Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics

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Optogenetics has revolutionized the experimental interrogation of neural circuits and holds promise for the treatment of neurological disorders. It is limited, however, because visible light cannot penetrate deep inside brain tissue. Upconversion nanoparticles (UCNPs) absorb tissue-penetrating near-infrared (NIR) light and emit wavelength-specific visible light. Here, we demonstrate that molecularly tailored UCNPs can serve as optogenetic actuators of transcranial NIR light to stimulate deep brain neurons. Transcranial NIR UCNP-mediated optogenetics evoked dopamine release from genetically tagged neurons in the ventral tegmental area, induced brain oscillations through activation of inhibitory neurons in the medial septum, silenced seizure by inhibition of hippocampal excitatory cells, and triggered memory recall. UCNP technology will enable less-invasive optical neuronal activity manipulation with the potential for remote therapy.

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**Fig. 1. UCNP-mediated NIR upconversion optogenetics for deep brain stimulation.**

(A) Schematic principle of UCNP-mediated NIR upconversion optogenetics. (B) Transmission electron microscopy (TEM) images of the silica-coated UCNPs. (Inset) High-resolution TEM image showing the core-shell structure. (C) Schematic design of a blue-emitting NaYF₄:Yb/Tm@SiO₂ particle. (D) Emission spectrum of the NaYF₄:Yb/Tm@SiO₂ particles under excitation at 980 nm. (Inset) Upconversion emission intensity of UCNPs [0.18 mg, 200 mg/ml in 900 nl of phosphate-buffered saline (PBS)] as a function of excitation intensity at 980 nm. (E) Size distribution of the UCNPs measured by dynamic light scattering. No aggregation is observed in water, PBS, or bovine serum albumin (BSA, 1 mg/ml in PBS) solution. (F) Scheme of in vivo fiber photometry for measuring UCNP-mediated NIR upconversion in deep brain tissue. The tip of an optic fiber transmitting NIR excitation light was positioned at various distances from the VTA where UCNPs were injected, and their emission was recorded by a second optic fiber. (G) Upconversion emission at the VTA upon 980-nm NIR irradiation (25-ms pulses at 20 Hz, 2.0-W peak power) from varying distances. (H) Measured (n = 4 mice) and simulated intensity of upconversion emission at the VTA as a function of the distance from the NIR irradiation source. Data are presented as mean ± SEM.

**Fig. 2. NIR excitation of VTA DA neurons in vitro.**

(A) Experimental scheme. AAV-DIO-ChR2-EYFP was injected into the VTA of TH-Cre transgenic mice for Cre-dependent expression of ChR2 in DA neurons. Four weeks later, 900 nl of 200 mg/ml blue-emitting NaYF₄:Yb/Tm@SiO₂ UCNPs was injected into the VTA. Horizontal acute slices containing the VTA were prepared, and in vitro whole-cell recordings were performed. (B) Electron micrographs of UCNPs distributed in the VTA tissue. Black arrows indicate clusters of UCNPs. The upper image shows the distribution of the majority of UCNPs in extracellular space, and the lower image shows the uptake of UCNPs by an axon. (C) Voltage-clamp traces of neurons from VTA slice preparations in response to 100-ms NIR stimulation at various intensities. NIR light triggered photocurrents only in ChR2-transfected neurons in the presence of UCNPs. The traces for ChR2(−) and UCNP(−) controls in black were recorded under 8.22-W/mm² NIR irradiation. (D) Increase in photocurrent amplitude with elevated intensity of the NIR stimulation (n = 6 cells). (E) Current-clamp traces of a ChR2-expressing DA neuron in response to trains of 10 NIR pulses at different frequencies (20-ms pulses at 10 and 20 Hz, 10-ms pulses at 50 Hz, 8.22-W/mm² peak power) in the presence of UCNPs. Brief red lines indicate NIR pulses. (F) Spike probability as a function of the frequency of NIR stimulation (n = 6 cells). Data are presented as mean ± SEM.
the stimulation parameters should always be optimized for a balance between safety and efficacy.

