INTERNEURONAL ORGANIZATION IN THE FLIGHT SYSTEM OF THE LOCUST

R. M. ROBERTSON and K. G. PEARSON

Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2C6

Abstract—In this paper we describe the characteristics, connections, resetting properties and organization of some identified interneurones in the flight system of the locust. The major conclusions are that: (1) the flight rhythm is generated at the interneuronal level and the flight oscillator is not continuously active (2) the interneurones in the flight pattern generator are distributed within at least 6 segmental ganglia (three thoracic and three fused abdominal ganglia) and are not organized into two homologous groups for the separate control of the forewing and the hindwing (3) this distribution of flight interneurones has no obvious functional significance but could be a consequence of flight having evolved from a segmentally distributed motor behaviour (4) there may be a functional hierarchy among flight interneurones such that premotor interneurones are separate from those generating the rhythm.

INTRODUCTION

Insects today provide some attractive preparations for the study of the organization of interneurones and their role in various behaviours (Burrows and Siegler 1978, Pearson *et al.* 1980, Burrows 1980, 1982, Nolen and Hoy 1982, Pearson and Robertson 1981, Robertson and Pearson 1982a). This is due to the relative ease of recording intracellularly from interneurones and of identifying them structurally. Moreover patterned motor activity expressed by tethered and dissected insects can be reasonably complex thus allowing an intracellular analysis of interesting behaviours such as locust flight (Robertson and Pearson 1981, 1982a,b, 1983 Robertson *et al.* 1982).

Locust flight has been the subject of a great deal of study in the past and a large amount of information has gathered about many aspects of flight and its control (for reviews see Wilson 1968, Burrows 1976, 1977). It has been shown that the flight rhythm is centrally generated (Wilson 1961) and initial theories attributed the rhythmicity to connections and properties of motoneurones (Wilson and Waldron 1968). Notwithstanding the demonstration of delayed coupling between flight motoneurones (Burrows 1973, however see Robertson and Pearson 1982a for critique) it is now clear that connections and properties of interneurones are responsible for generating the basic flight rhythm (Burrows 1977, Robertson and Pearson 1982a). The challenge is to be able to understand how the pattern of motoneuronal activity is produced by interactions among interneurones. Until the development of our preparation (Robertson and Pearson 1982a) no flight interneurones had been identified although some information on interneurones was inferred from their common synaptic input to different motoneurones (Burrows 1975b,c). Using our preparation we have identified more than 50 interneurones that are phasically active with the flight rhythm; we have shown that 10 of these have constant short-latency connections (excitatory or inhibitory) with identified flight motoneurones; we have further shown that some other interneurones can reset the flight rhythm and thus are members of the interneuronal network generating the flight rhythm (Robertson and Pearson 1982a,b, 1983, Robertson *et al.* 1982). In addition some flight premotor interneurones receive direct connections from the forewing stretch receptor indicating a role for interneurones at a reflexive, compensatory level as well as at the central generating level (Pearson *et al.* 1983). What follows is a brief review of what we now know about interneurones in the locust flight system.

INTERNEURONAL PROPERTIES

Our technique has been to record intracellularly from the neuropile processes of motoneurones and interneurones in the thoracic ganglia using Lucifer Yellow filled glass microelectrodes (Robertson and Pearson 1982a for details of the preparation and procedure). In this way it is possible to determine the structure of particular neurones as well as their activity during flight sequences induced by blowing air on the head of the animal. The search for flight neurones is aided by the fact that the flight neuropile is predominantly confined to a layer approximately 50 μm thick just beneath the dorsal ganglionic sheath.

