The Influence of Wingbeat Synchronous Feedback on the Motor Output Systems in Flies

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Action potentials have been recorded from fibrillar and from non-fibrillar flight muscles of tethered flying flies *(Calliphora erythrocephala* and *Musca domestica)*.

Analyses of the spike-trains from the fibrillar muscles reveal a clear preference of the spikes to appear at a special phase with respect to the wingbeat cycle. This holds true even in cases of experimentally changed motor output patterns. There seems to be some kind of wingbeat-synchronous feedback which influences the output system phasically.

Crosscorrelations between spike-trains from the fibrillar muscles on the one hand and the non-fibrillar muscles on the other give evidence that there are strong interactions between the output producing neurons of both systems with at least one inhibitory pathway.

Introduction

It has been shown by several workers, that the output pattern of the flight motor neurons in insects is produced endogeneously in the CNS and that the timing of the output pulses is not dependent on sensory feedback. This has been stated both for neurogenic fliers (locusts: Wilson [1], Waldron [2], — moths: Kammer [3], Hanegan [4]) and for myogenic fliers (flies, fibrillar flight muscle system: Wilson and Wyman [5], Wyman [6-8], Mulloney [9], Levine [10]).

There are, however, other findings which give evidence that rhythmical afferences are able to influence the output pattern of the motoneurons phasically (bees, fibrillar flight muscle system: Bastian and Esch [11], — flies, non-fibrillar flight muscle system: Heide [12], — locusts: Wendler [13]).

In the present paper the non-fibrillar flight muscles are referred to as N-muscles, and the fibrillar flight muscles as F-muscles. Because there is a strong tendency of the spikes from N-muscles in flies to be phase-locked with the rhythmic wing movements, a further investigation has been done to search for phase preferences of the spikes from the flies' F-muscles with respect to the wing-beat. In addition, it has been determined if there are any interactions between the output producing neurons of the F-muscle system on the one hand and the output producing neurons of the N-muscle system on the other hand.

Methods

Recordings of action potentials have been made from units of the fibrillar dorsoventral muscles dvm1 and dvm2 and from the non-fibrillar steering muscles b1 and III1 of tethered flying dipterans *(Calliphora erythrocephala* and *Musca domestica)*. The N-muscles are numbered according to an anatomical study on *Calliphora* (Heide [14]). The recordings were performed using non insulated tapered metal electrodes (tip diameter about 10 μm) which were placed into a muscle, the long axis of the electrode parallel to the muscle fibres.

Some of the N-muscles of flies are active only during turning reactions (Heide [15, 12]). In order to study the activity of these muscles, turning reactions have been elicited by means of a striped pattern which was moving with constant velocity in front of the fly. (Fixed boundary-moving pattern, after Bishop, Keehn, and McCann [16].)

Because *Calliphora* is a bad flier, some flights of this species have been elicited by electrical stimulation of the fly's brain (Heide [12]). In most cases the stimulating current is turned off a few seconds after the flight has been initiated. In order to be able to record the action potential when the stimulation current is on (prolonged stimulation) the stimuli have been fed to the stimulation electrodes via a stimulus isolation unit (Monsanto opto-isolator MCT 2).

The swinging wing of the flying fly was crossing a light beam which was mounted perpendicular to the stroke plane in a fixed position relative to the fly. The light beam thus marks a fairly constant phase with respect to the wingbeat cycle. Every second impulse coming from the photodiode which

sensed the light beam had to be eliminated because of the fact that the swinging wing was crossing the light beam twice in a cycle.

The action potentials, the pulses from the photodiode and the pulses which control the movements of the striped pattern have been stored on tape. From the tape all electrical events have been played back to a threshold detector and from there to an interface which digitized the time of the occurence of an event, which is the time when the rising edge of an analog signal crosses a given threshold of the detector. The digitized data have been transmitted to the discs of an IBM 360-44 Computer. Data analyses were done by use of the Biological Data Processing System of the California Institute of Technology (Lockemann and Knutsen [17]).

Relations between pulse-trains have been studied by means of post-stimulus time histograms (PSTs) and by cross-correlations (Moore *et al.* [18]). In the PSTs the value on the ordinate gives the number of pulses per second for a given lag time with respect to the reference-pulses at time 0. The reference-pulses mark the start of the pattern movement in a given direction. In the cross-correlation histograms the value on the ordinate gives the number of occurences (OCC) of action potentials for a given lag time with respect to the occurences of reference-pulses, the latter appearing at time zero in the plots. (Actually lag time means lag bin of resolution width.) In the figures, a cross-correlation is simply indicated as cross-correlation of X-spikes relative to Y-pulses.

