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Evidence of neuron sensitivity to ultraweak electromagnetic fields

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Abstract

The paper describes an experiment carried out activating a laser source in the near of a culture of neural stem cells. The experiment was performed after three years of researches where the experimental conditions were made more and more controlled. The neural signals show spikes in correspondence of the laser pulses, even under strict electromagnetic and optical shielding. No electrical reactions are shown by the control basins. Experimental set-up and bench-tests on the instrumentation used in the experiment are described in detail. A discussion of the results is presented.

1. Introduction

Our group started three years ago researches on wetware systems, interfacing electronics and human neural cells (Pizzi, 2004a; Pizzi, 2006a; Pizzi, 2006b). During the experiments several anomalies in the electrical signals recorded from separated basins of neurons have been found out, that encouraged us to investigate the source of the anomalies by means of a series of dedicated experiments.
To this purpose we arranged 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 electrical and laser emissions, wheras the other MEA, separated by several centimeters, was shielded electrically by a Faraday cage 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 (Pizzi, 2004b). During the last three years we prepared and carried out several other experiments, improving both the hardware/software 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. The paper describes our latest experiment, that replicates and confirms the findings showed in the previous ones.

2. Materials and Methods

2.1 The biological system

Our biological system is constituted by two or more networks of neural stem cells cultured on a set of microelectrode arrays (MEAs). Each MEA is constituted by a glass cylindrical chamber endowed with 64 ITO (Indium Tin Oxide)-Platinum electrodes. The microelectrode size is 50 μ, the interpolar distance 150 μ. Our neurons are cultured starting from human neural stem cells extracted by a human embryo. The culture method adopted in our experiments has been well established in time by Prof. Angelo L. Vescovi's team (Vescovi, 2002).
Cells are plated at a density of 3500 cells/cm2 in suspension in a chemically defined, serum-free medium containing 20 ng/mL of human recombinant epidermal growth factor (EGF) and 10 ng/mL of fibroblast growth factor (FGF-2). After 3-5 days the cultures are harvested and the cells are mechanically dissociated and replated under the same conditions. Our experiments are performed 4

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weeks after seeding our neural stem cells onto MEA surfaces previously coated with a matrigel adhesive substrate, composed by mouse laminin (2 ng/ml) , human fibronectin (2 ng/ml) and other proteins.

