NEURONS AS BIOANTENNAS

R. Pizzi, D. Rossetti, G. Cino and A. Vescovi*
Living Networks Lab
Department of Information Technologies - University of Milan
*Stem Cells Research Institute - DIBIT S.Raffaele Milan

Our group started three years ago researches on the interface between electronics and human neural cells . During the experiments several anomalies in the electrical signals coming from neurons have been found out, that could suggest non-classic origin.
Many theoretical models in the past decade have been proposed on this issue [HAM96,MAT99,HAG02,STA93], in order to clarify the advanced brain functionalities that have not a full neuropysiological explanation yet.
However, none of these models had up to now a significant experimental verification.

Our researches began with a tentative experimental set-up constituted by two networks of human neural stem cells cultured on separated MEAs (Micro Electrode Arrays). One of the MEAs was stimulated with a laser emission wheras the other MEA, separated by several centimeters, was shielded bot electrically and optically by means of a thick aluminium cap.

The first results showed very high values of crosscorrelation and frequency coherence during the laser impulse [PIZ04]; These results encouraged us to continue the experimentations.

During the last three years we prepared and carried out several other experiments, improving both the hardware detection and controlling system and the shielding techniques. We also took the maximum care in preparing the experimental protocols, devoted to exclude possible biases and alternative hypotheses.

We present below the results of our last experiment, that on one side replicate and confirm the previous ones, on the other side address towards a plausible physical model of the observed phenomenon.

Materials and Methods

Our system is constituted by two or more networks of neural stem cells cultured on a set of 2□ microelectrodes . From the neurons, adhering to the microelectrodes, an electrical signal is collected by means of a shielded cable. The signal enters a high impedance amplifier, then passes through two 50 Hz Notch filters , in order to eliminate a possible presence of the power supply disturbances.

After the filters the signal is further amplified and by means of isolate couplers (ISO) is transferred to a National Instrument acquisition card, installed on a shielded PC that manages and records the signals.

All the circuit is completely isolated and closed into a thick metallic box connected to the ground.

In order to avoid that possible spurious signals influence the amplification system, we fully isolated the whole preamplifier circuit.

The input (acquisition) and output (after amplification) analog signals are completely isolated by means of special Texas Instruments electronic circuits, that avoid any possible coupling between external and internal circuits.

The digital signals are also completely uncoupled from the internal circuit by means of photocouplers.

In this manner the electrodes connected to the cells are by no means in touch with the external measure/control environment.

Four rechargeable Lithium batteries provide power supply to all the circuits, ensuring in this way a “clean” tension.

The laser power supply circuit, that is completely separated from the controller, is supplied by a d.c. 8 V negative stabilizer.

All the MEA basins (blu circles) are put inside of a brass Faraday cage with a 1mm grid (orange square in fig. 1).

One of the basins is covered by a thick black plastic box wrapped with a double aluminium foil (red circle). We also closed it inside a thick opaque cardboard box (3.5 mm) as a further optical shielding.

One of the basins is left inside the brass Faraday cage but free to directly receive light impulses coming from a laser diode (670 nm) put outside the Faraday cage.

The laser diode (blu arrow) sends to the first basin a random set of 2.5 sec bursts of 1 ms pulses .

In the described experiments we have three basins, one is filled with neurons (cultured in matrigel and culture liquid) whereas two control basins are filled one with matrigel , the other with culture liquid.

All the basins are in turn put under the laser impulse or under the plastic/aluminium/cardboard shielding.

In a second phase, maintaining the previous shielding procedures, the laser emission is covered by a double aluminium foil. In a further phase the laser is put more than 1 meter far from the Faraday cage and the emission is directed to a direction opposite to the basins.

