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Coupling MEA Recordings and Optical Stimulation: New Optoelectronic Biosensors

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1. Introduction

In the last twenty years the efforts in interfacing neurons to artificial devices played an important role in understanding the functioning of neuronal circuity. As result, this new *brain technology* opened new perspectives in several fields as neuronal basic research and neuro-engineering. Nowadays it is well established that the functional, bidirectional and real-time interface between artificial and neuronal living systems counts several applications as the brain-machine interface, the drug screening in neuronal diseases, the understanding of the neuronal coding and decoding and the basic research in neurobiology and neurophysiology. Moreover, the interdisciplinary nature of this new branch of science has increased even more in recent years including surface functionalisation, surface micro and nanostructuring, soft material technology, high level signal processing and several other complementary sciences.

In this framework, Micro-Electrode Array (MEA) technology has been exploited as a powerful tool for providing distributed information about learning, memory and information processing in cultured neuronal tissue, enabling an experimental perspective from the single cell level up to the scale of complex biological networks. An integral part in the use of MEAs involves the need to apply a local stimulus in order to stimulate or modulate the activity of certain regions of the tissue. Currently, this presents various limitations. Electrical stimulation induces large artifacts at the most recording electrodes and the stimulus typically spreads over a large area around the stimulating site.

Compound optical uncaging is a promising strategy to achieve high spatial control of neuronal stimulation in a very physiological manner. Optical uncaging method was developed to investigate the local dynamic responses of cultured neurons. In particular, flash photolysis of caged compounds offers the advantage of allowing the rapid change of concentration of either extracellular or intracellular molecules, such as neurotransmitters or second messengers, for the stimulation or modulation of neuronal activity. This approach could be combined with distributed MEA recordings in order to locally stimulate single or few neurons of a large network. This confers an unprecedented degree of spatial control when chemically or pharmacologically stimulating complex neuronal networks.

Starting from this point, the main objective of this chapter is the discussion of an integrated solution to couple the method based on optical stimulation by caged compounds with the technique of extracellular recording by using MEAs.

2. Scientific background

In the second half of the last century the functional properties of neurons, e.g. receptor sensitivity and ion channel gating, have been investigated providing a detailed picture of the neuronal physiology. In fact, some peculiar behaviors, e.g. plasticity, have been deepened down to the different molecular mechanisms underlying this function. Nowadays the high level of knowledge about single neuron functioning does not reflect an high level of understanding of the complex way of intercommunication between neurons in neuronal networks. The need of learning the neuronal language and the desire to bidirectionally communicate with neurons encouraged the development of new technologies, as MEA devices, focused to this purpose.

MEAs have been proposed more than thirty years ago (Gross, 1979; Pine, 1980; Thomas et al., 1972) for the study of electrogenic tissues, i.e. neurons, heart cells and muscle cells. Nowadays, they represent an emerging technology in such studies. In the last thirty years, MEAs have been exploited with various preparations such as dissociated cell cultures (Marom & Shahaf, 2002; Morin et al., 2005), organotypic cultures (Egert et al., 1998; Hofmann et al., 2004; Legrand et al., 2004) and acute tissue slices (Egert et al., 2002; Kopanitsa et al., 2006) for a large variety of applications, such as the study of functional activity of larger biological networks (Tscherter et al., 2001; Wirth & Lüscher, 2004), as well as applications in the fields of pharmacology and toxicology (Gross et al., 1997; 1995; Natarajan et al., 2006; Reppel et al., 2007; Steidl et al., 2006). Recently, MEA biochips have also been used as *in vitro* biosensors to monitor both acute and chronic effects of drugs and toxins on heart/neuronal preparations under physiological conditions or pathological conditions modelling human diseases (Stett et al., 2003; Xiang et al., 2007).

Referring to neuronal preparations, a major distinguishing feature of the nervous system is its ability to inter-connect regions that are relatively distant from each other, via synaptic connectivity and complex circuits/networks. Consequently, when studying the nervous system and its complex circuitry *in vitro*, it is necessary and desirable to be able to provide a given stimulus (typically electrical or chemical/pharmacological in nature) at a well-defined point of the circuit and subsequently monitor how it propagates through the circuit. The MEA technology provides key advantages for carrying out such studies. It allows the possibility to record electrical activity at multiple sites simultaneously, thereby providing information about the spatio-temporal dynamics of the circuit. Moreover the usefulness of MEAs comes also from the possibility to electrically stimulate cells cultured on top of them.

However MEA applicability in cell culture/tissue electrical stimulation could not be simple as it sound. Usually the amplitude of stimulation is at least an order of magnitude bigger than the cell spiking activity, thus making impossible the detection of activity during the stimulation. Moreover, the stimulation produces large electrical artefact lasting on most channels for milliseconds after the real stimulus, making uncertain the interpretation of data in the first period after stimulation. Some attempts to remove the stimulus artifacts from the recordings have been recently proposed using off-line or on-line blanking methods (Jimbo et al., 2003; Wagenaar & Potter, 2002) partially solving this problem.

Another important disadvantage is related to the poorly controlled spatial distribution of the electrical stimuli. In fact, it has been demonstrated that electrical stimuli spread to the whole biological preparation with amplitude decreasing with the square of the distance from the stimulation site (Heuschkel et al., 2002); in fact, electrical stimuli can directly activate a large number of cells distributed in a quite large area (hundreds of microns) around the stimulation electrode also in the presence of synaptic blockers (Darbon et al., 2002). The reason

of that is unknown, but probably due to several axons passing through the region of the stimulating electrode. Varying the stimulation protocol (i.e. amplitude, polarity, waveform or duration of the pulse) the number of cells directly responding to the electrical stimulus could be adjusted, however the classification of responses detected at different electrodes surrounding the stimulating electrode in directly elicited or due to synaptic transmission remains uncertain. Finally, electrical stimulation needs care to use voltages or current densities that do not harm the electrode.

Some attempts have been done in order to keep down the extension of electrical stimulation. Clustering structures have been proposed (Berdondini et al., 2006) showing a clear difference in the Post-Stimulus Time Histogram (PSTH) between traditional and clustered MEAs. Whereas the traditional MEA shows a the dominance of the early responses (mean latency of 10 ms), the different clusters show a great variability in mean latency (from 10 ms to 100 ms). Unfortunately, the use of clustering structures as well as network patterning structured PDMS layers or neurocages (Erickson et al., 2008) can relatively limiting the random nature of the network and its functional plasticity.