Results

The presented results are computed from 35 flights of 10 blowflies and from 14 flights of 7 houseflies. The mean wingbeat frequencies of the flights has been taken from interval histograms of the impulses coming from the photodiode which sensed the light beam. Wingbeat frequencies for different specimens are in the range of $105 - 164$ Hz for *Calliphora* and of $131 - 164$ Hz for *Musca*.

1. Remarks to the basic output pattern: Action potentials up to 82 mV positive in sign have been recorded from the F-muscles. The spikes from N-muscles reach amplitudes up to 15 mV. With regard to the spike trains from the F-muscles the ratio of the mean interspike interval to the mean wingbeat period varies in different recordings from $9.1 : 1$ to $18.8 : 1$ in *Calliphora* and from $5.7 : 1$ to $9.7 : 1$ in *Musca*. The distribution of the spike intervals in an interval histogram is roughly bell-shaped in

cases of smooth flights. The N-muscles most often fire once in a wingcycle or once in every second wingcycle.

The spike frequency in the F-muscles increases very often when a turning reaction is induced. This can be seen in PST-histograms in which the frequency of the dvm-spikes is plotted against time (Fig. 1). The increase in the spike frequency is a
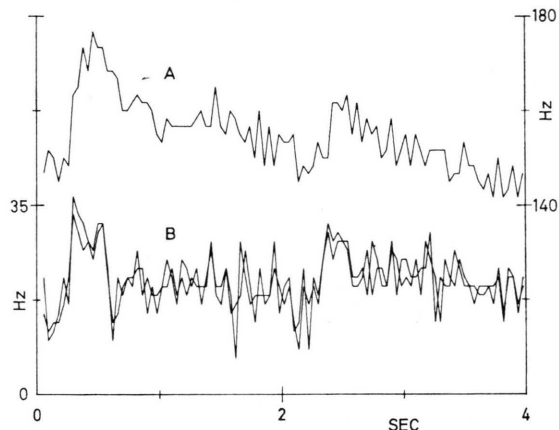

Fig. 1. PST of the wingbeat frequency (A) and the spike frequencies of the left and right dvm1 (B) from *Musca* during induced turning reactions. The values on the right ordinate are for A, the values on the left ordinate are for B. A striped pattern is repeatedly moving in front of the fly, 2 sec from right to left inducing a left turn reaction and subsequently 2 sec from left to right inducing a right turn reaction. The values from the frequencies are averaged over 15 left turn reactions and 15 right turn reactions. Irrespective of the sign of the turning reactions the spike frequencies of the right and left dvm1-muscle are about the same or are changing in the same direction. — One flight, 9919 wingbeats, 1362 spikes from the unit of the left dvm1, 1341 spikes from the unit of the right dvm1.

transient phenomenon occuring immediately after the moment when the induced turning reaction changes its sign. In the same preparation the N-muscle III1 is active during the whole time when the striped pattern is moving in a given direction (Fig. 2). During induced turning reactions changes of the spike frequencies of the dvm1-muscles of both sides of the thorax are about the same confirming the results of Smyth and Yurkiewicz [19], Nachtigall and Wilson [20] and Heide [15, 12], that the F-muscles of flies do not control yawing. Changes in the dvm1-spike frequency are positively correlated with changes in wingbeat frequency which is in accordance with the findings of Wilson and Wyman [5] and Nachtigall and Wilson [20].

