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A Custom Multielectrode Array with Integrated Low-Noise Preamplifiers

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Abstract— **Multielectrode arrays (MEAs) have emerged as a leading technology for extracellular neural recording and stimulation. Their large number of recording sites promises to yield important insight into neural systems. As the density of recording sites increases, interfacing to each electrode becomes increasingly difficult. Introducing electronics onto the MEA substrate provides a technique for preliminary signal conditioning to take place at the MEA itself, reducing the complexity of off-package electronics. This paper introduces a custom MEA system with integrated preamplifiers. MEA fabrication, cell-culturing, and electrical performance are discussed.**

Index Terms— **electrode, multielectrode arrrays, MEA, MEMS, extracellular recording, low-noise.**

I. Introduction

Neural systems depend on parallel, distributed processing involving many different neurons. Many standard intracellular techniques investigate only small numbers of cells and cannot determine network properties adequately. Research techniques that are capable of recording from and stimulating multiple cells are necessary to investigate the properties of large networks of neurons. A common approach to such investigation is to culture a neural sample on a multielectrode array (MEA), facilitating extracellular recording and stimulation in a small area [1] [2].

One of the difficulties encountered in extracellular recordings is the small magnitude of the signal. A common technique to record such signals is to connect a preamplifier to the MEA. The preamplifier provides a modest gain for the signal while introducing a small amount of noise. Literature has presented the recording systems that pair an MEA with external preamplifiers [3] [4]. The preamplifiers that interface directly to the electrode must meet the stringent requirements of high input impedance and low noise if they are to faithfully record neural signals. Integration of the preamplifiers onto the MEA substrate relaxes the performance required of external circuits that connect to the MEA, thereby providing an effective intermediary between the electrodes and external measurement systems.

Standard complementary metal-oxide-semiconductor (CMOS) fabrication processes provide a convenient method for realization of the preamplifiers because of existing design techniques for a wide variety of amplifiers, signal processing circuitry, and multiplexers [5]. The capability to construct such a variety of circuitry in a very small area leads to the possibility of incorporating large arrays of circuitry and large numbers of electrodes on the MEA.

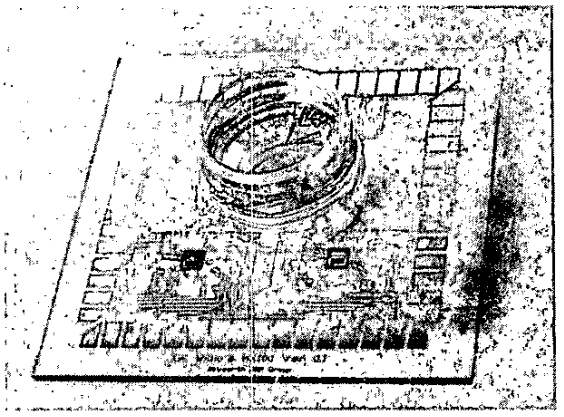

Fig. 1. Photograph of the MEA with integrated electronics. Each integrated circuit contains two preamplifiers, for a total of four amplified channels. Additional electrodes that connect directly to the contact pads are included for testing purposes.

One long term goal of the research effort presented here is to fabricate an MEA with 1024 electrodes and circuitry, such as spike detection and artifact removal, that interfaces to the electrodes. An initial step towards this research goal is the creation of a prototype MEA that demonstrates packaging of the electrodes and electronics together. This paper introduces a single package MEA, shown in Fig. 1, that includes preamplifiers bonded to the custom designed MEA substrate. The single package MEA requires that the micromachined structures and CMOS integrated circuits are compatible with each other. We discuss these components' design and performance, and demonstrate the use of the complete system for neural recordings.

II. Preamplifier Design and Performance

Preamplifiers that are suitable for use in a single package system must meet additional design requirements beyond low-noise and high input impedance, as the area and power budgets are tightly constrained. These requirements are contradictory, because designs can typically achieve lower noise at the cost of larger area and higher power consumption. A suitable design

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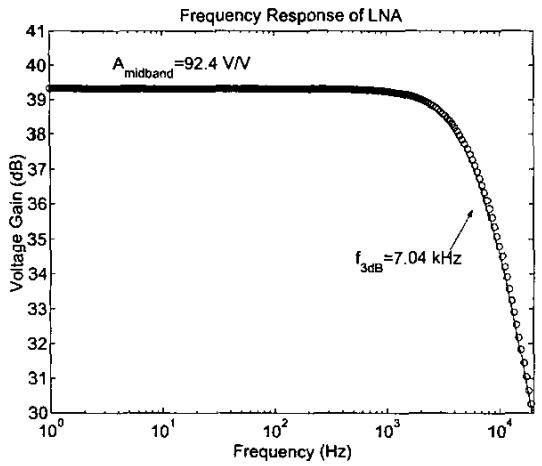

