

SHORT COMMUNICATION

INTRACELLULAR RECORDINGS FROM INTACT LOCUSTS FLYING UNDER CLOSED-LOOP VISUAL CONDITIONS

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The flight path of locusts is under permanent visual and mechanoreceptive control (the latter evaluates changes in wind direction). Involuntary course deviations are compensated immediately by a neuronal feedback system or 'autopilot', which induces corrective steering movements of the wings, abdomen and legs and which are generally accompanied by compensatory head movements (Taylor, 1981; Hensler and Robert, 1990). During apparently straight flight the flight path is not perfectly straight, but instead the insect oscillates slightly along a mean course (Robert, 1988; Hensler and Robert, 1990).

The principal neural events underlying corrective steering have been unravelled: sensory information on course deviations (coming from the eyes, ocelli and wind-sensitive hairs on the head) and proprioceptive information caused by head movements are coded in a class of descending deviation detector neurones (DDNs). 15 DDNs have already been identified. Most of them are multimodal and respond in a direction-specific manner to deviations from course (e.g. Rowell and Reichert, 1986; Hensler, 1988, 1992). Information from DDNs is further processed and transmitted to motoneurones *via* a class of thoracic interneurones (Reichert and Rowell, 1986; Rowell and Reichert, 1991; Hensler, 1992).

Corrective steering stabilizes the locust's orientation in space. A new orientation in space is acquired by a behaviour which I call 'voluntary' steering (this does not imply a conscious act by the locust). 'Voluntary' course deviations produce the same sensory inputs as involuntary course deviations; hence, the 'autopilot' has to be disabled during voluntary steering otherwise corrective steering would restore the initial flight path. In principle, voluntary steering could be accomplished in two ways: (1) by modulating the neurones of the 'autopilot' in order to produce the 'voluntary' course change, or (2) by controlling motoneurones through a parallel neuronal pathway, while the 'autopilot' is temporarily suppressed or overridden. To investigate this, intracellular recordings of steering

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neurones are required during the performance of 'voluntary' steering. The key problem of such an investigation is to distinguish between 'voluntary' and corrective steering in an experimental set up.

The usual set up for making intracellular recordings of steering neurones used either reduced (Rowell and Reichert, 1986) or intact (Hensler and Rowell, 1990) locust preparations, which were fixed in front of an artificial horizon. The former animals performed fictive flight (i.e. flight muscles were denervated, but wing motoneurones were active with a motor pattern resembling that of normal flying), the latter performed normal flight behaviour. Under such open-loop visual conditions, corrective steering is easily induced by rotating the artificial horizon in order to simulate course deviations for the locust (Rowell and Reichert, 1986; Hensler, 1988, 1992), but 'voluntary' steering cannot be determined unequivocally. It can only be detected when the locust adopts and maintains a new orientation with respect to its visual surroundings, either spontaneously or in response to an attractive or repellent stimulus. This situation may be simulated in a so-called closed-loop compensator: this set up has been used for the investigation of visual orientation in flies (e.g. Götz, 1987) and recently also in locusts (Robert, 1988). The present report introduces a method which enables intracellular recordings to be made from flight neurones of intact locust preparations flying in a closed-loop compensator. In the set up used here, the feedback loop for visual inputs signalling rotation around the roll axis (longitudinal body axis) was closed (in principle, the same method works for rotation around the yaw and pitch axes).

Locusts (*Locusta migratoria*) were tethered on a flight balance in front of an artificial semihorizon (diameter 15 cm; the posterior halves of the eyes were covered). The flight balance consisted of a holder connected to a rotatable metal rod fixed on ball bearings. The axis of rotation corresponded to the longitudinal body axis of the animal (Fig. 1). The rod was held in place by an elastic element (a strip of Sylgard), which restricted rotation to less than 1° when the locust produced maximum torque. A small permanent magnet was fixed to the metal rod in order to record (as a measure of torque) minute rotations using a Hall detector. Flight behaviour was induced and maintained by a frontal airstream of about 2 m s^{-1} . Turbulence was reduced by blowing the airstream through a bundle of pipes (diameter of each pipe 4 mm; diameter of the whole bundle 7 cm; length 6 cm). Course deviations were simulated by rolling the artificial horizon (e.g. horizon rolling to the left simulated roll deviation of the locust to the left).

