganglion (Iwasaki & Wilson, 1966). Bursts of stimuli to the wing nerve containing the axon of the stretch receptor produced depolarizing potentials with a minimum latency of 7–8 msec upon unidentified motoneurones, but single stimuli had little effect (Kendig, 1968). The results of experiments such as these in which the neurones were not identified are difficult to interpret and afford little insight into the way sensory input influences central neurones during flight.

In an attempt to resolve the role played by sense organs of the wing, I have made use of the fact that the spike of the stretch receptor can be identified in extracellular recordings from the nerve of the wing hinge to ask if the stretch receptor synapses upon motoneurones of power-producing flight muscles. The motoneurones can be identified by intracellular recording from their somata, a procedure which also reveals at least some of their synaptic inputs. This type of experiment should allow the connexions of a known sensory neurone with known elements of the central neurones system to be determined and may reveal any influence upon the central

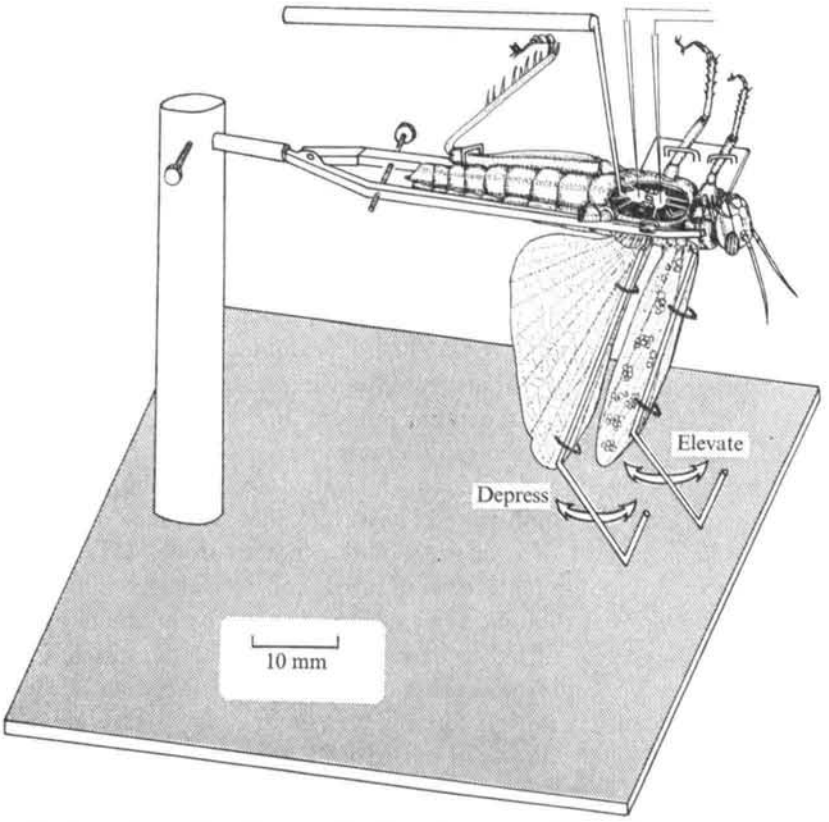

Fig. 1. The locust in position for recording from the somata of its flight motoneurones during imposed movements of the wings. An adjustable clamp grips the locust along its sides and the legs are fixed to laterally protruding platforms. In the diagram, but not in the experiments, the nearside legs are removed. The leading edge of the wing is clipped to one arm of a U-shaped bar, the other arm of which is attached to a potentiometer. A rigid platform slipped beneath the meta- and mesothoracic ganglia allows stable recordings to be made from the ventrally situated somata of the motoneurones by microelectrodes, two of which are shown.

pattern generator. It may also allow predictions to be made about the role of the sense organ in the flying locust. I will show that the stretch receptor synapses directly upon the motoneurones forming part of a negative feed-back loop which excites depressor and inhibits elevator motoneurones.

MATERIALS AND METHODS

Eighty-five adult *Schistocerca gregaria* were obtained from culture. A locust, mounted ventral side uppermost, was secured along the sides of its thorax with the proximal joints of the legs restrained but with the head and the abdomen free to move (Fig. 1). One limb of a U-shaped bar was attached to the leading edge of each wing, the other to the spindle of a potentiometer. This arrangement allowed forced movements of a wing to be made and monitored at the same time over a range of 80°, from the horizontal to the fully elevated position.

Four pairs of 50 μm silver wires insulated but for the tip were implanted into the dorso-ventral muscles of both the meso- and metathoracic segments. They were used

to record extracellular muscle potentials or to stimulate the axon terminals and hence evoke antidromic spikes in motoneurones impaled centrally. Four pairs of platinum hook electrodes insulated by a petroleum jelly and oil mixture were used to record the sensory inflow from the wings, or for stimulation. They were placed on the two first nerves of the mesothoracic ganglion (or the two sixth nerves of the prothoracic ganglion) and on the two first nerves of the metathoracic ganglion distal to their fusion with the sixth nerves of the mesothoracic ganglion (Fig. 2).

Stable intracellular recording from the somata of the motoneurones was possible when the meso and metathoracic ganglia were stabilized against movements of the body by passing a wax-covered platform between the abdominal connectives and under the ganglia. Full details of the method are given by Hoyle & Burrows (1973). The electrodes, filled with 2 M potassium acetate and with resistances of 50–80 MΩ had to pass through the intact sheath of the ganglia before penetrating the somata of the motoneurones. The thorax was perfused with a constant flow of saline (Usherwood & Grundfest, 1965). To extract those synaptic potentials linked to the sensory spikes from the background of other synaptic potentials, signal averaging was sometimes necessary using a Biomac 1000 (Data Laboratories Ltd., London). Averaged signals were displayed on an *x–y* plotter. All experiments were performed at 19–22 °C.

The central projections of the stretch receptor neurones were revealed by staining with cobaltous chloride (Pitman, Tweedle & Cohen, 1972) carried into the ganglion from the cut end of nerve 1 by a procedure similar to that described for procion dyes by Iles & Mulloney (1971). The whole mount of a ganglion was drawn using a 40 × oil-immersion lens and a Wild drawing tube.

The numbering of the nerve trunks is taken from Campbell (1961) and of the muscles from Snodgrass (1929).

RESULTS

Identification of motoneurones

The somata of motoneurones innervating flight muscles are located on the ventral surface of the three thoracic ganglia (Fig. 2). A particular motoneurone was identified after penetration with a microelectrode by: (*a*) evoking an antidromic spike from its axon terminals on the muscle which it innervates; (*b*) by correlating muscle potentials with soma spikes during spontaneous activity; (*c*) by depolarizing the soma to evoke orthodromic spikes and observing correlated muscle potentials and movements of a wing. In some motoneurones orthodromic and antidromic spikes could be made to collide. Identification using these criteria is nevertheless difficult because the muscles are closely packed. The motoneurones identified and used here are shown in Fig. 2, data for which are drawn from Bentley (1970) for the mesothoracic and my own observations for the other ganglia. All the motoneurones shown have somata ipsilateral to the muscle which they innervate.

Identification of the stretch receptor

Upon elevation of a wing, sensory spikes of large amplitude are recorded in nerve 1 (N 1) innervating that wing. These spikes can be followed in the branch ($N1D_2$) which innervates the unicellular stretch receptor at the wing hinge. The spikes were assumed to originate from the stretch receptor (Wilson, 1961; Gettrup, 1962; Wilson

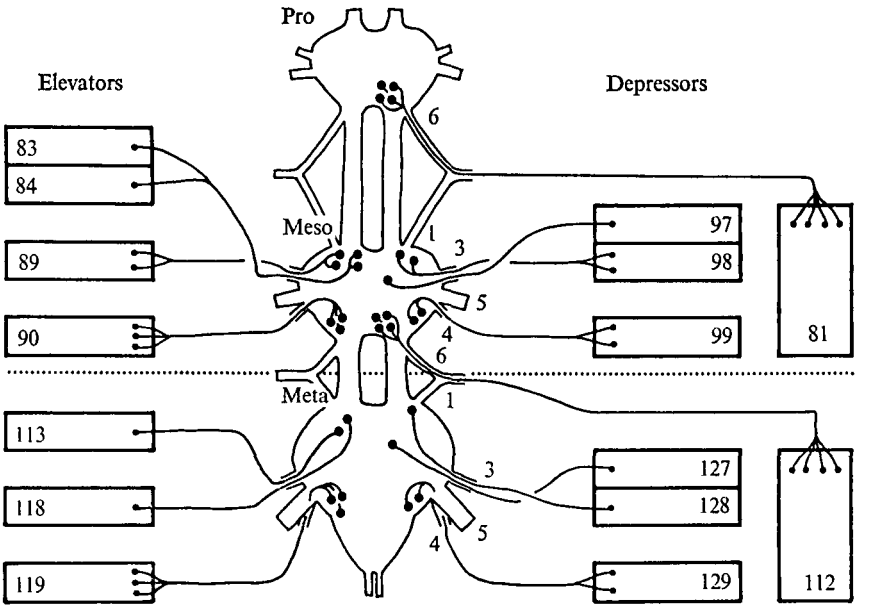

Fig. 2. The positions of the somata and pathways of the axons of the flight motoneurones described in this paper. Depressor motoneurones and the muscles they innervate are shown on the right, elevators of the left. The dotted line separates forewing from hindwing muscles. Note that the dorsal longitudinal muscles are innervated by motoneurones of the next anterior segment. The names and numbers of the muscles are given in Table 1 (page 211).

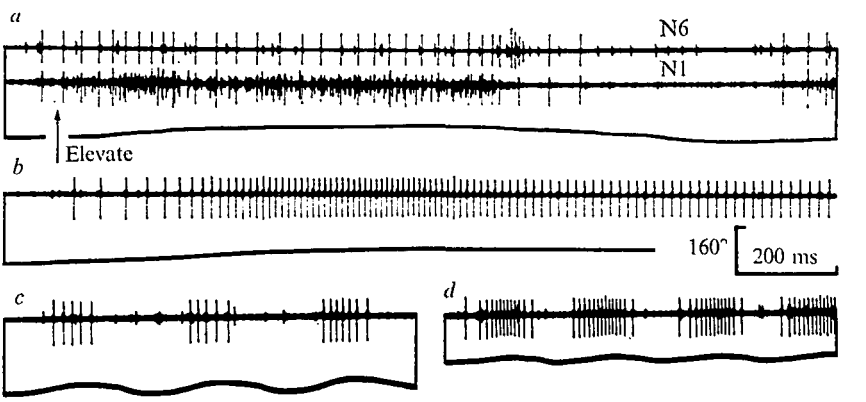

Fig. 3. The response of forewing sense organs to forced movements of the forewing. (*a*) Upon elevation (upwards on the lower trace) many neurones are activated whose axons run in N1 (second trace) of the mesothoracic ganglion. Only one sensory spike is recorded in N6 of the prothoracic ganglion (first trace) and which is phase locked to one in N1. These spikes are from the single sensory neurone of the stretch receptor and for ease of recognition are best recorded from N6 as in (*b–d*). (*b*) Elevation of the wing causes a gradual increase in the frequency of stretch receptor spikes. If the wing is held elevated a tonic discharge is maintained of a frequency lower than during the preceding dynamic phase. (*c*) An imposed oscillation of the wing about the horizontal causes a phasic response of the stretch receptor. (*d*) When elevated 70 ° above the horizonal and then oscillated the frequency and number of spikes upon each additional elevation is increased. Record (*a*) is from one locust (*b–d*) from another.

& Gettrup 1963; Pabst, 1965), but the more convincing evidence which would be provided by an intracellular recording from the peripherally located soma and the axon is lacking. Some of the other sensory axons in $N1D_2$ come from the multicellular scolopidial organ also at the wing hinge and have spikes of smaller amplitude as recorded extracellularly, which correlates with their smaller diameter; they are 2·5–4 μm as compared with the 6 μm axon of the stretch receptor (Gettrup, 1962). The relatively large amplitude of the spike of the stretch receptor means that it can be distinguished from other sensory neurones activated by movements of a wing in recordings from N1 (Fig. 3*a*). As it approaches the central nervous system, however, N1 branches to form N6 to the next anterior segmental ganglion and N1 to its own ganglion. Recordings from N6 show that the only large sensory axon from the wing which it contains is from the stretch receptor (Fig. 3*a*). A recording of the stretch receptor spike alone is obtained when motor activity in this nerve is abolished by cutting it central to the electrodes. No other sensory neurones have been observed whose axons branch into N1 and N6 but if there are any, their axons must be of considerably smaller diameter than that of the stretch receptor. This is an important conclusion for some of the arguments which follow (cf. Fig. 18). A further advantage of using N6 to record the spike of the stretch receptor is that the electrodes will not impede its passage into the mesothoracic ganglion but electrodes on N1 might.

