A Flexible Perforated Microelectrode Array for Extended Neural Recordings

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Abstract—**A flexible and perforated 32-element planar microelectrode array has been fabricated and used to measure evoked potentials in brain slices. Electrodes are spaced 200 μm apart in a 4 × 8 array and are sandwiched between layers of insulating polyimide. The polyimide sandwich is lifted off its substrate, making it flexible so that it could shape to contoured tissues. Prior to liftoff, holes are etched to expose recording sites 15 μm in diameter and to create perforations which allow increased circulation of artificial cerebrospinal fluid to the recording surface of the tissue and, hence, increased viability. Comparisons of evoked potentials measured over time showed an average increase of 10 h to the viability of the slice while using the perforated versus nonperforated arrays.**

Introduction

THE need for multiple electrodes spatially distributed over various dimensions has spawned numerous designs in the form of probes [1]–[3] and planes [4]–[6]. Probe-style devices typically have an in-line arrangement of electrodes used to record from various depths as the probe is inserted through cortical layers. Planar electrode arrays have been used for surface recordings from isolated ganglia, brain slices, and cultured neuron populations. Characteristic of all these arrays has been the use of techniques borrowed from integrated circuit technology to fabricate devices with linewidths comparable to sizes of individual neurons.

The planar arrangement allows for a high density of electrodes in a given area. Previous arrays, including those developed in our laboratory, are built with a solid, rigid substrate. When used with thicker tissue preparations, such as brain slices, oxygen and nutrient-rich fluids are prevented from reaching the tissue surface from which recordings are being made, leading to early tissue death and limited experimental time [7], [8].

To minimize this problem we fabricated a perforated array consisting of gold conductors sandwiched between layers of polyimide. Reactive ion etch techniques permitted precisely defined perforations to be etched adjacent to the recording sites. When removed from the substrate the array is also flexible and able to conform to neural surfaces.

The perforations surrounding the electrodes increase the circulation to the recording surface of the tissue or slice. In the work reported here, the extended viability of the brain slice tissue was assessed by recording evoked potentials from rat hippocampal slices over time, with the result being a substantial increase in viability using perforated versus nonperforated arrays.

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Fabrication of the Electrode Array

A mask set was produced from array designs drawn on the LASI (LAyout System for Individuals) [9] CADD system and fabricated using the Cambridge EBMF-6 Electron Beam Fabricator at the UIUC Microelectronics Laboratory. This set consists of three masks including the metal pattern, the insulation holes, and the perforations.

Substrates of 24 × 40 mm no. 2 Corning glass coverslips are used as rigid support during array fabrication. The lower polyimide layer (DuPont Pyralin 2525) is spun on the coverslips (3000 rpm; 30 s) with rapid acceleration to prevent edge-bead effects. The polyimide is cured on a hot plate by ramping to 350°C over 45 min and holding at 350°C for an additional 45 min, resulting in a 10 μm layer resistant to high temperatures and the wet etchants used in the array fabrication process.

Next the conducting layers of metal are patterned on the polyimide. To increase the adhesion of gold, 30 Å of Ti is evaporated first, followed by 750 Å of Au without breaking vacuum. The gold is patterned using standard photoresist (PR; Shipley 1450J) and wet etching techniques. The gold is etched for 30 s in a solution of 80 g KI and 20 g I in 450 mL of deionized (DI) water. The titanium is etched for 30 s in a 2% solution of HF and DI water. After removal of the PR and baking to remove moisture, the insulating layer of polyimide (DuPont Pyralin 2555) is spun on the array (3000 rpm; 30 s) to form a 2 μm layer after curing. Experience shows it is not necessary to use an additional titanium layer for adequate adhesion of the top layer of polyimide to the gold.

