LOCAL CHEMICAL STIMULATION OF NEURONS USING FLUIDFM TECHNOLOGY COMBINED WITH MICROELECTRODE ARRAYS

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ABSTRACT

It is still unclear how networks of neurons in the brain carry out individual functions such as information processing and storage. Better understanding these is a challenge of this century. We report the combination of microelectrode arrays to measure *in vitro* neuronal activity with the recently developed FluidFM technology acting as a force-controlled nanopipette to stimulate a neuron with the controlled release of a neurotransmitter. We show the possibility of localized release of glutamate on top of the target cell with precise control over force and spatial position therefore acting as an "artificial synapse".

KEYWORDS: Chemical stimulation, neurotransmitter, glutamate, localized stimulation, primary neurons, electrophysiology, microelectrode array, MEA, atomic force microscope, AFM, FluidFM, nanopipette

INTRODUCTION

Networks of neurons can be cultured *in vitro* on microelectrode arrays (MEAs) to record the network activity. Investigating how a network reacts to external stimuli is essential for understanding the network behavior. However, the current stimulation options are limited. Electric stimulation generates large artifacts and its spread is poorly understood. Dispensing stimulants by glass pipettes resembles the physiological conditions but the localization and dosing are difficult to control [1]. Photo-stimulation techniques are emerging but rely on compounds that can be uncaged such as MNI-glutamate [2].

Here, we present an experimental platform for stimulating and recording neuronal networks based on the integration of a MEA with the FluidFM [3,4,5] which combines an atomic force microscope (AFM) with nanofluidics. The force-control of the AFM allows for exact positioning of the nanopipette at the location of choice and the pressure-based flow precisely controls the dosing out of the 300 nm aperture. This combination of an AFM with nanofluidics is a drastic improvement over the traditional glass pipette and makes it possible to stimulate a single neuron with precise spatio-temporal control.

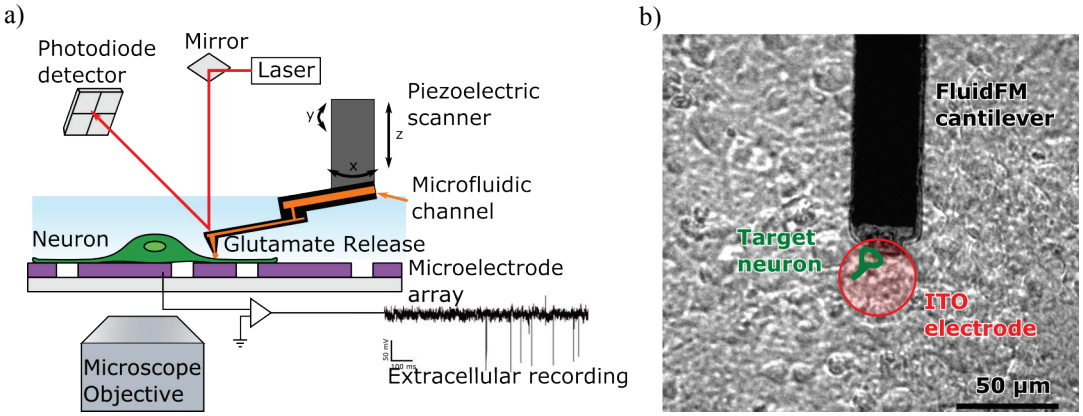

Figure 1: Experimental platform for localized chemical stimulation of neurons. a) A culture of neurons on a MEA is placed in a custom headstage on an inverted microscope. On top is the FluidFM which creates an environmental chamber (not shown). b) Brightfield image of the FluidFM cantilever positioned over the target neuron growing on the ITO electrode.

EXPERIMENTAL

A Multichannel System MEA2100 was modified to be operated together with the AFM in a Zeiss AxioObserver inverted fluorescence microscope. The FluidFM experiments were carried out on a Cytosurge FluidFM system with either a Nanosurf or JPK AFM using 300 nm aperture cantilevers. A fluorescent PEG from Nanocs was used as a tracer molecule to visualize the dispensing. Z-stack images were obtained on a ZEISS LSM 510 laser scanning confocal microscope.

Primary rat hippocampal neurons were prepared from E17 embryos and cultured on PDL coated MEAs from Qwane Biosciences with neurobasal medium containing 2% B-27, 1% pen-strep, and 1% GlutaMAX. Experiments were conducted after 2 weeks in vitro with a stimulation solution of 5 mM glutamic acid solution in neurobasal medium adjusted to a pH of 7.3.