The response properties of the stretch receptor have been described in detail (Gettrup, 1962; Pabst, 1965) and they correspond with those of the neurone which is assumed here to be the stretch receptor. It is sufficient in the present context to point out that the stretch receptor is activated upon elevation of the wing (Fig. 3*b*). If the wing is held elevated, the initial dynamic response during the movement declines to a static one whose frequency depends upon the extent of elevation. The number and frequency of spikes evoked during a rhythmic elevation of the wing, such as occurs during flight, depends upon the extent (Fig. 3*c*, *d*) and angular velocity of the elevation. The spike of either the fore- or hindwing stretch receptor is conducted at a velocity of 2·0–2·5 m^{s-1}. In an adult locust with a metathoracic tibia 28 mm long the length of nerve from the stretch receptor itself to the thoracic ganglia is about 13 mm, so that the spike will take 5–6 msec to travel this distance at 20 °C.

The pathway of the axon of a stretch receptor can be traced within the thoracic nervous system by extracellular recording of its spike (Fig. 4). The axon of a *forewing stretch receptor* branches into N6 of the prothoracic ganglion (Fig. 4*a*) where it seems to terminate because no spike can be recorded from the ipsilateral pro-suboesophageal connective. The branch which enters the mesothoracic ganglion from N1, continues into the ipsilateral meso-metathoracic connective where its spike can be revealed after averaging (Fig. 4*b*). Failure to observe a spike in recordings from a connective posterior to the metathoracic ganglion suggests that the axon terminates here (Fig. 4*c*). The single neurone of a forewing stretch receptor thus projects into three ganglia, the pro, meso and metathoracic.

The axon of a *hindwing stretch receptor* branches into N6 of the mesothoracic ganglion (Fig. 4*e*) where it probably terminates since its spike cannot be recorded, even with averaging, from the ipsilateral meso-prothoracic connective (Fig. 4*d*). The branch which enters the metathoracic ganglion from N1 seems to terminate within this ganglion as inferred from the failure to record its spike in the ipsilateral posterior

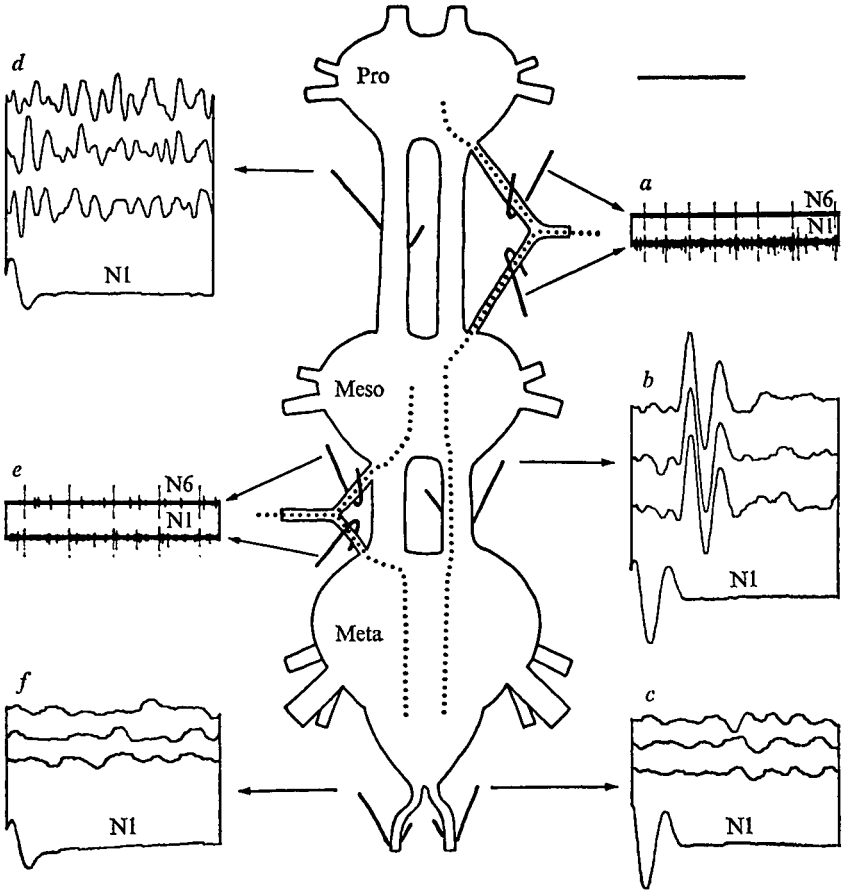

Fig. 4. The pathways of the right forewing and the left hindwing stretch receptors as determined by extracellular recording of their axon spikes with two sets of electrodes. (*a*) The axon of the *forewing stretch receptor* bifurcates to enter N6 of the prothoracic and N1 of the mesothoracic ganglion as shown by simultaneous recording from both nerves. (*b*) The projection of the axon into the metathoracic ganglion can be revealed only by averaging the signal, recorded extracellularly from the whole meso-metathoracic connective. Each trace represents the average of 64 occurrences triggered from the spike in mesothoracic N1. A spike in the connective is clearly linked to the one in N1 and three traces are aligned to show its consistent occurrence. (*c*) Averaging fails to demonstrate a projection into the abdominal connectives. The *hindwing stretch receptor* bifurcates to enter N6 of the mesothoracic and N1 of the metathoracic ganglion (*e*). Averaging fails to show a projection into the meso-prothoracic (*d*), or abdominal connectives (*f*). Calibration: oscilloscope records 200 msec, averaged records 5 msec.

connective (Fig. 4*f*). The single neurone of a hindwing stretch receptor thus projects into only two ganglia, the meso- and the metathoracic.

The central projection of a prominent axon of N1 can be described anatomically when cobaltous chloride is allowed to diffuse into the cut axons of that nerve. It is similar to the one described in *Locusta* (Bentlage, 1973). Peripherally this axon enters branch $N1D_2$ and terminates at the wing hinge. Centrally this axon in N1 of the *forewing* branches to enter N6 of the prothoracic and N1 of the mesothoracic ganglion. The mesothoracic branch passes through that ganglion and into the metathoracic ganglion where it terminates (Fig. 21, Plate 1). In no ganglion is the axon seen to be connected with a soma. If cobaltous chloride is allowed to enter the distal ends of

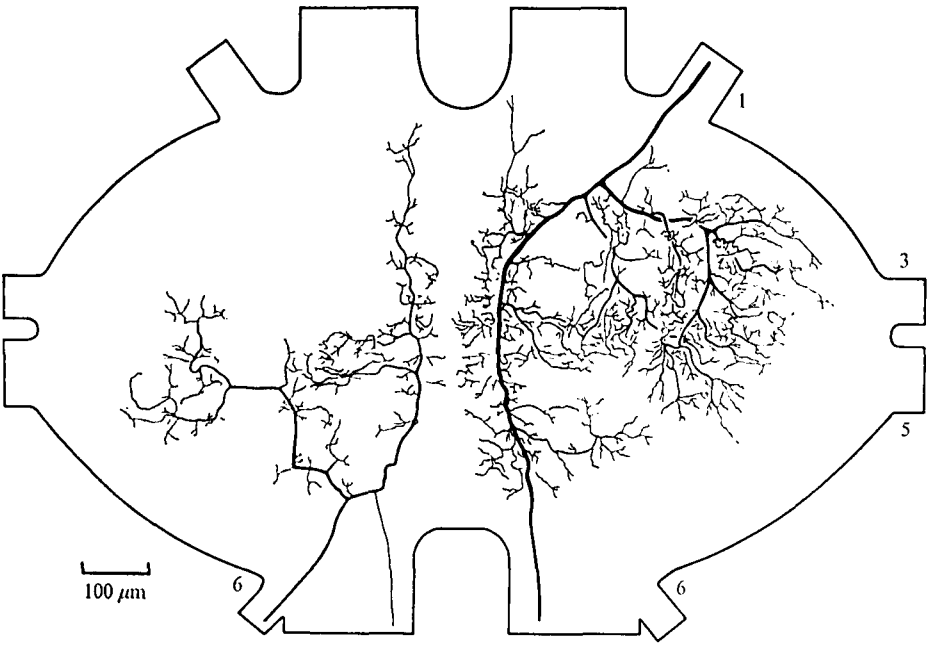

Fig. 5. Drawing of the projections of the right *forewing* and the left *hindwing* stretch receptors into the mesothoracic ganglion. The axon of the forewing stretch receptor enters through N1, that of the hindwing stretch receptor through N6. The branches project only to the ipsilateral side of the ganglion. No branches emerge through the anterior connectives, but the axon of the forewing stretch receptor enters a posterior connective. The drawing was made from the dorsal surface of the ganglion but not all the branches are shown.

axons of prothoracic N6, a branch of one axon continues peripherally but a second branch enters N1 and the mesothoracic ganglion. Here it follows the same pathway and has the same pattern of branching as the axon stained from the central end of N1, peripheral to its bifurcation into N1 and N6. The only sensory axon from the wing identified physiologically in N6 is from the stretch receptor. No other sensory neurones have the same anatomical projection as this axon.

A prominent axon of N1 of the *hindwing* can be described by a similar procedure. It enters the mesothoracic ganglion through N6 and the metathoracic ganglion through N1 and is not seen to connect with a soma in either ganglion. These sensory axons from the fore- and hindwings thus have the same anatomical projections as the physiologically determined projections of the stretch receptors. It is thus a reasonable assumption that the axon characterized physiologically to be from the sensory neurone of the stretch receptor is the axon whose central projection is described anatomically.

The anatomical projection of the stretch receptors within the thoracic nervous system

The projections as revealed by allowing cobaltous chloride to enter N1 are shown in Fig. 21, Plate 1 and Figs. 5 & 6. Fig. 21, Plate 1 shows a photograph of a ganglion so filled but not all the branches can be focused in one picture. Drawings therefore were made of the complex projections (Figs. 5, 6), and only some salient features will be indicated. The axon of a *forewing stretch receptor* gives off a prominent

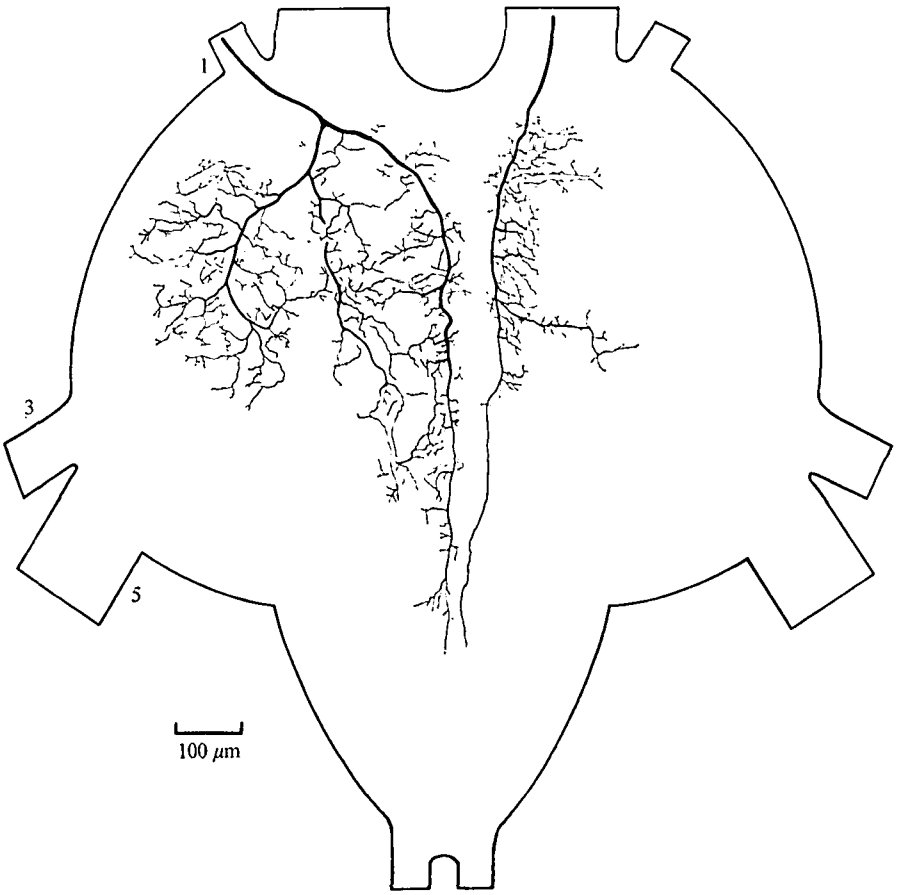

Fig. 6. Drawing of the projection of the right *forewing* and the left *hindwing* stretch receptor into the metathoracic ganglion. The axon of the forewing stretch receptor enters through the anterior connective, branches to the ipsilateral side of the ganglion but was not observed to enter the posterior connectives. The axon of the hindwing stretch receptor enters through N1, branches profusely on the ipsilateral side of the ganglion but was not observed to enter either the anterior of posterior connectives. The drawing was made as in Fig. 5.

branch soon after entering the dorsal neuropile. From this side branch arises a mass of finer branches which extends from the lateral edge of the dorsal neuropile almost to the midline and posteriorly beyond the emergence of N5 to the leg (Fig. 21 *a*, Plate 1 and Fig. 5). The main axon continues toward the midline and gives off a smaller diameter branch which turns posteriorly and again forms a profuse network of fine branches (Fig. 5). At the level of emergence of N3, the main axon turns posteriorly to continue through the ganglion close to the midline and into the ipsilateral meso-metathoracic connective. As it passes through the ganglion numerous fine branches emerge along its length (Fig. 21 *b*, Plate 1). One or two branches of larger diameter emerge laterally at about the level of N5 and their branches become intermingled with those of the posteriorly running branches. At the posterior edge of the neuropile a further profusion of branches emerges. All the fine branches are characterized by a beaded appearance, and some seem to end abruptly in knobs but whether these represent synaptic regions is unknown.

