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Starr, A
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Publication Date

DOI

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Peer reviewed

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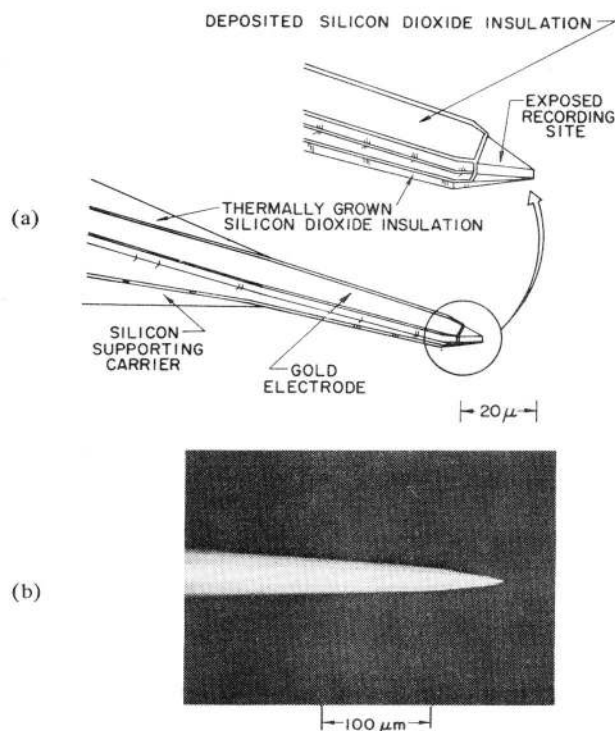

Fig. 1. (a) Microprobe tip structure. The entire probe is fabricated using integrated-circuit photoengraving technology. (b) Photomicrograph of a single-electrode microprobe suitable for single-unit recording.

An Evaluation of Photoengraved Microelectrodes for Extracellular Single-Unit Recording

ARNOLD STARR, KENSALL D. WISE, AND JOHN CSONGRADI

Abstract—Microelectrodes fabricated using integrated-circuit technology are shown to be suitable for extracellular single-unit recording, and the factors influencing electrode-cell coupling are discussed. Electrode tip size as well as location is found to be critical in recording high spike amplitudes, suggesting that the paths for current flow in central nervous system (CNS) tissue may be extremely small.

Introduction

The use of microelectrodes for recording electrical events generated in the central nervous system (CNS) is an important technique for studying living systems at the cellular level. Conventional microelectrodes [1], [2] are subject to great variability in their physical and electrical characteristics, making an understanding of electrode-cell coupling difficult. Furthermore, conventional electrodes are difficult to fabricate in multielectrode arrays, and such arrays, when realized, contain appreciable capacitive coupling between electrodes.

Some time ago we reported [3] work on a microelectrode structure based on integrated-circuit photoengraving technology. At that time, such photoengraved microelectrodes had successfully recorded spontaneous single-unit spike activity in cat hippocampus, and several potential advantages of such structures had been demonstrated. There has been considerable interest in this approach, and we have continued to refine the probe structure. Additional studies have been conducted to evaluate these microelectrodes for single-unit recording, and this communication will describe the results achieved. Our goals were: 1) to determine if such photoengraved probes are capable of isolating single units in the brain; and 2) to gain a better understanding of how electrode dimensions (especially recording area size) affect extracellularly recorded unit potentials. We feel the results are important not only because they provide an evaluation of this type of microelectrode, but also because knowledge of the requirements for a useful probe structure provides clues to the mechanisms of extracellular current flow and electrode-cell coupling—topics of fundamental importance in electrophysiology.

Methods

A total of approximately 75 electrodes of various geometries have been used *in vivo* to arrive at the basic probe structure shown in Fig. 1(a). The gold electrode is formed on a thermally oxidized silicon carrier by electroplating, and it is insulated from the surrounding electrolyte by a thin layer of deposited silicon dioxide (fused quartz). The insulation is removed from the electrode tip to define the recording area using precise photoengraving techniques. Probe size is set by the the size of the supporting carrier, while electrode parameters such as recording area may be set independently. Furthermore, multielectrode structures can be realized with no increase in processing complexity over that for a single-electrode probe. The thermal oxide layer under the electrodes is 1 μm thick, allowing the silicon carrier to act as a ground plane and greatly reduce interelectrode coupling [3]. The total capacitance from electrode to ground is nevertheless small (typically under 2 pF) due to the narrow metal lines used to transmit the signal. Using both isotropic and anisotropic etchants for shaping the silicon carrier, a few microns of silicon can be retained under the thermal oxide at the probe tip for increased mechanical strength. Fig. 1(b) shows a single-electrode probe fabricated using these techniques.