2.2 The hardware system

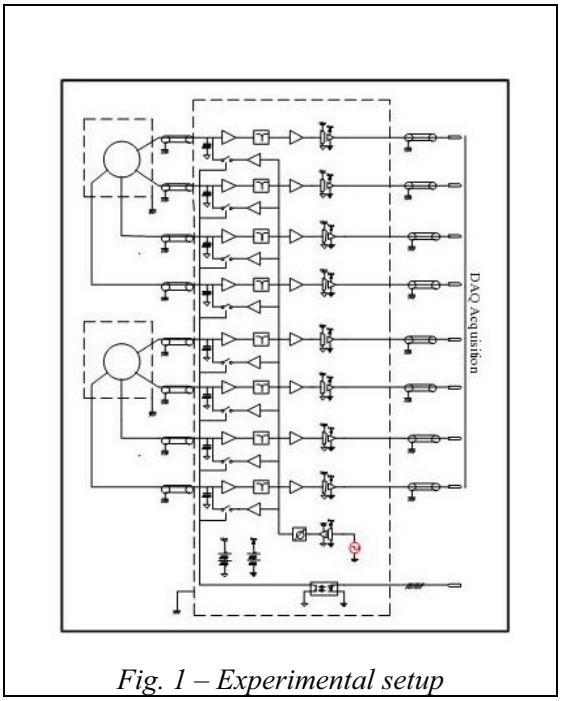

Fig. 1 – Experimental setup

The schema of our experimental set-up is depicted in Fig. 1. The MEAs (a) are put inside a plexyglass incubator. Heating resistances realized by coal have been put inside the incubator. They are d.c. supplied and ensure steady temperature in the incubator, allowing a longer survival of cells. The whole plexyglass incubator is completely wrapped by a brass 1 mm net that is connected to the ground and constitutes an effective Faraday cage. The MEAs rest on separated copper boards. From the neurons, adhering to the microelectrodes, bioelectrical signals are collected at a frequency of 10 kHz by means of shielded cables (b). Small capacitors (~18 pF) (c) have been placed on the inputs in order to avoid instabilities in the amplifiers and reject RF frequencies. Then the signals enter a high impedance custom preamplifier (d). The preamplifier, provided with 8 analog acquisition channels, has been designed to interface the MEAs with the DAQ acquisition card (e) taking into account the particular needs of this kind of experiment. Thus we developed the system in such a way as to obtain the maximum amplification avoiding at the same time any kind of spurious signal coming both from the outside and from the inside of the circuit. After the preamplifier the signals pass through two 50 Hz Notch filters (f), in order to eliminate a possible presence of power supply disturbances. Then the signals enter a high impedance custom preamplifier (d). The preamplifier, provided with 8 analogue acquisition channels, has been designed to interface the MEAs with the DAQ acquisition card (e) taking into account the particular needs of this kind of experiment. Thus we developed the system in such a way as to obtain the maximum amplification avoiding at the same time any kind of spurious signal coming both from the outside and from the inside of the circuit. After the preamplifier the signals pass through two 50 Hz Notch filters (f), in order to eliminate a possible presence of power supply disturbances. After the filters the signals are further amplified and are transferred to a National Instrument 16 bitPCI-6036E acquisition card after a complete isolation by means of special Texas Instruments (ISO124) electronic circuits (g) , that avoid any possible coupling between external and internal circuits. The ISO124 isolation amplifier uses an input and an output section that are galvanically isolated by means of matched 1pF isolating capacitors built into a plastic package. The input section is dutycycle modulated and transmitted digitally across the barrier. The output section receives the modulated signals, converts them back to an analogue voltage and removes the ripple component inherent in the demodulation. In order to avoid that possible spurious signals influence the amplification system, all the electronic circuits are completely isolated and closed into a thick metallic box connected to the ground (h) The DAQ acquisition card is installed on a shielded PC that manages and records the signals. The power supply is furnished by rechargeable Lithium inside battery (i). The stimulation is supplied by a visible light 2 mW laser diode, with 630 nm wave length and peak-to-peak modulation of 500 mV. The emission power has been reduced in order to avoid lesions of the cells. The laser circuit, that is completely separated from the above described circuits, is supplied by a d.c. 8 V negative stabilizer. The laser synchronicity signal is generated by a separated continuous voltage circuit and injected in a

channel of the acquisition card after the preamplifier. In this way we can verify the laser activation without interfering with measures.

2.3 The experiment

In our last experiment we used three MEA basins: one basin is filled with neurons (cultured as described), whereas two control basins are filled one with matrigel, the other with culture liquid. All the MEAs are put inside the brass Faraday cage. One of the basins is covered by a thick black plastic box wrapped with a double aluminium foil. The other basins are left inside the brass Faraday cage but free to receive light impulses coming from the above described laser diode, put outside the Faraday cage. The two basins not hit by the laser are also closed inside a thick opaque cardboard box (3.5 mm) as a further optical shielding (Fig. 2 and 3). During the experiment all the basins exchange in turn their positions and shielding conditions. The laser diode sends to the non-covered basin a random set of 1-2.5 sec bursts of 1 ms pulses. In the phase a) all the basins in turn are put first without shielding under the laser pulses, then under the plastic/aluminium/cardboard shielding, with the laser directed to the other basins.

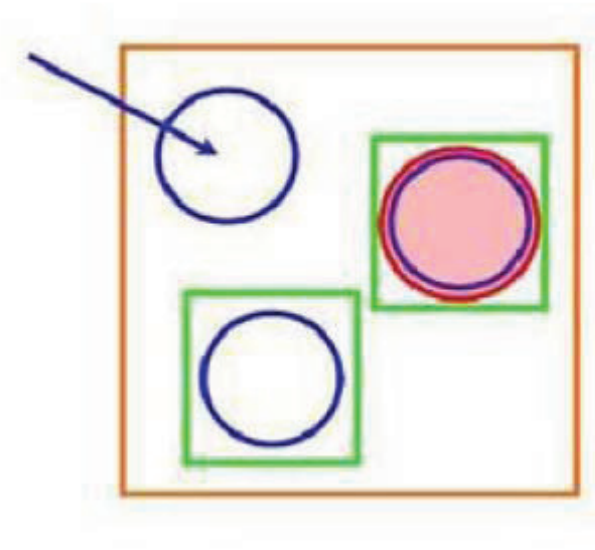

Fig. 2. Schema of the experiment . The three basins are closed into a brass Faraday cage (yellow square). One basin is hit by the laser (blue arrow), one is inserted in a cardboard box (green square), the third one is covered by a thick plastic cap (red circle) wrapped with a double aluminium foil and inserted in a cardboard box.