The perforations and electrode sites are defined by reactive ion etching (RIE) using a PlasmaLab μP RIE, for which an etch rate of 0.05 μm/min had been determined for cured polyimide in an oxygen plasma (gas flow 30 sccm; 200 mtorr; RF power 50 W). First, a 1000 Å thick masking layer of Ti is evaporated over the polyimide, pat-

terned, and etched using the 2% HF solution. The RIE etched through the 12 μm of polyimide in 4.0 $\pm$ 0.5 h. After a second photopatterning of the same Ti mask layer, the electrode site holes are etched through the upper 2-μm-thick polyimide layer in 40 $\pm$ 10 min. The Ti mask is stripped with the 2% HF solution. The resulting sandwich of materials is shown in Fig. 1.

To loosen the array from the glass coverslip, the arrays are autoclaved for 30 min at 120°C in a steam environment. This helps to cleave the bonds holding the polyimide to the glass. Any moisture at this interface induces separation. As the arrays are soaking in DI water, a corner of the coverslip is broken with tweezers. Water quickly enters the interface between the polyimide and the coverslip and the array floats to the surface. The hydrophobic polyimide allows easy drying and maintains its planar shape even after being lifted free. A flexible, perforated array lifted free of its rigid substrate is shown in Fig. 2(a).

To reduce electrode impedance, the electrodes are individually plated for 30 s at 1 μA in a solution of 3% platinum chloride and 0.025% lead acetate in 0.025 N HCl. Individual electrodes are monitored microscopically to ensure the quality of the deposited platinum black. Arrays are thoroughly rinsed with water and conditioned in ACSF following plating.

The finished array has perforations of sizes 30 $\times$ 30 μm, 30 $\times$ 100 μm, and 30 $\times$ 200 μm, which occupy 6% of the central recording area of the array. The diameter of the electrode sites was consistently between 13 and 15 μm with an interelectrode spacing of 200 μm. The 32-electrode sites are arranged in a 4 $\times$ 8 array shown in Fig. 2(b). The electrode sites are connected to 0.75 $\times$ 5-mm readout pads, located at the edge of the array, via 20 μm wide lines.

Electrical Characteristics of Array

Electrical characteristics of the electrodes were either estimated or measured to ensure that they were likely to record small, low-frequency neural signals. The shunt impedance to ground (the bathing media) was estimated, using a simple parallel plate capacitor model, to be 0.5 pF or 321 MΩ at 1 kHz for a 2 μm insulating layer of polyimide of permittivity 3.5 [10]. Similarly, capacitive coupling between adjacent electrodes through the electrolyte was estimated to be less than 835 fF or greater than 190 MΩ at 1 kHz.

The impedances of unplated electrodes at 1 kHz averaged 3.4 MΩ (range 1.5–9.2 MΩ), with a phase angle of $-70° \pm 5°$, due to the small (13–15 μm diameter), specular surface of the electrode. For comparison, electrodes defined using wet chemical etching were much larger (the 30–50 μm holes exceeded the metal pattern size) and had impedances ranging from 0.8 to 1.8 MΩ. After electroplating, the 32 RIE-defined electrodes had impedances ranging from 5.1 to 17.8 kΩ with phase angles between $-14°$ and $-27°$. The impedance values of both the perforated and the nonperforated arrays were comparable to each other as well as to those of rigid electrode arrays previously fabricated in our laboratory [7]. Electrode impedances were checked prior to placing a slice on the array. The electrodes required plating after every two to three slices. No attempt was made to track electrode stability over time.

Supporting Equipment

The mount for the array consisted of: 1) an etched circuit board to which the array's gold readout pads were press fit; 2) a Plexiglas chamber clamped beneath the array, with a milled slot along which ACSF could flow and contact the underside of the perforated arrays; 3) a matching chamber which rested above the array, with a slot permitting ACSF and gas flow on top of the array, and a small circular port for viewing and insertion of the bipolar electrode; and 4) a Plexiglas base to which the first three items are mounted and which sat in contact with a heated pool of water. The ACSF was dripped onto a filter paper manually placed either in the bottom trough, or on top of the array. The paper was cut away over the region of the electrodes. The circuit board has 34 copper lines (32 for electrodes, 2 for grounds; 0.05 in wide; 0.10 in centers) configured on a double-sided printed circuitboard. Seventeen pass to the second side and run to an edge where a 34-pin slide-on connector with ribbon cable is easily attached. Press-fitting the flexible array proved adequate since the array readily conformed to the raised copper lines.

