4.8 CENTRAL NEURONAL INTERACTIONS IN THE FLIGHT SYSTEM OF THE LOCUST

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ABSTRACT

Central and sensory neuronal elements interact to produce the flight behaviour of a locust. The central component is capable of producing a flight motor pattern in isolation from phasic feedback from the periphery. This central motor pattern is produced primarily as a result of the properties and interactions of interneurons located in the thoracic ganglia. Interneurons phasically active with the flight rhythm can be identified both morphologically and physiologically. This paper reviews the properties and interconnections of identified flight interneurons. An interneuronal network which can account for certain features of the flight motor pattern recorded in deafferented preparations is presented.

INTRODUCTION

Locomotor behaviours of insects are particularly enticing for neurobiologists who wish to understand how nervous tissue controls behaviour. This is evident in the content of this volume. Such behaviours despite being agreeably complex have proved amenable to analysis from various approaches. The approach which interests me is to determine the neuronal circuitry underlying behaviour by recording from identified neurons. Is it possible to describe behaviour in terms of the properties and interactions of identified neurons? Locust flight is a behaviour which lends itself to such an approach not only because intracellular recordings can be made during the expression of a flight motor pattern but also because there exists a wealth of background information on this behaviour (e.g. Wilson, 1968; Burrows, 1976, 1977).

It is clear from the preceding papers in this volume that sensory input has a major role to play in controlling the flight of locusts (Möhl, 1985; Neumann, 1985; Horsmann & Wendler, 1985; Rowell & Reichert, 1985). In fact some sense organs fulfill the criteria necessary for inclusion in the rhythm generator for flight (Bacon & Möhl, 1983; Horsmann et al., 1983; Pearson et al., 1983). Notwithstanding these demonstrations, it is also clear and has been known for some time that there is an element of the flight rhythm generator in the thoracic ganglia which can function and produce rhythmical activity without phasic input from the periphery (Wilson, 1961; Robertson & Pearson, 1982). This central element is revealed by the pattern of activity of wing motoneurons which can be recorded in essentially deafferented preparations under the appropriate stimulus conditions, e.g. after presentation of a wind stimulus to the head of the dissected animal. The motor pattern recorded from deafferented preparations (Hedwig & Pearson, 1984) is not identical to that recorded in intact flying locusts (Wilson & Weis-Fogh, 1962) but it is sufficiently similar to recognize it as a flight motor pattern. The rationale behind the work described here is that an understanding of how the central flight rhythm is generated will be a major step towards understanding how flight itself is controlled at the cellular level. Indeed, to understand precisely how sensory information is integrated it will be necessary to know the neural substrate upon which it acts.
My intention here is to describe some synaptic connections between identified neurons in the flight system of the locust and show how they might account for features of the flight motor pattern recorded in deafferented preparations.

FLIGHT INTERNEURONS

Early models of the rhythm generating mechanism underlying locust flight gave a prominent role to motoneurons (WILSON & WALDRON, 1968). Subsequently interneurons were found to be important (BURROWS, 1977) and it is now believed that motoneurons act as little more than output elements with no immediately obvious part to play in controlling the motor pattern (ROBERTSON & PEARSON, 1982). To characterize in any detail the interneurons that are involved it is necessary to record intracellularly from them during the expression of a flight motor pattern. K. G. PEARSON and I developed a preparation with which this type of recording is possible (ROBERTSON & PEARSON, 1982). In brief the thoracic ganglia of a dissected locust whose wings and legs have been amputated are stabilized on a stainless steel plate. All thoracic nerve roots are cut except those innervating the dorsal longitudinal muscles (DL, indirect wing depressors) and electromyographic (emg) recordings taken from these muscles are used as a monitor of the time of depessor activity during flight sequences. Flight sequences are induced by blowing wind on the head of the animal. Our experimental strategy was to use glass capillary microelectrodes filled with Lucifer Yellow to penetrate the neuropile processes of individual neurons. In this way both the morphology and the physiology of the interneurons we found could be determined. Only physiological data are presented here but in all cases these were obtained from identified interneurons whose structures are known. These structures and the system for allocating identity numbers to interneurons are described in a previous paper (ROBERTSON & PEARSON, 1983; see also Fig. 4 of DELCOMYN, 1985, introductory chapter to this volume).

