Applications of Nanoparticles

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Wireless Activation of Neurons in Brain Slices Using Nanostructured Semiconductor Photoelectrodes**

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Understanding the spatio-temporal function of the brain is one of the most complex challenges in science.[1–5] For example, how brain regions process synaptic inputs to generate defined responses is still an unsolved question. While the function of neural circuits is traditionally assessed by electrically charging a sharp metal electrode inserted into brain tissue, the resulting focal activation pattern is highly unnatural. Even in topographically organized brain regions, like the primary visual cortex, activating a small receptive field results in a modulated spatial pattern of synaptic inputs that impinge on many neurons. So far, activating neural circuits using traditional single-site stimulation electrodes cannot reproduce the complex spatial patterns that natural stimuli evoke and, therefore, cannot recreate the biological input that local neural circuits normally process. The inability to drive neural circuitry with appropriate stimuli is one of the most important limitations to understanding the cellular basis of neural computation. Currently available methods to solve this problem—electrode arrays,[1] caged glutamate,[2] and genetically encoded "light switches"[3–5]—have significant limitations and have not succeeded in reproducing natural activity patterns. Traditional metal electrodes also introduce tissue damage by electrochemical side reactions that occur during the stimulation process on the electrodes.[6,7] Therefore, replacing existing current-injection methods with photoinduced depolarization provides a potentially clinically relevant strategy to avoid tissue damage from electrochemical side reactions.

Here we present nanostructured photoelectrodes, which offer an alternative for generating inputs with high temporal resolution. Using nanoparticle-coated photoelectrodes, we have produced photoelectrical activation patterns in slices of rat hippocampus and olfactory bulb by addressing the photoelectrode (PE) with focused near-infrared (NIR) light signals at $\lambda = 830$ nm.

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Earlier work on the photoelectric excitation of cultured cells used mercury-based thin-film electrodes, in which ensembles of cultured neurons grown on the thin films were activated through photoinduced currents.[8] In the present report, a light-activatable nanoparticle thin film was coated on the inside surface of a pulled-glass microtip, which could eliminate any observable neural toxicity during the experiment. With these photoelectrodes, no molecular biology manipulations are necessary, and no direct contact of the neurons with nanoparticle-coated surfaces is required (with the inside-coated tips). Unlike conventional stimulating electrodes, this method requires no wiring or electrical power and instead relies only on infrared light to stimulate synaptic inputs to neural circuits. In the present study, neurons in brain slices could be directly stimulated. In addition to using photoelectric stimuli to define specific neural processing steps in brain slices, the same technology will likely prove useful clinically to activate brain regions and damaged nerves. The schematic diagram and video micrograph of the photoelectrical activation of neurons in a brain slice is shown in Figure 1.

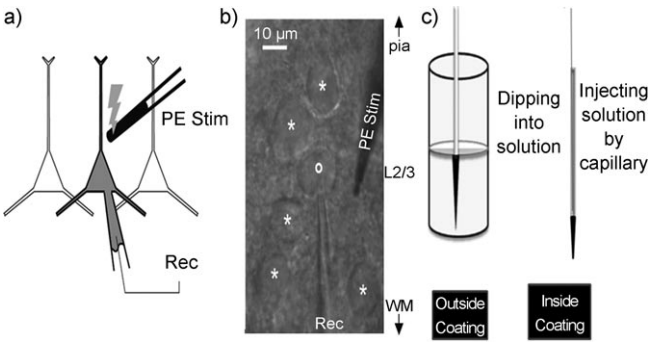

Figure 1. Schematic diagram (a) and video micrograph (b) of a neuron recorded through a recording electrode (Rec) contacting a L2/3 pyramidal cell (PC) and nanostructured photoelectric stimulating electrode (PE Stim). c) Schematic description of the process for coating the outside (left) and inside (right) surfaces of glass microtips with nanoparticle thin films through chemical bath deposition (CBD). WM = white matter, pia = pial surface.

