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Synchronous activity of flight neurons in the mesothoracic ganglion of the locust

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Summary. 1. Coordinated movements of the wings during flight in the locust result from coordinated activity of flight neurons in the thoracic ganglia. Many flight interneurons and motoneurons fire synchronous bursts of action potentials during the expression of the flight motor pattern. The mechanisms which underlie this synchronous firing were investigated in a deafferented preparation of *Locusta migratoria*.

2. Simultaneous intracellular recordings were taken from flight neurons in the mesothoracic ganglion using glass microelectrodes filled with fluorescent dye.

3. Three levels of synchronous activity between synergistic motoneurons and between the right and left partners of bilaterally symmetrical pairs of interneurons were observed: bursting which was loosely in phase but which showed little correlation between the temporal parameters of individual bursts in the two neurons; bursting which showed synchrony of the beginning and end of bursts; and bursts which showed highly synchronous spike-for-spike activity.

4. Direct interactions between the neurons had little or no part to play in maintaining any of the levels of synchrony, even in instances of very close synchrony (spikes in different neurons occurring within 1 ms of each other). Highly synchronous firing was a consequence of common synaptic input impinging on neurons with similar morphological and physiological properties.

Key words: Insect-Flight – Interneuron – Rhythm – Synchrony

Introduction

Flight motor patterns of the locust are produced in part by a network of interneurons and motoneurons located within the thoracic ganglia (Robertson 1986; Reichert and Rowell 1986). Some features of flight motor patterns require sensory feedback for their expression (Pearson and Wolf 1987; Wolf and Pearson 1987). Indeed, precise synchronization of right and left motor units in intact animals requires proprioceptive feedback and the variability of the motor pattern of tethered locusts is thought to reflect the operation of a flexible system capable of continuously modifying itself in response to changing conditions (Möhl 1985, 1988). Nevertheless, most of the major features can be generated by a circuit of interneurons isolated from sensory information (Wilson 1961; Robertson and Pearson 1982; Stevenson and Kutsch 1987). The interneuronal circuit produces a motor pattern in which equivalent motoneurons of the right and left side are active in phase and hindwing motoneurons lead equivalent forewing motoneurons by the appropriate phase delay.

The published work on interneurons known to be rhythmically active during the expression of flight sequences has concentrated on their structure and other individual properties (Robertson and Pearson 1983; Pearson and Robertson 1987; Robertson and Wisniowski 1988) and on their integration of sensory information (Reichert and Rowell 1985; Reye and Pearson 1987; Pearson and Wolf 1988). There is some information on the interactions between interneurons and how these may contribute to pattern generation (Robertson and Pearson 1985; Robertson and Reye 1988). However, most attention has been paid to how interneuronal interactions coordinate the alternation between the elevator and depressor phases of the rhythm. Relatively little information is available on interactions which might mediate synchronous activity within one phase of the cycle.

The locust and its flight mechanism are bilaterally symmetrical and all the flight neurons so far described exist as bilaterally symmetrical pairs. In other bilaterally symmetrical systems, in phase activity of the two sides is aided by direct electrical or chemical synapses between neurons of the right and left sides (Hagiwara and Morita 1962; Stuart 1970; McCrohan and Winlow 1985; Satterlie 1985) although this is not always the case (Kandel

Abbreviations: *DL* dorsal longitudinal; *E* elevator; *EMG* electromyographic; *epsp* excitatory postsynaptic potential; *psp* postsynaptic potential; *Tst* tergosternal

and Tauc 1966; Gardner 1971). In the locust the interconnections underlying synchronous activity of bilaterally symmetrical neurons are unknown.

Locust flight motoneurons are organized such that there are separate groups for each wing and coordination of the right and left sets of motoneurons is necessary to ensure that the wings on the right and left sides beat in synchrony. With the exception of the contralateral motoneuron of the dorsal longitudinal muscles (Tyrer and Altman 1974), there is little opportunity for direct interaction between motoneurons of the right and left sides because their processes do not overlap. Thus their coordination in deafferented preparations must be mediated by interneurons (e.g. Burrows 1975). Flight interneurons of a bilaterally symmetrical pair have equivalent outputs and during generation of flight rhythms they are active in phase. Longitudinal hemisection of the mesothoracic ganglion does not obviously disrupt the normal coordination of the motor patterns, even in the absence of phasic information from wing proprioceptors (Ronacher et al. 1988). This interesting observation suggests that direct interactions between right and left interneurons of mesothoracic pairs are not necessary for coordination of the right and left wings, but it leaves open the question of whether such interactions exist.

The recordings described here were made primarily to determine the extent to which direct interactions between the right and left partners of bilaterally symmetrical pairs of neurons are involved in coordinating the synchronous activity of the two sides of deafferented preparations. Synchrony of action potentials and synchrony of bursts were observed and the mechanisms by which each level of synchrony was maintained were investigated.

Materials and methods

Specimens of *Locusta migratoria* were obtained from a crowded colony maintained in the Biology Department at Queen's University. Both males and females were used, although males were preferred. The sex of the animal did not affect the results in any noticeable way. Experiments were performed at room temperature (22–25° C).

The preparation used for recording from flight neurons during the expression of the flight motor pattern has already been described (Robertson and Pearson 1982). Briefly, the meso- and metathoracic ganglia were exposed dorsally and stabilized on a stainless steel plate. The nerve roots to most of the wing muscles were cut to reduce mechanical interference and prevent phasic afferent feedback from the wing stumps. The exception was the nerve root innervating one of the dorsal longitudinal muscles (DL) which provided an EMG monitor of the time of the depressor phase of the rhythm. The thoracic cavity was flooded with standard saline (in m*M*: 147 NaCl, 10 KCl, 4 $CaCl_2$, 3 NaOH, 10 HEPES buffer). Wind stimuli delivered to the head of the animal induced brief sequences of a deafferented (from phasic proprioceptive information) flight motor pattern.