There are a large number (n > 50 to date) of thoracic interneurons which give discrete bursts of spikes at different phases of the flight rhythm. Interneuronal bursts can relatively easily be distinguished from those of motoneurons because typically they exhibit a greater intraburst spike frequency (200-250 impulses/s in interneurons compared with 60-80 impulses/s in motoneurons) and their spike amplitudes tends to be smaller. The amplitude of the membrane potential waveform underlying these bursts can be as large as 25mV and often it is possible to discern discrete excitatory and inhibitory postsynaptic potentials (epsps and ipspss) contributing to the membrane potential changes. There is so far no evidence that single interneurons possess endogenous properties which would allow their membrane potentials to oscillate without the appropriate phasic input from other interneurons. One way of gauging the possible importance of a particular interneuron is to determine whether perturbing the activity of the interneuron by current injection during flight sequences has any noticeable effect on the recorded rhythm. Using a simple monitor of the rhythm (the DL emg) relative phasing of the activity of different motoneurons can not be examined. However in some cases (Fig. 1) brief stimuli can alter the rhythm by increasing or decreasing the period of the cycle in which the stimulus is presented without affecting the period of subsequent cycles. This is resetting and in conjunction with rhythmical activity it is used as evidence for a neuron's involvement in rhythm generation (KRISTAN et al., 1977; WEEKS, 1981; ROBERTSON & PEARSON, 1983). Currently we know of 5 interneurons (excluding homologues) which are capable of resetting the rhythm. The quality (advance or delay of the next cycle) and quantity (magnitude of the induced phase change) of the resetting effect are dependent upon the phase of the cycle at which the stimulus is delivered. This relationship can be plotted as a phase response curve (PINISNER, 1977) which thus illustrates the influence of a single interneuron on the timing of the rhythm. An example of such a phase response curve is shown in Fig. 2 for the interneuron numbered 301 more of whose properties are presented below. Another way of gauging an interneuron's possible importance is by determining its synaptic connections with motoneurons and with other interneurons. In this way
Fig. 1. Resetting the flight rhythm with short pulses of depolarizing current delivered to single interneurons. a) In the lower pair of traces a 100 ms pulse of depolarizing current (about 10 nA) delivered to 501 causes the flight rhythm to be reset by increasing the period of the cycle in which the stimulus occurs (compare with upper pair of traces). b) A similar pulse of depolarizing current delivered to 301 also increases the period of the cycle in which it falls (compare lower three traces with upper three traces). DL, dorsal longitudinal muscles. From ROBERTSON & PEARSON, 1983.

networks of connectivity among flight neurons can be mapped and the extent to which these networks might account for features of the flight motor pattern can be assessed.

SYNAPTIC CONNECTIONS BETWEEN INTERNEURONS

It is possible by simultaneously recording from two neurons to find neuronal connections which appear to be monosynaptic. For technical reasons, conclusive evidence that an observed connection is monosynaptic, such as can be obtained in other systems (BERRY & PENTREATH, 1976; GETTING, 1981), is difficult or impossible to gather in this system. However, psp's recorded in one neuron can be observed to follow action potentials recorded in another neuron in a one-to-one fashion even at high frequencies of discharge and with a short (approximately 1 ms after subtracting conduction delay) fixed latency. For convenience, connections with these characteristics will be referred to as monosynaptic.

In general the membrane potential waveform underlying the phasic activity of flight neurons is formed by the summed effects of a variety of synaptic inputs (ROBERTSON & PEARSON, 1983, 1984b). Individual psp's are commonly between 1 and 4 mV in amplitude whereas membrane potentials can oscillate through 25 mV during flight sequences. This can be accounted for by temporal summation of psp's during a burst of action potentials, facilitation, drive from all members of a homologous set of presynaptic interneurons and drive from several different presynaptic interneurons. Furthermore, both excitatory and inhibitory psp's contribute to the membrane potential changes and these are produced by separate presynaptic interneurons. There is currently no evidence of a single interneuron having more than one postsynaptic effect at monosynaptic connections.
Fig. 2. Structure and phase response curve of interneuron 301. - A) Drawing of the structure of 301 in the mesothoracic (upper) and metathoracic (lower) ganglia. B) Phase response curve for 30 ms depolarizing current pulses (about 10 nA) delivered to 301. Time of stimulation was taken as the start of the stimulus pulse and cycle periods were measured from the beginning of successive bursts of dorsal longitudinal activity. The latency of the stimulus was defined as the interval from the start of the perturbed cycle to the start of the stimulus. Stimulus phase was measured as the ratio of the latency to the average period of the two preceding unperturbed cycles. Phase shift was calculated as the ratio of the difference between the period of the perturbed cycle and the average period, to the average period. Data are from a representative single experiment and plotted as the mean ±1 standard deviation for all stimuli falling in bins of 0.05 stimulus phase. Note that pulses to 301 can reliably delay and advance the occurrence of the subsequent cycle. From ROBERTSON & PEARSON, 1984b.