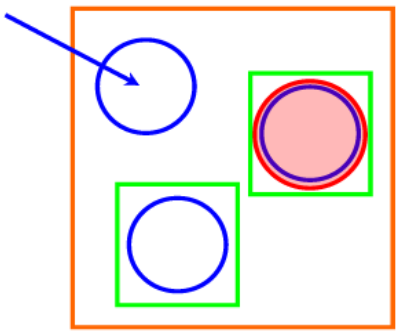

fig. 1

Results

In all the above described experimental phases the laser pulse (red channel) arouses a simultaneous spike in the neural basin (gray and lilac channels) (see fig. 2). In the figures the x values are expressed in seconds, the y valus in mV.
The spike, that for lasting and amplitude is characteristic of the TEP (transducted extracellular potential) action potentials measured with the MEA procedure, is absent in the channels derived from the control basins.

In the second and third phase the neural spike is also present, although attenuated (fig. 3).

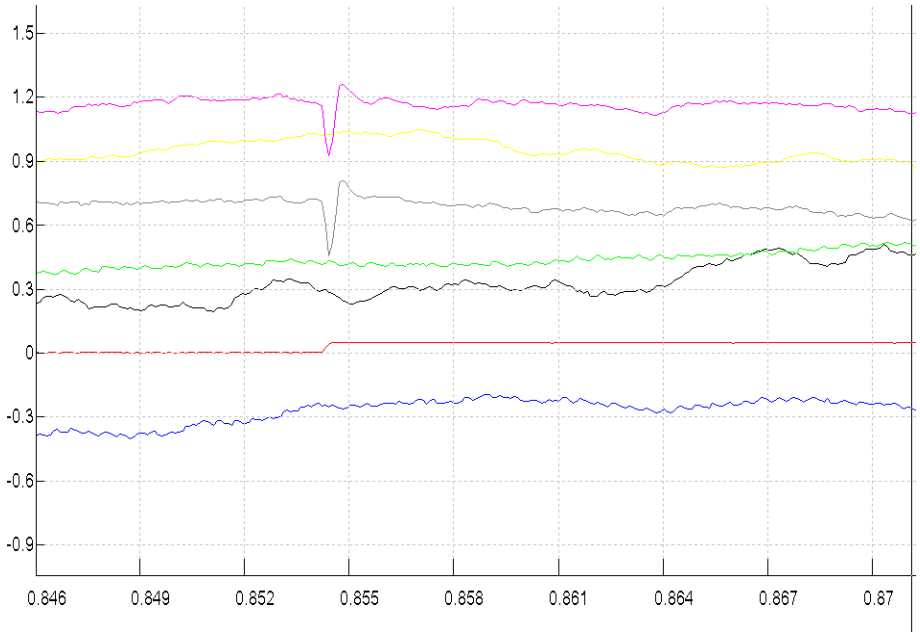

fig. 2

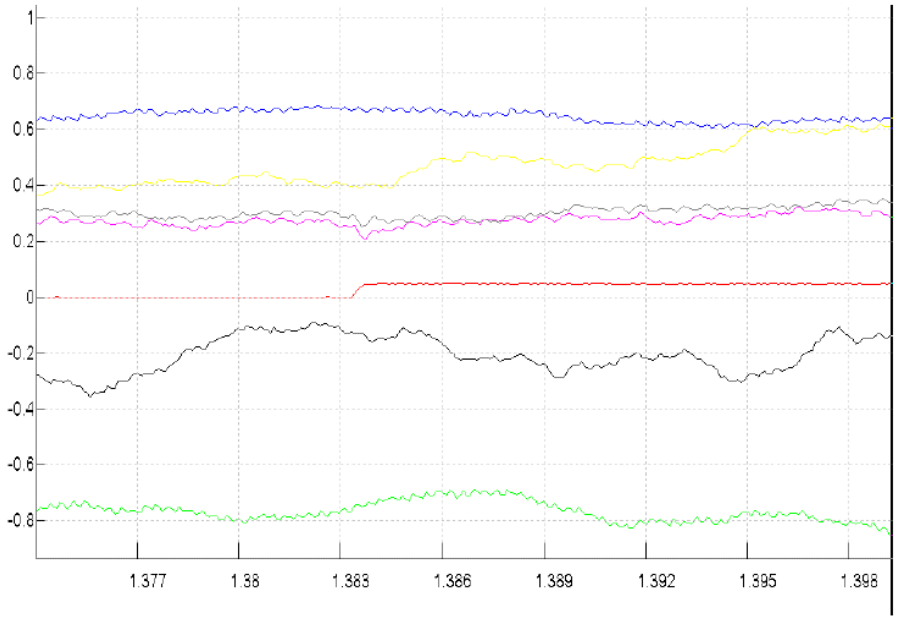

fig. 3

Discussion

Several test have been performed and recorded to verify the effectiveness of the methods adopted during the design and implementation phase in order to isolate the hardware components .

The noise measures have been performed closing the inputs of all the channels on 120 Kohm resistances . The noise measured at the output of each channel has been always lower than 2 mV.

Other bench tests have been performed in order to check that the preamplifier circuit did not pick up inductor-generated peaks .

The Notch filters have been measured using a sweep generator calibrated between 30 and 80 Hz, with a 10 mV output amplitude . We measured at the output an attenuation of –20dB at the 50Hz+/- 1 Hz frequency.

Cross-talk tests have been performed injecting a signal with variable frequencies from 100 Hz to 500 Hz and amplitude of 1, 5, 30 and 80 mV into each channel, measuring the outputs on all the channels.

On the basis of the experimental findings and of the bench tests it is possible to state that the spikes appearing in the neural signals simultaneously with the laser impulse are not due to interferences.

On the other hand, by definition, interferences should be present simultaneously on all the channels. Moreover, the spikes in the neural channels have not the characteristic features of induction-generated peaks. Instead, they have the known shape of a TEP-measured action potential.

