Sep 15, 2022

Protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice

DOI
dx.doi.org/10.17504/protocols.io.rm7vzbn94vx1/v1

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Protocol Citation: Mark Bevan 2022. Protocol for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice. **protocols.io https://dx.doi.org/10.17504/protocols.io.rm7vzbn94vx1/v1**

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

Created: September 15, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 70102

Keywords: ASAPCRN, optogenetic manipulation of basal ganglia neuron, basal ganglia neuron, optogenetic manipulation, neuron, mice step by step procedure, fixed mice step, fixed mice, awake head

Abstract

Step by step procedure for in vivo electrophysiological and optogenetic manipulation of basal ganglia neurons in awake head-fixed mice

Materials

All animal procedures follow the policies of the National Institutes of Health and have been approved by the Institutional Animal Care and Use Committee of Northwestern University.

Reagents

- 0.1 m phosphate buffer (PB), pH 7.4
- 4% paraformaldehyde (PFA)
- Filtered HEPES-buffered synthetic interstitial fluid (HBS)
- Isoflurane
- Ketamine
- Nolvasan surgical scrub
- Phosphate buffered saline (PBS)
- Trypsin

Instruments and Materials

- 473 nm or 633 nm diode laser (LuxX, 100mW)
- Anesthetic induction chamber
- Autoclaved fine science surgical tools, including scalpel, scissors, forceps
- Blackfly S USB3 (522 fps)
- Cheetah Data Acquisition software
- Computer with monitor and software
- Cotton tipped applicators
- Digidata 1440A digitizer
- Digital Lynx SX experiment control system
- Head-restraint assembly
- Headpost holders
- Laser safety goggles
- LinLab software
- Microelectrode holder
- Motorized micromanipulator
- Needles and syringes
- Neuralynx Headstage
- Optical patch cables with ceramic sleeves
- Omicron laser control software
- PatchPad
- pClamp software
- Replacement treadmill belts
- Stereotaxic frame and instruments for mice
- Silicon neural probes / optrodes
- SpeedBelt linear treadmill apparatus
- SpinView software
- Surgical cellulose eye spears

Upright microscope

Troubleshooting

In vivo recording preparation

1 Before and after any procedures, spray down working lab surfaces and equipment with 5% Nolvasan to disinfect the surgical area.

2 Autoclave all surgical instruments and ensure that sterility is maintained throughout the procedure.

3 Ensure at least 1 mL of filtered HEPES-buffered synthetic interstitial fluid (HBS) has been thawed. Fill sterile syringe with HBS, label, and store on ice.

4 Place the mouse in an anesthetic induction chamber and lightly anesthetize with 2-3% isoflurane.

5 Transfer the lightly anesthetized mouse to the SpeedBelt linear treadmill apparatus. The treadmill consists of a fabric belt (60cm length x

6 5cm width) wrapped around ball bearing mounted pulleys that ensure low friction gliding support for the animal.

7 Gently raising the animal's head, firmly screw and secure the animal's headplate into the headpost holder brackets. The head-restraint assembly is custom made using components from Thorlabs and Luigs & Neumann for maximum stability and durability. The headplate should be raised approximately 2.5 cm (per 25g mouse) above the treadmill belt.

8 Using fine point forceps grasp onto the Kwik-Sil mount within the headplate window and firmly pull off to remove from the skull. Both bregma and the in vivo recording craniotomy should be visible.

9 Irrigate the surface of the brain with HBS to wash away any blood or debris. If bleeding occurs during this or subsequent steps, place a surgical cellulose eye spear over the area to absorb the blood while gently applying pressure.

Probe implantation

10 Draw away excess HBS from the in vivo recording craniotomy. Using a bent injection needle carefully remove the dura within the craniotomy site. Removal of the dura requires extreme care and patience to avoid unnecessary complications. When performed correctly the brain will appear to be unblemished with distinct blood vessels.

