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Mouse EEG implantation and monitoring

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We use this protocol and it's working

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Abstract

Monitoring cortical EEG is an efficient means of identifying and quantifying epileptiform activity in mouse models, especially in cases where behavioural correlates of seizures are subtle (e.g. absence seizures). This protocol describes EEG implantation surgery and EEG data collection for mouse models.

Troubleshooting

Safety warnings

Isoflurane is a halogenated anesthetic gas associated with adverse health outcomes in humans and must be handled according to governmental and institutional regulations. To reduce the risk of occupational exposure during rodent anesthesia, waste gas was collected in a biosafety cabinet using a charcoal scavenging system as approved by the California Institute of Technology.

Ethics statement

Animal husbandry and all procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee (IACUC) and by the Office of Laboratory Animal Resources at the California Institute of Technology.

Before start

Unless necessary, keep animals on a reverse light cycle and perform behavioural tests during the mouse's dark phase. Ensure that animals have ample time to entrain to a new light cycle, if changing light cycle or transferring animals to new spaces.

Avoid conducting EEG recordings and other behavioural analyses on the same day, or on cage change days.

EEG headmount implantation surgery

1 Note on EEG headmounts

> Note
>
> Though it is possible to construct electrodes and headmounts in lab, we recommend obtaining headmounts and electrodes from commercial suppliers. These can improve surgery speed and decrease variability in resultant EEG signals.
>
> We have used EEG headmounts (Cat. No. 8201) and electrodes (Cat. Nos. 8209 and 8212) from Pinnacle technologies with good success.

2 Note on surgical procedures

> Note
>
> Follow institutional and veterinary guidelines, and approved protocol(s) for your lab. This protocol is meant to give a general idea of how the surgery is done. We will not go into detail about particular anaesthesics used, as these are likely to vary between labs and institutions.
>
> This surgery is quick (20-30 min), and it is possible to perform the surgery on 2 animals simultaneously, provided you are set up to do so.
>
> Ensure that you have the necessary approvals and training(s) before starting this protocol.

3 Anaesthesize the animal, provide analgesic(s) and other necessary medications according to approved protocol. Shave the animal's scalp, then transfer to a stereotaxic frame, with a heating pad to maintain body temperature. Once sufficient time has passed for anaesthesia and analgesia, fix the animal's head in the stereotaxic frame and level the head with the stereotax.

4 Clean the animals' scalp according to approved protocol. If applicable, apply local anaesthetics.

Make a 1.5 cm anterior to posterior incision to expose the animal's skull. Use sterile cotton swabs and sterile saline to clean the incision and skull, and to gently part the skin to reveal the animal's skull. Ensure that the skull is completely dry before proceeding.

5 With a scalpel, score the animal's skull in a crosshatch pattern. If necessary, clean any resulting blood with a sterile cotton swab and sterile saline.

Note

Scoring the animal's skull will provide greater surface area for adhesives to attach the headmount to the skull.

6 Apply a small amount of cyanoacrylate glue to the bottom of the EEG headmount, and place onto the surface of the skull. The anterior edge of the headmount should be 3.5 mm anterior to bregma. Hold the headmount on the skull surface until it feels firmly attached (about 5 min).

7 Use a sterile 23g needle to pierce the skull underneath each hole in the EEG headmount. Place the needle through the hole, then gently turn the needle until the needle pierces through the skull. Be careful not to push too hard down on the needle, as you do not want the needle to go into the brain.

8 Once a hole is made, put screw electrodes through the holes and, using a screwdriver, thread them through the holes in the skull. If bleeding occurs, use an absorbent spear to stop bleeding. Stop advancing the screw once it is halfway, and then move onto the next screw.

Once all the screws are positioned halfway in the holes, apply a small amount of silver epoxy on the threading of each screw, just under the head of the screw. Finishing threading the screws into the headmount and skull.

(Optional) Once all the screw are in place, use a multimeter to check the electrical connection between screws and corresponding metal contacts on the top of the headmount.