Another method commonly used to stimulate or modulate in vitro neuronal preparations is the application of chemical or pharmacological compounds, e.g. neurotransmitters, ion-channel blockers etc. The problem here is that the chemical/pharmacological compound traditionally is applied over the whole culture preparation through bath addiction, and thus affects almost the entire culture/circuit. Local drug delivery has been proposed in several fashions, from the use of glass pipettes placed near the target cell to dedicated Lab On Chips (LOCs). Glass pipettes are widely used in neuroscience for the local delivery of chemical compounds, but this method is limited by the time needed for the pipette placing and the impossibility to perform parallel multipoint delivery. On the contrary, several publications report on microfluidic devices making possible to transport molecules to cells in a spatially resolved way, i.e. multiple laminar flows (Takayama et al., 2003). Unfortunately, a few systems have been reported where MEAs were combined with microfluidic devices for the testing of toxins (DeBusschere & Kovacs, 2001; Gilchrist et al., 2001; Pancrazio et al., 2003) but without efforts towards the localization of the delivery or complete characterization. A dispensing system for localised stimulation was recently designed to be combined with a MEA chip (Kraus et al., 2006) but not yet completely implemented.

A useful method to combine local neuronal stimulation and local drug delivery involve the use of optical techniques. In principle, different works report on methods for optical stimulation of neurons (Callaway & Yuste, 2002), including direct (Fork, 1971) or dye-mediated laser stimulation (Farber & Grinvald, 1983), direct two-photon excitation (Hirase et al., 2002), endogenous expression of molecules sensitive to light (Zemelman et al., 2002) and caged neurotransmitter activation (Callaway & Katz, 1993). Among the above, the use of caged compounds seems to be the most physiologically suitable approach for the coupling of light with either neuronal excitation, e.g. with caged glutamate (Wieboldt et al., 1994), or modulation, e.g. with caged intracellular second messengers (Nerbonne, 1996).

Caged compounds are characterized by the presence of a blocking chemical group that can be removed by ultra-violet (UV) light pulses (Ellis-Davies, 2007). In this manner, a rapid increase in the concentration of the desired molecule can be obtained by switching the caged analogue into its active form through the cleavage of its blocking group. However, while the process of compound uncaging can be well controlled temporally, the spatial control of this process is limited by the width of the light beam and by light diffraction effects between the light source and the biological preparation, as well as by compound diffusion in the medium around the

site of stimulus application.
In these years, various methodological solutions have been adopted to optically stimulate neurons by caged compounds going from the use of UV sources (e.g. xenon flash lamps) coupled to the port of an epifluorescent microscope (Callaway & Katz, 1993), to the use of laser scanning approaches (Shoham et al., 2005) or digital holographic microscopes (Lutz et al., 2008). Moreover, also external devices such as optical fibres (Bernardinelli et al., 2005) or semiconductor UV light-emitting diodes (Venkataramani et al., 2007) have been used.
The idea of coupling MEAs and optical uncaging has been explored (Ghezzi et al., 2008) using a micro-actuated optical fibre that is able to activate a single site of a cultured neuronal network. In that work, the evaluation of the compound diffusion and of the uncaging efficiency confirmed the applicability of this approach to the local excitation of a selected region of a neuronal network cultured on a MEA device.

3. Evaluation of photostimulation with PhotoMEA

The novel PhotoMEA platform (Ghezzi et al., 2007) combines the standard MEA features, i.e. electrical monitoring, with local chemical stimulation through compound uncaging. In comparison to electrical stimulation, where the electrical stimulus spreads over the whole biological preparation with an amplitude decreasing with the square of the distance from the stimulation site (Heuschkel et al., 2002), the optical stimulation scheme of caged compounds is limited to areas that are exposed to light pulses with sufficient energy to uncage the compounds and diffusion of the compounds in the medium, i.e. uncaging takes place only in a well defined volume (Ghezzi et al., 2008). In order to allow local chemical stimulation, the spatial control of light, i.e. the propagation of light through MEA biochips, has to be defined carefully to reduce the stimulation area to an electrode location and its close surroundings.
Thus, in order to achieve local chemical stimulation, the PhotoMEA platform introduces two novel features to conventional MEA-based data acquisition systems, i.e. the use of specific MEA biochips that integrate a metal shadow mask and the addition of an optical fibre bundle specially designed to fit to the PhotoMEA electrode layout. This allows unprecedented highly localised chemical stimulation at a single electrical recording site, while monitoring the overall culture preparation. Moreover, the use of a multiple-fibre bundle system gives some advantages respect to the use of a single fibre. In fact, it avoids the movement of an optical fibre above the culture plane, thus protecting the culture from possible damages and reducing the experimental time needs for the alignment. In addition, the automatic alignment of multiple fibres to the electrode layout allows patterned stimulation at each electrode of the PhotoMEA biochip, improving the ability to release compounds in multiple sites in parallel.

3.1 The PhotoMEA biochip

Basically, the PhotoMEA biochip is based on a glass substrate, transparent Indium-Tin Oxide (ITO) recording electrodes, a titanium shadow mask that blocks light and thus prevents chemicals from uncaging in undesired regions, and an SU-8 epoxy insulation layer.
Fabrication of the PhotoMEA biochip is made using micro-fabrication technologies, i.e. positive and negative photolithography and wet chemical etching. It is built from a float glass wafer (diameter: 4 inch, thickness: 700 μm) covered with 100 nm ITO and 100 nm titanium (Fig. 1A1,B1). First, the titanium layer is patterned using Microposit S1805 positive tone photoresist (Shipley, Marlborough, USA) and wet chemical etching in 1 % HF solution for 5 s (Fig. 1A2,B2). This step defines the shadow mask of the PhotoMEA (opening diameter of 80 μm). The Microposit S1805 photoresist is then stripped away. The ITO layer is then patterned

