A PIEZOELECTRIC DEVICE TO AID PENETRATION OF SMALL NERVE FIBERS WITH MICROELECTRODES

ROLAND HENGSTENBERG

Max-Planck-Institut f. Biologische Kybernetik, Spemannstrasse 38, 72076 Tübingen (G.F.R.)
(Received November 10th, 1980)
(Revised version received January 23rd, 1981) (Accepted February 8th, 1981)

Key words: electrode jolter — neuron impalement — penetration aid — piezoceramic — electrode insertion
A small piezoelectric device for cell penetration is described. It retracts the micropipette slowly by electrostriction, and pushes it very fast (<5 µsec) forward by shortcircuiting the transducer. The design, operation circuit, and performance under test conditions are described. Penetration examples from small nerve fibers (<5 µm) show that membrane puncture occurs only with the fast forward push. Cells are not noticeably damaged, even if the device is repeatedly operated after cell penetration.

INTRODUCTION

It is a common experience that disappointingly few neurons are impaled when microelectrodes are smoothly advanced through nervous tissue. The reasons for this are not well understood, but the finite ‘sharpness’ of microelectrodes, the fluidity of cytoplasm and of membrane material, and the difference between the hydrophilic glass surface and the hydrophobic cell surface may contribute to this effect. In single cells like echinoderm eggs (Tyler and Monroy, 1955), or isolated neuron somata, one can directly observe the deep indentation of the cell membrane as the electrode advances, its sudden yielding, and usually cell death, resulting from too deep a penetration. In solid tissue, cells are partly displaced, and partly distorted in front of a slowly advancing electrode. At a critical strain, the tissue yields, and plunges in an uncontrolled manner against the electrode. Such ‘stick-and-slip motion’ may cover several tens of microns and usually does not end with a satisfactory cell penetration.

The best way of electrode advancement would be in small, purely axial steps of infinite speed. Tissue inertia and the viscosity of cytoplasm and cell membranes would render the tissue effectively stiff against such movement. Step width must be larger than membrane thickness (10 µm), but much smaller than cell diameter in order to avoid excessive damage. Unfortunately, no mechanical drive is yet known which meets these and other requirements for electrode advancement. Staircase motion can, however, be approximated if an instantaneous ‘pecking movement’ of high velocity and small amplitude is added to the slow electrode advancement.
KNOWN PROCEDURES

Various means of facilitating cell penetration have already been reported: (1) manual tapping of the experimental set-up (Tyler and Monroy, 1955); (2) electromagnetic jolting of the preparation (Tomita, 1965), (3) electromagnetic tapping of the microelectrode (Frank and Becker, 1964; van der Pers, 1980; Hamdorf personal communication); (4) magnetostrictive tapping of the microelectrode (Weiler and Zettler, 1976); (5) electrostrictive tapping of the microelectrode (Pascoe, 1955; Ellis, 1962; Lassen and Sten-Knudsen, 1968; Rikmenspoel and Lindemann, 1971; Chen, 1978; Fromm et al., 1980); (6) DC impulse through microelectrode (Zettler and Järvelä, 1971); (7) high frequency current through microelectrode (DeVoe, 1975), (8) electrostrictive vibration of microelectrode (Ellis, 1962; Chowdhury, 1969).

Procedures 1 and 6 utilize more or less a single shock to relieve tissue strain, and to pierce cell membranes. Procedures 7 and 8 aim at purely axial vibration like a percussion drill. All procedures have been developed and shown to work for particular preparation and experimental set-ups. Their relative efficiency is therefore difficult to judge without systematic study.

Intracellular studies an visual interneurons of flies, where small (2-5 µm) fibers have to be penetrated deeply within the neuropil, require a suitable penetration aid. Procedures 1, 5, 6, 7 and 8 were not found to be fully satisfactory: 2 is not applicable, 3 and 4 required too much space. Therefore, a new instrument has been developed to meet the following requirements: size, small, lightweight, robust; control, simple, variable, remote operated; energy dissipation, instantaneous, just sufficient for membrane puncture; piercing motion, small, fast, forward step, no transverse or residual vibration; recording state, inherently stable, no mechanical drift or electrical interference.