Multicomponent psp's similar to those described underlying swimming in Tritonia (GETTING, 1981) have not yet been found. Also interneurons have either excitatory or inhibitory monosynaptic connections with other neurons but not both.

The monosynaptic connections described here are dependent on impulse-mediated synaptic transmission, and in most cases the pre- and postsynaptic neurons are located in ganglia separated by a distance which normally precludes passive propagation of membrane potential changes. However, there is indirect evidence that graded, non-spiking, interactions between neurons in a single ganglion may have a role in setting up and maintaining membrane potential oscillations in flight neurons. First, local interneurons which have the characteristics of nonspiking interneurons (BURROWS, 1981) and whose membrane potentials oscillate in phase with the flight rhythm have been identified (ROBERTSON & PEARSON, unpublished observations). Such interneurons are rare (2 identified to date) but necessarily the postsynaptic effects of these neurons must be mediated by graded release of transmitter.

The second piece of indirect evidence for graded interactions in the flight system pertains to the proposed mechanism underlying delayed epsps (ROBERTSON & PEARSON, 1984b). This type of psp is commonly observed in flight neurons and, at least in one instance, such a psp seems to be of fundamental importance in controlling the relative phase of firing of different neurons (see below). An example of a delayed epsp in 501 following spikes in
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Fig. 3. Delayed and direct excitation of 501. A) Spikes in 301 produce a delayed excitation of 501. B) 201 has a monosynaptic connection with the same 501. Note that the 301-501 connection has some monosynaptic characteristics (constant latency; one for one following) but that the 201-501 connection has a markedly shorter latency and time course (compare A and B). The conduction delays from both neurons to 501 are assumed to be similar (see text). In this and following figures the method used to display neuronal connections is to overlay multiple sweeps of the oscilloscope triggered off the rising phase of the presynaptic spike. From ROBERTSON & PEARSON, 1984b.

Fig. 4. A disynaptic pathway from 301 to 501 via 511. A) Diagrammatic representation of the pathway. B) Simultaneous recordings of 511 and 301 during a flight sequence. C) Simultaneous recordings of 511 and 501 during a flight sequence. The arrow under the trace marks the onset of the hyperpolarization caused by the wind stimulus that initiated flight. D) Spikes in 301 produce an ipsp in 511. E) Spikes in 511 produce an ipsp in 501. In this and following figures inhibitory connections are diagrammed as filled circles and excitatory connections are diagrammed as T-bars, and the DL trace indicates the time of firing of dorsal longitudinal motoneurons (depressor phase) recorded either electromyographically or en passant from the nerve roots containing their axons. From ROBERTSON & PEARSON, 1984b.
postsynaptic cell membrane. Two possible mechanisms could account for these observations: the delayed epsp could result from a monosynaptic, decreased conductance connection (e.g. WEIGHT & VOTAVA, 1970; COLE & NICOLL, 1983) or it could result from a disynaptic disinhibitory connection via neuron(s) which release inhibitory transmitter in a tonic and graded manner. The reasons for favouring the second of these possibilities (disynaptic disinhibition) are presented fully in another paper (ROBERTSON & PEARSON, 1984b). Briefly we know that, in the case of the delayed excitatory connection from 301 to 501, 301 causes monosynaptic ipsps in other neurons (e.g. Fig. 4D) and we have identified a disynaptic disinhibitory pathway from 301 to 501 via 511 (Fig. 4) although we do not yet have direct evidence that the 511-501 synapse releases transmitter in a tonic and graded manner. Moreover, the application of approximately 1.5 X 10^{-6} M picrotoxin (known primarily as a blocker of inhibitory synapses) will block both the monosynaptic ipsps and the delayed epsps following spikes in 301.

INTERNEURONAL CIRCUITS

It is shown above that 301 has a delayed excitatory connection to 501. It is particularly interesting that 501 feeds back to 301 with a monosynaptic ipsp thus forming a simple burst generating circuit (Fig. 5A), i.e. activity in 301 will excite 501 which will feed back to terminate activity in 301. This simple circuit is not the sole basis for rhythmicity in the flight system for we know that picrotoxin can disrupt the circuit without eradicating rhythmicity (ROBERTSON & PEARSON, 1984b). However it may contribute to rhythm