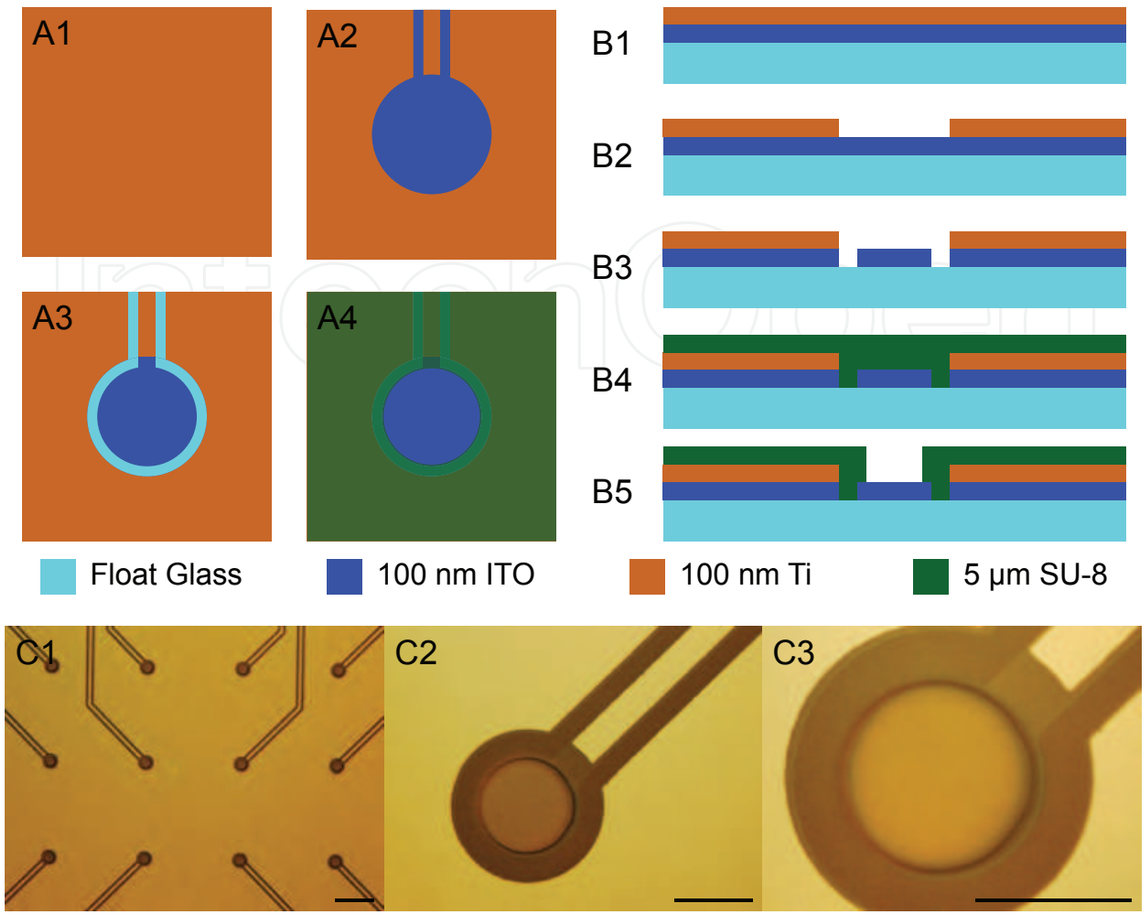

Fig. 1. (**A**) Top view of the fabrication process regarding electrodes and conductive leads. The basic design consists of ITO leads and electrodes (Blue) on a glass substrate (Cyan) covered by a titanium shadow mask (Orange). The titanium mask covers the entire region between electrodes (**A1**). To avoid shorts between the leads and the metal mask, titanium (**A2**) and ITO (**A3**) are patterned creating two small separations along each electrode lead. The insulating layer is composed of SU-8 epoxy covering all the area except for electrodes and contact pads (**A4**). (**B**) Cross-section view of the electrode fabrication process. (**B1**) Cleaning of the glass wafer covered with 100 nm ITO and 100 nm titanium. (**B2**) Patterning of the titanium layer by lift-off. (**B3**) Patterning of the ITO layer by lift-off. (**B4**) Deposition of the SU-8 epoxy insulation layer by spin-coating. (**B5**) Photolithography and opening of the insulator layer by lift-off. (**C**) Partial view of the PhotoMEA biochip workspace. Transparent electrode sites allow both local chemical stimulation and electrical readout. The space around the electrodes is covered by an integrated thin film titanium shadow mask in order to avoid unwanted uncaging of compounds (**C1**). High magnifications of one electrode site show the opening into the shadow mask (**C2**) and the real ITO electrode (**C3**). The scale bar is 200 μm in **C1** and 40 μm in **C2,3**.

using also Microposit S1805 photoresist and wet chemical etching in 37 % HCl for 150 s. This step defines the locations and wires of the ITO electrodes (diameter of 55 μm). The Microposit S1805 photoresist is then stripped away (Fig. 1A3,B3). The next step is the fabrication of SU-8 epoxy insulation layer. SU-8 GM1060 negative tone resist (Gersteltec, Pully, Switzerland) is

coated (5000 rpm for 40 s) and baked for solvent evaporation (15 min at 95 °C) in order to obtain a 5 μm thick layer (Fig. 1B4). It is then exposed to UV light at 365 nm (120 mJ/cm^2) and cross-linking of the illuminated SU-8 parts is achieved by a polymerisation bake (15 min at 95 °C). Unexposed parts, i.e. effective electrode areas (diameter of 50 μm) and connection pads, are released in SU-8 developer (poly-glycol-methyl-ether-acetate) for 1 min (Fig. 1A4,B5). An oxygen-plasma (500 W, 1 min) and a hard bake (2 hrs at 140 °C) insure well definition and good adhesion of the SU-8 insulation layer. Finally, the PhotoMEA chips were released by wafer dicing.

The obtained PhotoMEA chips are then assembled onto a printed circuit board using silver-epoxy glue E212 (Epotecny, Levallois Perret, France) and are sealed using EPO-TEK 302-3M epoxy (Epoxy Technology Inc., Billerica, USA). A glass ring (internal diameter of 19 mm, external diameter of 24 mm and height of 6 mm) defining the culture chamber is finally mounted on top of the PhotoMEA assembly using Sylgard 184 silicone elastomer (Dow Corning, Seneffe, Belgium).

The electrode layout is based on an 8x8 matrix without corner electrodes, whit an electrode spacing of 500 μm (Fig. 1C1). The space between the recording-sites is covered with titanium in order to avoid unwanted chemical stimulation. The electrode leads are also made of ITO covered with titanium in order to limit the area where light can pass through the PhotoMEA biochip. To avoid shorts between the electrode leads and the titanium mask, ITO and titanium are patterned creating to small separations along the electrode lead (Fig. 1C2). The electrode shape is circular with a diameter of 50 μm and an opening in the metal shadow mask with a diameter of 80 μm defines the actual chemical stimulation area (Fig. 1C3).

