PhotoMEA: An opto-electronic biosensor for monitoring in vitro neuronal networks activity

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SUMMARY

PhotoMEA is a biosensor useful for the analysis of an in vitro neuronal network, fully based on optical methods. Its function is based on the stimulation of neurons with caged-glutamate and the recording of neuronal activity by fluorescence Voltage-Sensitive Dyes. The main advantage is that it will be possible to stimulate even at sub-single neuron level and to record with high resolution the activity of the entire network in the culture. A large-scale view of neuronal intercommunications offers a unique opportunity for testing the ability of drugs to affect neuronal properties as well as alterations in the behaviour of the entire network. The concept and a prototype for validation is described here in details.

Keywords: neuronal information processing, technology for neuropharmacology, neural-based biosensor, bio-information processing system, computational biology

INTRODUCTION

The most important function of the neurons and neuronal networks is to process and transmit electrical signals. The ability to modulate and record this activity is fundamental to the understanding of how the central nervous system (CNS) works. Therefore one of the most relevant topic in neuroscience is the better understanding of the functional dynamics that govern a neuronal network activity and the electro-chemical variations at the base of the modulation of neuronal physiology.

There are two possible approaches in studying neuronal functions. On one hand, a large-scale approach aims at understanding a more or less synchronized activity of the whole neuronal system and, on the other hand, a micro-scale approach studies detailed behavioural models, not only of every single cell, but also of molecular systems which, interacting with each other, actively contribute towards the generation and modulation of the whole neuronal activity.

Brain functions are exerted by the coordinate activity of the discrete regions involved in the information process where a single neuron may influence the activity of many other neurons in the neuronal network. Thus, the study of the modulation of neuronal physiology that regulates neuronal activity must necessarily be inserted into a large-scale view of the neuronal network dynamics. A new breakthrough into neuroscience will be the possibility to stimulate and modulate a single neuron and study its response and that of the entire network.

There is a growing need to observe smaller and smaller systems, kept alive in vitro, at a proper spatially and temporally resolution level. At the same time, it is necessary to monitor neuronal activity of the whole network in order to understand how every single neuron is able to modulate the whole network activity.

At the current state of the art, in most cases, these investigations have been carried out by electrically stimulating and recording the tissue of interest. Methods which employ intra-cellular or extra-cellular electrodes or arrays have yielded important results in neurobiology, but nowadays these technologies are showing some important limits. The stimulation of a single neuron can be traditionally performed by intra-cellular electrodes with the disadvantage of the mechanical damage of the cell and the consequent alteration of the entire network. Thus intra-cellular electrodes do not allow studies of the whole neuronal network. In contrast the extra-cellular electrodes, as micro-electrode array devices (MEA) (Thomas et al., 1972; Gross, 1979; Pine, 1980), are not suitable for local stimulations and high-resolution recordings, because of the interference generated by electrical fields which spread in the medium. They can be used in order to monitor the electrical activity of the entire network, but with the limitation of recording some discrete regions only.

Besides traditional electrophysiology, optical methods for stimulating and recording neuronal activity have been used for a long time (Kotter et al. 1998; Kandler 1998; Zecevic, 1996; Antic et al. 1995; Antic et al. 1999).

The ability to use light provides a non-invasive method for precise temporal and spatial activation of different regions of a neuronal network, which can be used to stimulate single neurons as well as discrete regions of the neuron itself. In addition, optical methods allow to monitor the entire network activity. However, at the current state of the art, optical methods are not yet independent from electrical measurements, but they provide only a useful support for neurons stimulation, or alternatively for recording membrane electrical activity.

Our work provides a technical solution for the stimulation and recording of the activity of an in vitro neuronal network entirely based on optical methods. The proposed biosensor, PhotoMEA, is capable to combine an optical stimulation of neural activity with high spatial resolution in addition to recording the activity of the whole network.

The PhotoMEA concept (Patent Pending number MI2005A000114) is currently under testing.

METHODS

Stimulation of neurons with light

Light stimulation of neurons can be performed by different optical methods, including direct two-photon excitation (Hirase et al. 2002), endogenous expression of molecules sensitive to light (Zemelman et al. 2002) and caged-glutamate activation (Callaway et al. 1993). These and other methods have been recently reviewed (Callaway et al. 2002).

Since light can be focused with high spatial and temporal resolution, optical methods are the best solution to provide local and controlled stimulations to neurons or to any part of them. In particular, the most physiological way used in order to couple light and neuronal excitation is based on the use of the caged neurotransmitter glutamate. Glutamate, the most important and widespread excitatory neurotransmitter in the brain, can be caged by a chemical group which can be removed by light absorption.