In the phase b) the phase a) procedures are repeated, maintaining the previous shielding procedures, but the laser emission is further covered by a double aluminium foil. In the phase c) 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.

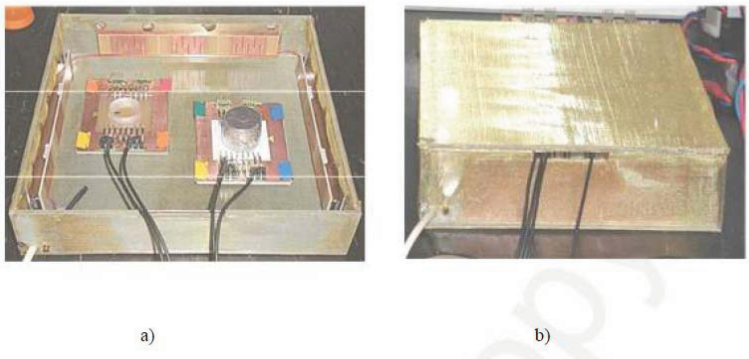

Fig. 3. The Faraday cage: a) open, with two basins , one covered with the optical and electromagnetic plastic/aluminium shielding; b) closed.

3. Results

During several experiments we collected and analysed more than 1 Gb of data. We will examine in more detail the results of our last experiment, whose set-up was described above. In all of the described experimental phases the laser pulse arouses a simultaneous spike in the neural basin (see fig. 4), both when it is left without shielding and receives directly the laser pulse, and when the laser is directed to other basins and the neural basin is electrically and/or optically shielded. In the following figures the x values are expressed in seconds, the y values in V. The amplifier gain is g=20.

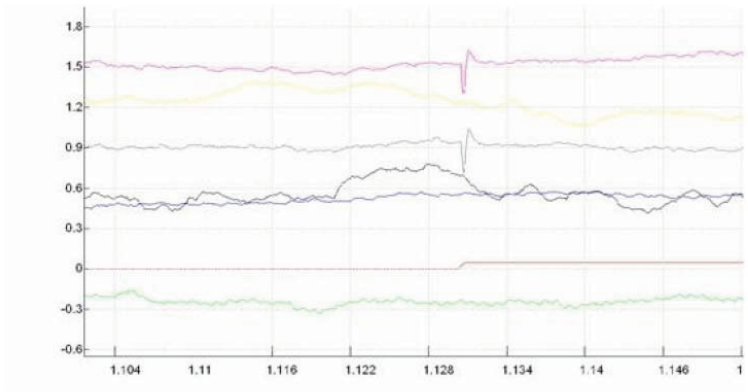

Fig. 4. Phase a) graph of the signals before and during the laser pulse. Red channel: laser gray and lilac channels: neural basin, shielded by brass Faraday cage, plastic/aluminium cap, cardboard box; other channels: control basins, put inside the brass Faraday cage, one free and the other inside a cardboard box.

The spikes, that for lasting and amplitude are characteristic of the TEP (transducted extracellular potential) action potentials measured with the MEA procedure, are always absent in the channels derived from the control basins.

In the b) and c) phases of the experiment 2 the neural spikes are also present, with the same intensity as in phase as), except for some spikes that show an attenuation (Fig. 5, 6, 7). It must be said that after around half an hour from the beginning of the experiment, the delicate stem cell cultures, exposed to an inadequate atmosphere, start a quick degeneration, detach from the electrodes, attenuating their electrical response, and die. As a consequence, the spike attenuation sometimes verified in the b) and c) phases could be due to the beginning of cell detachment from the electrodes. In several experiments performed in the past we compared the effect of the laser pulses with a random LED emission (non polarized, 430 nm) generated by the same circuit with the same random set of bursts. We never found spikes generated during the LED emission, whereas during the same experiments the laser emission gave raise to the usual concomitant spikes in the neural basins.

