Redshifted optogenetic excitation:
A novel tool for fast neural control derived from *Volvox carteri*

Feng Zhang, Matthias Prigge, Florent Beyrière, Satoshi P. Tsunoda, Joanna Mattis, Ofer Yizhar, Peter Hegemann, and Karl Deisseroth

SUPPLEMENTARY METHODS

Genomics

We identified VChR1 through BLAST searches on the U.S. Department of Energy Joint Genome Institute's server (http://genome.jgi-psf.org). We then used the identified protein sequence to generate and synthesize (DNA 2.0, Menlo Park, CA) a mammalian codon-optimized cDNA sequence (GenBank accession number 1080996).

Hippocampal neuron culture and electrophysiology

We removed hippocampi from postnatal day 0 (P0) Sprague-Dawley rats (Charles River) and treated the isolated hippocampi with papain (20 U/ml) for 45 min at 37°C. The digestion was stopped with 10 ml of MEM/Earle's salts without L-glutamine along with 20 mM glucose, Serum Extender (1:1000), and 10% heat-inactivated fetal bovine serum containing 25 mg of bovine serum albumin (BSA) and 25 mg of trypsin inhibitor. The tissue was triturated in a small volume of this solution with a fire-polished Pasteur pipette, and ~100,000 cells in 1 ml plated per coverslip in 24-well plates. Glass coverslips (prewashed overnight in HCl followed by several 100% EtOH washes and flame sterilization) were coated overnight at 37°C with 1:50 Matrigel (Collaborative Biomedical Products, Bedford, MA). Cells were plated in culture medium: Neurobasal containing 2x B-27 (Life Technologies) and 2 mM Glutamax-I (Life Technologies). One-half of the medium was replaced with culture medium the next day, giving a final serum concentration of 1.75%. No all-*trans* retinal was added to the culture medium or recording medium for any of the neuronal experiments here described.

For whole-cell recording in cultured hippocampal neurons, the intracellular solution contained 129 mM K-Gluconate, 10 mM HEPES, 10 mM KCl, 4 mM MgATP, and 0.3 mM Na_3GTP, titrated to pH 7.2. Tyrode's solution was employed as the extracellular solution (125 mM NaCl, 2 mM

KCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$, 30 mM glucose, and 25 mM HEPES, titrated to pH 7.3). Recordings were conducted on an upright Leica DM-LFSA microscope equipped with a 40X water-immersion objective. Borosilicate glass (Sutter Instruments) pipette resistances were ~5 MΩ, with a range of 4–6 MΩ. Access resistance was 10-30 MΩ and monitored for stability throughout the recording. All recordings were conducted in the presence of synaptic transmission blockers: 20μM gabazine, 10μM CNQX, and 25μM D-AP5.

Lentiviral vector construction

VChR1-EYFP was constructed by fusing *VChR1*(1–300) with EYFP via a *Not*I restriction site. We ligated the fusion gene subsequently into the *Age*I and *Eco*RI sites of lentiviral backbone to generate the *pLenti-CaMKIIa-VChR1-EYFP-WPRE* vector. Construction of the *pLenti-CaMKIIa-ChR2-EYFP-WPRE* vector was previously described[1]. Briefly, we PCR amplified *ChR2-EYFP* with *Age*I and *Eco*RI flanking the 5` and 3` ends respectively. Then we purified the PCR product and digested both the purified PCR product as well as the FCK(1.3)GW backbone[2] (a gift from Dr. Pavel Osten, Northwestern University) with *Age*I and *Eco*RI. We ligated *ChR2-EYFP* into the *FCK(1.3*)-W backbone. Detailed sequence information can be found at http://www.optogenetics.org.

VSVg pseudotyped recombinant lentiviruses were produced as previously described[1] and detailed (www.stanford.edu/group/dlab/optogenetics/expression_systems) by triple-transfection of 293FT cells (Invitrogen) with pLECYT, pVSVg, and pCMVdeltaR8.7. Virus supernatant was collected 40 hours after transfection and applied to neurons to facilitate gene transfer.

Optics

For hippocampal neuron photostimulation, the following four filters were used in the Lambda DG-4 optical switch (Sutter Instruments) with a 300W Xenon lamp: 406nm (FF01-406/15-25), 438nm (FF01-438/24-25), 531nm (FF01-531/22-25), and 589nm (FF01-589/15-25) (Semrock).

At 589 nm no ChR2 spikes were observed even at maximal power density, and indeed the action spectrum as measured in Figure 1 drops to 0 for ChR2 at 589nm. Further molecular engineering is required at the blue end, as we did not find a better choice for differential activation than 406 nm. Even at the next significantly longer wavelength with typical bandpass filters (438 nm) VChR1 already begins to drive spiking robustly (3.6 mW/mm^2 light: 82.2 ± 8.37% spike fidelity; 7.2 mW/mm^2 light: 96.7 ± 3.2% spike fidelity; 23.8 mW/mm^2 light: 99.0 ±

1.0% spike fidelity; *n* = 10 neurons), indicating that longer wavelengths will not provide improved blue-end separation. We did not extensively explore near-UV wavelengths shorter than 406nm as scattering and phototoxicity will become increasingly problematic, but VChR1 spiking can still be readily observed at 380nm as with ChR2.

