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Prototyping a memristive-based device to analyze neuronal excitability

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ABSTRACT

Many efforts have been spent in the last decade for the development of nanoscale synaptic devices integrated into neuromorphic circuits, trying to emulate the behavior of natural synapses. The study of brain properties with the standard approaches based on biocompatible electrodes coupled to conventional electronics, however, presents strong limitations, which in turn could be overcame by the in-situ growth of neuronal networks coupled to memristive devices. To meet this challenging task, here two different chips were designed and fabricated for culturing neuronal cells and sensing their electrophysiological activity. The first chip was designed to be connected to an external memristor, while the second chip was coated with TiO_2 films owning memristive properties. The biocompatibility of chips was preliminary analyzed by culturing the hybrid motor-neuron cell line NSC-34 and by measuring the electrical activity of cells interfacing the chip with a standard patch-clamp setup. Next, neurons were seeded on chips and their activity measured with the same setup. For both cell types total current and voltage responses were evoked and recorded with optimal results with no breakdowns. In addition, an external stimulation was applied to cells through chip electrodes, being effective and causing no damage or pitfalls to the cells. Finally, the whole bio-hybrid system, i.e. the chip interconnected with a commercial memristor, was tested with promising results. Spontaneous electrical activity of neurons grown on the chip was indeed present and this signal was collected and sent to the memristor, changing its state. Taken together, we demonstrated the ability of memristor to work with a synaptic/plastic response together with natural systems, opening the way for the further implementation of basic computing elements able to perform both storage and processing of data, as in natural neurons.

1. Introduction

The field of information technologies as well as neurosciences developed rapidly in the last years, demanding among others more performant tools for data processing. In particular, tools able to emulate human brain functionalities, effectiveness and capacity would add great value to both the study and the applications of new electronic and computing technologies. The study of the interface between neuronal cells and artificial systems has therefore rapidly grown, especially those focused on the development of neuro-bio-inspired electronics systems based on elements mimicking the function of components of the nervous system. Recent works on neural networks [1,2] attempt to build more efficient elements which partially mimic nature. A novel computational architecture enabling adaptations and learning at the level of hardware is indeed highly desirable. Systems exploiting this architecture can be founded on a basic computing element that performs both storage and processing of data, as in natural neurons – the reason why this approach can be termed “neuro-morphic”. Fundamental bricks

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are memristive devices, whose existence was theorized in 1971 as a new two-terminal circuit element called “memristor” from the contraction of the terms memory and resistor [3–5]. Memristors are indeed resistors whose present conduction state depends on their history or, as for our study, on the voltage that has previously been applied to them. Such devices are prepared in a more or less conductive state, resulting in changing configurations of either preferential or disadvantageous pathways within an electrical network.

Different materials have been studied for the preparation of memristive devices, including inorganic materials and even crystals [6]. One of the firstly studied inorganic material is based on titanium: a titanium dioxide (TiO_2) film sandwiched between two platinum electrodes was utilized by Strukov [4] to model a memristive behavior and the electrical behavior of TiO_2 films was then deeply studied [7,8]. Moreover, memristors offer a solution as an ultrahigh-density memory layer that can be directly integrated on the processor chip, thus significantly reducing the memory bottleneck and improving the energy efficiency and speed of the system [9]. According to Zidan and colleagues [9], three categories may significantly benefit from memristor developments: on-chip memory and storage, biologically inspired computing and in-memory computing. In the context of biologically inspired computing, memristors can be compared to natural synapses, which are variable weight connections: learning means, physically speaking, repeatedly activating the right connections to reinforce them over the wrong ones.

The functional characterization of memristor-based bio-hybrids, in which artificial devices are interfaced with living systems, has rapidly grown in last years, above all to determine the parameters required to reproduce or mimic neuromorphic architectures [10]. In fact, the functional activity between neuronal and non-cellular models adhering to organic and inorganic memristive surfaces has been successfully achieved by means of advanced electrophysiology and vibrational spectroscopy. In particular, bio-hybrids composed by organic memristors like PANI and PEDOT and neuronal and neuronal-like cells have been studied and proposed as reliable and putatively suitable for further application in integrated neural systems [11–13]. Above all applications, those in which extrinsic (and/or artificial) modulation of synaptic circuitries is required to afford to the neuronal cross-talk properties of learning, memory and computation have great potential [14]. Inorganic TiO_2 biohybrids, in particular those supporting the growth of murine cortical neurons, have been recently analyzed and represent the seminal study for the current work [15].

Here, two different chips were designed and fabricated for culturing neuronal cells and sensing their electrophysiological activity. Two chips, one connected with an external memristor and one coated with TiO_2, were tested with neurons grown directly on the devices. The electrophysiological activity was measured with a dedicated setup, successfully testing the memristive properties of the new device. Our results might open the way towards the realization of an innovative bio-electronic device exploiting the adaptive behavior of memristor networks to interact with the adaptive abilities of neurons. Moreover, this work can be considered as a proof of the ability of memristors to elicit a synaptic/plastic response together with natural systems (i.e. neuronal cells).

2. Materials and methods

2.1. Materials

DMEM, glutamine, penicillin/streptomycin, poly-D-lysine, Cytosine -D-arabinofuranoside, DNase, DPBS, salts and all reagents were purchased from Sigma-Aldrich. Neurobasal medium and B27 supplement were purchased from Life Thecnologies (USA), while papain solution for cells dissociation was purchased from Worthington Biochemical Corporation (USA).

2.2. Design of chips

2.2.1. Standard chip

The final device used for cell experiments is reported in Fig. 1. In order to obtain a simple coupling with the memristive devices, chips were fabricated in the inner part of a square die of 1.5 cm of side (Fig. 1A). The dimension and the arrangement of the micro electrode array for extracellular recording are reported in Fig. 1B. The electrode dot (90 μm diameter) array was fabricated depositing a 5 nm chromium adhesive layer, a 120 nm gold layer and on the top a 120 nm platinum layer, isolating the guides with a passivating layer ($SiO_2/Si_3N_4/SiO_2$).

The packaged device was assembled bonding a cell culture Petri dish (35 mm diameter, ThermoFisher Scientific), through a PDMS layer, on top of the chip previously mounted on a PCB board. Before bonding, a suitable hole was drilled in the Petri dish, connecting the chip area where cells are seeded and the dish used as a reservoir for cell culture medium. The final device is shown in Fig. S1A.