Intracellular recordings were taken from the neuropil segments of different neurons using glass microelectrodes pulled so that if filled with 1 *M* potassium acetate their resistance was 30–60 MΩ. The tips of these electrodes were filled with 4% Lucifer Yellow CH (Aldrich, Milwaukee) in 0.5 *M* lithium chloride, and the shafts were filled with 0.5 *M* lithium chloride. The dye was injected into neurons using 5–10 nA of constant hyperpolarizing current for 2–10 min. The structures of the neurons were determined either during the experiment using a Leitz epifluorescence illuminator attached to the binocular microscope, or by viewing them in whole mount on a Leitz Diaplan fluorescence microscope after the ganglia containing the neurons were excised, fixed in 4% paraformaldehyde, dehydrated in an ethanol series and cleared in methyl salicylate. Particular neurons were identified on the basis of their characteristic morphology and physiology and numbered according to Robertson and Pearson (1983). Four interneuron pairs are dealt with in this paper and their primary characteristics have already been described (Robertson and Pearson 1983, 1985). In brief, 201 is active in depressor phase, excitatory, and premotor to depressor motoneurons; 301 is active in elevator phase, inhibitory, and in the rhythm generator; 302 is active in depressor phase, inhibitory, and premotor to elevator motoneurons; 701 is active in depressor phase, excitatory, and premotor to depressor motoneurons.

The intracellular recordings were amplified and stored on standard FM tape for subsequent analysis. To determine the relationship between the times of occurrence of single action potentials in different neurons it was necessary to filter the recordings so that the spikes were more distinct from the slow membrane potential oscillations that caused them. The signals were filtered by passing them through a Grass P15 preamplifier (gain ×10) with the low and high frequency cut-offs at 100 Hz and 50 kHz, respectively. This sufficiently eradicated the slow oscillation (approximately 12 Hz – the repetition frequency of the deafferented flight motor pattern) to enable each successive spike in a burst to trigger the output of a window discriminator (WPI, model 121). For the analysis two simultaneous intracellular recordings were taken from the tape, filtered and directed into two window discriminators the outputs of which were fed into the input lines of the Game-I/O connector of an Apple-IIe compatible microcomputer. The relative times of occurrence of spikes in the two traces were measured and analyzed using the REAL-TIMER software (Gras and Hörner 1985) and some custom written software. The timing resolution of the REAL-TIMER is 0.2 ms. The resolution of the measurements was improved to 0.1 ms by playing the tapes back at half speed. The limit to reliable measurements was imposed by slight variation in the triggering of the window discriminator by different spikes. Results of this analysis are presented as cross-correlation and latency histograms. For the cross-correlation histogram the latencies from each successive spike on one trace to all spikes on the other trace within a preset limit (e.g. 50 ms before and after in Fig. 2B) were measured. For the latency histogram the latencies from each successive spike on one trace to only the following spike on the other trace were measured. The latencies are presented as frequency histograms with bin widths of 1.0 or 0.2 ms.

The synaptic connections that are described here as monosynaptic are considered to be so for the following reasons: the branches of the pre- and postsynaptic neurons overlap in the neuropil and the estimated synaptic delay from spike to PSP is constant and short (less than 0.8 ms). The measured latencies which include conduction times along the axons were approximately 0.8 ms between neurons in the same ganglion and approximately 1.8 ms between neurons in adjacent ganglia. Immunocytochemical studies of inhibitory neurons have supported the use of these criteria to establish probable monosynapticity, at least for flight interneurons in the locust (Robertson and Wisniowski 1988).

The results were obtained during the course of investigations of the interactions between flight interneurons over several years. The particular connections and phenomena described here were each seen in at least 3 different preparations and each has been indirectly confirmed in many others.

Results

Prime candidates for neurons whose synchronous activity might be mediated via a direct interaction would be 2 motoneurons innervating the same muscle. Indeed the

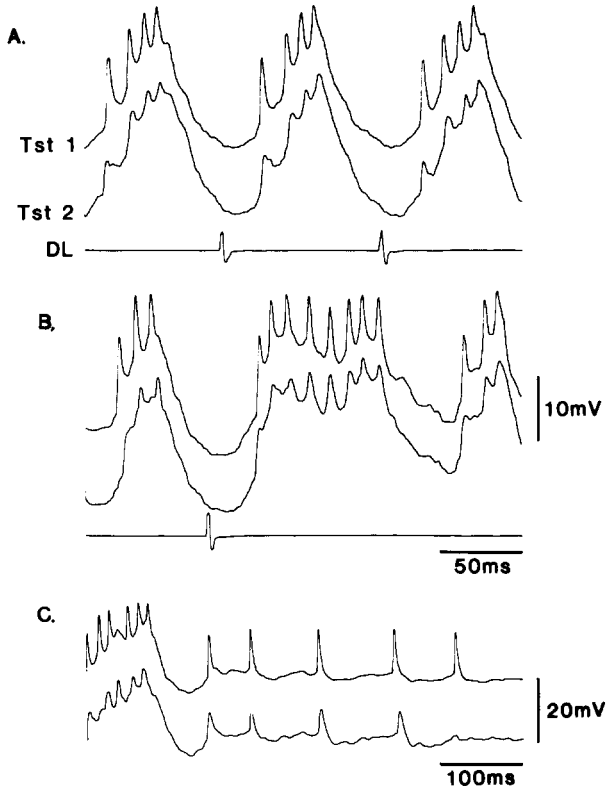

Fig. 1A–C. Synchronous activity of synergistic elevator motoneurons. Simultaneous intracellular recordings from the 2 motoneurons (Tst 1 and 2) innervating the right mesothoracic tergosternal muscle during: **A** a normal flight sequence; **B** a disrupted flight sequence; and **C** the final portion of a flight sequence. Note the concurrence of action potentials in the 2 neurons, especially after rhythmical activity has ceased in **C**. In this and subsequent figures the trace labelled DL is an electromyographic monitor of the time of depressor muscle activity taken from the dorsal longitudinal muscle

only described example of an electrical synapse between neurons in the thoracic ganglia of the locust is one between a metathoracic tarsal levator motoneuron and a supernumerary partner innervating the same muscle (Siegler 1982). One of the locust flight muscles innervated by two motoneurons is the tergosternal muscle (an elevator). Simultaneous recordings from both of the motoneurons innervating a mesothoracic tergosternal muscle showed that their firing patterns were highly synchronous during normal flight sequences (Fig. 1A), during atypical flight cycles (Fig. 1B) and after rhythmicity had stopped at the end of a flight sequence (Fig. 1C). There was a high degree of simultaneous spiking with spikes in one motoneuron occurring within 1 ms of spikes in the other (Fig. 2A for a single burst; Fig. 2B for several flight sequences). Nevertheless, there was no indication of any obvious interaction between the two neurons. There was much common synaptic input to the two motoneurons (Fig. 3A) but neither spontaneous action potentials (Fig. 3B) nor experimental stimulation (Fig. 3C) of one motoneuron had any effect on the membrane potential of the other.