3.2 The PhotoMEA platform set-up

The basic idea of the PhotoMEA platform is the combination of a standard MEA data acquisition system (Multi Channel Systems MCS GmbH, Reutlingen, Germany) with an optical fibre bundle (Ceramoptec, Bonn, Germany) coming from its bottom side (Fig. 2A). The fibre bundle is composed of 64 optical fibres (UV 50/120/150, NA0.12) arranged in an 8x8 square matrix (Fig. 2B). This arrangement was designed to match the exact geometry of the PhotoMEA biochip electrode layout. A spacing of 500 μm was achieved by positioning the fibres in a special mount made in Arcap AP1D alloy (Fig. 2C) where 160 μm holes were done by precise mechanical drilling (Fig. 2D). Each fibre was glued in the corresponding hole using the semi-rigid optical grade epoxy resin Epo-Tek 305 (Epoxy Technology Inc.).

A TTL controllable 375-nm UV laser source (Coherent Italia, Milano, Italy) was used to generate UV pulses coupled to the selected optical fibre via a 20x objective lens (Thorlabs Inc., Newton, USA). The laser can be alternative coupled to every optical fibre by moving the input side of the fibre bundle through a M105.3 DC motorised 3-axes micropositioning stage (Physik Instrumente SrL, Milano, Italy), controlled by a LabView (Teoresi SrL, Torino, Italy) custom application. On the other side, the exact alignment between the fibres of the bundle and the electrodes of the PhotoMEA biochip was obtained using another M105.3 DC motorised 3-axes micropositioning stage (Physik Instrumente SrL) controlled by the same LabView custom application. The alignment was optimized exactly matching at least 4 optical fibres with the corresponding electrode site (Fig. 2E).

3.3 Neuronal cultures

Low-density primary cultures of hippocampal neurons were prepared from embryonic day 18 rat embryos (Charles River Laboratories Italia SrL, Calco, Italy), essentially as previously

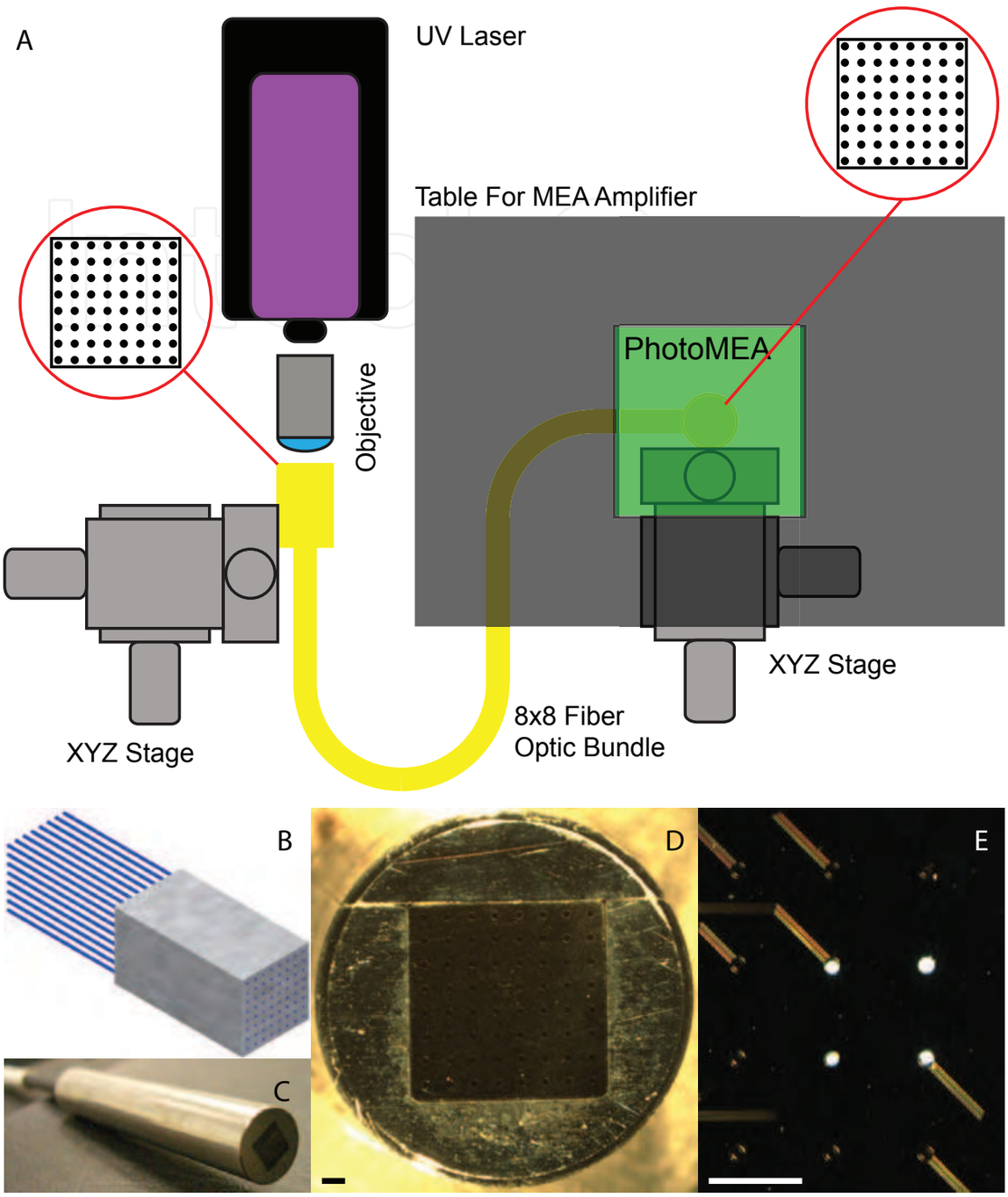

Fig. 2. (**A**) Scheme of the PhotoMEA platform set-up. (**B**) Drawn of the fiber arrangement in the bundle bundle. (**C**) Picture of the bundle head. (**D**) Magnified picture of the bundle head. (**E**) Fiber bundle aligned with the PhotoMEA biochip.