2.2.2. Chip coated with TiO_x film

A 10 mm × 10 mm quartz substrate was used for the deposition by evaporation of 32 Cr/Au/Pt dots (300 μm diameter). Each dot had a passivated gold guide that permits the collection of the signals far from the main electrode area, avoiding formation of unwanted electrical contacts. A 50 nm TiO_2 thin film was then grown by pulsed microplasma cluster source (PMCS) on the substrate, coating part of the patterned circuits, in particular the dots (Fig. 1C), as already described [15]. Briefly, this deposition process occurred in vacuum, based on the ablation of a titanium rod by a pulsed plasma, a process created by the ionization of an inert gas trigged by an electric discharge [16]. After the ablation of the cathode, metallic ions thermalized with gas and achieved clusterization. The mixture of clusters and the inert gas was extracted in vacuum through a nozzle to form a supersonic molecular beam seeded by nanoparticles. Film growth is then achieved by deposition on the substrate, at room temperature. We selected pure helium as inert gas, because it gave the best performances in terms of neuronal cell biocompatibility, as reported in our previous work [15]. The immediate exposition of the deposited titanium thin film to air at room temperature led to transition from metallic phase to metal oxide structure.

In order to achieve the memristive properties, a single memristor was realized on each dot by a specific electroforming procedure using a platinum wire as top electrode (Fig. S2).

The substrate was then mounted on a specific larger chip holder and each memristor/dot was bonded to an external electrode by using a gold wire. As for the standard chip described in the previous paragraph, the device was packaged in order to let the memristor area free for cell culture, while having a cell medium reservoir on the top of cell layer (Fig. S1B).

2.3. Cell culture

The Neuroblastoma Spinal Cord-34 (**NSC-34**) is a mouse motor neuron-like hybrid cell line [17]. NCS-34 cells were grown with standard methods under sterile conditions at 37 °C (humidity, 5% CO_2), using the DMEM culture medium (supplemented with 2 mM glutamine, 10% FBS and 1% of penicillin/streptomycin). Cells seeded on chips were grown at least four days until used. All chips were sterilized with an air plasma for 2 min at 4 mbar and 18 W just before use.

Primary cultures of murine cortical neurons were prepared as previously described [15]. Briefly, cortices were extracted from mouse embryos at stage E15.5. Brain tissues were dissociated with papain solution for 20 min at 37 °C, and then treated with DNase. Dissociated cells were resuspended in pre-warmed plating medium, passed through a 70 m mesh strainer, counted to assess cell density and diluted to

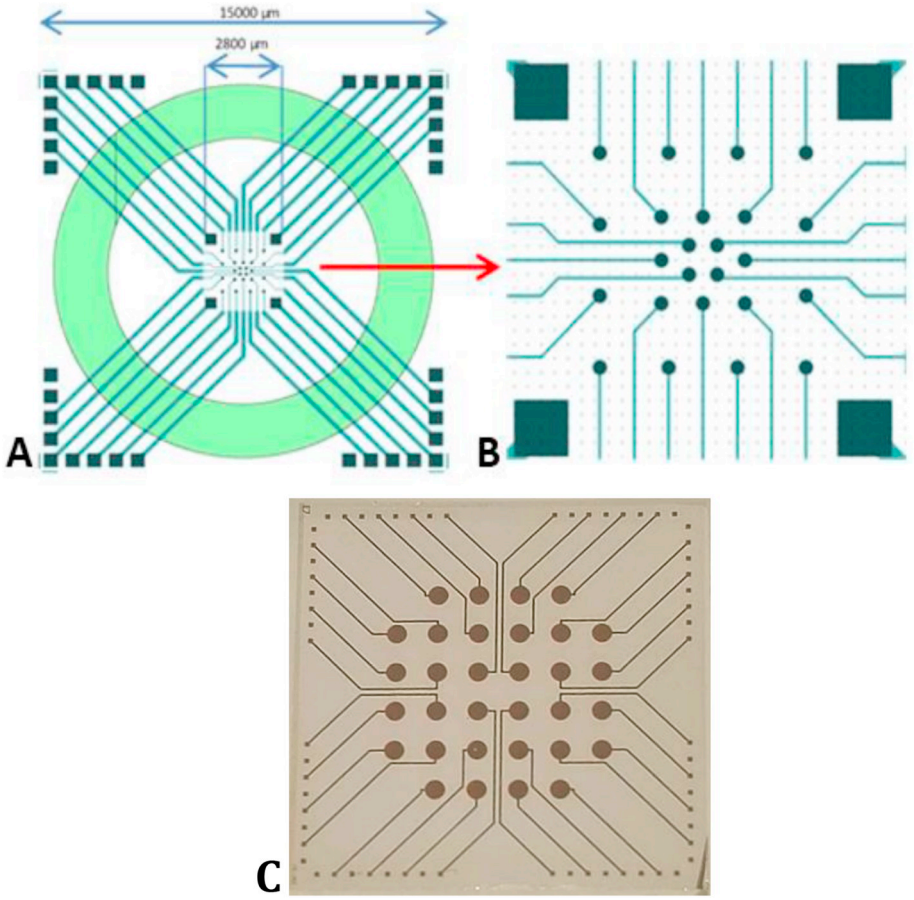

Fig. 1. Standard chip for neuronal cells analysis (A and B). Schema of electrodes and connections of the chip for monitoring electric cell signals (A) and an enlargement of the central chip area (B) are shown. C: chip coated with TiO_x film. 10 mm × 10 mm quartz substrate, on which 32 large dots Cr/Au/Pt are deposited.

3×10^5 cell/mL. Neurons were then seeded on the chips at a final density of 100×10^3 cell/cm^2 and kept in incubator at 37 °C and 5% CO_2. The following day and every 3–4 days, 50% of plating media was replaced with Neurobasal complete medium (i.e. Neurobasal medium plus B27 supplement 1X, 1 M Cytosine -D-arabinofuranoside and 1% of penicillin/streptomycin).

The chips were sterilized with an air plasma and treated with 0.01 mg/mL poly-D-lysine in DPBS buffer for 1 h at 37 °C. The poly-D-lysine solution was successively removed by washing several times with PBS.

Cell imaging was performed with an Olympus CX41 inverted microscope equipped with a digital camera.

2.4. In vitro patch-clamp electrophysiology

The following protocols have been adapted from standards already effectively applied to primary cortical neurons [15], motor neuron-derived MN-1 cells [18] and SH-SY5Y human neuroblastoma cells [11].

Patch-clamp recordings in whole-cell configuration [19] were performed in non-sterile conditions and at room temperature (about 20 °C) on cells cultured as described in the previous paragraph. The chip was allocated and mounted on a NIKON FN1 upright microscope. The generated stimulation signals were fed to the chip. In this configuration, from one to four electrodes can be simultaneously connected to the Sourcemeter output. Typically, a single NSC-34 cell patched was about 30 µm distant from the electrode (Figs. S3A–B and 5) as well as individual cells from primary cortical neurons from mouse (Figs. S3C–D and 9A).