Fig. 2. Frequency response of the low noise amplifier. The best fit, single-pole transfer function has a midband gain of 92.4 and a high frequency corner at 7.04 kHz. The amplifier also has a low frequency corner below 0.1 Hz, which is not shown

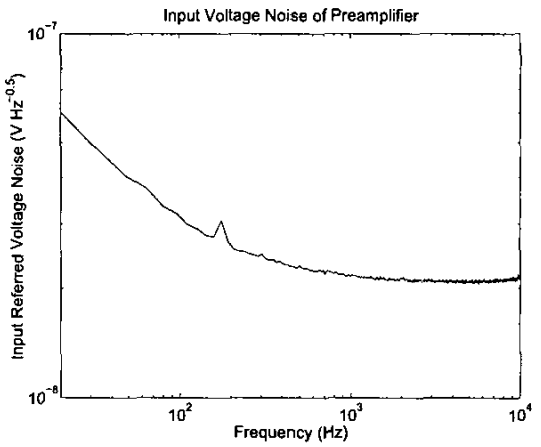

Fig. 3. Input referred noise of the low noise amplifier. The input referred thermal noise is $22.59\,\mathrm{nV}/\sqrt{\mathrm{Hz}}$. The total input noise is $2.27\,\mu\mathrm{V_{rms}}$. A peak is visible at 180 Hz, corresponding to the third harmonic of 60 Hz line noise. The noise due to lower harmonics is not visible; this is probably due to dominant $\frac{1}{f}$ noise.

strikes a reasonable balance among the requirements for noise, power, and area.

The preamplifier design chosen for the MEA is especially suited to integration because of its small size, low noise, and low power consumption [6]. The design is fabricated using the AMI Semiconductor 1.5μ process available through the MOSIS brokerage service. The preamplifier has noise performance close to the minimum possible for a desired power dissipation. The frequency response, shown in Fig. 2, exhibits bandpass response with a low frequency corner below 0.1 Hz and a high frequency corner at 7.04 kHz. The low frequency pole blocks the dc offset commonly found in electrodes. The input-referred noise is shown in Fig. 3. The total input noise within the noise bandwidth of the amplifier is $2.27\,\mu\mathrm{V_{rms}}$. The midband noise corresponds to a thermal noise level of $22.59\,\mathrm{nV}/\sqrt{\mathrm{Hz}}$.

III. Microfabrication and Packaging

In order to facilitate the integration of electronics onto an MEA, it is first necessary to create a custom MEA that is optimized for low noise performance and physical compatibility with the preamplifiers. This MEA is produced locally in the cleanroom at the Microelectronics Research Center (MiRC) of the Georgia Institute of Technology. The fabrication of this custom MEA, which employs only two masks, uses conventional surface micromachining technology, as illustrated in Fig. 4. First, a polished glass wafer is prepared as a substrate with sequential rinses of trichloroethylene, acetone, methanol, and deionized water followed by nitrogen jet drying and dry baking. NR9-8000 photoresist is patterned to define the MEA wiring. A 200 Å thick layer of titanium, followed by 4000 Å of gold, is e-beam evaporated with the undesired portions lifted off in acetone. SU-8 is patterned to form the openings to the wire bonding pads, contact pads, and electrodes. The SU-8 serves as both the electroplating mold for the platinum black electrodes and as the final insulation layer. The electrode region has an area of 900 μ^2. The platinum plating solution consists of 1% H_2PtCl_6 (hydrogen hexachloroplatinate IV), 0.01% $Pb(CH_3COO)_2$ (lead acetate) and 0.0025% HCl [7]. Platinum black electrodes are electroplated into the SU-8 molds under ultrasonic conditions which effectively remove loosely adherent platinum deposits to insure long lasting adhesion [8]. The thick SU-8 insulator, coupled with the low impedance of the platinum electrodes, provides an excellent signal-to-noise ratio. In the final step, preamplifiers, fabricated using a commercial complementary metal-oxide semiconductor (CMOS) process, are bonded onto the MEA substrate using ultrasonic wirebonding techniques. For testing purposes, two of the amplifier inputs each connect to both an electrode and a contact pad. Sylgard 186 is used to encapsulate the electronics and bind the culture dish.

Impedance spectroscopy provides a common measure of electrode performance. Measurements of six fabricated electrodes are presented in Fig. 5. The magnitude of the impedance at 1 kHz varies from 9.46 kΩ to 20.1 kΩ, with an average of 16.8 kΩ. This impedance compares favorably with that of commercially available MEAs.

