Experimental Details for

Intracellular photoswitchable neuropharmacology driven by luminescence from upconverting nanoparticles.

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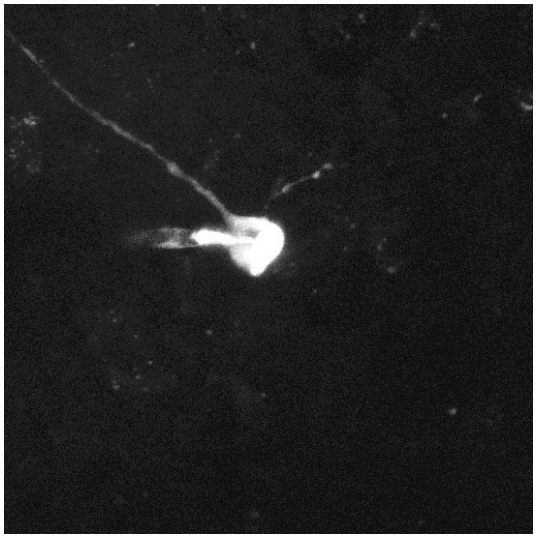

Supporting Figure S1. Image of luminescence from UCNPs loaded via a patch pipette into a CA1 neuron in hippocampal brain slices. Emission from the UCNPs was produced by excitation using NIR light. No light could be collected with a blue blocking filter.

Materials and methods.

All chemical were obtained from commercial sources and used without further purification. AAQ was from Tocris Bioscience (Bristol, UK), and the $NaYF_4$:TmYb nanoparticles from Creative Diagnostics (cat. no. dni-f001, Shirley, NY, USA).

UV-Vis absorption spectra were recorded using a Cary 50 spectrophotometer (Agilent, Santa Clara, CA, USA). Photolysis used UV or green LED (M365LP1, M530L3, Thorlabs, NJ, USA), and an Ultra II laser (Coherent, Palo Alto, CA, USA). For experiments shown in Fig. 2c, we dissolved the NPs in HEPES buffer at a concentration of 0.8 mg/mL with AAQ (0.040 mM) in a quartz cuvette (1 cm pathlength, volume 50 μL).

All animal studies were approved by Mount Sinai IACUC review. C57BL/6J mice (8-12 weeks.) were anaesthetized with isoflurane and the brain was quickly removed. Horizontal slice sections (350 μm) were then made in ice-cold cutting solution containing (in mM): 60 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 0.5 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, 100 sucrose, 3 sodium pyruvate, 1.3 sodium ascorbate equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). The brain slices were then incubated for 15 min at 33°C in artificial cerebrospinal fluid (ACSF, mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, 3 sodium pyruvate, 1.3 sodium ascorbate; 95% O_2 and 5% CO_2, pH 7.4). After incubation, all brain slices were held in ACSF at room temperature > 1 hour before patch clamp recording.

Brain slices were transferred to a submerged chamber on a BX-61 microscope (Olympus, Penn Valley, PA, USA) and perfused with ACSF (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, 95% O_2 and 5% CO_2, pH 7.4) at room temperature. Hippocampal CA1 pyramidal neurons were visualized with a 40X objective (Olympus) and infrared differential interference contrast optics. Whole-cell recordings were made with an EPC-10 amplifier (HEKA Instruments, Bellmore, NY, USA) in voltage-clamp mode (Vm holding at −60 mV) or current-clamp mode. The electrical signals were recorded at 20 kHz and filtered at 3 kHz with Patchmaster (HEKA). Recording pipettes (3-5 MΩ) were filled with an internal solution (in mM): 135 potassium gluconate, 4 $MgCl_2$, 10 HEPES, 4 Na_2-ATP, 0.4 Na_2-GTP, 10 Na_2-phosphocreatine, (pH 7.35). Glutamate receptor antagonists CNQX (10 μM) and DL-AP5 (100 μM) and the $GABA_A$ receptor antagonist bicuculline (20 μM) were added to the ACSF during experiments.

UV and green LEDs (365 and 530 nm, respectively) were mounted on the epifluorescence port of the microscope and light beams were combined and directed to the objective by standard long-pass dichroic mirrors. Light power was controlled via the LED driver (LEDD1B, Thorlabs) by external voltage modulation. Timing and duration of LED lights were controlled by TTL signals and synchronized with the EPC-10. NIR excitation used 975 nm output from a Vision II laser, directed onto the patch-clamped cell via an Ultima scan head (Prairie Technologies, Middleton, WI, USA).