The design is based upon electrostriction of a piezoceramic transducer. In contrast to other instruments, it uses the fast relaxation from electrostriction, rather than its comparatively slow build up. Very high step velocity is obtained this way.

DESIGN

The design is simple and straightforward: a small piezoceramic tube (Vernitron, Thornhill, U.K., Type BMT-8-8031-5 H) is attached by its rear end to the micromanipulator and carries the microelectrode at its front end (Fig. 1). The tube changes length when an electrical field is applied across the tube wall: \[ \Delta L = L \times V \times \frac{d_{1,3}}{W} \], where \( L \) = length, \( V \) = voltage, \( W \) = wall thickness, and \( d_{1,3} \) = transverse piezoelectric constant. For PZT-5H ceramics, \( d_{1,3} = -274 \times 10^{-12} \text{ m/V} \), i.e. for the above tube \( \Delta L = -4.4 \text{ nm/V} \).

The tube expands (\( \Delta L > 0 \)) if the electrical field is positive on the inner face of the tube and contracts (\( \Delta L < 0 \)) with reverse polarity. High precision tubes are recommended to achieve best straightness of motion.
With PZT-5H ceramics, the field applied must not exceed 400 V/mm rms. The maximum steady excursion achievable with this transducer is 2.0 µm at 450 V DC. Temperature must not exceed 200°C, otherwise the ceramic becomes depolarized. Care should therefore be taken when soldering leads and during assembly with thermosetting epoxy glue.

OPERATION

The electrical circuit is equally simple (Fig. 1b), but provides several advantages: in the resting state (e.g. during intracellular recording), switch S is closed, the piezoceramic transducer C is grounded, and the charging current $I_c$ is short-circuited via S. The polarity of the voltage $U$ is such that the transducer tube contracts when S is opened. Due to $RC \approx 50$ msec, the microelectrode tip retracts smoothly by 0.5µm, if C is charged to -100 V. Closing S thereafter discharges C within less than 5 nsec, and restores the grounded resting state. The velocity of the forward step is therefore only limited by the mass of the electrode and the elastic compliance of the piezoceramic material and the electrode, but not by the finite slewrate of a driving amplifier. Notice that the initial discharge current $I_d = 100$ V/0.1 Ω = 1000 A!

A convenient voltage source to cover the whole range of allowed excursions can be made by a photomultiplier high voltage supply, with an appropriate current limiter/voltage divider in series.

With electrode holders of $m \approx 2$ g, $-100 \text{ V} < U < -50 \text{ V}$, i.e. step widths of 0.2-0.5 µm have been found empirically to be most satisfactory.
PERFORMANCE

Fig. 2 shows the performance of the piezoceramic jolter. The microelectrode is replaced by a razorblade, which intersects a narrow beam of light up to its axis. Light flux modulation by jolting is recorded by a calibrated Phototransistor and a voltage-biased operational amplifier. Fig. 2a shows the whole cycle of operation, with slow retraction (upwards) and fast expansion.

Fig. 2. Performance of the piezoceramic jolter. The jolter moves a razor blade in a narrow light beam, and the light flux modulation is recorded by a phototransistor. a: slow retraction (upwards) and fast expansion of the jolter at \( U = 120 \, \text{V} \). b: fast forward step at \( U = 140 \, \text{V} \). The apparent time constant of 5 \( \mu \text{sec} \) is due to the limited recording bandwidth of 30 \( \text{kHz} \). The actual movement is presumably much faster.
Notice that very little residual oscillation occurs after the fast forward jump. This shows that the stored energy is almost instantaneously released during the fast forward step. Such movement should be close to ideal for cell impalement, because the risk of damaging the cell by vibration after membrane penetration is minimal. Fig. 2b shows the fast forward step on an expanded time scale. The apparent time constant of 5 µsec is determined by the limited recording bandwidth of 30 kHz. The actual step velocity must therefore be even faster.