The noise present in the total system is the limiting factor for the minimum detectable signal. For the purpose of noise characterization, the input to the amplifier is connected to ground. This connection is accomplished by filling the well with Hanks' Balanced Salt Solution (Gibco, Grand Island, NY), which is used as a medium for neural cultures. The electrolytic solution forms electrochemical interfaces with the input electrode and the ground electrode. As shown in Fig. 6, the noise from the electrodes and solution is $6.58\,\mu\mathrm{V_{rms}}$. This input noise is larger than the inherent amplifier noise, indicating that the amplifier is not the dominant noise source in the system.

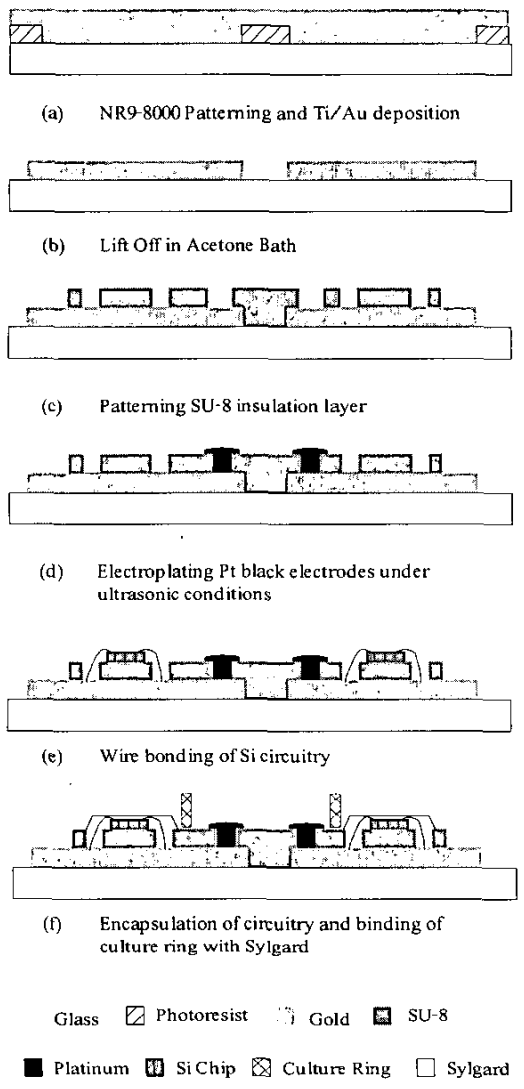

Fig. 4. Side view of MEA fabrication process. The process is based on conventional surface micromachining technology and uses only two masks.

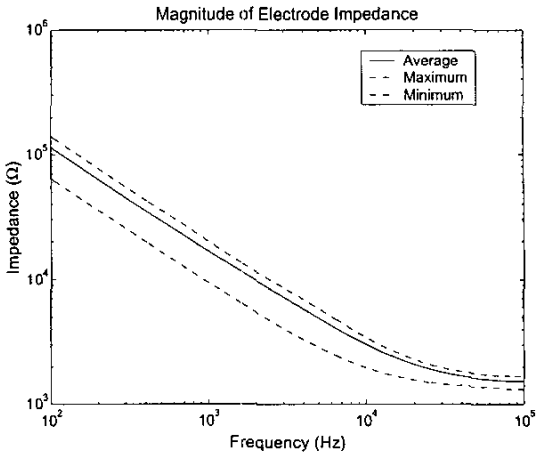

Fig. 5. Magnitude of the electrode impedance as a function of frequency. Measurements are taken for six platinum plated electrodes. Each electrode is 900 μ^2 in area. The impedance at 1 kHz ranges from 9.46 kΩ to 20.1 kΩ, with an average of 16.8 kΩ.

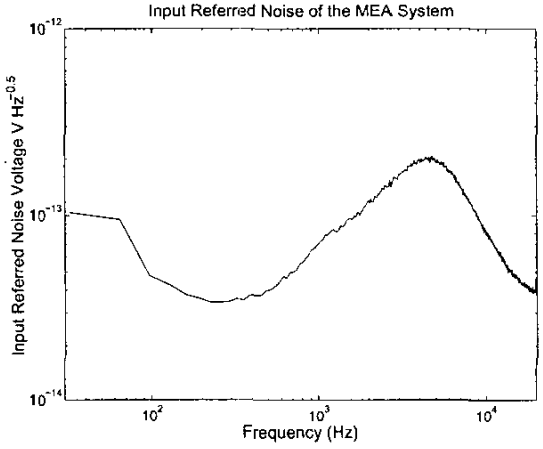

Fig. 6. Input referred noise of the system consisting of the preamplifier and MEA. The input noise is 6.58 μV_{rms}. This is significantly larger than the noise introduced by the preamplifier.

