

## SHORT COMMUNICATION

### WIRELESS TRANSMISSION OF MUSCLE POTENTIALS DURING FREE FLIGHT OF A LOCUST

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Accepted 13 August 1993

Scientists have long been interested in recording data from freely moving animals. For larger animals, several telemetric techniques are available not only for following the movement of unrestrained animals in the wild (White and Garrott, 1990) but also for transmitting measures of heartbeat, body temperature, wingbeat, respiration, etc. (e.g. Lord *et al.* 1962; Butler and Woakes, 1980; Funk *et al.* 1993; for an overview, see Amlaner and Macdonald, 1980). Because of the size of such transmission devices, however, data acquisition has been restricted to larger animals. The development of lightweight batteries and microchips has only recently facilitated efforts to transmit data from smaller animals such as insects. Such data should greatly enhance our understanding of the processes involved in the neuronal control of unrestricted behaviour. Eventually it should be possible to monitor the activity of individual units (neurones, muscles) under closed-loop conditions, which closely resemble free movement. It is to be expected that this new approach will surpass previous studies involving intact but surface-bound animals implanted with long flexible electrodes (for crickets, see Kutsch, 1969) or animals tethered in a windstream (for improvement of the flight balance device, compare Weis-Fogh, 1956, with Dombrowsky, 1991). Increased freedom has been achieved in experiments on 'free flight' of large insects (Möhl, 1988; Stolley, 1990), although even these animals were restricted to a short or stationary flight handicapped by several implanted flexible electrodes.

We report here a new technique which allows data such as electrical signals generated by muscles to be transmitted and analysed during true free flight over distances of at least 20m.

All tests were carried out with female *Schistocerca gregaria* Forskål. Mature females weighing 3.0–3.5g can carry a load of up to 0.5g without marked impairment of free-flight performance (W. Kutsch, personal observation). This sets the weight limit for the selection of components to be mounted on the locust. The weight of currently available quartz crystal oscillators made them unsuitable for use. We therefore constructed an LC-oscillator circuit (Fig. 1) which generated a carrier frequency of 140–150MHz. This carrier frequency varied more than that of a quartz crystal oscillator but, considering the

Key words: telemetry, *Schistocerca gregaria*, free flight, locust, muscle potentials.

