A MICROFLUIDIC MICROELECTRODE ARRAY FOR EXTRACELLULAR RECORDINGS AND FOCAL STIMULATION OF BRAIN SLICES

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ABSTRACT

We present a microfluidic multi-electrode array (MEA) for studying development in mouse cortical slices. The device is designed to facilitate integrated multimode measurement with the capability to perform simultaneous multi-site extracellular recording, electrical stimulation, and focal chemical stimulation. It is compatible with brightfield and fluorescence microscopy, enabling simultaneous electrical, optical, and chemical manipulation and characterization of slices with high spatial and temporal precision.

KEYWORDS: Electrophysiology, Multielectrode Array, Focal Stimulation, Cortical Slices

INTRODUCTION

Spontaneous synchronous activity (SSA) during early stages of brain development is important for processes such as circuit formation and cortical neuron migration [1]. Although this activity has been observed for some time, the mechanisms of SSA and its influence on development are not well understood, in part due to the difficulty of characterizing the complex temporal, spatial, and biochemical characteristics of the process. Techniques for simultaneously measuring and manipulating electrical, optical, and chemical properties of tissue are needed to elucidate the mechanisms of SSA. Calcium imaging can be used to provide information about global behavior of the tissue or to isolate activity of specific neuron sub-populations, however it is an indirect measure of electrophysiology and has very limited temporal resolution due to the slow nature of calcium and dye kinetics. Electrical recordings offer exceptional temporal resolution and more direct measurement of activity, however typical recordings have limited spatial resolution and can be difficult to interpret. Moreover, these techniques should be combined with controlled focal chemical stimulation to determine the spatial and temporal influence of specific neurotransmitters. Traditional tools such as electrophysiology recording glass pipettes, calcium imaging, and multi-electrode-arrays (MEAs) offer only a subset of these functions.

Microfluidic platforms have shown promise for enhancement and automation of electrophysiology measurements. Patch-clamp chips have demonstrated that high quality electrical recordings can be obtained using a microfluidic chip platform [2,3]. Microfluidic perfusion and focal chemical stimulation systems have been combined with traditional electrophysiology techniques such as recording pipettes and Ca imaging, demonstrating the potential utility of microfluidics for controlling the biochemical environment of brain slices [4]. We introduce a polymeric microfluidic MEA on a chip that enables simultaneous high quality multi-site electrical recording and stimulation, imaging, and focal chemical stimulation.

EXPERIMENTAL

Figure 1: Side-view schematic (a) and optical micrograph (b) of electrochemical electrode array device. Photographs of devices without (c) and with (d) focal chemical stimulation capabilities are shown.
The device, shown schematically in Figure 1, is fabricated using standard photolithographic, PDMS replication, and bonding techniques. The chip has multiple apertures for recording and stimulation. Microfluidic channels filled with electrolyte solution are used to interface the recording sites to off-chip Ag/AgCl electrodes connected to differential amplifiers. The amplifier signals are digitized and recorded using standard electrophysiology instrumentation and software. Electrical stimulation is performed using a pulse generator. Each aperture/channel offers the electrical and chemical functionality of a micropipette electrode and the chip format offers an integrated multi-site measurement with well-defined, stable geometry. The apertures are surrounded by a thicker PDMS region that keeps the tissue slice raised from the chamber surface to enable effective perfusion and promote sealing of the tissue to the aperture sites, thereby reducing leakage current from the apertures to the bath. For chemical stimulation, it is necessary to exchange reagents rapidly without significant flow in or out or the aperture. The design is modified to enable focal chemical stimulation by extending the channel and adding a well for exchanging reagent as shown in Figure 1d. The stimulating solution is introduced by micropipette and suction is used to move the chemical under the aperture. The agent is allowed to diffusively deliver to the slice for a period of time. To end the stimulus, the stimulating solution is pipetted out of the reagent exchange chamber and buffer is added. Suction is applied to move the buffer under the aperture, thereby ending the chemical stimulus.

The microfluidic MEA is being used to study SSA in cultured mouse brain slices at different stages of development. The channels are filled with artificial cerebrospinal fluid (ACSF) and the chamber is continuously perfused with ACSF that has been bubbled with 5% CO₂. A mouse brain slice that has been cultured on a permeable membrane for 1-5 days off chip is placed on the chamber of the device and mechanically held down by the membrane using a metal disk or harp. For calcium imaging experiments, the slice is loaded with Fluo-4 prior to placing it in the measurement chamber.

RESULTS AND DISCUSSION

Figure 2: Typical recording of SSA using microfluidic MEA. A brightfield optical micrograph of the P1 slice is shown in (a). Single-unit recordings from position 1 are shown in (b). An example of a fully propagating event is shown in (c).

The microfluidic MEA produces high-quality electrical recordings, as shown in Figure 2. In typical experiments, local field potentials (LFP) can be clearly observed within 10-30 minutes of placing the slice in the chamber. The signal to noise ratios obtained with the device are as good or better than those obtained in the same experimental apparatus using glass micropipette extracellular recording electrodes. This suggests that the PDMS surface of the device interacts sufficiently with the tissue to seal in place and prevent excessive leakage current from the recording aperture to the bath. In the chip, slices are routinely measured for periods of more than 5 hours with no degradation in signal. In addition to LFP signals, single-unit recordings are obtained when an aperture is sealed to a neuron that is bursting asynchronously. To validate device performance, SSA in mouse cortical slices at various stages of development from E18 to P6 was studied. Perfusing the slice with Picrotoxin and CNQX to block GABAergic and glutamatergic activity stops SSA. The activity is restored after washout with ACSF. Patterns of local and propagating waves and pharmacology are consistent with prior Ca imaging experiments of similar slice preparations, validating the performance of the MEA [5].
PDMS has exceptional optical clarity and does not autofluoresce, therefore the microfluidic MEA is an ideal platform to combine with microscopy. Figure 3 shows an example of data from a simultaneous calcium imaging/electrical recording experiment. It is clear that global features of SSA propagation can be inferred from the calcium traces while the details of temporal features such as wave duration and propagation velocity can only be seen in the multi-site electrical recording.

CONCLUSION
We present a versatile microfluidic MEA for simultaneous electrical recording, optical imaging, and focal chemical stimulation of mouse cortical slices. These techniques provide complementary information about the spatial, temporal, and biochemical nature of brain activity. We are currently studying SSA in developing cortical slices using the unique combination of capabilities on this chip.

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