inadequate in predicting cell type. Although all frankly pseudomonopolar cells did innervate OHC's, the bodies of some of the cells giving rise to OSF's (such as 75L-1 and 78L-1) (Fig. 1d) were indistinguishable from those that gave rise to RF's.

The most reliable indicator of cell type across animals is the caliber of the processes, measured as they leave the cell body (Fig. 1c). As illustrated in Fig. 1e, the peripheral processes of cells giving rise to RF's were extremely thin in the vicinity of the cell body (*13*). Thus, the ratio of central process diameter to peripheral process diameter near the cell bodies was always greater for neurons innervating IHC's than for neurons innervating OHC's (Fig. 2a). The separation of cell types may be clearer with this measure than with absolute cell size because the ratio measure eliminates the problem of systematic size bias across animals.

Measurements of cell size and process ratios for a larger sample of spiral ganglion cells (including untraced cells) appear in Fig. 2b. These data suggest that in normal adult cats it should be possible to predict the peripheral termination of spiral ganglion cells with a high degree of certainty. These criteria should be of considerable practical importance in experimental work, since they can make tracing of peripheral processes unnecessary.

We conclude that at least two different types of spiral ganglion cells send projections deep into the internal auditory meatus. One group innervates OHC's by means of thin OSF's and the other innervates IHC's by means of thicker RF's. The two groups can be distinguished by certain morphological characteristics visible under the light microscope, and are generally consistent with Spoendlin's (*8*) descriptions of type I and type II ganglion cells.

To be entirely satisfied with such a view of the afferent innervation pattern, one needs to explain why these HRP data, which clearly support Spoendlin's conjectures, do not agree with the existing Golgi descriptions. Retzius (*1*), Lorente de Nó (*10*) (working with newborn mice), and Perkins and Morest (*11*) (working with newborn kittens) show drawings of large bipolar neurons traceable to OHC's. These neurons appear to be indistinguishable from those traceable to IHC's. Perkins and Morest also report the existence of single fibers sending branches to both IHC and OHC regions, a situation looked for but not found in our HRP data. Assuming that all the observations have been accurate, the simplest explanation of these discrepancies is that the morphology of spiral ganglion cells in the neonatal animal is significantly different from that in the adult. Specifically, during development the pseudomonopolar (type II) neurons of the spiral ganglion may pass through a transitional bipolar form similar to that described for the pseudomonopolar neurons of spinal ganglia (*14*).

The suggestion that the thin central axon of the OSF's corresponds to unmyelinated fiber components previously described for the auditory nerve (*15*) has two important implications. First, it means that we know nothing about the physiological response properties of these neurons, since small unmyelinated fibers are almost certainly not recordable with the techniques usually applied to the auditory nerve. Second, it suggests that little is known about the central terminations of these fibers, since relevant anatomical studies have concentrated on the larger fibers. Thus two avenues for future studies are clearly defined.

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13. The narrowing of the peripheral process of the type I neuron where it meets the cell body raises an interesting physiological issue. According to current ideas, this arrangement of a small fiber leading into a large cell body would reduce the safety factor for action potentials propagating through the cell body. Since the peripheral process is always large (diameter > 2 μm) until just before it reaches the cell body, one wonders why this arrangement occurs. A low safety factor would be useful if there were a need to inhibit impulses at this location. Perhaps the thin fiber region should be explored systematically for specialized structures, such as synaptic contacts.
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Flight Interneurons in the Locust and the Origin of Insect Wings

Abstract. *Interneurons involved in the generation of motor activity for flight in the locust were found in the first three abdominal ganglia as well as in thoracic ganglia. The evidence that sets of homologous flight interneurons occur in abdominal and thoracic ganglia supports theories that insect wings originated from movable appendages which were serially distributed along the thorax and abdomen and which were under central nervous control.*