9 (Optional) If using a headmount with EMG leads, place these leads into the neck muscles. Gently separate the muscles, and bend the EMG leads into the resulting pocket. Ensure that the EMG leads are straightened out and will not rub against the skin once the incision is closed.

10 Apply a coating of adhesive cement to around the base of the headmount, to secure the screws and headmount to the skull.

Once the adhesive cement is dry, apply dental cement to cover the edges of the headmount. Try to construct a smooth, rounded surface for the skin to lay against.

Note

When applying the dental cement are applied, ensure that there are no sharp or rough edges that may irritate the animals skin.

Also ensure that no cement is applied to the top of the headmount, as this may interfere with attaching the pre-amplifier.

11 Close the incision with suture, ensuring that the skin is firmly closed around the headmount, but is still able to move slightly.

12 Transfer animal back to home cage, and provide any necessary post-operative medications. Allow at least 1 week of recovery before attaching animal to recording system or collecting data.

EEG recording

13 The testing room should be lit by red light, and should be free of sources of non-red light (computer screens), sounds, and odours.

Note on required equipment

Note

The specific equipment used for EEG data acquisition will vary depending on the specific headmounts and electrodes used. If using commercial headmounts and electrodes, the supplier may have pre-amplifiers and data acquisition systems appropriate for their headmounts and electrodes.

We have used pre-amplifiers (Cat. No. 8208-SL), commutators (Cat. No. 8204), data acquisition systems (Cat. No. 8206), as well as cage stands and mounting plates from Pinnacle technologies.

In addition to EEG acquisition equipment, you will need a cage or large cylinder in which to conduct the recordings. This cage or cylinder should be free of edges that the EEG wires can be caught on and should have a hole for access to drinking water.

Depending on experiment scale, it may be useful to have multiple recording chambers. To mitigate confounds due to non-cagemate odours, we recommend only recording from cagemates in parallel.

14 Habituate animals to EEG equipment and the recording chamber for at least 1 session. Testing can start at least 1 day after habituation.

Before beginning, transfer animals from holding room into the testing room and allow them to acclimate to the space for at least 30 mins before beginning. Use a peroxide-based cleaner to clean and deodourize cylinders, then provide a fresh bedding in the bottom of the recording chamber.

On both habituation and testing days:

1. Gently pick up animal by base of tail and place onto a stable surface (such as a cage lid). Gently hold the animal's head and the headmount with your thumb and index finger. Attach the pre-amplifier, ensuring that it is oriented correctly and is firmly attached to the headmount.
2. Transfer the animal into the recording chamber and attach the preamplifier to the commutator. If possible, visually inspect the EEG signal to verify quality.
3. If testing, begin data acquisition process. Data acquisition is not necessary if habituating animals. For detection and quantification of spontaneous absence seizures, we acquire 90 min of recording.
4. Following the habituation or test period, disconnect the pre-amplifier from the commutator. Gently transfer the animal to a stable surface (such as a cage lid), and stabilize head and headmount with your thumb and index finger. Gently detach the preamplifier from the animal, then return the animal to the home cage.

Depending on the experimental question, it may be necessary to clean and deodorize the recording chamber between each animal.

EEG data analysis

15 Commercial or custom software can be used to analyze EEG data. Appropriate analysis will depend on the experimental question.

For quantification of spike-and-wave discharges in absence epilepsy, we used Sirenia Seizure Pro (Pinnacle Technology). Raw EEG was first bandpass filtered (1-25 Hz), and absence seizures were detected in a sliding window (0.8s wide, moving in 0.4s increments) using the following criteria: a root mean square (RMS) power exceeding 50 µV in the 5-8 Hz band and a mean amplitude at least 2-fold higher than the baseline defined during the pre-injection recording session. Though 90 min of EEG recording was collected, only the final 60 min was analyzed.

Note that the particular parameters used will depend on the surgeries, the animal model, and the recording set-up.