To optimize the biocompatibility and long-term utility of UCNPs, we decorated the core-shell NaYF₄:Yb/Tm nanocrystals with silica (NaYF₄:Yb/Tm@SiO₂) or poly(acrylic acid) (PAA) (NaYF₄:Yb/Tm@PAA). Both coating strategies resulted in monodispersed UCNPs of ~90-nm diameter (Fig. 1, B and E, and figs. S1 and S2) with similar luminescence profiles (Fig. 1D and fig. S1). One month after injection, UCNPs could still be observed at the target site, regardless of the type of coating (figs. S10 and S11), suggesting their long-term stability and low dispersion in tissue. We selected silica-coated UCNPs for in vivo upconversion optogenetics because they showed minimum cytotoxicity compared to the PAA-grafted ones, as indicated by less glial activation and lower macrophage accumulation in the VTA over prolonged exposure (figs. S10 and S11). This is likely a result of the silica coating that chemically stabilizes the nanoparticles and prevents direct contact of their lanthanide-doped core to cells within the tissue (29).

We chose the VTA for an initial examination of NIR stimulation because it is a deep brain region with a well-characterized anatomy and function. An adeno-associated virus (AAV) encoding ChR2-EYFP (enhanced yellow fluorescent protein) in a double-floxed inverted open reading frame (DIO) was injected into the VTA of tyrosine hydroxylase (TH)-driven Cre recombinase (TH-Cre) transgenic mice, resulting in Cre-dependent expression of ChR2 in dopamine (DA) neurons (Fig. 2A). Four weeks later, 900 nl of 200 mg/ml blue-emitting NaYF₄:Yb/Tm@SiO₂ UCNPs was injected into the VTA. We first assayed UCNP-mediated optogenetic activation of DA neurons in acute slices. Electron microscopy (Fig. 2B and fig. S12) showed that the UCNPs were localized in the injection area without extensive diffusion. The majority were distributed in extracellular spaces in the vicinity of cell membrane and synaptic clefts. A small fraction of UCNPs were taken up by neurons, mainly localized to axons, as well as by microglia. The confinement of UCNPs in the target brain region agrees with our light microscopy results showing that UCNPs exhibited minimal dispersion 1 month after injection (figs. S10 and S11). The 980-nm NIR pulses triggered membrane depolarization sufficient to generate photocurrents and evoke spikes in VTA DA neurons. The photocurrent amplitudes of ChR2 increased in response to elevated intensity of the incident NIR light under voltage clamp (Fig. 2, C and D). In the absence of UCNPs, no inward photocurrent was detected upon NIR irradiation (Fig. 2C). The activation kinetics of ChR2 by NIR upconversion was slower than that of blue light-activated ChR2 (fig. S6) (30) but comparable to that of recently reported red-shifted rhodopsins (10). NIR irradiation could also evoke action potentials of DA neurons, as shown in current-clamp traces. Trains of NIR illumination at 30 to 50 Hz elicited multiple spikes (Fig. 2E). The spike probability showed no significant dependence on the frequency of the NIR light (Fig. 2F).

We next tested the in vivo utility of UCNP-mediated NIR upconversion optogenetics. We sensitized VTA DA neurons of TH-Cre mice to transcranial NIR stimulation through viral delivery of ChR2 followed by bilateral UCNP injection (Fig. 3A). Anesthetized mice were exposed to transcranial pulses of NIR irradiation (15-ms pulses at 20 Hz, 3 s every 3 min for 30 min, 30-W peak power, 15-mW average power) delivered from an optical fiber (200 μm in diameter) placed 2 mm above the skull (1.4-W/mm² NIR on the skull surface). NIR-activated DA neurons
were mapped by imaging the expression of c-Fos (Fig. 3, B and C, and fig. S13). Neuronal excitation was only triggered by NIR light in ChR2-transfected mice in the presence of UCNPs, as indicated by the significantly higher proportion of c-Fos-positive cells in areas where UCNP injection and ChR2 expression overlapped. We injected UCNPs to just one side of the VTA and observed NIR-induced c-Fos expression only in the injected hemisphere (fig. S14). We also observed up-regulation of c-Fos expression in the ventral striatum (fig. S15), which receives inputs from VTA DA neurons (37), indicating NIR-evoked excitation of postsynaptic structures of the targeted neurons. Control mice with UCNP injection, ChR2 expression, or NIR stimulation alone showed no significant c-Fos expression in either VTA or ventral striatum.

