Nerve Growth Factor Dependent Changes in Electrophysiology Measured in a Neurotrophic Electrode.

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Abstract - **We describe here a neurotrophic electrode designed to improve long-term reliability and signal-to-noise in a brain-device interface. Our electrode consists of a fine microwire inside a polyimide tube. The polyimide tube is filled with nerve growth factor (NGF) incorporated into a fibrin matrix. Our goal is to have a neurite grow into the tube. The close apposition between neurite and the recording wire should enhance signal-to-noise. The physical connection between device and tissue should improve reliability. We have implanted complete devices in 4 rats. Each rat gets a four-electrode device implanted in the barrel cortex of each hemisphere. Each electrode is filled with a different test substance. One control tube contains only saline, and another contains only the fibrin matrix. The other two electrodes in each array are filled with different concentrations of NGF. Once weekly following the implant, each rat is sedated, and electrical activity in each of the electrodes recorded. Electrodes in 5 of the 8 implants exhibited multi-unit spindling activity. A direct comparison of the amplitude of action potentials over time in each of the types of implants shows that the presence of NGF within the polyimide tube leads to an increase in action potential amplitude.**

I. Introduction

The technology required for neuroprosthetic systems is developing rapidly. We have systems capable of simultaneously recording 10s of channels of neuronal data, computers easily fast enough to analyze that data and transmit it to prosthetic devices, and devices that can do useful tasks ranging from communication to grasping and retrieving objects in peri-personal space. One crucial link though, is still lacking: a reliable, high signal-to-noise ratio, brain-electronics interface.

One solution that has been proposed is to use a biologically active neuronal interface [1]. A device like this could establish a physical connection with a neuron by 'capturing' a neurite (Fig. 1), and encouraging growth of that neurite to a recording surface.

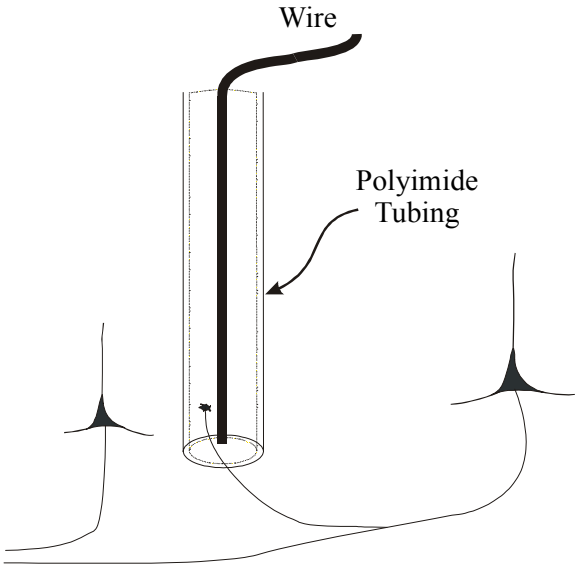

Fig. 1 Schematic diagram of our neurotrophic electrode with a neurite growing into a polyimide tube.

A previously developed neurotrophic electrode used the cone portion of a typical glass micropipette with two attached leads and filled with a neurotrophic slurry [1]. The "cone" electrode then captured neurites growing into the neurotrophic medium, so that the investigators were able to record neuronal signals with a large signal-to-noise ratio over a long period [2].

The cone electrode, however, has some limitations. For one, the space in which the neurites can grow is rather large: 50 micron at the narrow end, over 400 micron at the fat end. This enough to support the growth, easily, of tens of neurites. Isolating the electrical signal from a single one of those neurites would be quite difficult.

Another problem is that, while the cone electrode does encourage growth, the neurotrophic

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Abstract

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media used in the electrode have not been well-characterized. We would like to be able to make more careful engineering assessments of possible neurotrophic media.

We have been developing a device which addresses these two problems, and have been using the device to assess the efficacy of one particular neurotrophic medium. We are using a bio-engineered fibrin gel which binds NGF [3], and releases the NGF by the action of extracellular enzymatic activity [4].

At early stages in the study, we have been able to use the device to see clear changes in recording quality develop over time in a manner that depended on the presence of nerve growth factor.

II. Methodology

A. Device Construction

Each device is a four electrode array. Each electrode in the array consists of two leads inside a section of polyimide tubing.

