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AN INTEGRATED MICROELECTRODE ARRAY AND MICROFLUIDIC PLATFORM FOR STIMULATING AND RECORDING RECONSTRUCTED NEURONAL NETWORKS

Eve Moutaux\textsuperscript{1,2}, Benoit Charlot\textsuperscript{3,4}, Fabrice Bardin\textsuperscript{3,5}, Frederic Saudou\textsuperscript{1,2,6} and Maxime Cazorla\textsuperscript{1,2,*}

\textsuperscript{1}Grenoble Institute des Neurosciences, Univ. Grenoble Alpes, F-38000, Grenoble, France, \textsuperscript{2}INSERM, U1216, F-38000, Grenoble, France \textsuperscript{3}IES, Institut d’Electronique et des Systèmes, CNRS UMR 5214 \textsuperscript{4}Université de Montpellier, Montpellier, France \textsuperscript{5}Université de Nîmes, Nîmes, France \textsuperscript{6}CHU Grenoble Alpes, F-38000, Grenoble, France

ABSTRACT

Compartmentalized microfluidics are devices that allow the in vitro reconstitution of neuronal circuits using primary cultures of different neuronal populations (1, 2). These systems can be used to build thousands of specific neuronal junctions and to study intracellular dynamics using selective markers and live fluorescent reporters. In this work we added a dedicated Micro Electrode Array that fits the microfluidics design to record and control electrical activity of pre- and postsynaptic neurons while simultaneously monitoring intracellular dynamics. Because microfluidics are fluidically isolated, each compartment can be independently manipulated to determine their contribution to network functions.

KEYWORDS: Micro Electrode Arrays (MEA), Microfluidics, Neurons, Electrophysiology, Calcium imaging

INTRODUCTION

The microfluidic circuit is based on the design of Taylor et al. 2010 (3) for the reconstruction of cortico-cortical networks in which each compartment (presynaptic, synaptic and postsynaptic) is identified (space compartmentalization) and in which the progression from axonal growth to synapse regulation is controlled (time compartmentalization). The device shown in Figure 1(a,b,c) consists of two opposite neuronal chambers that communicate through an intermediate synaptic chamber through long microchannels on one side (to select axons only) and short ones on the other side (to allow dendrites to reach the synaptic chamber). This configuration allows the reconstruction of physiological axodendritic contacts between two cortical populations such as those found between cortical layers in vivo.

In order to extend the functional analysis of reconstructed cortico-cortical networks, we developed a specific Micro Electrode Array (MEA) substrate that fits to the microfluidic geometry to stimulate and record pre- and post-synaptic neurons (Figure1(c)). Presynaptic electrodes were disposed at the entrance of axonal channels in order to stimulate the axon initiation segment that will generate physiological action potentials and trigger release of neurotransmitter in the synaptic chamber. Postsynaptic electrodes are located under the cell bodies to concomitantly stimulate or record electrical activity of target neurons. Combined with high-resolution fluorescence videomicroscopy and fast calcium imaging (Figure1(e)), this system allows monitoring intracellular dynamics in response to different patterns of neuronal activity.

EXPERIMENTAL

The microfabrication process for building the MEA chips (Figure1(a)) are made of a two-step lithographic process on top of 4.9 x 4.9cm, 170\textmu m thin glass substrates. The first step consists in the deposition of micro electrodes by a lift-off of 10/200nm Ti/Pt metal over an AZ 2020 patterned photoresist. Next, a 500nm thick Silicon Nitride (Si$_3$N$_4$) passivation layer is deposited by Plasma Enhanced Chemical Vapor Deposition (PECVD) process at 280\textdegree C. A second photolithographic step is done to define openings in the Si$_3$N$_4$ passivation layer on the micro-electrodes and connexion pads. These openings are made by Reactive Ion etching with a mix of CHF$_3$ and O$_2$ plasma. This process has been stabilized in order to produces hundreds of devices with a fabrication yield of more than 80%. The microfluidic circuit is made of PDMS using a standard dual thickness SU8 on silicon process that is aligned on top of the MEA and bonded by oxygen plasma activation of the surface. Microelectrodes show electrical impedances of 235 and 165k\textOmega (for 50 and 30\textmu m diameter) in PBS 1X medium. The MEA-microfluidic
platform is connected to a 60-channels MEA workstation (MultiChannel Systems) and is then mounted on an inverted confocal spinning-disc videomicroscope (CSU-W1, Yokogawa).

Rat primary neurons are cultured into their respective chambers and are infected with lentiviruses to express markers of intracellular dynamics such as the fast calcium indicator GCaMP6f. Spontaneous electrical activity and calcium dynamics in pre- and post-synaptic neurons can be followed throughout network maturation (Day 4 to 21). Specific stimulation protocols mimicking different patterns of neuronal activity can then be applied to study calcium dynamics in presynaptic axons and in postsynaptic dendrites. Synaptic transmission between the two populations can also be assessed by applying selective drugs in the synaptic compartment (Figure 2(d,e)).

CONCLUSION
By combining space-time compartmentalization of neuronal populations, electrophysiological stimulation and recording, and high-resolution videomicroscopy, our integrated platform allows to decipher the cellular events that are involved in synaptic transmission and plasticity within neuronal networks. This device can be applied to virtually any type of neuronal circuits, in normal and pathological conditions.

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CONTACT
* B.Charlot; phone: +33-467144654; benoit.charlot@um2.fr
* M.Cazorla; phone: +33-456520669; maxime.cazorla@inserm.fr