ELSEVIER

Journal of Neuroscience Methods 155 (2006) 296–299

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Short communication

Neural recording and stimulation of dissociated hippocampal cultures using microfabricated three-dimensional tip electrode array

Yoonkey Nam[a,*], Bruce C. Wheeler[a], Marc O. Heuschkel[b]

[a] *Department of Bioengineering, University of Illinois at Urbana-Champaign, 405 N. Mathews Ave., Urbana, IL 61801, USA*

[b] *Ayanda Biosystems SA, PSE Parc Scientifique, EPFL, CH-1015 Lausanne, Switzerland*

Received 8 December 2005; received in revised form 16 January 2006; accepted 17 January 2006

Abstract

There is increasing interest in interfacing dissociated neuronal cultures with planar multielectrode arrays (MEAs) for the study of the dynamics of neuronal networks. Here we report on the successful use of three-dimensional tip electrode arrays (3D MEAs), originally developed for use with brain slices, for recording and stimulation of cultured neurons. We observed that many neurons grew directly on protruding electrode surface, appearing to make excellent contact. A larger than usual portion of extracellular spikes had large positive peaks, while most of the spikes from conventional two-dimensional electrode arrays had large negative spikes. This may be due to the direct capacitive coupling situation provided by relatively large electrode surface area.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Multielectrode array; Three-dimensional tip electrode; Cultured neurons; Extracellular recording; Electrical stimulation; Spike shapes

1. Introduction

A planar multielectrode array (MEA) is a culture dish with embedded microelectrodes for the purpose of recording and stimulating interfaced tissues, e.g., acute slices (Wheeler and Novak, 1986; Boppart et al., 1992; Hiroaki et al., 1999) or dissociated cultures (Pine, 1980; Gross et al., 1982; Jimbo et al., 1993). This has been extensively used to investigate the learning and memory paradigm in neuroscience (Jimbo et al., 1999; Shahaf and Marom, 2001) or to build cell-based biosensors for drug screening (Gross et al., 1995; Pancrazio et al., 2003).

Recently, a three-dimensional tip electrode array (3D tip MEA) has been developed to enhance the quality of the recording signal for acute slice experiments (Heuschkel et al., 2002). Protruding 3D tip electrodes allow access through surface dead cell layers to the intact cell layer in the brain slice allowing the detection of larger signals than can be obtained with conventional 2D type electrodes. From acute slice preparations, single unit extracellular action potentials as well as evoked excitatory postsynaptic potentials were recorded. The low electrode impedance due to the relatively large surface area resulted in a low noise level and higher safe-charge injection limit for electrical stimulation.

To date this new type of MEA has been used principally for acute slice experiments, and to our knowledge, no reports have been made of its use with dissociated neuronal cultures. Hence, we were interested in demonstrating basic biocompatibility as well as recording and stimulation capability. It was expected that one could grow cells on these MEAs. However, in as much as an electrode integrates the potential present at its surface, it was expected that these larger than average surface area electrodes would report smaller than average signals from neurons that happened to grow nearby. Previously, we have reported empirical data indicating that somata needed to be within 20 μm of an electrode to be recordable in sparse dissociated culture (Nam et al., 2004). As shown in the report below, we were surprised by the tendency of neurons to adhere tightly to these electrodes and for the recorded signals to be easily large enough to warrant use in dissociated culture.

* Corresponding author. Tel.: +1 217 244 2693; fax: +1 217 244 5180.
E-mail address: ynam1@uiuc.edu (Y. Nam).

0165-0270/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2006.01.014

2. Methods

2.1. Multielectrode arrays and culture

3D tip MEAs were received from Ayanda Biosystems (Lausanne, Switzerland). Electrodes were made of platinum (60 electrodes per MEA, geometric area: 60 μm × 60 μm, height 50–70 μm) and they were insulated with SU-8. Two different types of conventional MEAs with two-dimensional electrodes served as control groups. One had a thin silicon nitride insulator (0.5 μm) which provided a flat type electrode (TiN, 10 or 30 μm in diameter, Multi Channel Systems, Reutlingen, Germany). The other had a relatively thick polymer insulator (SU-8, 5 μm) which formed a recessed electrode (indium–tin oxide, 40 μm × 40 μm, Ayanda Biosystems).