The short ribbon cable runs to a bank of 32 separate multistage amplifiers. Each channel is buffered by a high impedance, unity gain JFET, and ultimately amplified by either 2000 or 5000. A novel feature of the system is a track/hold circuit which disconnects the high-gain amplifiers during the stimulation pulse [7]. Without it the stimulation artifact often causes saturation in the second stage, high gain amplifiers, lasting 10–20 ms. All 32 channels are sampled simultaneously with a high-speed A/D converter [11] and analyzed using specially designed software on an AT&T 6300 PC. The typical display in Fig. 3 not only demonstrates the array's functionality, but also illustrates the 4 $\times$ 8 display of 32 channels. The row and column orientation match that found on the electrode array.

Experiment

To demonstrate the effectiveness of the perforations etched through the array, evoked potentials were recorded from rat hippocampal slices on both flexible perforated and flexible nonperforated arrays. Arrays were clamped in the array mount which was then placed in an environmental chamber. The chamber allowed a warmed, humidified gas mixture of 95% O_2/5% CO_2 to pass into the chamber and over the surface of the slice. Oxygenated ACSF was gravity-fed at 1 mL/min through a warmed

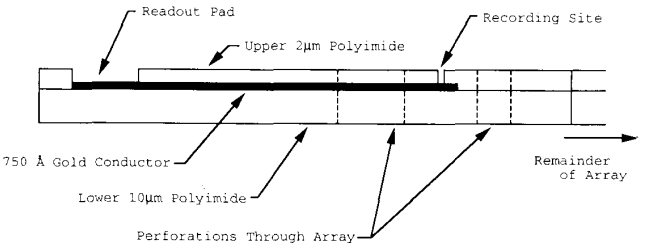
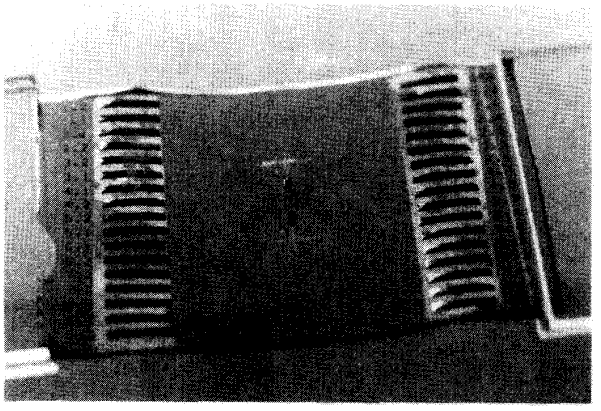
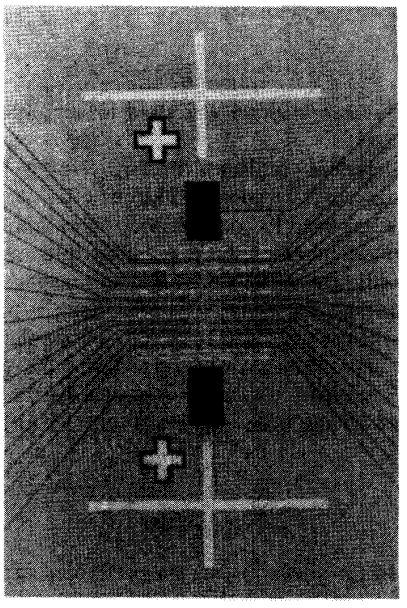


Fig. 1. Side view of a flexible perforated electrode array, in the absence of a rigid substrate, showing polyimide sandwich of Au/Ti conductor and RIE-formed holes and perforations.