Experiments were performed on six adult cats anesthetized with pentobarbital and placed in a sound attenuating chamber. The animals were held in a head clamp, and a 3-mm hole was drilled in the bone overlying the primary auditory cortex (5 cats) and precruciate sulcus (1 cat). The dura was removed and the exposed brain covered by a solution of agar. The microelectrode was lowered through the cortex by a hydraulic microdrive controlled from outside the experimental chamber. For the experiments in auditory cortex, clicks or tone bursts

were presented into the contralateral ear by an insert earphone (Audio-vox 9C). The maximum intensity of signal used was approximately 80 dB above the experimenters' thresholds. For the experiment in precruciate sulcus, a conventional bipolar stimulating electrode was inserted into the pyramidal tract in the brain stem to aid in locating units by antidromic activation. Electrical activity recorded at the microelectrode was amplified by a source follower in series with a Grass P511 amplifier and was displayed in the adjacent room on an oscilloscope. The bandpass of the system used was from 7 Hz to 10 kHz. Photographs were made of each unit studied, and measurements of spike amplitude, polarity, and duration were made from these records. Our procedure was to lower the microelectrode through the cortex while presenting stimuli at 1/s. When a unit was encountered that was either spontaneously active or driven by the stimuli, photographs were made. We then attempted to see how long the unit could be recorded without moving the microdrive to approximate conditions during an experiment. The electrode was then advanced to find another single unit. In two of the cats, histology was carried out to define the tissue damage that resulted from the electrode. Standard histological methods were used consisting of frozen sections 25 μm in thickness stained with cresyl violet.
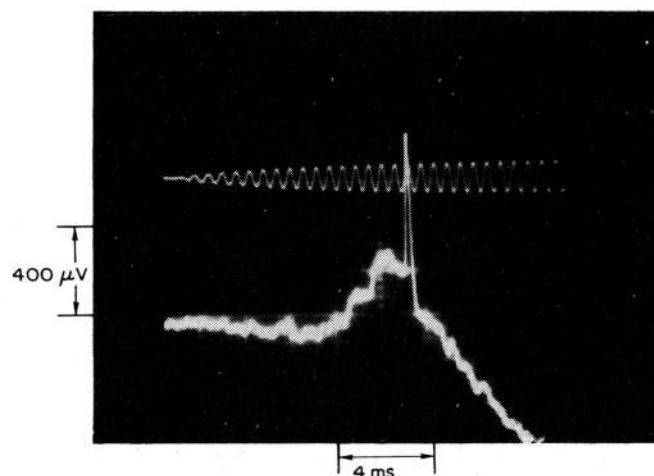

Fig. 2. Single-unit activity recorded from cat auditory cortex using a single-electrode microprobe. Negative polarity upward. Upper trace—input tone burst (stimulus); lower trace—evoked neural activity (scales as noted).

RESULTS

A total of 15 single-electrode probes were tested, ranging in impedance from 3 to 14 MΩ at 1 kHz. Electrode impedance was measured by passing a known current (1-nA peak amplitude or less) through the electrode in a buffered saline electrolyte [3] and measuring the resulting voltage. The geometrical recording areas, as set by the photoengraving masks and confirmed by microscopic observation, ranged from 15 to 75 square microns. Larger recording areas were not generally successful in isolating single units, while smaller recording sites could not be realized with the control desired for these experiments. The surface roughness of these plated electrodes, which relates impedance to geometrical recording area, can be varied significantly depending on the plating current density used. However, on a given silicon wafer, where all probes are plated simultaneously, the roughness is virtually constant for all electrodes, and at a given current density, the reproducibility from wafer to wafer is typically better than ±15 percent. The surface roughness (and thus impedance per unit area) was similar for all probes used in these studies.

Twenty-eight neuronal units were classified in auditory cortex. All were either biphasic (polarity being initially negative) or negative only, and spike durations were about 1 ms. Their peak amplitudes ranged from 40 to 600 μV, averaging 290 μV. All of the units could be held for periods greater than 30 min. There was a tendency for the higher impedance probes to record the larger amplitude spikes; five electrodes with impedance levels from 3 to 6 MΩ recorded units which averaged 210 μV in spike amplitude, while five probes with impedances from 9 to 14 MΩ recorded units averaging 360 μV in amplitude. In contrast, the slow-wave evoked responses were of similar amplitude for all probes, independent of their impedance levels. Fig. 2 is an example of a unit event in auditory cortex recorded with these probes.