Poly-D-lysine (PDL, 0.1 mg/ml in deionized water, MW 70,000–150,000, Sigma–Aldrich) was coated for 2 h. After the coating, the MEA surface was rinsed with deionized water once and sterilized with 70% ethanol for 30 s and stored in sterile-petri dish.

2.2. Cell culture

Dissected hippocampal tissues (18-day gestation Sprague/Dawley or Fisher 344 rat hippocampus) were purchased from Brain Bits^{TM} (http://www.brainbitsllc.com). Tissues were mechanically dissociated and plated in serum free B27/Neurobasal medium (Invitrogen, Gaithersburg, MD) with 0.5 mM glutamine and 25 μM glutamate at the density of 100 cells/mm^2. Cultures were stored in an incubator at 37 °C, 5% CO_2 and 9% O_2. After 4 days in vitro (DIV), the media was changed to serum free B27/Neurobasal medium with 0.5 mM glutamine. Thereafter, half of the media was changed weekly or after recording if necessary. Cultures were inspected under transmitted light inverted microscope (phase contrast) and scanning electron microscope (SEM).

2.3. Neural recording and stimulation

MEA 1060 (gain 1 200, bandwidth 10–3000 Hz, Multi Channel Systems) and MC Rack software (Multi Channel Systems) were used for data acquisition. Raw data were digitized and stored at 40 kHz for off-line analysis. Each recording session lasted for 2 min and seven cultures were used to collect data from 11 recording sessions (5 recordings from 14 DIV, 6 recordings from 21 DIV).

For electrical stimulation of the culture, voltage pulses were delivered by STG-1008 (Multi Channel Systems). We chose positive first biphasic stimulus (voltage controlled, ±0.8 to ±1.4 V, pulse width 200 μs), as recommended for effectiveness in stimulating cultured neurons (Wagenaar et al., 2004). The intensity of the stimulus was selected such that stimulation artifact permitted monitoring of evoked responses as early as 5 ms after stimulus delivery.

Raw data were filtered using the second order Butterworth digital filter and spikes were detected by setting the threshold level at five to seven times the standard deviation of background noise level. Channels that exceeded spike rate of 0.1 Hz were regarded as active.

3. Results

3.1. Biocompatibility of 3D tip MEAs

The insulator and electrode tip material was completely compatible for short (hours to days) and long (weeks) term. Neurons showed normal cell adhesion and neurite extension after the plating and formed a dense neural network after a few weeks in vitro. Most of the cultures in this study remained healthy for at least a month (one survived for 7 weeks). Data reported below are from cultures in either the third or fourth week since plating.

In order to observe cell growth on top of the electrode, we used scanning electron microscopy. Images showed that somata as well as neurites adhered to the surface of the electrode and grew without any noticeable difference when compared to cell growth on flat surfaces. Some electrodes were completely covered by the mixture of somata and entangled neurites (Fig. 1(a)). Others had a single soma or multiple somata with extending neurites