Figure 1 shows typical recordings from flight motoneurones (Fig. 1a) and flight interneurones (Fig. 1b,c) during flight sequences. It is usually possible to differentiate motoneuronal and interneuronal penetrations solely on the basis of the activity recorded. Motoneurones have a larger resting membrane potential and a greater spike amplitude which is presumably a reflection of the larger diameter of motoneuronal processes and thus a smaller electrotonic distance from the point of penetration to the spike
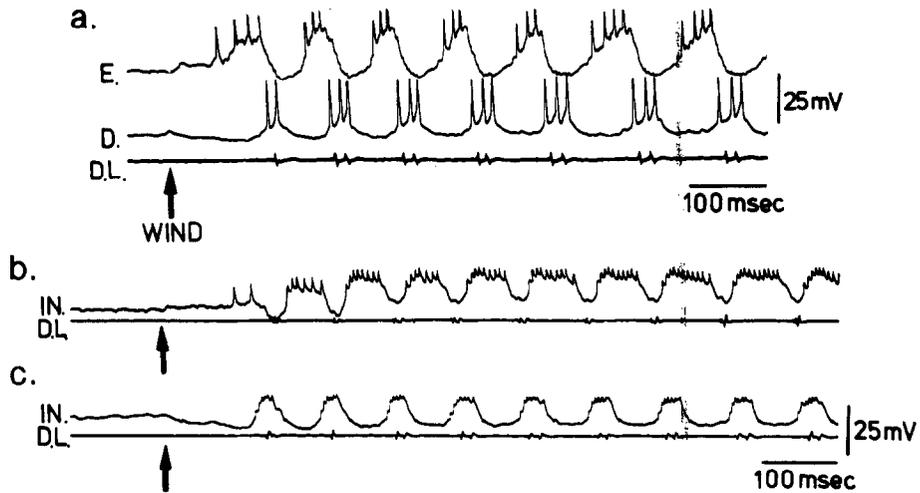
Fig. 1. Intracellular recordings from flight motoneurones and interneurones. (a) Simultaneous recordings from unidentified elevator (E) and depressor (D) motoneurones during a flight sequence. (b) Recording from an elevator-type interneurone (IN). (c) Recording from a depressor-type interneurone (IN). b and c are from different experiments. In this and all subsequent figures the arrow under the trace indicates the time of wind onset and the DL trace is an electromyographic recording from the dorsal longitudinal muscles to monitor the time of depressor activity during flight sequences.
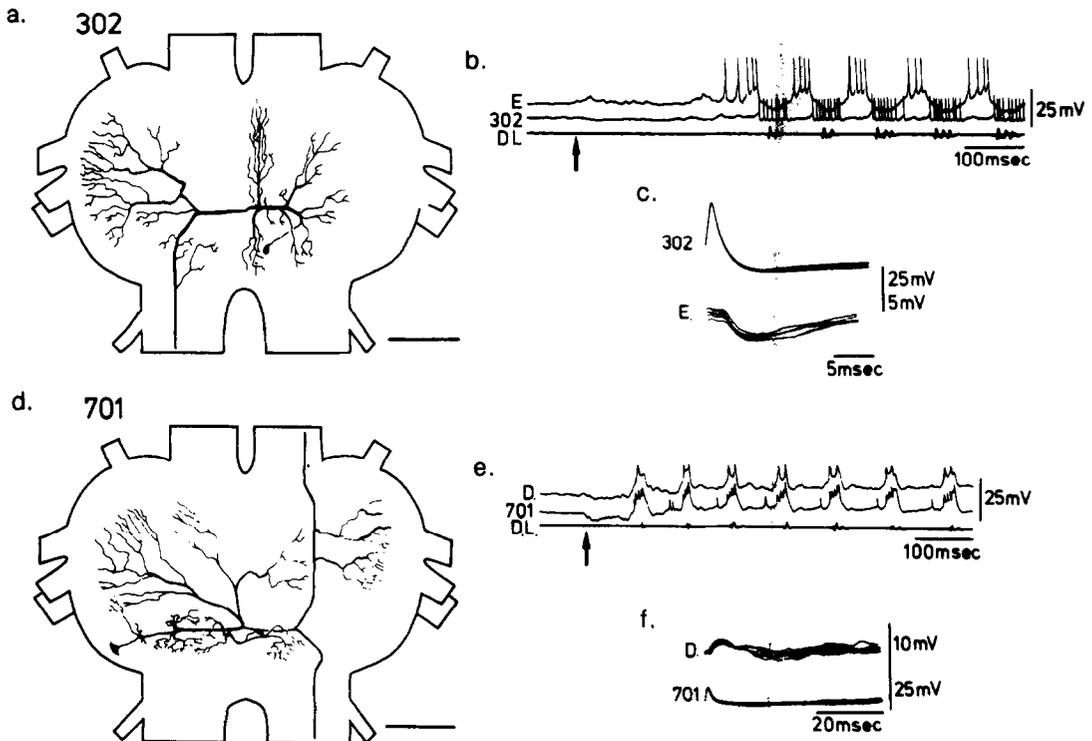