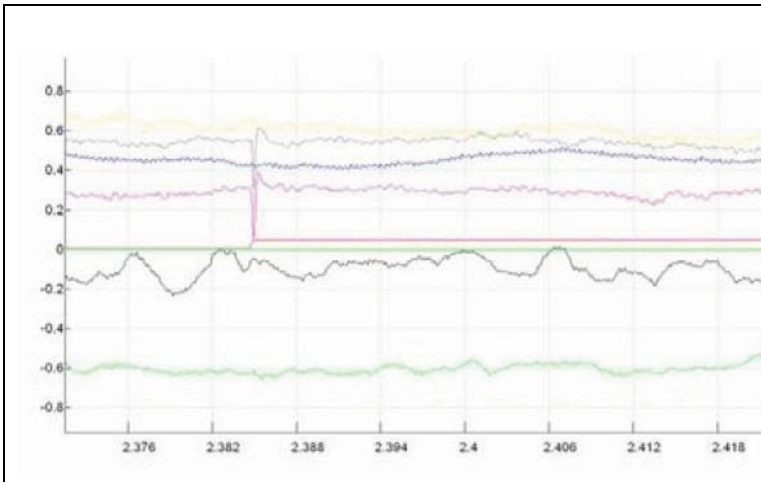

Fig. 5. Phase b) graph of the signals before and during a laser pulse. The laser source is covered with a double aluminium foil. Red channel: laser; gray and lilac channels: neural basin, shielded by brass Faraday cage, plastic/aluminium cap, cardboard box; other channels: control basins, put inside the brass Faraday cage, one free and the other inside a cardboard box.

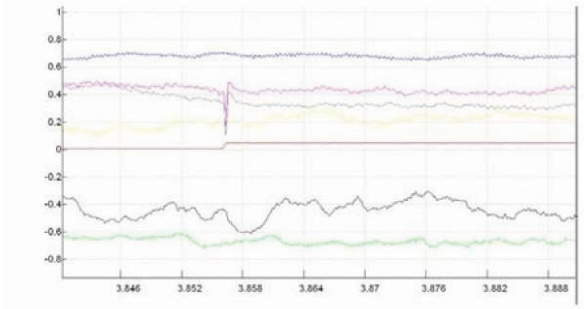

Fig. 6. Phase c): graph of the signals before and during a laser pulse. The laser is more than 1 meter apart and directed to an opposite direction. Red channel: laser; gray and lilac channels: neural basin, shielded by brass Faraday cage, plastic/aluminium cap, cardboard box; other channels: control basins, put inside the brass Faraday cage, one free and the other inside a cardboard box.

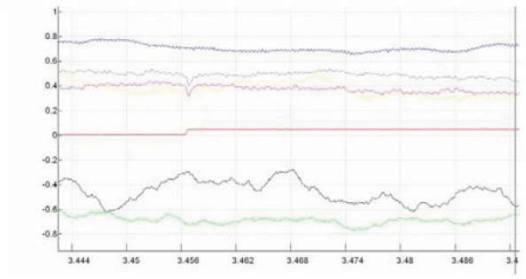

Fig. 7. Phase c): the same as in Fig. 6. The graph shows an example of spike attenuation. This kind of attenuation is sometimes present also in phase b).

4. Discussion

Maximum care was put in testing all the hardware components of our experimental set-up, in order to exclude the presence of artefacts in the recorded signals.

4.1 DAQ and MEA test

The test on the acquisition card was performed by injecting up to 1 V signals on more input channels and verifying possible cross talks on the remaining channels: we verified that the channelto-channel cross talk is < -110 dB (from DC to 100 Hz; up to 10 kΩ source resistance). We also generated high current sparks to simulate spikes and check possible propagation to other channels. All the test completely excluded possible cross talks. With the same method we also tested the insulation of the MEA electrodes: the electrode-to-electrode cross talk is < - 80 dB (from DC to 1000 Hz; up to 10 kΩ source resistance). On the other hand the MEAs have a glass support that ensures perfect insulation, and the distance between wires in the MEAs are widely dimensioned to avoid mutual capacitance.

4.2 Preamplifier test

Cross-talk preamplifier tests were 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. 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 were performed in order to check that the preamplifier circuit did not pick up inductor-generated peaks. We generated high current sparks as in the DAQ test. No interferences were shown. The load resistors were chosen inside the range of resistivity offered by both the neuron and the control basins. 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