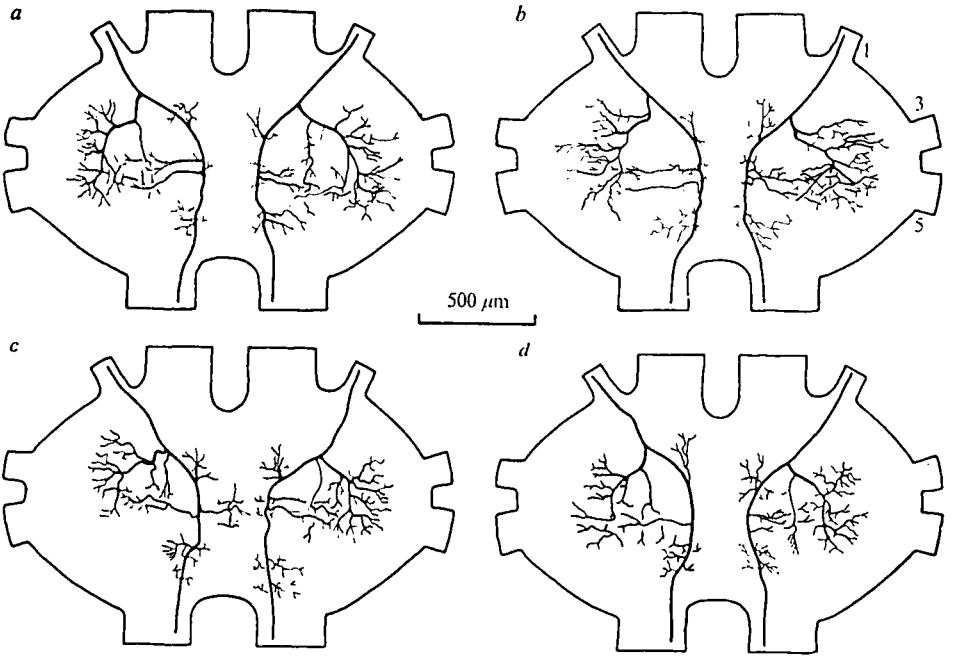

Fig. 7. Types of variation seen in the projections of eight different forewing stretch receptors into the mesothoracic ganglion. In (*a* and *b*) the projections of the left and right stretch receptors from different locusts have been combined in the same drawing. In (*c* and *d*) the left and right projections are from the same locust. Note the consistency of the main branches, the variability in the smaller ones and that in the left projection of (*c*) a prominent contralateral branch is present.

The main axon gradually becomes thinner as it passes through the meso-metathoracic connectives and enters the metathoracic ganglion. The reduced diameter is not attributable to failure of staining because the conduction velocity of the stretch receptor spike, measured on either side of the mesothoracic ganglion, falls to 1·5–2·0 m^{s-1}. Within the metathoracic ganglion the axon runs close to the midline of the dorsal neuropile and numerous fine branches emerge along its length most of which run laterally and dorsally (Fig. 6). One branch can usually be seen to extend further than the rest. Beyond the level of N_5 the staining is usually faint but the axon can be traced into that part of the ganglion which contains the abdominal ganglia. I have been unable to trace the branch in prothoracic N6 into the prothoracic ganglion.

Within the metathoracic ganglion the axon of a *hindwing stretch receptor* has a branching pattern similar to that of a forewing stretch receptor in the mesothoracic ganglion (Fig. 6). A prominent lateral branch emerges as the axon enters the dorsal neuropile which runs posteriorly and forms a profuse tree. The main axon continues towards the midline, then runs posteriorly to end at about the same level as the axon of a forewing stretch receptor. Along its length the axon gives rise to a mass of fine, mostly laterally and dorsally directed branches. The axonal branch in N6 of the meso-thoracic ganglion branches as it enters the dorsal neuropile of that ganglion (Fig. 5). One branch passes laterally to form a lateral tree at the level of N_5 and the other runs close to the midline and ends near the emergence of the ipsilateral anterior connective. Along its length many fine branches emerge. In some preparations a recurrent branch

of small diameter enters the meso-metathoracic connective and can be traced back to the metathoracic ganglion.

The central branches of both the fore and hindwing stretch receptors are confined to the ipsilateral side of the ganglion. In only one (Fig. 7*c*) of more than fifty fillings of a stretch receptor axon was a contralateral branch revealed. In different locusts the main branches of the stretch receptor can be consistently recognized but there are differences (Fig. 7). For example the second posteriorly running branch of the forewing stretch receptor in the mesothoracic ganglion may arise from the main axon (Fig. 5, 7*c*), from the first posterior branch (Fig. 7*a*, *d*) or apparently be absent (Fig. 7*b*). The ramifications of the fine branches make it impossible to establish criteria by which a consistent pattern could be recognized. The overall shape of the neurone is nevertheless characteristic and no other sensory neurones of the wing have a similar shape.

Motoneurones innervating muscles of both the fore and hindwings of the Australian locust *Chortoicetes* have an extremely complex pattern of branches within the neuropile (Burrows, 1973*b*, 1975). Fillings of the homologous neurones in *Schistocerca* reveal similar patterns (Burrows, unpublished). Motoneurones innervating the muscles of the forewing have axons in nerves 3, 4 and 6 and those with axons in a particular nerve have their branches in the same region of the ipsilateral dorsal neuropile; N3 motoneurones towards the anterior of the ganglion, N6 motoneurones posteriorly and toward the midline and N4 motoneurones between the other two. There is much overlap between the groups but they correspond roughly to some of the stretch receptor projections as follows: in the large posteriorly directed tree with the projection of N3 motoneurones; in the lateral branches and the more posterior branches of the above tree with N4 motoneurones; and in the tufts of branches near the posterior connective with the N6 motoneurones. The extensive projections of both motoneurones and stretch receptors leads to the conclusion that branches of the two are intertwined. The anatomical study thus establishes that there are numerous possible sites at which the sensory and motoneurones could come into contact. It cannot reveal the function of possible contacts or even if contacts are functional, but it does indicate where to look for these contacts physiologically. Contralateral connexions would not be expected but interganglionic ones would.

Connexions of a forewing stretch receptor with motoneurones

A spike of the stretch receptor produces a depolarizing synaptic potential in the ipsilateral mesothoracic first basalar motoneurone (Fig. 8*a*). The synaptic potential typically has an amplitude of 4–5 mV as recorded in the soma. The potential follows each observed spike with a constant latency which is less than 1 msec as measured from the time of entry of the spike into the ganglion and the appearance of the potential in the soma (Fig. 8*b*). Within the ganglion, in addition to any synaptic delay, time must be allowed for the spike of the stretch receptor to travel along an axon of unknown diameter and length. The frequency of stretch receptor spikes increases when the wing is raised slowly and the amplitude of the potentials in the soma declines (Fig. 8*c*). At least three factors may contribute to the decline in the amplitude of the evoked potentials. First, there may be rectification at non-synaptic membrane. A depolarization of 5–15 mV applied to the soma is not rectified by an amount that would explain

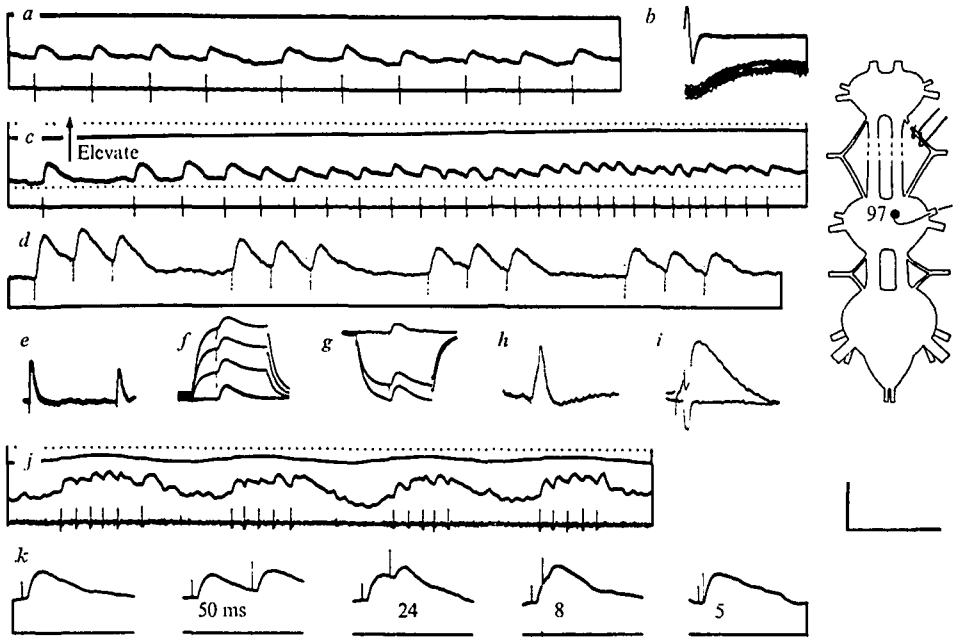

Fig. 8. Connexion of the forewing stretch receptor with the ipsilateral first basalar motoneurone (muscle 97). (*a*) Each stretch receptor spike (third trace) causes an EPSP in the motoneurone (intracellular recording from its soma on the second trace) when the wing is held elevated but stationary 20° above the horizontal (first trace). (*b*) Multiple superimposed sweeps triggered from the stretch receptor spike show that the latency to the potential is constant. (*c*) A slow elevation of the wing produces an increase in the frequency of stretch receptor spikes (displayed at a lower gain) and of the potentials which decrease in amplitude as the membrane potential of the motoneurone falls. (*d*) Groups of three stimuli delivered to N6 of the prothoracic ganglion. Note the reduction in the amplitude of the initial potential of the first three groups. (*e*) The second potential of a pair is reduced in amplitude even when the interval separating them is 800 msec. (*f, g*) Depolarizing or hyperpolarizing the soma affects the amplitude of the evoked potential. (*h*) The potential causes a spike when added to an applied d.c. depolarization of the soma. Two electrodes were inserted into the soma in (*f–h*), one to record, the other to pass current. (*i*) Two superimposed sweeps, in one of which a potential was evoked. The voltage change caused by a constant current pulse is reduced when the potential occurs. (*j*) Rhythmic movements of the wing cause rhythmic depolarizations of the motoneurone. (*k*) Pairs of stimuli, the interval between which is progressively reduced, evoke two potentials provided they are separated by more than 5 msec. Records (*a–c*) are from one locust, (*d–i*) from another and (*j, k*) from a third. The diagram on the right in this and subsequent figures shows the position of the impaled soma and of the recording and stimulating electrodes. Calibration: vertical, voltage (*a–e, h, i*) 5 mV, (*f, g, j, k*) 8 mV, wing movement 80°; horizontal (*a–d, j*) 200 msec, (*b*) 16 msec, (*e*) 800 msec, (*f–h*) 160, (*i, k*) 80 msec.

all the reduction of the potentials. The current voltage relationship of the soma membrane is approximately linear in this range, but this of course gives no indication of the properties of the membrane which lies between the synaptic site and the soma. Secondly, there may be a process which can be loosely called fatigue at the synapse. Fatigue can be demonstrated by applying groups of stimuli to nerve 6 of the prothoracic ganglion to evoke the potentials in the first basalar motoneurone. The initial potential of each group is reduced sequentially until an equilibrium is reached, even though each of these potentials arises from the same membrane potential (Fig. 8*d*). When separated by 800 msec the second potential of a pair is of reduced amplitude (Fig. 8*e*). The large amplitude of the first potentials would provide an amplification

mechanism at the start of flight increasing the probability that the motoneurones will spike in response to the first stretch receptor spikes. Thirdly, the reduced membrane potential may affect a conductance mechanism at the synapse. Depolarizing (Fig. 8*f*) or hyperpolarizing (Fig. 8*g*) pulses applied to the soma cause respectively a reduction or an increase in the amplitude of the evoked potential. The potential cannot be reversed but can be shown to be excitatory because it will lead directly to a spike when the soma is depolarized by d.c. current (Fig. 8*h*). The voltage change caused by a constant current pulse applied during the rising phase of the potential is reduced compared with that applied when no potential is evoked (Fig. 8*i*). This indicates that the resistance of the membrane has fallen during the potential. In a Ringer solution containing 20 mM-Mg^{2+} the amplitude of the potential is reduced (Fig. 9). The results are consistent with the potential being due to a conductance change in the membrane caused by the release of a chemical transmitter. The potential can thus be called an excitatory postsynaptic potential (EPSP). Upon repeated elevation of the wing the EPSPs summate to cause a rhythmic depolarization of the membrane (Fig. 8*j*). One EPSP follows each spike of the stretch receptor at frequencies of 125 Hz. When the axon of the stretch receptor is stimulated with pairs of shocks a second EPSP always results provided that the interval between the stimuli is above 5 msec. (Fig. 8*k*). There is no apparent facilitatory effect of the first EPSP upon the second, but the two sum.

