INVARIANCE OF OSCILLATOR INTERNEURONE ACTIVITY DURING VARIABLE MOTOR OUTPUT BY LOCUSTS

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Summary

Simultaneous intracellular recordings were made in locusts from (a) flight motor neurones and (b) output interneurones of the flight oscillator. The insects were mounted with the head at the centre of rotation of an artificial horizon. During fictive flight, these animals responded to simulated deviations from course with the changes in motor output appropriate to course-correction manoeuvres, as previously described. In the motor neurone of depressor muscle MN98 (meso-thoracic second basalar) these changes take the form of systematic variation in amplitude in the cyclical depolarization seen in the neurone in flight which, in turn, leads to variation in the number of action potentials per cycle (from 0–3) and in the latency of the first spike (up to 19 ms difference). These changes are closely related to the perceived movement of the horizon. The oscillator output, as recorded in metathoracic interneurone 511, shows, in contrast, very little change. The fraction of its variation which is correlated with horizon movement is vanishingly small (e.g. for number of action potentials per burst $r^2 = 0.008$). The exteroceptive sensory inputs which modify motor output during steering do not, therefore, affect the oscillator appreciably. Thus, by exclusion, the motor patterns of compensatory steering are due exclusively to summation of the oscillator drive with the sensory inputs. This takes place in the motor neurones and especially in the premotor interneurones, as previously described.

Introduction

Most rhythmic behaviours rely on neuronal oscillators for the generation of a basic premotor rhythmicity (Delcomyn, 1980). The oscillator-derived signal is usually modified to a greater or lesser extent by sensory input, especially phase-related proprioceptive inputs, but also those derived from other sources. The point in the motor circuit at which this sensory input acts is variable. In some cases it affects the oscillator itself or even pre-oscillator elements, as in the stomatogastric ganglion of Crustacea (reviewed by Nagy & Moulins, 1986). In others it appears that it affects only postoscillator circuitry, perhaps only the motor

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neurones. This was first suggested, on the basis of indirect evidence, for proprioceptive feedback to the lobster swimmeret system (Davis, 1968a), and subsequent work (e.g. Heitler, 1986) appears to confirm this. In the same system, however, input from the statocysts appears to modify tonically the segmental oscillators (Davis, 1968b). Locust flight is similarly complex. Phase-related proprioceptive feedback is important in the generation of the flight motor pattern (Wilson & Gettrup, 1963; Möhl & Nachtigall, 1978; Wendler, 1978; Neumann et al. 1982; Pearson, 1985; Stevenson & Kutsch, 1987; Wolf & Pearson, 1987a,b,c; Reye & Pearson, 1988) and acts not only on the motor neurones directly (Burrows, 1975; Kien & Altman, 1979; Peters et al. 1985; Elson, 1987a; Wolf & Pearson, 1987a,b,c), but also on the premotor and oscillator interneurones (Pearson et al. 1983; Elson, 1987b,c; Reye & Pearson, 1987). Our recent studies of corrective steering in flight by locusts (Reichert & Rowell, 1985; Reichert et al. 1985; Rowell & Reichert, 1986) suggest that a range of adaptive motor outputs is produced by exteroceptive inputs without altering the operation of the oscillator. This is because in this system the output from the oscillator interneurones is fed to the flight motor neurones (FMNs) in large part via a population of premotor interneurones, rather than directly (Robertson & Pearson, 1985a,b). At this stage it is mixed with sensory inputs, both exteroceptive and proprioceptive, and it is this modified output which drives the FMNs. Further, the FMNs themselves receive exteroceptive sensory inputs (Simmons, 1980; Tyrer, 1981; Rowell & Pearson, 1983) which, by summation with the already modified drive from the interneurones, further influence the pattern of firing of the FMNs. In contrast, we have not found sensory inputs related to flight steering in oscillator neurones. In view of this circuitry, stereotyped operation of the oscillator might well be compatible with variable motor output during steering. The following report is of experiments to test this hypothesis. By recording intracellularly and simultaneously from oscillator interneurones (INs) and flight motor neurones, we show that the activity of oscillator interneurones indeed remains virtually unchanged, although the motor output varies dramatically and predictably in response to external stimuli.