Total currents were evoked preparing solutions with different ion concentrations, prepared using ultrapure water in order to secure the maximum purity and filtered with 0.22 µm filters (Millex filters-GP, non sterilized). Finally, solutions were adjusted to pH 7.4 using NaOH or KOH and stored at 4 °C temperature until used. Solutions were used at room temperature (20 °C) and were changed between patch experiments maintaining them fresh with an automated perfusion system by ALA Instruments. All reagents were from Sigma Aldrich. Composition of bath solution: NaCl 136.4 mM, KCl 5.4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 0.53 mM, Hepes 5.5 mM, Glucose 5.5 mM. Composition of pipette solution: potassium acetate 130 mM, KCl 20 mM, KH_2PO_4 1 mM, $MgCl_2$ 1 mM, Hepes 5 mM, EGTA 1 mM, ATPNa 1 mM, GTPNa 1 mM.

Patch micropipettes were fabricated from GB150-8p (OD 1.5 mm, ID 0.86 mm) borosilicate glass capillaries (Science Product, Hofheim, Germany) using a PIP6 temperature controlled pipette puller (HEKA, Lambrecht/Pfalz, Germany). Pipettes were filled with the use of syringes and plastic tubes and inserted in the holder/headstage bnc-pinned (swi, x0.1/x1, npi, Tamm, Germany). The current at the pipette tip was nulled before stablishing the seal by injecting voltage via the amplifier circuitry as in most of the cases the use of high K^+ concentration gave a positive voltage of around +38.51.5 mV.

Whole-cell configuration was obtained by standard procedures accessing to cell interior after the rupture of the patch seal (electric resistance > 1 GΩ). In all experiments, the holding potential was set to -40 mV and then 15 steps of 10 mV/each (1 Hz frequency) were applied starting at -100 mV. The voltage drift was eliminated computationally as no bridge electrodes were used. Bioelectric signals were picked up using a universal voltage/current clamp amplifier (4 pole-bessel, ELC-03XS, npi, Tamm, Germany) connected to a PC computer via a breakout-box interface (INT-20X, npi, Tamm, Germany). High-precision positioning was provided by a piezo-drive micromanipulator (Sensapex, Oulu, Finland). Instant patch clamp parameters (e.g. $R_{pipette}$,

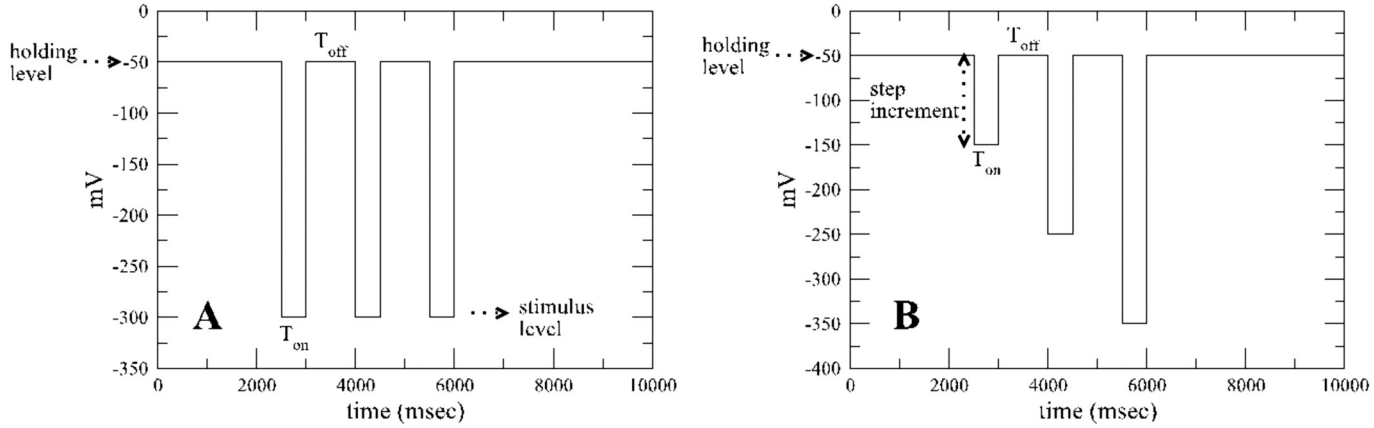

Fig. 2. Examples of train of voltage pulses (A) and steps (B) used to stimulate neurons directly cultured on chip.

R_{seal}, C_m, G_{access}, G_m) were monitored with the signal acquisition software (WinWCP Electrophysiology Software, ©John Dempster, University of Stratchlyde, Scotland). Voltage commands and current responses values were stored in a PC. Each stimulation/response window had 2048 fixed data points with a sampling frequency dependent on time window duration. Once stored, signals and monitored parameters were processed off-line and IV curves were calculated.

2.5. Protocols for neurons stimulation

To stimulate neurons directly cultured on chip, several protocols were implemented, programming a signal generator (Keithley 2601 Sourcemeter). This instrument can act as voltage or current source. The instrument was programmed (using the Keithley Test Script Processor language) to feed the chips with the desired stimuli. Scripts were developed to feed neurons with train pulses with the same amplitude or series of steps, both using the generator as voltage or current source, see Fig. 2 for typical examples. Opportune parameters that can be selected by the user during the experiment are the baseline (holding level), the pulse height (or the step increment), the duration of the pulse (T_{on} in Fig. 2) and the interval between pulses (or steps), T_{off} in Fig. 2.

2.6. Interfacing neurons with a memristive device

A general scheme of the setup used to interface neurons with a memristive device is summarized in Fig. 3. The connection from neuronal cells (growing on the chip with gold or platinum electrodes) - numbered as 1 in Fig. 3, to the memristive device (number 5 in Fig. 3; BS-AF-W chip, KNOWM, USA) is shown as well as the chain of amplifiers (2 and 4 in Fig. 3), which increase the level of the collected neuronal signals to match the memristor input. During experiments, devices 1 and 2 were inserted into a Faraday cage, to reduce the pick-up of external interference. A commercial amplifier for cell recording (ISO-80, World Precision Instruments, Germany) was used as first stage amplifier. Typically, gain 1000 was set on the WPI ISO-80 amplifier and gain 20–50 on the Princeton Applied Research PAR model 113 PRE-AMP, used as second stage amplifier. Two oscilloscopes (Agilent Technologies MSO6034A Oscilloscope (number 3 in Fig. 3) and LeCroy 324A Oscilloscope) were used for checking the experimental conditions, while a waveform generator (Agilent Technologies 33500B Waveform Generator) was used to produce signals used to set and check the memristor state.