described (Kaech & Banker, 2006). Some modifications were introduced to adapt the method to the PhotoMEA biochip (Ghezzi et al., 2008).
Rat hippocampal neurons can be cultured over the MEA and PhotoMEA biochip for up to several weeks, making large neuronal network characterized by dense synaptic

interconnections and huge spontaneous electrical activity detected at MEA electrode sites. Using conventional transparent MEAs, images of neurons can be easily acquired either

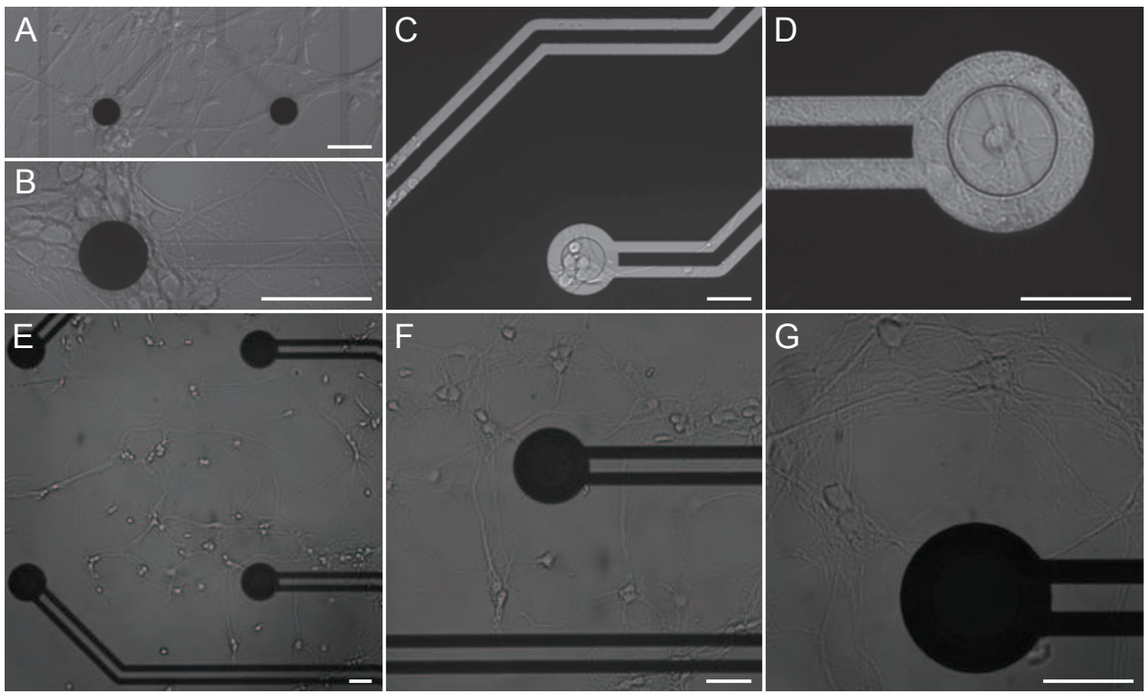

Fig. 3. **(A)** Transmitted light microscopy picture of neurons cultured on a commercially available ThinMEA. **(B)** Enlargement of an electrode site of the ThinMEA. **(C)** Transmitted light microscopy picture of neurons cultured on the PhotoMEA biochip. **(D)** Enlargement of an electrode site of the PhotoMEA. **(E-G)** Reflected light microscopy image of a portion of the PhotoMEA biochip covered with hippocampal neurons at different magnifications. Scale bar is 50 μm.

using inverted or upright microscope in transmitted light microscopy (Fig. 3A,B). Images of neuronal cultures were taken by an Axiovert 200 inverted epifluorescence microscope (Carl Zeiss SpA, Arese, Italy) positioned over an anti-vibration table and equipped with a 20x/0.8NA Plan-Apochromat short distance objective lens, a 40x/1.3NA EC Plan-Neofluar oil immersion objective lens and a an ORCAII CCD camera (Hamamatsu Photonics Italia SrL, Arese, Italy).
On the contrary, the titanium mask of the PhotoMEA biochip hampers the observation of the entire network in transmitted light microscopy. In fact, only neurons at the electrode sites (not covered by the titanium mask) are clearly visible (Fig. 3C,D).
Working with non-transparent substrates, neurons cultured on top of them can be observed using an upright microscope in reflected light mode. Images of neuronal cultures were taken by a FN1 upright microscope (Nikon Instruments SpA, Calenzano, Italy) positioned over an anti-vibration table and equipped with a 4x/0.1NA and a 10x/0.25NA long distance objective lenses, a Brightfield filter (Chroma Technology Corporation, Rockingham, USA) and a an ImagEM CCD camera (Hamamatsu Photonics Italia SrL). This method allows us to observe the entire network cultured covering the PhotoMEA chip with the exception of the transparent electrode sites (Fig. 3E,G). In conclusion, the titanium mask does not block the possibility to observe the development and the vitality of the neurons in culture.

3.4 Electrical properties

To completely characterize our fabricated PhotoMEA chips, we measured characteristic 1 kHz impedances of all electrodes. Mean measured electrode impedance is 1015 kΩ ± 112 kΩ, with a minimum value of 780 kΩ and a maximum of 1420 kΩ. Moreover, electrical recordings were performed in the culturing medium at 37 °C using the MEA1060 system (Multi Channel Systems MCS GmbH). Data recorded at 25 kHz/ch from the 60 channels were then filtered from 10 Hz to 3 kHz and spikes were sorted using a threshold algorithm included in the MC Rack software (Multi Channel Systems MCS GmbH). The threshold was defined as a multiple of the standard deviation of the biological noise computed during the first 500 ms of the recording (-5 * SD_{noise}). PhotoMEA electrodes showed a noise level appropriate to spike detection (Fig. 4) during recordings.