In freely flying locusts a steering manoeuvre produces torque, which reorients the animal in space. This mechanism was simulated in the closed-loop compensator by electronically integrating the torque signal and setting the roll orientation of the artificial horizon in proportion to the resulting voltage level (Fig. 1). Accordingly, a pulse of torque resulted in a new orientation of the artificial horizon and constant torque produced constant rotation of the artificial horizon. The locust's incentive to steer was controlled by feeding a bias voltage into the integrating element. The bias voltage caused horizon rotation with an angular velocity of 90° s^{-1} when steering effort ceased. Transient course deviations were

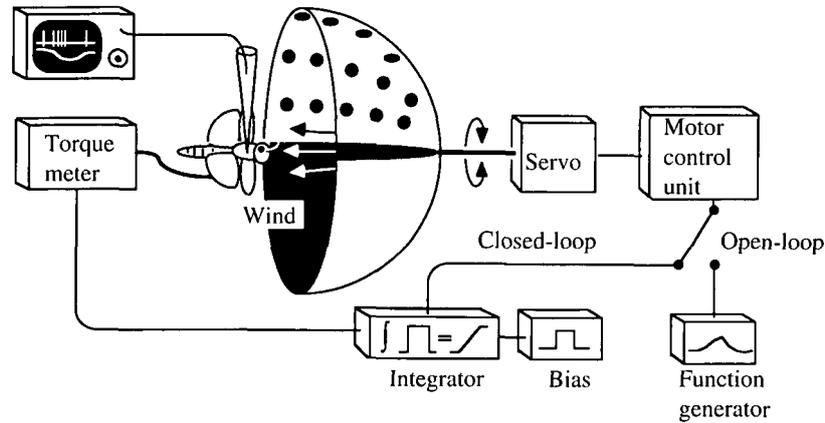


Fig. 1. Diagram of the experimental set up. A locust faced an artificial horizon. It was fixed on a flight balance recording torque around the roll (longitudinal) axis. Flight behaviour was induced and maintained by frontal wind. The horizon position (which simulated course deviation to the locust) was controlled by a servo either *via* a function generator (open-loop) or *via* the torque signal produced by steering manoeuvres (closed-loop). The torque signal was integrated electronically and the horizon position was set in proportion to the resultant voltage level. Course deviations were simulated by feeding transient signals into the integrator. Steady steering was maintained by a bias voltage which rotated the horizon at 90°s^{-1} if the locust did not produce correcting torque. Intracellular electrodes were inserted into the neck connectives through a small hole in the pronotum. Locusts could move their head, wings, legs and abdomen in a normal fashion.

simulated by feeding short voltage pulses into the integrating element (in the open-loop mode, the horizon position was controlled by a function generator).

Flight performance was monitored electromyographically from the first basalar muscles of both forewings (M97). Head rolling was recorded using a capacitive device (Hensler, 1988). DDNs were recorded intracellularly from the neck connectives through a small window cut into the posteriodorsal portion of the pronotum. The anterior part of the gut was removed to expose the neck connectives, which were then fixed with tissue glue onto a supporting metal platform. Locusts were otherwise intact and were capable of performing normal flight activity and normal head movements. Details of the preparation method are described elsewhere (Hensler and Rowell, 1990).

The results shown in this paper are taken from DNC, a DDN that has already been described under open-loop visual conditions (Rowell and Reichert, 1986). DNC was phasically excited by course deviations towards the side contralateral to the axon (in the following this is called the 'preferred direction') and phasically inhibited by simulated course deviations towards the side ipsilateral to the axon (the 'antipreferred direction'). It was tonically excited by frontal wind. The anatomy of DNC has been described by Griss and Rowell (1986).

