

Neuronal circuits controlling flight in the locust: central generation of the rhythm

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How is the nervous system organized to produce the complex motor patterns which underlie behaviour? Can behaviour be adequately described in terms of the properties and interactions of single neurons? These questions have been addressed in a number of motor systems and some progress has been made in describing central neuronal mechanisms generating motor patterns (e.g. Ref. 1). However, behaviour involves an interaction of an animal with a changing environment and thus sensory as well as central processes must be investigated before complete success in answering such questions can be claimed. Locust flight lends itself particularly well to both such investigations (e.g. Ref. 2) and this system may prove to be the most tractable for a cellular analysis of behaviour. In this article I review recent results concerning the central generation of the flight motor pattern of the locust^{3–5} and in the following article Reichert and Rowell review their advances in understanding of how information from the periphery modifies this centrally generated motor pattern^{6,7}.

Locust flight is by no means a simple behaviour. However, it is a behaviour that is powered by relatively few peripheral components. Two pairs of wings, about 40 muscles and about 80 motoneurons keep a locust aloft. Each wing beat is neurogenically initiated and hence the sequence of contraction of the wing muscles is a direct result of the pattern of activity of wing motoneurons. This complex motor pattern has been likened to a piece of music, indeed to a fugue, for the themes expressed in the forewing and hindwing segments are similar but not identical and the hindwing theme is expressed slightly before that of the forewing⁸. The flight motor pattern has been studied for some time but it is only recently that we are coming to understand how it is neurally composed.

Flight motor patterns

The motor pattern recorded from intact locusts is characterized as an alternation of activity in elevator and depressor motoneurons repeating at about 20 cycles/second (Fig. 1)⁸. Activity in hindwing motoneurons leads equivalent activity in forewing motoneurons by between 5 and 10 ms. Each motoneuron tends to fire only once in each cycle except during flight manoeuvres which are driven by changes in the relative strengths and phasing of activity of different motoneurons^{9–11}. An early and significant finding was that rhythmic motor activity resembling flight could be produced from a deafferented nervous system, indicating that a substantial proportion of the motor pattern is centrally generated¹². However, deafferentation does alter the motor pattern that is recorded. The major changes are that repetition

frequency drops to about 12 cycles/second and that each motoneuron now tends to fire about three times in each cycle (Fig. 1B). In spite of the difference between the motor patterns recorded from intact and deafferented preparations one can be confident that the latter is a representation of the operation of the central component of the flight rhythm generator. With standard intracellular techniques and

some patience, the network of connections among flight neurons can be worked out. A knowledge of this central circuitry is essential as a basis for understanding how flight is controlled by the nervous system.

Flight neurons

In several rhythmic motor systems the motoneurons themselves are intimately involved in generating not only the precise phasing of the output pattern but also the timing of the rhythm^{13–15}. This seems not to be the case for locust flight motoneurons which act as simple output elements, the motor pattern being generated at the interneuronal level. Given the existence of output synapses on the dendrites of at least one flight motoneuron, it is possible that motoneurons have some role to play other than merely as the output pathways¹⁶. However, such a role is not obvious in the deafferented preparation. Thus the search for the neuronal