The basic approach is to switch caged-glutamate into active glutamate by ultraviolet light pulses with a single-photon method. In order to achieve high spatial resolution, light can be focused by means of two-photon microscopy.

Currently, optical stimulations of neurons are matched with electrical recording of neuronal activity performed by intra or extra-cellular electrodes, with the consequence that the local stimulation performed by caged-glutamate is usually limited to the study of one single neuron (using an intracellular electrode for recording) or it is accompanied by a spread recording of a network activity with low resolution (using MicroElectrode Array devices).

Neurons activity recording by optics

Changes of neuronal membrane potential can be measured optically by different methods, either directly or by a variety of molecular probes. Intrinsic optical signals are generated by nerve activity which induces changes in light scattering. However, such changes are usually small, therefore they are not applicable to the study of in vitro neuronal networks (Grinvald et al. 1988).

Using molecular probes, several optical properties, which are sensitive to membrane potential, can be detected, e.g. fluorescence, absorption, dichroism, birefringence, fluorescence resonance energy transfer, nonlinear second harmonic generation, and resonance Raman absorption (Zochowsky et al. 2000).

In particular, fluorescence is the most common property used in optical imaging. Therefore, optical recording of neural activity is possible thanks to Voltage-Sensitive Dyes (VSDs) acting as transducers which convert voltage changes into optical signals recorded by a proper optoelectronic sensor. Furthermore, VSDs have the ability to bind the membrane of neurons and change their fluorescence emission according to the variations of the membrane potential.

VSDs open new perspectives for the study of neuronal communication, thanks to their ability in revealing membrane potential changes along neuronal processes and the whole networks with spatially high-resolution imaging. Actually, optical recordings are matched only with electrical stimulations, either low-resolved (MEA) or limited to single neuron (intracellular electrodes). Therefore, this method currently provides a tool suitable only for spatially high-resolution imaging of neuronal activity, without the possibility to perform highly-controlled stimulations.

RESULTS

Concept of PhotoMEA

PhotoMEA is a biosensor which uses optical methods both to stimulate and to record the activity of an in vitro neuronal culture. The idea is to integrate the glutamate caged stimulating method with the use of Voltage-Sensitive Dyes for recording network activity.

The combination of the two mentioned technologies is not straightforward. As they have been so far used, they are not adapted to be integrated. The main problem concerns the working volumes of the two. Indeed, caged-glutamate is released using a confocal microscope focused on the point of stimulation, hence at single neuron or sub-single neuron level. On the other side, if we want to record the activity of the whole neuronal culture by VSDs, a fluorescence microscope with CCD is required. The two microscopes are not usable together and make the two current optical technologies not compatible.

PhotoMEA proposes a solution for the integration of the two methods by avoiding the use of both microscopes. The innovative concept (Patent pending number MI2005A000114) is based on the use of optical fibres, which are used to lead the stimulation light directly on defined positions of the coverslip and to record the fluorescence modification from the same areas.

In the culture medium, both caged glutamate and VSDs are added. The size of the fibre could be micrometers, the UV light is brought right under the culture, stimulating glutamate in the medium right outside the exit of the fibre. In this way, depending on the size of the optical fibre, parts of neurons (dendrites or axon hillock) can be selectively stimulated. At the same time, the fibres are used to record the fluorescence activation signal. This solution is simple from the technological point of view, but allows only a discrete recording of the network activity depending on the number of the fibres and their interspacing.

Figure 1 shows the operation principles of the PhotoMEA system.