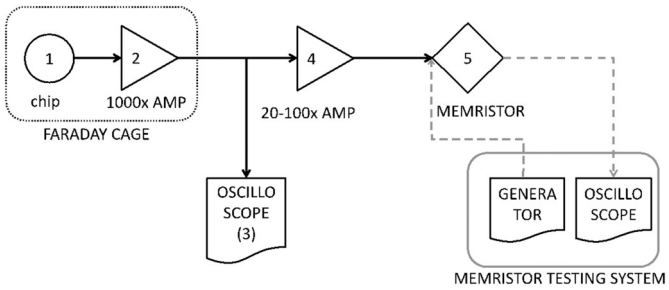

Fig. 3. Set-up for the analysis of the hybrid bio-memristor system. General scheme of the experimental setup used to establish the neuronal cells-memristor communication. Meaning of symbols: 1) standard chip, 2) first stage-high impedance amplifier, 3) control oscilloscope, 4) second stage amplifier, 5) memristive device. Black dotted box (top left) is the Faraday cage, grey box (bottom right) represents the instrumentation used for setting and checking the state of the memristive device. When memristive state being set or checked, the connection between 4 and 5 is interrupted and the connections shown as broken lines are instead activated.

3. Results and discussion

Two different types of chips were designed and fabricated for culturing neuronal cells and sensing their electrophysiological activity. The bio-compatibility of chips was preliminary tested by culturing neuronal-like cells NSC-34 and by measuring their electrical activity with a standard patch-clamp setup, both with or without the application of external stimuli by interfacing the chip with an external generator. Cortical neurons were then seeded on chips and their activity was measured with a dedicated setup. Finally, the whole hybrid bio-memristor system was tested with promising results.

3.1. Memristive properties of TiO_x films produced by PCMS technique

In order to achieve the desired and necessary memristive properties, a single memristor was realized on each dot using the electroforming procedure described in the Supporting Information (Fig. S2 legend). Since the variability of memristive devices based on metal oxides is well known [20,21], we carefully checked the best current compliance (I_{compl}) and the switch reset voltage (V_{reset}) to improve reliability in our specific working conditions. In particular, we set the system to work with low current during voltage pulse stimulation of neurons. After the electroforming procedure, the TiO_2 thin film showed a good memristive response. The typical current/voltage pinched hysteresis loops are shown in Fig. 4, with a V_{SET}/V_{RESET} of about −0.8/1.1 V, with ± 0.1 V stability for tens of cycles. The ON-OFF ratio is higher than one order of magnitude, confirming the good memristive quality of this system.

Every memristor was characterized and left in a specific resistivity states: high resistance state (R = 10-100kΩ), low resistance state (R = 0.1-1kΩ), short circuit state (R = 12-15 Ω). The first and the second state correspond to OFF and the ON memristor conditions,

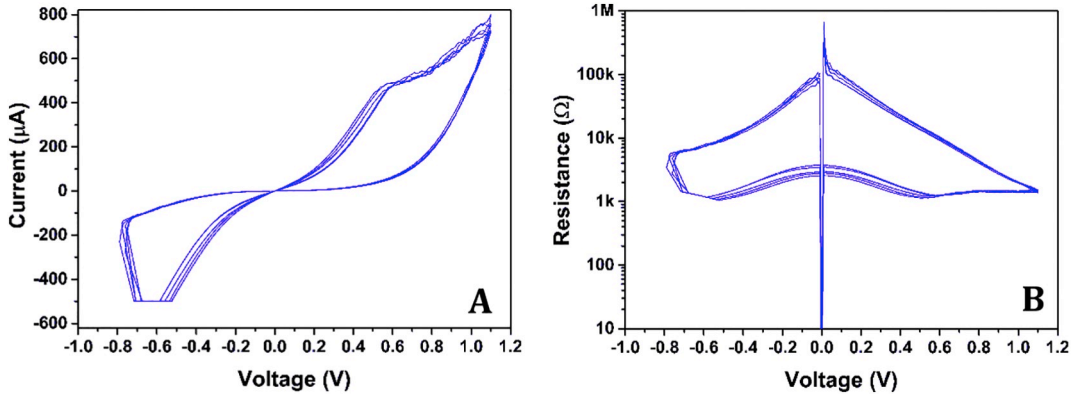

Fig. 4. Memristive behavior of PMCS TiO_2 thin films. Plots show: A): current vs voltage; B): resistance vs voltage.

respectively, while the third corresponds to the resistance value right after the electroforming.

It has to be noted that here, for the first time, the PMCS (pulsed microplasma cluster source) deposition technique was successfully employed for the synthesis of nanostructured TiO_2 thin films for memristive applications.

Due to the good memristive properties of TiO_2 thin films, the realization of a hybrid device based on a suitable electrode array of platinum dots with a 50 nm TiO_2 PMCS film deposited on the top was pursued and further explored via patch-clamp (see below).

3.2. Setup of cell growth on chip

In order to perform the electrophysiological measurements, a suitable number of cells was seeded on chip. This number ensured reliable electric measurements by preventing too many cell to cell interactions, which in preliminary tests produced noisy measurements and complicate analysis. To set up all cell growth conditions before using primary neurons and for preliminary electrophysiological measurements, NSC-34 (Neuroblastoma Spinal Cord) cells were selected. NSC-34 cells are a neural hybrid cell line, derived by fusion of motor neuron enriched embryonic mouse spinal cord cells with neuroblastoma [17]. These cells are considered as a suitable model for motor neuron associated disorders as well as a suitable model for studying the intrinsic electrophysiological properties also using MEA systems [22]. Here, NSC-34 cells were seeded in a low number (250 cells/mL) directly in the central area of the chip, without any surface treatment or coating.

After 24 h culturing at 37 °C and 5% CO2 atmosphere in complete medium, cells were imaged (Fig. 5A). Cell adhesion and morphology were perfectly comparable with control cells seeded in standard conditions (i.e. cell culture dish). The possibility to differentiate NSC-34 cells growing on the chip was also evaluated (Fig. 5B). After three days of serum starvation, cells were imaged and the presence of neurite-like processes was observed, confirming that the chip also ensured the cell differentiation.

Finally, long-term on-chip cell growth was evaluated. After 6 days of culture, cells were imaged (C), confirming the presence of adherent and viable cells with the proper morphology. Longer culturing times gave similar results, confirming the suitability of both the chip sterilization protocol employed and general growing conditions (data not shown).

3.3. Electrophysiological analysis - via patch-clamp - of cells grown on-chip

In order to test the functionality of cells grown on chip, the electrical cell properties were tested in terms of membrane ionic currents, using patch-clamp electrophysiology in whole-cell configuration.

Under standard electrophysiological conditions (physiological intra and extra-cellular solutions, see Materials and Methods), NSC-34 cells were tested with positive results in evoking total currents. Fig. 6 shows macroscopic cell currents after stimulation with a wide-range voltage step protocol (−50 mV to +90 mV). Recorded currents show canonical inward and outward components in the range of nano amperes. This result demonstrates that cell-chip interface preserve the physiological conditions for a proper membrane excitability.

