Jan 31, 2024

Chronic optrode recordings from the Locus coeruleus (Optrode construction, implantation, and recording)

DOI
dx.doi.org/10.17504/protocols.io.rm7vzxd45gx1/v1

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Protocol Citation: Ernesto Durán, Cristian González-Cabrera, Matthias Prigge 2024. Chronic optrode recordings from the Locus coeruleus (Optrode construction, implantation, and recording). **protocols.io**
https://dx.doi.org/10.17504/protocols.io.rm7vzxd45gx1/v1

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

Created: January 30, 2024

Last Modified: May 31, 2024

Protocol Integer ID: 94426

Keywords: Optrode, OpenEphys, Optogenetics, chronic recordings, ASAPCRN, chronic optrode construction, optrode construction, optrode, electrode insertion, electrode, opto, electrode interface board, attaching electrode, recordings from the locus coeruleus, careful electrode positioning, minute sessions with opto, implantation, identification of neuron response, recording, neuron response

Funders Acknowledgements:
ASAP
Grant ID: 020505

Abstract

This protocol covers chronic optrode construction, implantation, and recording in the Locus coeruleus (LC). It begins with building the optrode, involving assembly of a plastic housing, screw, thumbnut, and electrode interface board (EIB-32), followed by attaching electrodes and optic fibers to the EIB-32. The electrodes are then glued and trimmed, ensuring protection from animal-related damage.
Implantation involves anesthesia, craniotomy, and careful electrode positioning. A gradual approach is recommended for electrode insertion to minimize tissue damage. The optrode is secured with cement and vaseline for stability.
For recording from the LC, an OpenEphys system with omnetics headstage and acquisition software is used. Animals undergo 30-minute sessions with opto-tagging via red or blue light pulses. The setup ensures precise data acquisition and identification of neuron responses.

Troubleshooting

Building Optrode

1 **Body parts the optrode**

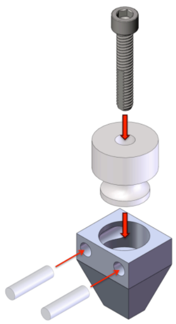

Figure 1. Overview of the driver. From top to bottom. Drive screw, thumbnut, and plastic housing (black part) with the interference dowel pins (two white pieces).

1. Screw the vented screw into the thumbnut halfway, so that the bottom half is protruding from the thumbnut by 2-3 mm.
2. Insert the screw-thumbnut assembly into the plastic housing. It will only fit in the designed orientation in which flattened facets of the vented screw align with the flat sides of the tunnel of the plastic housing.
3. Fix the thumbnut to the plastic housing with the interference dowel pins. From this point turn the thumbscrew counterclockwise to lower the screw through the housing. Lower the screw to near the end of its range of motion (see Figure 1).

2 **Assemble of parts**

1. Positioning the EIB-32 button-up direction (e.g using a holder). Insert the tube into the assembling drive and put straight the screw in the base of EIB-32 using one of the middle holes of the EIB-32 (where do you order).
2. Glue the head of the screw to the EIB-32. Here you can use a bit of UV glue to attach the screw to the EIB-32 rapidly. Then use epoxy to ensure long-lasting attachment. Epoxy needs ~90 min to cure but ~24 hrs are highly recommended.

Figure 2. Head of the screw glued to the EIB-32.

3 **Electrodes and optic fibers**

1. Use epoxy to glue ~16 mm long plastic tubing to the screw hole next to the ground pinhole of the EIB-32. Plastic tubing should rise ~0.3-0.5 mm above the EIB-32 to prevent the glue from getting inside. Plastic tubing should protrude ~14 mm under the EIB32. Let the epoxy dry for at least 90 minutes (ideally 24 hrs).
2. Affix the microwires?tetrodes to the EIB-32. Feed the 32 individual ends of the wires into the 32 pinholes of the EIB-32 following the bottom-up direction. Fix the wires to the EIB-32 with gold pins. The EIB-32 should now have a guiding tube and the tetrodes hanging underneath (figure 3).
3. Repeated the same procedure for the rest of the 32 electrodes
4. Use a fiber-ferrule cut to the length appropriate for the experiment. The fiber length should be sufficient to protrude from the vented screw and reach the area of interest within the traveling distance of the vented screw.
5. Lower the fiber-ferrule into the plastic tubing.
6. Use UV glue or equivalent to attach the electrodes to the fiber. At this point, the electrodes should still be significantly longer than the fiber-ferrule. Use a small amount of glue to fix the fiber-ferrule to the guiding tube and EIB. It is important to ensure that some glue penetrates inside the vented screw; this will ensure a good seal.