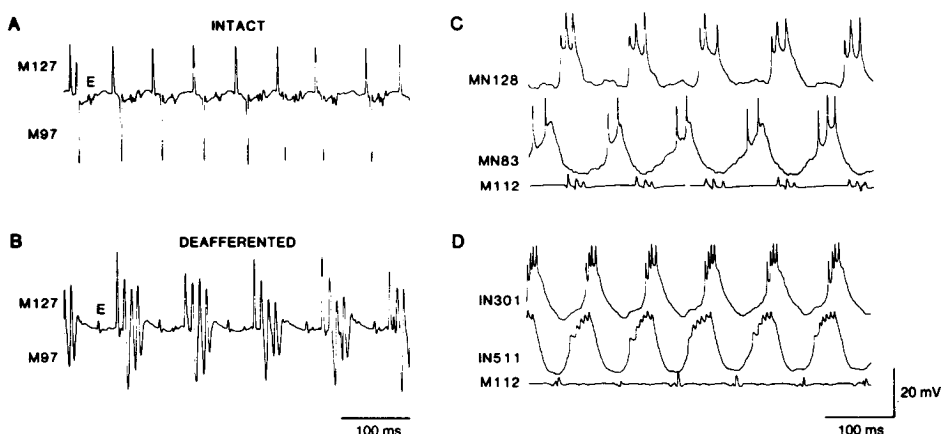


Fig. 1. Flight motor patterns and flight neurons. (A) Electromyographic recordings of wing muscle activity in an intact, tethered locust. Copper wire (0.05 mm diameter) electrodes insulated except at the tip were placed in muscles 97 and 127 (forewing and hindwing 1st basalars respectively) of the same side. The electrodes were connected to the two inputs of a differential amplifier which resulted in deflections caused by electrical activity in 127 to be directed upwards and those caused by activity in 97 to be directed downwards. Both electrodes fortuitously picked up the activity of elevator muscles thus allowing them to be monitored on the same trace. Note the lag in activity of depressor muscles from hindwing to forewing, the alternation in activity of elevator and depressor muscles and the repetition frequency of about 17 cycles/second. (B) Recordings from the same muscles in the same preparation after severing all thoracic nerve roots with the exception of nerves 3 of the meso- and meta-thoracic ganglia (innervating the 1st basalar and tergosternal muscles). Note the reduced repetition frequency (about 10 cycles/second), the increase in the number of impulses/burst and the fact that the basic pattern of alternation of antagonists with a hind-to-fore lag is still evident. (C) Simultaneous intracellular recordings from a 2nd basalar motoneuron of the left hindwing (MN 128; depressor) and a tergosternal motoneuron of the left forewing (MN 83; elevator) during a flight sequence. (D) Simultaneous intracellular recordings from interneurons 301 and 511 during a flight sequence. In both (C) and (D) the electromyographic recording from the dorsal longitudinal muscle (M 112; indirect depressor) indicates the time of the depressor phase of the cycle. Note the higher intraburst spike frequency and smaller spike amplitude in the recordings from the interneurons compared with those from the motoneurons. E, elevator; IN, interneuron; M, muscle; MN, motoneuron. Scale bars are 100 ms and 20 mV.

controllers of locust flight must be among interneurons. Proper identification of interneurons and motoneurons depends on establishing their morphology as well as their physiology and the distinctive structures of insect interneurons have helped greatly in giving flight neurons individuality and allowing data on each to be more readily accumulated. The structures of four typical flight interneurons are shown in Fig. 2. These are the interneurons some of whose synaptic interactions are shown in Fig. 3A, B and C.

The bursts of spikes recorded in both motoneurons and interneurons are caused by large oscillations in their membrane potentials (Fig. 1C and D). There is no evidence to date that any part of these oscillations is generated endogenously. Rather they seem to be the result of phasic excitatory and inhibitory synaptic input from a variety of presynaptic neurons, i.e. of networks interactions in the system. The connections that are observed between neurons are fairly straightforward. Chemically mediated excitatory and inhibitory postsynaptic potentials (EPSP and IPSP) of short latency predominate (Fig. 3A, B). The fact that the latency between the presynaptic spike and postsynaptic potential is constant and less than 1 ms (if conduction time is subtracted) warrants considering these connections as monosynaptic although conclusive evidence that this is the case is not available. A third type of postsynaptic neuron (i.e. caused by a presynaptic spikes with a longer latency (Fig. 3C) and in some preparations it can have approximately twice the duration of the short constant latency connections. These potentials are excitatory and depolarizing but, in contrast with standard EPSPs, are reversed by passing hyperpolarizing current into the postsynaptic neuron (i.e., caused by a decreased conductance at the postsynaptic membrane). This and other evidence supports the conclusion that they result from a circuit of di-synaptic disinhibition with graded release of transmitter at least at the second synapse⁵. Because the exact nature of the pathways underlying these delayed excitatory connections is unknown they are represented functionally in Fig. 3D as excitatory connections incorporating a delay box. No electrical connections and no multicomponent postsynaptic potentials have been seen and spikes in single neurons result in the same sign of short constant latency post-synaptic potential in all follower neurons.