Materials and methods

Our experimental set-up has been previously described (Robertson & Pearson, 1982; Reichert et al. 1985). In brief, adult locusts (Locusta migratoria L.) were dissected dorsally to expose the thoracic nervous system, and placed with the head at the centre of an artificial horizon which enabled deviations from course (i.e. yaw, pitch and roll movements relative to the normal flight position) to be simulated via the ocelli and the compound eyes. Fictive flight activity was induced by a frontal stream of wind to the head. In the present experiments the horizon was moved sinusoidally through ±20° about the animals' longitudinal axis at 2·5 Hz to simulate roll. During fictive flight this stimulus causes responsive animals to produce an altered motor output, described below. In intact animals this altered pattern results in a compensatory roll torque (Thüring, 1986; Schmidt & Zarnack,

1987; Robert, 1988), causing them to follow the rotation of the horizon. The lateral nerves of the pterothoracic ganglia were cut, with the exception of nerves 1, which allowed the myograms of the dorsal longitudinal muscles to be used as an indicator of fictive flight. The pterothoracic ganglia were stabilized on a grounded metal spoon, and the activity of interneurones and motor neurones recorded with intracellular electrodes filled with Lucifer Yellow solution, according to standard techniques. Dye injection allowed the morphological recognition of the recorded neurones. Physiological data were stored on magnetic tape for subsequent display and statistical analysis. It will be noted that the thoracic proprioceptive inputs characteristic of intact flying animals are lacking. These inputs significantly affect the form of the final motor output (Wolf & Pearson, 1987a,b; Stevenson & Kutsch, 1987), and they are probably modified during normal steering manoeuvres. Thus, the real motor output is more complex than that achieved in our experiments, but this does not vitiate the points made in this article.

Robertson & Pearson (1983, 1985a,b) have given a preliminary account of the neuronal oscillator responsible for flight in *Locusta*. It consists of interconnected interneurones, some with resetting properties, modulated at flight frequency during fictive flight, and with typical intermittent discharge patterns. We have recorded from a number of these interneurones; the examples illustrated below come from IN511. This is a metathoracic interneurone which fires in the elevator phase of the wingbeat cycle. It is part of a cyclically inhibitory circuit of interneurones, consisting of three successive stages of inhibitory connections $(511 \rightarrow 501 \rightarrow 301 \rightarrow 511)$. Of these, 501 has resetting properties. 511 itself has not been seen to reset the oscillator; additionally, it makes inhibitory connections with premotor interneurones and FMNs active during the depressor phase. Although its feedback connections suggest that it may play some minor part in the oscillator itself it is best regarded as an output cell of the oscillator. Accordingly, it is well suited for use as a monitor of the oscillator as a whole. It does not receive input from the descending neurones mediating steering behaviour.

Steering in flight by locusts is largely the consequence of phase shifts in the firing time of the FMNs within the wingbeat cycle (Möhl & Zarnack, 1975, and many subsequent workers; literature reviewed by Rowell, 1988). One of the best studied motor neurones is FMN98, used in the experiments described below. It is a mesothoracic depressor motor neurone innervating the second basalar muscle of the forewing. It is known to be involved in flight steering (Möhl & Zarnack, 1977; Baker, 1979; Thüring, 1986). The experiments described below are derived from this pair of neurones, IN511 and FMN98. Less complete experiments with other pairs gave comparable results.

Results

Three types of output alterations are typical of rhythmically active motor systems which respond to the error signal content of afferent input with compensatory changes of behaviour (Stein, 1978). These are: (1) changes in the

