Photostimulation for optogenetics or uncaging V.2

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Protocol status: Working
We use this protocol and it's working

ABSTRACT

Protocol for photostimulation, to be combined with any other electrophysiology/imaging recording approach.
Once the desired patch clamp recording (e.g., cell attached, whole cell, perforated patch) and/or imaging (e.g., genetically encoded sensor or synthetic dye) configuration is achieved and a stable recording is established, photostimulation can be used to stimulate presynaptic terminals/cell expressing an opsin of interest (e.g., Chronos or Channelrhodopsin) or to release a photo-sensitive caged compound (e.g., RUBI-GABA, RUBI-Glutamate, MNI-glutamate...), and test the effect on the experimental outcome of interest (e.g., excitatory/inhibitory response).

GUIDELINES

Follow institutional guidelines.

MATERIALS

See protocols for slicing and electrophysiological/imaging recordings for the reagents and the equipments needed in order to set up the desired experiment.

Below are indicated materials/equipment specifically required for the photostimulation protocols here described:

Wide-field LED stimulation:

Larger area photo-stimulation is available with the CoolLED pE-100 blue LED (470nm, 540mW; via Tek5 Systems in USA) with 1% intensity via remote control and 3mm light guide optical coupling to the Olympus BX-51WIF rear epi-fluorescence port. The blue light is routed to the sample with a Chroma 49002 ET eGFP large (BX2) Olympus filter cube. A manual shutter on the microscope epi-fluorescence turret is used in series to block exposure; the LED can be remotely and synchronized activated by a TTL signal from *WinFluor* software and the NI-6713 output card. The minimum response timing pulse is ~1/3ms. The maximum exposure field of view with the 60x/1.0NA objective is ~415mm diameter spot; this area can be reduced by using the condenser field iris inside the reflected fluorescence RFA module of the Olympus

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BX-51 upright microscope.

Targeted laser stimulation

Prairie Aurora Launch (473nm, 50mW rated laser, with ~80mW when delivered) with AOTF intensity control (sub-ms
responses) and metal clad fiber optical coupling to the Ultima scan head. A mechanical hard shutter is located within the Aurora launch (Uniblitz) to remove sample laser exposure when not photo-stimulating. The 473nm shutter is controlled by th *WinFluor* software via a NI-6713 card output; the control is separate in *PraireView* 5.2. The launch optics are designed to deliver a sub-um spot size at the focal plane of the 60x/1.0NA objective lens, but due to the 1P source there will also be lower intensity stimulation above and below the focal plane fanning out in an hourglass shape. The typical sample maximum power is ~18mW, but most active workstation systems have reduced this power to ~1.8MW maximum
with a 1.0 N.D. filter to better mimic biological responses when using sub-mm spot illumination and to move the required drive signals to a more robust region of the SIN^2 voltage-power calibration curve. *Inserting the silver rod in the launch adds a lens into the blue light path which will increase the sample exposed area to ~10mm diameter at the focal plane.* The Helios laser launch has the same system details for 473nm photo-stimulation, but the new launch uses the Coherent IBIS
laser system for the light source and is also designed from the block up to be much more stable with thermal changes.

Caged compounds:

Caged compounds (e.g., RUBI-GABA, RUBI-glutamate, MNI-glutamate) are dissolved in stock solutions in the dark to the desired concentration according to the manufacturer's instructions.

Caged compounds can be diluted and delivered in the recording solution, via **peristaltic pump** or **gravity flow** (depending on the preferred electrophysiology/imaging setup).
Alternatively, they can be delivered via a **microinjector** with the outlet positioned in immediate proximity of the recording area.

SAFETY WARNINGS

Follow institutional guidelines.
Use appropriate PPE.
Check reagents SDS and equipment's operating instruction manual.
Highly photosensitive reagents are used in this protocol - take precaution to limit light exposure.

BEFORE START INSTRUCTIONS

This protocol assumes that slices have already been obtained (see for example dx.doi.org/10.17504/protocols.io.dm6gp328jvzp/v2) and that an experimental recording approach has already been established (see for example dx.doi.org/10.17504/protocols.io.36wgq3y9olk5/v1 or dx.doi.org/10.17504/protocols.io.eq2lyj8zrlx9/v1), and it is meant only as an add-on to those protocols.

Preliminary steps

1 Obtain brain slices of region of interest according to existing protocols

1.1 Optogenetic experiments require the expression of genetically-encoded opsins in neurons projecting to the area of interest. Mice lines expressing opsins under cell-specific or inducible promoters are available. Alternatively, opsin expression can be induced via injection of appropriate viral vectors (see existing protocols for viral injections). For the specific case we are considering experiments on slices expressing the excitatory opsin Chronos, which is stimulated by blue light (~470nm). For slices expressing opsins, limit exposure to intense light during slice preparation and incubation steps.

1.2 Uncaging experiments can be performed on naive slices, and different caged compound can be applied to the slice of interest and photostimulated as needed. For this specific case, we are considering experiments using RUBI-GABA, which is stimulated by blue light (~470nm). If the experiments include imaging, plan experiments accordingly so that compatible excitation wavelengths and/or intensity probes/compounds are employed. Caged compounds can be bath applied or microinjected over the site of recording.Caged compounds are intrinsically **light sensitive**, so any light exposure should be strictly avoided during reconstitution, and particular attention should be paid to shielding all solutions, tubings, and equipment from light.

2 Turn on hardware (including the light source for the desired photostimulation approach) and software

required for the electrophysiology/imaging experiment of choice.

3 Identify/patch neurons of interest and adjust settings according to the desired experimental protocol .

4 Once a recording on a cell of interest is established, set up the stimulation protocol.

4.1 When performing photostimulation combined with imaging experiments, if needed the photostimulation protocol should include a protective step for the light detectors (e.g., the closure of a shutter synchronized with the photostimulation pulse).

Stimulation protocol

5 Either a wide-field LED stimulation or a target focal spot blue laser can be utilized, depending on the preferred experimental approach. **Note that the excitation wavelength might vary with the opsin/compound utilized.**

5.1 Wide-field LED stimulation is achieved with the CoolLED pE-100 blue LED (470nm) activated via the recording/controlling software.

5.2 For target focal spot blue laser (473nm) stimulation, stimulating spots (sub-micron size) were positioned adjacent to individual spines. The laser power was calibrated to evoke a somatic postsynaptic potential of 2-5mV. For simultaneous stimulation of 5-10 spines, a larger blue spot (8 microns) is used.

6 Synchronize desired stimulation protocol with recording.

7 Start recording/imaging acquisition synchronized with a stimulation protocol.

7.1 **For uncaging:** if the caged compounds are bath applied, allow sufficient time for full replacement of the solution in the recording chamber and permeation in the slice before initiating the stimulation protocol (at least ~5minutes, but this might vary based on the length of tubing, flow rate, size of the chamber). If the caged compounds are microinjected over the site of recording, the stimulation protocol can be initiated shortly after the application of the compounds.

8 Adjust stimulation settings based on the desired experimental outcomes.

After the experiment

9 Terminate experiment, remove electrode and slices, and shut down software/hardware according to the experimental protocol used.

10 Discard waste according to institutional guidelines.

11 Clean up work station.