We insert two pieces of 50mm insulated tungsten wire into a 250mm ID polyimide tube, which is beveled at the tip. One wire is the length of the tube, the other, 1mm shorter with 1mm of insulation removed at the tip. Each of the four polyimide tubes is epoxied into a small feline catheter, and the catheters are fixed to a 10-pin connector (GF-10, Microtech Inc, Boothwyn, PA). Each of the eight wires is then soldered to a pin on the connector. The four polyimide tubes are fixed into a square pattern with PMMA. Shortly before implanting, the tubes are injected with 1) saline, 2) fibrinogen 3) fibrinogen with 1250ng/l NGF, and 4) fibrinogen with 2500ng/l NGF. The tip of a completed electrode is show in Fig. 2.

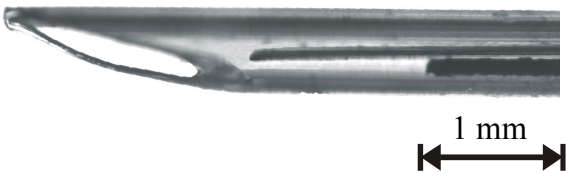

Fig. 2 The tip of a neurotrophic electrode with a beveled tip and two wires inside the tubing.

B. Implant Protocol

The animals were initially anesthetized with an IM injection of (7.0)ketamine/(1.0)xylazine cocktail (0.1 ml/100 g), shaved and scrubbed, and then placed into a stereotactic frame. Areflexic anesthesia was mantained with ketamine updates.

An initial incision was opened along the midline, and small screws were placed at the front and rear of the skull for securing the implants. A craniotomy was cut at 2 mm posterior and 6 mm lateral to bregma. The dura underlying the craniotomy was dissected, and localization determined by means of vascular landmarks. The implant positioned over the craniotomy, and driven into the cortex to a depth of 1.0 to 1.5 mm.

C. Recording Protocol

We recorded electrophysiological signals from the implants once weekly using a Multichannel Acquisition Processor (Plexon, Inc., Dallas, TX).

Initially, we scanned the channels and identified any isolatable units as we did a somatosensory evaluation. We would isolate action potentials using either template matching or a two-window time-amplitude discrimination procedure.

Any other activity remaining on the channel was then recorded as a threshold crossing and isolated using template matching.

Once the channels corresponding to all 8 electrodes had been examined, we would record one to two minutes of simultaneous activity from all of the channels.

Data were saved as waveform shapes and time of occurrence.

III. Results

We have implanted 8 devices in 4 rats. In five of the devices we have been able to record multi-unit activity over the course of several weeks (see Fig. 3 and Table 1). In all of those implants, the activity has been dominated by low frequency spindling, a characteristic of barrel cortex under general anesthesia [5].

Most of the devices were successfully implanted into the whisker barrels of Sm1, but we have also observed neurons with receptive fields elsewhere on the face, on the shoulder or torso, and a few on the hind limb.