IV. Cell Preparation and Recording

In order to test the functionality of the MEA system, it is necessary to culture a neural sample on the MEA and to record extracellular signals from the sample. MEA preparation and cell plating is performed according to a protocol modified from [9]. Briefly, the MEA is cleaned using 3% BM cleaning solution (MultiChannel Systems, Ruetlingen, Germany) and rinsed with deionized water. For sterility purposes, the MEA is soaked in 75% ethyl alcohol for at least 2 hours. When the ethyl alcohol is aspirated and the MEA is completely dry, 100 μL of 0.05% polyethyl imine solution (Clonetics, Finland) is applied to the electrode region. After 1 hour, the solution is aspirated and the MEA is rinsed several times with autoclaved deionized water and allowed to dry. Twenty minutes before the addition of disassociated cells, 15 μL of laminin (Sigma, St. Louis, MO) is applied to the electrode region.

Cells are harvested from either the hippocampus or the cortex of embryonic day 18 Sasco Sprague Dawley rats and disassociated in papain solution at 37 °C. After 30 minutes, papain is removed and 1 mL of Neurobasal (Invitrogen, Carlsbad, CA) is added. The solution is then gently triturated several times and filtered to remove any remaining cell aggregates. Fifteen μL of the resulting cell solution is added to the laminin drop on the MEA at a density of 3000 cells/ μL. After 20 minutes, 0.75 mL of plating medium containing 90% Neurobasal, 10% horse serum (Invitrogen), glutamax, and B27 (Invitrogen) is added to the culture ring. The MEA is stored in a 94% humidified incubator maintained at 37 °C, 20% O_2, and 5% CO_2. After 48 hours, the plating medium is aspirated and culturing medium is added, as described by [10]. The

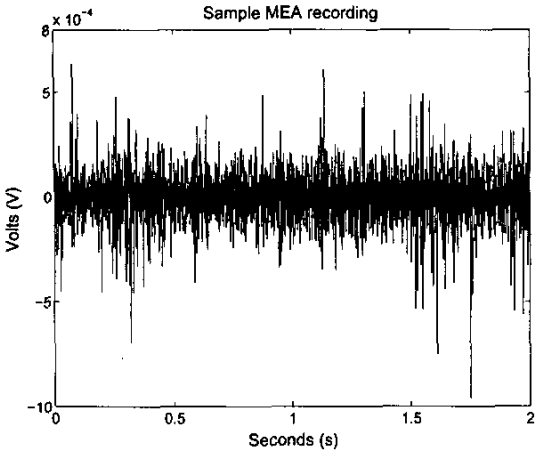

Fig. 7. Preliminary recording from rat hippocampus recordings using the MEA system. Both the on-package preamplifier and an external low noise amplifier provide gain for the signal.

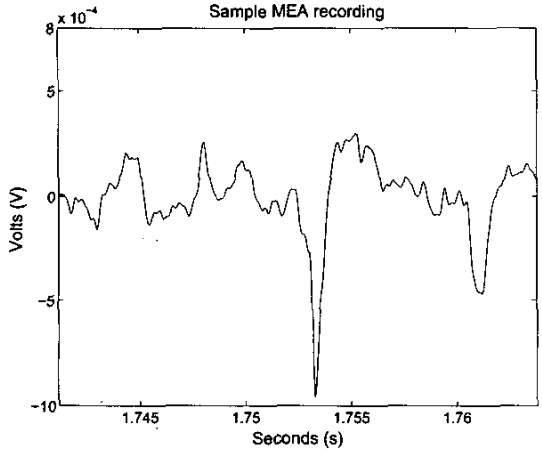

Fig. 8. Detail of the recording data shown in Fig. 7, showing a 2.5 ms interval.

medium is exchanged every 4 days as the culture remains in an incubator for 7 days, allowing adequate time for neurite extension and the formation of functional neural networks. After the incubation period, the MEA is ready for neural recordings.

In order to verify the functionality of the MEA, we record and present preliminary data. The MEA, with the live neural culture, resides in a shielded enclosure, minimizing the coupling of external noise sources, and connects to an external measurement system. onto the system. The measurement system external to the MEA package consists of a commercial low noise amplifier and a computer data acquisition card. The data acquisition system recordings, Fig. 7 and Fig. 8, show evidence of neural activity.

V. Conclusions

Incorporation of electronics on a single package with a planar array of microfabricated electrodes has been introduced. This system offers performance that is competitive to commercial systems. The preamplifiers buffer the weak neural signals, allowing for successful recordings even with low performance external circuitry. Neural recordings have been demonstrated using this system. In addition to providing a useful tool, this method shows future potential as the integration of electronics on the package raises the possibility of signal processing at the MEA. Future work focuses on using integrated electronics to support increased electrode density.

Acknowledgment

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