PhotoMEA: A new optical biosensor for neuronal networks analysis

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Light stimulations and optical recordings of neuronal activity are two promising approaches for investigating the molecular mechanisms at the basis of neuronal physiology. In particular, flash photolysis of caged compounds [1] offers the unique advantage of allowing to quickly change the concentration of either intracellular or extracellular bioactive molecules, such as neurotransmitters or second messengers, for the stimulation or modulation of neuronal activity. Moreover optical recordings of neuronal activity by Voltage-Sensitive Dyes (VSDs) [2] allow to follow changes of neuronal membrane potential with high-spatial resolution. This enables the study of the subcellular responses and that of the entire network at the same time.

In the last decade, studies on neuronal physiology and plasticity have provided a detailed picture of the molecular mechanisms underlying the modulation of neuronal activity; on the other hand, the molecular mechanisms which control the network properties remain poorly understood, and represent a new frontier in neuroscience. Two different approaches can be followed for the study of neuronal functions: a large-scale approach aiming at understanding the activity of many neurons interacting in a complex network and a micro-scale approach aiming at providing detailed behavioural models of the molecular systems which actively contribute to the generation and modulation of the neuronal activity.

A new breakthrough in neuroscience would be the possibility to stimulate and modulate a single neuron, or selected parts thereof, and study its influence over the functioning of the entire network. In this manner, the micro-scale meets the large-scale approach, allowing the understanding of how the mechanisms that influence the physiology of single neuronal units are able to alter the behaviour of the entire network.

At present, experiments are carried out by electrical stimulations and recordings using intracellular or extracellular electrodes as well as MicroElectrode Array devices. These systems have yielded important results but show some limits, e.g. in terms of mechanical damage of the cell (intracellular electrodes) and poor spatial resolution both in stimulation and recording (MEA).

In addition to traditional electrophysiology techniques, optical methods for stimulating (using Caged Compounds) and recording (using Voltage-Sensitive fluorescent Dyes) neuronal activity have been used separately for a long time. Typically light stimulations are combined with electrical recordings, whereas optical recordings with electrical stimulations.

First experiments of optical stimulation and imaging were done with a microscope-based set-up (Figure 1).

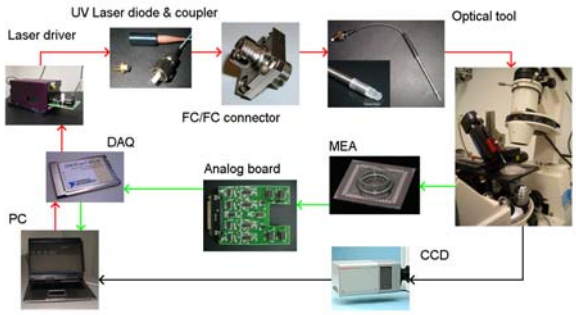

Figure 1: This figure represents the set-up for a total optical analysis of neuronal networks. Three pathways were sown: that of light stimulation (Red line), that of MEA recordings (Green line) and that of optical recordings (Black line).

In this set-up MEA recordings were used as validation of the optical stimulations. Figure 2 (Top panel) shows a neuronal spike evoked with a 100msec UV stimulus for activating the caged-glutamate (MNI-caged-L-glutamate, Tocris Bioscience, Bristol, UK) used in a 100uM concentration. Figure 2 (Bottom panel) shows the Ca_i^{2+} variations measured using the Ca^{2+}-sensitive fluorescent ratiometric indicator fura-2AM (Invitrogen, San Giuliano Milanese, Italy). This panel shows the temporal analysis obtained both for a neuronal cell (Black line) and a glial cell (Red line). The first period of 2min shows that the intensity of light for fluorescent excitation of fura-2 (340/380nm)

is too low for activating caged glutamate.