All the flight interneurons described so far are bilaterally repeated with right and left symmetrical partners. Simultaneous recordings from the right and left partners of different mesothoracic flight interneurons showed that they fired synchronous bursts of spikes. In the majority of cases no direct interaction between the two interneurons of a bilaterally symmetrical pair could be demonstrated. This is perhaps not surprising for inhibitory interneurons (e.g. 302, Fig. 4A) but more interesting for excitatory interneurons (e.g. 201, Fig. 4B). In the case of interneuron 701 (Fig. 5), small amplitude (<1 mV) excitatory connections could be demonstrated reciprocally between the right and left partners (e.g.

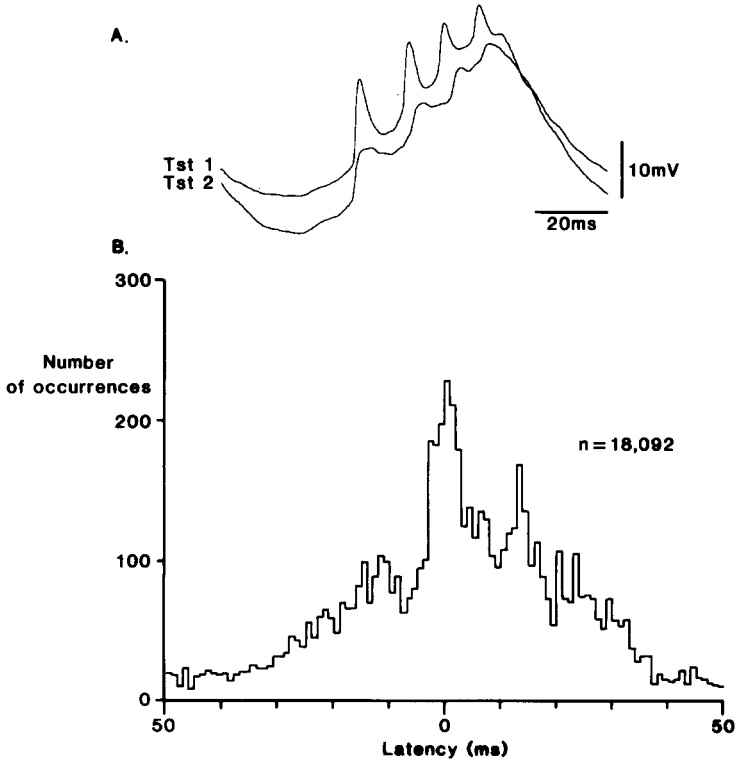

Fig. 2A, B. Cross-correlation analysis of synchrony between synergistic elevator motoneurons. **A** Simultaneous intracellular recordings from the two motoneurons (Tst 1 and 2) innervating the right mesothoracic tergosternal muscle. A single burst during a flight sequence. Note that spikes occurred in each motoneuron almost simultaneously. In both traces the spikes are relatively small due to the recording site being distant from the site of spike initiation. This is more apparent for the Tst 2 trace. **B** Cross-correlogram of latencies to spikes in Tst 2 in the period 50 ms on either side of each spike in Tst 1. Bins are 1 ms in duration. The strong central peak indicates a pronounced synchronous spiking in the 2 motoneurons. Smaller peaks can be seen on either side at times which are determined by the intraburst spiking frequency of the neurons. The extended low peak between 40 ms before and after zero latency arises because the neurons are firing in bursts and its duration is twice the normal burst length. Data were taken from several different flight sequences in different preparations

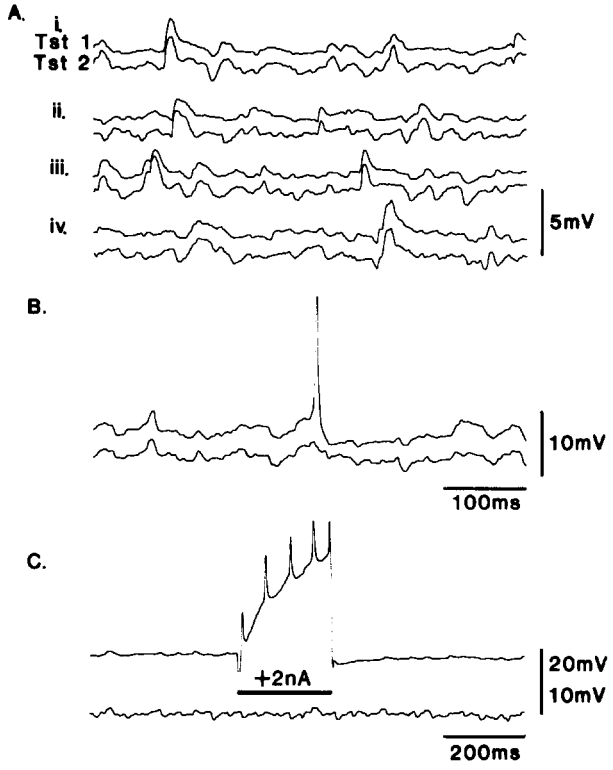

Fig. 3A–C. Common synaptic input to synergistic elevator motoneurons but no direct interaction between them. **A** Simultaneous intracellular recordings from the 2 motoneurons (Tst 1 and 2) innervating the right mesothoracic tergosternal muscle. i–iv are different short sequences demonstrating common synaptic input to the 2 motoneurons when the preparation was not expressing the flight rhythm. Note the strong similarity between the traces from Tst 1 and Tst 2. Slight differences in the relative amplitudes of individual synaptic potentials can be discerned. **B** A spontaneous spike in Tst 1 had no obvious effect on the membrane potential trace of Tst 2. **C** A depolarizing current pulse delivered to Tst 1 causing it to fire a burst of spikes revealed no evidence for electrical or chemical interaction with Tst 2. Duration of the stimulus is indicated by the length of the black bar. Note the artifacts associated with the beginning and end of the stimulus pulse, that the bridge was not balanced and that the amplitude scales for the two neurons are different

