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Optogenetic Manipulation (Mouse)

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ABSTRACT

This protocol describes the steps for in vivo ontogenetic manipulation in mice, including assembly of fiber-ferrules, surgical implantation of fibers, and testing procedure.

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1 Fiber-ferrule implant assembly.

1.1 To make your own optical fiber-ferrule assemblies, you will need a reel of cladded optical fiber (ThorLabs, 0.39 NA, 200 micron multimodal fiber diameter, #FT200UMT), ceramic ferrules (ThorLabs, 6.4 mm ceramic ferrule, 230 micron bore, #CFLC230-10), a fiber stripping tool, a fiber cleaving tool, a petri dish, 5 minute epoxy, and optical sandpaper (see ThorLabs “fiber polishing supplies”).

1.2 Cut approx 2 cm lengths of fiber by using the cleaving tool to create cut, and then breaking cleanly on edge of the counter. Cut about 10 such lengths at a time.

1.3 Slide ceramic ferrules over the end of these lengths of fiber and lay in the bottom of a petri dish.

1.4 Prepare 5 minute epoxy in a small weigh boat, stirring with a 10 microliter pipet tip thoroughly. Use this tip to apply a small amount of epoxy along a few mm in the upper half of the fiber (below the ferrule), and slide the ferrule down over the epoxy, leaving at least the length of fiber you want to implant below this level untouched by epoxy and exposed below the bottom (flat) portion of the ferrule. There should be some excess fiber above the top (rounded) portion of the ferrule. Lay this into another petri dish to dry/set. Repeat process on other fibers/ferrules until the epoxy has hardened in the weigh boat, and then make another batch as needed until you have glued all the fibers/ferrules you want to make.

1.5 Let sit for >2 hours or overnight.

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1.6 Once set, break off the excess fiber above the top (rounded) end of the ferrule and fit the ferrule into a polishing puck or pen-type device.

1.7 Start with the coarsest polishing film, making figure 8 movements with the top of the ferrule against the film, while holding the puck or pen-type device as vertical as possible. When you no longer see clear figure 8s on the film, move to the next finer film, then repeat on this film. Finally, move to the finest film.

1.8 Once you feel you have polished the fiber well, test its transmittance. Attach a fiber optic patch cable to a laser or LED driver, and measure the output at the tip of the patch cable using an optical power meter. Adjust light power so this is about 10 mW. Now fit your fiber-ferrule assembly in the tip of the patch cable and re-measure the light power. If it is 8 mW, that is 8/10, or 80% transmittance. If that is 3 mW, that is 3/10, or 30% transmittance, and so on. If the fiber is >70% transmittance, it is likely acceptable and you can place it in a petri dish with a line of dental wax down the center. Place a piece of tape adjacent to the dental wax and use a sharpie to write down the transmittance adjacent to the place you put this fiber-ferrule assembly. Repeat for each fiber until you have made the number you need. Place the top over this petri dish and store in a drawer until needed.

2 Surgery.

A full detailed protocol for performing stereotaxic surgery is available here:
dx.doi.org/10.17504/protocols.io.n2bvj6qynlk5/v1
The steps below describe specific considerations needed to implant optical fibers.

2.1 Drill hole over target structure.
Lab-fabricated optical fiber-ferrule assemblies are 200 microns in diameter (Thorlabs), for which a single drill hole is used.

2.2 Inject virus at the target site before implanting fibers.

2.3 Note the transmittance of the fiber implant. If you are using 2 fibers, choose two with similar transmittance.

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2.4 Insert the fiber-ferrule assembly in a ferrule “stick” made of old/empty ceramic ferrules and sleeves, and place this stick into the grooved wand which can be attached to the stereotax. A little wax can help keep the stick on the wand, but you can also use the clamp to keep the stick attached to the wand.

2.5 Lower the fiber over about 10 seconds to 100 microns above the coordinates at which you injected the virus.

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

3 Habituation.

3.1 Habituate the mouse to tethering and the behavioral chamber for 30 minutes/day for two days prior to starting testing sessions. To habituate to tethering, scruff the mouse and attach the optical fiber patch cable(s) to the mouse’s implant(s) and place in the behavioral chamber, eg a clear acrylic cylinder, 25 cm in diameter. The patch cables should be plugged into an optical commutator (Doric Lenses) located above the chamber to allow freedom of movement.

3.2 Monitor in 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.

4 Computer and optical setup. You can send TTL pulses to the laser device via several systems (Noldus, Master8, Arduino).

4.1 Turn on the laser device and turn the key to ON.

4.2 Initially, set to manual control to test the light power output (if you have not calibrated it recently). Measure the power output using the patch cable(s) you will be using for the experiment and adjust so the power output from the tip of the patch cable x the transmittance of your fibers will yield the desired light delivery to the brain, eg 1.2 mW tip of cable x 80% transmittance fiber = 1.0 mW in brain.

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4.3 Change so the laser is set to TTL control (light should be off).

4.4 Turn on the computer that runs one of these systems and choose the appropriate routine or template. You may wish to test that it delivers light pulses at the appropriate time/frequency before you plug in the mouse. If simultaneously performing electrophysiology with Noldus video tracking, you will want to verify that TTL inputs are also being sent by Noldus/received by electrophysiology system for subsequent alignment.

5 Testing. These steps are specific to the type of experiment you are running.

5.1 Scruff the mouse and attach optical cable(s) to the optical implant(s), and place in his/her home cage, adjacent to but not in the open field. In most cases, you will be obtaining behavioral data with video.
- If using Noldus, choose the appropriate template, capture a background image, and place the mouse in the behavioral setup to optimize detection settings.
- If doing simultaneous electrophysiology, start this recording first, as it will serve as the master data file, collecting timestamps from all other devices.

5.2 Start the Noldus recording, which will both capture video and send TTLs to the laser at specified times. Alternatively, you can use other devices to drive video capture and/or laser pulses (eg Arduino, Raspberry Pi, Master8), and likewise make sure these TTLs are captured by your recording system for later alignment with video and/or electrophysiology system.

5.3 Once session is completed, stop recordings in reverse order (ending with the “master” recording device, eg electrophysiology).

6 Cleanup.

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

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

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6.3 When experiments are completed for the day, turn off the laser, close Noldus, Arduino/Pi, and/or electrophysiology programs. Transfer and back up your files to the server.

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