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Fiber Photometry (Mouse)

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ABSTRACT

This protocol describes the procedure for fiber photometry in awake behaving mice. It includes details on the surgical implantation of fibers.

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1 Surgery.

A full protocol for stereotaxic surgery is available separately here:
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This section describes special considerations for implanting fibers.

1.1 Acquire fibers.

- Prefabricated optical fibers are used for photometry, which are 200 or 400 microns in diameter (Thorlabs)
- Keep a record of the specs of each fiber (diameter, length)

1.2 Drill holes over target structure.

- Use a single drill hole for 200 micron fibers
- Use a “cloverleaf” drill pattern for 400 micron fibers - this is achieved by drilling a single hole at the desired AP/ML coordinates, then moving the drill bit 0.2 mm in each direction (anterior, posterior, lateral, and medial) to create a larger hole for the fiber to be inserted

1.3 Implant optical fibers.

- Inject virus before implanting fibers
- Implant the fiber tip 100 microns above the coordinates at which virus was injected

1.4 Testing should occur a minimum of 2 weeks after surgery, to allow for viral expression.

2 Habituation.

Habituate the mouse to tethering and the behavioral chamber for **30 minutes/day for two days** prior to starting testing sessions.

2.1 Scruff the mouse and attach the optical fiber patch cable(s) to the mouse’s implant(s). The patch cables should be plugged into an optical commutator (Doric Lenses) located above the chamber to allow freedom of movement.

2.2 Place mouse in the behavioral chamber, e.g. a clear acrylic cylinder, 25 cm in diameter.

2.3 Monitor the mouse for the duration of the session to ensure it does not become tangled by the patch cable(s) and moves freely about the chamber.

3 Computer and optical setup.

3.1 Turn on the computer.

3.2 Open Arduino.
If just performing fiber photometry, without other stimulation, choose “SendTTLpulse”. This will send a single pulse to the Synapse (photometry) software at the start of the recording and with any other user-identified event times (such as levodopa injection).

3.3 Open Synapse. Select Experiment (top left pull-down menu) and the mouse name.

3.4 Turn on TDT RZ5P (power button on top left of box, blue light turns on).

3.5 On the Photoreceiver there are only 2 switches, the one on the left is red. Only touch this one! Flip it towards you to turn the photoreceiver on. Turn on the LED Driver (switch on left side of box). You should see the screen turn on.

3.6 There are 2 knobs on the tops of the LED Driver. Press and hold the left knob by pushing on the top for ~4 sec (the light on the top of the button should turn on, then off, when it turns off you can release). An asterisk should appear next to the “1 LED” line. Do the same for the right knob.

3.7 Now, just press (without additional hold) the left knob 3 times. Under MODE, you should see it switch from OFF to MOD. Do the same for the right knob.

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3.8 Rotate the left and right knobs clockwise until the numbers under I(mA) read 200.

4 Testing.
These steps are specific to the type of experiment you are running. For a simple pharmacological experiment, e.g. injection of levodopa in the open field while monitoring dyskinesias, follow the instructions below.

4.1 Scruff the mouse and attach optical cable to the photometry implant, and place in his/her home cage, adjacent to but not in the open field.

4.2 Press the red “Record” button in Synapse. This will start the experiment. It is important that you start the photometry recording before any other signals, as it will serve as the master data file, collecting timestamps from all other devices (TTLs from the device controlling video camera frames, Master8, etc).

4.3 Check to make sure the cameras are in a good position to see the whole arena and then place the mouse into the open field. This is to ensure that there is an empty open field frame at the beginning of the video for subsequent behavior tracking.

4.4 To check the fiber signal, right click the axis on the computer screen and select auto scale to bring signal into frame.

4.5 Wait 15 min for the mouse to acclimate further, and for the fiber signal to bleach. Then run sendTTLPulse in Arduino to signal the start of the experiment.

4.6 Wait 30 min to establish a baseline.

4.7 Inject mouse with levodopa (or other pharmacological agent) and run “sendTTLPulse” again to signal the injection timing.

4.8 Wait 2 hours, recording AIMs for one minute every five minutes.

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4.9 Wait an additional 30min (assuming mouse has returned to baseline behavior), then stop experiment in Synapse.

5 Cleanup.

5.1 Remove mouse from chamber and unplug optical cable(s). Return to home cage.

5.2 Clean chamber with 70% ethanol between mice and at end of the day.

5.3 When experiments are completed for the day, turn off the laser, close Arduino and Synapse programs. Transfer and back up files.

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