Fig. 2. Interneurones with direct connections to flight motoneurones. (a) Structure of an interneurone (302) in the mesothoracic ganglion. (b) Simultaneous intracellular recordings from 302 and a tergosternal elevator motoneurone (E) in the metathoracic ganglion. 302 fires high frequency bursts of spikes in phase with depressor activity. (c) Successive oscilloscope sweeps triggered by the rising phase of the action potential of 302 show that each spike in 302 is followed after a short and constant latency by an inhibitory postsynaptic potential in an elevator motoneurone. c is from a different preparation to b. (d) Structure of an interneurone (701) in the mesothoracic ganglion. (e) 701 fires in phase with depressor motoneurones during flight. (f) Each spike in 701 is followed after a short and constant latency by an excitatory postsynaptic potential in a mesothoracic dorsal longitudinal motoneurone of the hindwing (D in both e and f). The scale bars in a and d and all subsequent neurone drawings indicate 200 μm.

initiation zone. In addition motoneurones have a lower intraburst spike frequency producing 2–3 spikes per burst at 60–80 impulses/s compared with 5–10 spikes per burst at 200–250 impulses/s for interneurones (compare Fig. 1a with Fig. 1b,c). There is no evidence that any flight neurone is continuously active with the flight rhythm. That is to say the flight oscillator is not continuously active as previously suggested (Burrows 1975b).

The flight motor pattern in dissected preparations consists of alternating bursts of activity in elevator and depressor motoneurones with the latency from depressor to elevator being longer than the latency from elevator to depressor (Wilson and Weis-Fogh 1962, Waldron 1967, Wilson 1968) and the wingbeat frequency is about half normal (i.e. about 10 Hz). Burst activity in motoneurones is produced by large oscillations in membrane potential (10–25 mV) consisting of alternating excitatory and inhibitory phasic synaptic potentials (Robertson and Pearson 1982a). Corresponding to this pattern of motoneuronal activity most flight interneurones discharge maximally either in phase with elevator activity or in phase with depressor activity. Interestingly the elevator-type interneurones, like elevator motoneurones, tend to be depolarized by wind onset whereas the depressor-type

interneurones, like depressor motoneurones, tend to be hyperpolarized by wind onset (Fig. 1). A number of other physiological features are shared by corresponding types of interneurones and motoneurones. The forewing stretch receptor which makes direct connections to depressor motoneurones (Burrows 1975a) also makes direct connections to flight interneurones that are active at the depressor phase (Pearson et al. 1983, e.g. the interneurones shown in Fig. 2 receive direct stretch receptor input), and most elevator-type interneurones and motoneurones show similar depolarizing responses to auditory (a hissing sound) and visual (a decrease in the ambient light intensity) stimuli whereas most depressor-type neurones show hyperpolarizing responses to the same stimuli.