(a)



(b)

Fig. 2. (a) Microelectrode array exhibiting flexible nature. (b) Presence of holes in insulating polyimide layer. Notice size, number, and locations of perforations relative to the electrodes in recording region. Size of array and features can be inferred from the 7 mm separation between the large alignment crosses.

water bath and then either below the perforated array or above the nonperforated array. ACSF passed below the perforated array flowed through the perforations and over the top surface of the array as it bathed the slice. With the nonperforated array, ACSF was passed directly to the top surface of the array since flow beneath the array would be of no use. The water bath was warmed to 33°C by conduction coils filled with heated circulating water. This lower temperature was used to keep slices in a healthier state for maximum viability [12].

Solutions of normal ACSF were prepared containing (in mM) 117 NaCl, 5 KCl, 1 NaH_2PO_4, 26 $NaHCO_3$, 10 Glucose, 1.6 $MgSO_4$, and 2.5 $CaCl_2$. Low magnesium ACSF solutions omitted the $MgSO_4$. Solutions were bubbled with the gas mixture for 1 h prior to dissection to ensure complete oxygenation [13] and to lower the pH of the media to 7.3–7.4. One-half of the normal medium was transferred to the slice holding dish while the other half dripped through to wet and condition the array and chamber.

Seven Long-Evans Hooded rats (three female, four male) were sacrificed according to LACAC and approved protocols over the course of the experiment. They ranged in age from 6–9 weeks and weighed 200–275 g. Rats were kept on a 12 : 12 light : dark cycle. From seven rats, five slices were used on a perforated array and five on a nonperforated array. Slices were 400 μm thick and taken from the mid-region of the left hippocampus by way of the procedure described by Teyler [14]. Slices were allowed to incubate in oxygenated normal medium for 1 h prior to any testing for recovery from the depressed state induced by the slicing procedure.

Following incubation, the normal medium dripping through the chamber was replaced by the low magnesium ACSF to induce seizure-like activity [15], which was easily detected with the array. Slices were placed on the array using a fine sable brush and aligned with the Schaffer collaterals parallel to the long axis of the array while the short axis spanned strata oriens, pyramidale, and radiatum. This orientation allowed for the observation of propagating signals across several distinct layers as observed by Novak and Wheeler [8]. A bipolar stimulating electrode with 300 μm electrode separation was placed in the stratum radiatum at the CA3/CA1 border.