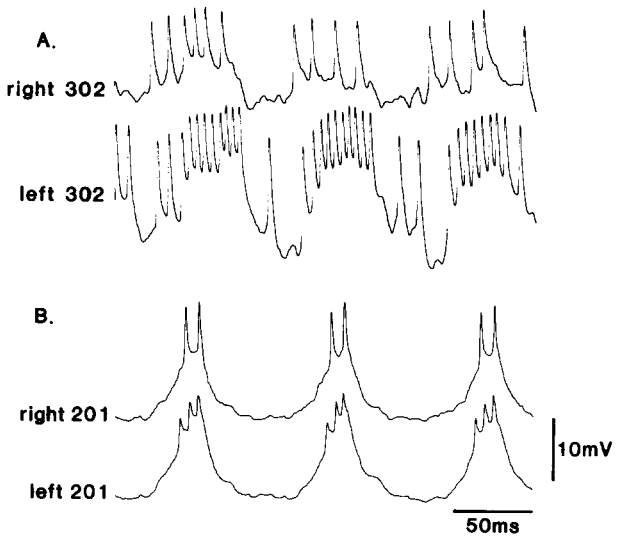

Fig. 4A, B. Synchronous bursting of right and left partners of bilaterally symmetrical pairs of interneurons. **A** Simultaneous intracellular recordings from right and left 302 during a flight sequence. **B** Similarly for right and left 201. Note that although the right and left partners were active in phase there was no indication of spike-for-spike synchrony within bursts

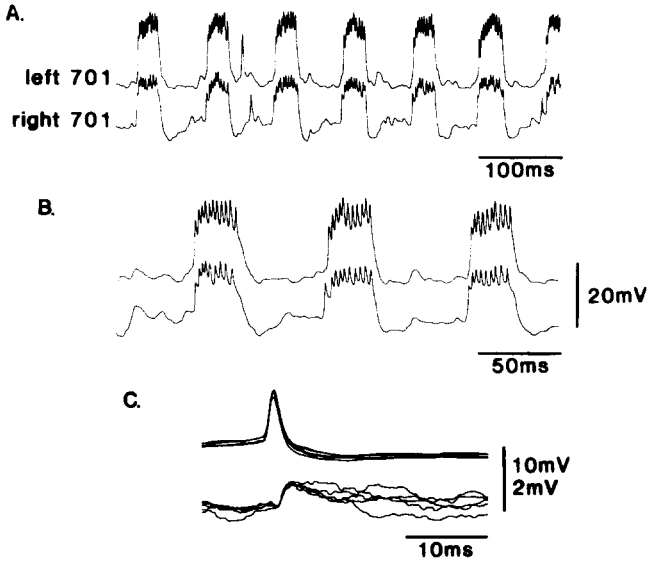

Fig. 5A–C. Synchronous bursting and reciprocal interaction of right and left interneuron 701. **A** Simultaneous intracellular recording from right and left 701 during a flight sequence. **B** Same as **A** at a faster time scale. **C** Spikes in the left 701 were followed after a short (<0.8 ms) and constant latency by small (<1 mV) epsps in right 701. The reciprocal connection was similar (not shown)

Fig. 5C), although not all double penetrations of the two 701s showed these connections. However several observations suggest that the synchronous firing arose as a result of common synaptic input rather than of the reciprocal connections. Successive bursts were outlined by inhibitory input at the beginning and end and this input appeared common to both the right and the left 701. In most cases this only became apparent in the relatively few cases when the inhibitory input was insufficient to prevent spiking completely (Fig. 6A). Although the inhibition sharpening the beginning of each burst was more variable in its occurrence, it is clear that the rapid repolarization at the end of each burst was driven by inhibitory synaptic input. Double penetrations of 301 and 701 showed that a prominent inhibition of 301's membrane potential (arrowhead in Fig. 6C) was concurrent with the rapid repolarization in 701. In slow flight sequences the loss of this prominent inhibition was always accompanied by a loss of the rapid repolarization in 701 (Fig. 6D). Indeed inhibition at this time, concurrent with the end of depressor muscle activity, was common to many flight neurons (not shown). In addition, it is clear that the direct excitatory interaction between the two interneurons was not sufficient to ensure their synchronous bursting because the right and left 701s could be rhythmically active out of phase (Fig. 7).

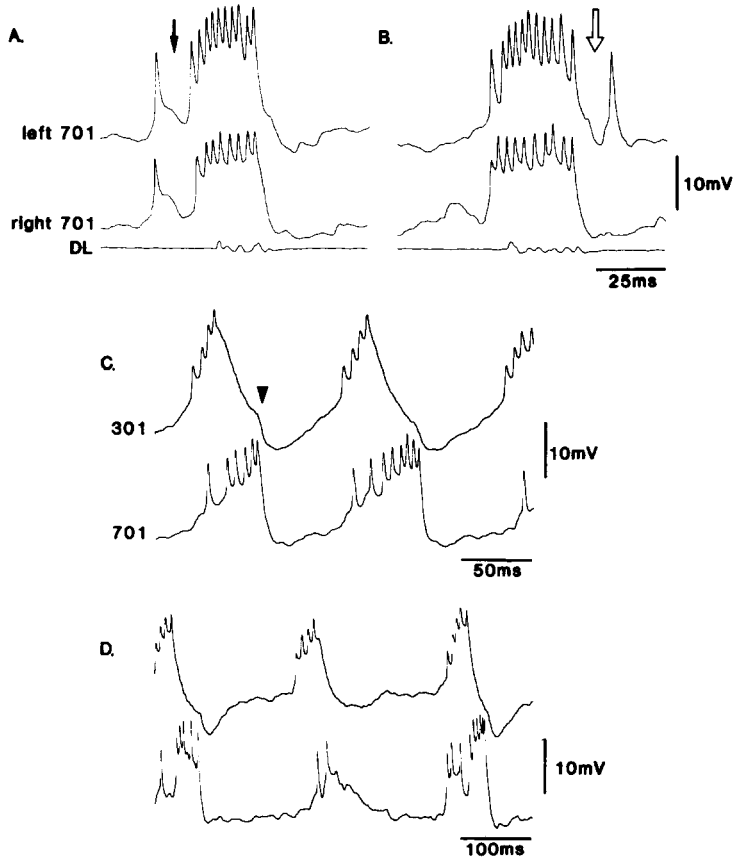

Fig. 6A–D. Common input to the right and left 701. **A** and **B** Recordings from the right and left 701 showing that common inhibitory input occurred at the beginning (filled arrow) and end (open arrow) of each burst. **C** Recordings from 301 and a 701 showing that the rapid repolarization of 701 at the end of a burst was associated with a prominent inhibition of 301 (arrowhead). **D** In slow rhythms loss of this inhibition of 301 (e.g. in second cycle) was accompanied with loss of the rapid repolarization of 701. Note that time scales of **C** and **D** are different