The large number of interneurones we found in the flight system ($n = 61$ including serial homologues) meant that we had to devise a numbering scheme to refer to them. This scheme is described in detail elsewhere (Robertson and Pearson 1983) but, in short, the first numeral of three is assigned on the basis of the axon pathway and the subsequent numerals are assigned arbitrarily. For example 201 refers to an interneurone with an axon that descends the cord in the connective ipsilateral to the cell soma. In the
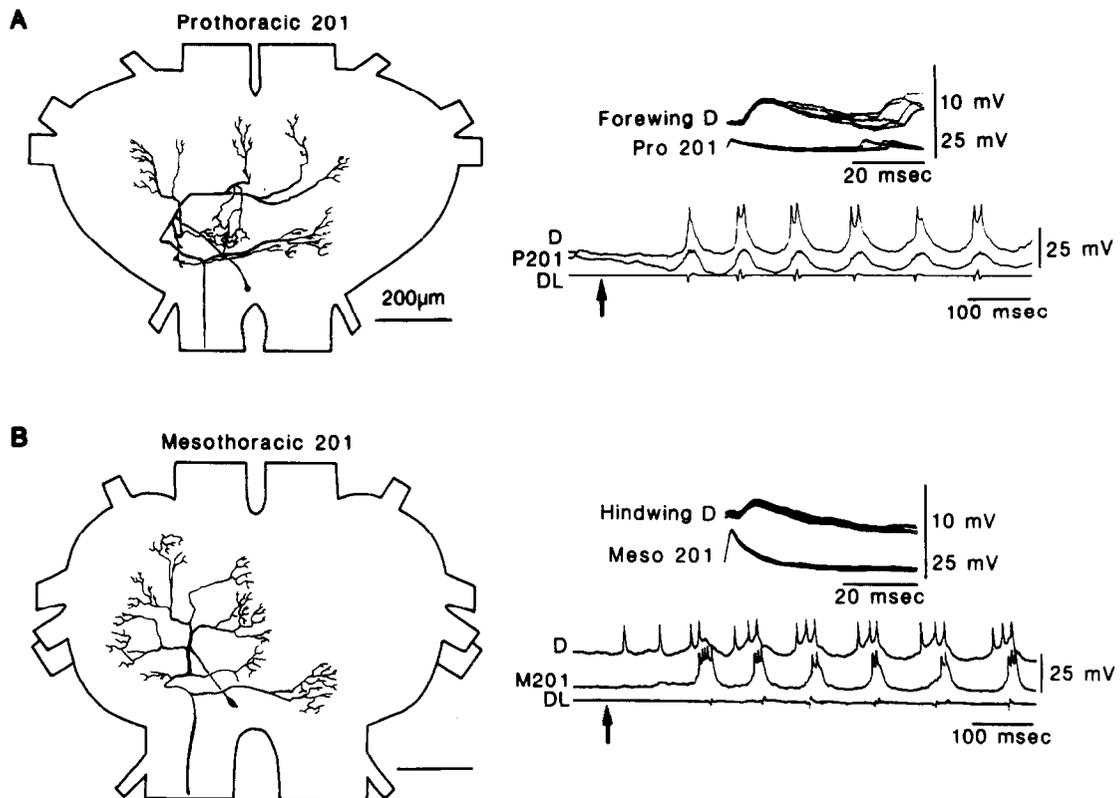

Fig. 3. Homologous interneurones in the prothoracic and mesothoracic ganglia which separately excite depressor motoneurones innervating forewing and hindwing muscles. (a) An interneurone in the prothoracic ganglion (201) which fires in phase with depressor motoneurones and has a constant latency excitatory connection with a forewing 1st basalar motoneurone in the mesothoracic ganglion (D) (b) The homologous interneurone in the mesothoracic ganglion which fires in phase with depressor motoneurones and has a constant latency excitatory connection with a hindwing 1st basalar motoneurone in the metathoracic ganglion (D). Note that there is a slight difference in structure: 201 in the prothoracic ganglion has dendritic branches in the contralateral hemiganglion.

remainder of this paper we will describe the properties of identified interneurones and refer to them by the number defined in Robertson and Pearson (1983).

INTERNEURONAL CONNECTIONS

One of our aims has been to identify the interneurones responsible for the membrane potential changes in the motoneurones. To do this we simultaneously recorded from motoneurones and interneurones. Interneurones with short constant latency connections to flight motoneurones can be found (Fig. 2) and the connections made by a single interneurone are either inhibitory (Fig. 2a–c) or excitatory (Fig. 2d–f) but not both.