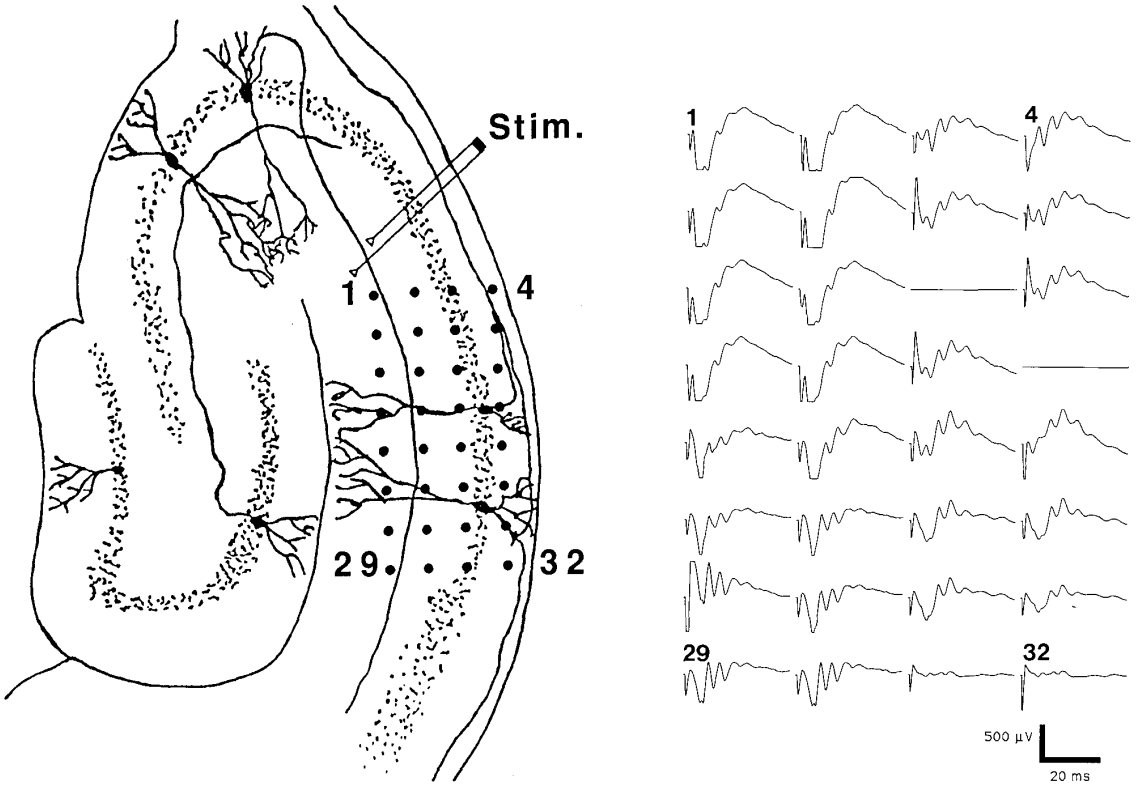


Fig. 3. Rat hippocampal CA1 region data obtained simultaneously from 32 channels following stimulation of Schaffer Collaterals in slice. Electrodes on channels 3,3 and 4,4 were not functional during the recording. Diagram of hippocampal slice illustrates relative positioning of stimulating and array electrodes.

Recordings

Under computer control, 100–400 μA pulses were delivered at 10 min intervals to the slice with the bipolar electrode. The population responses were digitized at 8 kHz, displayed, and saved. If no bursting activity was observed after an initial 20 min, the inactive slice was removed and replaced. If a slice did exhibit a response, the stimulations were allowed to continue until neural responses were no longer evident and the slice was considered no longer viable.

The signals obtained over the lifespan of a slice were examined to record the peak-to-peak amplitude of the first major population spike, the period of bursting, and the duration to the first major potential following each stimulation pulse. These values, for a single channel, were plotted over the lifespan of the slice. As expected, as the slice viability declined, the amplitude decreased, while the period of oscillation and latency increased. The remaining channels were examined to confirm that these changes did occur in other areas of the slice. For comparison of results from the perforated and nonperforated arrays, slices were manually positioned in the same position relative to the array as determined through a microscope. The same channel was used to acquire the comparative data.

The conventional indicator for slice viability is the amplitude of its evoked response. Plots of relative amplitude over time were used to compare viabilities among slices. Fig. 4 includes plots of the population spike amplitudes obtained while the slices were alive and responsive.

To determine the viability of a slice, a third-order polynomial curve was fitted to the plot of population spike amplitude versus time. From this curve, the maximum value was identified. The slice was regarded as no longer viable when its response amplitude fell to half this maximum value.