PbSe is a Group IV–Group VI semiconductor with a narrow bulk band-gap energy of 0.26 eV and is commonly used for IR photodetectors.[9–11] The film's composition and structure were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD), respectively. XRD reveals a rock salt structure known for bulk PbSe (Figure 2a). Scherrer analysis of the XRD provides an estimate of the grain sizes of approximately 100 nm as confirmed by the

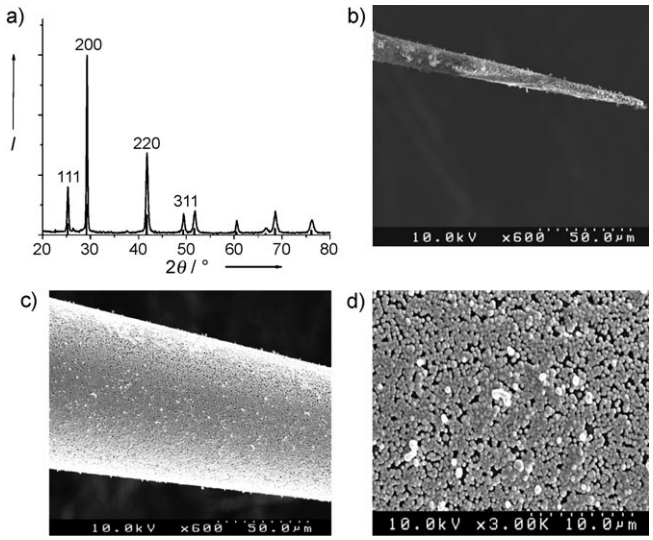

Figure 2. a) XRD of the thin film of PbSe nanoparticles coated on a glass substrate (the bars below the signals correspond to the standard JCPDS standards PbSe [78-1903]). b–d) SEM images of the PbSe nanoparticle film coated on the outside of the glass microtip: b) overview, c) side view, d) enlargement showing the PbSe nanoparticles.

SEM; this corresponds to an absorption onset well beyond the excitation wavelengths used in this study.[10] The SEM images of the photoelectrodes (Figure 2b–d) show that a uniform thin film of PbSe nanoparticles was coated on the outside surface of the glass microtips. Earlier measurements show that in PbSe films the majority charge carriers are holes, leading to intrinsic *p*-type nanostructures.[12]

PbSe absorbs broadly in the visible to infrared spectral range,[11–13] and it is convenient to use 830 nm light from a mode-locked Ti:sapphire laser for photoexcitation since these experiments can also involve two-photon visualization of the neurons filled with Alexa or Ca^{2+}-sensitive dyes. Because of the low excitation energy of PbSe, a one-photon excitation process at conditions of low photon flux was sufficient to trigger the neuron response. We developed both outside- and inside-coated microtip pipettes (Figure 1), principally for defining the physical and biological properties of the photoinduced effects, such as absorption, neural triggering, and phototoxicity, since we can position them close (≈ 10 µm) to defined neuronal components like cell bodies and dendrites.

Outside-coated photoelectrodes with exposed PbSe nanoparticle films did not show any noticeable toxicity in these experiments, probably owing to the low solubility constant of PbSe ($K_{sp} = 3 \times 10^{-28}$) and the relatively short exposure time. To provide a completely nontoxic solution, inside-coated glass microtips were also developed as photoelectrodes. This led to a nanostructured photoelectrode surrounded by a thin glass layer. The inside-coated photoelectrode was also tested for another more fundamental reason. The question arose regarding whether the neuron stimulation results from a chemical electron-transfer process,[8,14] which would require direct contact through the solution phase, or if it results from an electric field effect. This question is relevant in order to understand the underlying phototriggering process. If inside-coated photoelectrodes that were enclosed by glass would produce a phototrigger for nearby neurons, evidence for a local electric field effect would be obtained. Indeed, the inside-coated microtips (Figure 1c, right), with which there is minimal chemical contact between the nanostructured semiconductor thin film and the neuron cells, show the same effect in phototriggering neurons as the outside-coated photoelectrodes. The formation of a photoinduced electric field was tested by electrical potential measurements near glass microtips coated with PbSe nanoparticles (Figure 3); in these

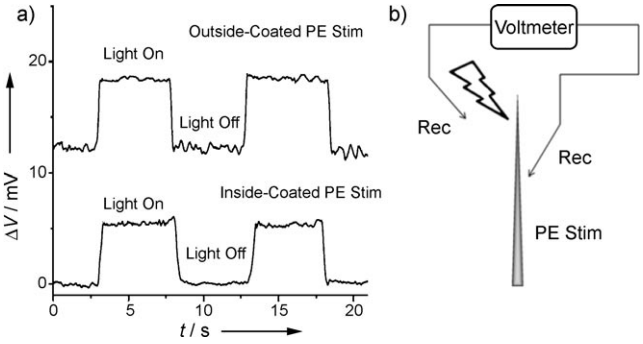

Figure 3. a) Photopotential recorded from the photoexcited stimulation photoelectrode (PE Stim) using two recording microelectrodes (Rec) placed near the photoelectrodes. Traces are offset for clarity. b) The schematic setup for photoinduced electrical field measurement.

measurements two recording tungsten metal electrodes (Rec) were placed near the stimulating microelectrode (Stim), and the voltage differential was measured. The results showed that there is a potential formed between the two recording electrodes when the stimulating photoelectrode was irradiated with light. The photoinduced potential and lack of measurable photocurrents together suggest that it is a light-induced field effect from the nanoparticle film which causes the stimulation of neurons. The same setup was also used to measure the photoinduced potential on glass microtips without PbSe nanoparticle coating, which failed to induce a detectable photopotential. The results suggest that the phototriggering could be activated by a photoinduced electric field caused by trapped charge carriers in the PbSe nanoparticle film. Of course, the possibility of small currents, which would originate from photogenerated surface charges, cannot be excluded completely.