The size of the post synaptic potentials recorded from motoneurones in response to single spikes of interneurones varies between 1 and 4 mV. Even taking into account facilitatory connections (e.g. Fig. 5b,c of Robertson and Pearson 1983) it is unlikely that any one interneurone is entirely responsible for the 10–25 mV oscillation in the membrane potential of motoneurones during flight sequences. This fits well with our observation that motoneurones are driven by sets of homologous interneurones (see below and Robertson and Pearson 1982a, Robertson et al. 1982) as well as several other interneurones (Robertson and Pearson 1983).

One of the questions that we hoped to answer was whether the strict serial homology seen at the periphery (two pairs of wings, two sets of wing muscles, two sets of thoracic motoneurones) is mirrored centrally; i.e. at the premotor or rhythm generator level. So far we have only one example of serially homologous flight interneurones which pattern, in part, activity in motoneurones to only the forewing or only the hindwing.

This is interneurone 201 (Fig. 3). We have based our conclusion that interneurone 201 in the prothoracic ganglion (premotor to forewing depressor motoneurones, Fig. 3a) is homologous to interneurone 201 in the mesothoracic ganglion (premotor to hindwing depressor motoneurones, Fig. 3b) on their structural similarities (soma position, major dendritic branching, pathway of the axon) and their physiological similarities (response to wind onset, phase of activity during flight, sign of connection, type of motoneurone driven). In most cases, however, the premotor interneurones we find (e.g. 302 and 701 in Fig. 2) have no known homologues in thoracic ganglia and the structure of their axonal branching indicates that they connect with motoneurones of both the forewing and the hindwing muscles. We observe a similar phenomenon for another interneurone (503) which connects directly with a premotor interneurone of a hindwing depressor mo-
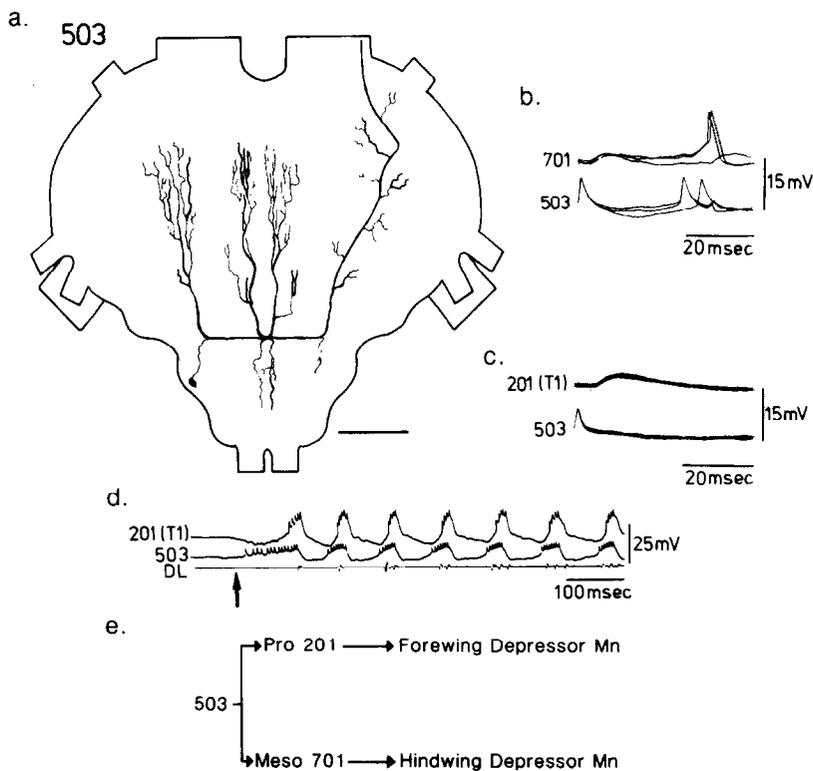