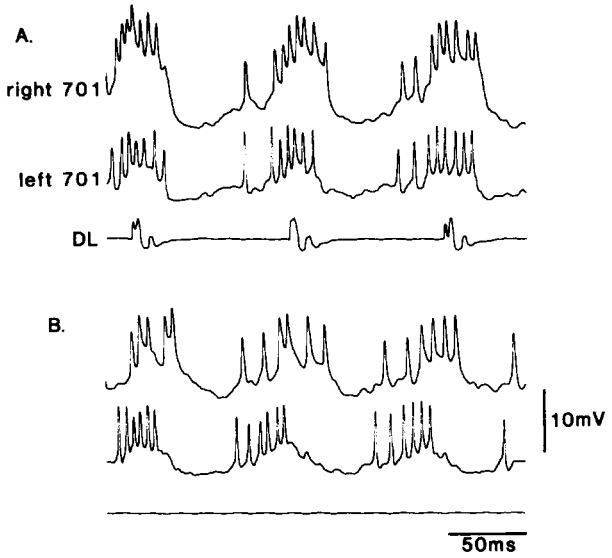

Fig. 7A, B. Reciprocal excitatory interactions between the 701s were not sufficient to ensure synchronous bursting. **A** In a normal flight sequence the bursts in left and right 701 are in phase. **B** Strong rhythmical bursting of the right and left interneurons could be asynchronous. Note that this sequence is additionally abnormal in that the oscillation frequency is higher than in **A**, and the dorsal longitudinal motoneurons were not rhythmically active

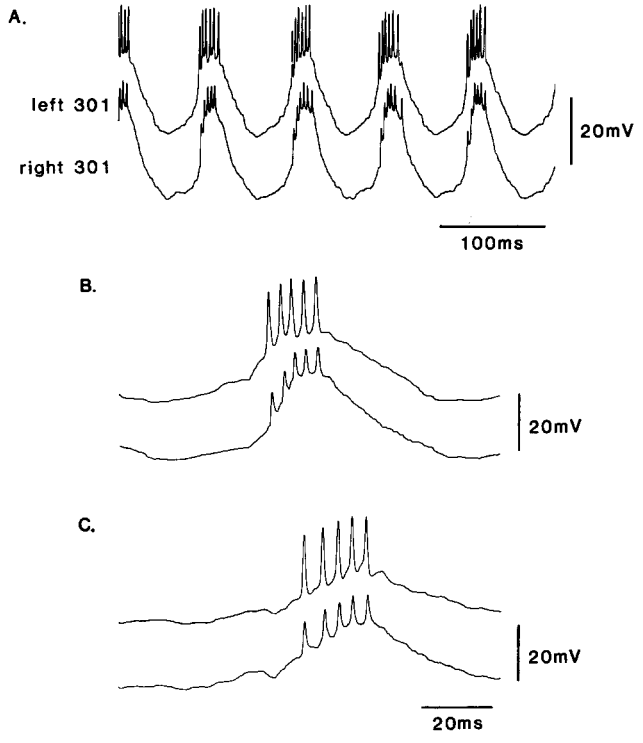

Fig. 8A–C. Synchronous activity of the right and left 301. **A** Simultaneous recordings from the right and left 301 during a flight sequence. **B** and **C** Same as **A** but at a higher sweep speed. **B** was taken from the middle of a flight sequence whereas **C** was taken towards the end of a flight sequence. Note that spikes occur in the 2 neurons within 1 ms of each other

Simultaneous intracellular recordings from the right and left 301s (Fig. 8A) showed a striking degree of synchrony in their firing patterns (Fig. 8B, C). For the first spike in each burst (Fig. 9A) and for all spikes in several

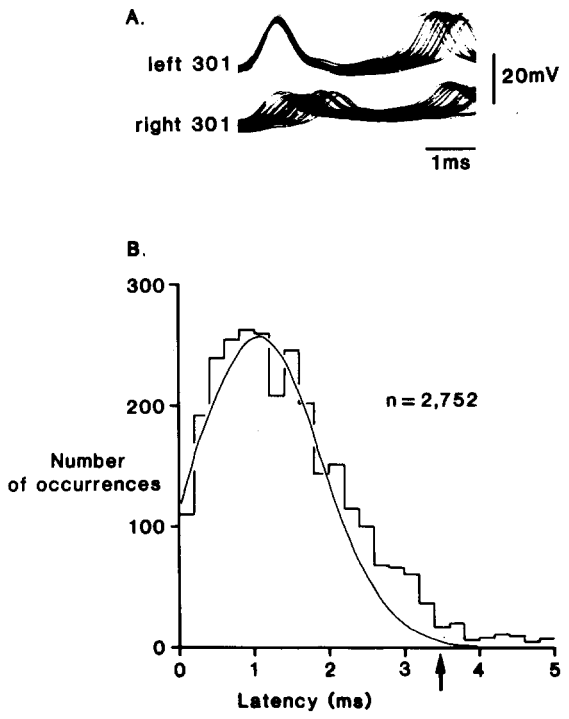

Fig. 9A, B. Characterization of the synchrony between right and left 301. **A** Multiple oscilloscope sweeps triggered off the rising phase of the first spike in different bursts of the left 301. Note the close synchrony with the first spike of the right 301 in the same bursts. **B** Histogram of latencies from spikes in the leading 301 (either left or right) to spikes in the following 301 (either right or left) for several different flight sequences in different preparations. Bin size 0.2 ms. Note the close synchrony such that most spikes in the following 301 occur within 1 ms after spikes in the leading 301. The arrow under the abscissa indicates the mean time of occurrence of the subsequent spike in the leading 301. The solid curve connects the peaks of 0.2 ms bins of a normal distribution of 2,752 values with a mean of 1.15 ms and a standard deviation of 0.85 ms. Note that the distribution of values in the histogram of real values resembles a normal distribution

different flight sequences from different preparations (Fig. 9B) there was a strong tendency for a spike in one 301 to occur within 1 ms of a spike in the other. In different preparations there was no consistent bias for the right or the left 301 to lead, although within a single preparation one 301 consistently led the other. The 301s have been shown to be inhibitory interneurons (Robertson and Pearson 1985; Robertson and Wisniowski 1988) but indirect reciprocal excitatory connections between the two 301s have also been shown (Robertson and Reye 1988). However, these indirect connections had latencies which were too long (> 5 ms, Fig. 10A, B, C) for the connections to underlie the spike-for-spike synchrony.

