DEVELOPMENT OF A NEW DEVICE TO COMBINE LOCAL OPTICAL STIMULATION AND MEA RECORDINGS

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INTRODUCTION

Since the introduction of the Micro-Electrode Array (MEA) technology [1], it has been exploited as a powerful tool providing distributed information about learning, memory and information processing in cultured neuronal networks, changing the field of view from single cell level to the scale of complex biological networks. Despite the great advantage of the MEA technology in recording extracellular activity, its applicability to cell culture/tissue stimulation presents some important limits related to the use of electrical stimulation in a conductive volume. Its major limits are the presence of large stimulus artefacts and the poorly controlled spread of electrical stimuli in the medium. Although some of the problems of stimulus artefacts have been recently solved using blanking circuits [2] and algorithms [3], the spreading of electrical signals remains a limitation of MEA technology. In fact, it has been demonstrated that electrical stimuli spread to the whole biological preparation with an amplitude decreasing with the square of the distance from stimulation site [4]. To overcome these limitations affecting the MEA-based electrophysiological analysis of network properties, alternative approaches based on optical technologies can be investigated as tools for the stimulation of neurons. In the last two decades several methods to couple light and neuronal excitation have been proposed [5], but above all the use of caged compounds seems to be the most physiological approach for the coupling of light with either neuronal excitation, e.g. with caged neurotransmitters, or modulation, e.g. with caged intracellular secondary messengers. Here we present the use of MEA biochips coupled to the local optical uncaging in order to obtain high localised stimulation at single electrical recording sites while monitoring the overall preparation. Moreover, we describe a new technological improvement, called PhotoMEA, aimed to integrate in the MEA biochip optical waveguides to address directly the UV light on the neuronal cultures in a very localised manner.

METHODS

Cell cultures. Low-density primary cultures of hippocampal neurons were prepared from embryonic day 18 rat embryos, essentially as previously described [7]. Hippocampi were dissociated by a 15min incubation with 0.25% trypsin at 37°C, and cells were plated at a density of 450-550cells/mm^2 on poly-L-lysine (1mg/ml)-treated devices in MEM, supplemented with 10% horse serum, 2mM glutamine , and 3.3mM glucose. After allowing neurons to adhere to the surface for 3-4hrs, a coverslip with astrocytes was turned upside down, with astrocytes facing down towards neurons. Physical contact between neurons and glial cells was avoided by placing paraffin dots in the MEA culture dish. Cells were then cultured in serum-free MEM supplemented with 1% N2 supplement, 2mM glutamine, 4mM glucose, and 1mM sodium pyruvate. Evaporation of the medium was avoided using a membrane-sealed culture chamber [8]. Experiments were performed after 14 days in vitro (DIV).

Fluorescence Imaging. A drop of CMNB-caged fluorescein (100µM) dissolved in glycerol was deposited on a180µm-thin glass coverslip. The fluorescence emission of uncaged fluorescein was measured using an Axiovert 200 inverted epifluorescence microscope equipped with a metal-halide fluorescence illuminator x-cite120, a 40x\1.3NA objective, a FocusScope V-200i CMOS camera and a dedicated FITC filter set (ex: BP450/490, dic: FT510, em: BP515/565).

Electrical recordings. Recordings were performed in the culturing medium supplemented with MNI-caged glutamate (100µM) at 37°C using the MEA system. Data recorded at 25kHz/ch from the 60 channels were then filtered from 200Hz to 3kHz and spikes were sorted using a threshold algorithm included in the MC Rack software. The threshold was defined as a multiple of the standard deviation of the biological noise computed during the first 500ms of the recording ($-5*SD_{noise}$).

RESULTS AND DISCUSSION

Uncaging geometry and efficiency. The most important feature of this set-up is the capability to stimulate small regions of a neuronal network. To demonstrate the potential of an adequate geometry for single cell stimulation the area of uncaging was evaluated using CMNB-caged fluorescein.