We evaluated the real-time efficacy of NIR-evoked excitation of VTA DA neurons with fast-scan cyclic voltammetry (FSCV) (Fig. 3D). Striatal DA transients reflect the phasic spike activity of VTA DA neurons (31) and have therapeutic implications for the treatment of major depression. In nomifensine-pretreated mice with both UCNP injection and ChR2 expression in VTA, we detected DA release that was temporally locked to transcranial NIR stimulation (15-ms pulses at 20 Hz, 700-mW peak power) (Fig. 3F). After a 2-s NIR stimulation, striatal DA release lasted for more than 15 s and peaked at ~5 s after light onset (Fig. 3, F and G). We detected no significant DA release in control mice with omission of NIR stimulation, UCNP injection, or ChR2 expression (Fig. 3, G to I). We compared the efficacy of NIR with blue light in evoking DA release by VTA DA neurons (Fig. 3E). The tip of an optic fiber transmitting NIR or blue light was positioned at various distances from the VTA target for optogenetic activation of DA neurons. When illuminating from a distance of 0.5 mm, NIR and blue light triggered similar amounts of DA release in ventral striatum (Fig. 3E and fig. S16). However, transcranial application of blue light did not result in striatal DA release (Fig. 3, E and I). Furthermore, NIR stimulation showed significantly slower attenuation in DA release with the increase of the distance from fiber tip to VTA (Fig. 3E).

We next expanded the application of in vivo upconversion optogenetics to multiple neural systems. A schematic of a green-emitting NaYF4:Yb/Er@SiO2 particle. (B) Illustration of transcranial NIR inhibition of hippocampal (HIP) activity during chemically induced seizure. (C) Confocal images of the hippocampus following transcranial NIR stimulation under different conditions. Significant decrease in c-fos (red expression) was observed only in the presence of both UCNPs (green) and Arch expression (labeled with EYFP, blue). Scale bars: 400 μm. (D) c-Fos expression under the four conditions presented in (C) (n = 3 mice each, F3,28 = 94.02, P < 0.0001). (E) Illustration of transcranial NIR stimulation of medial septum (MS) for generation of theta oscillations. (F) Confocal images showing the overlap between ChR2 expressing PV interneurons (labeled by EYFP, green) and UCNPs (blue) in the MS of a PV-Cre mouse. Scale bars: 50 μm. (G) Hippocampal LFP in response to 8-Hz transcranial NIR stimulation (15-ms pulses, 10 s, 3.0-W peak power, 360-mW average power) of MS under different conditions. Top: Raw LFP trace from mouse with both UCNP and ChR2 injection. Bottom: Z-scored power in the theta range averaged across 30-s trials in all three conditions. (H) Transcranial NIR entrainment of hippocampal theta in a frequency-dependent manner. (I) Illustration of transcranial NIR stimulation of hippocampal engram for memory recall. (J) Confocal image showing the overlap between UCNPs (blue) and ChR2 expression (labeled with EYFP, green) in the DG of a mouse that underwent habituation, fear conditioning, and test sessions presented in (K). Scale bar: 200 μm. (K) Mice were on Dox food and habituated with NIR stimulation (15-ms pulses at 20 Hz, 250-mW peak power) in context A for 5 days, then off Dox food for 2 days and fear conditioned in context B. Mice were put back on Dox food and tested for 5 days in context A with transcranial NIR stimulation. (L to N) After fear conditioning, only c-fos-TTA mice with both ChR2 expression and UCNP injection showed increased freezing during 3-min NIR-on periods. Orange lines indicate the NIR-on epochs. (O) Summary of freezing levels of the three groups during test NIR-on epochs (F2,25 = 105.9, P ≤ 0.0001). Data are presented as mean ± SEM.
anesthetized and received the excitotoxin kainic acid (KA) at a dose used to induce seizure. We then applied transcranial low-intensity chronic pulsed NIR irradiation (3-ms pulses at 10 Hz, 120 min, 750-mW peak power, 22.5-mW average power) to inhibit neural activity in the hippocampus. Compared to all controls, mice with Arch expression, UCNP injection, and NIR stimulation showed a significant decrease in KA-induced c-Fos expression in the granule cells of the DG (Fig. 4, C and D), indicating effective silencing of hippocampal neurons by UCNP-mediated Arch activation. When NIR irradiation or Arch delivery was only unilaterally applied, distinct levels of c-Fos expression were observed between the two hemispheres (fig. S17).