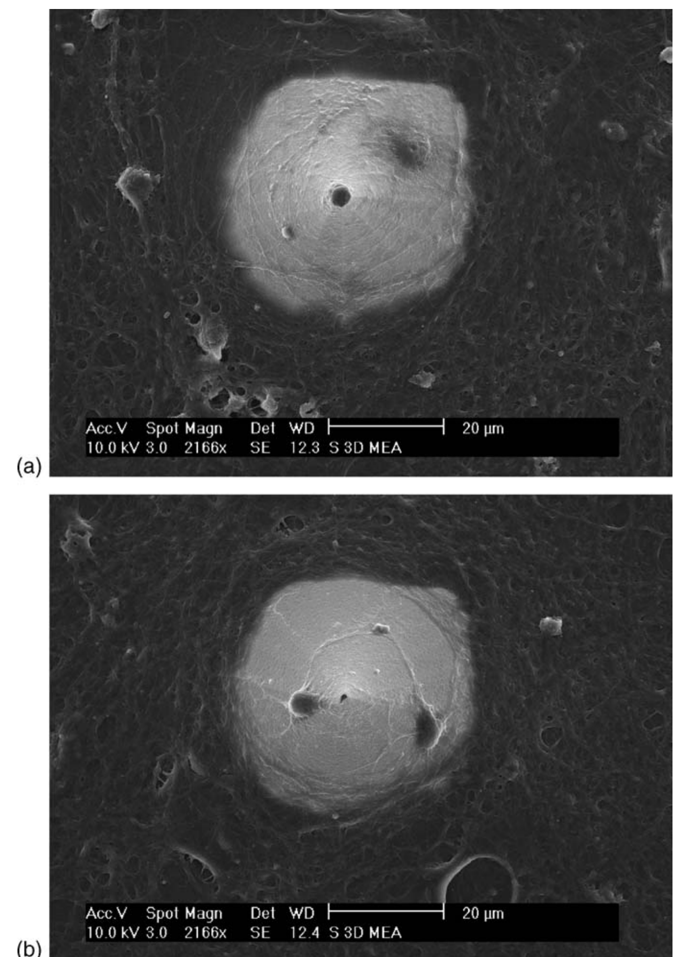

Fig. 1. Scanning electron micrograph of neuronal growth (hippocampal neurons) on the surface of the 3D tip electrode at 24 DIV. Positive (a) or negative going spikes (b) were recorded from these electrodes (see text). Top view. Scale bar = 20 μm.

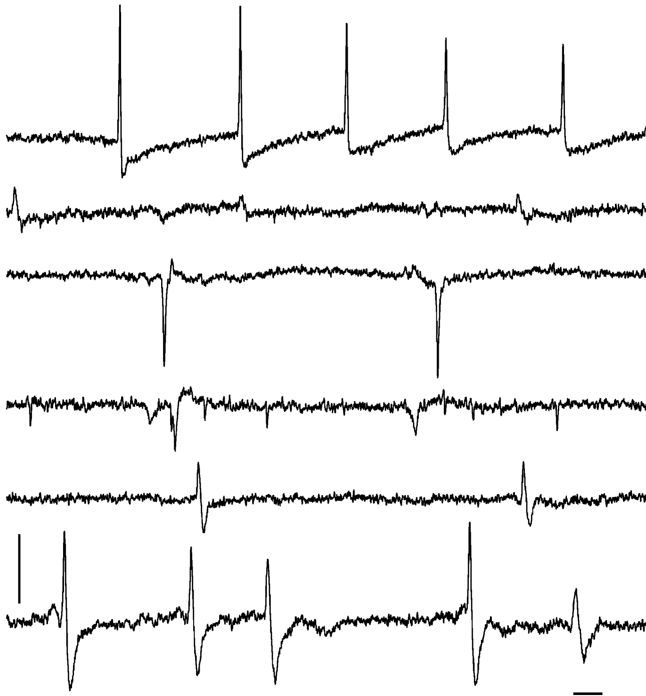

Fig. 2. Representative multichannel recording from hippocampal cultures at 21 DIV. Scale bar = 5 ms, 100 μV.

(Fig. 1(b)). Judging from light and electron microscope images, we concluded that 3D tip MEAs were suitable for dissociated neuronal cultures.

3.2. Neural recording

Electrode impedances were 400 ± 28 kΩ at 1 kHz (mean ± S.D., $N = 60$ electrodes). We observed extracellular spikes from 3D tip electrodes after approximately 2 weeks in vitro. Spike amplitude ranged from 15 μV_{pp} to 1 mV_{pp} and background noise level was 2–3 μV_{rms}. Some recording sessions had more than 30 active channels, which clearly demonstrated multichannel recording capability. Some representative traces of active channels are shown in Fig. 2.

Most of the spike amplitudes (70%) were less than 100 μV_{pp}, though some electrodes recorded spikes larger than 200 μV_{pp}. The distribution of the amplitude of recordable spikes was similar to that of conventional 2D MEAs (electrode area 10 μm × 10 μm or 30 μm × 30 μm). Considering the electrode surface area (approximately 60 μm × 60 μm), we only expected small spikes. Although most of the spikes were small, we often encountered large spikes up to 1 mV_{pp}. The origin of such large spikes is not clear, but we speculate that there was a direct capacitive coupling between cell and electrode surface when neurons adhere to the electrode surface. Direct capacitive coupling has been known to produce large extracellular spikes in certain conditions (Fromherz et al., 1991).

