Light-Evoked Modulation of Neuronal Activity with Conjugated Polymers

Elisabetta Colombo, Paul Feyen, Duco Endeman, Mattia Nova, Maria Rosa Antognazza, Guglielmo Lanzani, Fabio Benfenati, and Diego Ghezzi, *Member, IEEE*

Organic bioelectronics [1] aims at the intimate integration between organic materials and biological tissues in order to obtain a targeted functional outcome. In the last decades, conductive polymers have been successfully employed to interface excitable cells through biocompatible probes and devices [2, 3]. We previously reported the possibility to culture primary neurons on photosensitive conjugated polymers, such as poly(3-hexylthiophene) (P3HT), without altering either the polymer or the neuronal properties [4]. These neurons displayed a depolarizing response to a brief (< 50 ms) green light stimulus and a consequent control over the action potential firing rate. The same interface was also shown to elicit activation of retinal circuitry and ganglion cell firing in explanted degenerate retinas upon light stimulation [5]. However, while neuronal excitation has been extensively proved via planar or protruding electrodes [6], carbon nanotubes [2], and conjugated polymers [7], the hyperpolarization of neurons and silencing of their firing activity has been achieved with optogenetics [8], but rarely documented for functionalized planar devices and structures [3]. We show here a method to induce both depolarization and hyperpolarization of cells interfaced with conjugated polymers upon prolonged (500 ms) light stimulation. Patch-clamp experiments showed that HEK293 cells grown on P3HT displayed a biphasic response composed of an initial depolarization followed by a sustained hyperpolarization during light illumination. We also documented that the amplitude of the light-induced hyperpolarization was reduced in the absence of intracellular potassium, but was insensitive to changes in the intracellular chloride concentration. A prolonged illumination was equally able to hyperpolarize primary neurons cultured over P3HT and significantly reduced both spontaneous and electrically elicited action potential frequency. We further assessed the translatability of our findings to tissue stimulation by describing the modulation of ganglion cell firing in explanted blind retinas from Royal College of Surgeons rats, a model of *Retinitis pigmentosa*. By coupling conjugated polymers with multi-electrode arrays and conventional metal electrodes, single-unit activity of ganglion cells was inhibited by prolonged illumination. We similarly investigated the phenomenon in acute hippocampal brain slices placed onto a conjugated polymer thin film. These results indicate that conjugated polymers can be used to non-invasively excite and inhibit the electrical activity of cells cultured on its surface. Furthermore, the translation of our findings from *in vitro* cultures to different central nervous system tissues, suggests a potential application of these organic devices as a tool for modulation of neuronal activity *in vivo*.

References

[1] M. Berggren and A. Richter-Dahlfors, "Organic bioelectronics," *Advanced Materials*, vol. 19, pp. 3201-3213, Oct 19 2007.
[2] L. Bareket, N. Waiskopf, D. Rand, G. Lubin, M. David-Pur, J. Ben-Dov, *et al.*, "Semiconductor nanorod-carbon nanotube biomimetic films for wire-free photostimulation of blind retinas," *Nano Lett*, vol. 14, pp. 6685-92, Nov 12 2014.
[3] V. Benfenati, S. Toffanin, S. Bonetti, G. Turatti, A. Pistone, M. Chiappalone, *et al.*, "A transparent organic transistor structure for bidirectional stimulation and recording of primary neurons," *Nature Materials*, vol. 12, pp. 672-680, Jul 2013.
[4] D. Ghezzi, M. R. Antognazza, M. Dal Maschio, E. Lanzarini, F. Benfenati, and G. Lanzani, "A hybrid bioorganic interface for neuronal photoactivation," *Nature Communications*, vol. 2, Jan 2011.
[5] D. Ghezzi, M. R. Antognazza, R. Maccarone, S. Bellani, E. Lanzarini, N. Martino, *et al.*, "A polymer optoelectronic interface restores light sensitivity in blind rat retinas," *Nature Photonics*, vol. 7, pp. 400-406, May 2013.
[6] M. E. Spira and A. Hai, "Multi-electrode array technologies for neuroscience and cardiology," *Nat Nanotechnol*, vol. 8, pp. 83-94, Feb 2013.
[7] V. Gautam, D. Rand, Y. Hanein, and K. S. Narayan, "A Polymer Optoelectronic Interface Provides Visual Cues to a Blind Retina," *Advanced Materials*, vol. 26, pp. 1751-1756, Mar 2014.
[8] F. Zhang, L. P. Wang, M. Brauner, J. F. Liewald, K. Kay, N. Watzke, *et al.*, "Multimodal fast optical interrogation of neural circuitry," *Nature*, vol. 446, pp. 633-9, Apr 5 2007.

This work was supported in part by Compagnia di San Paolo under Grant ID 4191 (to DG), Fondazione Cariplo under Grant n. 2013-0738 (to MRA and DG), EU under Grant FP7-PEOPLE-212-ITN 316832-OLIMPIA (to FB and GL), and Telethon – Italy under grant GGP12033 (to GL and FB).

E. C., P. F., D. E., M. N., F. B., and D. G. authors are with the Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, 16163 Genova, Italy.
D. G. author is with the Center for Neuroprosthetics, School of Engineering, École polytechnique fédérale de Lausanne, CH-1015 Lausanne, Switzerland. (Corresponding author D. G.; e-mail: diego.ghezzi@epfl.ch).
E. C., M. R. A., and G. L. authors are with the Center for Nano Science and Technology, Istituto Italiano di Tecnologia, 20133 Milano, Italy.