The short, constant latency and the ability of the EPSP to follow spikes of the stretch receptor at high frequencies are features consistent with the connexion being monosynaptic. As a further test of this assumption the following prediction was made and tested. If transmitter release is blocked presynaptically by raising the level of Mg^{2+} in the saline, the amplitude of the EPSP should decline slowly with time, if the connexion is monosynaptic. An abrupt decrease to zero would be expected at some stage, if the connexion were through an interneurone, as the EPSP falls below the threshold for spike initiation at that interneurone. Interpretation of the experiment requires that two assumptions be made. First, that the Mg^{2+} has free access to all synapses in the pathway and secondly that the putative interneurone produces spikes. Interneurones which do not produce spikes occur in the thoracic ganglia of the cockroach (Pearson, Fourtner & Wong, 1973).

The branch of the stretch receptor in N6 of the prothoracic ganglion was stimulated at 1 Hz and EPSPs recorded from the soma of the first basalar motoneurone. The amplitude of the EPSPs declines gradually when the normal Ringer which contains 4 mM-Ca^{2+} but no Mg^{2+} is replaced with one containing no Ca^{2+} but 20 mM-Mg^{2+} (Fig. 9). The amplitude of the EPSPs gradually resumes its original level upon return to the normal Ringer. The rise time of the EPSP slows and the decay time lengthens as the amplitude decreases, features which are more easily seen in the averaged EPSPs (Fig. 9) and which are consistent with an increased membrane resistance and reduced current flow. The gradual decline in the amplitude of the averaged EPSPs is not due to an increase in the number of failures to evoke an EPSP because an EPSP follows each stimulus. The result is consistent with a monosynaptic connexion between the stretch receptor and the first basalar motoneurone.

The forewing stretch receptor also makes excitatory synaptic connexions upon other ipsilateral, mesothoracic depressor motoneurones (Fig. 10). In a second basalar

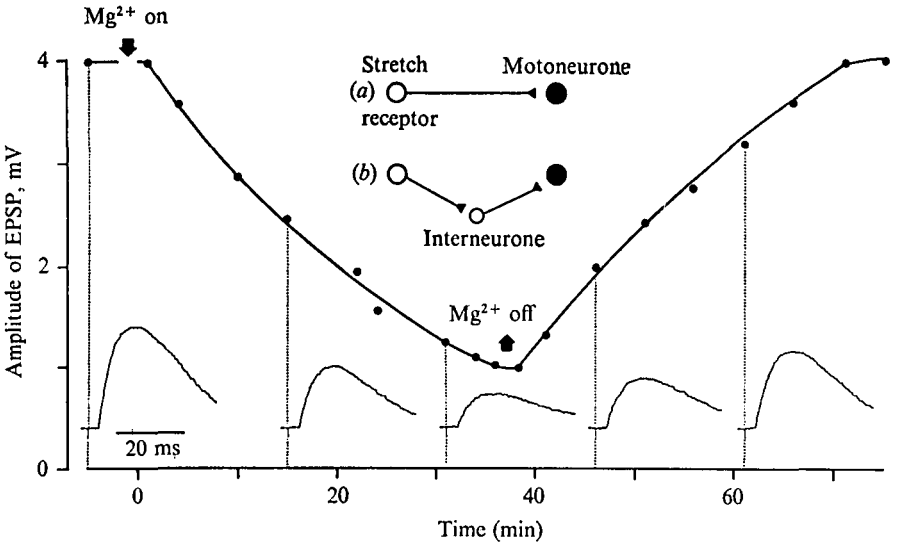

Fig. 9. A test to determine the number of neurones in the pathway between the stretch receptor and motoneurones. The axonal branch of the forewing stretch receptor in the prothoracic N6 is stimulated at 1 Hz and EPSPs recorded in the ipsilateral 1st basalar motoneurone of the mesothorax. The normal Ringer solution is replaced (downward arrow) by one containing no calcium but 20 mM magnesium whereupon the amplitude of the EPSP declines. The EPSP gradually resumes its original amplitude when the test Ringer is removed (upward arrow). The average of 32 occurrences of the EPSP at the times indicated are shown. The experiment is interpreted to indicate that pathway (*a*) rather than the pathway (*b*) exists.

motoneurone the EPSP has an amplitude of 1–3 mV and follows each spike of the stretch receptor at frequencies of 125 Hz with a constant delay of 1–1·5 msec (Fig. 10*a*). The delay to the EPSP in a subalar motoneurone is 0·5–1·0 msec longer than that to the EPSP in the 1st basalar recorded simultaneously (Fig. 10*b*, *e*). Upon elevation of the wing the EPSPs summate and decrease in amplitude in both subalar (Fig. 10*c*) and second basalar motoneurones (Fig. 10*a*). In neither of these motoneurones is the EPSP as readily distinguishable as in the first basalar and frequently, either because the EPSP is of small amplitude, or because there are a large number of other synaptic potentials, it cannot be linked to each spike of the stretch receptor by visual inspection alone. On these occasions signal averaging was used. Each trace of Fig. 10(*d*) or 10(*e*) shows the average of 64 occurrences of the spike of a stretch receptor and the resulting synaptic potential in the motoneurone. The reason for presenting six traces is to show that the potential occurs always with the same latency and has the same amplitude. This implies that the potential consistently follows a spike of the stretch receptor and that its shape, when averaged, is not influenced by randomly occurring potentials of large amplitude. To show that the potential is linked only to the spike of the stretch receptor, the signal averager was triggered randomly from the white noise of an amplifier. No potential was linked to this noise.

The value of signal averaging in revealing connexions of the stretch receptor which cannot be observed by visual inspection is illustrated well for the dorsal longitudinal motoneurones of the forewing, four of which have their somata in the prothoracic ganglion (Fig. 11). In some of these motoneurones the EPSP caused by a spike of the ipsilateral, forewing stretch receptor appears to be of variable amplitude because there

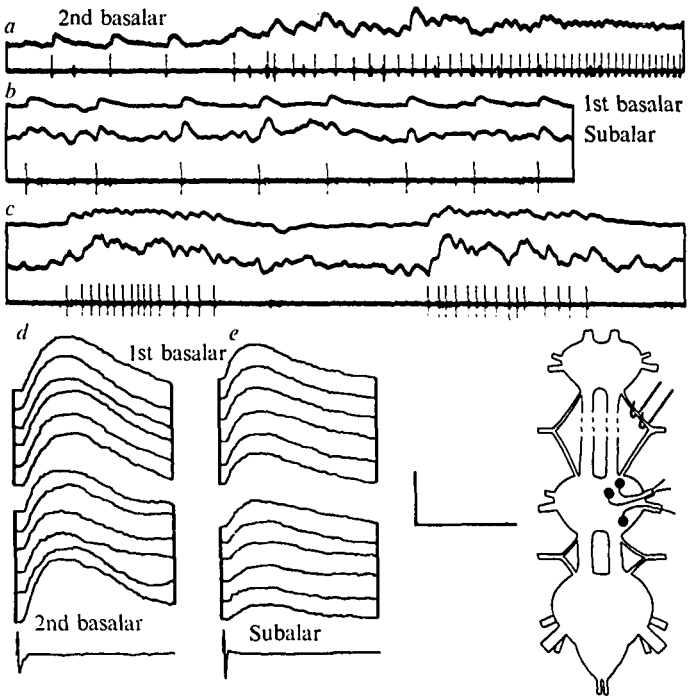

Fig. 10. Connexions of a forewing stretch receptor with ipsilateral depressor motoneurones. (*a*) Stretch receptor spikes cause EPSPs in one of the two motoneurones to the second basalar muscle of the mesothorax (upper trace). The increase in the frequency of spikes is caused by an elevation of the wing which is not indicated. (*b* and *c*) Spikes of the stretch receptor (lower trace) cause EPSPs in the first basalar (upper trace) and in a subalar motoneurone (second trace) recorded together. In (*b*) the wing is held still but in (*c*) is elevated twice. (*d* and *e*) The EPSPs in most recordings from the subular and second basalar motoneurones can be revealed only after averaging. Each trace represents 64 occurrences of the stretch receptor spike and shows the consistency of the EPSP in the motoneurone. In both (*d* and *e*) the upper block of traces are from the first basalar, the lower from the second basalar (*d*) or subalar (*e*). The single trace is the spike of the stretch receptor. Records (*b*, *c*) are from the same locust, the remaining ones each from different locusts. Calibration: vertical (*a*) 5 mV, (*b*, *c*) first trace, 10 mV, second trace, 5 mV; horizontal (*a–c*) 200 msec, (*d*, *e*) 26 msec.

are many other synaptic inputs (Fig. 11*a*). As for other depressor motoneurones, elevation of the wing causes a depolarization with consistent following of the EPSP after each spike (Fig. 11*b*). Signal averaging shows that the stretch receptor connects with at least three of the four motoneurones which were recorded sequentially in one locust (Fig. 11*c–e*). The delay between the spike as it enters the ganglion through N6 and the appearance of the EPSP in the soma is 1·5–2 msec.

The ipsilateral forewing stretch receptor causes inhibitory postsynaptic potentials (IPSPs) in mesothoracic elevator motoneurones. Each spike of the stretch receptor causes an IPSP in the first tergosternal (elevator) and an EPSP in the first basalar (depressor) motoneurones (Fig. 12*a*). The IPSP follows each spike with a consistent but longer latency than for the EPSP (Fig. 12*c*), which is 4–5 msec for the first tergosternal and 5–6 msec for the first posterior tergocoxal. Part of this delay may be accounted for by the time taken for electrotonic conduction of the IPSP from synapses upon branches of the motoneurone distant from the soma. The small amplitude of

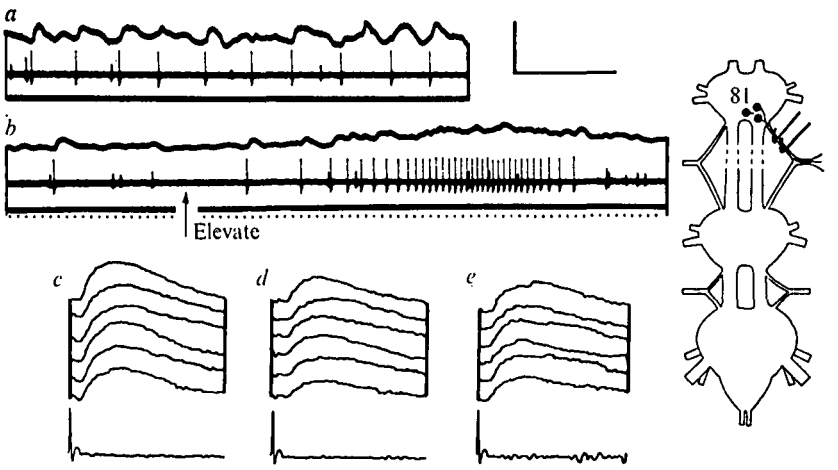

Fig. 11. The forewing stretch receptor synapses upon ipsilateral dorsal longitudinal motoneurones of the forewing which have their somata in the prothoracic ganglion. (*a*) Each stretch receptor spike (second trace) causes an EPSP upon one of the motoneurones (first trace) when the wing is stationary or (*b*) when elevated (upwards on third trace). (*c–e*) Averaged records of the EPSP in three different motoneurones of the same locust. Records (*a*, *b*) are from the same locust, (*c–e*) from another. Calibration: vertical, (*a*) 6 mV, (*b*) 10 mV, wing movement 80°; horizontal (*a*, *b*) 200 msec, (*c–e*) 26 msec.

the IPSP and its slow rise time is consistent with this. It is therefore assumed that the excitatory synapses upon depressors are closer to the soma than are the inhibitory synapses upon elevators, but other explanations may be equally valid as nothing is known about the time constants of various parts of a motoneurone or about the action of the transmitter. The IPSP is probably chemically mediated because an applied depolarization increases its amplitude and a hyperpolarization decreases it. In a saline containing 20 mM-Mg^{2+} the amplitude of the IPSP declines gradually but recovers upon return to the normal saline. The decline occurs at about the same rate as that of the EPSP in a first basalar motoneurone recorded at the same time. No effect was observed on the resting potential of either motoneurone.