Finally, each inductive phenomenon should be present both when a circuit is turned on, and when the same circuit in turned off: whereas we verified the neural reaction only when the laser emission is activated, but no reaction was shown every time the laser was turned off.

Thus the neural spikes seem, by exclusion, due to the action of the photons coming from the laser, even though each one of the optical shielding procedures, in good dark conditions, was enough to prevent the naked-eye perception of the laser emission.

A verification of the quality of the optical shielding have been performed using a WATEC super-high sensitivity camera (0.0003 lux).

The laser emission have been shielded using the four shielding procedures altogether: aluminium foils around the laser, cardboard wrapping, plastics cover with aluminium foil , and laser emission opposite to the videocamera direction.

The experiment has been carried out in a dark room, where a used-to-dark eye did not perceive any luminescence coming from the shielded laser emission.

However, each time the laser was turned on, the camera showed a weak but neat luminescence. As the human used-to-dark eye can perceive around a tenth of photons, and each one of the four shielding procedures was enough to prevent the eye from perceiving any light, this experiment showed the emission of <<10 photons even with the maximum optical shielding conditions.

Conclusions

The above presented experimental results, supported by all the experimental results recorded by our group during the past three years, show a very high sensitivity of neurons to the visible light radiation. This sensitivity seems to depend on the characteristic of light: in fact, in previous experiments where the effects of laser and led light (non polarized, 430 nm) were compared, even

without any kind of shielding over the MEAs the neurons did not show any reaction to the led stimulation. (see fig. 4). The led was activated by the same circuit as the laser.