18. Let the probe rest in place and wait for at least 10-15 minutes for the tissue to settle.

11 Keep craniotomy irrigated with HBS throughout the remainder of the experiment to prevent drying.

12 If performing optogenetic experiments, 473 nm or 633 nm light can be delivered through an optical fiber using a laser (LuxX diode laser, 100mW). Interconnect the laser light source to the fiber cannula on the silicon probe using an optogenetic patch cable. For best performance, choose a patch cable and cannula with the same fiber core diameter and numerical aperture. Use the key switch to turn off the interlock. The laser output shutter and laser attenuation are each controlled from computer signals using a Digidata 1440A digitizer, pClamp software, and Omicron laser control software. Laser intensity should be calibrated as power at the tip of the optrode before probe implantation and verified at the conclusion of each experiment. Laser safety goggles should be worn during laser calibration procedures. The laser should remain shuttered until stimulation is required.

13 Firmly connect the silicon probe onto the Neuralynx Headstage and electrode holder by using the pin adapters.

14 Affix the electrode holder (with connected probe and headstage) onto the motorized micromanipulator. Mount the micromanipulator onto a stereotaxic arm positioned within the head-restraint assembly. The micromanipulator can be used to generate precise and reliable movements with the silicon probe in the vertical axis. The direction, distance, and speed of these movements can be controlled through a PatchPad device and LinLab software.

15 Lower the electrode holder so that it is 10-15 mm above the animal's head. Attach the reference electrode pin to the socket connector on the Neuralynx Headstage.

16 Gently lower the silicon probe and measure bregma carefully using the tip of the electrode. Use stereotaxic coordinates to locate your target position and move the silicon probe accordingly into position for in vivo recording.

17 Gently lower the silicon probe to the surface of the brain and identify the z-coordinate to approximate the depth of recording.

18 Using the PatchPad control device slowly lower the silicon probe into the brain until it is resting 1 mm above the target structure. During brain tissue entry, if there is bleeding or if the electrode bends, remove the silicon probe, clean the site, and verify that dura has been properly removed.

In vivo electrophysiological recording

19 Turn on Cheetah Data Acquisition software. This is the main user interface for the graphic display, acquisition, and control of electrophysiological recording. Signals are sampled at 40 kHz, with a gain of 14x. Online digital finite impulse response filters are applied. Single-unit activity is bandpass-filtered between 200 and 9000 Hz, and local field potential signals are bandpass-filtered between 0.1 and 400 Hz.

20 To monitor the mouse's movements on the treadmill load the velocity encoder configuration file using pClamp software. The SpeedBelt linear treadmill apparatus consists of several electrical components and the position of the mouse is determined by converting the output voltage of the rotary encoder to the mouse's velocity. Signals are sampled at 1 kHz. This can be used for the measurement of speed and distance of locomotion during electrophysiological recording. For synchronizing the recording devices, a transistor-transistor logic (TTL) signal from the digitizer to the Cheetah Data Acquisition system is used.

21 Turn on SpinView software. This is the main user interface for the graphic display, acquisition, and control of high-speed video capture. Video recordings are collected with two high-definition cameras mounted in front and on the side of the head-fixed apparatus. Frames are sampled at 100 fps. This can be used to track the kinematics of each movement during electrophysiological recording. Acquisition is controlled by a TTL signal from the digitizer and this signal is used to synchronize the behavioral data with neural recordings.

22 Using the PatchPad control device slowly lower the silicon probe into the region of interest. Move the probe through the entire depth stopping to record neural and behavioral data.

23 If performing optogenetic experiments, use pClamp software and Omicron laser control software to regulate the laser output and laser attenuation.

Experiment completion and removal from treadmill apparatus

24 At the end of each experiment, unscrew the animal's headplate from the headpost holder brackets and euthanize the animal with a lethal dose of ketamine. Perfuse transcardially with ~5-10 ml of 0.01 m phosphate buffered saline (PBS) followed by 15-30 ml of 4% paraformaldehyde (PFA) in 0.1 m phosphate buffer (PB), pH 7.4. Remove the brain and postfix overnight in 4% PFA (in 0.1 m PB, pH 7.4).

25 Clean the treadmill belt with soap and water and replace treadmill belts regularly.

26 Clean the silicon probe by soaking in Trypsin (diluted 1:10 in distilled water) overnight. Rinse well with distilled water the next day and allow to air-dry.