Any overall theory of the evolution of insect flight must deal with the origin of the wings and their precursors, the "prowings" (*1*). The paranotal lobe theory (*2*) proposes that wings were derived from rigid expansions of the thoracic terga, which had a protective function. Through various possible functions such as epigamic display, thermoregulation, and parachuting (*3*), these paranotal lobes are believed to have become adapted first for gliding flight and then for flapping flight. The main alternative to this paranotal lobe theory is the pleural appendage theory (*4*) according to which wings were derived from movable and articulated pleural structures that were serially repeated along the thorax and abdomen. It has been proposed that the wings originated as protective covers for the spiracles (*5*) or the gills (*6*), or that they originated, from an uncertain beginning, as fins (*7*). After a stage in which they were used in ventilation and swimming movements, these precursors developed as flapping flight appendages—gliding flight being a subsequent sophistication to conserve energy (*4*). It is gener-

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ally accepted that wings evolved only once (*1*). Therefore, since different evolutionary pathways to attain flight would probably have resulted in different neural circuits to control flight, the evolutionary origin of the wing might be revealed by investigating the central nervous control of insect flight. We present physiological and anatomical evidence that interneurons, which are important components of the flight motor, are serially homologous and are located in the abdominal ganglia as well as in the thoracic ganglia.

The preparation (*8*) essentially consisted of a locust, *Locusta migratoria*, with wings and legs amputated and the meso- and metathoracic ganglia exposed and supported on a stainless steel plate. The flight rhythm could be induced by blowing on the head of the animal (*9*). Electromyographic (EMG) recording from the dorsal longitudinal muscles indicated the time of wing depressor activity during flight. Conventional electrophysiological techniques were used to record, display, and store EMG activity and intracellular activity from the neuropil processes of flight neurons and to fill these neurons with the fluorescent dye Lucifer yellow (*10*). The adult metathoracic ganglion in the locust is a composite of what, in the embryo, were four

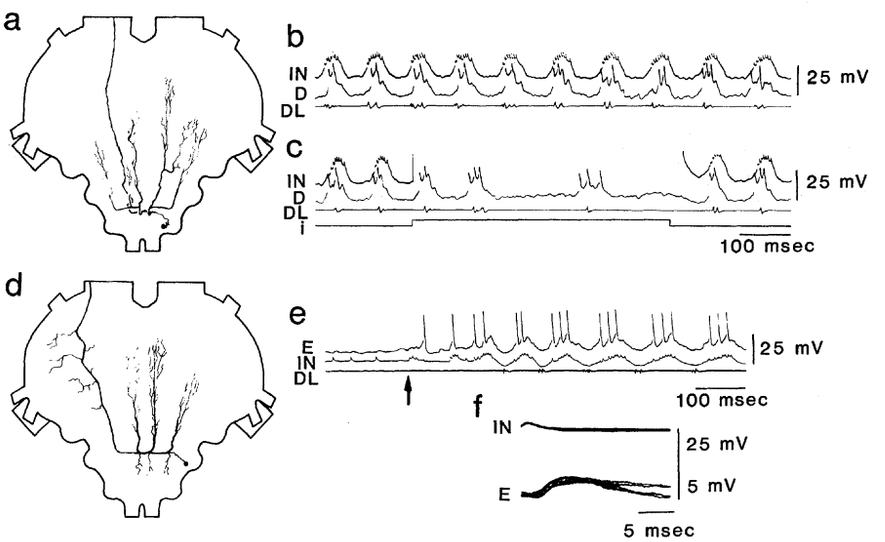

Fig. 1. Interneurons located in the abdominal ganglia and involved in patterning motor output during flight. (a to c) Structure and activity of an interneuron involved in the generation of the basic flight rhythm. (a) Drawing of the structure of the neuron in the metathoracic ganglionic mass. The cell body is located in the third abdominal ganglion. (b) During flight the interneuron (*IN*) produces high frequency bursts of spikes in phase with depressor motoneurons. Depressor activity is monitored intracellularly from an unidentified depressor motoneuron in the mesothoracic ganglion (*D*) and extracellularly by an electromyographic recording of the metathoracic dorsal longitudinal muscle (*DL*). (c) A 500-msec pulse of depolarizing current [approximately 10 nA; the lowest trace (*i*) was used to monitor duration] passed into the interneuron causes the flight rhythm to slow dramatically and the duration of the depressor motoneuron burst to increase. The normal flight rhythm resumes after the depolarization. (d to f) Structure and activity of an interneuron with an excitatory projection to a flight motoneuron. (d) Drawing of the structure of the interneuron in the metathoracic ganglionic mass. The cell body is located in the second abdominal ganglion. (e) During flight the interneuron gives high frequency bursts of spikes in phase with elevator motoneurons [monitored intracellularly from a mesothoracic tergosternal motoneuron (*E*)]. (f) Successive oscilloscope sweeps triggered by the rising phase of the interneuronal spike reveals an excitatory postsynaptic potential (EPSP) in the tergosternal motoneuron (*E*) at a short and constant latency after the interneuronal spike. These EPSP's can also be seen at the beginning of the trace in (e). The characteristic difference between the activity of motoneurons (low frequency, large spike amplitude) and interneurons (high frequency, small spike amplitude) are seen in (b) and (e).