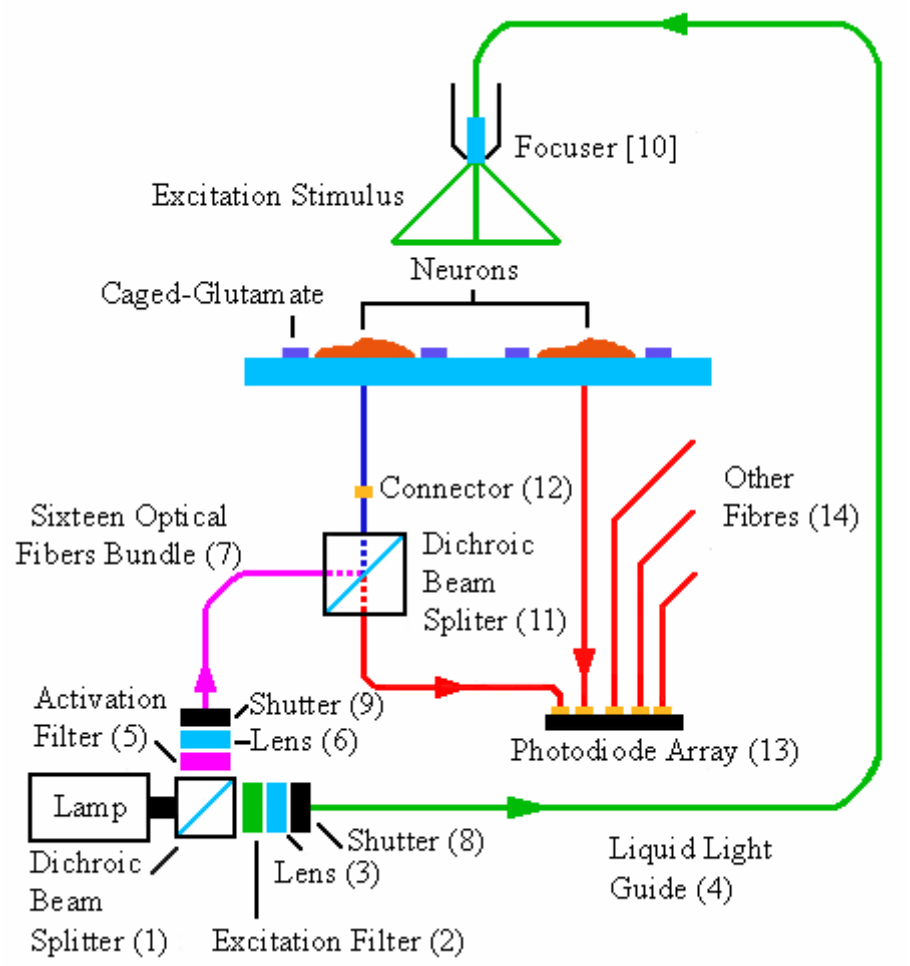

Figure 1: Principles of functioning of the PhotoMEA system. For detailed description see text.

Description of the prototype

Sixteen multimodal optical fibres (OZ Optics, BC1259), are glued on the bottom surface of a simple microscope coverslip. Neuronal network with caged-glutamate and VSDs (Molecular Probes) can be cultured on this coverslip. These fibres can be used both to active glutamate and to record fluorescent signals.

A 100W mercury arc lamp is used to activate caged-glutamate and to excite fluorescence spready above the culture, by using a dichroic filter to split the light in two components at the proper wavelength. This system is shown in figure 2.

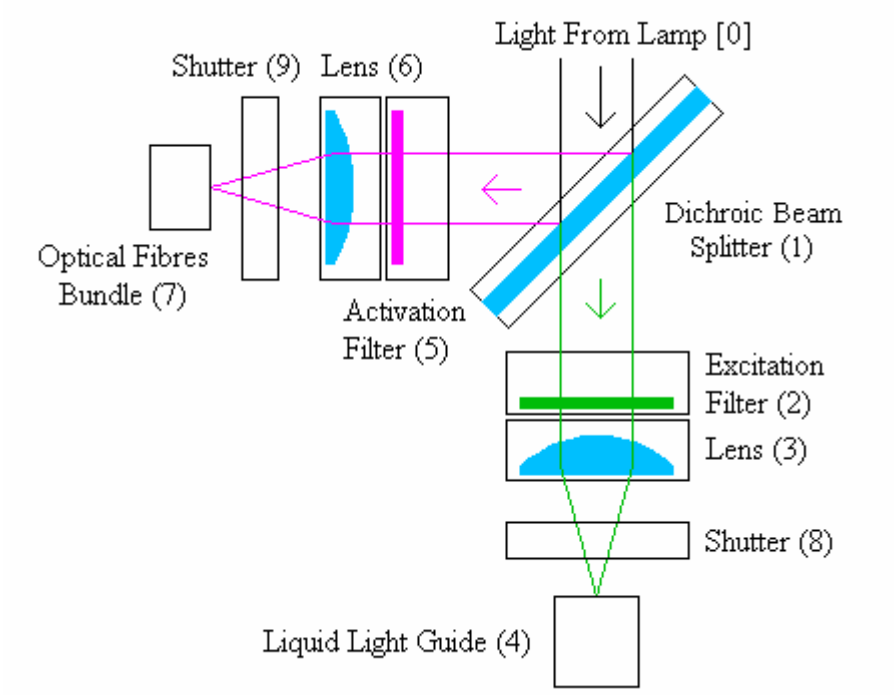

Figure 2: First stage of the PhotoMEA system, which allows to active caged-glutamate and to excite fluorescence by means a single 100W mercury arc lamp. For detailed description see text.