Fig. 2 shows the activity pattern of the DNC with its axon in the left-hand

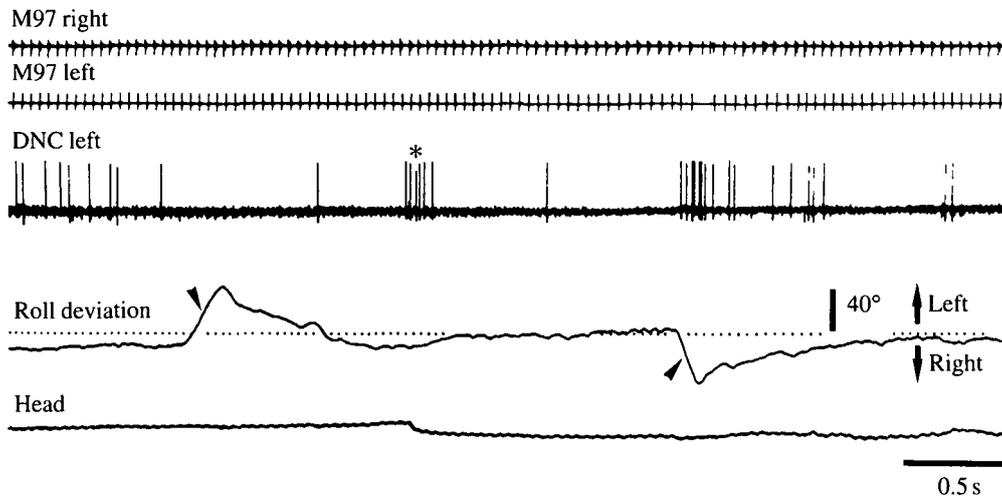


Fig. 2. A typical flight sequence under closed-loop visual conditions. The left and right muscles M97 (depressor muscle of the forewing) served as monitors for existing flight activity. The left DNC (one of a group of descending deviation detector neurones) was recorded intracellularly. Initially, the locust stabilized a static deviation of about 15° to the right (the dotted line represents the normal orientation, i.e. the horizon was set parallel to the horizontal plane of the locust's thorax). Left arrowhead: simulated roll deviation to the left reduced tonic discharge in DNC. Right arrowhead: simulated roll deviation to the right elicited a burst of action potentials in DNC. Simulated deviations were counteracted by steering effort, which restored the normal orientation of the horizon within 400–800 ms. Asterisk: a burst of action potentials without discernible external input.

connective during an episode of closed-loop flight. Initially the horizon was stabilized at a roll angle of about 15° right of the normal orientation. This stabilization resulted from active steering because the horizon would otherwise have rotated at 90° s^{-1} as a result of the bias fed into the horizon-controlling servo. DNC spiked at 11 Hz during this period. Simulated deviation of 65° to the left, i.e. in the antipreferred direction of the DNC under investigation (Fig. 2, left arrowhead), induced steering which restored the normal position within 400 ms. DNC did not spike during this period except for one action potential (AP) shortly before the normal orientation was regained. An overshoot of the steering reaction caused slight deviation to the right. Shortly after the overshoot had passed its maximum (a point not easily definable), there was a burst of APs in DNC (40 Hz for 150 ms; asterisk in Fig. 2). This burst seemed to be of central origin since it was neither evoked by visual input (during this period the visual image moved in the antipreferred direction) nor could any other obvious external input be correlated with it. After 1 s of stable, straight flight, a course deviation of 60° to the right was simulated, i.e. in the preferred direction of the DNC under investigation (Fig. 2, right arrowhead). This induced a burst of APs (45 Hz, 150 ms) followed by spiking at about 10 Hz until the normal flight course had been attained after about 800 ms. Note that the peak deviation was associated with the failure of one AP in the left