Having confirmed the membrane electrical activity, the effects under external non-patch-clamp stimulation were evaluated. NSC-34 cells grown on a standard chip were patched to record simultaneously the current cell response (Fig. 7A, upper panel) and an increasing external voltage input (Fig. 7A, lower panel). The results show a perfect correspondence between external stimulation and cell response. Recognizable outward currents appear after a −500 mV stimulus, after which the outward current increased in a voltage dependent manner. The neuronal response to an outer-chip stimulus, demonstrates that an independent electrical communication has been established between the cellular and the electronic components of the chip. The cell recorded was in close proximity to the chip-electrode and the stimulus

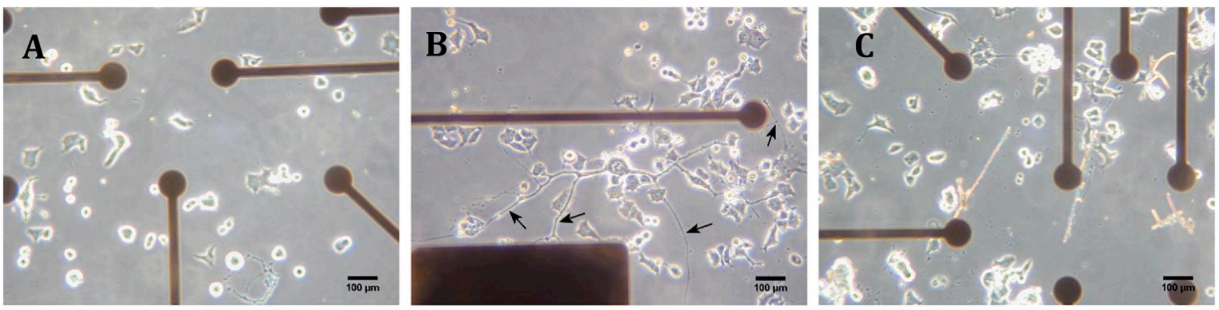

Fig. 5. NSC-34 cells growing on a standard chip. Picture of cells after 24 h growth (A), after 3 days of serum starvation (B) and after 6 days culture (C). Bright field images were taken with an optical microscope with 10× objective. Arrows in panel B highlight the neurite-like processes appearing after serum starvation. Bars = 100 μm.

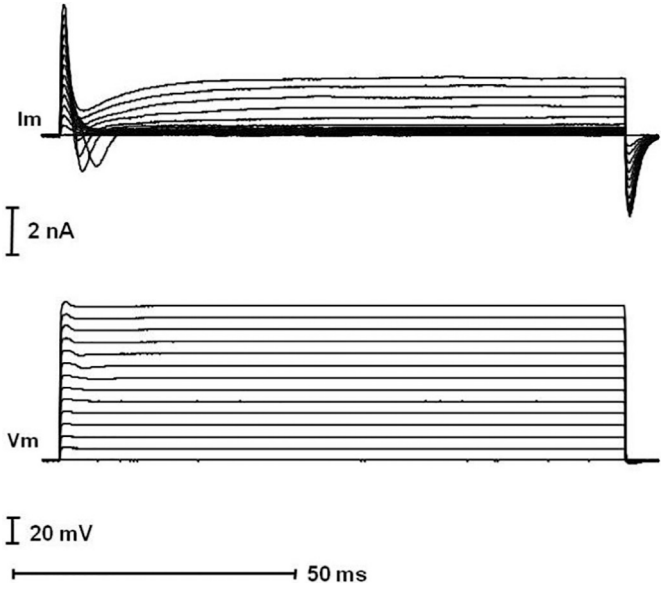

Fig. 6. Retained neuronal activity of NSC-34 cells grown on a standard chip. Patch-clamp recording of ionic currents (upper traces) from a cell cultured on chip in the whole cell configuration with standard solutions. Voltage clamp mode with a stimulation protocol from −50 mV to +90 mV for 100 ms (lower traces). Inward and outward currents are shown.

was increased by a 10× factor, compared to a usual patch-clamp stimulus (−50 mV compared to −500 mV), which is a good starting point to determine the electrical attenuation of the system. Measuring the distance between the neuron and the chip it is possible to know precisely how much stimulation every neuron in the surrounding area undergoes.

The neuronal networks were next evaluated. Fig. 7B shows primary culture of cortical neurons in the whole-cell configuration employed to record simultaneously the current cell response (Fig. 7B, upper trace) and the external voltage input (Fig. 7B, lower trace) Stimuli were consecutive −500 mV steps for 2000 ms and elicited voltage-dependent outward currents in the nano ampere range.

Fig. 7C–D show the spontaneous activity of 14 DIV and 21 DIV cultured neurons measured by patch clamp in CC configuration. Maximum firing activity of action potentials was recorded at 14 DIV.

This section has shown that neurons grown on a chip respond to stimuli either evoked by an external source or by the neuronal network established in the culture.

Once assessed that neuronal cells were functional, connected and responsive on a standard chip, we moved forward to evaluate the same conditions on a chip designed as memrisitve being coated with TiO_2 films. Cortical networks grown on TiO_2 chips were recorded above all in current clamp mode in order to evaluate the firing activity.

Differences in current amplitude compared to standard chip cannot be assumed as a TiO_2 effect, since the exact cell-electrode distance was not measured in every experiment. However, with a less steep stimulus, currents show a higher current increase per step (data not shown). Roncador et al [15] have already shown that TiO_2 surfaces increase current amplitude in primary cortical neurons, which is in good agreement with these results.

Fig. 8 shows spontaneous spike activity recorded from cortical neurons cultured in TiO_2-coated chips. 14 DIV neurons (Fig. 8A) show a stronger burst activity of action potentials although a good spike activity was also observed also at 21 DIV maturation stage (Fig. 8B). The