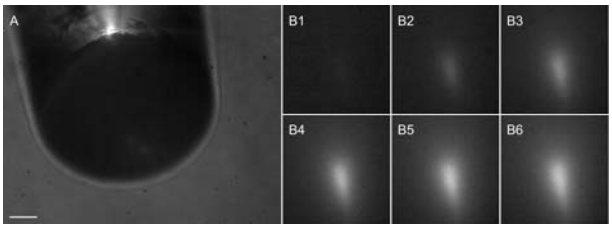

Fig.1 Uncaging geometry and efficiency measure using the caged fluorescein. Scale bar is 20µm

Photolysis of caged fluorescein was induced by placing the optical fibre near the coverslip surface (Fig. 1A) and illuminating the sample by single light pulse with a pulsewidth ranging from 1ms to 25ms. Fig. 1B1-6 shows the fluorescence generated by laser pulses of 1, 5, 10, 15, 20, 25msec. The fluorescence intensity increased proportionally with the length of the pulse while the uncaging area remained relatively constant for pulses longer that 15ms. In fact, the efficiency of uncaging is sufficient to produce a detectable fluorescence and the size of the activated region becomes stable, ranging from 33µm to 35.5µm.

Efficiency of local uncaging measured by electrical recordings. In order to evaluate the efficiency of glutamate uncaging in inducing electrical responses we performed experiments of optical stimulation of neurons cultured on MEAs. Thus the optical fibre for glutamate uncaging was placed closed to a group of neurons in the proximity of an electrode of the MEA (Fig. 2A) used to monitor the neuronal responses. Two optical stimuli of 50ms were delivered to the neurons and the responses were recorded before and after the UV stimulation. As shown in Fig. 2B, each light pulse was followed by a transient increase in the electrical activity, thus suggesting that this pulsewidth is sufficient for neuronal excitation. In addition, it can be appreciated that the optical stimulation did not cause a stimulus artefact which is a typical defect of electrical stimulation using MEA electrodes. After demonstrating the possibility to stimulate a single region of the culture, it was important to test the capability of the system to monitor the responses from the entire network after a local stimulation. As shown in Fig. 2C, after stimulation of a single site of the network, responses could be detected in several electrodes, located at various distances from the stimulated region.

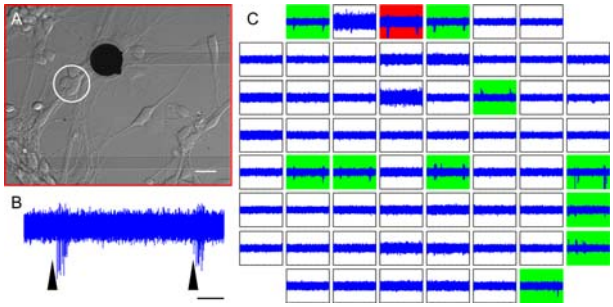

Fig.2 Electrical activity recorded by the MEA system after optical uncaging. The scale bar is 20µm in panel A and 1s in panel B

Development of the PhotoMEA biochip. Finally we are working on a device integrating the optical tool to stimulate neurons. Optical waveguides, able to address the UV light, are directly wrote in the glass substrate where the culture will take place. By using the capabilities of a femtosecond laser for the microfabrication of an integrated photonic device we produce a device able to uncage compound in spatially confined regions. In fact, the cutting capabilities are used to create a pit in a glass sample where the cells can grow, whereas optical waveguides are then directly inscribed in the glass sample for a multipoint and monolithically addressing of the culture in the pit. The advantage of this approach is the complete absence of any micromanipulation of optical elements by the end-user with the significant possibility of automating the measurements. Next step will be the combination of this device with a traditional MEA biochips for neuronal electrical recordings.

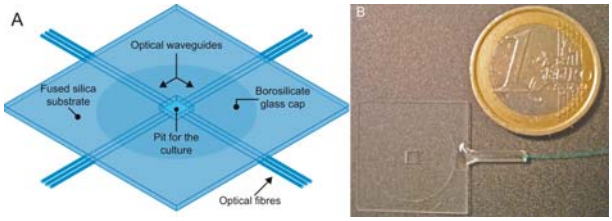

Fig.3 PhotoMEA chip for optical uncaging.

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