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We use this protocol and it's working

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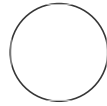
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## Photostimulation for optogenetics or uncaging

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### ABSTRACT

Protocol for photostimulation, to be combined with any other electrophysiology/imaging recording approach.

Once the desired patch clamp recording and/or imaging configuration (cell attached, whole cell, perforated patch) 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...).

### 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 BX-51 upright microscope.

#### Targeted laser stimulation

Prairie Aurora Launch (473nm, 50mW rated laser, with ~80mW when delivered) with

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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 the *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<sup>2</sup> 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.

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](https://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](https://doi.org/10.17504/protocols.io.36wgq3y9olk5/v1) or [dx.doi.org/10.17504/protocols.io.eq2lyj8zrlx9/v1](https://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 line expressing opsins under cell-specific or inducible promoters re 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). 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 Identify and patch neurons of interest according to the desired experimental protocol.
- 3 Once a recording on a cell of interest is establish, set up the stimulation protocol.

### Stimulation protocol

- 4 Either a wide-field LED stimulation or a target focal spot blue laser can be utilized, depending on the preferred experimental approach.

- 4.1 Wide-field LED stimulation is achieved with the CoolLED pE-100 blue LED (470nm) activated via the recording/controlling software.
- 4.2 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.
- 5 Synchronize desired stimulation protocol with recording.
- 6 Start recording.

## After the experiment

- 7 Terminate experiment, remove electrode and slices, and shut down software/hardware according to the experimental protocol used.
- 8 Discard waste according to institutional guidelines.
- 9 Clean up work station.