VChR1 expression and electrophysiology in *Xenopus laevis* oocytes

For oocyte experiments, we subcloned a synthetic DNA sequence corresponding to $VChR1_{1\text{-}313}$ into pGEMHE and pEGFP. cRNAs encoding $ChR2_{1–315}$[3] and VChR1, synthesized *in vitro* from pGEMHE-plasmid by T7 RNA polymerase (mMessage mMachine, Ambion), were injected into the oocytes (50 ng/cell). The oocytes were stored for 3–7 days in the dark at 18°C in Ringer solution (96 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM MOPS-NaOH, pH 7.5) in the presence of 1 mg/ml penicillin, 1 mg/ml streptomycin, 1 µM all-*trans*-retinal (stock: 1 mM in 2-propanol), and 0.5 mM theophylline.

Two-electrode voltage clamp measurements on *Xenopus laevis* oocytes were performed with a Turbo Tec-05X (NPI Electronic, Tamm, Germany). Data acquisition and light triggering were controlled with pCLAMP software via DigiData 1322A or 1440A interfaces (Molecular devices, Sunnyvale, USA). The microelectrodes were fabricated by pulling borosilicate glass capillaries (1.5 mm outer diameter. and 1.17 mm inner diameter) using a micropipette puller (model P-97, Sutter instrument, Novato, USA) and filled with 3 M KCl. The resistances of microelectrodes were 0.5-1.5 MΩ.

Action spectra were recorded using tunable 10 ns laser flashes (400-620 nm). 4–9 x 10^{19} photons s^{-1} m^{-2} (at the surface of the oocyte) from a Rainbow OPO (OPOTEK, Carlsbad, CA) pumped by the third harmonic of a Brilliant b Nd-YAG-Laser (Quantel, Les Ulis Cedex, France) were applied to the oocyte via a 1 mm light guide. The laser intensity varied from flash to flash within a range of 5%. For action spectrum generation, amplitudes were normalized to represent equal photon exposure. The amplifier Tec-05X (NPI Electronic, Tamm, Germany) was compensated to keep the voltage change below 0.05 mV at a half-saturating laser flash. Data acquisition and light triggering were controlled with pCLAMP software via DigiData 1440A interfaces (Molecular Devices, Sunnyvale, USA). Data (averages of 10 to 30 recordings) were recorded at high gain with a sampling rate of 250 kHz in external solution containing 100 mM NaCl, 1 mM $MgCl_2$ and 0.1 mM $CaCl_2$, with 5 mM glycine (pH 9.0), 5 mM Mops (pH 7.5 and 6.5) or 5 mM citrate (pH 5.5).

VChR1 expression and electrophysiology in HEK cells

For expression of VChR1 in HEK cells, the EGFP-encoding region in pEGFP-N1 (Invitrogen) was replaced with the mCherry gene, and this modified plasmid was used to subclone *VChR1*. HEK293 cells were cultured in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum, 2mM glutamine (Biochrome, Berlin, Germany) and penicillin/streptomycin. Cells were seeded on cover slips at a concentration of 0.4 x 10^6 cells/ml and transient transfection was performed by using Transpass COS/293 (New England Biolabs, UK). Whole-cells patch clamp was performed 48h post-transfection.

Photocurrents of transient transfected HEK293 were recorded with conventional whole-cell patch-clamp method. The external solution contained [mM]: 140 NaCl, 2 $CaCl_2$, 2 $MgCl_2$ with 10 HEPES (pH 7.2), 10 Tris (pH 8.5) or 10 citrate (pH 5.0 and pH 6.0). The internal solution contained [mM]: 120 NaCl, 10 EGTA, 2 $MgCl_2$ and 10 HEPES (pH was adjusted to 7.2 either using CsOH or HCl). For ion selectivity experiments, to record pure proton photocurrents, NaCl and $CaCl_2$ were substituted with N-Methyl-Glucamine; intracellular solution was 110 mM NMG-Cl, 2 mM $MgCl_2$, and 10 mM EGTA at pH 7.2, and extracellular solution was 10 mM HEPES, 2 mM $MgCl_2$, and one of 140 mM NMG, 140 mM NaCl, 140 mM KCl, or 70 mM $CaCl_2$. A 75 W Xenon lamp (Jena-Instruments, Jena, Germany) was used for the source of light pulses. The light was passed through a K50 filter (Balzers, Liechtenstein) and applied to the oocytes by using a light guide (diameter of 2 mm). 100% light intensity corresponded to 4.5 x 10^{21} photons m^{-2} s^{-1} at the cell surface; under these conditions, mean τ_{off} was found to be 96±31ms.

References

1. Zhang, F.*, et al.* Multimodal fast optical interrogation of neural circuitry. *Nature* **446**, 633-639 (2007).
2. Dittgen, T.*, et al.* Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 18206-18211 (2004).
3. Nagel, G.*, et al.* Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc Natl Acad Sci U S A* **100**, 13940-13945 (2003).