The neuropil segments of the right and left 301s lie close together in the 5th dorsal commissure of the mesothoracic ganglion and another means by which the two interneurons could directly influence each other would be via ephaptic interactions through their extracellular field potentials (not detected in intracellular recordings referenced to ground). Arguing against this possibility are two observations. First, spikes in the following interneuron were normally distributed (Fig. 9B). Thus there was no indication of a spike in one 301 having a short range influence to advance the time of occurrence of the following spike in the other 301. If there were a short latency influence one might expect that the distribution of latencies to following spikes would be skewed towards the left with a relative lack of latencies following the peak. Second, a more direct demonstration of a lack of direct influence between the two was that short pulses of current capable of disrupting the normal pattern of firing of one 301 had no effect on the firing pattern of the other 301 (Fig. 11A–E).

Discussion

The purpose of the work described here was to investigate the nature and basis of the synchronous activity of flight neurons in the mesothoracic ganglion during the expression of the deafferented flight motor pattern in the locust. Motoneurons which innervate the same muscle, and bilaterally symmetrical interneurons were synchronously active. The tightness of the synchrony ranged from bursting in phase without precise temporal correlation (e.g. interneurons 201), through a situation in which the start and end of bursts in partner neurons occurred at the same time (e.g. interneurons 701), to a tighter, spike-for-spike synchrony (e.g. interneurons 301). In all cases I found that, whatever the extent of

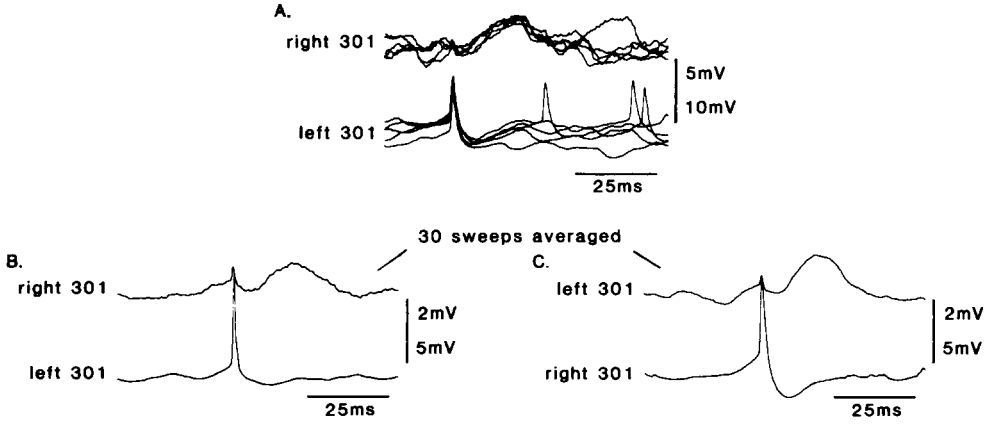

Fig. 10A–C. Delayed reciprocal interactions between right and left 301. **A** Each spike in the left 301 was followed after a relatively long latency (4–6 ms) by a delayed epsp. **B** and **C** Averages of the traces of 30 oscilloscope sweeps triggered from spikes in left 301 **B** and from right 301 **C** indicate that interaction was reciprocal

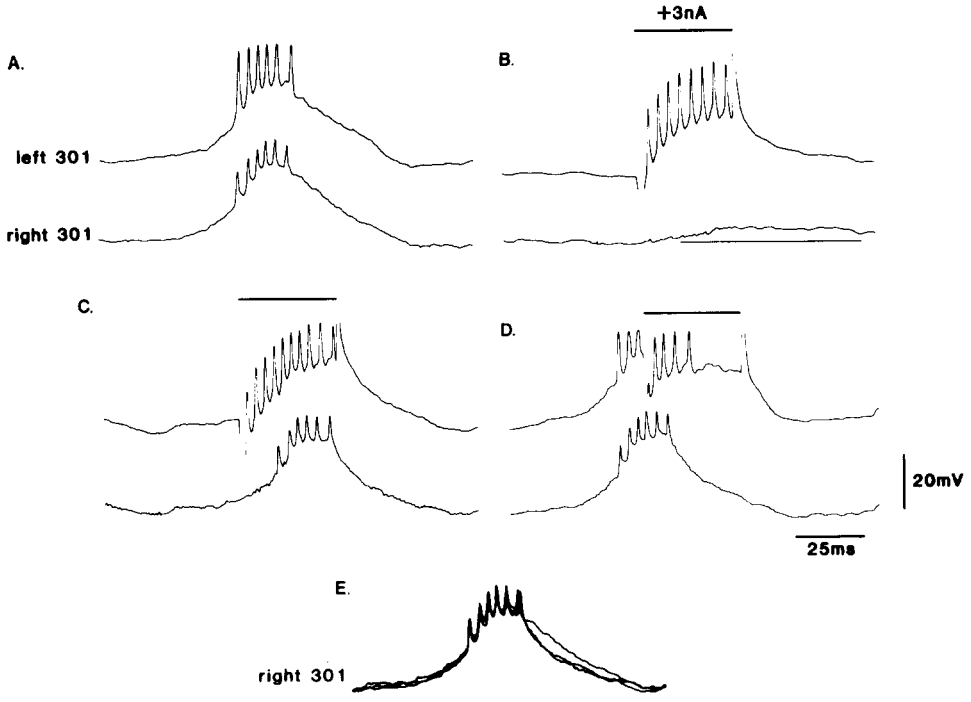

Fig. 11A–E. Perturbation of the firing pattern of one 301 has no effect on the firing pattern of the other 301 during flight sequences. **A** Recordings of left 301 and right 301 during a single flight cycle. **B** A pulse of depolarizing current (duration indicated by the bar) delivered to the left 301 causing it to fire a high frequency burst of spikes had only a moderate slow depolarizing effect on the right 301. The line under the right 301 trace is at the level of the pre-stimulus membrane potential. **C** and **D** The same stimulus delivered to perturb the firing pattern of the left 301 during flight sequences had no obvious effect on the firing pattern of the right 301. This is made clearer in **E** in which the 3 traces of the right 301 taken from **A, B,** and **C** have been overlaid. Note that the stimulus to left 301 had no effect on the firing of right 301

the synchrony, direct interactions between the neurons involved played little or no part in maintaining the synchrony. It is important to distinguish the separate concerns arising from these findings given that the findings were of synchrony between synergists and, separately, of synchrony between bilateral homologues. The first concern is the nature of the processes underlying the synchrony. The second is the possible functional role of spike-for-spike synchrony. The last is the significance of the lack of direct interaction between bilateral homologues with respect to right/left coordination of the wingbeat. These are treated separately below.