RESULTS AND DISCUSSION

Neurons were repeatedly stimulated by locally dispensing the neurotransmitter from the 300 nm aperture directly above the cell membrane of the preferred neuron as shown schematically in Fig. 1. The simultaneous extracellular recordings in Fig. 2 show that the stimulation successfully induced action potentials corresponding to the individual stimulations.

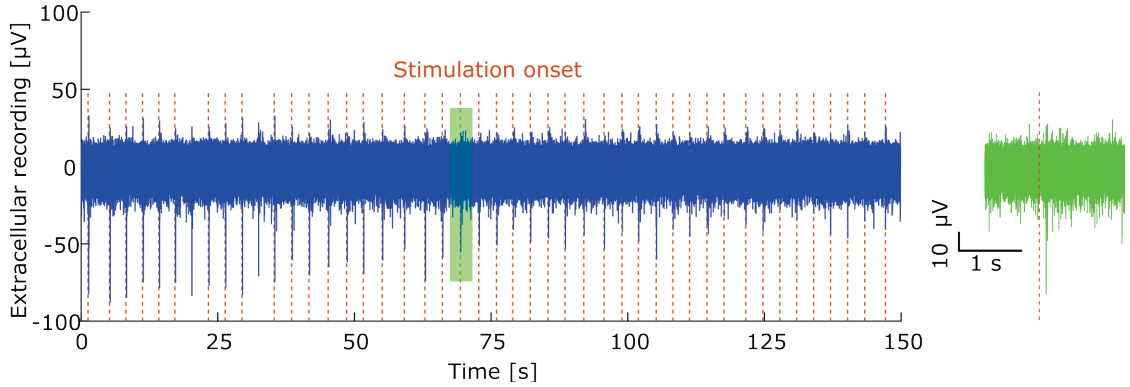

Figure 2: Direct response to stimulation. Glutamate is locally dispensed by applying pressure pulses of 100 mbar for 300 ms on top of the target neuron. The extracellular recording clearly shows a direct response to the chemical stimulation. The green inset shows one stimulation pulse with the corresponding induced action potentials.

Unlike conventional glass pipettes, the precise positioning with the AFM system makes it now possible to determine the local neurotransmitter concentration responsible for the induced activity. By combining the experimental characterization in Fig. 3a with numerical simulations, the local distribution of glutamate is determined and shown in Fig. 3b for different pressures.

CONCLUSION

For the first time, neurons were chemically stimulated with precise control over dosing and location with simultaneous MEA recordings. The characterization of the dispensed neurotransmitter shows that the stimulation dose can be adjusted as desired. This novel setup makes it possible to directly interact with a network of neurons via this “artificial synapse”.

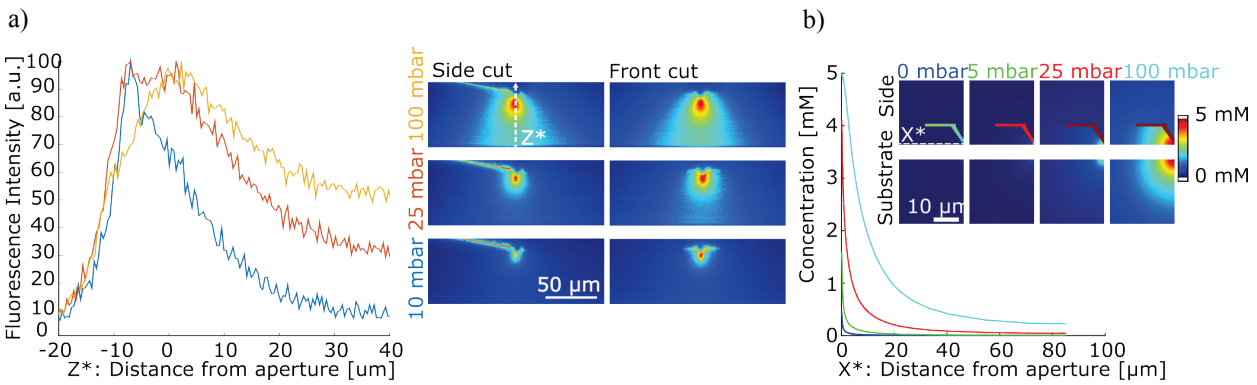

Figure 3: a) Characterizing the dispensing from the 300 nm FluidFM aperture 50 µm above the substrate. Using a confocal microscope, z-stack images are obtained with pressures from 10 to 100 mbar applied to the microchannel. b) Determining the local concentration of glutamate during stimulation with numerical simulations. The concentration profile applied to a neuron when 1 µm above the membrane is characterized by numerical simulation. Due to the small molecular weight of glutamic acid the local concentration distribution cannot be directly measured by fluorescence imaging. The fluorescent intensity profiles and confocal images clearly show the ability to regulate the dosing by applying different pressures.

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