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Plug Removal for acute in vivo Electrophysiology Experiments

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Ryan Gillis[1], Mikayla Carlson[1], Severine Durand[1]

[1]Allen Institute for Neural Dynamics

Allen Institute for Neural...

Ryan Gillis
NPX

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We use this protocol and it's working

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Abstract

This protocol describes the procedure for removing the SORTA-clear plug used in acute *in vivo* electrophysiology experiments in whole-hemisphere craniotomy (WHC) preparation mice with Neuropixels probes. This exact methodology is crucial to these experiments due to the role the SORTA-clear plug plays in preserving the brain prior to the described procedure. After the mice undergo a WHC surgery, the implant used to replace the skull on the left hemisphere is covered with a SORTA-clear plug, which keeps the brain from potentially interacting with air or debris, as the SORTA-clear seals the holes in the implant and thus ensures the brain stays healthy between surgery recovery, training, and subsequent experiments. However, we cannot insert Neuropixels probes through SORTA-clear, meaning we must remove it before the experiment. After the SORTA-clear is removed, we place another temporary seal over the brain - Kwik-Cast. This is a more temporary measure and acts as another easily removable seal to preserve the brain just prior to recording. We recommend doing this procedure 1 day prior to the procedure outlined in **Neuropixels Data Collection: Whole Hemisphere Recordings** as to not expose the mouse to anesthesia on the day of recording, but if necessary, this procedure can be done on the same day as a recording as long as the mouse is given two hours to recover from the anesthesia.

Guidelines

Only perform this procedure in accordance with IACUC and veterinary requirements.

Materials

Anesthesia

Isoflurane **Patterson Veterinary Catalog #**07-890-8115

Reagents

	Material	Vendor	Part #	Specifications
	ACSF.V	In house	n/a	10mL

Surgical tools and supplies

	Material	Manufacturer	Part #
	45° forceps*	Dumont	Fine Science Tools 11251-35
	Glad Press'n Seal*	Glad	78616
	Alcohol Wipes*	BD	326895
	I-DROP 0.25% Viscoadaptive hyaluronan*	VET PLUS	261
	C Universal 4-META Catalyst, 0.7 mL	Parkell	S371
	B Quick Base for MetaBond, 10 mL	Parkell	S398
	Radiopaque L-Powder, white, 5g	Parkell	S396
	Kwik-Cast Sealant	World Precision Instruments	KWIK-CAST
	Ceramic mixing dish*	Parkell	S387
	Plastic surgical	In house	N/A

	Material	Manufacturer	Part #
	drape		

*or equivalent

Troubleshooting

Before start

Please note that this protocol is meant for mice that have previously undergone and recovered from a Whole-Hemisphere Craniotomy surgery.

Graphical overview of procedure

1

Prepare the surgical rig and anesthesia system

2 Cover heating pad with Press'n Seal and ensure that the heating pad is turned on to 36-37 °C

3 Obtain a container of ice and place the Metabond tray and a 10 mL syringe of ACSF on top of the ice

4 Ensure the vacuum gauge is set to 10-10 pounds per square inch (PSI) and that all vacuum lines are functioning correctly for both the induction chamber and nosecone scoop.

5 Connect induction chamber to the isoflurane and oxygen (if not already connected).

6 Ensure the oxygen regulator is set to 0.8-1 L/min.

7 Double check all the gas tubing to ensure the system is connected correctly.

Anesthetize the mouse

8 Open the vacuum valve and isoflurane valves. Direct the flow to the induction chamber.

9 Remove the animal from its experimental cage and place the mouse into the induction chamber

10 Turn the isoflurane regulator to setting [M] 5 % (v/v)

11 Once the mouse is fully unconscious, turn off the isoflurane. Leave the oxygen and vacuum lines open.

12 Transfer and position the mouse on the heating pad and attach the headpost into the clamp.

13 Secure the nose cone over the mouse's snout. Make sure the body of the mouse is on top of the thermometer on heating pad.

14 Redirect isoflurane flow from the induction chamber to the surgical rig.

15 Turn the isoflurane regulator to [M] 1-2 % (v/v)

16 Turn off the vacuum line of the induction chamber and close the lid.

17 Continue to monitor the mouse's breathing throughout the procedure

Note

If the breathing is rapid and shallow, increase the isoflurane level as needed in ~.25% increments. If the breathing is too deep, decrease the isoflurane level as needed in ~.25% increments.