Fig. 4. Disynaptic connection from a single interneurone to depressor motoneurones innervating the hindwing and the forewing muscles. (a) Structure of an interneurone (503) in the metathoracic ganglionic mass. (b) 503 has a direct excitatory connection to interneurone 701 which connects to hindwing depressor motoneurones (see Fig. 2f). (c) 503 also has a direct excitatory connection to 201 in the prothoracic ganglion which connects to forewing depressor motoneurones (see Fig. 3a; note that P201 and 201(T1) are synonymous, T1 and P both refer to the prothoracic or 1st thoracic ganglion). (d) 503 fires in phase with depressor motoneurones. (e) Diagram of the pathway of connections from 503 to motoneurones of both the forewing and the hindwing.

toneurone (Fig. 4b and Fig. 2f) and also with a premotor interneurone of a forewing depressor motoneurone (Fig. 4c and Fig. 3a).

Thus this interneurone causes movements of both the forewing and the hindwing (Fig. 4e). Data of this kind indicate that rhythmical activity of forewing and hindwing motoneurones is largely generated from the same source. Thus the flight rhythm generator appears to be a single entity distributed among several segmental ganglia and operating as a unit. Support for this conclusion is that the interneurones which influence the timing of the rhythm are also *not* organized into two homologous systems for the separate control of forewing and hindwing.

INTERNEURONES WHICH AFFECT TIMING OF THE FLIGHT RHYTHM

The criteria used to establish that a neurone is a member of a flight rhythm generator are that it is phasically active with the rhythm and that transient perturbations of its activity affect the timing of the rhythm (Kristan *et al.* 1977, Weeks 1981, Robertson and Pearson 1983). We tested flight neurones by passing pulses of depolarizing current (up to 10 nA, 100 ms − 1 s) into their neuropile processes during flight activity. Stimulation of motoneurones never had an effect on the timing of the rhythm. By contrast a number of identified interneurones do reset the rhythm when depolarized. An example is shown in Fig. 5.

By passing depolarizing pulses of long duration into this interneurone the frequency of the flight rhythm was reduced for the duration of the stimulus. The frequency returned to normal immediately after the pulse (see e.g. Fig. 1c of Robertson *et al.* 1982). The reduction in the frequency was approximately linearly related to the magnitude of the injected current up to a value of 3 nA at which point flight was abolished for the duration of the current injection (Fig. 5b). Pulses shorter than an cycle length could reset the flight rhythm by delaying the subsequent burst (Fig. 5c).

To date we have identified 3 sets of interneurones that are members of the rhythm generator. Two of the sets are composed of 8 interneurones each (paired homologues in 4 segmental ganglia, see below) and the other exists as a bilateral pair of interneurones in the mesothoracic ganglion. This gives a total to date of 18 interneurones capable of influencing the timing of the rhythm. An interesting observation is that although we have tested all of the premotor interneurones so far identified ($n = 10$) none of them is able to reset or modulate the rhythm in any way. Conversely we have been unable to show that any of the rhythm generator interneurones make direct connections to flight motoneurones. Despite the negative nature of this data they do suggest a functional hierarchy of interneurones in the flight system such that separate sets of interneurones generate the rhythm and drive the motoneurones. Such an arrangement would certainly be useful in allowing
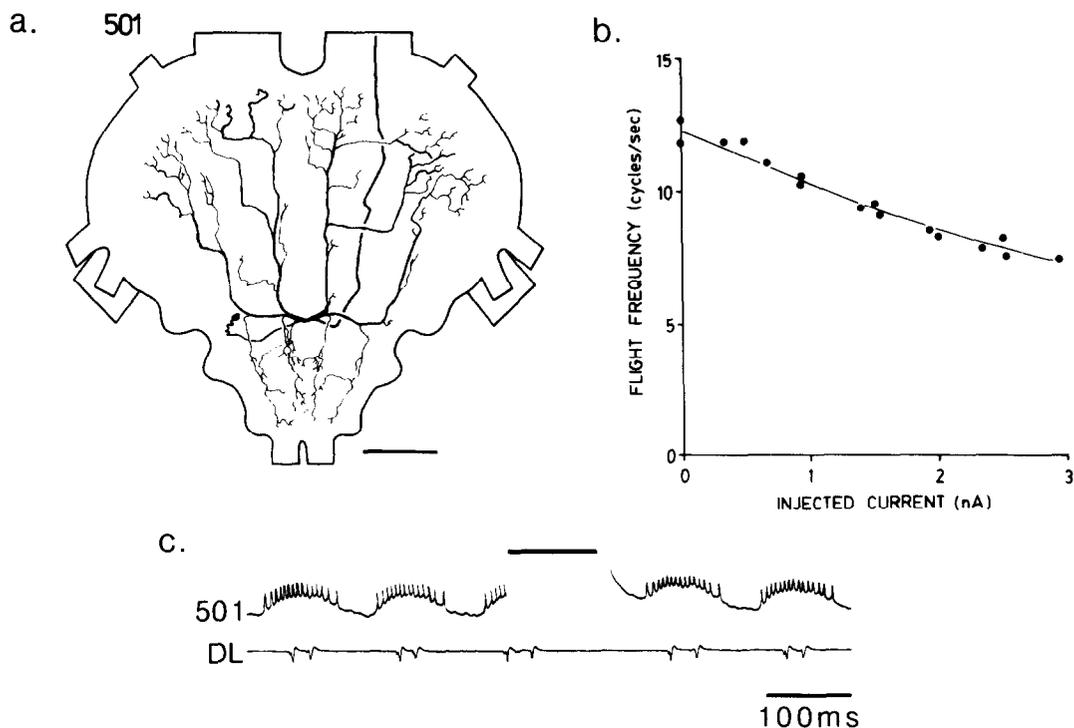