latency of motor neurone firing in a given activity cycle; (2) changes in the number of spikes per cycle; (3) silencing or recruitment of motor neurones. All three are observed in the responses of FMN98 to simulated roll (Fig. 1). In this preparation they are of large size, giving variation in the range 0–3 spikes per burst and up to 20 ms shift in latency (see below). These changes are appropriate for compensatory behavioural reactions and are similar to those recorded electromyographically from FMNs in intact tethered locusts (Möhl & Zarnack, 1977; Elson & Pflüger, 1985; Thüring, 1986; Schmidt & Zarnack, 1987). In contrast, the rhythmic firing of IN511 remains apparently constant throughout. No signs of inhibition or excitation are seen by simple inspection. To test this impression of constancy
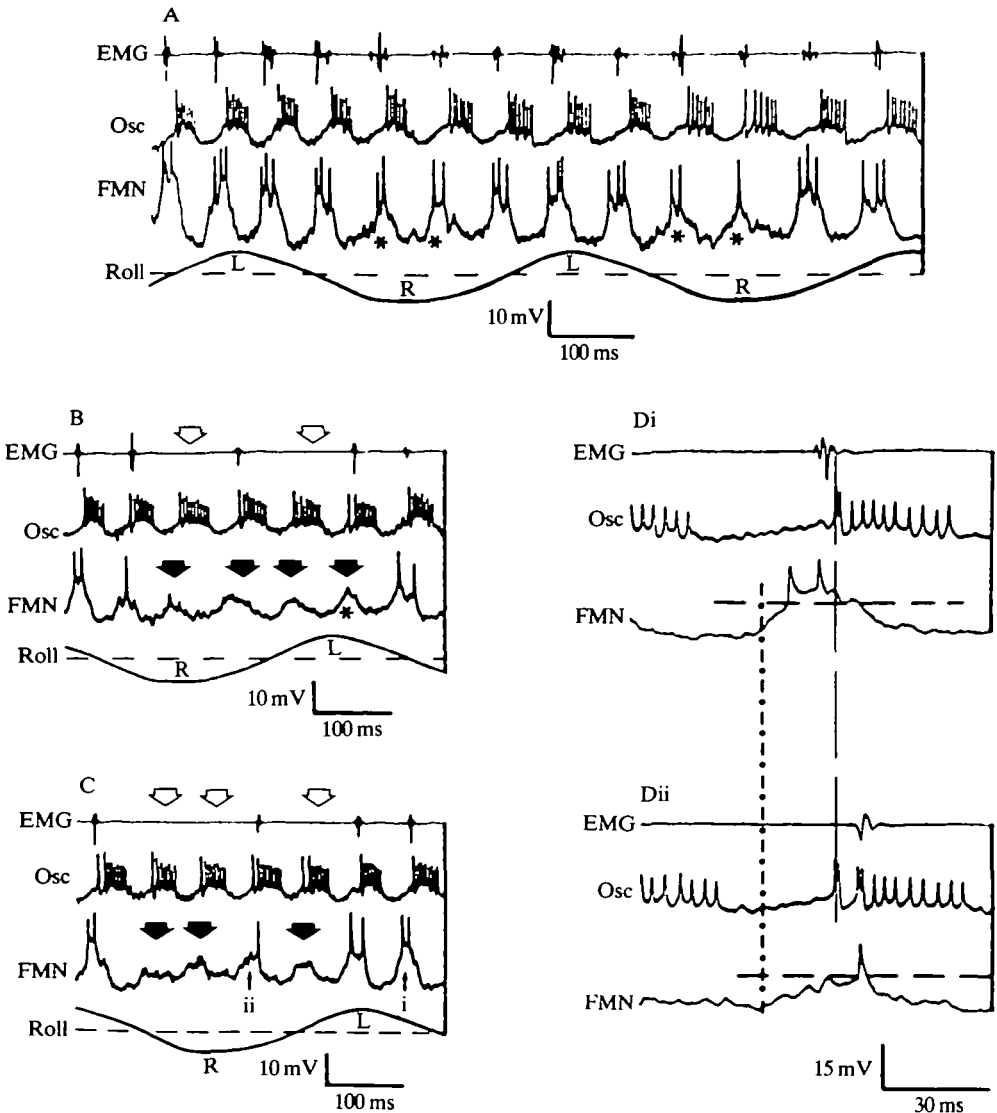

Fig. 1

rigorously, a quantitative analysis was performed, based on 107 oscillator cycles during four longer sequences of flight activity with simulated roll (Fig. 2). We measured the cell's oscillation period, burst duration and the number of action potentials per burst. All three parameters were slightly modulated at the frequency of horizon movement, by ±5%, ±7% and ±2%, respectively, indicating an effect of exteroceptive sensory input on the oscillator. Values of r^2 for the three parameters (fourth-order polynomial regression, approximating a sine curve similar to that driving the horizon) were, however, only 0·1, 0·08 and 0·008, respectively. This indicates that the oscillator is modulated by the steering input to only an extremely small extent, in no way comparable in magnitude to the large alterations in motor neurone firing: the sinusoidal modulation accounts for only a tiny fraction of the variation in the performance of the interneurone. Thus obvious and behaviourally appropriate changes in motor neurone firing can occur with very little change in the activity of oscillator interneurones.