It has been empirically observed using conventional electrodes that high-impedance electrodes are more effective in recording higher amplitude spikes than low-impedance electrodes [1], [4]. In order to investigate the relationship of spike amplitude to electrode impedance, multielectrode probes having carrier tip widths (specified 50 μm behind the recording sites) of 40 μm and containing two electrodes were fabricated as shown in Fig. 3(a). The impedance of the smaller electrode was higher by a factor of from two to five over that for the larger electrode, which had an impedance of about 3 MΩ at 1 kHz. The interelectrode separation was 7.5 μm. Four of these probes were used to record from single units in the cortex of four cats, and results from one of the units sampled are shown in Fig. 3(b). For 23 single-unit events studied (19 in auditory cortex, 4 in precruciate cortex), the spike amplitudes recorded from the higher impedance electrodes were larger in every case than those from the corresponding lower impedance electrodes, whereas the amplitudes of the slow-wave evoked re-
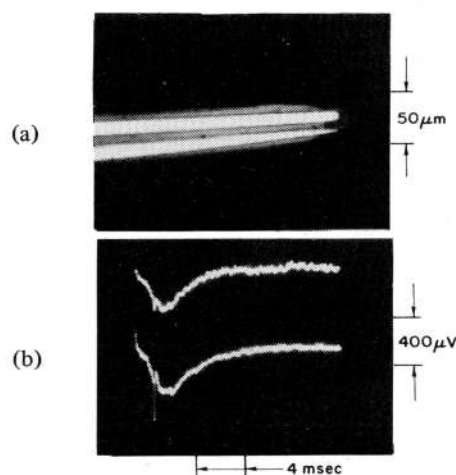
Fig. 3. (a) Photomicrograph of a double-electrode microprobe used for single-unit recording. Interelectrode separation is 7.5 μm. (b) Single-unit activity recorded from cat precruciate cortex with the double-electrode probe shown in Fig. 3(a). Upper trace—recorded activity from the larger electrode (3 MΩ at 1 kHz); lower trace—recorded activity from the smaller electrode (16 MΩ at 1 kHz). Voltage and time scales as noted. Positive polarity upward.

sponses were virtually identical. For these 23 units, the average of all spike amplitudes for the higher and lower impedance electrodes was 182 μV and 80 μV, respectively. In contrast, when these same probes were placed in saline and sinusoidal currents (30-nA peak amplitude) of different frequencies (10 Hz–10 kHz) and were passed through a set of closely adjacent stimulating probes, no differences were observed between the voltages detected by the high- and low-impedance electrodes.

DISCUSSION

The results indicate that these probes are capable of recording single-cell discharges in both auditory and precruciate cortices of the cat and are probably suitable for recording from a wide variety of CNS tissue. The recordings were stable with good signal-to-noise ratios, and cell discharges could be maintained for periods sufficiently long to make detailed physiological correlations. Examination of histological sections of the studied areas showed tissue damage by the probes to be minimal.

Our data clearly indicate that tip size is a critical factor for obtaining large-amplitude cell discharges. Results from the present study in which two electrodes of different impedances were maintained at the same distance from the cell indicate that the larger amplitude spikes recorded with electrodes of higher impedance cannot be attributed entirely to the advantage that higher impedance electrodes might have in

more closely approaching the cell. Rather, the data suggest that the paths of current flow through the brain for cell discharges may be extremely small and that electrodes of small dimensions can more effectively sample these paths than can larger electrodes. This observation is in agreement with Robinson [1], who suggests that the extraneuronal current may flow principally in the narrow extracellular clefts between glial cells. The tissue would then constitute a relatively high impedance between the cell and the electrode. It is likely that, except perhaps at the very tip, a sheath of fluid exists close to the surface and along the length of a microelectrode in tissue. Such a sheath could be expected to offer a relatively low-impedance path to remote ground, and thus the voltage sensed by the electrode would be determined by the ability of the probe tip to enter these channels or clefts and by how well the tissue can seal around the electrode near the tip. We have observed that unless the recording sites are within about 20 μm of the probe tip, these photoengraved microelectrodes are not able to record spike activity effectively. This observation lends further support to the above ideas. Certainly the size differences of these electrodes do not cause any frequency response differences *in vitro* and stray capacitive attenuation would produce an opposite effect from that observed.