Fig. 5. The circuit formed by 301 and 501. - A) Diagrammatic representation of delayed excitation from 301 to 501, and feedback inhibition from 501 to 301. Delayed excitation, here represented as an excitatory connection incorporating a delay box, probably results from a disynaptic disinhibitory pathway (see text). The insets show examples of the ipsp in 301 following each spike in 501 (left) and the delayed excitatory potential in 501 following each spike in 301 (right). B) Simultaneous intracellular recordings of 301 and 501 during a wind-induced flight sequence. C) Stimulation of 301 with a long duration pulse of depolarizing current (about 10 nA, duration monitored with current trace, l) induces rhythmical bursting activity in 501 and dorsal longitudinal motoneurons. Note that the cycle frequency and the phase relationships of the bursting neurons are similar to those induced by the wind stimulus (compare with B). From ROBERTSON & PEARSON, 1985.
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generation and if this is the case then tonic activation of 301 might be expected to induce rhythmic activity. This is demonstrated in Fig. 5B, C. The rhythm induced by driving 301 has a frequency similar to the frequency normally recorded in deafferented preparations and the phase relationships of the activity of the monitored neurons are similar to normal. We are unable to rule out the possibility that unknown components of the central flight circuitry are contributing to the induced rhythm and indeed other neurons will be activated by driving 301, however the known circuit with 501 appears likely to play a part.

Interneuron 301 also has a delayed excitatory connection with an interneuron numbered 503. The significance of this connection is that it is the first step in a pathway from 301 (an interneuron known from its phase response curve to be involved in timing the flight rhythm) to depressor motoneurons of both the forewing and the hindwing (Fig. 6; ROBERTSON & PEARSON, 1984a). Depressor motoneurons of the hindwing receive monosynaptic connections from 201 in the mesothoracic ganglion (T2). To date monosynaptic epsps have been seen in the 1st and 2nd basalar, subalar and dorsal longitudinal motoneurons following each 201 spike (ROBERTSON & PEARSON, 1983; ROBERTSON, unpublished observations). An homologous interneuron in the prothoracic ganglion (201(T1)) drives forewing depressor motoneurons. In this case it is only known to connect to 1st basalar motoneurons but it is probable that the distribution of its output will mirror that of its homologue in the mesothoracic ganglion. 503 has monosynaptic excitatory connections both with 201(T1) (ROBERTSON & PEARSON, 1983, 1984b).

Fig. 6. Multisynaptic pathway from 301 to depressor motoneurons. - A) Diagrammatic representation of the pathway of connections from 301, through 503 and 201 to depressor motoneurons. B) Rhythmic bursting activity of these neurons during flight sequences. (note that 301 fires at elevator phase whereas the other neurons fire at depressor phase). C) Multiple oscilloscope sweeps demonstrating each of the connections: upper pair of traces - delayed excitation from 301 to 503 (note that the time scale for this pair of traces is twice that for the other two pairs); middle pair of traces - epsp recorded in 201 after each spike in 503; lower pair of traces - epsp recorded in a depressor motoneuron (metathoracic first basalar) after each spike in 201. Modified from ROBERTSON & PEARSON, 1983, 1984b.
& PEARSON, 1983) and with 201(T2) (ROBERTSON & PEARSON, 1984b). Thus activity in 301 will drive 503 which in turn will drive the premotor interneurons for the depressor motoneurons of the fore- and the hindwing. It is noteworthy that of these neurons (301, 503, 201 and motoneurons) only 301 fires during the elevator phase of the flight rhythm; the others fire during the depressor phase (Fig. 6B). The shift in the phase of activity may be introduced at the delayed excitatory connection between 301 and 503.

It can be demonstrated that some of the drive for both elevator and depressor motoneurons originates in a single source (Fig. 7). Elevator motoneurons of the forewing receive monosynaptic excitatory connections from interneuron 504. Judging by the pathway of the axon of 301 it is probable that it also excites elevator motoneurons of the hindwing (ROBERTSON & PEARSON, 1983). The same 504 produces epsps in 301 as well as in elevator motoneurons (Fig. 7C). Thus a burst of activity in 504 will first activate fore- and hindwing elevator motoneurons and then activate fore- and hindwing depressor motoneurons after a relatively constant delay introduced by the indirect pathway (504-301-503-201-depressor motoneurons) which also incorporates a delayed excitatory connection. Activity in 504 thus results in an elevator-depressor depolarization sequence.

Activity in elevator motoneurons is terminated due to a monosynaptic inhibitory connection from 501 (Fig. 8). It is shown above that 501 is driven by 301. Thus 504 simultaneously drives elevator motoneurons and 301, and subsequently 301 simultaneously drives 503 (to activate depressors) and 501 (to inhibit elevators).