2. Distribution of the occurences of dvm-spikes with respect to the wingbeat period: Preferences of
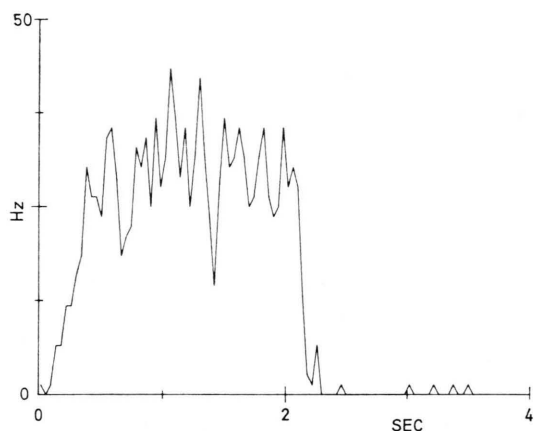
Fig. 2. PST of the spike-frequency of the right IIIl from the same preparation as in Fig. 1 during induced turning reactions. Pattern movement as in **Fig. 1**. The values for the frequencies are averaged over 16 left turn reactions and 16 right turn reactions. The right IIIl is active only during turning reactions to the left. The computation yielded rather low values for the averaged spike frequencies because during some of the turns the muscle reacted only sporadically. — One flight, 10974 wingbeats, 976 spikes from the right IIIl.

the action potentials to appear at a special phase within the wingbeat cycle have been checked by means os cross-correlation-computations. As can be seen from Figs 3 and 4 action potentials appear at
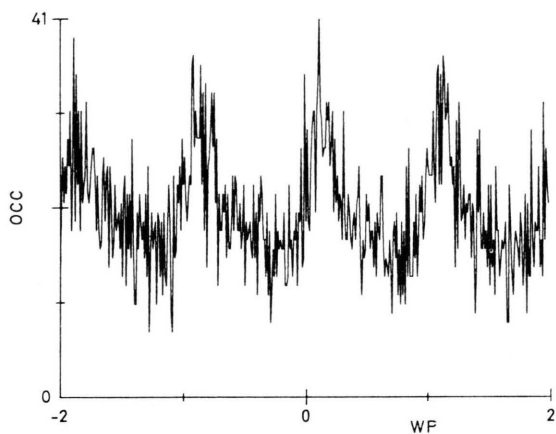

Fig. 3. Crosscorrelation of dvml-spikes from *Calliphora* relative to the pulses of the wingbeat sensing photodiode. The time axis is scaled in mean wingbeat periods (WP). One peak in every cycle indicates increased probability of occurence of a dvml-spike. — 3 added flights with no induced turning reactions, 24490 wingbeats, 2031 spikes from a unit of the right dvml.

any possible phase within the wingbeat cycle, but there is a clear phase preference indicated by one peak in every cycle. Such phase preferences have been found in most dvm-spike trains which have
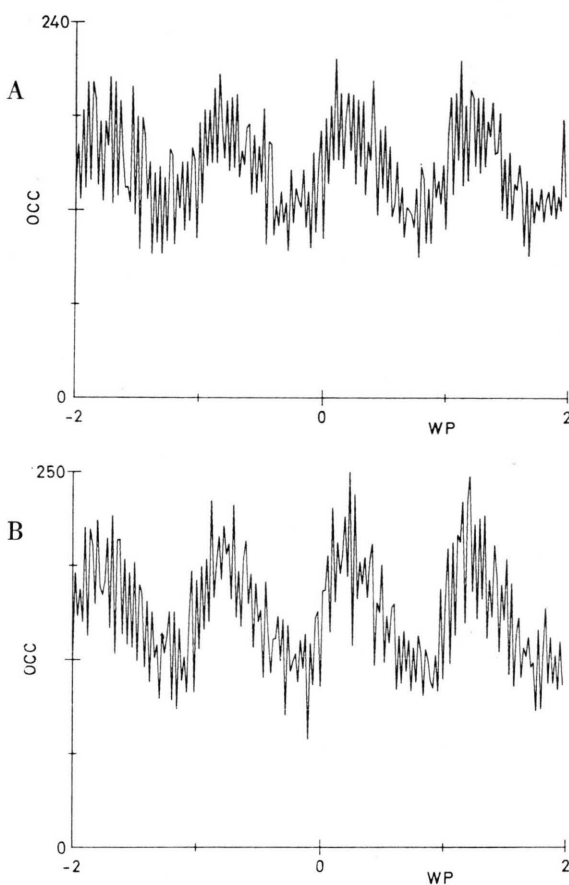

Fig. 4. Crosscorrelations of dvml-spikes from *Musca* relative to the pulses of the wingbeat sensing photodiode. The time axis is scaled in mean wingbeat periods (WP). Both correlations are for simultaneously recorded spike-trains from a unit of the left dvml (A) and from a unit of the right dvml (B). They both show one peak at the same phase in every cycle indicating increased probability of occurence of the spikes at this phase. — One flight with repeatedly induced turning reactions, 44251 wingbeats, 7284 spikes from the unit of the left dvml, 7744 spikes from the unit of the right dvml.

been recorded from *Calliphora* and from *Musca*. Similar results are obtained from flies which are flying straight ahead and from flies which are flying under the conditions of induced turning reactions. Simultaneously recorded spike trains from the right and left dvml-muscle of a fly both show a peak at the same phase in the cycle (Fig. 4). This holds true even in cases of no synchrony between the spike trains themselves.