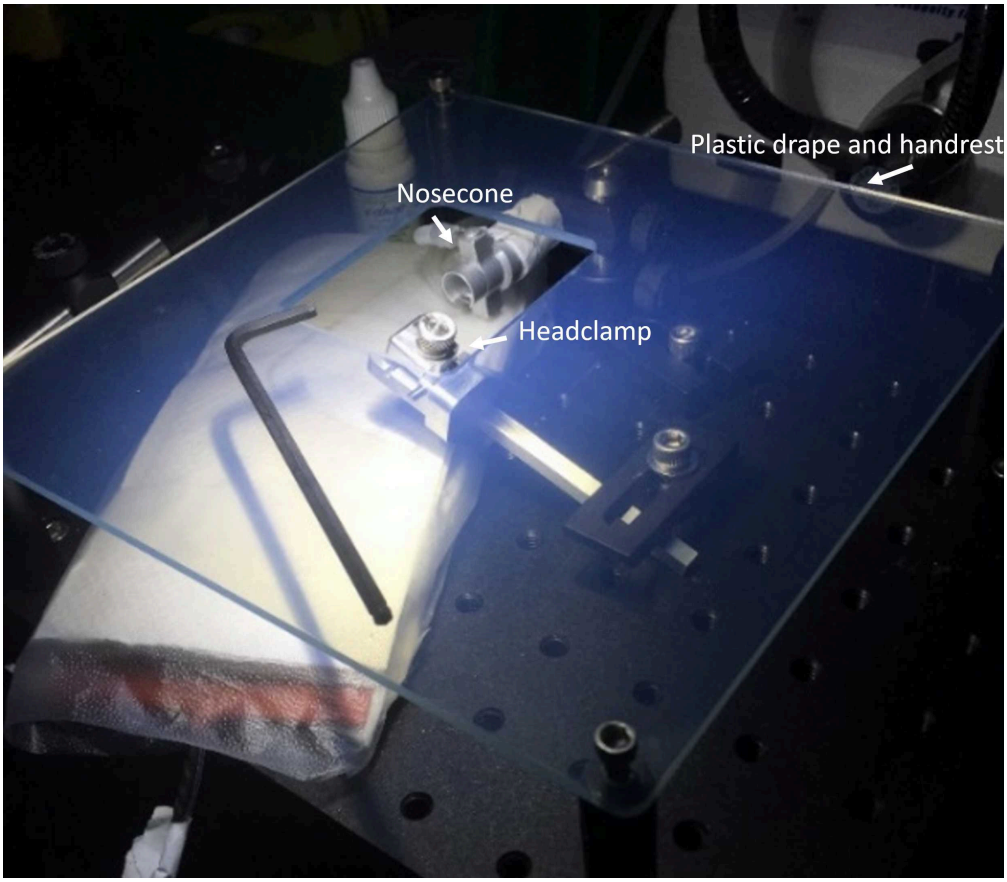
18 Cover the mouse's eyes with a drop of I-DROP on each eye to keep them moist.

Note

These mice are used for experiments involving visual stimuli, so their vision needs to be unobstructed by the time of recording. Any eye lubricant is appropriate if you do not have these experimental constraints.

19 Place plastic surgical drape over mouse and screw the drape into place with an Allen key

19.1



Paint the inside of the well with white Metabond to facilitate probe visibility during insertion

20 Remove the plastic snap-on cap from the well

21 Gently swab the inside of the well three times with ethanol wipes to remove debris and dust

22 While the ethanol evaporates, prepare the white Metabond

22.1 Mix 4 drops of Quick Base for MetaBond, 1 drop of Universal 4-META Catalyst, and 2 even scoops of Radiopaque L-Powder Metabond in the ceramic plate

22.2 Stir well with a toothpick

23 Apply a thin layer of Metabond in a ring around the edge of the implant with a toothpick. The working time of Metabond ranges from 2-3 minutes from the time the powder is mixed with the base and catalyst to the time it begins setting, so it is important to work quickly during this step. The Metabond will thicken during the working time but should remain a liquid.

Note

Take care not to get any Metabond on the SORTA-clear plug

24 Wait for the Metabond to dry completely. Use a toothpick to poke the Metabond - advance if it feels hard to the touch

Note

Metabond drying time is variable and depends largely on both the mixture quality and temperature. The best way to see if it is dry is feel it harden with a toothpick.

Remove the SORTA-clear plug

25 Ensure the well is clear of excess debris

Note

Rinse the well with ACSF if necessary

26 Fill the well with enough ACSF to cover the plug, around 3-4 drops.

27 With forceps, find the anterior or posterior edge of the SORTA-clear plug

Note

You can poke the edge of the SORTA-clear plug to find the location where you can best grip the SORTA-clear with the forceps (this can vary from mouse to mouse).

28 Gently peel the SORTA-clear plug off the implant, generally from the posterior or anterior edge.

29 Once the plug has completely separated, comb the edge of each hole in the implant with small forceps to ensure that the SORTA-clear plug successfully and cleanly detached from the implant

After SORTA-Clear plug has been removed

30 When all implant holes are clear of any debris or SORTA-clear, remove the ACSF from the well with 1-2 Sugi spears

31 Fill the well with Kwik-Cast

Note

Be careful not to submerge the ground wire too deep in Kwik-Cast as it may detach if pulled too harshly

32 Wait for the Kwik-Cast to dry, then replace the snap-on cap on the well

Surgery takedown

33 Unscrew the plastic hand rest

34 Turn off the isoflurane and oxygen

35 Remove the mouse and recover it in its cage

36 Clean surgery station with 70% ethanol

Protocol references

Neuropixels Data Collection: Whole Hemisphere Recordings