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Tetherless near-infrared control of brain activity in behaving animals using fully implantable upconversion microdevices

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ABSTRACT

Many nanomaterials can be used as sensors or transducers in biomedical research and they form the essential components of transformative novel biotechnologies. In this study, we present an all-optical method for tetherless remote control of neural activity using fully implantable micro-devices based on upconversion technology. Upconversion nanoparticles (UCNPs) were used as transducers to convert near-infrared (NIR) energy to visible light in order to stimulate neurons expressing different opsin proteins. In our setup, UCNPs were packaged in a glass micro-optrode to form an implantable device with superb long-term biocompatibility. We showed that remotely applied NIR illumination is able to reliably trigger spiking activity in rat brains. In combination with a robotic laser projection system, the upconversion-based tetherless neural stimulation technique was implemented to modulate brain activity in various regions, including the striatum, ventral tegmental area, and visual cortex. Using this system, we were able to achieve behavioral conditioning in freely moving animals. Notably, our microscale device was at least one order of magnitude smaller in size (~100 μm in diameter) and two orders of magnitude lighter in weight (less than 1 mg) than existing wireless optogenetic devices based on light-emitting diodes. This feature allows simultaneous implantation of multiple UCNP-optrodes to achieve modulation of brain function to control complex animal behavior. We believe that this technology not only represents a novel practical application of upconversion nanomaterials, but also opens up new possibilities for remote control of neural activity in the brains of behaving animals.

1. Introduction

Electrical, optical, and chemical manipulations of neural circuits have been major approaches used to elucidate the functions and connections of the nervous system. Recently, optogenetics has become a versatile and transformative tool for neuroscience studies [1,2]. The technique is based on optical stimulation of light-sensitive ion channels genetically expressed on cell membranes, and thus allows for spatially and temporally precise control over neural activity [3–5]. Since the first demonstration using channelrhodopsin-2 (ChR2), various photosensitive proteins have been developed to provide flexible options for optogenetic applications [6–9]. Most of these proteins are activated by light within the visible spectrum (VIS), which has limited tissue penetration and can lead to great photo-toxicity following prolonged exposure [10]. A typical optogenetic experiment usually requires light delivery by implanted optic fibers connected to a light source [11]. In many behavioral tests, such tethered systems prevent animals from moving freely and impose significant constraints on experimental design. These systems also complicate the analysis of animal behavior. Recently, great efforts have been made to develop tether-free optogenetic strategies [12–15]. Many of these techniques utilize wirelessly powered light-

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emitting diodes (LEDs) to deliver visible light for optogenetic stimulation of deep brain regions. Even though implantable LED devices and associated powering circuits have become smaller, lighter, and more versatile [12,15], the requirement for electronic components still complicates the fabrication and implementation procedures. Thus, a simple, all-optical, tetherless optogenetic method remains attractive and is yet to be developed.

Conceptually, light in the near-infrared (NIR, 700-1100 nm) range would be a preferable source for tether-free optogenetic applications, given its advantages, which include deep tissue penetration, low absorbance by biomolecules, and minimal photo-induced damage to mammalian cells [16]. However, direct excitation of ChR proteins at wavelengths in the NIR spectrum is challenging despite tremendous efforts in the development of red-shifted ChR variants through intricate protein engineering [6,8,17]. Two-photon fluorescence microscopy (TPFM) has been adopted for optogenetic analysis of neural circuitry using NIR to stimulate specific ChR variants [18–21]. However, TPFM-based optogenetics techniques are limited by drawbacks, such as small illumination volume, complex optical setup, and low compatibility with *in vivo* experiments, especially in freely moving animals [22,23]. Alternatively, transducing elements, such as upconversion nanoparticles (UCNPs), can be used to bridge the spectral gap between NIR and VIS. Upon excitation by lower-energy NIR photons, UCNPs can reliably emit shorter wavelengths within the VIS spectrum [24]. Recently, UCNPs have been used in different *in vivo* applications, such as non-invasive imaging of deep tissues, drug delivery, and photodynamic therapy [25–27]. When combined with different opsin proteins, UCNP techniques may have unique potential as novel and straightforward methods to achieve tetherless optogenetic control in deep brain regions [28,29].