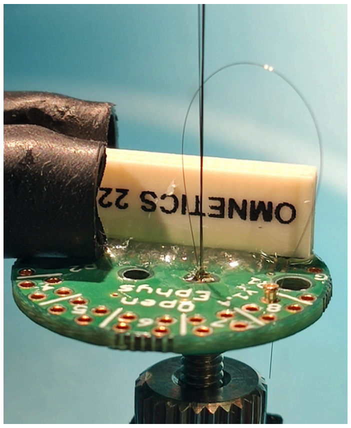

Figure 3. Top view of the EIB-32 to show the way to connect the electrodes by using the gold pins.

4 **Finishing**

1. Insert the ground wires in the corresponding ground holes (Gr) of the EIB-32.
2. Perform a final cut of the tetrodes <1 mm below the fiber (that distance may vary depending on the purpose of the recording).
3. Use UV glue to cover the EIB-32 and all the exposed wires. This will protect the device from animal-related damage while ensuring structural integrity.

Implanting Electrode

5 **Overview**

The following instructions assume the animal is placed in a stereotaxic frame under general anesthesia (Isoflurane / Oxygen recommended) and that the skin, fascia, and periosteum overlying the skull have been appropriately removed with the skull landmarks bregma and lambda properly accounted for.

6 **Surgery and implantation**

1. Wash the skull screw (s) / bolt (s) with 100% alcohol and air-dry them.
2. Drill a small craniotomy at the entry point of your interest; be sure to remove all bone fragments. Carefully clean the remainder of the skull surface and lightly score/scratch with a scalpel or drill to provide a key for the RelyX cement.
3. Drill small holes in the skull at a reasonable distance from craniotomy at the entry point of your interest and using a screwdriver insert 3 to 4 screws to anchor your

implant. This step is an alternative but highly recommendable to get a long-lasting and stable recording.

4. Carefully lower the electrode tips to touch the brain surface. Observe each electrode while advancing to ensure a smooth advance without significant electrode bending or deviation.
5. When setting the initial implanted position, it is recommended that you implant the multi-electrode a few hundred microns above your target and then spend a couple of days slowly descending to the target. This approach is intended to reduce the chances of local tissue damage/micro-bleeding due to the relatively fast insertion employed during surgery compared to 50 microns per day descent after that.
6. Using the low-temperature cautery, carefully apply molten vaseline to the polyamide tube. Avoid pushing the drive and similarly avoid direct contact with the probe electrode when applying vaseline. The objective is to form a column of vaseline around the polyamide tube. Use the cautery to gently heat the vaseline so it runs down the polyamide tube to create the desired column. Excess vaseline can be easily removed with the spatula or cautery once cooled.
7. Apply the Relyx cement carefully to the Drive housing and vaseline column, making sure to run enough cement between the drive and the nearest clean skull surface. Use the curing light to set the cement as you work.
8. Take the loose end of the skull screw soldered to a wire for the ground. Carefully hold the loose end of the wire and solder it to the ground wire of the probe.
9. Carefully loosen the probe connector from the drive holder and position it to your desired location in the implant.
10. Cover the probe cables, loop them with cement, and build the cement up over the loop.

Recording LC

7 **Overview**

We use an OpenEphys acquisition board, an omnetics headstage, and OpenEphys acquisition software. Animals were recorded for 30 minutes, and opto-tagged neurons were identified with short 10ms pulses of red light (Chrimson-expression) or blue light (ChR-expression).

8 **Attachment and Acquistion**

Animals are firmly grasped and pressed down to attach omnetics connectors. Additional fiber optics are attached to implanted optical fiber through a tight sleeve (Thorlabs, Doric).

Animals are placed into an open field arena, and the software is started.

The low pass filter should be adjusted to see individual spikes.

Light pulses are delivered every 10 seconds to identify responses.

Protocol references

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