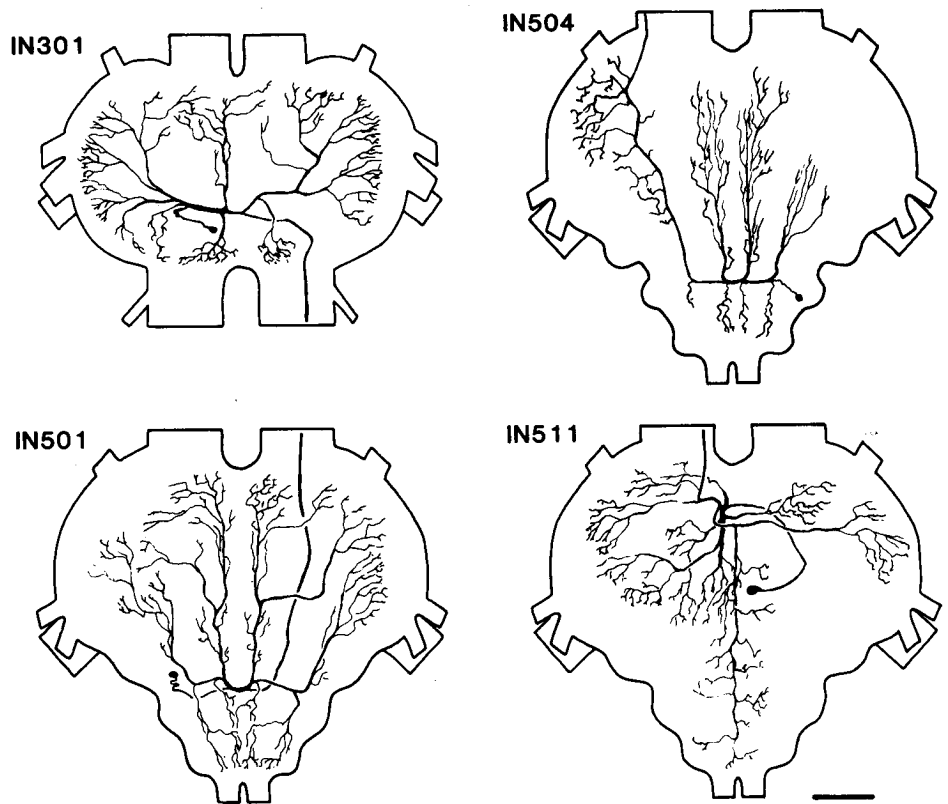


Fig. 2. Drawings of the structures of four flight interneurons. The cell body of interneuron 301 is located in the mesothoracic ganglion whereas the cell bodies of the other three interneurons (501, 504, 511) are located in the metathoracic ganglionic mass which comprises four fused neuromeres. For all these neurons the cell body is on the ventral surface of the ganglion and the dendritic branching is primarily confined to a dorsal sheet of neuropile just under the sheath. Scale bar is 200 μm .

Flight neural circuits

Fig. 3D summarizes many of the connections among flight neurons that have been described⁵. Not all known connections and not all identified flight interneurons have been included. Moreover no distinction between different types of elevator or depressor motoneuron has been made. This makes the figure a gross simplification. However, the circuit presented here can account for the prominent features of the motor pattern recorded in deafferented preparations¹⁷. Furthermore this sort of investigation has enabled us to make a number of statements concerning the organization and operation of the system.

(1) The drive for forewing and hindwing motoneurons originates in the same source. Although the wings, wing muscles and motoneurons are serially duplicated for forewing and hindwing, the same is generally not true of flight interneurons. Specific examples of interneuronal duplication can be found. For example interneuron 201 has prothoracic homologues which drive forewing depressor motoneurons, and mesothoracic homologues which drive hindwing depressor motoneurons. But both prothoracic and mesothoracic 201

are driven by the same (i.e. not duplicated) group of excitatory interneurons (503). Similarly 504 has an excitatory connection with forewing elevator motoneurons and in all probability has one with hindwing elevator motoneurons. The flight pattern generator therefore appears to be a single system rather than being separated into two systems for the separate control of the forewing and the hindwing although its components are distributed among the three thoracic ganglia comprising six embryologically distinct neuromeres¹⁸.

(2) The circuitry which interconnects interneurons 301 and 501 probably has oscillatory properties. Circuits of delayed excitation and feedback inhibition such as exist between 301 and 501 have been shown to result in oscillatory activity in which burst duration is independent of cycle period¹⁹. This property has been demonstrated for depressor depolarizations in the deafferented flight preparation of the locust¹⁷. Consistent with the idea that the 301–501 circuit is oscillatory is the observation that stimulation of 301 can induce a flight-like rhythm in a resting preparation. However, the fact that blocking the delayed excitation with