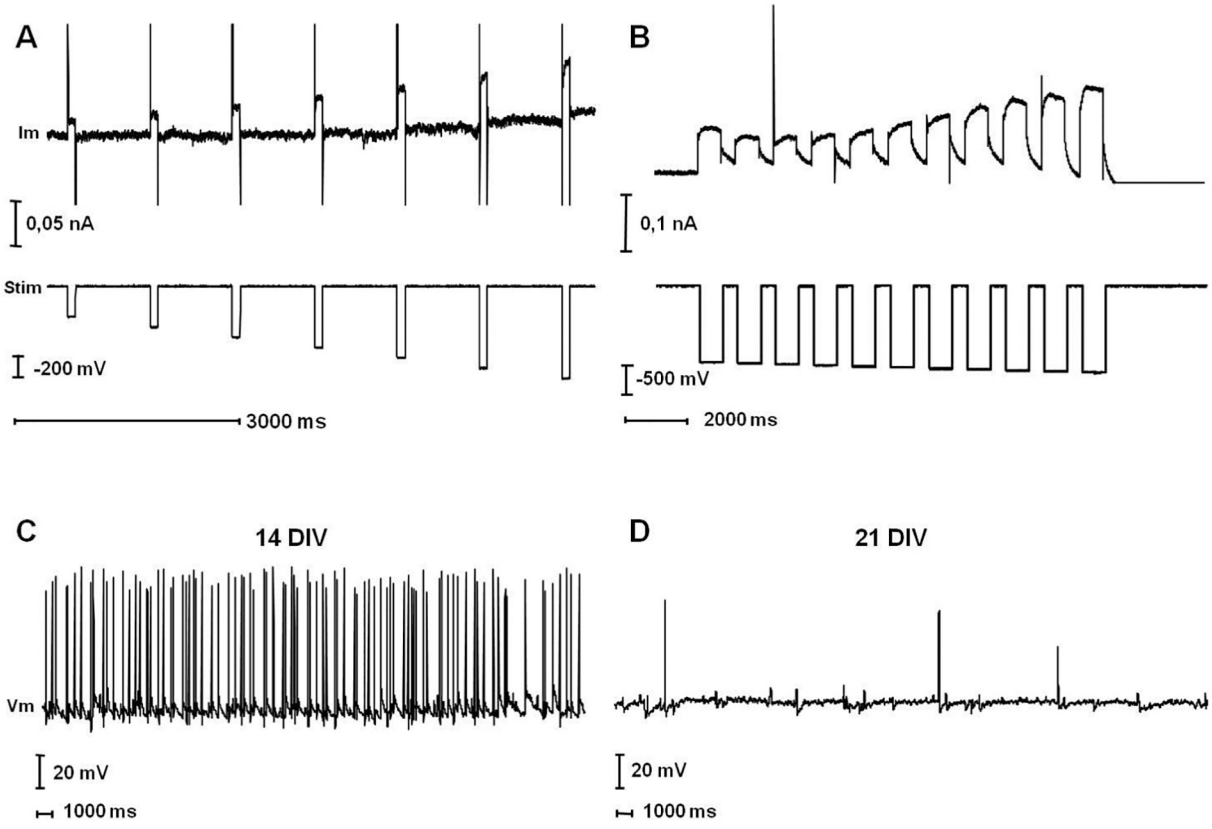

Fig. 7. Neuronal response to stimuli on standard chip A) NCS-34 cells on standard chip, increasing stimulation voltage steps from −300 mV to −900 mV for 100 ms (upper trace), which show the voltage dependency of the recorded outward currents (lower trace). B) Primary culture of mouse cortical neurons on standard chip, consecutive external stimulation −500 mV voltage steps for 2000 ms (lower trace), which shows the voltage dependency of the recorded outward currents (upper trace). C) - D) Recordings of spontaneous activity in CC (current clamp) mode from mouse primary cortical cells cultured on standard chips.

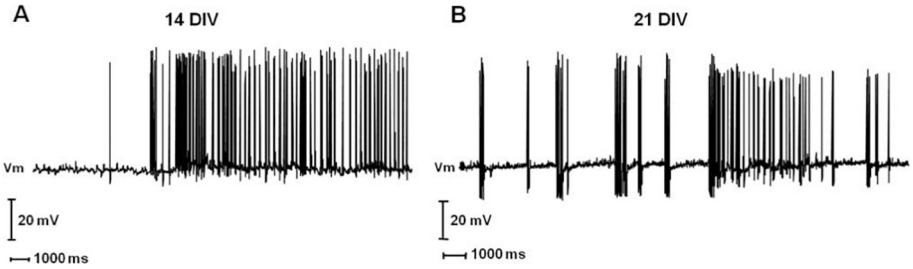

Fig. 8. Neuronal activity on memristive surface. A) - B) Recordings of spontaneous activity in CC (current clamp) mode from primary cultures of murine cortical neurons grown on TiO_2-coated chips at different maturation stages.

difference among maturation stages of the firing activity overlaps that recorded on cell at the same maturation stages on standard chips and confirm once again the enhancement role of the bioelectrical activity exerted by the TiO_2 surfaces [15].

Taken together, our results support the reliability of the customized standard chip, which assures the full cell viability as well as the physiological electrical excitability in terms of ionic currents behavior and action potentials firing. Also in the TiO_2-coated chip, current and voltage physiological parameters of the cells are retained.

For both the above chip configurations the recorded intrinsic ionic conductances, ascribed to voltage-gated ion channels, and firing activity patterns (i.e. action potentials – spikes – shape, size and frequency) are in agreement with similar activities reported for other neurons belonging to cortical and neocortical districts [23,24].

These successful data encouraged next steps, i.e. the measure of neuronal electrical cross-talk activity within the hybrid bio-memristor system in order to functionally unravel neuromorphic architectures.

3.4. Interfacing neurons with a memristive device

3.4.1. Electrophysiological analysis of neurons grown on-chip

Neurons cultured on chip were firstly checked for the presence of a diffuse neuronal network, as a preliminary step (Fig. 9A). Then, a chip was placed in the Faraday cage and one of its electrodes was connected with the ISO-80 amplifier probe (item 2 in Fig. 3). The signal was sent to the oscilloscope for checking the presence of spontaneous neuronal firing. Cell-free chips filled with only neurobasal medium were also tested for the presence of non-specific electrical signals (Fig. 9B). The typical traces recorded in these control conditions were very flat, showing no spurious signals. Once established the optimal experimental conditions, for example neurons showing a suitable network with firing ability and no spurious signals due either to culture medium or to the set-up system, spontaneous electrical signal of neurons on-chip was acquired (Fig. 9C). An evident electrical signal was recorded, indicating that neurons maintained not only their morphological features (Fig. 9) but also their physiological activity. The measured signal is attributed to the activity of the several neurons lying in close proximity of electrodes, since no signal is present without neurons (Fig. 9B). The recorded neuron firing represents the collective activity of the network and is compatible with the spontaneous neural activity measured in vivo [25].

These results prompted us to investigate the connection between neurons and the memristive device.

3.4.2. Control of a memristor trough neurons grown on chips

In the context of exploiting the adaptive behavior of memristors to interact with the adaptive abilities of neurons, a direct connection between primary cortical neurons grown on standard chips and a commercial memristor was tested. At the beginning of each experiment the memristive device was prepared in its closed state, applying a suitable signal. The resulting closed state was always checked (Fig. 10B) before proceeding with the experiment. The signal recorded from neurons grown on the standard chip, was conditioned through a further amplification step using a second stage amplifier (Fig. 10A), in order to reach the necessary value able to change the memristor state. This signal was then sent to the memristive device for a definite period of time (i.e. 4 min). At the end of the experiment, the memristor state was checked again (Fig. 10C). As clearly visible in Fig. 10, a state change was obtained, due to the feeding of signals from the spontaneous firing of the neuronal cells. The memristor indeed changed from its closed state to about 70% of open state. The spontaneous electrical activity of primary cortical neurons was therefore able to induce a measurable change, as previously reported in the literature [26,27], indicating that a direct connection between biological and artificial systems is not only possible but also functional.