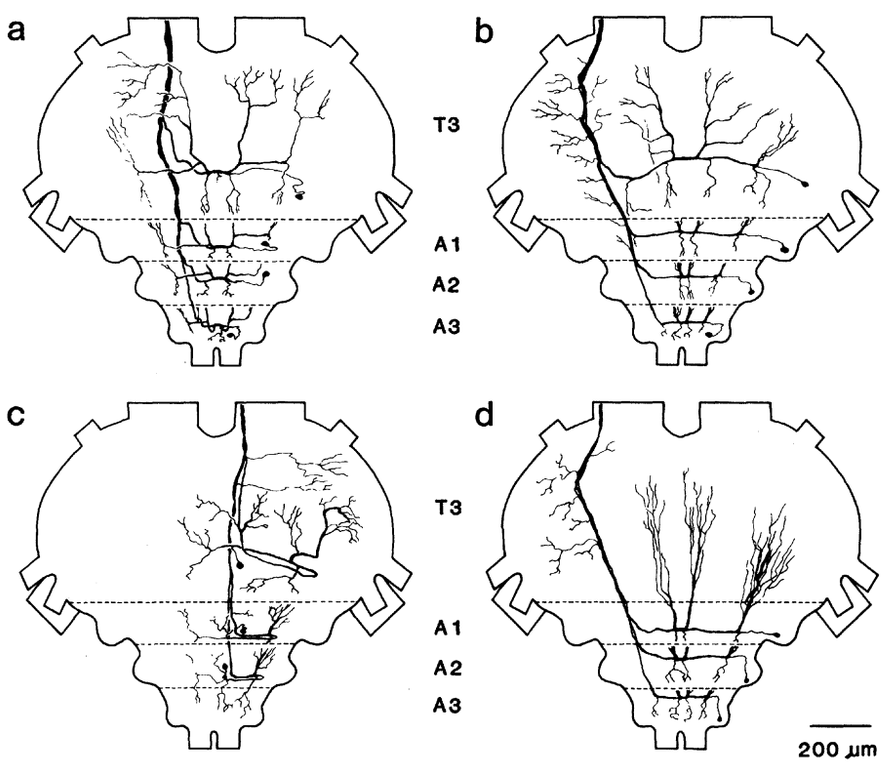

Fig. 2. Structure of homologous sets of interneurons phasically active during flight. They have been assigned to different sets on the basis of similarities in their structure and physiology. The metathoracic ganglionic mass can be divided into regions originating from the four embryologically distinct ganglia (*T3*, metathoracic; *A1*, *A2*, and *A3*, first, second, and third abdominal, respectively). Except for the course of the axon, which is shown in full for each neuron, the structure of an interneuron is depicted only in that area of the metathoracic ganglionic mass corresponding to the ganglion containing the cell body of that interneuron, unless the adjacent ganglia are unoccupied [as in (c) and (d)]. Only the interneurons on the right side are shown. (a) Depressor-type interneurons, depolarization of which slows the flight rhythm. (b) Elevator-type interneurons, some of which have connections to elevator motoneurons in the mesothoracic ganglion. (c) Elevator-type interneurons, depolarization of some of which disrupts the flight rhythm. (d) Depressor-type interneurons. In (c) and (d) the sets are not complete. Within each set, the locations of the cell bodies, courses of the axons, and dispositions of the primary neurites and major dendritic branches of all the interneurons are almost identical.

distinct ganglia, the metathoracic and the first three abdominal ganglia (*11*). However, the characteristic location of any of the interneuron cell bodies described in this report could be assigned easily to the different ganglia.