4.3 Shielding test

In order to verify the quality of the shielding systems used in the experiments we introduced an antenna inside the brass Faraday cage. The antenna has been connected to a spectrum analyzer to verify the presence of possible frequencies in the range 100 KHz -3,5 GHz. The antenna has been also connected to an oscilloscope to verify the presence of frequencies in the range 0 −100 KHz. We put the laser device in contact with the brass cage and activated it several times. The instruments did not detect any activity. On the basis of the experimental findings and of the bench tests it is possible to affirm that the spikes appearing in the neural signals simultaneously with the laser impulse are not due to interferences or cross-talk. On the other hand, by definition, interferences should be present simultaneously on all the channels and be identical in shape and amplitude, whereas in the presented experiment the spikes are present just in the neural channels and have not the characteristic features of induction-generated peaks. Moreover, they neither resemble the laser synchronicity signal and have a higher amplitude. In particular , they have the known shape of a TEP-measured action potential. Finally, we verified the neural reaction only when the laser emission is activated, but no reaction was shown every time the laser was turned off (Fig. 9). We considered the possibility that the neural spikes could be due to the action of the photons constituting the laser beam. In a specific test we verified that each one of the four optical shielding systems (double aluminium foil, dark plastic box, cardboard box, further double aluminium foil on the laser source) , in a dark room and used-to-dark eyes, was enough to prevent the naked-eye perception of the laser emission. A further verification of the quality of the optical shielding has been performed using a WATEC super-high sensitivity camera (3 x 10^{-4} lux). The laser emission was shielded using double aluminium foil, dark plastic box, and cardboard box altogether. The test was carried out in a dark room, where a used-to-dark eye did not perceive any luminescence coming from the shielded laser emission. Each time the laser was turned on, the camera showed no luminescence. This test cannot completely exclude that one or few polarized photons could hit the shielded neurons during the described experiment, however it shows that this event was quite unlikely. It should be taken into account that in the phases 2b) and 2c), with a further optical shielding on the laser or putting it at a larger distance and directed to an opposite direction, the phenomenon presented itself nearly always without attenuation.

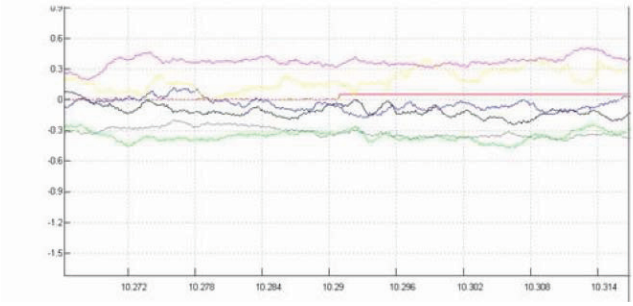

Fig 8. Typical graph of the signals before and during a LED pulse. Red channel: LED; gray and lilac channels: optically/electrically shielded neural basin; yellow and green channels: non-shielded neural basin; other channels: non-shielded control basin.

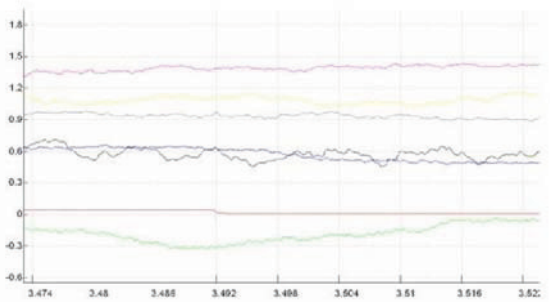

Fig. 9. Graph of the signals during and after the laser pulse. Red channel: laser; gray and lilac channels: neural basin, shielded by brass Faraday cage, plastic/aluminium cap, cardboard box; other channels: control basins, put inside the brass Faraday cage, one free and the other inside a cardboard box.

4.4 Evidence of a neural reaction to ultraweak electromagnetic field

During one of the experiments we substituted the laser with a dummy load in order to simulate the current absorption equivalent to the one generated by the laser and we found the same peak was present. On the other hand, in the experiments where the effects of laser and LED light (activated by the same circuit as the laser) were compared, even without any kind of shielding over the MEAs the neurons did not show any reaction to the LED stimulation. This could be explained by the fact the LED current absorption is far lower than the laser one. As a demonstration, no peaks were present using a dummy load equivalent to the LED absorption. We concluded that the phenomenon should not be due to the laser itself but to an electromagnetic field coming from the laser supply circuit. Neurons appear to receive and amplify a signal whose value through the air , measured with a filar antenna (suitable to detect electromagnetic frequencies), and before reaching the Faraday cage, is under 2 mV (sensitivity threshold of our oscilloscope). The value of the electric field under the double Faraday cage is under the sensitivity of our instrumentation but is estimated to be at least one order of magnitude less. It must be stressed that in order to cause an action potential (spike), a neuron needs to be stimulated inside the cell with a 30 mV pulse. In order to evaluate the intensity of the magnetic field we used a high-sensitive Gaussmeter, whose sensitivity threshold is around 70 μG. The laser supply circuit, when turned on, generates in the near of the Gaussmeter around 0.002 G, but when moving away the Gaussmeter beyond 30 cm, the field intensity gets under the Gaussmeter sensitivity. During the experiments the laser circuit was at least 50 cm far. We could not assess the intensity of the magnetic field (if any) received by the neurons during the experiments because it is so weak that it gets under both the oscilloscope and the Gaussmeter sensitivity.