In most recordings from elevator motoneurones the IPSP caused by the stretch receptor is revealed only after averaging (Fig. 12*b*, *e*) but the consistency of the averaged waveform implies that the IPSP follows each spike with a constant latency. The ability of the IPSP to follow spikes at high frequency is more difficult to assess because the summed IPSPs rapidly approach their reversal potentials. Each stimulus at a frequency of 50 Hz to the branch of the stretch receptor in N6 is followed by an IPSP in the first tergosternal motoneurone. The amplitude of the IPSP as recorded in the soma may be small, but the conductance change which underlies it is effective at the spike initiating zone as shown by the following observation. One of the three first posterior tergocoxal motoneurones may sometimes spike tonically. Elevation of the wing produces an increase in the frequency of spikes of the stretch receptor and IPSPs upon the motoneurone which abolish its spikes (Fig. 12*d*). When the wing is returned to its original position the motoneurone spikes resume.

No connexions of a forewing stretch receptor have been found upon contralateral pro or mesothoracic motoneurones. An EPSP in the first basalar motoneurone results

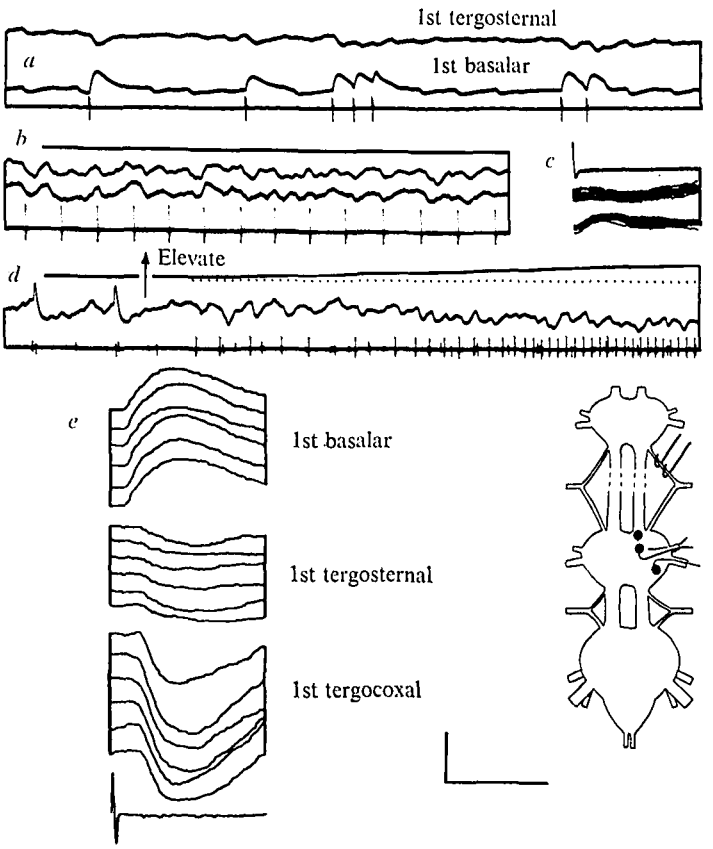

Fig. 12. The forewing stretch receptor input to ipsilateral mesothoracic elevator motoneurones is inhibitory. (*a*) The first tergosternal motoneurone (first trace) has IPSPs coincident with EPSPs recorded in the first basalar motoneurone (second trace) and caused by the spike of the stretch receptor (third trace). An IPSP follows each spike. (*b*) IPSPs occur in both the first posterior tergocoxal (second trace) and the first tergosternal (third trace). (*c*) Multiple sweeps triggered from the spike of the stretch receptor show the constancy of the latency to the IPSP in a first posterior tergocoxal (second trace) and the EPSP in the first basalar motoneurone (third trace). (*d*) Elevation of the wing (first trace) causes an increase in the frequency of stretch receptor spikes and abolishes spikes in a posterior tergocoxal motoneurone. (*e*) Averaged PSPs from the first basalar, first tergosternal and a first posterior tergocoxal motoneurone of the same locust. Calibration: vertical, voltage (*a*) first trace 5 mV, second trace 10 mV, (*b*, *c*) 5 mV, (*d*) 10 mV, wing movement 80°; horizontal (*a*, *b*, *d*) 200 msec, (*c*) 20 msec, (*e*) 26 msec.

from a spike in the ipsilateral stretch receptor and never from a contralateral one (Fig. 13*a*). Elevation of a forewing to give a high frequency of spikes of the stretch receptor has no effect on the membrane potential of the contralateral first basalar motoneurone (Fig. 13*b*). Averaging the waveforms in any of the elevator or depressor motoneurones has failed to reveal a potential linked to the spike of a contralateral forewing stretch receptor (Fig. 13*c–e*). Anatomy shows that neither the motoneurones nor the stretch receptor typically have contralateral branches. The forewing stretch receptor would thus appear to synapse only upon ipsilateral motoneurones, and these connexions are summarized in Table 1. Effects upon motoneurones of leg muscles, such as the flexor and extensor tibiae, levator and depressor tarsus, although sought, have not been found.

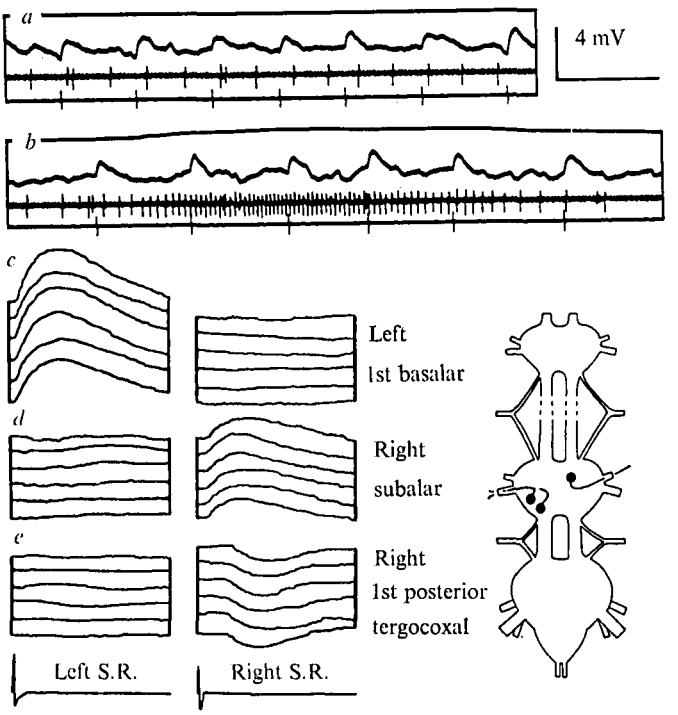

Fig. 13. The forewing stretch receptor does not synapse upon contralateral mesothoracic motoneurones. (*a*) The stretch receptor ipsilateral (fourth trace) or contralateral (third trace) to the impaled first basalar motoneurone (second trace) spike tonically when the forewings are held stationary (first trace), but only the ipsilateral stretch receptor evokes EPSPs. (*b*) Elevation of the contralateral wing causes a high frequency of spikes in its own stretch receptor but no EPSPs in the motoneurone. (*c–e*) Averaged PSPs from the left first basalar (*c*), a right subalar (*d*) and a right first posterior tergocoxal motoneurone (*e*) of the same locust. The left-hand column shows potentials linked to the spike of the left forewing stretch receptor, the right hand column to that of the right stretch receptor. There are no contralateral effects. Calibration, horizontal: (*a*, *b*) 200 msec, (*c–e*) 26 msec.

Connexions of a hindwing stretch receptor with motoneurones

The connexions which a hindwing stretch receptor makes with ipsilateral metathoracic motoneurones are similar to those which a forewing receptor makes with mesothoracic motoneurones; EPSPs are produced upon depressor and IPSPs upon elevator motoneurones (Fig. 14). The EPSP upon the first basalar motoneurone of the hindwing is 3–4 mV in amplitude at a repetition rate of less than 0·5 Hz. When the wing is elevated and the frequency of stretch receptor spikes increases, the EPSPs sum and decrease in amplitude. An applied d.c. depolarization of the soma reduces the amplitude of the EPSP and a hyperpolarization increases it, which is again consistent with it being chemically mediated. The EPSP follows each spike of the ipsilateral, hindwing stretch receptor with a constant latency of about 1 msec as measured from the entry of the spike into the ganglion and the appearance of the EPSP in the soma (Fig. 14*b*). These properties are consistent with the connexion being monosynaptic. The EPSP upon the second basalar or upon a subalar motoneurone has similar properties (Fig. 14*d*, *e*). IPSPs upon elevator motoneurones can usually be revealed only after averaging (Fig. 14*f*, *g*), and follow a spike with a longer latency and have

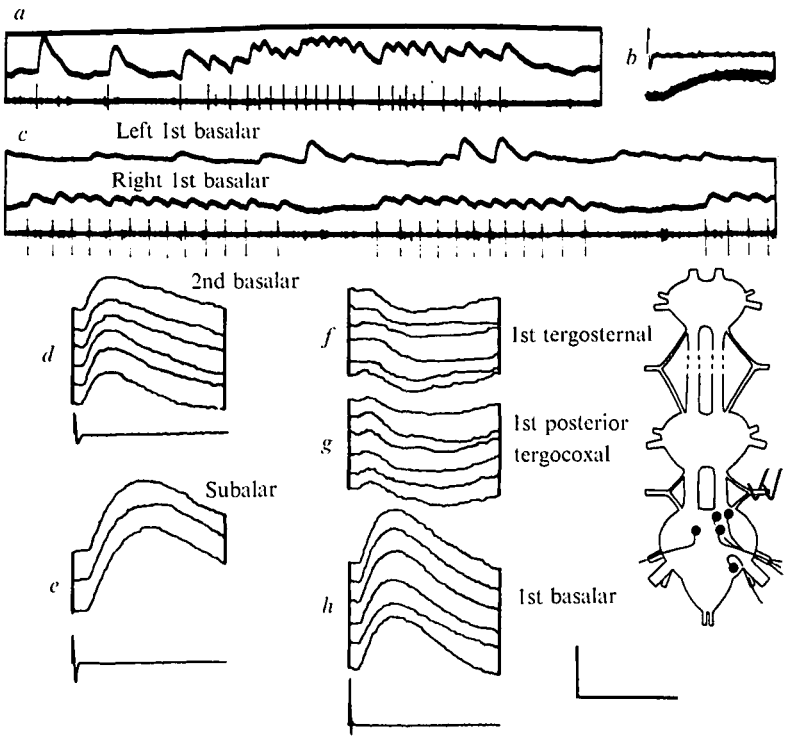

Fig. 14. The connexions of the *hindwing stretch* receptor with ipsilateral metathoracic motoneurones. (*a*) Each spike of the stretch receptor (third trace) causes EPSPs in the first basalar motoneurone (second trace) upon elevation of the wing (first trace). (*b*) The EPSPs follow with a constant latency as revealed in multiple sweeps triggered from the spike of the stretch receptor. (*c*) The left (first trace) and right (second trace) first basalar motoneurones recorded with the right stretch receptor (third trace). There are no contralateral effects. (*d–h*) Averaged PSPs in ipsilateral motoneurones. (*d*) A second basalar. (*e*) A subalar. (*f*) The tergosternal. (*g*) A first posterior tergocoxal. (*h*) The first basalar. (*f–h*) are from the same locust. Calibration: vertical (*a–c*) 4 mV, wing movement 80°; horizontal (*a*, *c*) 200 msec, (*b*) 16 msec, (*d–h*) 26 msec.

a smaller amplitude. From the averaged waveforms it can be inferred that the IPSPs follow each spike with a constant latency and that each has a similar amplitude.

