JAN 24, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5jyl8pxz6g2w/v1

Protocol Citation: Mai-Anh Vu, mwhowe 2024. Targeted optogenetic stimulations. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8pxz6g2w/v1

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Protocol status: Working

Created: Jan 09, 2024

Last Modified: Jan 24, 2024

Targeted optogenetic stimulations

In 1 collection

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ABSTRACT

We have developed a new micro-fiber array approach capable of chronically measuring and optogenetically manipulating local dynamics across over 100 targeted locations simultaneously in head-fixed and freely moving mice, enabling investigation of cell-type and neurotransmitter-specific signals over arbitrary 3-D volumes . This protocol includes the steps for targeted optogenetic manipulation. Please contact us (mwhowe@bu.edu) if you are interested in using this technique.

PROTOCOL integer ID: 93204

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020370

Targeted optogenetic stimulations

1 To couple light into individual optical fibers in our array for targeted optogenetic manipulations, simultaneously with imaging, we integrated a programmable digital mirror device (DMD, Mightex Polygon1000 Pattern Illuminator DSI-K3-L20) into the light path of our imaging microscope (see Multi-color fiber array imaging protocol) as detailed via the substeps below:

1.1 Excitation light was provided by a 3.2W, 465nm laser (Mightex, LSR-040-0465), which was coupled to the DMD with an optical fiber.

1.2 Light from the DMD was coupled into the objective path by a dichroic (Chroma 570lpxr).

1.3 570nm excitation and emission filters (see Multi-color fiber array imaging protocol) enabled simultaneous imaging of red fluorophores during stimulation.

2 Control of light patterning and stimulation parameters was achieved with PolyScan2 control software (Mightex) and custom MATLAB functions.

3 A calibration step was performed prior to stimulations to align the camera view with the PolyScan2 software, allowing us to design patterns of circular light (~40µm diameter for each spot) to target individual fibers.

4 Transmission efficiency through 50µm diameter fibers in our arrays was calculated at approximately 39% based on comparisons between power at the objective and transmitted light through individual fibers.

4.1 For stimulations of dopamine release, a light spot of ~750 µW (measured at the objective) was used, resulting in an estimated power density of 175 mW/mm2 (0.29 mW total power) at each implanted fiber tip.

4.2 For stimulations of D1 expressing neurons, a light spot of ~600 µW at the objective was used, resulting in an estimated power density of 141 mW/mm2 (0.23 mW total power) at the fiber tip.

5 We estimated relative excitation light intensity and excitation area as a function of distance from the fiber tip by applying a light scattering model developed by Yona et. al 2016 with a scattering coefficient of 140 cm-1 approximated for the striatum based on Azimipour et al 2014 and Al-Juboori et al. 2013 and an activation threshold for ChR2 of 1 mW/mm^2 70,71.

6 Light pulse trains for stimulation (30Hz, 4ms pulse width, 1s or 5/10s durations for DA and D1 neuron stimulation respectively) were programmed in PolyScan2 and triggered with 5V digital pulses controlled via MATLAB and sent from a NIDAQ board (National Instruments PCIe 6343) to the Polygon. Stimulations were triggered randomly (inter-stimulation interval 30-60 seconds) for 5-10 minutes per session for all stimulation experiments.