In this study, we demonstrated all-optical tetherless brain stimulation using upconversion based NIR-actuated micro-devices. $NaYF_4$-based nanoparticles were used as optical transducers to convert NIR energy to visible light used to stimulate neurons expressing opsin proteins. The fully implantable microscale device was based on a UCNP-embedded glass micro-optrode, which was excited by an NIR laser (980 nm) to emit green or blue light, depending on different dopants in the nanoparticles. When the optrodes were implanted into the brain infected to express ChR2 or C1V1, NIR illumination reliably stimulated the neurons to fire action potentials. The upconversion-based device was then integrated with robotic instrumentation to implement an all-optical tetherless neural stimulation strategy. To demonstrate the unique advantages of the system for behavioral experiments in live animals, the UCNP-optrodes were implanted at various locations in mouse or rat brains. These locations included the cortical striatum, ventral tegmental area (VTA), and visual cortex. Our technique was successfully utilized to condition motor or learning behavior in freely moving animals. Because of the microscale size (~100 μm in diameter) and extremely light weight (less than 1 mg) of the UCNP-based implants, multiple devices could be simultaneously placed in a rodent brain to achieve sufficient activation to modulate complex brain function. We also demonstrated the long-term biocompatibility and functionality of the UCNP-optrode device, which remained effective even six months after surgical implantation. In sum, the upconversion-based tetherless neural stimulation technology opens up new possibilities and creates flexibility for remote control in deep brain regions, and can be easily adopted by other laboratories.

2. Results

2.1. System design

The emission of visible light from upconversion results from sequential discrete absorption of two or more lower-energy photons. Though this process can potentially enable the implementation of a method using NIR to stimulate neurons expressing commonly used light-sensitive ion channel proteins (e.g., ChR and C1V1, Fig. 1a), several technical challenges must be overcome before any practical implementation. First, the UCNPs need to be implanted in a biocompatible format with sufficient concentration, so that the implants are not toxic and the upconversion emission is sufficient to evoke optogenetic response. Second, the NIR irradiation has to target a specific part of the body of the freely moving animal (e.g., head for brain stimulation) for consistent tetherless delivery of stimulus signals. Accordingly, we made a fully implantable transducer device by sealing dry UCNPs in a glass micro-pipette to form an optrode (Fig. 1b and c). This package guarantees that there is no direct contact between the nanoparticles and neurons, effectively providing the UCNP-optrode with the same biocompatibility as an optical fiber, which is widely used in traditional optogenetic experiments. The micro-device was only ~100 μm in diameter and less than 1 mg in weight, which is greatly beneficial in preserving tissue integrity, as it leads to negligible brain lesion during surgical implantation. For remote delivery of NIR to the targeted body part of a behaving rodent (Fig. 1d), we further designed a robotic laser projection system, which was capable of automatically tracing the animal's head. We were able to place a single NIR illumination spot (3 cm in diameter) on the head of the rodent in real-time so that consistent NIR stimulation could be achieved (Fig. 1e). In this system, two rotational motors and a 3D-printed holding adaptor were assembled to form a robotic arm, which was placed ~50 cm above the arena and was used to project NIR illumination at arbitrary coordinates in a 40 × 40 cm experimental field. The control of the robotic arm and subsequently the placement of illumination spot were achieved using a custom image recognition program. The implantable UCNP-optrode and the autonomous laser projection system thus form a novel all-optical solution for tetherless control of brain function in freely moving animals.

2.2. Characterization of the upconversion-based transducer device

Engineered core-shell nanoparticles were used to make the UCNP transducer device (Fig. 2a). The nanostructure produces unique optical properties that are inaccessible by its bulkier counterparts [30]. UCNPs composed of lanthanide-doped $NaYF_4$ were fabricated via a layer-by-layer growth process and used as light transducers in this study. Our sophisticated nano-synthesis protocol offered exquisite control over particle size, morphology, and doping strategy with high reproducibility. The uniform spherical shape of the core, which had an average size of ~20 nm, was seen in transmission electron microscopy images in Fig. 2b. The particle size was slightly increased with some morphological variation in the corresponding core-shell UCNPs used in this study (Fig. 2c). Upon NIR (980 nm) illumination, the UCNPs comprising Tm^{3+} (or Er^{3+}) dopants in the core emitted blue (or green) light peaking at 470 nm (or 540 nm). After being packaged in a micro-optrode, the device typically produced an upconversion efficiency of ~4%, and the emission power of blue or green light was positively correlated with the input NIR power (Fig. 2d and e). Though this efficiency is relatively low, the intensity of visible light emission could be sufficient for regular ontogenetic simulation, which has a power density requirement of 1–5 mW/mm^2 [1].

2.3. Sufficient tissue penetration under safe NIR irradiation

As NIR energy is not strongly absorbed by water or biomolecules, NIR can penetrate much deeper into tissue than visible light [16], which is highly phototoxic and may cause serious damage