Light emitted from mercury arc lamp is filtered by a dichroic beam splitter (Fig. 1 and 2, (1)) (Chroma Technology, 400dclp) for separating the two optical components needed. Light transmitted is filtered by an excitation filter (Fig. 1 and 2, (2)) (Chroma Technology, 535/50x) and focused by a BK7-Plano convex lens (Fig. 1 and 2, (3)) (Thorlabs, LA1422) into a liquid light guide (Fig. 1 and 2, (4)) (Lotoriel Italia, 77567). Output of the guide is focused (Fig. 1, (10)) (Lotoriel Italia, 77646) above the culture. Light reflected by the dichroic beam splitter (Fig. 1 and 2, (1)) is filtered by an activation filter (Fig. 1 and 2, (5)) (Chroma Technology, 360/80x) and focused by a UV fused silica-plano convex (ThorLabs, LA4725) into a sixteen optical fibres bundle (OZ Optics, BC1259).

Two electro-mechanical shutters (Fig. 1 and 2, (8,9)) (Sunex, SHT934) have been used to generate short pulses for glutamate uncaging and for fluorescence activation respectively. One shutter (Fig. 1 and 2, (9)) generates short pulses for uncaging caged-glutamate, whereas the other (Fig. 1 and 2, (8)) avoids optical damage of the culture.

By means of a second optical system, shown in figure 3, each of the sixteen optical fibres glued to the coverslip can be used both to activate glutamate and to record fluorescent signals.

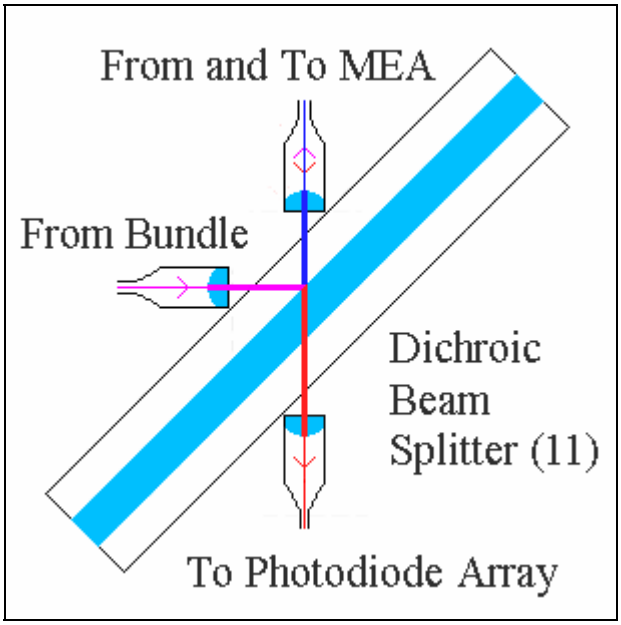

Figure 3: Second stage of the PhotoMEA System, which allows to use the same optical fibres glued on the bottom of coverslip both for to active caged-glutamate and to record optical signals in fluorescence. For detailed description see text.

Three pigtail style collimators (OZ Optics, LPC-04) have been used to couple optical fibres.

Light from one of the fibres of the bundle (Fig. 1, 2 (7)), used to active cage-glutamate, is reflected by a dichroic beam splitter (Fig. 2 and 3, (11)) (Chroma Technology, 400dclp) into one of the optical fibres glued on the bottom surface of the coverslip. Optical signals from this same fibre are transmitted by the same dichroic filter (Fig. 2 and 3, (11)) and lead to one photodiode of the array (Fig. 1, (13)). This second stage has been carried out for each of the sixteen fibres. By means of sixteen connectors (Fig. 1, (12)), the sixteen optical fibres can be used to stimulate and record or to only record action potentials. Fibres (Fig. 1, (14)), which are not linked to connectors (Fig. 1, (12)) can be directly connected to photodiode array (Fig. 1, (13)).

Prototype testing

In order to check the real effectiveness of the conceived method, a prototype has been built (Figure 4). It allows comparing the recordings of the PhotoMEA optical system with those of standard MEA system, assumed as validating reference.

