PRINTED CIRCUIT MICROELECTRODES AND THEIR APPLICATION TO HONEYBEE BRAIN

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SUMMARY

1. The application of printed circuit technology to the production of a new type of multi-channel micro electrode is described.

2. Recordings have been obtained from the protocerebrum of the honeybee Apis mellifera L. using printed circuit microelectrodes in both restrained and free-moving preparations.

3. These recordings are compared with those previously obtained from conventional probe microelectrodes and are found to have similar characteristics of transient voltage changes superimposed on an undifferentiated high frequency background.

4. A wide range of development possibilities for the printed circuit microelectrode are discussed.

INTRODUCTION

Conventional probe microelectrodes have played a vital role in the investigation of unit neuronal function in insects (Maynard, 1967; Collett, 1971; Rowell, 1971; Burrows, 1973). However, the relationship between unit function in the insect supraoesophageal ganglion and complex behaviour (such as that involving learning) remains difficult to establish with conventional stimulating and recording techniques (Huber, 1965; Howse, 1975). To approach an understanding of this relationship it may be necessary to record unit activity at large numbers of loci simultaneously in relatively free-moving animals. Printed circuit microelectrodes have been developed for this purpose. Multi-barrelled probe electrodes with more than ten channels are usually extremely bulky and their recording tips tend to be restricted to adjacent loci. Printed circuits can be designed to present any spatial array of recording sites to the tissue and even with a large number of channels (50) they remain relatively small and manageable.

The present account deals with the construction of printed circuit microelectrodes and their application to the supra-oesophageal ganglion of Apis mellifera L. Recordings are presented for comparison with those obtained by other workers from probe electrodes (e.g. Vowles, 1964; Kaiser & Bishop, 1970; Suzuki, 1975).

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**MATERIALS AND METHODS**

**Construction of the microelectrodes**

The technique used is based on that described by Hill & Rigby (1969) for the production of evaporation masks. Diagrams of different types of electrode circuit were prepared with Letra-set dry print on white card. These were photographed on Kodalith Ortho Type 3 film and the images were then reduced optically to the size required for electrode printing. The reduced images were printed on to a ‘master’ photographic plate (Ilford Formolith G72). The master plate carried several hundred positive images and was used to produce thousands of electrodes.

To transfer the reduced photographic images to a metal substrate, the master plate was laid on top of a sheet of copper foil (0.045 mm in thickness) previously coated with Kodak KPR photoresist (Fig. 1a). Master plate and foil were exposed to ultra violet light for 3 min in a vacuum printing frame (the photoresist polymerizes under u.v. light). The foil was then placed in Kodak KPR photoresist developer to remove unpolymerized resist and produce a hardened negative resist image over the copper surface (Fig. 1b). After washing in ethanol and drying, the foil was electro-
Fig. 2. Printed circuit microelectrodes photographed by transmitted light. (a) Type A electrodes. (b) Type B electrode, 1–8 channel numbers. (c) Recording tips of a four-channel type A electrode. (d) Eight-channel type A electrode illustrating printing resolution obtainable.
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plated in Watt's nickel plate solution (Fig. 1c). Because the total area of the electrodes was very small for consistent electroplating, it was necessary to bare about 1000 mm² of copper to more readily control the depth of nickel deposited. Under these conditions a current of 0.1 A for 15 min was sufficient to complete the plating process. Polythene (0.028 mm in thickness) was fixed to the resist/nickel surface of the copper foil with cyanoacrylate (Fig. 1d) and the copper was then completely etched away in ammonium persulphate solution leaving the finished nickel electrodes attached to the polythene sheet (Fig. 1e).

Fine copper wires (0.0508 mm in diameter) were used to connect the microelectrode output terminals to a multi-channel differential preamplifier (Palmer 8122). The copper wires were tinned and threaded individually through fine glass pipettes mounted on micro-manipulators. Each wire was positioned firmly on each output terminal and the joints were secured with an overlay of cyanoacrylate adhesive. The pipettes were then withdrawn and discarded. (Alternative methods of attaching copper wires involved the use of conducting adhesives, spot welding or electroplating the contact joint with copper.) The remaining circuitry was insulated to leave exposed recording tips of approximately 10 μm². The finished multi-channel circuit was then cut from the production sheet, complete with its polythene backing. Electrodes were checked microscopically before use and those showing any cross-linking of channels, caused by excessive nickel plating, were discarded.

