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Procedure for EEG surgery V.2

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We use this protocol and it's working

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Abstract

Standard Operating Procedure for EEG surgery

Materials

- Transponder- EET40 or EET20
- M4-threaded screw (agntho’s MCS1X2)
- Stereotaxic surgery frame and related equipment

Troubleshooting

Preparation of EEG transponder (DSI)

1 Prepare the transponder by twisting the excess wire in the ribs holder.

2 The transponder has 4 wires, 2 orange/orange-white and 2 blue/blue-white. It is important to keep the same convention for all experiments. By convention, we use the orange/orange-white for EEG and blue/blue-white for EMG. These correspond to channel RAW for EEG and RAW-1 for EMG.

3 Cut off a few millimeters of the plastic coating on the end of the wires and solder a M4-threaded screw (Agntho's MCS1X2) onto the exposed metal wire.

Anesthesia and Animal Preparation

4 Induce anesthesia using isoflurane in an induction chamber. We recommend to use between 2-3% isoflurane in 02.

5 Confirm the depth of anesthesia by checking the paw reflex.

6 Once adequately anesthetized, transfer the mouse to a stereotaxic frame and secure its head using ear bars. Here, we use the digital control Kopf stereotaxic instrument (69100 series).

7 Place the heating pad or lamp to maintain the mouse's body temperature throughout the procedure.

8 Shave the scalp area over the skull to expose the surgical site. Here we used epilation cream to provide better recovery.

9 Clean the exposed area with alternating iodine (50% in water).

Sub-chronic (internal) protocol (max 2-3 weeks)

10 Anesthetize the animal as usual with isoflurane (2% in O2) and fit into stereotaxic frame. Maintain
the head carefully and safely for proper surgery. Shave the animals' head and back using depilatory cream.

11 Make an incision on the back just large enough to insert the transponder. The incision should be around 1cm long and located on the thoracic level of the spines.

12 Make a separate incision on scalp from the frontal cortex to cerebellum.

13 Clear scalp of tissue using scalpel blade and scrape the surface to make it rough for the dental cement to adhere better. It is crucial that the skull is dry and scratched.

14 Insert forceps under the skin between the two incisions to make space for the wires of the ransponder.

15 Use the forceps to guide the wires from the transponder under the skin and out through the scalp
incision. Careful to prevent the wire to damage the skin.

16 Periodically flush the back incision with sterile saline to hydrate the tissue and remove debris.

17 **EMG wires:** Place a small piece of heat-shrink plastic tubing through the blue wire. Take a 27-gauge needle and insert it under/through the trapezoid muscle. The needle is used as a cannula to guide
the wires through the muscle: insert the exposed metal of the blue wire into the needle and then remove the needle. The wire is now through the muscle. Now stretch out the coiled wire, insert the end into the heat-shrink tubing, and heat the heat-shrink with the hand-held cauterizer to fasten the loop. Cut off the excess wire. The loop formed by the wire need to be a small as possible to avoid contact with the skin, but enough to allow muscle movements.

18 Repeat the same procedure with the blue/white wire, approx. 1-2mm caudally to the blue wire. **N.B:Make sure the metal wires cannot touch each other!**

19 **EEG wires:** Drill a hole (same diameter as the screws using a 0.7mm drill bit) at the level of the right PFC (coordinates approx. AP: +1,7-1,9 mm, ML: +1,1 mm) and one at the level of the right Snc (approx. AP:
-3,3, ML: +1,5). Gently screw the orange wire to the hole over the PFC, approximately 1,5 turns (approx. 0,6mm deep). Screw the orange/white wire to the caudal hole.

20 **NB: For both the color code is indicative, but requires the same for all experiment to record the signal in channel 1 and 2 from the same origin (muscle, brain).**

21 Add dental cement to the skull. It is important to completely cover the screws and wires in order to fasten and electrically insulate them. First. apply a layer of thin dental cement

to allow it to seep into crevices. Then, add thicker cement.

22 Allow for approx. 1 week recovery before recording.

Chronic (external) protocol (several months)

23 Prior to the surgery, an implant constituted by 4 pins will be connected to 4 silver wires with on the first 2 a EEG screw fixed on the other end. Each transponder is thus connected to a 10-15cm long color-coded
wire with a female 4pins connector on the other end.

24 Similar to the acute recording, the mice are anesthetized, shaved, and prepared for surgery.

25 A small incision (around 1cm) on the top of the scalp is done and the EMG wire placed as before. The EEG screw are thus implanted in the same coordinates as before. During surgery, a few centimeters long metal rod (for example a cutoff syringe) is also attached to the skull with dental cement to allow holding of the animal head during connection and thus reduce the stress linked with the holding process.

26 For each recording the mice placed in their home cage will be connected to the wire placed on the top lead of the cage and connected. The transponder will then be positioned on the top of the cage and can move freely with the animal.

Calibration

27 Before recording you need to calibrate the system.
- EEG/EMG are acquired at 500 Hz.
- Activity is acquired at 1Hz.
- Temperature is set at 35,36 and 39.
- For EEG and EMG calibration is set at 550.

Recording

28 **Following recovery, place the animals in their homecage on top of the receiver. We acclimate the animals for 48h before recording.**

29 During the recordings, group-house animals with 1-2 cage mates throughout the entire procedure, enabling the characterization of sleep independently of social stress factors.

30 On the day of the recording, activate the telemetry device using a strong magnet, and place the cages on the telemetry receiver (DSI,

https://www.datasci.com/products/software/neuroscore).

31 **A total of 16 animals can be recorded simultaneously.**