We interpret this result to mean that constancy of the oscillator and variability of the motor output are compatible during steering in flight. Although such experiments on single interneurones cannot logically disprove the hypothesis that other, unrecorded neurones to the oscillator might be more strongly influenced by sensory input, they render it very unlikely. Typically, alterations to one element of an oscillator circuit lead to corresponding effects in all interacting elements. The results, in short, support the hypothesis originally formulated.

Discussion

What is the mechanism by which varying drive to the FMN produces the observed changes of behaviour? Fig. 1B,C shows that even when the modulation of the motor neurone is sufficient to silence it for several cycles, it continues to

Fig. 1. Recordings from mesothoracic dorsal longitudinal depressor mescle, extra-cellular (EMG); metathoracic interneurone 511, intracellular (Osc); flight motor neurone of the mesothoracic second basalar muscle, intracellular (FMN); and movement of the artificial horizon around the roll axis (Roll). The voltage scale applies to the intracellular recordings only. Both neural recordings were made from cells with their axon on the left-hand side of the animal. The roll trace is marked L and R, according to whether the animal perceived that it had rolled involuntarily to the left or right, respectively. The motor pattern that this elicits is correctional: thus, for example, a perceived roll to the left elicits after a delay of 50–100 ms the motor pattern appropriate to an active rolling to the right. The basalar muscles depress and pronate the wing, and their stronger or earlier activation tends to decrease lift. In A–C it can be seen that in the left-hand side FMN98 active rolling to the right corresponds to a weakening of activity and thus an increase in lift on that side. This is expressed as a change in the number of spikes per burst (A; asterisks); in recruitment (B,C: filled arrows show cycles where the motor neurone did not spike, open arrows indicate a similar silencing of another depressor unit, driving the dorsal longitudinal muscle); and in latency of the first spike of the burst (C,D). In C two extreme cycles (i and ii) are indicated and are compared at greater time resolution in D. Further explanation of D is given in the text.
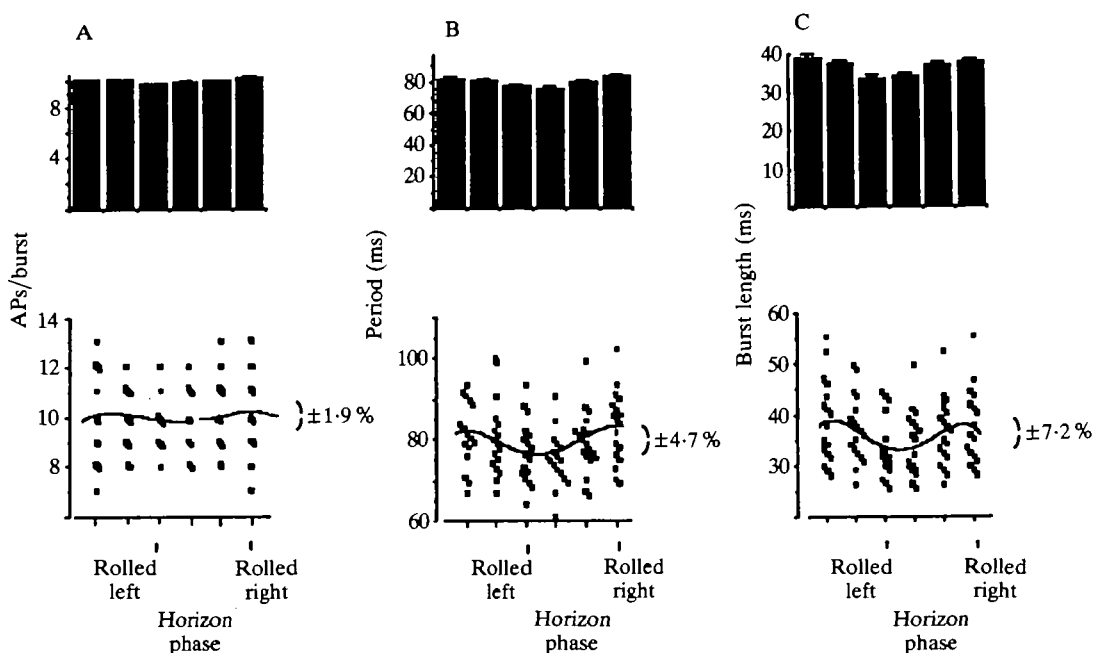
Fig. 2. Relationship between three parameters of the activity of IN511 and sinusoidal movement (±20°) of the artificial horizon around the roll axis. In all graphs the movement of the horizon is plotted along the x-axis, which represents one full wavelength. The points of maximum excursion to left and right (as perceived by the animal) are indicated. The three parameters measured are: (A) the number of action potentials in each burst; (B) period (the time from the first spike of one burst to the first spike of the next burst); (C) burst length (time from the first to the last spike of the burst). In each case the upper plot shows a histogram of the average values (bars show standard errors), and the lower plot (note expanded vertical scale) shows the curve of a fourth-order polynomial regression equation fitted to the individual data points (small squares), to permit an estimate of the size of the sine wave-related variation and of the amount of the total variation explained by this component. All plots are based on the same 107 cycles of activity. Note that B and C show very similar variation; A shows much less sinusoidal modulation. The coincidence of many values in the lower plots is due to their being measured to the nearest integer (number of action potentials in A; 0·5 mm of chart paper in B and C in which the variation is actually continuous). Coincident points have been displaced from each other along a 45° diagonal to allow their display.

