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An organic electronic biomimetic neuron enables auto-regulated neuromodulation

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ABSTRACT

Current therapies for neurological disorders are based on traditional medication and electric stimulation. Here, we present an organic electronic biomimetic neuron, with the capacity to precisely intervene with the underlying malfunctioning signalling pathway using endogenous substances. The fundamental function of neurons, defined as chemical-to-electrical-to-chemical signal transduction, is achieved by connecting enzyme-based amperometric biosensors and organic electronic ion pumps. Selective biosensors transduce chemical signals into an electric current, which regulates electrophoretic delivery of chemical substances without necessitating liquid flow. Biosensors detected neurotransmitters in physiologically relevant ranges of 5–80 μM, showing linear response above 20 μm with approx. 0.1 nA/μM slope. When exceeding defined threshold concentrations, biosensor output signals, connected via custom hardware/software, activated local or distant neurotransmitter delivery from the organic electronic ion pump. Changes of 20 μM glutamate or acetylcholine triggered diffusive delivery of acetylcholine, which activated cells via receptor-mediated signalling. This was observed in real-time by single-cell ratiometric Ca^{2+} imaging. The results demonstrate the potential of the organic electronic biomimetic neuron in therapies involving long-range neuronal signalling by mimicking the function of projection neurons. Alternatively, conversion of glutamate-induced descending neuromuscular signals into acetylcholine-mediated muscular activation signals may be obtained, applicable for bridging injured sites and active prosthetics.

1. Introduction

Disorders of neural function often involve abnormal electrical and neurochemical signalling in the central nervous system. To improve quality of life, patients undergo a variety of neuromodulation therapies. Deep brain stimulation is a commonly applied electrical technique with proven beneficial effects, despite some lack of understanding of its molecular mechanism of action (Kringelbach et al., 2007; Leeman and Cole, 2008; Olanow et al., 2004; Singh et al., 2007). Localized drug delivery can also be used, by means of fluidic systems (Rossi et al., 2013; Whitesides, 2006). However, these are often encumbered by accessory equipment (i.e., tubes, pumps, valves) and have difficulty in determining precise dosage.

Organic electronic electrophoretic transport devices – so called, iontronics – represent a new class of technologies that can provide spatiotemporal resolution and biochemical specificity. Based on polyelectrolytes and π-conjugated semiconducting polymers, iontronics exhibit a unique combination of ionic and electronic properties, enabling transduction between electronic impulses and biochemical signals (Larsson et al., 2013). This was first demonstrated in the organic electronic ion pump (OEIP), an electrophoretic delivery system which transports ions independently of liquid flow (Isaksson et al., 2007). The technology is based on thin films of the electrically conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) doped with the polyelectrolyte poly (styrenesulfonate) (PSS) (Heywang and Jonas, 1992) to provide

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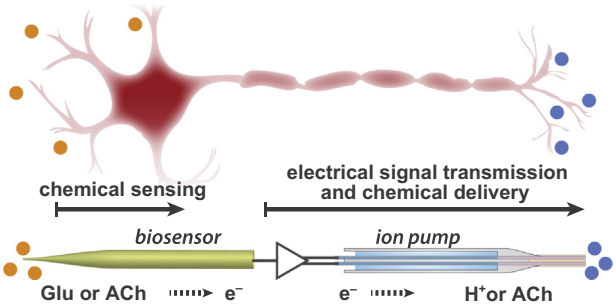

Fig. 1. Chemical-to-electrical-to-chemical signal transmission of a neuron. A neuron (upper panel) integrates chemical signals (left), triggers an electrical pulse of membrane depolarization (action potential) along the axon, causing chemical release at the axon terminals (right). This functional signal transduction can be mimicked by a biosensor connected to an organic electronic ion pump, thereby forming an organic electronic biomimetic neuron (lower panel). Both systems comprise a chemical sensing component detecting a neuroactive species (orange circles), an electrical signal transmission region, and a chemical output component delivering another species (blue circles).

enhanced electronic conductivity as well as cation-selective ionic conductivity. An OEIP consists of two PEDOT:PSS electrode strips on a plastic substrate, each passing through an electrolyte-filled reservoir (Fig. S1a). One end of each strip is connected to a power supply, while immersion of the other ends in the target system forms a salt-bridge. When voltage is applied, positively charged substances in the source (anodic) reservoir are electrophoretically "pumped" into the target solution, without liquid flow. Simultaneously, cations are transported into the cathodic electrode/reservoir system, completing the electrochemical circuit. The precise electrochemistry of PEDOT:PSS enables delivery of one positively charged molecule for each electron involved in PEDOT:PSS redox, leading to extremely high dosage precision (Isaksson et al., 2007; Simon et al., 2009).