Fig. 5. Stimulation of a single interneurone affects the timing of the flight rhythm. (a) Structure of an inerneurone (501) in the metathoracic ganglionic mass. (b) Graph showing that the flight frequency is reduced by injecting increasing amounts of depolarizing current into 501 in another preparation. Flight was abolished with depolarizing currents greater than 3 nA. (c) Injection of 501 with depolarizing current pulses of short duration (100 ms, duration indicated by bar above the trace) could reset the flight rhythm by delaying the occurrence of the subsequent burst.

changes in the phasing of muscle activity without changes in wingbeat frequency as seen during most flight manoeuvres in the pitch and yaw planes (Wilson and Weis-Fogh 1962, Möhl and Zarnack 1977, Zarnack and Möhl 1977, Baker 1979, Simmons 1980).

ORGANIZATIONAL FEATURES OF FLIGHT INTERNEURONES

The metathoracic ganglionic mass in the locust is formed by a fusion of the metathoracic and the first three abdominal ganglia (Albrecht, 1953). Since the wing musculature is located in only the meso- and metathoracic segments we were surprised to find numerous flight interneurones located in the fused abdominal ganglia. The most interesting of these are sets which have homologues in each of these ganglia and the metathoracic ganglion (Fig. 6).

Individuals in two of these sets influence the timing of the rhythm (one being the interneurone of Fig. 5, the other is described in Robertson and Pearson 1983), individuals in one set connect directly to elevator motoneurones (Robertson and Pearson 1982a) and the members of the last set have disynaptic connections to depressor motoneurones (Fig. 4). None of these interneuronal sets have

specific control over the forewing or over the hindwing. It is important therefore to distinguish this sort of homology from the serial homology corresponding to separate forewing and hindwing control discussed earlier (Fig. 3).

What is the significance of serial homologues in the metathoracic and fused abdominal ganglia? We have argued (Robertson et al. 1982) that this organization has no unique functional advantage but instead reflects the evolutionary history of the flight system. Our anatomical and physiological data on identified flight interneurones in combination with the fossil evidence of Kukalová-Peck (1978) argue in favour of the pleural appendage theory for the evolutionary origin of the insect wing (Robertson et al. 1982). This theory proposes that insect wings originated from appendages which had homologues on all abdominal segments. These appendages were probably involved in a behaviour such as ventilation or swimming.