The shockwave travelling through the micropipette might break its tip. Jolting very fine electrodes in saline (outer tip diameter: <0.1 µm; outer tip angle: 4-8°), does not, however, reveal recognizable changes in electrode

![Graph showing neuron penetrations with piezoceramic jolter.](image-url)

Fig. 3. Neuron penetrations with piezoceramic jolter. Three different types and parts of visual interneurons in the fly brain have been impaled. In a–c the slow retraction is not shown, but only the fast forward step (▼). The upper boundary line in each record gives the reference potential. Cell sizes were estimated by Procion yellow injection, cell reconstruction, and reference to the penetration site. a: interneuron soma of approximately 20 µm diameter. Notice that the discharge artifact (▼) of the jolter, and cell penetration coincide in a–c. b: tangential cell dendrite of approximately 5 µm diameter. Notice the almost immediate stability of membrane potential in a–c, indicating optimal impalement. c: tangential cell axon of approximately 3 µm diameter. Notice the lack of an 'injury burst'. d: same as a; 2 out of 20 additional shocks from the jolter after cell penetration. Notice charging (▲) and discharging (▼) transients, and the absence of apparent damage with repeated shocks.
resistance \((100 \text{ M\ohm} < R < 300 \text{ M\ohm})\), steady-state noise, or tip potential. Microelectrodes are therefore not damaged by jolting.

EXAMPLES

Fig. 3a-c show examples in which interneurons of the visual system of the blowfly have been impaled with the device described here, attached to a David Kopf hydraulic microdrive. The usual working cycle was: (a) retract microelectrode for 0.5 µm by opening switch S (remote actuated Reed relay); (b) advance electrode for 1-2 µm by hydraulic drive; (c) let microelectrode jump forward for 0.5 µm; and (d) advance 1-2 µm by hydraulic drive, if a cell has been penetrated. Step d is very much a matter of cell type and experience. All sample records in Fig. 3 show by the occurrence of the positive-going discharge transients (arrows) that penetration of the cell membrane does occur simultaneously with the small, fast forward jump. In many thousand trials, it has never been that a cell was penetrated during slow retraction of the jolter or by the comparatively slow hydraulic advancement. Should this ever happen, the fast forward step can still be released, without noticeably damaging the impaled cell. In Fig. 3d, several piezoelectric 'taps' have been applied after impalement of a small neuron soma without loosing the cell. This shows that the energy dissipated from the electrode tip is small enough not to cause cell or tissue damage.

Fig. 4 shows a heterolateral visual interneuron of the blowfly, as reconstructed from serial sections after Procion yellow injection. It connects the
left medulla with the right lobula plate. It is transiently excited by light on off, and responds to vertical pattern movement in the left eye's visual field. The fiber has been penetrated and injected at ▼, where the fiber diameter is about 3 µm.

With the piezoceramic jolter described here, it has been possible to penetrate and stain fibers as small as 1 µm, buried more than 100 µm deep in neuropil. The jolter is easy to manufacture, operate, and adapt to various needs. Its small, lightweight design allows its use in confined spaces, and finally, it has been proved to work under exacting requirements.

REFERENCES

Lassen, U.V. and Sten-Knudsen, O. (1968)
Direct measurement of membrane potentials and membrane resistance of human red cells, J. Physiol. (Lond.), 195: 689-696.
Pascoe, J.E. (1955)
A technique for the introduction of intracellular electrodes, J. Physiol. (Lond.), 128: 26P.
Tyler, A. and Monroy, A. (1955)
Tomita, T. (1965)
Weiler, R. and Zettler, F. (1976)
Electrophysiological and histological studies of the carp retina.
Zettler, F. and Järvilehto, M. (1971)
Decrement-free conduction of graded potentials along the axon of a monopolar neuron, Z. vergl. Physiol., 75: 402-421.