Fig. 7. Simultaneous drive of elevator motoneurons and 301 from 504. - A) Diagramatic representation of the connections from 504 to elevator motoneurons and to 301. B) Rhythmic bursting activity of these neurons during flight sequences. C) Multiple oscilloscope sweeps demonstrating each of the connections: upper pair of traces - epsp recorded in an elevator motoneuron (mesothoracic tergosternal) after each spike in 504; lower pair of traces - epsp recorded in 301 after each spike in 504. Modified from ROBERTSON & PEARSON, 1983, 1984b.
CONCLUSIONS

The easiest observation about the central flight circuitry that can be made at present is that it is fairly complex. This is illustrated by Fig. 6 of Delcomyn (1985) (introductory chapter to this volume; see also Robertson & Pearson, 1985) in which the majority of known connections are represented. Since that figure was made at least seven new connections have been found (Robertson, unpublished observations) but inclusion of these does not radically alter our understanding of how the circuit operates. Some of the interneurons are shown with no output connections and some with no input connections. Clearly a knowledge of their functional role is lacking. However, embedded in that large circuit is a network of interneuronal connections most of which are described above and with which it is possible to account for certain features of the flight motor pattern recorded in deafferented preparations. This smaller network represents the first steps towards a description of how the central nervous system controls flight in the locust (Fig. 9).

Hedwig & Pearson (1984) have characterized the flight motor pattern of deafferented preparations. Three features are prominent: 1) the basic unit of activity is an elevator depolarization followed after a relatively constant latency by a depressor depolarization. The connections of 504 described above ensure that elevator motoneurons and 301 are activated together. 301 then drives depressor motoneurons after a latency which is fixed by the synaptic delays in the indirect pathway between these neurons. - 2) The duration of the depolarization of depressor motoneurons is relatively independent of cycle time. Circuits of delayed excitation and feedback inhibition similar to the one described here (formed by 301 and 501) exhibit the property of burst duration being independent of cycle time (Getting, 1983). Depressor motoneurons may burst in this manner because the 301-501 circuit is, at least in part, responsible for their activation. Elevator motoneurons are inhibited by 501 for the same relatively constant duration but the time during which they are depolarized and can fire is variable and more dependent on the period of the cycle. - 3) Forewing and hindwing elevators are depolarized in-phase whereas hindwing depressor motoneurons are depolarized 5-15 ms prior to forewing depressor motoneurons. The premotor interneurons described here have widespread effects and probably excite elevator or depressor motoneurons as a group, i.e. making no distinction between those responsible for fore- or hindwing movements. The direct pathway from 504 to forewing elevators is very similar in length to that from 504 to hindwing elevators. However the indirect pathway from 503 to forewing depressors
is markedly longer than that to hindwing depressors due to the ganglionic locations of the relevant neurons. In fact this difference is great enough for conduction delay to impart the observed phase lag to forewing depressors (see ROBERTSON & PEARSON, 1985, for further details).

It should be pointed out that the interneurons described here are probably not the ones responsible for mediating the phase changes in motoneuron activity which are induced by sensory input and responsible for flight manoeuvres. The effects of these interneurons are widespread and it seems rather as if they act to set up and maintain the basic membrane potential oscillations in the system. It is more likely that the sensory induced modifications to the motor pattern are mediated by premotor interneurons (excitatory and inhibitory) which have more restricted ranges of action (i.e. to particular types of motoneurons). Some of these may already have been identified but their role cannot be assessed using the techniques described here. However, the thoracic ocellar interneurons described by ROWELL & REICHERT (1985) provide evidence that an analysis of the sensory control of the flight motor pattern in terms of identified neurons may be feasible.

Although the connections shown in Fig. 9 may contribute to rhythm generation they are not the only source of rhythmicity in the system. Indeed it may prove impossible to define the rhythm generator in anything but very general terms or to confine it to a particular locus. In other systems (e.g. MILLER & SELVERSTON, 1982a, b) several neuronal and network properties have been shown to contribute to rhythmicity and the concept that the oscillator for a particular rhythmic behaviour can be unequivocally identified might be outdated.

The network of Fig. 9 illustrates those connections which may be primarily responsible for switching from elevator to depressor phase through the actions of 301. What is lacking in the circuit is any means of reactivating the elevator phase save under the influence of tonic excitatory input such as might be provided
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by the wind stimulus. The recordings of many neurons active at either the elevator or the depressor phase show a strong, phasic inhibition at the time after depressor motoneurons have fired and before elevator motoneurons are active again. Interneurons that give a burst of spikes at this time have been identified although they are relatively rare. Discovering the interneuronal connections that would complete the cycle is, one hopes, just a matter of time.

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