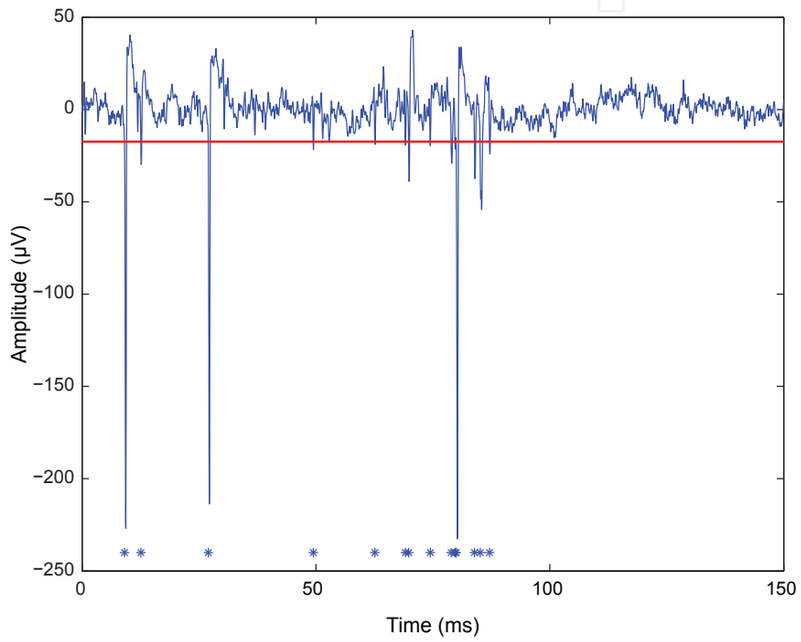

Fig. 4. Neuronal spikes can be easily detected with PhotoMEA electrodes, where root-mean square basal noise was measured as ±5.4 μV in a trace of 1 s without spiking activity. Threshold for detection was fixed to -17.5 μV by the software. Markers highlight the detected spikes after band-pass filtering.

3.5 Electrical stimulation

Hippocampal neurons (18 DIV) cultured on PhotoMEA biochips were electrically stimulated in order to illustrate the electrical stimulation disadvantages using conventional MEA technology. Biphasic, positive then negative, voltage pulses (amplitude of ±100 mV and pulse-width of 100 μs/phase) were applied to the neuronal network through one electrode of an PhotoMEA biochip (Fig. 5B). The electrical recording performed by the MEA system on all biochip electrodes shows that the stimulus spreads to the entire area of the culture, in spite of the large electrode spacing (Fig. 1). It results that the whole neuronal network could be electrically stimulated with an amplitude decreasing with the square of the distance from the stimulation site, affecting data quality as it is not known if the evoked responses detected at other electrode sites (Fig. 5C) correspond to direct cell stimulation due to the electrical

stimulus (the responses follow the electrical artefact) or to network activity (i.e. signals that were propagated within the cell culture by synaptic transmission). Moreover, often the stimulated electrode remains not available for a long time after the stimulus application.

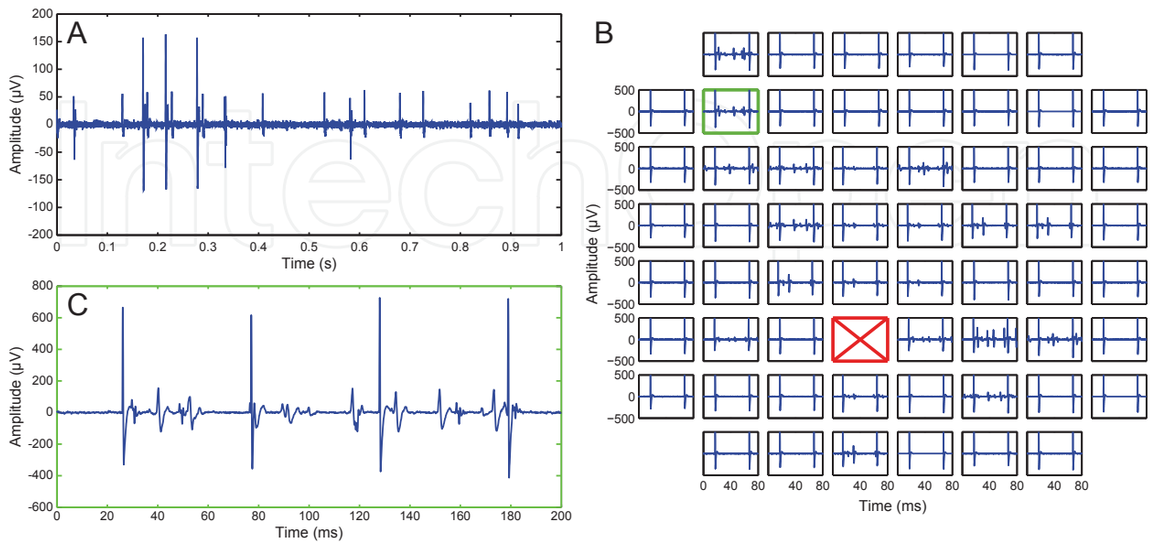

Fig. 5. (**A**) Recorded trace from one electrode of the PhotoMEA biochip showing a period of spontaneous spiking activity. (**B**) Applying a biphasic, positive then negative, voltage pulse (amplitude $\pm$100 mV and pulse duration 100 μs/phase) to electrode 46 (red cross) the stimulus artefact is recorded by all electrodes of the biochip. Evoked responses can be found at several electrodes of the recording space. (**C**) High resolution trace recorded at an electrode (green box in **B**) far from the recording site (red cross), when a train of four pulses is applied. The trace shows electrically evoked spikes directly coupled to the electrical artifacts.

3.6 Optical stimulation

The optical stimulation approach was first evaluated in its ability to stimulate a small region surrounding a recording electrode and then in its efficiency in stimulating neurons.

In order to demonstrate the compound uncaging principle in a small volume at an electrode location, optical pulses with different pulse duration were delivered to a fluorescent caged compound (CNB-caged fluorescein, Invitrogen SrL, Milano, Italy). Fluorescence images were taken with an MZ16F stereomicroscope (Leica Microsystems, Wetzlar, Germany) equipped with a Moticam1000 CMOS camera (Motic, Xiamen, China), a x-cite120 metal halide fluorescence illuminator (EXFO, Quebec, Canada) and a Leica blue filter set (ex: BP470/40, em: LP515). The stereomicroscope was positioned over the PhotoMEA experimental setup.