As mentioned above, in most cases no direct electrical or chemical psps could be discerned. For the one pair of interneurons (701) where direct chemical psps were demonstrated they were found to be relatively small (<1 mV) and to be variable in their occurrence (i.e. not capable of being demonstrated in all preparations). Also asynchronous bursting could be recorded from these interneurons indicating that the direct reciprocal connections were not capable of establishing synchrony. It was rather unexpected to find that particular neurons exhibited a synchronization that was remarkably precise-spiking within one ms of each other. This was not a widespread phenomenon among the flight neurons investigated. It was observed between the two motoneurons innervating the tergosternal muscle and between the right and left 301s. It is important to consider whether it was a real phenomenon or an artifact of prejudiced observation, especially given the lack of any evidence that the precise synchrony was directly controlled. The appearance of relatively tight coupling between the spikes of two neurons that are active in phase with bursts containing relatively few spikes at high intraburst frequency could be illusory. However, the facts, first that this type of firing could be attributed only to particular neurons and not to other flight neurons even though the latter had similar burst parameters, and second that the correlation analyses showed the phenomenon to be a robust property of these neurons for numerous cycles, in several different preparations, argue that it is indeed a real phenomenon. Accepting that, the closeness of the spike-for-spike synchrony suggested that it might be mediated by direct interactions between the relevant neurons. Spike-transmitting chemical (Auerbach and Bennett 1969; Rind 1984) and electrical (Watanabe and Grundfest 1962) synapses have been described. However, in the situation under discussion particularly powerful synapses may not be necessary because the membrane potentials of the relevant neurons would be already around threshold and a moderate direct interaction might be sufficient to trigger synchronous spikes. Other possibilities for interaction would include electrical connections and ephaptic interactions through the extracellular field potentials (e.g. Taylor and Dudek 1982; Traub et al. 1985; Yim et al. 1986). All the above possibilities can be excluded by the observations that no short latency postsynaptic potentials (psps) were observed either following spontaneous spikes or in response to electrical stimulation, and electrical stimulation to perturb the pattern of firing of one of a pair during flight sequences had no effect on the firing pattern of its partner. It must be concluded that the strict spike-for-spike synchrony that was observed was not a consequence of any direct interaction between the neurons.

Burrows (1975) had already demonstrated that flight motoneurons receive common psps although it is not certain whether the presynaptic neurons can be described as flight neurons. This study confirms his observations of extensive common psps in motoneurons when the preparation is not expressing a flight rhythm. Further-

more, the membrane potential traces of flight interneurons also showed common postsynaptic potentials. The above observations lead one to the conclusion that synchronous activity, even remarkably precise synchrony, in these flight neurons is most likely a consequence of common synaptic input.

It is clear that neurons with the same role such as bilaterally symmetrical interneurons and synergistic motoneurons should fire at the same phase. However, adaptive explanations for the precise timing mentioned above must remain speculative at the moment. Some bilaterally symmetrical neurons are presynaptic to the same neuron or neurons (Robertson, unpublished) and perhaps their combined effect might be greater if the occurrence of their presynaptic spikes was tightly coupled. Similar arguments could apply to the motoneurons supplying the same muscle. Moreover, the timing of muscular activity is critical for steering manoeuvres. Although shifts of 12–13 ms (Baker 1979) have been described, significant changes in the timing of electromyograms of 0.1 ms (Thüring 1986), between 1 and 2 ms (Zarnack and Möhl 1977), and around 3 ms (Elson and Pflüger 1986) have been correlated with steering manoeuvres. Correct timing of the spiking activity of two motoneurons supplying the same muscle would be important to avoid unintended shifts in the timing of muscle contractions.

The coordinated firing of bilaterally symmetrical motoneurons in the deafferented preparation must result from interactions between interneurons because the branches of motoneurons of the right and left do not overlap and direct interaction between them is impossible. The possibility that bilaterally symmetrical interneurons interact directly is not supported by the present results. That direct chemical interaction had no part to play in the coordinated firing of bilaterally symmetrical interneurons was not surprising for interneurons that are known to be inhibitory (e.g. 301, 302, Robertson and Pearson 1983). Also direct electrical interactions are relatively rare in insects (Robertson 1987) and for the locust thoracic nervous system have been described only in an anomalous situation (Siegler 1982). However, it was rather more surprising to find that direct chemical synaptic interactions had very little part to play in the coordinated firing of excitatory bilaterally symmetrical interneurons (e.g. 201, 701) in the deafferented preparation. Nevertheless, these results on the central circuitry operating in the deafferented preparation do fit well with what is known of the coordination of the right and left wings in intact animals. Möhl (1985, 1988) has shown that precise coordination of the electromyographic activity underlying the right and left wingbeat is dependent upon proprioceptive feedback. Also he proposes that the interaction of the periphery with the central circuitry is plastic and their conjoint operation is modifiable depending on prevailing internal and external conditions (Möhl 1988). Similar flexibility is necessary for the generation of steering manoeuvres which rely on differential activity across the animal (Zarnack and Möhl 1977; Baker 1979). Such flexibility of output in the intact animal can best be served by central circuitry with minimal constraints on the output pattern. In addition, the results here confirm a prediction which might have been made from the work of Ronacher et al. (1988). They found that longitudinal hemisection of the mesothoracic ganglion had no overt effect on flight ability or on the coordination of the 4 wings. Thus it could have been predicted that coupling between interneurons of the right and left sides of the mesothoracic ganglion would have little role to play in maintaining a coordinated flight motor pattern. It seems rather that appropriate timing arises as a result of multiple interactions among numerous interneurons.

The results described here were concerned with flight neurons in the mesothoracic ganglion. It is becoming increasingly obvious that the metathoracic ganglion has a more significant role in generating flight patterns. Longitudinal hemisection of this ganglion effectively disrupts the operation of the flight circuitry (Ronacher et al. 1988; Wolf et al. 1988). The fused neuromeres of the metathoracic ganglion contain sets of serially homologous, bilaterally symmetrical interneurons which have been shown to be important in generating flight rhythms (Robertson and Pearson 1983). The interactions between such neurons may be much more significant for the generation and coordination of flight patterns and the obvious next step is to continue this investigation in the metathoracic ganglion.