Implantation

A worker honeybee was mounted in an experimental holder without using any anaesthetic or damaging restraint (Pickard, 1975). An area of cuticle overlying the protocerebrum was depilated and cut out with a small spade drill (0.07 mm in diameter). The hypopharyngeal glands were displaced laterally and a printed circuit microelectrode was positioned either on top of the connective tissue sheath overlying the right corpus pedunculatum or directly beneath the sheath. The electrode was sealed in position and the wound closed with cyanoacrylate adhesive. Recordings were taken from both restrained and free-moving preparations (flying and walking in a plastic box, 178 mm x 114 mm x 38 mm). The electrode outputs were compared with each other or with an indifferent silver wire probe in the haemocoele and displayed on Dynamco 7130 and Telequipment DM64 oscilloscopes, and filmed with Nihon-Kohden PC-2A and Polaroid CR-9 cameras.

RESULTS

Two basic types of electrode were produced, type A presenting a row of recording tips to the tissue (Fig. 2a, c, d), type B presenting a group in two dimensions (Fig. 2b). Transient voltage changes were registered by both types of electrode. Electrodes placed on top of the connective tissue sheath produced outputs that were much more difficult to differentiate than those produced by electrodes trapped under the sheath. The individual traces (Fig. 3b-f) had similar characteristics to those produced by extracellular probe electrodes, of both the glass pipette (Vowles, 1964) and stainless steel or tungsten type (Kaiser & Bishop, 1970). A high level of background activity and the presence of low frequency pulses, accentuated after filtering out potentials
Fig. 3. Recordings from the protocerebrum of a stationary unrestrained worker honeybee. (a) Diagram of the brain (frontal aspect) showing the loci (1-4) over the right corpus pedunculatum (sheath intact) at which records (b) to (f) were obtained. (b-f) Recordings from the same preparation showing synchronous and asynchronous activity in different electrode channels. Traces 1-4 are recordings taken at loci 1-4 through channels 2, 3, 6 and 7, respectively, of an eight-channel type B electrode (Fig. 2b) compared with an indifferent probe also in the head capsule.

above 100 Hz, were constant features of the recordings. Controls indicated that such low frequency potentials did not represent the detection of muscular activity in the head capsule. Since the exposed area of each electrode tip was sufficient to cover several cells, each transient recorded could reflect the activity of one or several neurones functioning in synchrony.

In free-moving preparations, recording artifacts generated by movement of the electrodes were clearly discernible amongst neural activity. Cleaning of the antennae with the prothoracic legs produced most of these artifacts. Where eight-channel circuits were presented to the tissue there was a tendency for only three to five channels to monitor electrical activity at any one time (Fig. 3a, b). Both asynchronous (Fig. 3c) and synchronous (Fig. 3d) discharges were monitored. The possibility of synchronous discharges being the result of cross-talk between channels was investigated. Tests for electrical continuity between channels were negative and no synchronous pulses exhibited the degree of attenuation that would be associated with
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capacitance coupling where such good conducting pathways as the electrodes are present. It was concluded that recordings of synchronous transients reflected synchronized activity at the different electrode tips. Such synchronization may reflect associated activity between Kenyon cells which were situated beneath the printed circuit (Maynard, 1967; Schürmann, 1971).

Chronic preparations were viable for up to five hours. Neural activity was recorded during restricted flight; scenting with Nasanov's gland; walking; orientation to a light source; and cleaning of antennae, wings and metathoracic legs. Consideration of the relationship between recorded activity and behaviour is continuing at the present time.

DISCUSSION

The basic printed circuit microelectrode used in this preliminary study was successful as a surface recording electrode. There are, however, considerable possibilities for its development as a probing multi-channel electrode with projecting conical recording tips at right angles to the existing printed circuit. The printed circuits can be constructed from any of the conventional microelectrode metals such as silver, gold or platinum. With a given printed circuit the impedance characteristics can be controlled through the degree of electrode metal deposition and insulation. By microscopically masking the electrode tips to give the required area of exposure the insulation can be sprayed on to the circuit, preferably in a vacuum.

Since these electrodes are produced photographically from one master plate, it is possible to standardize a particular array of recording points in hundreds of circuits. Furthermore, the original circuit drawing can be accurately modified to change an electrode pattern to suit specific applications as an experimental investigation proceeds. This facility could be extremely useful in a statistical analysis of relative activity levels over a group of cells (Moore et al. 1970). With appropriate modifications it should also be possible to use these printed circuits for other specialized recording purposes such as monitoring tissue levels of pO₂ (Silver, 1965). With the increasing development of microelectronics it may soon be possible to incorporate the first stage preamplification on or very close to the printed microelectrode with considerable reduction in unwanted noise. Such a development would facilitate the current growth of telemetry in biological experimentation (Mackay, 1970).

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REFERENCES