Although the efforts reported here have concentrated on single-unit recording in order to evaluate how far the technology can be extended, probes having larger recording areas also have been fabricated. Such probes have performed well as population electrodes, and the fabrication of multielectrode arrays for population recording is one area for which we feel this technology is best suited. Finally, since the carrier is silicon, buffer amplifiers can be fabricated as an integral part of the probe structure, resulting in output impedances for the recording channels of less than 500 Ω. This makes possible the fabrication of arrays containing both stimulating and recording electrodes with essentially no crosstalk between the stimulation and recording channels [5].

The fabrication of these probes involves techniques that have not been available to physiologists in the past. A number of universities are now establishing integrated-circuit laboratories (some in connection with established bioengineering programs), and in such locations the further application of advanced technology to problems in medicine will undoubtedly occur. The further development and utilization of photoengraved probe structures in such centers is a necessary step if they eventually are to be made commercially available to physiologists in general.

SUMMARY

The types of single-unit events recorded from auditory and precruciate cortices of cats with photoengraved microelectrodes have been described. Spike amplitudes up to 600 μV of negative–positive polarity were recorded. Cell discharges could be maintained for over 1 h without loss of amplitude. The importance of electrode tip size in recording high-amplitude single-unit events appears to be dependent on the distribution of current fields around a cell. No evidence was found to support the contention that small electrodes record larger spikes only because they can approach more closely the cell soma. These new electrodes appear comparable to conventional metal microelectrodes in many respects. The new electrodes' advantages are in the control of tip sizes and spacings and in the feasibility of integrating buffer amplifiers close to the recording sites, making them suitable for a wide number of studies not practical with conventional technology.

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Microelectrode-Impedance Testing Swept-Frequency Technique

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Abstract—The selection of tungsten microelectrodes for cellular-level recordings is frequently based on simple testing techniques, such as microscopic examination and generation of gas bubbles on the exposed tip. A rapid technique, whereby the relative impedance magnitude may be determined using swept frequencies, is described.

BACKGROUND CONSIDERATIONS

The selection of tungsten microelectrodes for cellular-level recordings is a difficult task due to the electrical and mechanical properties of the device. It is fragile, having a tip dimension on the order of 10 μm, and is insulated with a coating not readily visible using conventional light microscopy. Since it is used to pick up cellular electrical activity, some means of evaluating electrical characteristics is desirable.

Microelectrode-impedance measurements have been made using specialized bridge techniques, such as those described by Gesteland and Howland [1]. Equipment of this type may consume 1 or 2 min for a given frequency-impedance measurement. Unfortunately, the microelectrode tends to be a nonlinear device requiring several measurements at different frequencies to establish a trend. Not only is it a time-consuming procedure, but the resulting impedance data are difficult to interpret relative to electrode performance. It is little wonder that routine electrode measurements are infrequently applied to mass screening.

The vector-impedance voltmeter could materially shorten the time required to measure impedance. Interpretation of the data is similar to that of the bridge technique, essentially interesting to the engineer, but unimpressive for the electrophysiologist. Not only are these equipments expensive, but most impress an unknown voltage across the test device, which, if not limited, could drive destructive currents through the electrode. These factors suggest conventional impedance-measuring techniques are unsuitable for microelectrode testing.

It was decided to find a measuring technique compatible with microelectrode characteristics. Design considerations are: 1) electrode test currents should be limited by generous series impedance (test voltages less than 100-mV peak and series impedance greater than 1 MΩ; 2) potential tip polarization suggests the use of ac test currents having a frequency range approximating physiological currents (nominally 100–10 kHz); 3) the measurement of impedance is not a necessity provided indirect measurements such as impedance magnitude is acceptable; 4) data readout providing a hard copy such as a Polaroid print is desirable to permit comparisons; and 5) the test system should be inexpensive and easily used.

The requirements for a microelectrode test system are considerable; therefore, the proposed test setup shown in Fig. 1 appears relatively simple. The circuit is straightforward; a constant oscillator voltage is impressed across the series load consisting of the test microelectrode and oscilloscope.

The scope input impedance, 1 MΩ shunted by 47 pF, represents a frequency sensitive input across which the oscilloscope voltage is measured. The horizontal axis of the scope parallels the oscillator voltage-control source (VCG input), hence the oscilloscope displays a plot of relative electrode impedance versus test frequency.

The circuit may be analyzed by applying Ohm's law which yields

$$\text{electrode } Z = \frac{(\text{oscillator } V)(\text{scope } Z)}{(\text{scope } V)} - (\text{scope } Z).$$