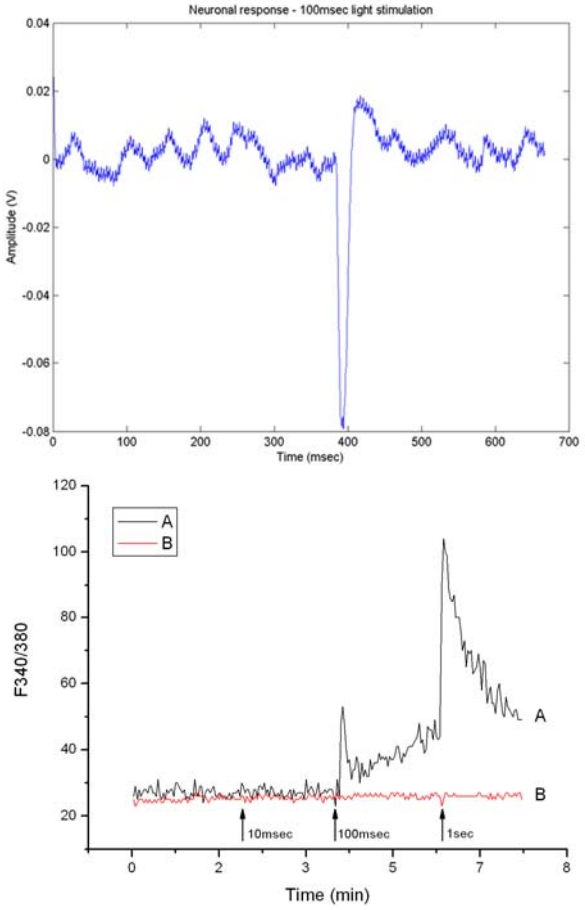

Figure 2: (Top panel) Neuronal spike evoked with a single UV light stimulus. (Bottom panel) the Ca_i^{2+} variations measured using the Ca^{2+}-sensitive fluorescent ratiometric indicator fura-2, evoked by three light pulse of different duration (10msec, 100msec, 1sec).

Figure 2 also shows that the elevation in Ca^{2+} recorded in the experiment was the effect of the optical stimulation and not a pure artefact of such stimulus. This appears from the tracking activity of a glial cell close to the neuron where no change was recorded (red trace in Fig. 2). This experiment shows that the optimal pulse duration for this set-up is about 100msec. Brief light pulses (10msec) could be unable to stimulate neuronal cells and too long pulses (1sec) could produce a saturated response of the neuron that could be not easy to recover.

Recently, a new device for neuronal cultures analysis, PhotoMEA, has been proposed (Italian patent pending number MI2005A000114) [3]. First prototype of PhotoMEA proposes a solution for the integration of the two optical methods by avoiding the use of both microscopes. The innovative concept 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 (Figure 3).

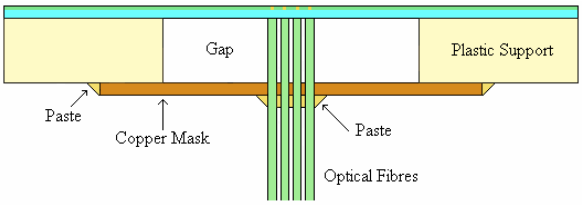

Figure 3: Prototype allows comparing the recordings of the PhotoMEA optical system with those of standard MEA system, assumed as validating reference. This is possible because, instead of the slide, we have used a MEA under which optical fibres are glued. Besides, in order to get an optimal check of the system's functioning, every optical fibre is glued exactly next to a MEA electrode, in such a way as to guarantee a high-correspondence between recorded signals.

Actual work is focused on the development of an improved version of the PhotoMEA device. In fact, the new biosensor will be based on innovative optical microtechnologies, such as integrated waveguides for the stimulation and detectors for the imaging, that combine in a single chip both local light stimulations and high-spatial resolution fluorescent optical recordings over the entire culture.

The main advantages of PhotoMEA arise from the possibility to avoid electrical stimulations and use light to achieve precise temporal and spatial activation of different regions of a neuronal network, which can be sized to single neurons or a part of it. Moreover, optical recordings allow the possibility to monitor at the same time the activity of the sub-neuronal compartments, the neuronal cell and the whole network with high-spatial resolution.

The merging of micro-scale and large-scale approach offers a unique opportunity to follow the effects of local neuronal pathways on neuronal network activity, for instance during pharmacological and toxicological treatments.

References

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[3] Patent pending number MI2005A000114, assigned to Politecnico di Milano, Technical University.