In 13 cases out of 47 the mentioned phase preferences are very weak or absent. Among simultaneously recorded spike trains there is one case showing a weak phase preference in the recording

from the left dvml but there is none in the recording from the right dvml.

Additional experiments have been done to look for preferences of the dvml-spikes to appear at a special phase in the wingcycle when the motor output pattern is changed experimentally. The output pattern can be changed in a predictable manner when the brain of a fly is stimulated with pulse trains from a pulse generator (4V-pulses, 0.5 ms in duration). During stimulation the action potentials in the F-muscles are fairly well synchronized with the stimulating pulses as long as the stimulation frequencies are in the range of $20-50$ Hz. The results shown in Figs 5 A and 5 B are obtained with a stimulation frequency of 46 Hz. Even under these conditions the cross-correlograms reveal again striking phase preferences of the spikes with respect to the wingcycle (Fig. 5 C).

3. Relations between spike trains which are recorded simultaneously from F-muscles and from

N-muscles: In the preceding section it has been shown that the spikes from the F-muscles accumulate more frequently at particular phases of the wingcycle than at others. Moreover it is known that there is a rather strong tendency of the action potentials from some N-muscles to be phase-locked with respect to the wingcycle (Fig. 6 and Heide [12]). These facts suggest that there might be some kind of coupling between the motoneurons of both flight muscle systems.

Cross-correlations between spike trains from both systems reveal the following results (Figs 7 and 8): A trough near the origin indicates a time interval of a reduced number of coincidences of action potentials which arrive in the F-muscle and in the N-muscle respectively. Because of the $1:1$ relation between nerve spikes and muscle action potentials, the exclusion zone reflects an interaction between the output producing neurons with at least one inhibitory pathway.
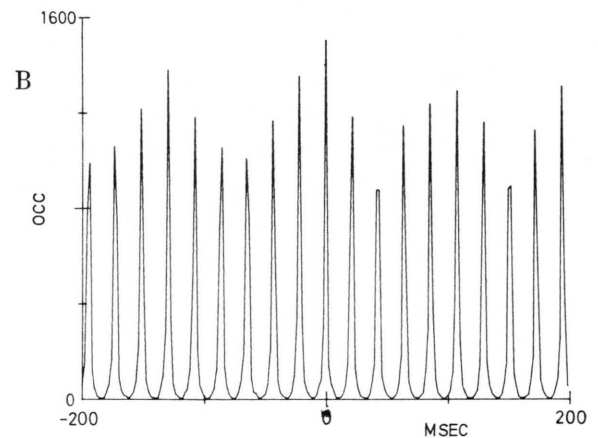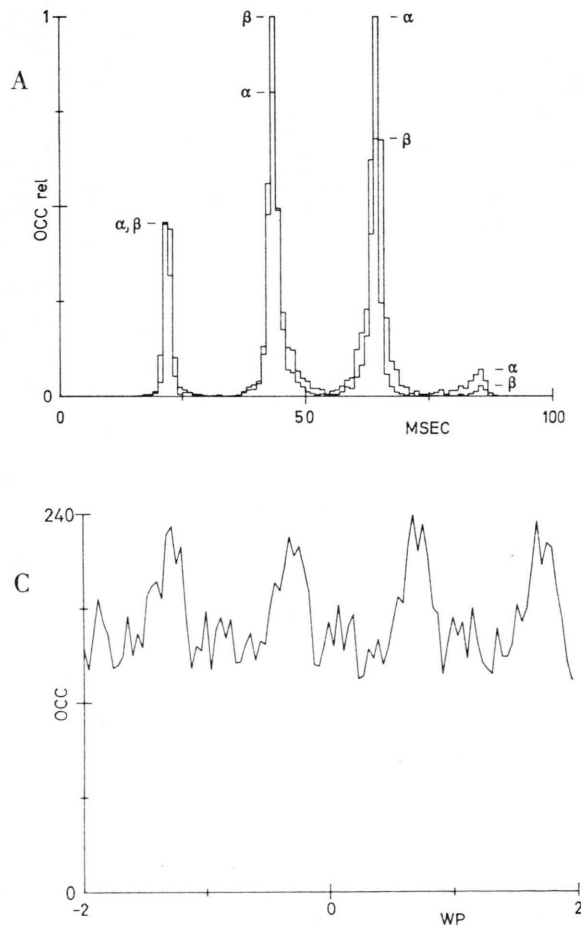