The neural stimulation could be modulated in time and intensity. In Figure 4a a comparison of photoelectric activation (top; 830 nm pulsed beam; 10 mW average illumination measured at the objective focal point; top trace) and depolarization of the same neocortical L2/3 pyramidal cell with the direct-current-injection step (bottom trace) is shown. Both stimulation methods depolarized the neuron to threshold and triggered two action potentials. The neural membrane potential recovered with the same time constant (≈ 30 ms) following photoexcitation and direct-current-injection steps, suggesting that the kinetics of both responses were governed by the membrane time constant.

Rapid laser scanning over the nanostructured stimulation photoelectrode effectively depolarized both neocortical pyr-

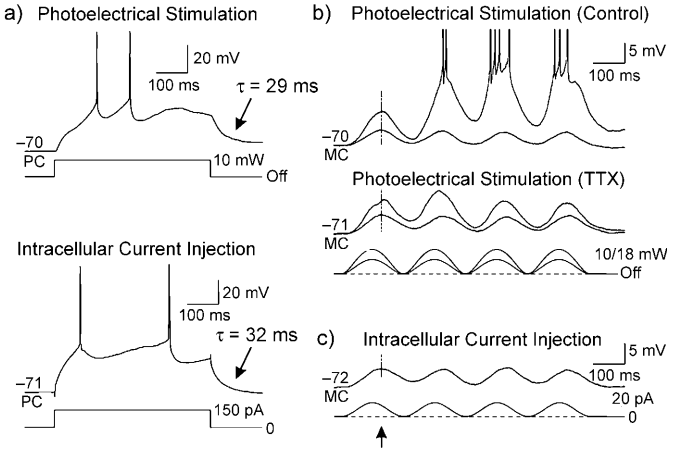

Figure 4. a) Comparison of photoelectric activation (top; 830 nm pulsed beam) and depolarization of the same L2/3 pyramidal cell with by direct-current injection (bottom); b) Comparison of rapid modulation of photoelectric excitation of mitral cell (MC) using sinusoidal light-intensity waveforms (top panel) with a direct-current injection step (bottom panel). The holding potential (in mV) is indicated above each trace. The top panel shows sub- and suprathreshold responses to 10 and 18 mW 5 Hz sinusoidal photoelectric stimuli, respectively; troughs between cycles correspond to 0 mW excitation. Action potentials truncated. The bottom panel illustrates the response of the same mitral cell to 10 mW modulated photoelectric excitation in the presence of 1 μM tetrodotoxin (TTX). Fast synaptic transmission blocked in these recordings using 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline (NBQX), D-(-)-2-amino-5-phosphonopentanoic acid (D-APV), gabazine. c) Response of the same neuron to 5 Hz sinusoidal current injection. Responses to both photoelectric (b) and current injection (c) show a similar phase lag (arrow and dotted vertical line).

amidal cells ($n = 11$) and olfactory bulb neurons ($n = 11$). Neurons could be activated repetitively without apparent damage such as long-lasting changes in resting membrane potential, input resistance, and action potential amplitude. Focal illumination of a similar area equidistant to the recorded cell but not covering the stimulation electrode failed to trigger intracellular depolarization (data not shown). No responses were detected when the laser beam was scanned over an uncoated patch pipette within 10–20 μm of the recorded neuron at similar illumination intensities (5–20 mW).

Photoelectric neuronal activation reflects predominately passive depolarization and can be elicited in the presence of blockers of fast synaptic transmission (5 μM NBQX, 25 μM D-APV, 10 μM gabazine; obtained from Sigma; Figure 4b, top). Neuronal responses could be patterned by applying arbitrary functions to the Pockels cell light intensity control, such as the responses to sine wave stimuli shown in Figure 4b. Photoelectric responses were graded with mean illumination intensity and subthreshold responses were unaffected by the Na^+ channel blocker tetrodotoxin (TTX, 1 μM; Figure 4b, bottom traces), suggesting that light-triggered responses were mediated directly, and not through activation of other nearby cells. Responses to sinusoidal illumination intensity modulation showed a similar phase lag to responses evoked by current waveforms injected intracellularly in the same neurons (28.6 ± 2.5 vs. 29.8 ± 0.7 ms, respectively; mean ± SEM; $p > 0.5$; Figure 4c), implying that the kinetics of both responses were governed by passive membrane properties.