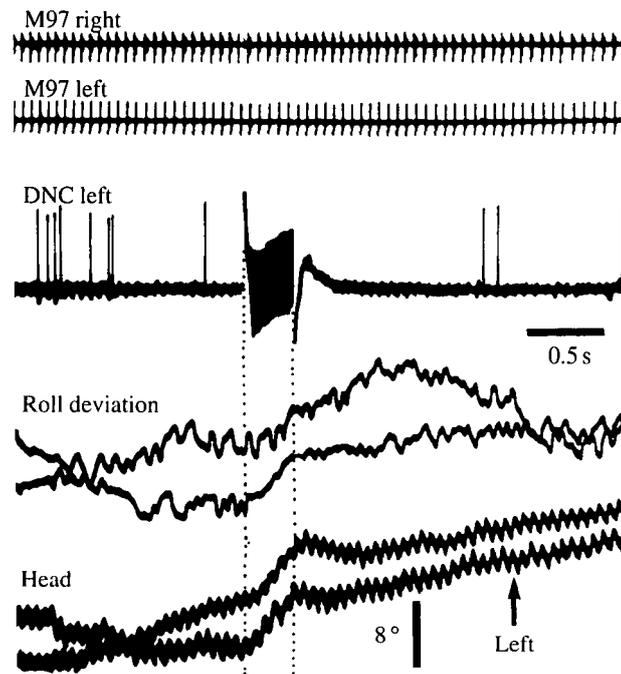


Fig. 3. The same experimental situation as in Fig. 2. Tracks of head and horizon movement from two successive trials are superimposed. The left DNC was stimulated electrically *via* the intracellular electrode. This evoked head rolling and roll deviation to the left, which is comparable to compensatory head rolling and corrective steering following course deviation to the right (i.e. the preferred direction of the DNC under investigation).

M97. The relative timing of APs in the left and right M97 plays an important role in steering (Möhl and Zarnack, 1977; Hensler and Robert, 1990). The head did not perform compensatory movements during the entire sequence, apart from a sudden roll of 13° to the right about 15 ms after the initial spike of the burst marked with the asterisk.

Under open-loop visual conditions, electrical stimulation of DNC induces shifts in the timing of wing motoneurons (Hensler and Rowell, 1990). Fig. 3 shows that such stimulation produces torque that relocates the horizon. Hence, the burst in Fig. 2 (asterisk) probably participated in the restoration of the normal flight course, which was stabilized almost perfectly afterwards. Because of the background of other horizon movements, the electrically induced roll manoeuvre in Fig. 3 was difficult to detect in individual observations. The overall tendency, however, was clearly discernible. Stimulation of DNC could be expected to have only a small effect, as DNC is merely one neurone of a bundle of at least 15 pairs of DDNs involved in course control (Hensler, 1992). In parallel with the production of torque, electrical stimulation of the left DNC induced head rolling to the left (Fig. 3; Hensler and Rowell, 1990, described the same effect, but their statement in the first paragraph of p.200 mistakenly exchanges left and right). The head

rolling in Fig. 2 (asterisk) could not have been initiated by the corresponding burst in DNC as it was directed to the right.

In summary, I have shown that it is possible to record intracellularly from neck connectives of locusts flying under nearly natural conditions, i. e. steering causes reafferent visual input. This method is suited to the investigation of the neural control of 'voluntary' steering, as the flight path is the only relevant measure that can be used to determine whether a steering movement is corrective or the basis of a 'voluntary' manoeuvre. Correlation of neurone activity with the flight path should reveal neurones involved in this behaviour. As far as visual responses in the closed-loop situation are concerned, DNC behaved qualitatively in the same way as it did under open-loop visual conditions during flight (Hensler and Rowell, 1990) and in resting locusts (Rowell and Reichert, 1986). The same holds for DNI, another DDN (K. Hensler, unpublished result). Most interesting was the occurrence of the 'spontaneous' burst (asterisk in Fig. 2), which may have been evoked by central input to DNC. Central excitation, inhibition and/or modulation seem to be a prerequisite for the induction of 'voluntary' steering by DNC or other DDNs.

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