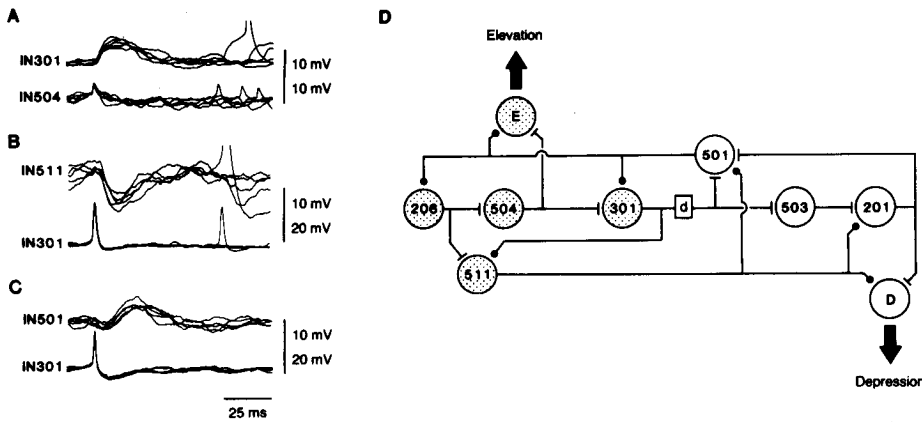


Fig. 3. Synaptic connections between flight interneurons. In (A), (B) and (C) postsynaptic potentials are demonstrated by overlaying 5 sweeps of the oscilloscope trace triggered from the rising phase of the presynaptic spike (the lower trace in each case). A digital storage oscilloscope was used, thus allowing the traces prior to the trigger point to be displayed. (A) Excitatory postsynaptic potential recorded in interneuron 301 a short and constant latency after a spike in interneuron 504. (B) Inhibitory postsynaptic potential recorded in interneuron 511 a short and constant latency after a spike in interneuron 301. (C) Delayed excitatory potential recorded in interneuron 501 after a spike in 301. Note that the latency is longer than for the possible monosynaptic connections of (A) and (B) but that it remains constant. (D) Summary diagram illustrating some of the connections that have been demonstrated between flight neurons. Excitatory connections are shown as 'T' bars, inhibitory connections are shown as filled circles and delayed excitatory connections are shown as excitatory connections with a delay box (d) in the connecting line. Neurons that are active during the elevator phase of the cycle are stippled. d, delay; D, depressor motoneurons; E, elevator motoneurons; IN, interneuron.

microtoxin does not eradicate oscillation in the system (although the correct phase relationships of the oscillations in different neurons are disrupted) indicates that probably the whole system relies on multiple oscillatory capabilities⁵.

(3) The output connections of most interneurons are widespread. This is particularly true of the inhibitory interneurons. Thus 511 promotes the elevator phase by inhibiting depressor motoneurons and excitatory premotor interneurons to depressor motoneurons and by disinhibiting elevator motoneurons rather than simply preventing depressor motoneurons from firing. Similarly 501 has widespread connection to terminate the elevator phase. This is also true of excitatory interneurons, e.g. 201 excites both depressor motoneurons and inhibitory premotor interneurons to elevator motoneurons. This reinforces the idea that the interneurons represented here are concerned with establishing the basic pattern of membrane potential oscillations in the flight system and that other interneurons will be found to be involved in controlling flight manoeuvres and mediating the effects of sensory stimulation.

(4) There are two partially segregated populations of premotor interneurons with prime responsibility for either driving the oscillations in membrane potential or for changing the relative timing of occurrence of these oscillations in different motoneurons. For example, interneuron 201 has been shown to have direct connections to all the different depressor motoneurons (dorsal longitudinal, 1st basalar, 2nd basalar and subalar). Therefore these premotor interneurons are acting to set up the basic membrane potential oscillation in all depressor motoneurons and it is unlikely that they would be involved in controlling the phase shifts between depressor motoneurons that underlie flight manoeuvres. Other interneurons are apparently much more restricted in the set of motoneurons they connect with and also receive the type of specific sensory input which is appropriate for their role in steering during flight. These interneurons are at the heart of the sensorimotor integration circuitry, which is the subject of the following article.

It is more than two decades since Wilson and Weis-Fogh first described the motor pattern of flying locusts and compared it with a fugue⁸. We are

beginning to understand how interactions among identified interneurons can produce a flight motor pattern in the absence of sensory information. The following article illustrates how it is possible to manipulate the sensory environment and investigate how individual interneurons can mediate the appropriate changes to the score that underlie steering.

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