These preliminary results are, therefore, promising for developing a more complex bio-hybrid neuromorphic device, where neurons could drive a memristive system. In other words, this device could be considered as a first step to satisfy the demand for platforms where to test model of brain functions and mechanisms. At the same time it represents the ability to develop bio-electronic devices and interfaces that could effectively interchange and transfer data information towards

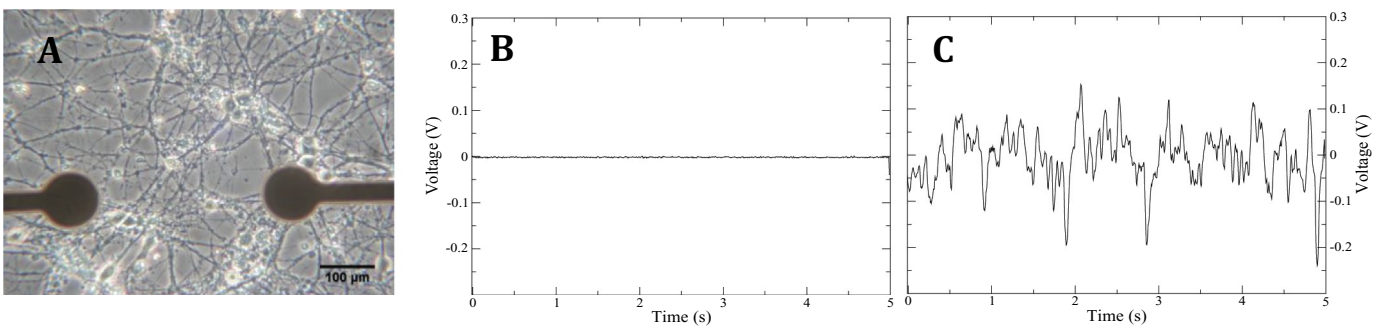

Fig. 9. Electrophysiological measures of neurons, recorded with the hybrid bio-memristor system. A: typical microscope image of the developed neuronal network on a standard chip after 14 days culture. Bar represent 100 µm. B: signal from a standard chip filled with complete neurobasal medium, without neurons (control). C: signal from mouse primary cortical neurons cultured on a standard chip. Signals were recorded using a WPI ISO-80 amplifier (gain 1000).

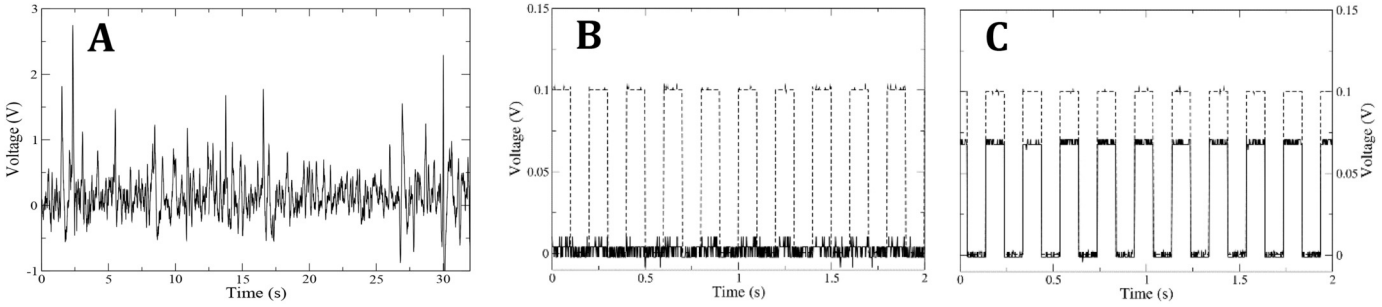

Fig. 10. A: Spontaneous signal of primary neurons grown on standard chip, further amplified trough a second stage amplifier. This signal was sent to a memristive device (in its closed state, B). B and C: Plots of signal applied to the memristor device to check its state. Dashed line: input signal. Solid line: output signal. B: memristor in its initial closed state. C: the memristor state at the end of the experiment.

hybrid bioelectronic systems, overcoming the present strong limits of an approach based on biocompatible electrodes coupled to standard electronics.

4. Conclusions

Two type of chips were designed, realized and tested, either in connection with a memristive device (standard chips) or as memristive by themselves (TiO_x-coated chips). The biocompatibility of chips was successfully assessed by growing both NSC-34 cell line and mouse cortical neurons. Electrophysiological recordings of the functional activity of neuron-like animal cells cultured on chips (either with or without a TiO_x coating) were then performed with whole-cell patch-clamp to detect and analyze the bioelectric activity (and therefore the viability) of the cells in order to ascertain the reliability of the device. Once set the electrophysiological conditions and the external stimulation protocols, we were able to evoke total current and voltage responses which were recorded, acquired, stored and analyzed. In both cell typologies we got optimal results with no breakdowns. In addition, the external stimulation (with fixed voltages) of the chip was effective and caused no damage or pitfalls to the cells.

The electrophysiological activity of primary cortical neurons grown on standard chips was next investigated with a dedicated setup. Firstly, the spontaneous electrical activity of neurons growing in close proximity of electrodes was successfully recorded showing current and voltage signals in agreement with the shape and the size of those already reported in the literature. Then, the chip was connected to a commercial memristor and the signal of neurons grown on the chip was sent to the memristor, which could change its state from closed to open. The ability of memristor to work with a synaptic/plastic response together with natural systems interfaced by contacts was therefore demonstrated, opening the way for the further implementation of basic computing elements able to perform both storage and processing of data, as in natural neurons.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2019.106212.

References

[1] P.A. Merolla, J.V. Arthur, R. Alvarez-Icaza, A.S. Cassidy, J. Sawada, F. Akopyan, B.L. Jackson, N. Imam, C. Guo, Y. Nakamura, B. Brezzo, I. Vo, S.K. Esser, R. Appuswamy, B. Taba, A. Amir, M.D. Flickner, W.P. Risk, R. Manohar, D.S. Modha, Artificial brains. A million spiking-neuron integrated circuit with a scalable communication network and interface, Science (New York, N.Y.) 345 (6197) (2014) 668–673 8.

[2] Z. Wang, M. Rao, J.-W. Han, J. Zhang, P. Lin, Y. Li, C. Li, W. Song, S. Asapu, R. Midya, Y. Zhuo, H. Jiang, J.H. Yoon, N.K. Upadhyay, S. Joshi, M. Hu, J.P. Strachan, M. Barnell, Q. Wu, H. Wu, Q. Qiu, R.S. Williams, Q. Xia, J.J. Yang, Capacitive neural network with neuro-transistors, Nat. Commun. 9 (1) (2018) 3208 8.

[3] L.O. Chua, Memristor-the missing circuit element, IEEE Transactions on Circuit Theory, CT-18 1971, pp. 507–519.

[4] D.B. Strukov, G.S. Snider, D.R. Stewart, R.S. Williams, The missing memristor found, Nature 453 (7191) (2008) 80–83 5.