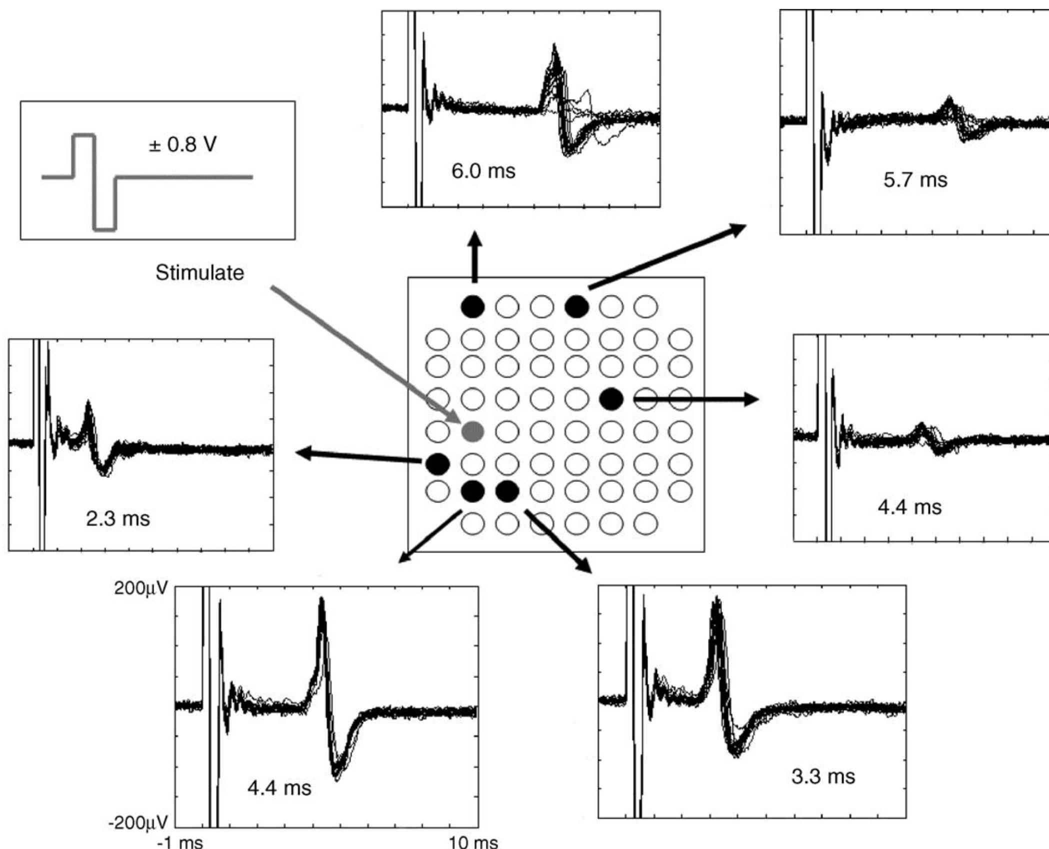

Fig. 3. Electrical stimulation (positive first biphasic pulse, ±0.8 V, pulse width 200 μs, 20 stimuli at 1 Hz) induced time-locked action potentials across the culture. Each inset is 20 traces overlapped and values on each plot are estimated delay of the first peak of the action potentials.

Table 1
Distribution of recorded spike shapes from three different electrode geometries

	Flat[a] (%)	Recessed[b] (%)	3D tip[c] (%)
Positive	16	0	56
Negative	79	100	33
Biphasic	5	0	11

[a] $N = 38$ electrodes (5 cultures, 5 recording sessions).
[b] $N = 33$ electrodes (2 cultures, 2 recording sessions).
[c] $N = 153$ electrodes (7 cultures, 11 recording sessions).

Recorded spikes had three distinctive shapes. Positive spikes (top two traces in Fig. 2) had a narrow and large positive peak followed by a wide negative peak. Negative spikes (middle two traces in Fig. 2) had a narrow large negative peak with or without a small and wide positive peak. Biphasic spikes (bottom two traces in Fig. 2) had a distinct positive peak followed by a negative peak with comparable amplitude and width. Of the active electrodes ($N = 153$ electrodes from 11 recording sessions), 56% had positive spikes and 33% had negative spikes and the rest were biphasic spikes. Table 1 summarizes the distribution of recorded spike shapes from 3D tip MEA and control groups (flat or recessed electrodes). Flat electrodes recorded mostly negative spikes with a few positive spikes, while recessed electrodes recorded exclusively negative spikes. A greater portion of spikes recorded from 3D tip MEA was positive or biphasic. More frequent recordings of positive spikes could be due to more frequent growth of passive soma or dendrites on electrode surfaces, which can affect the shape of recorded spikes (Claverol-Tinture and Pine, 2002).