Focal illumination of stimulation electrodes (PE Stim) coated on their inside surfaces also depolarized neocortical and olfactory bulb neurons ($n = 4$; 830 nm illumination; 5–20 mW average intensity), indicating that electrical fields, and not currents, mediate this effect. These results suggest that nanoparticle-coated micropipettes can effectively activate neurons in intact brain tissue in response to light pulses. This stimulation technique offers a simpler alternative to genetically encoded light switches or bath-applied caged compounds for neuronal stimulation. Nanoparticle-based photoelectric neuronal activation will likely prove useful clinically to activate brain regions and damaged nerves.

Experimental Section

Nanostructured PbSe films were deposited on glass microtips according to the procedures outlined in Refs. [15,16] First deposition bath solutions were prepared from 0.05 M lead acetate and 1.5 M citric acid in 7.5 mL deionized water; then 2.5 mL of 0.05 M sodium selenosulfate (Na_2SeSO_3 in excess Na_2SO_3) was added (prepared by refluxing 0.2 M Se with 0.5 M Na_2SO_3 for several hours until all Se powder had dissolved). Then the glass microtips were dipped into the bath solutions or the bath solution was injected into the microtip by a capillary; the microtips were then placed into an oven for curing at 80 °C. The prepared nanostructured PbSe-coated glass microtips were rinsed with distilled water and ethanol and dried under Ar.

The same PbSe nanoparticles were also deposited on a glass substrate under the same conditions by using the same precursor solution, and these nanoparticles were used for the XRD measurement. The glass substrate (22 × 40 mm), whose thickness is about 0.13–0.16 mm, was purchased from Corning Glass Works (USA) and cut into 11 × 20 mm pieces with a diamond cutter. Photopotential measurements of the PbSe-nanoparticle-coated photoelectrode were made by placing two tungsten microelectrodes near the photoelectrode and irradiating with visible light from a 75 W Xe lamp introduced by optical fibers under a microscope objective; the photopotentials were recorded on a CH instrument Electrochemical Station 6301B.

Acute brain slices were prepared from the hippocampi or olfactory bulbs of P14–21 Sprague Dawley rats using standard methods.[17–19] Slices (300 μm thick) from both brain regions were maintained in a submerged recording chamber at 30 °C and imaged on an upright fixed-stage microscope (Olympus BX51WI) using infrared differential interference contrast (IR-DIC) optics and a frame-transfer video camera (Cohu 6412–2000; Figure 1b). Slices were superfused with an artificial cerebrospinal fluid containing the following: 124 mM NaCl, 3 mM KCl, 1.23 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 26 mM $NaHCO_3$, 10 mM dextrose, and 2.5 mM $CaCl_2$ equilibrated with 95 % O_2/5 % CO_2. Whole-cell current-clamp recordings were made using an Axopatch 1D amplifier (Axon Instruments) and pipettes filled with an internal solution that contained: 140 mM methylsulfate, 4 mM NaCl, 10 mM HEPES, 0.2 mM EGTA (ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), 4 mM MgATP, 0.3 mM Na_3GTP, and 10 mM phosphocreatine (4–8 MΩ resistance). Electrophysiological records were low-pass-filtered at 2 kHz and digitized at 5 kHz (Instrutech ITC18) using custom Matlab software. A second patch-clamp photoelectrode (PE), which was coated either outside or inside with PbSe nanoparticles, was placed within the same field-of-view of the 60× water-immersion objective as the recording electrode. The nanostructured photoelectrode was positioned near the appropriate cell body (within 10–20 μm) under video microscopy (Figure 1b) after breakthrough to the whole-cell recording mode. We used a Verdi V10 pump laser and Mira 900

Ti:sapphire oscillator (Coherent) to illuminate the nanoparticles. High-speed XY galvanometer scanners (Cambridge Technology 6210) and custom Visual Basic 6, and Matlab software controlled the beam position within the microscope field-of-view. The laser beam was continuously scanned over the electrode tip in a Lissajous pattern that repeated at 1 kHz; the scan pattern typically covered 20 × 20 μm. Optical illumination was controlled by varying the control voltage to a Pockels cell intensity modulator (ConOptics) positioned in the laser beam path.

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