To date, we have recorded from a single implant

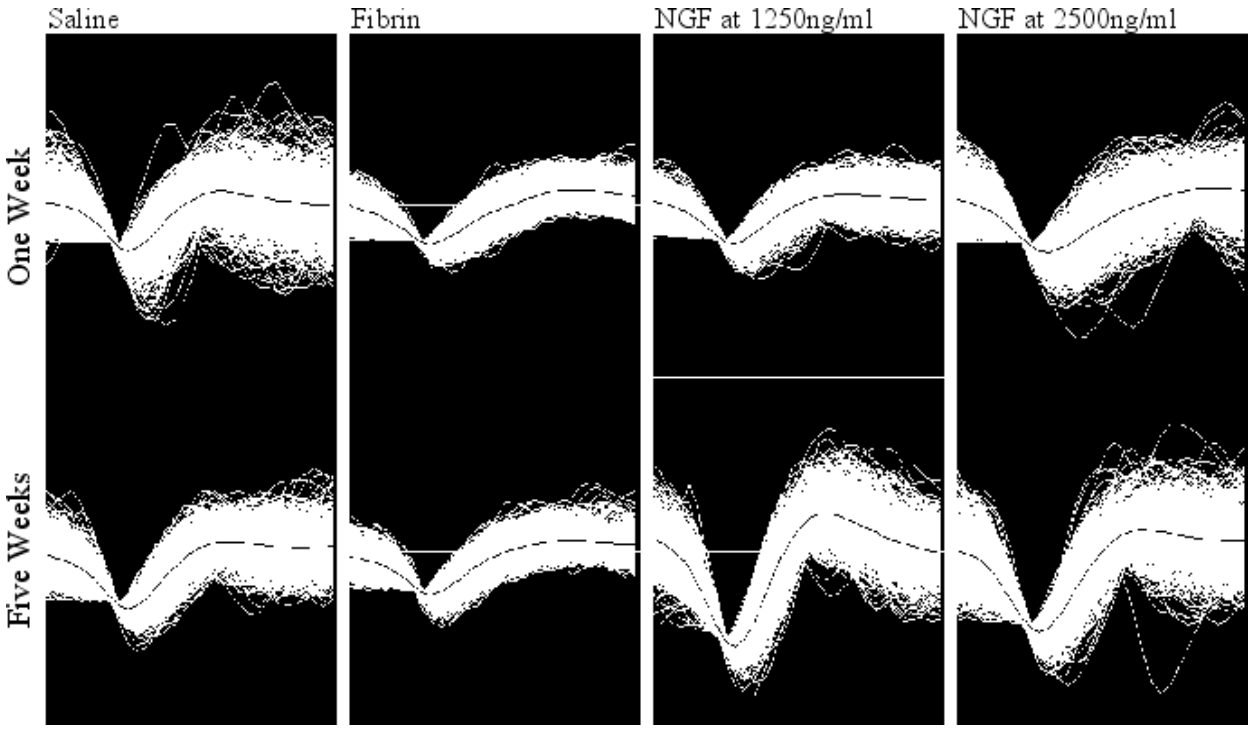

Fig. 3 Comparison of non-isolated neuronal activity across time and electrodes. Each plot shows 800 msec of overlain activity centered on a negative going threshold crossing. Th e upper row shows activity recorded one week post-implant. The lower row shows activity recorded five weeks post-implant. Each column is the data from a single electrode. Gain settings in the upper and lower rows are equal.

for as long as twelve weeks. At this stage, we have not yet seen activity that could be easily resolved into a single action potential.

TABLE I
OVERVIEW OF CURRENT IMPLANTS

Animal	Active Chans	Receptive Fields	Weeks
RNT-05	8	3 whiskers 1-2 face 2-3 torso 1 rear paw	12
RNT-06	7	3 whiskers 2-3 face 1 rear paw	8
RNT-07	8	7 whiskers 1 face	4
RNT-08	3	?	1

Over the course of five weeks of recording, we observed changes in the amplitude of the multi-neuronal hash recorded on some of the channels. This is illustrated in Fig. 3. The upper row are recordings taken from one hemisphere of rat RNT_06 one week after recording, and the lower row are recordings taken from the same implants four weeks later.

In the tubes containing saline and fibrin, the amplitude of the signal is unchanged after the four weeks. Both of the tubes containing NGF, however, exhibited striking changes in the amplitude of the multi-unit hash. The amplitude of the change was largest in the electrode associated with 1250 ng/mL of NGF, but the higher concentrations also produced an increase in signal amplitude. This is consistent with an earlier report [4] which showed that there was an optimal concentration of NGF in this system for producing neurite extension in a chick DRG preparation.

IV. DISCUSSION

One of the important advances required before neuroprosthetics can become a serious clinical tool is an improvement in the tissue-electronics interface. Current devices suffer from both a low signal-to-noise ratio and poor day-to-day recording stability.

A neuroprosthetic electrode has already shown promise in a clinical setting [6]. Investigators in that report describe training a patient to manipulate

a cursor on a computer screen using only brain activity: brain activity sampled using a single neurotrophic electrode. With careful engineering, it should be possible to design an electrode and accompanying neurotrophic medium that can be built as a multi-channel array. In fact, all of the recordings here were made using arrays of four separate electrodes.

A device like this would also be useful for long-term chronic neurophysiology. There are many open questions regarding changes in neural organization that accompany learning and memory. Many such questions could be addressed with a device that offers some degree of assurance that the same neuron is being recorded over the course of days or weeks [2]

We designed this device primarily as a means to assess various neurotrophic media. As yet, we do not know the extent to which it would produce stable recordings since we have not allowed for adequate survival times to assess long-term neurite health. Nonetheless, our evidence suggests that the device supports neurite growth, likely providing a stable recording environment at least over the course of weeks.

V. Conclusion

The field of neuroprosthetics needs a better interface device, and neurotrophic electrodes have shown promise. We are developing a device which we can use to test various neurotrophic media. This device also produces good quality recordings, and as such may also prove its usefulness as a long-term multi-channel recording interface.

Acknowledgment

The NGF - fibrin gel used in these studies was engineered by Shelly Sakiyama-Elbert of the Dept. of Bioengineering at Washington University, St. Louis.

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