receive (subthreshold) rhythmic input throughout the flight sequence. This input has the same temporal characteristics as the activity in the oscillator interneurone, indicating that even when motor units are functionally turned off during steering manoeuvres, they are not uncoupled from the central oscillator. Rather, the amplitude of rhythmic premotor drive seems simply to drop below the threshold for spike generation in the motor neurone (a similar phenomenon is seen at the end of flight periods; Burrows, 1973). Recruitment of the motor neurone is also

due to variation in the amplitude of rhythmic drive. For example, had this been marginally larger in the cycle marked with an asterisk in Fig. 1B, the motor neurone would have fired an action potential and have been recruited. The same process can explain both changes in latency of the first spike of a motor neurone burst and also changes in spike number within the burst. To demonstrate this, we took advantage of the constancy of the activity of IN511 and used the beginning of its burst as a fixed point, to establish when the motor neurone fired in relation to the oscillator during the different directions of simulated roll. In Fig. 1C, FMN98 is almost completely silenced following the simulated course deviation to the right. Two cycles occurring during opposing deviation periods (i and ii in Fig. 1C) are compared in Fig. 1D. In Fig. 1Di, FMN98 fires two spikes during a roll to the left; in Fig. 1Dii it fires only one spike during a roll to the right. Further, the first FMN spike in Fig. 1Di precedes the first spike in Fig. 1Dii by 19 ms. What causes the different phasing of the motor neurone spike in these two cases? In both it is receiving rhythmic drive, in both this drive begins to depolarize the motor neurone at the same time (vertical $-\cdot-$ line). In both cases the threshold value for spiking is the same (horizontal $---$ line). However, during a simulated roll to the left (Fig. 1Di), the depolarizing drive in the motor neurone is of larger amplitude. It reaches the threshold for spike initiation earlier than during a simulated roll to the right (Fig. 1Dii), and it remains suprathreshold long enough to cause a second spike. In contrast, in Fig. 1Dii the depolarizing drive is barely sufficient to cause a single spike. Had this drive been only slightly less, no spike would have resulted, as in fact occurred in the cycles preceding and following it (Fig. 1C). Thus not only recruitment, but also changes in spike number and changes in latency of motor units are related to the same phenomenon, namely, to changes in the amplitude of rhythmic depolarizing drive from the oscillator.

The PSPs evoked in the premotor interneurones by the descending deviation detector neurones are relatively modest (Reichert & Rowell, 1985); how do they cause such dramatic changes in the FMNs? Two factors contribute towards this. First, there is a sizeable population of descending neurones, acting as partial synergists (reviewed by Rowell, 1988), and convergence on premotor interneurones appears to be the rule rather than the exception. Second, the premotor interneurones vary considerably in their activity during 'normal' flight. Some fire a large burst in each cycle, and are likely to be only slightly modulated by summating sensory inputs; others, however, oscillate either near or even below their spiking threshold (for examples see fig. 5 of Reichert & Rowell, 1985). Summation of sensory inputs with the latter is likely to be especially important. It could bring such cells above or below threshold, and so contribute or remove a whole new element of rhythmic drive to or from the postsynaptic FMN. Several such cells in parallel would allow profound control of the drive to a given FMN.

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