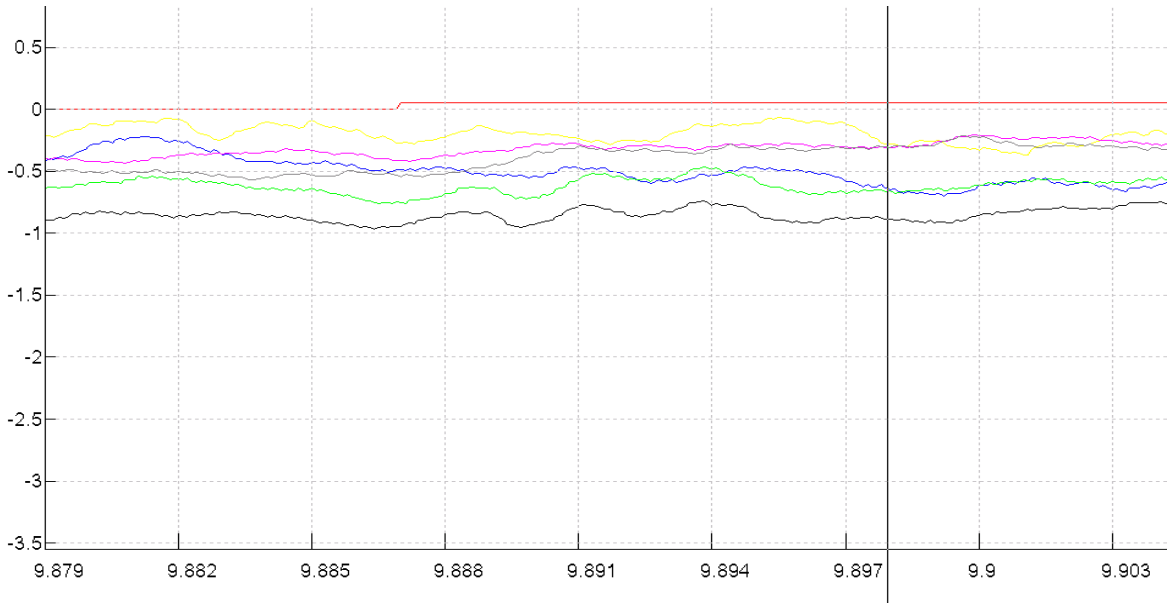

Fig. 4 a - Led stimulation, 6 neural channels. The led channel is the red one.The neural basins are not shielded.