[5] J.J. Yang, D.B. Strukov, D.R. Stewart, Memristive devices for computing, Nat. Nanotechnol. 8 (1) (2013) 13–24 1.

[6] G. Milano, M. Luebben, Z. Ma, R. Dunin-Borkowski, L. Boarino, C.F. Pirri, R. Waser, C. Ricciardi, I. Valov, Self-limited single nanowire systems combining all-in-one memristive and neuromorphic functionalities, Nat. Commun. 9 (2018) 5151.

[7] J.J. Yang, M.D. Pickett, X. Li, D.A.A. Ohlberg, D.R. Stewart, S.R. Williams, Memristive switching mechanism for metal/oxide/metal nanodevices, Nat. Nanotechnol. 3 (2008) 429–433.

[8] D.-H. Kwon, K.M. Kim, J.H. Jang, J.M. Jeon, M.H. Lee, G.H. Kim, X.-S. Li, G.-S. Park, B. Lee, S. Han, M. Kim, C.S. Hwang, Atomic structure of conducting nanofilaments in TiO2 resistive switching memory, Nat. Nanotechnol. 5 (2010) 148–153.

[9] M.A. Zidan, J.P. Strachan, W.D. Lu, The future of electronics based on memristive systems, Nat. Electron. 1 (2018) 22–29.

[10] P. Fattahi, G. Yang, G. Kim, M.R. Abidian, A review of organic and inorganic biomaterials for neural interfaces, Adv. Mater. 26 (2014) 1846–1885.

[11] S. Caponi, S. Mattana, M. Ricci, K. Sagini, L.J. Juarez-Hernandez, A.M. Jimenez-Garduno, N. Cornella, L. Pasquardini, L. Urbanelli, P. Sassi, A. Morresi, C. Emiliani, D. Fioretto, M. Dalla Serra, C. Pederzolli, S. Iannotta, P. Macchi, C. Musio, A multidisciplinary approach to study the functional properties of neuron-like cell models constituting a living bio-hybrid system: SH-SY5Y cells adhering to PANI substrate, AIP Adv. 6 (2016) 111303.

[12] G. Cellot, P. Lagonegro, G. Tarabella, D. Scaini, F. Fabbri, S. Iannotta, M. Prato, G. Salviati, L. Ballerini, PEDOT: PSS interfaces support the development of neuronal synaptic networks with reduced neuroglia response in vitro, Front. Neurosci. 9 (2016) 521.

[13] L.J. Juarez-Hernandez, N. Cornella, L. Pasquardini, S. Battistoni, L. Vidalino, L. Vanzetti, S. Caponi, M. Dalla Serra, S. Iannotta, C. Pederzolli, P. Macchi, C. Musio, Bio-hybrid interfaces to study neuromorphic functionalities: new multidisciplinary evidences of cell viability on poly(anyline) (PANI), a semiconductor polymer with memristive properties, Biophys. Chem. 208 (2016) 40–47 1.

[14] E. Juzekaeva, A. Nasretdinov, S. Battistoni, T. Berzina, S. Iannotta, R. Khazipov, V. Erokhin, M. Mukhtarov, Coupling cortical neurons through electronic memristive synapse, Adv. Mater Technol. 4 (2019) 1800350.

[15] A. Roncador, A.M. Jimenez-Garduño, L. Pasquardini, G. Giusti, N. Cornella, L. Lunelli, C. Potrich, R. Bartali, L. Aversa, R. Verucchi, M.D. Serra, S. Caponi, S. Iannotta, P. Macchi, C. Musio, Primary cortical neurons on PMCS TiO2 films towards bio-hybrid memristive device: a morpho-functional study, Biophys. Chem. 229 (2017) 115–122.

[16] F. Detto, N. Armani, L. Lazzarini, T. Toccoli, R. Verucchi, L. Aversa, M.V. Nardi, B. Rossi, G. Salviati, S. Iannotta, Excitonic recombination in superstoichiometric nanocrystalline TiO2 grown by cluster precursors at room temperature, Phys. Chem. Chem. Phys. 14 (2012) 5705–5710.

[17] N.R. Cashman, H.D. Durham, J.K. Blusztajn, K. Oda, T. Tabira, I.T. Shaw, S. Dahrouge, J.P. Antel, Neuroblastoma x spinal cord (NSC) hybrid cell lines resemble developing motor neurons, Dev. Dyn. 194 (3) (1992) 209–221 7.

[18] A.M. Jiménez Garduño, L.J. Juárez-Hernández, M.J. Polanco, L. Tosatto, D. Michelatti, D. Arosio, M. Basso, M. Pennuto, C. Musio, Altered ionic currents and

amelioration by IGF-1 and PACAP in motoneuron-derived cells modelling SBMA, Biophys. Chem. 229 (2017) 68–76 10.
[19] O.P. Hamill, A. Marty, E. Neher, B. Sakmann, F.J. Sigworth, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches, Pflugers Arch. - Eur. J. Physiol. 391 (2) (1981) 85–100 8.
[20] D. Ielmini, Resistive switching memories based on metal oxides: mechanisms, reliability and scaling, Semicond. Sci. Technol. 31 (2016) 063002.
[21] G. Baldi, S. Battistoni, G. Attolini, M. Bosi, C. Collini, S. Iannotta, L. Lorenzelli, R. Mosca, J. Ponraj, R. Verucchi, V. Erokhin, Logic with memory: and gates made of organic and inorganic memristive devices, Semicond. Sci. Technol. 29 (2014) 104009.
[22] K.R. Sabitha, D. Sanjay, B. Savita, T.R. Raju, T.R. Laxmi, Electrophysiological characterization of NSC-34 cell line using microelectrode Array, J. Neurol. Sci. 370 (2016) 134–139 11.
[23] P. Ghaderi, H.R. Marateb, M.-S. Safari, Electrophysiological profiling of neocortical neural subtypes: a semi-supervised method applied to in vivo whole-cell patch-clamp data, Front. Neurosci. 12 (2018).
[24] K. Ikeda, N. Suzuki, J.M. Bekkers, Sodium and potassium conductances in principal neurons of the mouse piriform cortex: a quantitative description, J. Physiol. 596 (22) (2018) 5397–5414 11.
[25] P.A. Kells, S.H. Gautam, L. Fakhraei, J. Li, W.L. Shew, Strong neuron-to-body coupling implies weak neuron-to-neuron coupling in motor cortex, Nat. Commun. 10 (1) (2019) 1575 4.
[26] I. Gupta, A. Serb, A. Khiat, R. Zeitler, S. Vassanelli, T. Prodromakis, Real-time encoding and compression of neuronal spikes by metal-oxide memristors, Nat. Commun. 7 (2016) 12805 9.
[27] D. Gater, A. Iqbal, J. Davey, E. Gale, Connecting spiking neurons to a spiking memristor network changes the memristor dynamics, 2013 IEEE 20th International Conference on Electronics, Circuits, and Systems (ICECS), 2013.