Results

Lifespans for slices resting on perforated arrays were significantly greater than those on nonperforated arrays. On the perforated array, slices were kept alive for 7.5, 24.5, 12.2, 15.5, and 9.5 h with an average of 13.8 h. For the nonperforated array, slices were viable for only 4.8, 6.7, 2.3, 2.5, and 2.5 h with an average of 3.8 h. Using the Student's t-distribution for the statistics of small

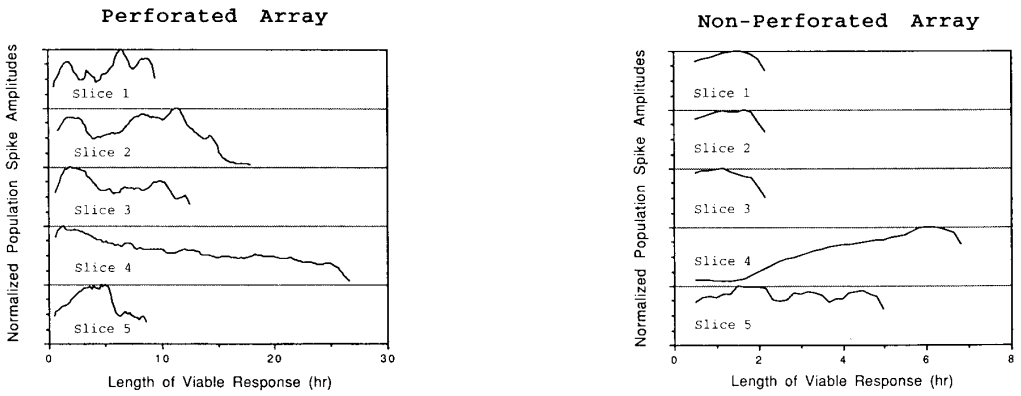


Fig. 4. Population spike amplitudes over time for rat hippocampal slices on perforated and nonperforated electrode arrays. (Note the significant difference between time scales.)

numbers of samples, the means were statistically different to the 2% confidence level.

Discussion

Microfabrication processes can be readily extended to the mass production of the flexible, perforated electrode arrays, especially when reactive ion etching is used to obtain greater etching control of the polyimide. The work here complements earlier efforts in fabrication of flexible microsensors, such as the polyimide encapsulated temperature sensors of Barth [16].

Polyimide appears to be a good but not perfect material for use in animal experimentation. Thorough investigations of polyimide coated wires in saline soak tests by Edell [17] showed that polyimide gradually deteriorates over many months under 5 V bias. Presumably the passive device reported here would remain intact longer.

Producing a flexible electrode array that is free of any rigid support offers opportunities for planar surface recordings from convoluted tissue surfaces. A flexible array could be laid over cortical regions during surgery to record responses or wrapped around peripheral nerves during experimental procedures. If adapted for implantation, its flexible nature would be more forgiving during tissue or electrode movement and prevent any severe dislocation, a common difficulty associated with present electrodes implanted for extended periods of time [18]. The perforations present might further serve as locations for tissue ingrowth and increase anchoring of the array to the tissue.

Hippocampal slices were utilized as a means of evaluating the array since their pathways of neural activity have been characterized extensively. The perforations etched through the flexible array have been shown to increase the viability of hippocampal slices. Planar arrays on rigid substrates have limited the recording and experimentation time with slices to 2 h [19]. Slices resting on perforated arrays were kept viable for an average of 10 h longer than those on nonperforated arrays. With increased viability noted, the experiment confirms that perforations in a planar electrode array lessen the adverse effects a large planar surface would have on occluding fluid flow and limiting oxygen diffusion to the tissue. This array and its improvements enable further studies of spatially distributed activity and extended slice responses to various drugs or stresses.

As the total area of the perforations increases, the array takes on the characteristics of a polyimide mesh. Further materials testing is required to determine the maximum dimensions and number of perforations for the given electrode area to maintain mechanical integrity and electrical recording fidelity. Such an array mesh or net could be not only a versatile tool for extended slice recordings, but also the basis for a chronically implanted device.

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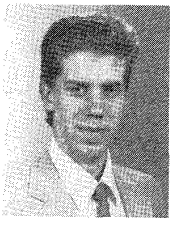

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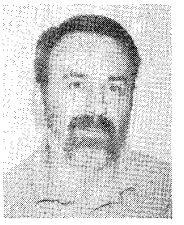

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