There is a corollary to our finding that the organization of interneurones is related to the evolutionary origin of the wings; namely that interneuronal systems may not be optimally constructed for a given function i.e. they may not contain the minimum numbers of neurones, connections etc. In the study of neural function it is common to attribute functional characteristics to various anatomical or
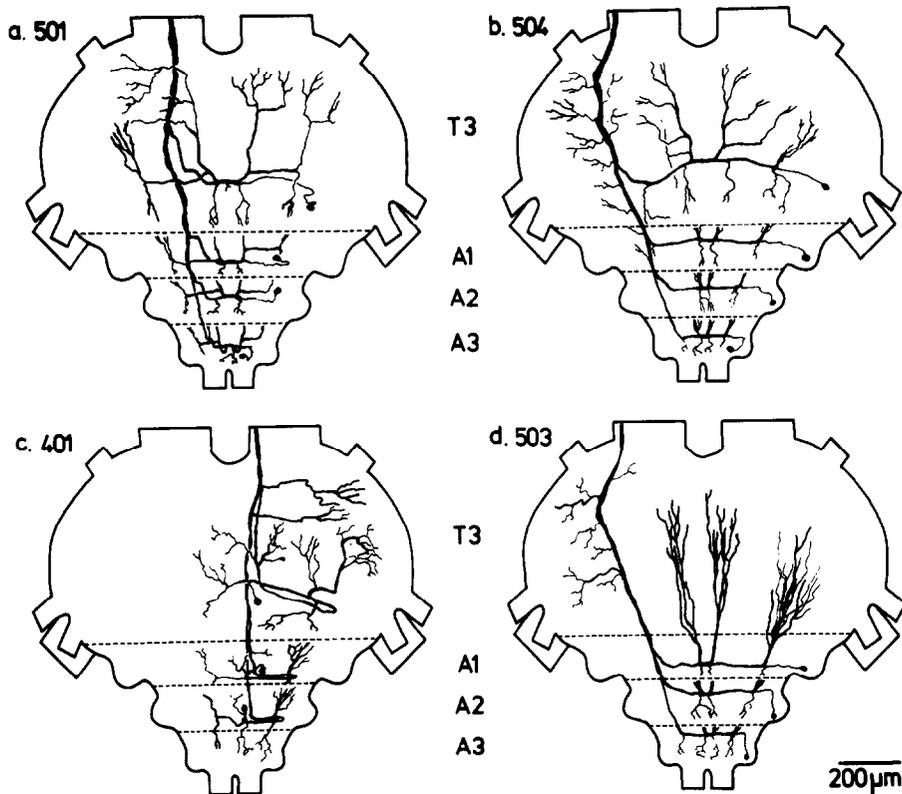

Fig. 6. Sets of homologous flight interneurones in the metathoracic and first three (fused) abdominal ganglia. Each interneurone in a set has the same structure and physiology. For clarity only one of each bilateral pair of interneurones is drawn and its dendritic branching is shown only in the ganglion containing the cell soma of the interneurone. (a) Interneurones with this structure can reset the flight rhythm. (b) Interneurones with this structure have direct connections to elevator motoneurones. (c) Interneurones with this structure can affect the timing of the flight rhythm. (d) Interneurones with this structure have disynaptic connections with depressor motoneurones (from Robertson and Pearson 1983).

physiological features. In many cases this can be done but it is not always possible. One reason might be that there are functional necessities which have not yet been identified. Another is that some features are evolutionary relics and serve no function. A third reason is that the relation between function and cellular properties is not straightforward and is obscured by the lack of optimization in the construction of neural systems. Given that evolution is a tinkerer and that natural selection can act only on the pre-existing phenotype (Jacob, 1977) it is not surprising that we find features of neurones which cannot be easily explained on the basis of good functional design. Therefore the search for general principles of neuronal organization and function may benefit from an evolutionary perspective.

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Since this paper was written we have discovered that the separation of interneuronal function is not as straightforward as suggested above. Rhythm generator interneurones can form direct connections with motoneurones. However, some form of hierarchy is present for we have established the existence of premotor interneurones at an intermediate level between the rhythm generator interneurones and motoneurones.