Fig. 5. A: Interspike-interval histogram of spikes from a unit of the right dvml (α) and a unit of the right dvm2 (β) from *Calliphora*. Ordinate: Relative number of occurences. The spike-trains have been recorded simultaneously from both units when the brain of the flying fly was stimulated with pulse trains from a pulse generator (46 Hz). The interspike-intervals are 1 to 3 times as large as the stimulus period.

B: Crosscorrelation of the dvm2-spikes relative to the dvml-spikes. Same data set as in A. As a result of the rhythmic stimulation of the fly's brain both units fire in strong synchrony.

C: Crosscorrelation of the dvml-spikes relative to the pulses of the wingbeat sensing photodiode. Same data set as in A. The time axis is scaled in mean wingbeat periods (WP). Again there is one peak in every cycle indicating increased probability of occurence of a dvml-spike at this phase of the cycle. (The dvm2-spikes show less preference to appear at a special phase with respect to the cycle.)

A—C: One flight, 35139 wingbeats, 4349 spikes from the unit of the right dvml.
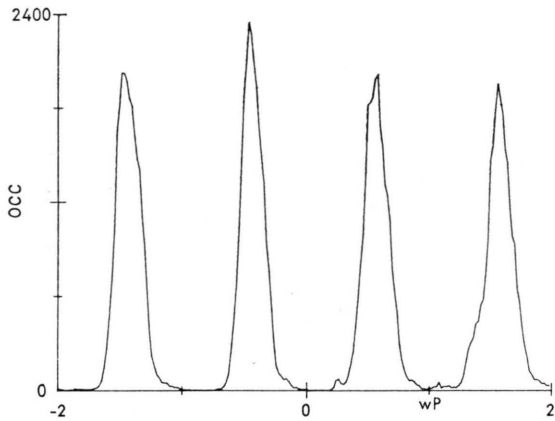
Fig. 6. Crosscorrelation of b1-spikes from *Calliphora* relative to the pulses of the wingbeat sensing photodiode. The time axis is scaled in mean wingbeat periods (WP). The b1-spikes appear strongly phase-locked with respect to the wingbeat cycle. — One flight with no induced turning reactions, 23902 wingbeats, 23824 spikes from right b1.
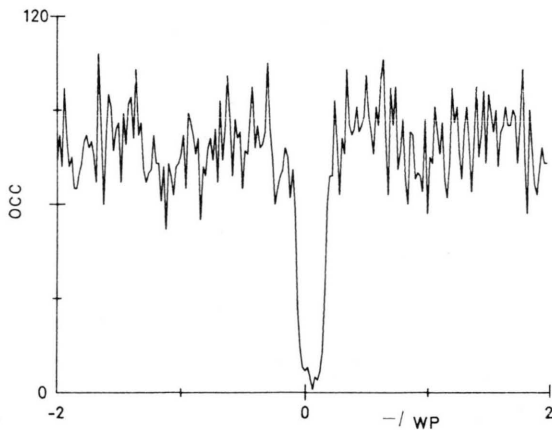

Fig. 8. Crosscorrelation of III1-spikes relative to dvm1-spikes from *Musca*. The time axis is scaled in mean wingbeat periods (WP). An exclusion zone near the origin indicates an inhibitory coupling between the output producing neurons which supply the III1 and the dvm1-unit respectively. — One flight with repeatedly induced turning reactions, 44251 wingbeats, 4725 spikes from right III1, 7284 spikes from left dvm1.
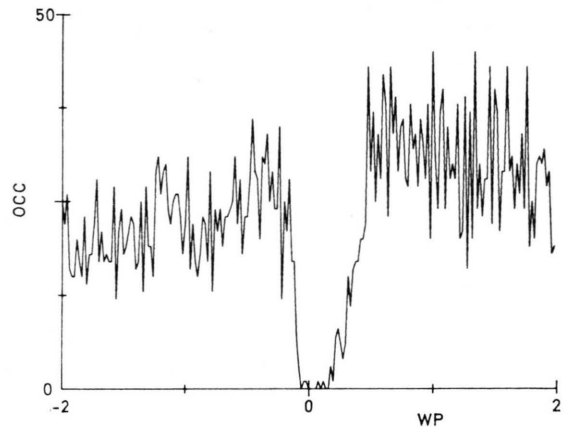