3.3. Electrical stimulation

The large surface area of the 3D tip electrode could provide large charge transfer capability due to low electrode–electrolyte impedance. Biphasic voltage controlled pulses with amplitude ranging from 0.8 to 1.4 V induced time-locked evoked spikes at neighboring electrodes. Fig. 3 shows an example of recording from six different electrodes after stimulating with 0.8 V voltage controlled pulses. This clearly demonstrates the capability of stimulation and recording of cultured neurons.

4. Discussion and conclusions

The three-dimensional tip shape provides a larger surface area than conventional planar shape electrodes. It has been shown that a large surface area provides a large electrode–electrolyte interface and lower impedance, which results in lower noise level, a higher safe-charge injection (Heuschkel et al., 2002).

In this study, we extended the usefulness of the 3D tip MEA to interfacing with dissociated neuronal cultures. Neural recording and stimulation was readily possible and the quality of the recorded signals was comparable to those from conventional 2D flat type electrodes with the advantage of reduced noise level. Biphasic voltage pulses at relatively low level (0.8 V) induced time-locked evoked responses.

Direct cell growth on electrode surfaces and more frequent recording of large positive spikes were unique observations in this work. The recording of large positive or biphasic spikes on large electrodes needs to be explored further using planar type electrodes with comparable geometrical size (for example, the lower impedance platinum black electrodes used in Hiroaki et al., 1999) to find if these observations are indeed unique for tip electrodes or not. Moreover, obtaining these in a controlled fashion may benefit the study of low density neuronal cultures using MEAs.

Acknowledgements

This material is based upon work supported by the National Institute of Health under Award No. R01 EB000786 and the National Science Foundation under Award No. EIA0130828.

References

Boppart SA, Wheeler BC, Wallace CS. A flexible perforated microelectrode array for extended neural recordings. IEEE Trans Biomed Eng 1992;39:37–42.

Claverol-Tinture E, Pine J. Extracellular potentials in low-density dissociated neuronal cultures. J Neurosci Methods 2002;117:13–21.

Fromherz P, Offenhausser A, Vetter T, Weis J. A neuron–silicon junction: a Retzius cell of the leech on an insulated-gate field-effect transistor. Science 1991;252:1290–3.

Gross GW, Rhoades BK, Azzazy HM, Wu MC. The use of neuronal networks on multielectrode arrays as biosensors. Biosens Bioelectron 1995;10:553–67.

Gross GW, Williams AN, Lucas JH. Recording of spontaneous activity with photoetched microelectrode surfaces from mouse spinal neurons in culture. J Neurosci Methods 1982;5:13–22.

Heuschkel MO, Fejtl M, Raggenbass M, Bertrand D, Renaud P. A three-dimensional multi-electrode array for multi-site stimulation and recording in acute brain slices. J Neurosci Methods 2002;114:135–48.

Hiroaki O, Ken S, Ryuta O, Hirokazu S, Makoto T. A new planar multielectrode array for extracellular recording: application to hippocampal acute slice. J Neurosci Methods 1999;93:61–7.

Jimbo Y, Robinson H, Kawana A. Simultaneous measurement of intracellular calcium and electrical activity from patterned neural networks in culture. IEEE Trans Biomed Eng 1993;40:804–10.

Jimbo Y, Tateno T, Robinson H. Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons. Biophys J 1999;76:670–8.

Nam Y, Chang JC, Khatami D, Brewer GJ, Wheeler BC. Patterning to enhance the activity of cultured neuronal network. IEE Proc Nanobiotech 2004;151:109–15.

Pancrazio JJ, Gray SA, Shubin YS, Kulagina N, Cuttino DS, Shaffer KM, et al. A portable microelectrode array recording system incorporating cultured neuronal networks for neurotoxin detection. Biosens Bioelectron 2003;18:1339–47.

Pine J. Recording action potentials from cultured neurons with extracellular microcircuit electrodes. J Neurosci Methods 1980;2:19–31.

Shahaf G, Marom S. Learning in networks of cortical neurons. J Neurosci 2001;21:8782–8.

Wagenaar D, Pine J, Potter SM. Effective parameters for stimulation of dissociated cultures using multi-electrode arrays. J Neurosci Methods 2004;138:27–37.

Wheeler BC, Novak JL. Current source density estimation using microelectrode array data from the hippocampal slice preparation. IEEE Trans Biomed Eng 1986;33:1204–12.