The resulting fluorescence intensity due to compound uncaging increased with the light pulse duration, indicating that the amount of uncaged compound increased with the energy delivered to the sample (Fig. 6A,B). On the other hand, the stimulated area measured as Full Width at Half Maximum (FWHM) also increased along with the pulse duration, but it did not spread widely over the size of the hole in the metal mask, even for long stimuli (Fig. 6C). The final experiment was aimed at demonstrating that neuronal activity can be locally evoked using the PhotoMEA platform. A UV light pulse was applied to cultured hippocampal neurons at 14 DIV (Fig. 7A) in the presence of MNI-caged-L-glutamate (Tocris Bioscience, Bristol, UK) at a concentration of 100 μM. When neurons were stimulated with

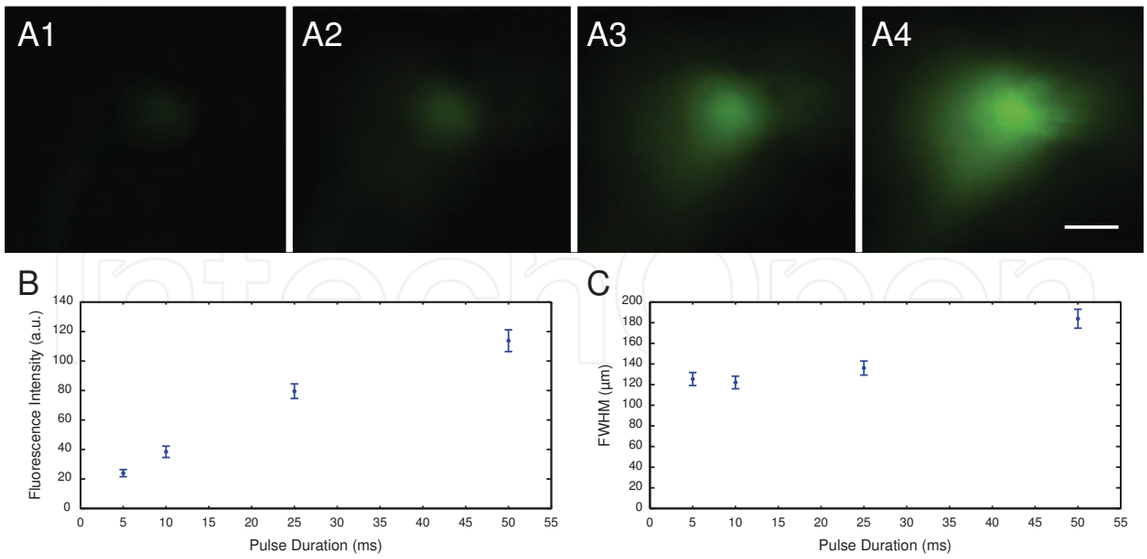

Fig. 6. (**A**) Pictures of the CMNB-caged fluorescein dissolved in glycerol at a final concentration of 100 μM activated by four optical pulses differing in their pulse duration: (**A1**) 5 ms, (**A2**) 10 ms, (**A3**) 25 ms and (**A4**) 50 ms. Measured maximum fluorescence intensity (**B**) and FWHM (**C**) of uncaged CMNB-caged fluorescein obtained by UV-light pulses with the same pulse durations as in A. The mean values ś standard deviations of the computed values are reported for every pulse duration (n = 5). Scale bars are 100 μm.

a UV pulse of 15 ms, evoked spikes were electrically detected at the stimulation electrode (Fig. 7B). Unfortunately, during the pulse, an interaction between the UV light and the stimulated electrode site was found. Similarly to what happens with electrical stimulation, the stimulated electrode site presented a stimulus artefact due to the optical pulse, which was not found on all other electrodes of the PhotoMEA biochip (Fig. 7C). These artifact seem to be related to the energy transferred by the UV light pulse as their duration is approximately twice the duration of the light pulse and increases with increasing pulse duration (mean $\pm$ standard deviation for n = 20 subsequent stimulation repeated for all pulse durations; 8.1 ms $\pm$ 0.59 ms at 5 ms, 18.8 ms $\pm$ 0.73 ms at 10 ms, 28.4 ms $\pm$ 0.62 ms at 15 ms, 35.2 ms $\pm$ 0.36 ms at 20 ms, 41.8 ms $\pm$ 0.61 ms at 25 ms, 1414.5 ms $\pm$ 215.16 ms at 50 ms and 2819.89 ms $\pm$ 274.53 ms at 100 ms). The physical nature of these artifacts and their possible influence on the neuronal activity are currently under investigation. However, the optical stimulation was found to work at the stimulated electrode site as evoked biological responses followed the chemical stimulation (Fig. 7B). At some other electrode sites, spontaneous and/or evoked activity appearing with a large delay and probably in response to a plastic effect of the network linked to the chemical stimulation were detected (Fig. 7C). We exclude that the activity at the other electrode sites can be evoked because of either direct local uncaging or diffusion of the glutamate. In fact, it has been demonstrated that the uncaging is localized to the close surrounding of the stimulated electrode (Fig. 6). Moreover, based on previous evaluation of the diffusion rate of the glutamate (Ghezzi et al., 2008), we can exclude that free glutamate affects electrodes far from the stimulation site.

As shown by a temporal representation of the network activity after stimulus (Fig. 7D), the activity is initially evoked at the stimulated site and after few tens of milliseconds it spreads