No connexions of a hindwing stretch receptor with contralateral metathoracic motoneurones have been revealed even when their waveforms were averaged. For example, the stretch receptor of the right hindwing causes clear EPSPs upon the right first basalar motoneurone but has no effect upon the left first basalar motoneurone recorded simultaneously (Fig. 14*c*). Anatomy fails to reveal any contralateral branches of motoneurones or of the stretch receptors. Those connexions of a hindwing stretch receptor found upon metathoracic motoneurones are summarized in Table 1. Effects upon the following motoneurones of the leg muscles have not been found although sought; fast and slow extensor tibiae, two fast and one slow flexor tibiae, a slow levator and a slow depressor tarsus.

Intergangliонic connexions

Four of the five motoneurones innervating the dorsal longitudinal muscle of the hindwing have their somata in the mesothoracic ganglion (Neville, 1963). Elevation of the ipsilateral forewing causes EPSPs upon two of these motoneurones impaled

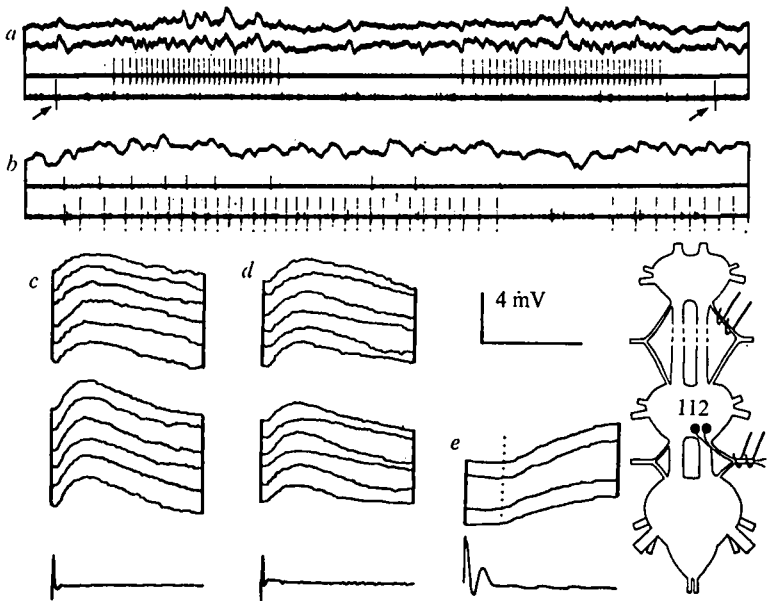

Fig. 15. Fore- and hindwing stretch receptors synapse upon ipsilateral dorsal longitudinal motoneurones of the mesothorax. (*a*) Two ipsilateral motoneurones are impaled together (first and second traces) and the *forewing* is elevated causing spikes in its stretch receptor (third trace) but not in that of the hindwing (fourth trace). EPSPs are evoked in both motoneurones. Arrows indicate two spikes of the hindwing stretch receptor which cause EPSPs upon both neurones. (*b*) A different locust in which only one motoneurone is impaled and in which the *hindwing* is moved. EPSPs can be distinguished after some of the spikes. (*c–e*) Averaged records showing clear EPSPs following the spike of the fore (*c*) or hindwing (*d*) stretch receptor. Two motoneurones were recorded simultaneously. (*e*) A dorsal longitudinal (upper two traces) and the ipsilateral metathoracic first basalar motoneurone (middle two traces) recorded with the hindwing stretch receptor (lower trace). Note the delay between the EPSP in the two motoneurones. Calibration, horizontal: (*a*) 400 msec, (*b*) 200 msec, (*c–d*) 26 msec, (*e*) 6 msec.

at the same time (Fig. 15*a*), which can be seen more clearly after averaging (Fig. 15*c*). The movement of the forewing does not cause spikes in the stretch receptor of the ipsilateral hindwing recorded at the same time. Elevation of the hindwing which activates the stretch receptor of the hind but not the forewing also causes EPSPs upon the motoneurones (Fig. 15*b*), which again are more clearly seen after averaging (Fig. 15*d*). As a further check that both ipsilateral stretch receptors synapse upon these motoneurones, the EPSP from the hindwing stretch receptor was observed before and after cutting mesothoracic N1 to abolish afferents of the forewing. The converse experiment, abolishing input from the hindwing whilst observing the EPSP from the forewing, was performed in another locust and with the same result. Therefore, both fore- and hindwing stretch receptors synapse upon the ipsilateral dorsal longitudinal motoneurones of the hindwing. The EPSP follows the spike of the forewing stretch receptor with a latency of about 1·5 msec when measured in the usual way. Similarly, the latency of the EPSP from the spike of the hindwing stretch receptor is about 1·5 msec. The EPSP from the hindwing stretch receptor, nevertheless, occurs after that upon the hindwing first basalar motoneurone, presumably because of the different routes taken by the axon of that stretch receptor; it branches to enter the mesothoracic ganglion by N6 and the metathoracic by N1.

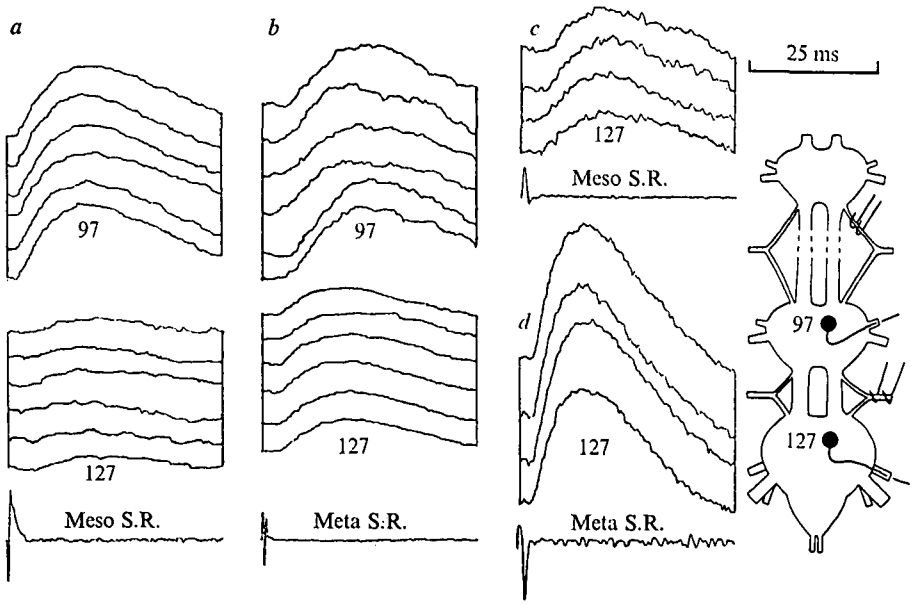

Fig. 16. Interganglionic connexions of the fore and hindwing stretch receptors. (*a*, *b*) Simultaneous recording from the mesothoracic (upper block traces in each) and metathoracic first basalar motoneurones on the same side of the body. The forewing (*a*) and the hindwing stretch receptor (*b*) cause EPSPs in both motoneurones. The EPSP caused by the stretch receptor of the different segment is displayed at 2·5 times the gain of the EPSP from the stretch receptor of the motoneurone's own segment. In (*c*, *d*) recordings at the same gain are made from the metathoracic first basalar in response to spikes of the ipsilateral fore (*c*) and hindwing (*d*) stretch receptors.

Other interganglionic connexions of a stretch receptor have been revealed only after averaging. The forewing stretch receptor has already been shown to synapse upon the mesothoracic first basalar motoneurone and the hindwing stretch receptor upon the metathoracic first basalar motoneurone; the EPSPs they produce are apparent with visual inspection alone (Figs. 8*a*, 14*a*). When recordings are made simultaneously from these two motoneurones on the same side of the locust, then each stretch receptor is seen to synapse upon both (Fig. 16*a*, *b*). The EPSP caused by the stretch receptor of the motoneurone's own segment is larger than that from the stretch receptor of the adjacent segment. For example, the EPSP from the hindwing stretch receptor upon the metathoracic first basalar motoneurone is almost four times the amplitude of that caused by the forewing stretch receptor (Fig. 16*c*, *d*). The potentials are recorded at the soma, however, which gives no indication of their likely effect at the spike initiating zone. The connexion between the forewing stretch receptor and the ipsilateral hindwing first basalar motoneurone is the only one so far revealed upon metathoracic motoneurones but the extensive anatomical projection within the metathoracic ganglion probably implies that more connexions are made. The hindwing stretch receptor, by contrast, synapses upon the subalar and the second basalar motoneurones of the forewing in addition to making those connexions already described (Table 1). No synapses have been revealed upon elevator motoneurones, upon motoneurones of leg muscles, or upon contralateral motoneurones of wing muscles.

Table 1 *The connexions of fore- and hindwing stretch receptors with ipilateral motoneurones innervating flight muscles*

Dashes indicate that no connexions were found, blanks that the observations were not made. No connexions from stretch receptors of the contralateral wings were found. The latency is measured from the time of entry of a stretch receptor spike into the ganglion of its own segment and the appearance of the PSP in the soma of the motoneurone. The latency for the dorsal longitudinal motoneurones is measured from the time when the spike enters the ganglion containing their somata.

			Stretch receptor input			
			Ipsilateral forewing		Ipsilateral hindwing	
Motoneurone	Muscle number	Nerve trunk	Potential	Latency (msec)	Potential	Latency (msec)
Prothoracic ganglion						
Dorsal longitudinal						
1	81	N6	EPSP	1·5–2·0	—	—
2	81	N6	EPSP	1·5–2·0	—	—
3	81	N6	EPSP	1·5–2·0	—	—
Mesothoracic ganglion						
depressors						
Dorsal longitudinal						
1	112	N6	EPSP	1·5	EPSP	1·5
2	112	N6	EPSP	1·5	EPSP	1·5
3	112	N6	EPSP	1·5	EPSP	1·5
First basalar	97	N3A	EPSP	1·0	EPSP	2·0–2·5
Second basalar	98	N3A	EPSP	1·0–1·5	EPSP	2·0–2·5
Subalar						
1	99	N4	EPSP	1·5–2·0	EPSP	1·5–2·0
2	99	N4	EPSP	1·5–2·0	EPSP	1·5–2·0
elevators						
First tergosternal	83	N3A	IPSP	4–5	—	—
Second tergosternal	84	N3A	IPSP	4–5	—	—
Anterior tergocoxal	89	N3A	IPSP	4–5	—	—
First posterior tergocoxal						
1	90	N4	IPSP	5–6	—	—
2	90	N4	IPSP	5–6	—	—
3	90	N4	IPSP	5–6		—
Metathoracic ganglion						
depressors						
First basalar	127	N3A	EPSP	3–4	EPSP	1·0
Second basalar	128	N3A	—	—	EPSP	1·0–1·5
Subalar						
1	129	N4	—	—	EPSP	1·5–2·0
2	129	N4	—	—	EPSP	1·5–2·0
elevators						
First tergosternal	113	N3A	—	—	IPSP	4–5
Anterior tergocoxal	118	N3A	—	—	IPSP	4–5
First posterior tergocoxal						
1	119	N4	—	—	IPSP	5–6
2	119	N4	—	—	IPSP	5–6

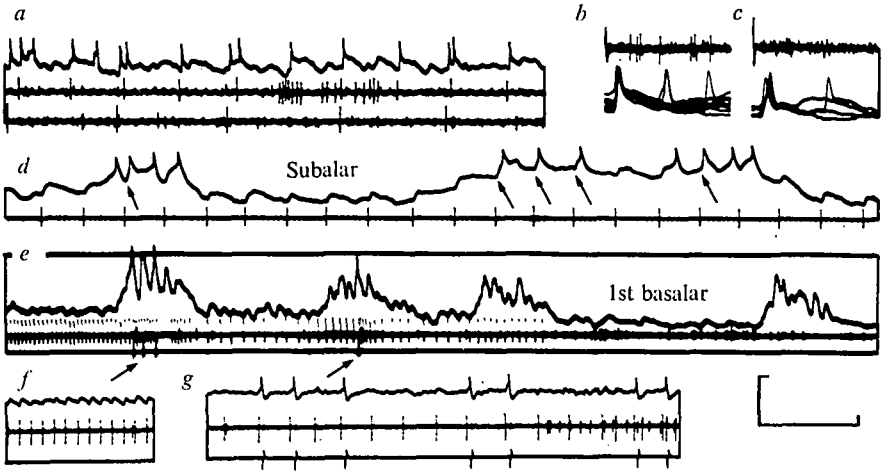

Fig. 17. The input from a stretch receptor can influence the production of spikes in central neurones. (*a–c*) An unidentified neurone in the mesothoracic ganglion in which the ipsilateral fore (second trace) or hindwing (third trace) stretch receptor evoke spikes. Multiple sweeps triggered from the fore (*b*) or hindwing (*c*) stretch receptor show the phase locking of the spikes. (*d*) EPSPs from the hindwing stretch receptor sum with other depolarizing inputs to evoke spikes (arrows) in a metathoracic subalar motoneurone. (*e*) The metathoracic first basalar motoneurone (second trace) and the muscle (fourth trace) during an imposed elevation of the contralateral forewing (first trace). Each movement causes a depolarization of the motoneurone but its effectiveness in producing spikes (arrows) depends upon the frequency of stretch receptor spikes (third trace) of the ipsilateral hindwing. (*f*) Spikes of the forewing stretch receptor cause EPSPs upon the ipsilateral mesothoracic first basalar motoneurone. (*g*) If a d.c. depolarization of 10 mV is applied to the soma then some spikes of the stretch receptor (second trace) evoke spikes recorded in the soma (first trace) and muscle (third trace). Each line of records is from a different locust. Calibration: vertical (*a–c*) 25 mV, (*d, f, g*) 20 mV, (*e*) 10 mV, wing movement 80°; horizontal (*a*) 200 ms, (*b, c*) 40 msec, (*d–g*) 400 msec.