By staining interneurons intracellularly after recording their activity, we identified more than 20 different flight interneurons in the mesothoracic ganglion and metathoracic ganglionic mass. For some of these interneurons, we could show that modulating their activity by passage of depolarizing current had a strong effect on the flight rhythm, an indication that the neuron under investigation was an important member of the neural circuit generating the flight rhythm (Fig. 1, a to c). By recording simultaneously from different neurons, we demonstrated that other interneurons had short constant-latency connections to flight motoneurons (Fig. 1, d to f).

We found four sets of homologous interneurons originating in the metathoracic and the first three abdominal ganglia (Fig. 2). Each of the neurons within a set had a structure that was almost identical to that of the others in the set, and their axons were located in a similar position in the meso-metathoracic connective. Furthermore, all of the neurons belonging to a single set had the same physiological characteristics in that they fired high frequency bursts of spikes at identical phases of the flight cycle. We do not yet have data on interneurons in the unfused abdominal ganglia.

Neurons of the first set had lateral cell bodies and characteristic longitudinal arborizations. Their axons arose from the primary neurite at about the midline and left the metathoracic ganglion in the middle of the meso-metathoracic connective contralateral to the cell body (Figs. 1a and 2a). Representatives of the first set were found in all four ganglia of the metathoracic ganglionic mass, and during flight they all were active in phase with depressor motoneurons (Fig. 1b). Passage of depolarizing current into members of this set could disrupt the flight rhythm by slowing the flight frequency (Fig. 1c).

The neurons of the second set had lateral cell bodies and longitudinally arranged arborizations. The axon arose from the primary neurite after the latter had crossed the midline, and it left the metathoracic ganglion in the extreme lateral edge of the meso-metathoracic connective contralateral to the cell body (Figs. 1d and 2b). Interneurons of this type were found in all four ganglia, and during flight they gave high frequency bursts of spikes in phase with elevator motoneurons (Fig. 1e). They had short-latency excitatory connections with tergosternal motoneurons (elevators) in the mesothoracic ganglion (Fig. 1f).

For the third and fourth sets, our data are less complete. We found representatives of these sets in only three of the four ganglia (Fig. 2, c and d). The third set contained elevator-type interneurons with a distinctive structure. They had medial cell bodies and predominantly unilateral arborizations. The primary neurite extended laterally from the cell body and then looped back on itself to give rise to an axon, which left the metathoracic ganglion in the extreme medial edge of the meso-metathoracic connective ipsilateral to the cell body (Fig. 2c). Strong depolarization of these neurons caused a transient slowing of the flight rhythm.

The structure of the interneurons in the fourth set (Fig. 2d) was similar to that of the interneurons of the second set (Fig. 2b), and descriptions of their salient features would be identical. However, the fourth set contained depressor-type interneurons, whereas those of the second set were elevator-type. We have no further physiological data for the interneurons of the fourth set, but predict that they have short constant-latency excitatory connections with depressor motoneurons.

The interneurons described above were located in the metathoracic and first three abdominal ganglia, and we have shown that they are involved in the generation of the flight rhythm and in driving motoneurons. In all four sets, these interneurons had axons projecting anteriorly; this suggests that these neurons were not merely relaying flight information to the abdomen, but were contributing to the thoracic flight mechanism. Phasically active interneurons of the metathoracic and abdominal ganglia with axons descending past the third abdominal ganglion could be found, but these were comparatively few.

In the locust, the flight motor is distributed between at least six embryologically distinct ganglia, three of which are abdominal ganglia. A characteristic of other motor systems that are distributed along segmental ganglia, such as those controlling leech heartbeat, leech swimming, and lobster swimmeret beating (*12*), is that the segmental nature of the control system is a reflection of the segmental nature of the appendages or structures being controlled. It is unlikely that a motor system that has controlled only thoracic structures, such as thoracic paranotal lobes, would be distributed in the abdominal ganglia. An explanation for the distributed segmental nature of the locust flight motor is that it reflects a prior evolutionary stage. Thus the evidence favors theories that wings originated from serially distributed segmental structures. Our data do not help in determining whether or not these structures were appendages. However, these data in conjunction with the accumulating fossil evidence (*4*) do help to make the pleural appendage theory the simplest explanation for the evolutionary origin of insect wings.

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