A neuron can be considered a chemical-to-electrical-to-chemical signal transduction unit (Fig. 1) able to convey information over long distances. Neurotransmitter binding incrementally depolarizes the cell membrane, generating an action potential of ionic currents at the axon hillock when threshold depolarization is reached. The action potential propagates along the cell membrane, leading to release of neurotransmitters at the axon terminal. Diffusion across the synaptic cleft enables neurotransmitter binding to receptors on the postsynaptic neuron. In this study, we developed an autoregulated electrochemical system, the organic electronic biomimetic neuron (OEBN), mimicking these fundamental aspects of neural function. Chemical-to-electrical signal transduction is achieved by selective amperometric biosensors, which in turn regulate electrical-to-chemical delivery of neurotransmitters. This is the first demonstration of a regulated biosensor-OEIP system with the ability to precisely modulate delivery of neurotransmitters based on sensing of endogenous biochemical signals, thus mimicking neural function. The unique electronic communication interface allows the sensing component to be located at an arbitrary distance from the point of delivery, thereby expanding future applications to a variety of medical areas.

2. Materials and methods

2.1. Manufacturing and operation of OEIP

OEIP devices (Fig. S1) were manufactured by screen-printing PEDOT:PSS (Clevios SV3, H.C. Starck) onto poly-ethylene terephthalate sheets pre-coated with PEDOT:PSS (Orgacon EL-350, Agfa-Gaevert). Double-electrode strips were prepared, and mounted in two-lumen polypropylene tubes, serving as anodic and cathodic chambers. The length of the delivery tip in final devices was ~7 mm. Upon electronic addressing of PEDOT:PSS, the anode is oxidized (1), and positively charged species (M^+) are liberated into the anode-side electrolyte. The cathode is reduced (2) and any cationic species (X^+) compensate the PSS^- in the target system.

$$PEDOT^0 + M^+: PSS^- \rightarrow PEDOT^+: PSS^- + M^+ + e^- \quad (1)$$

$$PEDOT^+: PSS^- + X^+ + e^- \rightarrow PEDOT^0 + X^+: PSS^- \quad (2)$$

The electrochemical potential established between the electrodes electrophoretically "pumps" the liberated M^+ into the target system, and X^+ is transported in toward the cathode. Devices were operated as described in Supplementary materials and methods.

2.2. Real-time pH recording

An OEIP filled with 100 mM HCl(aq) was immersed into a dish containing 100 mM NaCl(aq) and universal pH indicator (Fluka 36828) mounted on the stage of a stereo-microscope (Nikon SMZ1500). Colour change at the submerged delivery tip was recorded by time-lapse video. pH was approximated by comparing the blue channel intensity of the video over time with the calibration colours of the indicator (Isaksson et al., 2008).

2.3. Chemical sensing by amperometric biosensors

Commercial amperometric enzymatic biosensors utilize enzymatic reactions that oxidize or reduce the chemical component to be detected. This generates electrons available for electrical recording. The highly selective Glu sensor (7001, Pinnacle Technology) yields exactly two electrons for each Glu (3,4), and provides a sensor current directly proportional to [Glu].

$$Glu + O_2 + H_2O \xrightarrow{GluOx} 2\text{-xoglutarate} + NH_3 + H_2O_2 \quad (3)$$

$$H_2O_2 \xrightarrow{500\ mV} 2H^+ + O_2 + 2e^- \quad (4)$$

The ACh sensor (Sarissaprobe ACh, Sarissa Biomedical) yield four electrons from each ACh (5–7), and determines changes in [ACh] in environments lacking choline or with static [choline].

$$ACh + H_2O \xrightarrow{AChE} \text{acetate} + \text{choline} \quad (5)$$

$$\text{choline} + O_2 \xrightarrow{ChOx} \text{betaine aldehyde} + 2H_2O_2 \quad (6)$$

$$2H_2O_2 \xrightarrow{500\ mV} 4H^+ + 2O_2 + 4e^- \quad (7)$$

The Glu sensor was operated at 600 mV versus the built-in Ag/AgCl wire reference, and the ACh sensor at 500 mV versus an external Ag/AgCl reference electrode (Bioanalytical Systems). Voltage was applied and current recorded using a Keithley 2602 SourceMeter and custom LabVIEW software.

2.4. Cell cultivation and intracellular Ca^{2+} recordings

Human neuroblastoma SH-SY5Y cells (ATCC no. CRL-2266) were handled according to supplier's instruction, and ratiometric Ca^{2+} imaging was performed as previously described (Tybrandt et al., 2009) and detailed in the Supplementary Materials and Methods.