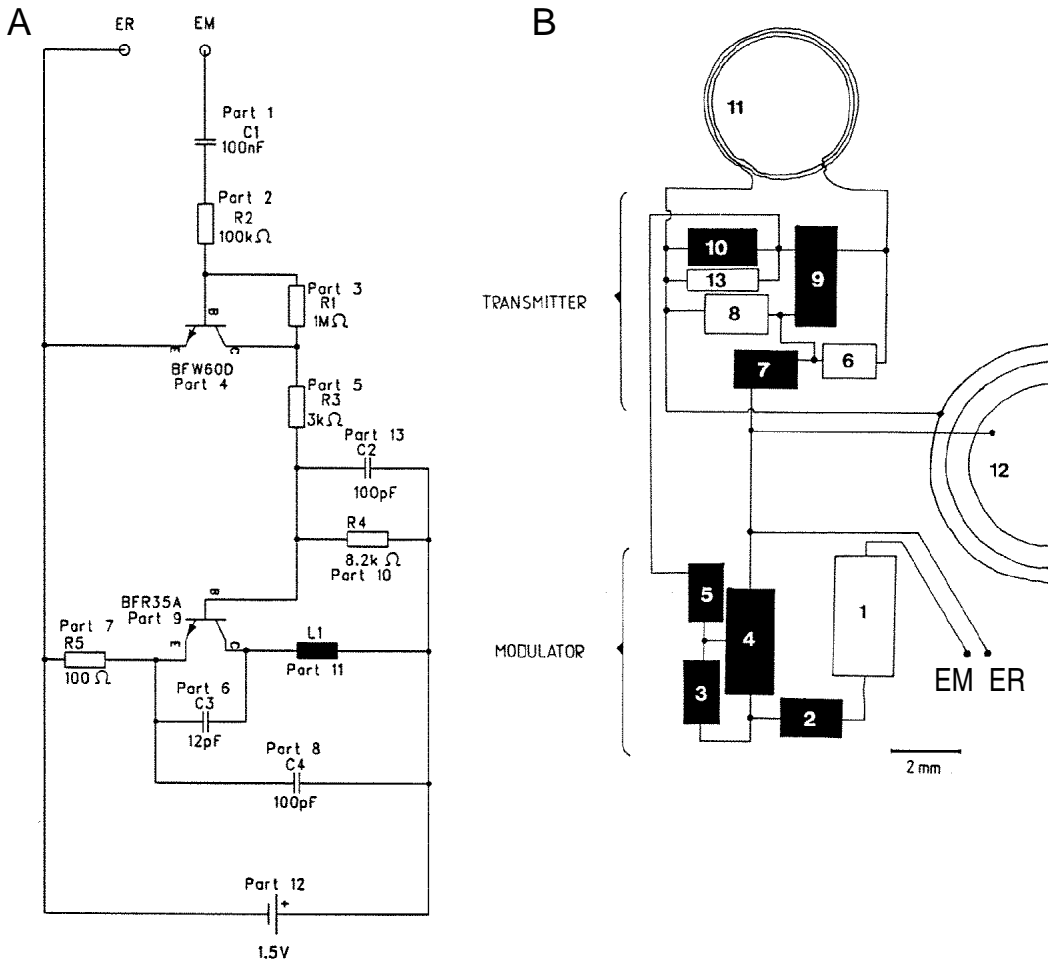


Fig. 1. The single-channel transmitter-modulator device. (A) Electronic circuit. (B) Approximate arrangement of the electronic elements. Part numbers as in A. EM, muscle electrode; ER, reference electrode; R, resistor; C, capacitor; L, inductor.

duration of free flights (1.5–2s), the drift was insignificant. The circuit was powered by a commercial silver oxide battery (Varta V319; 1.55V) weighing only 0.26g. The carrier frequency is frequency-modulated by the action potentials of flight muscles.

The various elements of the circuit (standard surface-mounted device; SMD, 0805; Roederstein, Landshut; transistors, SOT23; Valvo) were soldered together and the whole complex was fitted to the prothoracic shield of the locust (Fig. 2). The battery and coil were placed dorsally. The coil (part 11) was composed of insulated copper wire (200µm in diameter) coiled in three turns with a diameter of approximately 4mm. The other components of the transmitter and the modulator were mounted on the sides of the prothorax partly protected in natural hollows. These furrows usually accommodate the folded forelegs during flight; the imposed change of foreleg position did not appear to

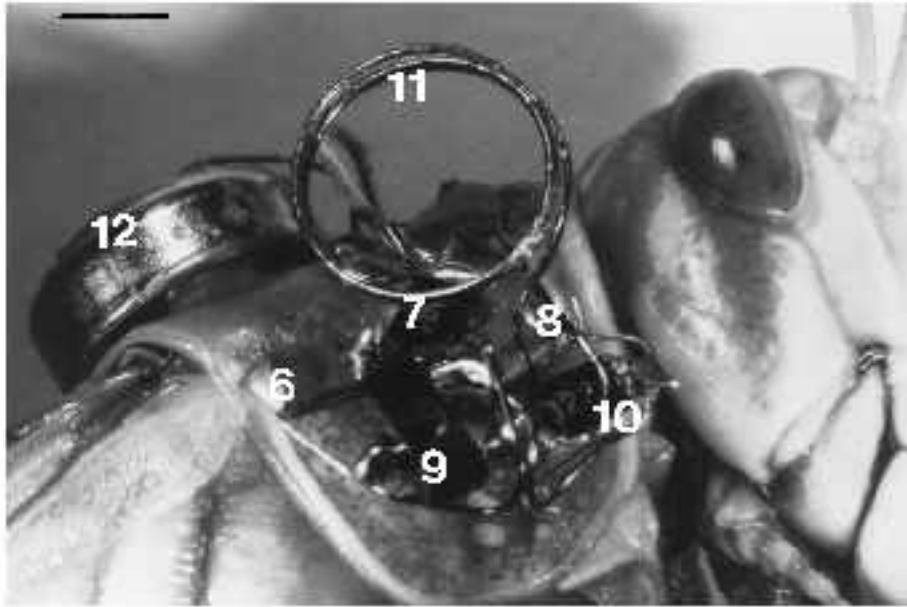


Fig. 2. Photograph of head and prothoracic shield of *Schistocerca gregaria* showing part of the transmitter-modulator circuit. For identification of components, see Fig. 1A. Scale bar, 2mm.