Fig. 7. Crosscorrelation of b1-spikes relative to dvm1-spikes from *Musca*. The time axis is scaled in mean wingbeat periods (WP). An exclusion zone near the origin indicates an inhibitory coupling between the output producing neurons which supply the b1 and the dvm1-unit respectively. — One flight, about 26500 wingbeats, about the same number of spikes from right b1, about 3100 spikes from left dvm1.

Up to now this type of interaction between the output systems of the F-muscles and the N-muscles has been found for the following muscle combinations: Left dvm1 — right b1, left dvm1 — right III1, right dvm1 — right III1. The depth of the trough varies in different computations of spike-trains from each choosen combination.

Discussion

The contraction frequency of the F-muscles and hence the wingbeat frequency of a flying fly is de-

termined by the mechanical properties of the vibrating thorax (review: Pringle [21]). One action potential occuring in an F-muscle fibre enables the fibre to contract at least 10-times in *Calliphora* and 6-times in *Musca* (p. 740). There is no need for the action potentials to occur in phase with the movements of the wings.

According to the work of Wyman the output pattern in flies is generated by the CNS in the way that the output units share common excitatory input and that some of these units are coupled by lateral inhibition. "The timing of events in the output pattern is not dependent on sensory feedback" (Wyman [8]).

However the results presented in the present paper give evidence that the spikes from the F-muscles do occur with particular phase preferences with respect to the wingbeat cycle: There seems to be some kind of wingbeat-synchronous feedback which influences the output producing system phasically.

For N-muscles of flies it has already been shown that wingbeat-synchronous afferences have a phase-locking effect on the output producing neurons (Heide [12]). The present results give evidence for strong couplings between the output patterns of the systems which supply the F-muscles and the N-muscles respectively. The question therefore is whether both output systems are influenced directly by wingbeat-synchronous afferences or whether the

phase preferences in the output pattern of the F-muscles is merely a consequence of the interactions between the output producing neurons of both muscle systems.

An exclusion zone in a cross-correlation may be caused by monosynaptic or polysynaptic inhibitions or by shared inhibition-excitation (Moore et al.[18]). If one speculates about the possible interactions between the motor output systems in flies one has to consider the fact that the frequency of the pulses which arrive at the N-muscles is about 10-times the frequency of the pulses which arrive at the F-muscles. Thereby the N-muscle spikes are more or less synchronous with wingbeat and fire with about wingbeat frequency. If the pulses from the output producing neurons of the N-system inhibit the output producing neurons of the F-system then the pulses of the F-system will be forced to appear between two pulses of the N-system. Consequently the spikes in the F-muscles will accumulate at preferred phases with respect to the wingcycle. The same output pattern will be produced if wingbeat synchronous afferences excite the output producing neurons of the N-system and simultaneously inhibit the output producing neurons of the F-system. The reverse situation would be that rhythmic afferences inhibit the N-system and simultaneously excite the F-system. The latter case might be excluded because of the fact that the output frequency of the N-system remains equal with wingbeat frequency even under the conditions of experimentally changed wingbeat frequencies (Heide[12]). If the pulses from the output producing neurons of the F-system inhibit the output producing neurons

of the N-system then about every tenth pulse of the N-system will be delayed or suppressed.

Unfortunately, from the exclusion zone in the presented cross-correlations one cannot deduce which muscle system might inhibit the other. The position of the trough in the plots depends on the transmission times which are needed to transmit the action potentials from the spike producing sites in the neurons to the membranes of the muscle fibres from which the recordings are made. However nothing is known about the transmission times in the output systems under investigation. Up to now it is not even possible to conclude whether the output patterns are produced at the level of the motoneurons or at the level of preceding interneurons. To obtain more knowledge about these systems it will be necessary to record directly from central neurons as has been done by Burrows[22] for the flight motor system of a locust.

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