Synaptic input from a stretch receptor influences the production of spikes in motoneurones

Elevation of a wing to produce a high frequency of stretch receptor spikes typically fails to evoke spikes in depressor motoneurones (Fig. 10*a*, 11*b*). On only two occasions was a single spike produced in a first basalar motoneurone of the forewing when spikes of the forewing stretch receptor reached a frequency of 100 Hz. The typical response to a rhythmical movement of a wing is a rhythmical, subthreshold depolarization of depressor motoneurones and hyperpolarization of elevators. The locust is not flying and the general level of sensory-input is low, so that the central excitatory state of the nervous system may be expected to be low. Under certain conditions, however, the stretch receptor can influence the production of spikes in central neurones. For example, an unidentified mesothoracic neurone, with its soma close to those of the dorsal longitudinal motoneurones, spikes tonically, but the EPSPs which it receives from both the ipsilateral fore- and hindwing stretch receptors lead directly to additional spikes (Fig. 17*a–c*). In other motoneurones the input from a stretch receptor must sum with other inputs before spikes can be evoked. For example, a hindwing stretch receptor causes EPSPs upon the ipsilateral, metathoracic subalar motoneurones which sum with a rhythmic, depolarizing synaptic input associated with ventilation to cause spikes (Fig. 17*d*).

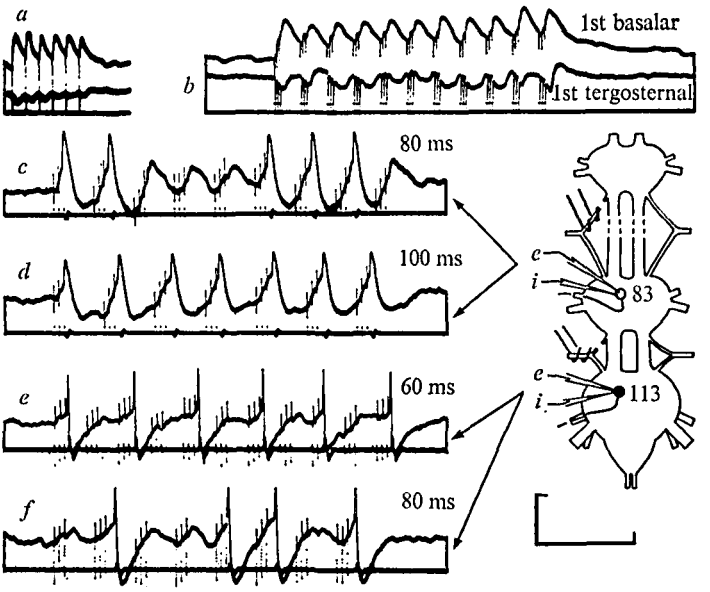

Fig. 18. Simulation of the pattern in which the stretch receptor is activated during flight showing that it could influence the production of depressor motoneurone spikes at each wingbeat. (*a*, *b*) Reciprocal inputs to the mesothoracic first basalar (first trace) and first tergosternal (second trace) motoneurones when the axonal branch of the forewing stretch receptor in N6 of the prothoracic ganglion is stimulated. (*a*) A PSP follows each stimulus repeated every 50 msec. (*b*) Bursts of three stimuli at 200 Hz repeated every 50 msec (the approximate wingbeat period) cause a rhythmic depolarization of the depressor and a hyperpolarization of the elevator, but no spikes are evoked. (*c–f*) Two electrodes are inserted into the first basalar of the mesothorax (*a*, *e*) or of the metathorax (*e*, *f*), one to pass a d.c. depolarizing current, the other to record voltage. When the soma is depolarized and the stretch receptor stimulated with a burst of 3 pulses at 100 Hz repeated at the intervals indicated, spikes are evoked on successive cycles. Muscle spikes are recorded on the second trace. Calibration: vertical (*a*) 25 mV, (*b*) first trace 30 mV, second trace 20 mV, (*c–f*) 10 mV; horizontal (*a*) 400 msec, (*b–f*) 200 msec.

The metathoracic first basalar motoneurone is depolarized by an elevation of the contralateral forewing, an effect which is not mediated by the stretch receptor of that wing. The effectiveness of this depolarization in evoking spikes in motoneurones is influenced by the input from the stretch receptor of the ipsilateral hindwing, whose frequency of spikes can be controlled by holding the hindwing at any desired elevation. Movement of the contralateral forewing evokes spikes only when there is a high frequency of spikes from the ipsilateral hindwing stretch receptor (Fig. 17*e*). Without the input from the hindwing, elevation of the contralateral forewing merely produces a subthreshold depolarization.

The forewing stretch receptor causes EPSPs upon the ipsilateral, mesothoracic first basalar motoneurone (Fig. 17*f*). If a second microelectrode is used to inject a d.c. current and depolarize the soma by 10 mV, then some spikes of the stretch receptor evoke spikes in the motoneurone (Fig. 17*g*). This shows that the EPSP from the stretch receptor has access to the spike initiating zone of the motoneurone.

It must now be asked whether the rhythmic input which a stretch receptor provides during flight could influence the production of spikes at each wingbeat. It is conceivable that the synapses of the stretch receptor are closer to the soma than the spike initiating site so that the membrane of the motoneurone then could act as a low-pass filter,

smoothing the rhythmic depolarization to a tonic one. The axonal branch of the forewing stretch receptor was stimulated in N6 of the prothoracic ganglion after cutting this centrally and cutting N1 peripheral to its bifurcation into N1 and N6. By this procedure it is believed that the only sensory axon stimulated will be that of the stretch receptor. Single stimuli repeated at intervals of 50 ms, which is the approximate wingbeat period during flight, cause EPSPs upon the first basalar (depressor) and IPSPs upon the first tergosternal (elevator) motoneurones (Fig. 18*a*). During flight, the stretch receptor normally spikes a few times at each elevation and to simulate this pattern, bursts of three stimuli at a frequency of 100–200 Hz were repeated at 50 ms intervals. As recorded in the soma, the stimuli cause a rhythmic depolarization of the depressor and a hyperpolarization of the elevator but no spikes are evoked (Fig. 18*b*). Two electrodes were then inserted into the soma of the first basalar motoneurone of the forewing, one to record voltage, the other to apply a d.c. depolarization. Bursts of stimuli repeated at 80 or 100 msec intervals cause spikes on successive cycles when the soma is depolarized by 10 mV (Fig. 18*c*, *d*). When the applied depolarization is reduced, the stimuli take several cycles to depolarize the motoneurone beyond its spike threshold but thereafter the spikes remain locked to the stimuli. The applied depolarization causes a large hyperpolarization after each spike which is not seen typically in spikes evoked by natural stimuli. This after-hyperpolarization often prevents the production of spikes on successive cycles when the bursts of stimuli are repeated at intervals of less than 60 msec. At this frequency the motoneurone spikes become phase locked for a few cycles, then drift relative to the stimuli before locking once more. A similar result is obtained for the first basalar motoneurone of the metathorax, but the interpretation is more difficult because the axon of the stretch receptor cannot be stimulated separately; N6 of the mesothoracic ganglion is too short to use easily. The soma of the metathoracic first basalar motoneurone was depolarized by 30 mV so that the hyperpolarization following the spike is more pronounced (Fig. 18*e*,*f*). At a repeat period of 60 msec the bursts of stimuli evoke spikes on alternate cycles, and at 80 msec intervals on successive cycles. The rhythmic depolarization caused by a patterned stretch receptor input is thus effective in evoking spikes in motoneurones phase locked to each group of stimuli. The experiment shows that subthreshold rhythmic input from the stretch receptor need only sum with an unpatterned input in order to cause a rhythmic pattern of motoneurone spikes.

DISCUSSION

The single neurone of the stretch receptor synapses upon motoneurones which innervate power producing muscles of the wing, forming a monosynaptic negative feedback loop (Fig. 19); elevation of a wing excites the stretch receptor which in turn inhibits the elevator motoneurones and excites the depressors. When the loop is closed, as in a freely flying locust, the effect will be to terminate the elevation and initiate the next depression. The time taken for information to traverse the loop, from the initiation of a spike in the stretch receptor to one in a depressor muscle, is about 10 msec in an adult locust at 20 °C. The thoracic temperature of a flying locust is some 10–15 °C higher than this. Branches of the sensory neurone of the stretch

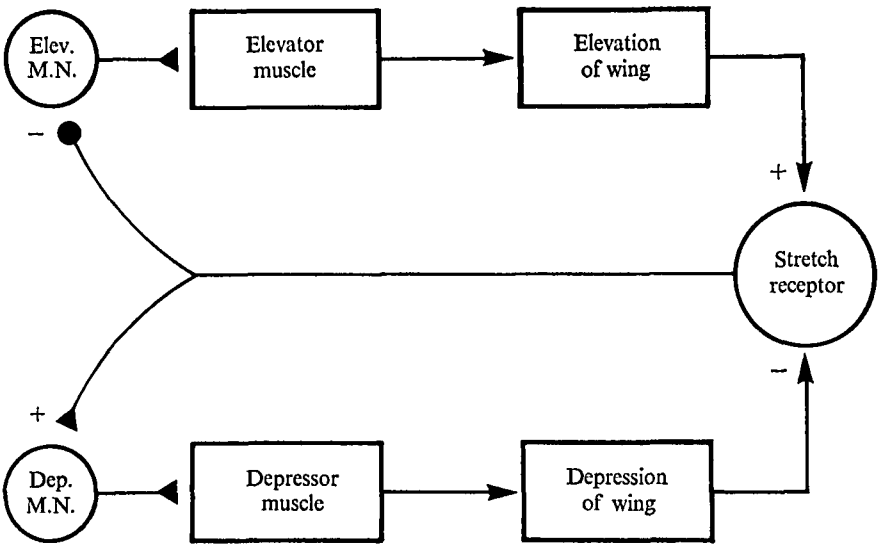

Fig. 19. The monosynaptic, negative feed-back loop between a stretch receptor and motoneurones of ipsilateral flight muscles.

receptor are thought to synapse directly upon the motoneurones for the following reasons:

(1) The EPSPs upon the depressor motoneurones follow each spike of the stretch receptor with a short and constant latency at frequencies of 125 Hz. The IPSPs upon elevators follow each spike at frequencies of at least 50 Hz but with a longer latency.

(2) Where tested, increasing the concentration of Mg^{2+} in the saline caused a gradual reduction in the amplitude of the EPSPs upon a depressor motoneurone and of the IPSPs upon an elevator. Both observations are consistent with the connexion being direct, but the demonstration of an anatomical synapse of the stretch receptor upon a motoneurone is lacking. The connexion of the stretch receptor with flight motoneurones is thus the first example of a two-neurone, reflex arc which has been revealed in an insect. Like a descending movement detector interneurone (Burrows & Rowell, 1973), the single neurone of the stretch receptor makes direct excitatory connexions with one set of motoneurones and inhibitory connexions with another.