result in an unstable flight performance. The battery was fixed to the pronotum by a drop of warm beeswax-resin mixture. The other components were carefully shaped around the prothorax by manipulating their short connections; they did not require additional fixation to the cuticle. The recording electrodes were inserted into muscles through the cuticle and were fixed with wax at the point of penetration. For our experiments, we chose the subalar muscle of either the fore- or hindwing (M99 and M129, respectively; nomenclature after Snodgrass, 1929), which are powerful wing depressor muscles. The reference electrode was inserted into the abdominal cavity. Finally, the chosen recording and reference electrodes were connected into the modulatory circuit. The mean mass of the load added to the animal was 0.42g. The centre of gravity lies about 22mm caudal to the front (see also Weis-Fogh, 1956). The prothoracic load displaces this approximately 6mm rostrally. We did not detect any change of free flight performance, but we cannot exclude the possibility that changes in the position of the centre of gravity are monitored by the flight system. However, any changes appear to be compensated immediately by the flight motor centres. Under normal conditions, such changes in the position of the centre of gravity will occur as a result of deposition of egg-pods, which weigh about 0.5g (Weis-Fogh, 1952). Instead of the conventional stainless-steel wire electrodes (e.g. Kutsch, 1971; Möhl, 1988), we used manganin electrodes (Isabellenhütte, Dillenburg; 50  $\mu\text{m}$  in diameter, insulated to the tip) whose low resistance ( $228 \Omega \text{m}^{-1}$ ) considerably enhanced the signal-to-noise ratio. Furthermore, these electrodes require a lower soldering temperature, reducing the chances of damaging the delicate electronic circuit.

A spectrum analyzer (Hewlett Packard, series 70000) was used as signal receiver. An antenna (HB9CV; Conrad electronic, Hirschau) constructed for receiving signals in the 145MHz band was used as a directional aerial. Its output was amplified so that the system was sensitive enough to detect signals at a distance of up to 25m. Manipulations of the transmitter (such as changing the orientation of the coil) resulted in small changes in the carrier frequency. Therefore, the optimal receiver frequency had to be adapted individually before each flight. The signals were displayed on an oscilloscope and stored on tape for further analysis.

In order to produce stable flight performance, locusts were thrown into the air in a dark corridor (6m length) with a lighted window at the end that attracted the flying animal.

In a preliminary test, we studied the signals transmitted from a tethered locust which was stimulated by a short wind-puff to perform a flight lasting several seconds. The signals (Fig. 3Aii) exhibited the typical frequency and form known from previous studies employing conventional extracellular recording techniques (Fig. 3Ai). To determine whether the beating wings or other oscillating body structures interfered with, or even produced, the received signals, we tested the electronic device in the usual way but without connecting the muscle electrodes to the transmitter (Fig. 3B). Occasionally, small oscillations related to the wingbeat were observed, but larger signals were never detected. This indicates that the transmitted signals represent the recordings of the muscle potentials. Even specific events such as single or multiple discharges of motor units, or recruitment of different motor units (see Wilson and Weis-Fogh, 1962), could be discerned (Fig. 3A).

When the animals were thrown into air in the direction of the lighted end of the corridor, they flew towards the window. The flight was reasonably regular (see also high-speed camera analysis; Kutsch and Stevenson, 1981) with only small changes in altitude and body position. The recordings showed distinct signals characteristic of muscle potentials (Fig. 4). In accordance with previous results (Kutsch, 1971; Kutsch and Stevenson, 1981), it was apparent that younger females had a lower wingbeat frequency (15–18Hz) than fully mature ones (approximately 21Hz). We also monitored several complete flight sequences (Fig. 5) lasting up to 1.7s. Such recordings occasionally showed changes in the duration of adjacent intervals which could be interpreted as steering responses (Zarnack and Möhl, 1977). Changes in the size of the potentials as well as fluctuations of the baseline were also observed.