Next, we examined if UCNP-mediated optogenetics could be used for noninvasive synchronization of neural activity. We targeted our NIR stimulation to inhibitory neurons in the medial septum, a key node in the network generating the theta oscillation (32, 33). In vivo recordings of hippocampal local field potential (LFP) were performed during transcranial NIR irradiation of anesthetized parvalbumin (PV)–Cre mice with ChR2 expression and UCNP injection targeted to the medial septum (Fig. 4, E and F). Pulsed NIR stimulation in the theta frequency range (6 to 12 Hz) entrained the hippocampal theta oscillation in a frequency-dependent manner (Fig. 4, G and H). Control animals without ChR2 expression or UCNP injection showed no oscillation entrainment upon NIR irradiation (Fig. 4G).

Finally, we used the UCNP-mediated optogenetics to alter behavior of an awake animal by targeting NIR excitation to granule cells in the hippocampus involved in memory recall. Recent studies have demonstrated the neuronal activity–dependent tagging of memory-encoding hippocampal neurons by ChR2 and subsequent optogenetic reactivation of these engrams (34). We injected blue-emitting UCNPs into the DG of c-fos-tTA (tetracycline transcriptional activator) transgenic mice and labeled active c-Fos–expressing DG granule cells with ChR2 during the encoding of fear memory in the absence of doxycycline (Dox) (Fig. 4, I to K, and fig. S18). We then applied transcranial NIR stimulation (15-ms pulses at 20 Hz, 250-mW peak power) to reactivate labeled granule cells. NIR irradiation increased freezing behavior of the mice during laser illumination in a safe context (Fig. 4L). Animals with no UCNP injection or ChR2 expression showed no significant change in freezing when NIR irradiation was applied (Fig. 4, M to O). Moreover, the behavioral effect of NIR stimulation in this experiment was observed 2 weeks after the injection of UCNPs, indicating their long-term in vivo utility. This timing is consistent with our findings that no extensive diffusion or degradation of UCNPs was observed 1 month after injection (figs. S10 and S11).

These findings demonstrate that UCNP-mediated optogenetics is a flexible and robust minimally invasive nanotechnology-assisted approach for optical control of in vitro and in vivo neuronal activity. We show spectral tuning of UCNPs for compatibility with the current toolbox of light-activated channels (9) that is sufficient for functional activation and inhibition across a variety of deep brain structures. Future characterization of the interaction of UCNPs with neural tissue will allow for better biocompatibility and long-term utility. In parallel, systematic optimization of the dose of UCNP injection and the parameters of NIR stimulation will provide improved accuracy and safety. Such data might also present an upper limit to the adaptability and efficiency of NIR stimulation. Furthermore, refinements of the nanoparticles to establish precise cell-type or intracellular targeting (17, 18), as well as improved delivery methods that would further reduce invasiveness (35), will advance the utility of the approach. These methods, combined with the enhanced ability to express light-sensitive channels in the brain, may allow UCNP-mediated neuronal control to complement or extend current approaches to deep brain stimulation and neurological disorder therapies.

REFERENCES AND NOTES

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SUPPLEMENTAL MATERIALS
www.sciencemag.org/content/359/6376/679/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S18
Tables S1 and S2
References (36–46)
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Stimulating deep inside the brain

Noninvasive deep brain stimulation is an important goal in neuroscience and neuroengineering. Optogenetics normally requires the use of a blue laser inserted into the brain. Chen et al. used specialized nanoparticles that can upconvert near-infrared light from outside the brain into the local emission of blue light (see the Perspective by Feliu et al.). They injected these nanoparticles into the ventral tegmental area of the mouse brain and activated channelrhodopsin expressed in dopaminergic neurons with near-infrared light generated outside the skull at a distance of several millimeters. This technique allowed distant near-infrared light to evoke fast increases in dopamine release. The method was also used successfully to evoke fear memories in the dentate gyrus during fear conditioning.

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