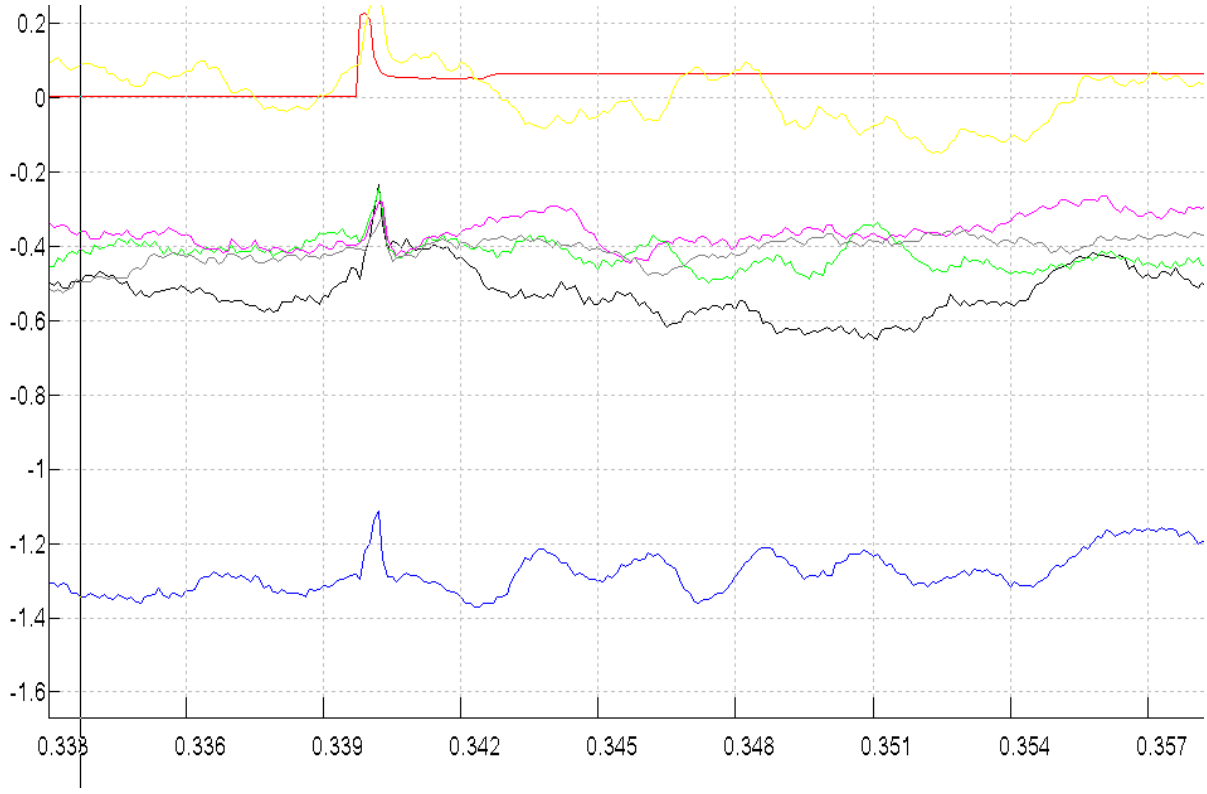

fig. 4b - Laser stimulation during the same experiment: 6 neural channels on three separated basins, one stimulated by the laser, the others shielded. The laser channel is the red one.

Although it was not possible to quantify the exact number of photons that hit the MEAs, the impossibility for the human eye to perceive them implies that their number was less than 10 units.

The reactivity of neurons to very weak light pulses could be due to the presence of microtubules in their cellular structure, for the reasons that we analyze below.

The microtubules, formed by wrapped tubuline molecules, are structurally similar to carbon nanotubes. Actually both structures are empty cilinders, the diameter of a microtubule is around 20 nm, its length is around some micron, whereas the carbon nanotubes dimensions can be similar or less than the microtubules ones.

Interesting optical , electrical and quantum properties of carbon nanotubes are known [GAO98,KAT99,LOV03,AND05]: in particular , recently it was found out [WAN04] that carbon nanotubes behave like antennas for the extremely high frequencies of the visible light radiation. Actually their tubular structure makes them ideal candidates to constitute cavity antennas, and their dimensions are suitable for receiving extra-high frequencies.

The amplification of the signal capted by neurons also requires an explanation.

Many hypotheses already present in the literature could be taken into account, in particular superradiance [DIC54,DEL88,HAM96] and stochastic resonance [DYK98,MOS95,REI04].

But an hypothesis that applies more easily to our experimental situation is that the microantennas constituted by microtubules can amplify the signal generated as a single antenna s they are aligned in schematically parallel configurations, creating an array of antennas that amplifies the signal.

It is also known that both microtubules and nanotubes behave as oscillators [SEP99,MAN94], and this could make them superreactive receivers able to amplify the signal

In conclusion, the above described work presents an experimental evidence of reactivity of neurons to ultra-weak light pulses.

The reproducibility of phenomenon has already been verified in a series of experiments during the past three years.

A hypothesis that must be verified is that the shown property extends to all the cellular and extracellular structures endowed with cytoskeleton or fiber structures, i.e. containing microtubules or microwires. The energy exchange in form of photons could imply several cellular functionalities that in the past have been hypothesized but never shown incontrovertibly [POP77, FIS89].

It is worth citing to this purpose that the ability for nanotubes to behave like antennas for light radiation is currently studied because of its possible technological exploitation both for the demodulation of optical signal and to make more effective the sunlight energy conversion : thus information processing and energy conversion are considered a possible consequence of the nanoantennas properties.

Acknowledgements

We are indebted to Prof. G. Degli Antoni (Dept of Information Technologies Un. of Milan) for his encouragement and support, to Prof. M.G.A. Paris (Dept of Physics Un. of Milan) and Prof. W. Baer (Naval Postgraduate School, Monterey) for their important suggestions, and to Dr. Daniela Marino (SCRI-DIBIT S. Raffaele Milan) for his valuable biological work. This research was partially supported by ST Microelectronics.

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