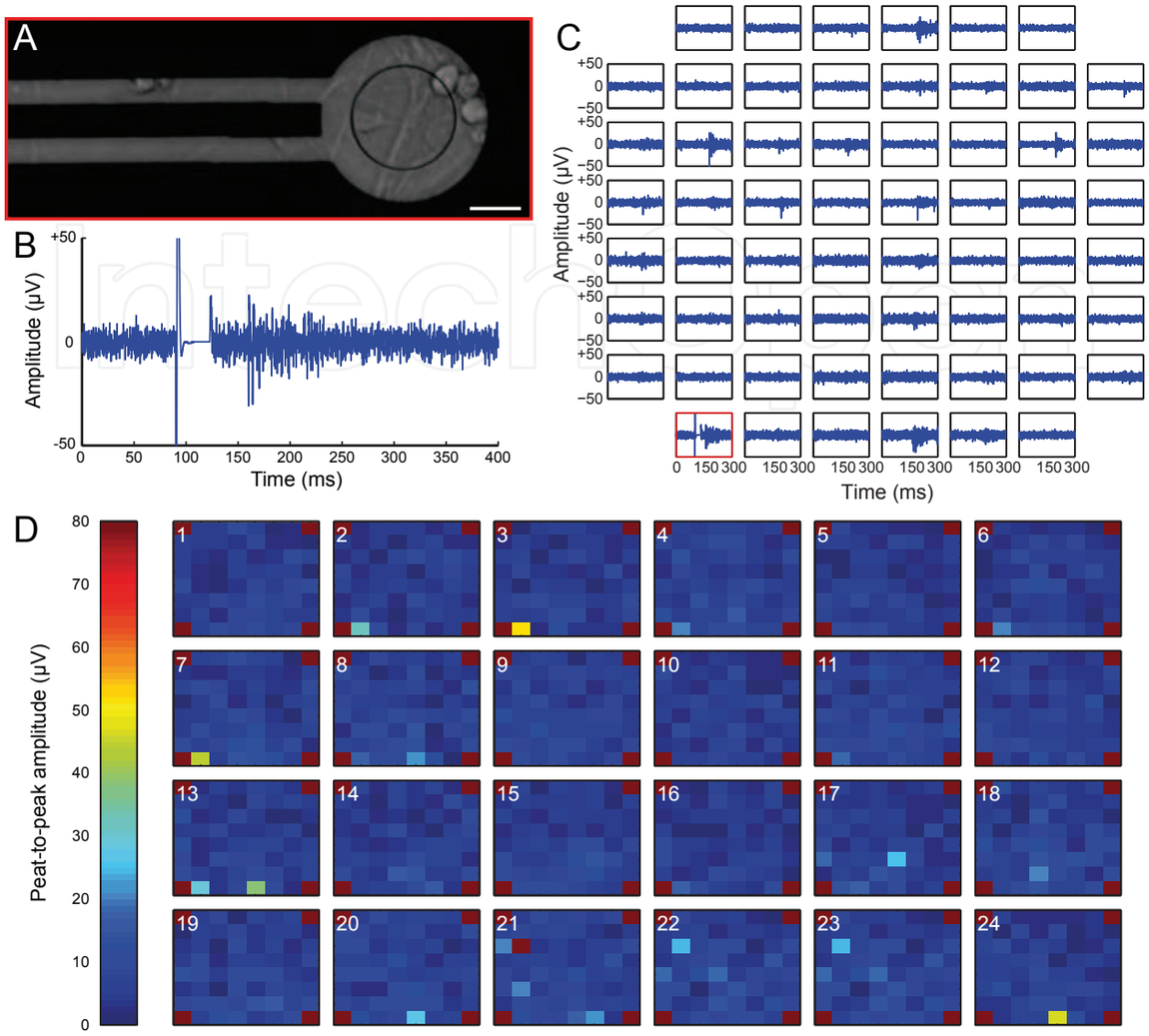

Fig. 7. (**A**) Picture of the optically stimulated neurons close to the ITO electrode of the PhotoMEA biochip. (**B**) Activity recorded at the stimulated site after an optical pulse of 15 ms. The artefact induced by the optical pulse and the following biological activity evoked by the glutamate uncaging is shown. (**C**) Activity recorded from the entire network after the UV pulse. The red box highlights the stimulated site. (**D**) Graphical representation of electrical activity spreading in the network after the optical pulse. Every frame, acquired with a sample rate of 1 kHz, represents a measure of the activity at every site. The color map represents a color representation of the peak-to-peak amplitude at every recorded site. Scale bar is 25 μm.

to other regions of the culture, thus revealing the interconnection between the different parts of the network. This also supports our conclusion concerning the localization of the stimulus.

4. Conclusion

Electrical stimulation on MEA presents certain experimental limitations, as it is difficult to prevent the electrical stimulus from spreading over the whole culture. Thus, the induction of evoked responses within the whole cell culture masks functional and network characteristics,

and makes difficult the proper evaluation of signal propagation. The same problem arises for chemical stimulation, as the chemical compound spreads throughout the culture medium, thereby also limiting the results to proper network behavioural observations. The goal of the development of the PhotoMEA platform was to generate a tool and method that would allow local chemical stimulation, in order to stimulate only a small portion of the biological preparation. It was expected that such device would facilitate the acquisition of more precise information about functional processes within complex biological networks.

In the current work, through the use of the novel PhotoMEA biochips combined with UV-light pulse stimulation, local chemical compound uncaging and hence local chemical stimulation was successfully achieved. The optical stimulation performed through the PhotoMEA platform limits the activation of the stimulus only to the area surrounding the electrodes, thus allowing the possibility to have a better defined study of information processing in neuronal networks with several independent inputs and outputs. Beside caged neurotransmitters, virtually every kind of signalling molecule or second messenger has already been caged, from protons to proteins, including also inositols, nucleotides, peptides, enzymes, mRNA and DNA (Ellis-Davies, 2007). This considerably widens the scope and potential impact of the PhotoMEA tool in cell signalling, systems biology and complex biological cultures, and makes it also amenable to use with non-neuronal cultures. In the field of pharmacology, the features of the PhotoMEA platform improve the possibility to create in a more controlled manner a spatial map of the drug effect's on the tissue preparation, in order to improve the evaluation of the drug's specificity, a result that cannot be easily achieved using conventional methods for the drug application, e.g. pipetting.

An important feature is that the PhotoMEA technology can be readily scaled up for higher throughput applications, and thus may provide opportunities in drug screening applications, especially for central nervous system (CNS) disorders. The CNS drug discovery industry currently has several high throughput tools (e.g. planar patch-clamp) for monitoring and testing drugs on single isolated cells. However, there are no suitable tools and methods, especially high-throughput, to evaluate drug activity on synaptic biology, i.e. at the network level (Dunlop et al., 2008) and on real neurons, thereby presenting an excellent opportunity for PhotoMEA tools in CNS drug screening community. In addition to caged compounds, the PhotoMEA system is also expected to rise considerable interest for applications using photosensitive tissue preparations, such as retinal tissue. There are already several studies that have used MEAs with retinal explants for electrical recording and stimulation (Puchalla et al., 2005; Segev et al., 2004). The combination of the standard MEA electrical recording feature with the PhotoMEAA capability to optically uncage a signalling molecule and/or optically stimulate light-sensitive retinal neurons, promise to provide an unparalleled information-rich paradigm for investigating the complex information processes that take place in the mammalian retina.

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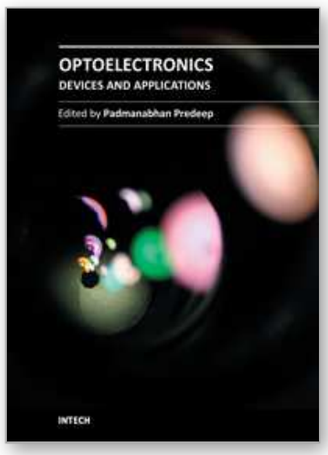

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