Each stretch receptor connects only with ipsilateral motoneurones (Fig. 20). Physiologically no direct, contralateral connexions have been revealed, even with waveform averaging, and the anatomy typically shows that there are no contralateral branches of either sensory or motoneurones. The connexions of a stretch receptor nevertheless are extensive. A forewing stretch receptor synapses upon each of the ipsilateral mesothoracic flight motoneurones examined and in addition synapses upon motoneurones in the pro- and metathoracic ganglia. A single sensory neurone thus synapses upon motoneurones in three ganglia. The connexions which a particular stretch receptor makes are not confined to motoneurones innervating muscles of the same wing. A forewing stretch receptor synapses upon one at least of the hindwing motoneurones, and a hindwing stretch receptor synapses upon several motoneurones of the forewing. There is therefore a two-way exchange of sensory information between fore- and hindwings.

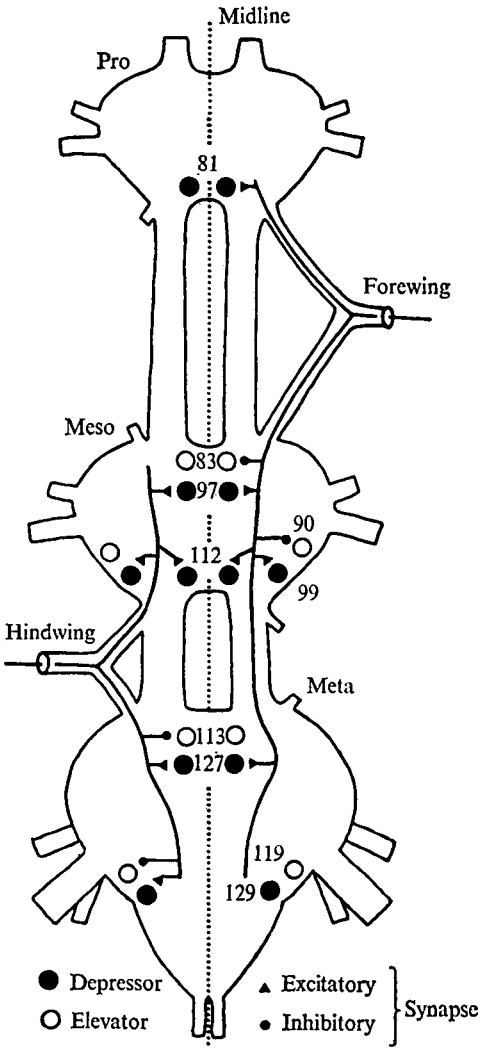

Fig. 20. The connexions of the left fore- and right hindwing stretch receptors with some flight motoneurones. Excitatory synapses are made upon depressor, inhibitory synapses upon elevator motoneurones. No contralateral connexions have been revealed. The simplified diagram includes only the following motoneurones: depressors, dorsal longitudinal (81 and 112), first basalar (97 and 127) and subalar (99 and 129); elevators, tergosternal (83 and 113) and first posterior tergocoxal (90 and 119). They can be identified by reference to Fig. 2 and Table 1.

The types of experiment reported here reveal functional connexions between neurones but give no information as to how these connexions operate in the flying locust. Some profit might, nevertheless, be gained from a consideration of what behavioural observations the connexions do or do not explain and what testable predictions can be made about their role in a flying locust. The difficulties in attempting such rationalizations of function are compounded by the fact that there is no detailed information about the activity of the stretch receptor in flight, only when the wing is forcibly moved (Gettrup, 1963; Pabst, 1965). Secondly, knowledge of the way in which the motor output is generated by the central nervous system is

scanty. Inferences about the central nervous system from observations made only in the periphery suggested that the pattern could be generated by connexions between the motoneurones themselves (Wilson, 1966). Now that a role for interneurones has been implicated in recordings from the central nervous system (Bentley, 1969; Burrows, 1973*a*), it would seem more likely that they are responsible for the generation of the pattern, although spikes of motoneurones may be able to influence them (Burrows, 1973*a*).

Indiscriminate removal of many sense organs at the base of three or four wings reduces the frequency of wingbeats by half. This effect was said to be mediated by the stretch receptors (Wilson & Gettrup, 1963), but I have argued that this interpretation is unjustified because they were not considering the stretch receptors alone. It is therefore unreasonable to expect that the connexions of the stretch receptors with motoneurones described here should explain their results. If the frequency of the wingbeats is to be reduced so drastically by lack of sensory input, then it must be inferred that afferents directly affect those neurones generating the rhythm. These are believed to be interneurones not motoneurones.

Forcibly moving a wing of a flying locust at a frequency slightly different to the natural one causes all wings to adopt the imposed rhythm (Wendler, 1972, 1974). For the sake of the following argument the input from the stretch receptor of the wing which is forcibly moved can be regarded as sinusoidal. We can propose that the input to the motoneurones from the pattern generating interneurones is also sinusoidal but because the frequency of the two sinewaves is different, they will inevitably drift. For the two inputs to become phase locked it must be supposed that sensory input, in addition to affecting the motoneurones, also influences the pattern generating interneurones. Alternatively, spikes of the motoneurones themselves could directly affect the interneurones. A third possibility can be proposed in which a large component of the input from the interneurones would be a tonic depolarizing one with only small cyclical fluctuations. Given this restraint, the input from the sense organs would then impose its own rhythm upon the motoneurones. The stretch receptor, however, connects only with ipsilateral motoneurones but an imposed movement of one wing affects the contralateral wings as well. There is no suggestion, however, that Wendler's result can be attributed to the stretch receptor alone. Forcibly moving a wing must activate many sense organs in addition to the stretch receptor, some of which have contralateral effects (cf. Fig. 17*e*) and at the same time opens any feedback loops which they form. Moreover, it is not suggested that all connexions of the stretch receptor have been revealed. For example, they may synapse upon interneurones which have bilateral outputs to motoneurones.

An effect which may be explained by the pathways shown is that described by Waldron (1967*a*). If the wings of a flying locust are held in an elevated position, elevator activity stops but the depressors continue to spike rhythmically. Waldron believed that with the wings in this position, input from the stretch receptor would be reduced but in a non-flying locust a maintained elevation of a wing causes a tonic discharge of the stretch receptor. This would inhibit elevator motoneurones but tonically excite the depressors which would continue to express the centrally determined pattern.

Stimulation of the axon of the stretch receptor in a dissected locust fails to evoke

spikes in motoneurones. If the stimuli are given in groups and another unpatterned input is added, spikes are evoked which are phase locked to each group of stimuli. When the interval between the groups is about the same as the wingbeat period, spikes usually occur only on alternate cycles. The hyperpolarization which follows a spike means that the next wave of EPSPs from the stretch receptor fails to reach the motoneurone's spike threshold. Increasing the level of the unpatterned input would allow spikes to be evoked on each cycle. It can be inferred that in the flying locust, input from the stretch receptor would influence the production of spikes in depressor motoneurones if these receive additional depolarizing inputs. It has been shown that the inhibitory connexions of the stretch receptor can abolish spikes in elevator motoneurones. The connexions of the stretch receptor with motoneurones can thus provide rapid cycle by cycle adjustments of the motor output. For example, an increased elevation of the wing, perhaps caused by an air current, will be signalled as an increased frequency of spikes of the stretch receptor whose central connexions will tend to bring the elevation to an end. The loop could thus act to stabilize the frequency of wingbeats by correcting the amplitude of each wingbeat. During flight the sinusoidal input from the interneurones will interact at the motoneurone level with the sinusoidal input from the stretch receptors. The two will sum, but slight variation in the time of arrival of the sensory input will determine when a particular motoneurone may spike. In this way some aspects of the co-ordination of both synergists and antagonists could be theoretically under the control of afferents. The increased variance in the intervals between antagonistic motoneurones upon the abolition of sensory input from three or more wings is evidence in favour of this inference (Kutsch, 1974).

The results imply that the feed-back loop formed by the stretch receptor must be regarded as an integral part of the flight system. It should be capable of reinforcing the centrally determined rhythm, a role already implicated for afferents in the endogenously controlled movements of lobster swimmerets (Davis, 1973), and provide fine control over some aspects of the pattern. Experiments carried out during free flight are now required so that the influence of the stretch receptor can be ascertained during normal behaviour.

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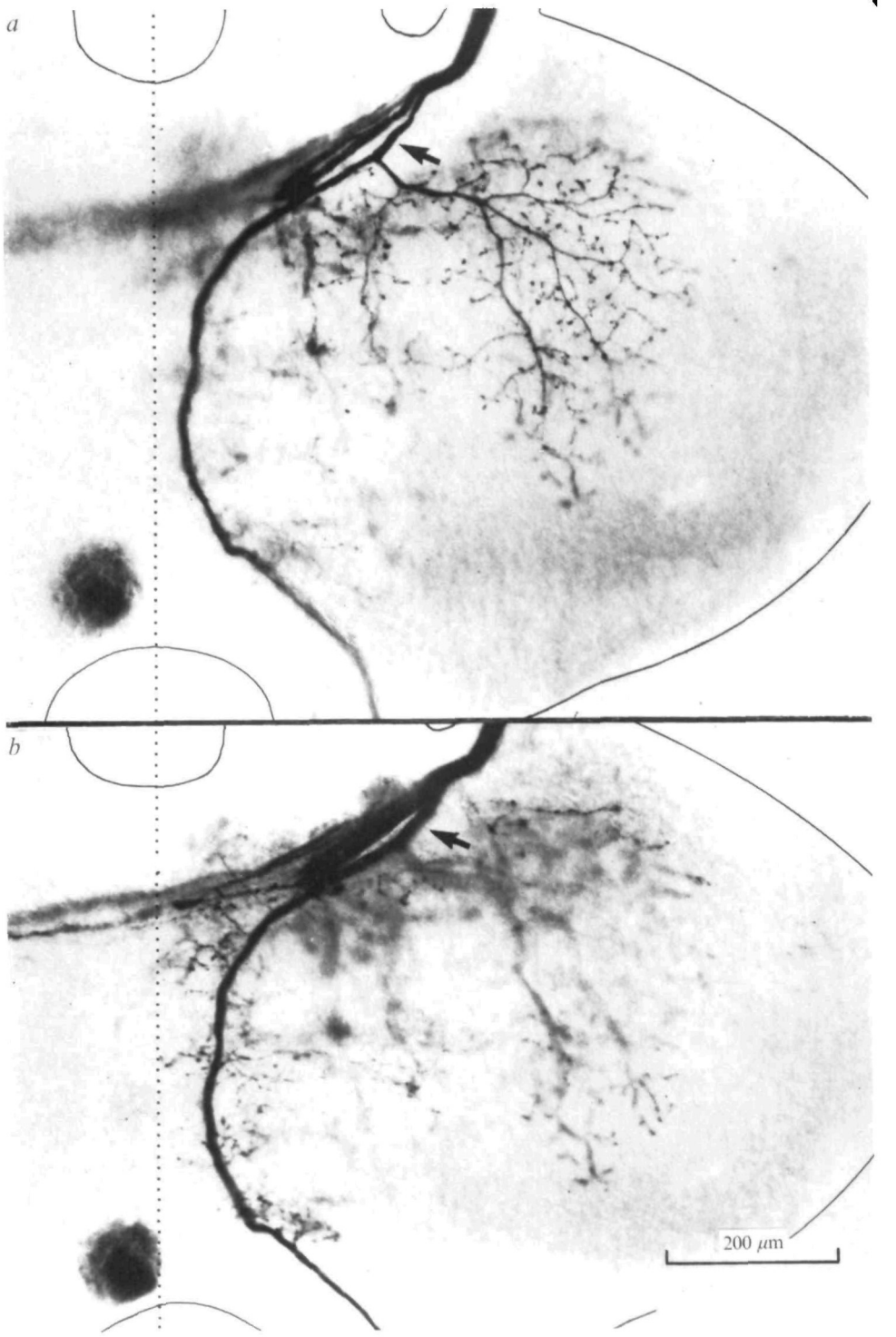
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EXPLANATION OF PLATE

Fig. 21, plate 1. Photographs of a whole mount of the mesothoracic ganglion at two focal planes (*a* and *b*) to show the projection of a forewing stretch receptor. The ganglion, whose outline is indicated, is viewed dorsally with anterior at the top. Cobaltous chloride was introduced into the ganglion from the cut ends of axons of nerve 1, so that neurones other than the stretch receptor are also filled; for example, the black circle out of focus at the posterior of the ganglion on the midline (dotted) is the soma of a neurone with its axon in N1. The axon of the stretch receptor can be seen entering the ganglion (arrows) and its branches unequivocally identified.