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References

Auerbach AA, Bennett MVL (1969) Chemically mediated transmission at a giant fibre synapse in the central nervous system of a vertebrate. J Gen Physiol 53:183–210

Baker PS (1979) The role of forewing muscles in the control of direction in flying locusts. J Comp Physiol 131:59–66

Burrows M (1975) Co-ordinating interneurones of the locust which convey two patterns of motor commands: their connexions with flight motoneurones. J Exp Biol 63:713–733

Elson R, Pflüger H-J (1986) The activity of a steering muscle in flying locusts. J Exp Biol 120:421–441

Gardner D (1971) Bilateral symmetry and interneuronal organisation in the buccal ganglia of *Aplysia*. Science (NY) 173:550–553

Gras H, Hörner M (1985) A program for the time control and measurement in electrophysiological experiments with an Apple II microcomputer. J Electrophysiol Tech 12:197–208

Hagiwara S, Morita H (1962) Electrotonic transmission between two nerve cells in leech ganglion. J Neurophysiol 25:721–731

Kandel ER, Tauc L (1966) Input organization of two symmetrical giant cells in the snail brain. J Physiol (Lond) 183:269–286

McCrohan CR, Winlow W (1985) Interganglionic coordination and bilateral symmetry in the nervous system of gastropod molluscs. In: Bush BMH, Clarac F (eds) Coordination of motor behaviour. Cambridge University Press, Cambridge UK, pp 33–62

Möhl B (1985) The role of proprioception in locust flight control. I. Asymmetry and coupling within the time pattern of motor units. J Comp Physiol A 156:93–101

Möhl B (1988) Short-term learning during flight control in *Locusta migratoria*. J Comp Physiol A 163:803–812

Pearson KG, Robertson RM (1987) Structure predicts synaptic action of two classes of interneurons in locust thoracic ganglia. Cell Tissue Res 250:105–114

Pearson KG, Wolf H (1987) Comparison of motor patterns in the intact and deafferented flight system of the locust. I. Electromyographic analysis. J Comp Physiol A 160:259–268

Pearson KG, Wolf H (1988) Connections of hindwing tegulae with flight neurones in the locust, *Locusta migratoria*. J Exp Biol 135:381–409

Reichert H, Rowell CHF (1985) Integration of nonphaselocked exteroceptive information in the control of rhythmic flight in the locust. J Neurophysiol 53:1201–1218

Reichert H, Rowell CHF (1986) Neuronal circuits controlling flight in the locust: how sensory information is processed for motor control. Trends Neurosci 9:281–283

Reye DN, Pearson KG (1987) Projections of the wing stretch receptors to central flight neurons in the locust. J Neurosci 7:2476–2487

Rind FC (1984) A chemical synapse between two motion detecting neurones in the locust brain. J Exp Biol 110:143–167

Robertson RM (1986) Neuronal circuits controlling flight in the locust: central generation of the rhythm. Trends Neurosci 9:278–280

Robertson RM (1987) Insect neurons: synaptic interactions, circuits and the control of behavior. In: Ali MA (ed) Nervous Systems of Invertebrates. Plenum Press, New York, pp 393–442

Robertson RM, Pearson KG (1982) A preparation for the intracellular analysis of neuronal activity during flight in the locust. J Comp Physiol 146:311–320

Robertson RM, Pearson KG (1983) Interneurons in the flight system of the locust: distribution, connections and resetting properties. J Comp Neurol 215:33–50

Robertson RM, Pearson KG (1985) Neural circuits in the flight system of the locust. J Neurophysiol 53:110–128

Robertson RM, Reye DN (1988) A local circuit interaction in the flight system of the locust. J Neurosci 8:3929–3936

Robertson RM, Wisniowski L (1988) GABA-like immunoreactivity of identified interneurons in the flight system of the locust, *Locusta migratoria*. Cell Tissue Res 254:331–340

Ronacher B, Wolf H, Reichert H (1988) Locust flight behavior after hemisection of individual thoracic ganglia: evidence for hemiganglionic premotor centers. J Comp Physiol A 163:749–759

Satterlie RA (1985) Reciprocal inhibition and postinhibitory rebound produce reverberation in a locomotor pattern generator. Science (NY) 229:402–404

Siegler MVS (1982) Electrical coupling between supernumerary motor neurones in the locust. J Exp Biol 101:105–119

Stevenson PA, Kutsch W (1987) A reconsideration of the central pattern generator concept for locust flight. J Comp Physiol A 161:115–129

Stuart AE (1970) Physiological and morphological properties of motoneurones in the central nervous system of the leech. J Physiol (Lond) 209:627–646

Taylor CP, Dudek FE (1982) Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. Science (NY) 218:810–812

Thüring DA (1986) Variability of motor output during flight steering in locusts. J Comp Physiol A 158:653–664

Traub RD, Dudek FE, Taylor CP, Knowles WD (1985) Simulation of hippocampal afterdischarges synchronized by electrical interactions. Neurosci 14:1033–1038

Tyrer NM, Altman JS (1974) Motor and sensory flight neurones in a locust demonstrated using cobalt chloride. J Comp Neurol 157:117–138

Watanabe A, Grundfest H (1962) Impulse propagation at the septal and commissural junctions of crayfish lateral giant axons. J Gen Physiol 45:267–308

Wilson DM (1961) The central nervous control of flight in a locust. J Exp Biol 38:471–490

Wolf H, Pearson KG (1987) Comparison of motor patterns in the intact and deafferented flight system of the locust. 2. Intracellular recordings from flight motoneurons. J Comp Physiol A 160:269–279

Wolf H, Ronacher B, Reichert H (1988) Patterned synaptic drive to locust flight motoneurons after hemisection of thoracic ganglia. J Comp Physiol A 163:761–769

Yim CC, Krnjevic K, Dalkara T (1986) Ephaptically generated potentials in CA1 neurons of rat's hippocampus in situ. J Neurophysiol 56:99–122

Zarnack W, Möhl B (1977) Activity of direct downstroke flight muscle of *Locusta migratoria* (L.) during steering behaviour in flight. I. Patterns of time shift. J Comp Physiol 118:215–233