Such changes may be associated with the position of the animal with respect to the receiver. Pitch, yaw and roll movements distorted the potentials and could be associated with sudden changes in the baseline (Fig. 5B). As the animal approached the receiver antenna, the potentials became more distinct.

We report here a new technique in neuroethology which allows transmission of electrical signals from small unrestrained animals, such as free-flying insects. At the moment, this approach allows only a single channel to be transmitted. However, improvement in microchip design and a reduction in the mass and size of the power supply should facilitate the transmission of several signals in parallel, perhaps also over longer distances. With respect to free-flying locusts (or other species of similar or even smaller size), we envisage that it will become possible (a) to record simultaneously the

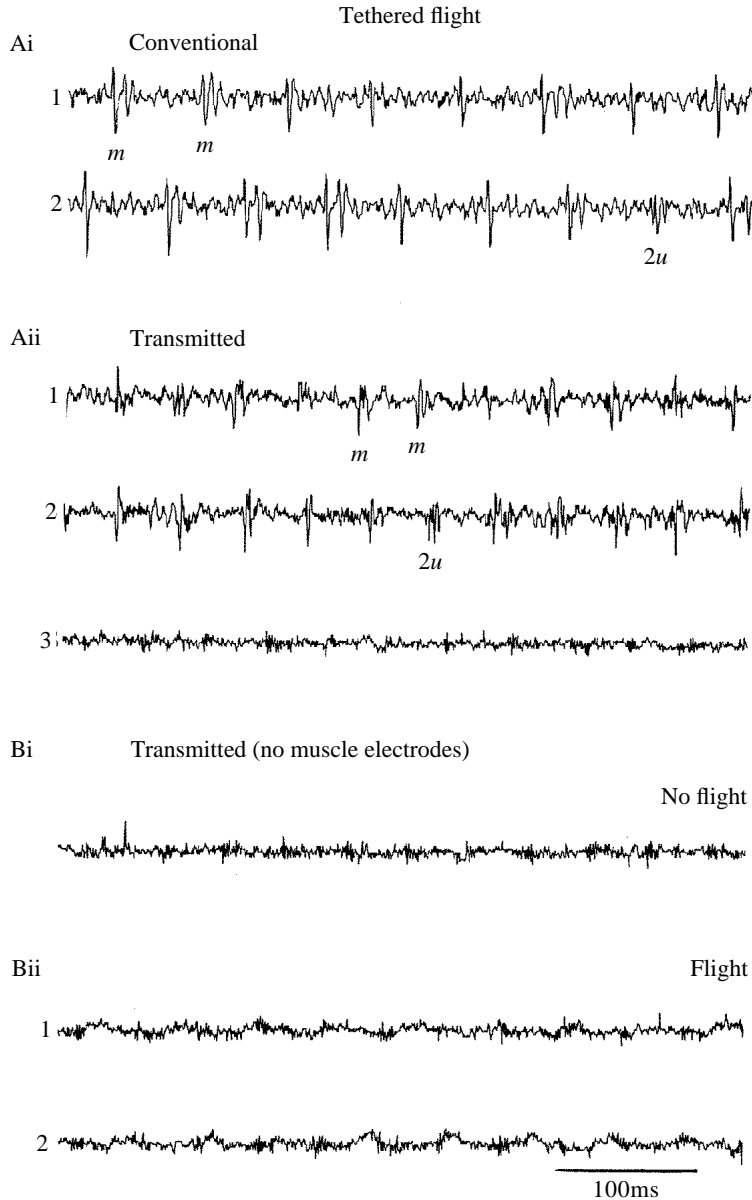


Fig. 3. Recordings of muscle potentials (M129) from tethered animals. (Ai) Recordings during tethered flight using a conventional equipment; i.e.  $30\ \mu\text{m}$  steel wires connected directly to the recording equipment. (Aii) Recordings during tethered flight using wireless transmission of muscle potentials. For each condition, two sequences are shown. *m*, multiple discharges of a motor unit; *2u*, instances of non-synchronous excitation of both subalar motor units. (Aii, record 3) Record made while the animal was not flying. At a distance of about 1m, the amplitude of the muscle potentials is approximately 0.7mV and that of the noise is approximately 0.1mV. (B) Signals were recorded in the absence of muscle electrodes. (Bi) Record from a flying locust. (Bii) Two sequences recorded during flight, showing regular small (up to  $\pm 0.12\text{mV}$ ) oscillations synchronous with the wingbeat.