5. Conclusions

In summary, in the described experiments we observed the following facts:

- The described effect is visible only in neural MEAs
- The matrigel MEAs don't show the effect
- The culture liquid MEAs don't show the effect
- The optical shielding does not inhibit the effect
- The electromagnetic shielding does not inhibit the effect
- The distance from the laser source (up to 1.5 meters) does not inhibit the effect
- The LED light does not induce the effect.
- Separated neural MEAs show similar spectra

Looking at the whole series of experiments, we note that we changed many times completely the experimental set-up: biological lab, PC, Acquisition card, MEAs model, Hardware controller, Faraday cage. Nonetheless, we always verified the same reactions of the neural MEAs to the laser pulses. After substituting the acquisition card with a more powerful one, a series of spikes appeared simultaneous to the laser pulses, and this effect appeared hundreds of times, it is perfectly repeatable and happens every time the laser circuit is turned on.

The above presented results have been supported by other experimental results recorded by our group during three years. The observed phenomena repeated themselves despite robust multiple optical and electromagnetic shielding system and several constant controls devoted to check the existence of possible crosstalks or interferences. It must be stressed that the MEA control circuit and the activation circuit are completely separated, the MEA basins are connected to the ground, their shape

is not suitable to act as antenna and the spikes observed in the neural basin are never present in the other control basins. Though the exact mechanism for the observed neural response has not been identified, we can at the moment hypothesize that neurons are active receiving elements, acting as antennas for extremely weak electromagnetic fields. Several approaches involving quantum theory have been proposed in the past decade in order to clarify neural functionalities that have not a full neurophysiological explanation yet (Hameroff, 1987; Stapp, 1993; Penrose, 1994; Hameroff, 1996; Josephson, 1991; Matsuno, 1999; Hagan, 2002, Hameroff, 1998; Pribram, 1999, Bohm, 1990; Hameroff, 2003; Thaheld, 2005). A consistent amount of authors directed their attention to the cellular structure of the neuron, in particular to the microtubules as possible actors of non-classical phenomena inside the cell. However, none of these models had up to now a significant experimental verification. 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 lower than the microtubules ones. Interesting optical, electrical and quantum properties of carbon nanotubes are known (Gao, 1998; Katura, 1999; Bachtold, 1999; Lovett, 2003; Andrews, 2005): in particular , recently it was found out (Wang, 2004) 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 captured by neurons in our experiments also requires an explanation. Stochastic resonance (Moss, 2005; Dykman, 1998; Reinker, 2004) could be taken in account. A simpler hypothesis is that the microantennas constituted by microtubules could amplify the signal generated as a single antenna as 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 (Sept, 1999; Marx, 1994; Insinna, 1992), and this could make them superreactive receivers able to amplify the signal. Other hypotheses on this issue are already present in the literature, in particular superradiance (Dicke, 1954, Del Giudice, 1988, Hameroff, 1996; Meier, 1997) could amplify specific frequencies (in particular the Fröhlich frequencies (Fröhlich, 1986) received by the microtubules. Quantum electrodynamical coherence and vector potential (Aharonov, 1959; Jibu, 1994; Del Giudice, 1985; Del Giudice, 2006), that extends beyond the electromagnetic field boundaries, have been hypothesized to play a role in the biological structures. The described neural reactivity may be due to the presence of microtubules in their cellular structure. Microtubules are structurally similar to carbon nanotubes, whose tubular shape makes them natural cavity antennas. However, the nature of the above presented experimental results requires extreme caution in drawing hypotheses and consequent models, and needs further investigation and confirmation by other research groups. In the next future our group aims to replicate the above described experiments using a different source of ultraweak electromagnetic field, varying distances between source and basins, in such a way as to identify general laws that could rule the phenomenon under investigation: in particular, its dependence from the distance , the intensity and the frequency of the electromagnetic source.

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