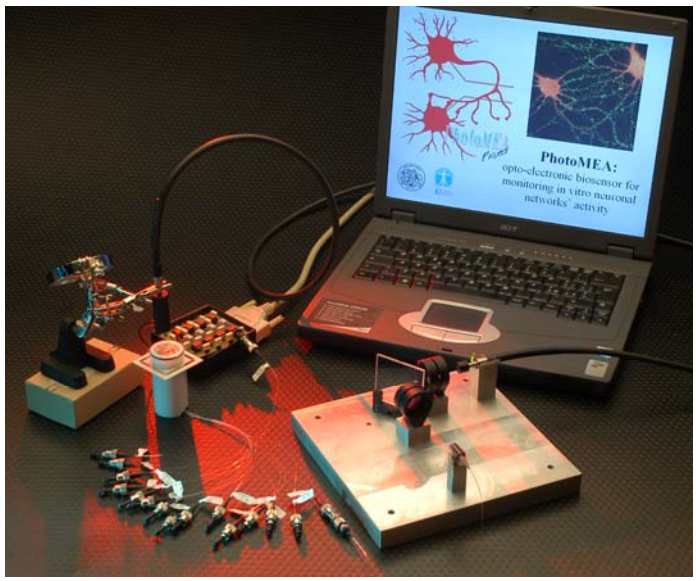

Figure 4: Prototype of the PhotoMEA system.

This is possible because, instead of the slide, we have used a MEA under which optical fibres are glued. Besides, in order to get an optimal check of the system's functioning, every optical fibre is glued exactly next to a MEA electrode (Figure 5), in such a way as to guarantee a high-correspondence between recorded signals.

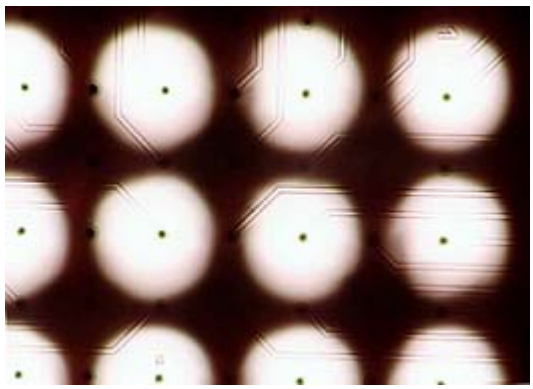

Figure 5: Alignment of a copper mask the MEA electrodes. Copper mask is used to glue optical fibres right next to MEA electrodes.

For the first tests, we create neuronal networks as controlled as possible from a topological point of view, i.e. neuronal networks in which the distribution of cells and the spatial development of neuronal processes are italics determined parameters. This way, it is possible to simplify considerably the network's features in order to make the correlation among recording signals easier for validation test of PhotoMEA functioning.

Lithographic methods of microtechnologies allow to create preferential areas of neuronal adhesion and connection leads on which they can develop their axonal processes [Wyart et al. 2002].

DISCUSSION

PhotoMEA is a biosensor useful for the analysis of an in vitro neuronal network, fully based on an optical method. Its function is based on the stimulation of neurons with caged-glutamate and the recording of neuronal activity by fluorescence recording.

The methods for stimulating and recording the neural activity were well known in literature even if their combination was not straightforward. PhotoMEA is a try in this direction.

The main advantages of PhotoMEA are the following:

1) The possibility to stimulate a neuron or part of it with very high resolution and in a physiological way by using caged-glutamate.
2) The possibility of a simultaneous recording of the activity of the whole neuronal network with high resolution by using VSDs probes.
3) The possibility to perform acquisitions under the laminar-flow cabinet, making the system totally independent from a microscope, hence simplifying the experimental setup.

PhotoMEA is a prototype suitable for testing the fully optical method proposed. The testing prototype described here allows a good validation of the PhotoMEA concept proposed, thanks to its coupling with a MEA system. On the contrary, the current prototyping solution does not overcome the main limit of the MEA devices which is the low resolution both in stimulation and in recording. Anyway, the concept of PhotoMEA theoretically permits higher resolution and better stimulation and recording as well as, from a technical point of you, other improvement solutions are feasible.

The main step forward is in the choice of smaller optical fibres (available off-the-shell down to 2μm) and the reduction of their interspacing. Of course, this solution is applicable as well as the present prototype but it does not allow the validation by MEA. After the complete validation testing, currently ongoing in our laboratory, prototypes with smaller and denser fibres will follow.

This novel device opens exciting perspectives in many fields of neurobiology. Thus, it will be possible to analyze in great details the activity of the neurotransmitter glutamate in the stimulation of selected neuronal compartments and to reveal the extent of spreading of a localized signal. In addition, the possibility of studying the relative contribution that an identified neuron has in the economy of an entire neuronal network may help in elucidating whether different sub-regions or neuronal subtypes play distinct roles in the flux of electrical information flowing within the meshwork. Finally, a large-scale view of neuronal intercommunications is of great interest in the elucidation of the mechanism of action of neurotropic drugs and offers a unique opportunity for testing their ability to affect neuronal properties as well as alterations in the behaviour of the entire network.

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