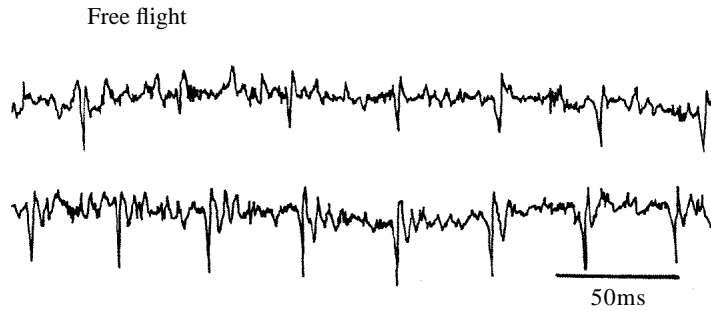


Fig. 4. Transmission of muscle potentials (M129) during two sequences of free flight from two different female *Schistocerca gregaria*.

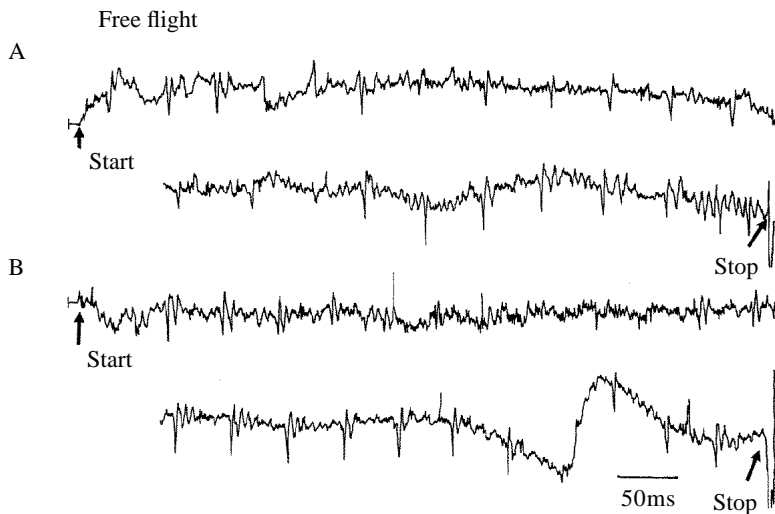


Fig. 5. Transmission of muscle potentials (M129) from two different females in free flight showing the beginning and end of flight. Flight duration (A) 1.2s, (B) 1s.

activity of several muscles; (b) to relate muscle activity to the position of wings or other body parts (Nolen and Hoy, 1986; Jensen, 1956; Zarnack, 1972); and (c) to transmit the activity of receptor cells and identified interneurons (e.g. wing stretch receptor, Wilson and Gettrup, 1963; Möhl, 1985; tritocerebral commissure giant, Bacon and Möhl, 1983; descending movement detector neurones, Rowell, 1971).

It may become possible to use a similar equipment in the 'reverse' direction, i.e. to stimulate the animal through a wireless transmission device. Development of the present method should give us a powerful tool with which to approach the ultimate objective of neuroethology, the understanding of the neuronal circuits underlying the behaviour of freely moving animals.

We greatly appreciate the continuous interest of Diplom Ingenieur O. Marschall and

other members of the Electronics Department in developing the device used here. We thank M. A. Cahill and Dr T. Matheson for improving our English.

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