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Mouse Perfusions

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Schonhoff, A.M., Figge, D.A., Williams, G.P. *et al.* Border-associated macrophages mediate the neuroinflammatory response in an alpha-synuclein model of Parkinson disease. *Nat Commun* **14**, 3754 (2023). https://doi.org/10.1038/s41467-023-39060-w

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We use this protocol and it's working

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Abstract

This protocol outlines the procedure to ethically euthanize/sacrifice a mouse using sterile, cold PBS and paraformaldehyde (4%). Following this perfusion, organs including the brain can be retrieved and fixed for further experiments.

Materials

The fume hood
Protective gear (gloves, goggles, masks, lab coat)
Collecting dish
Styrofoam Surgical stage
Cold phosphate buffered saline (Millipore, catalog number: 6506-1L, diluted to 1x)
Cold Paraformaldehyde (PFA) solution 4% in PBS (Thermo scientific, catalog number: J19943-K2)
Heparin (Sigma, catalog number: H3149)
Two Syringes, 20ml (one for PBS, and one for PFA)
Two glass beakers
30 gauge infusion needle
Tissue dissecting scissors
Forceps
Micro spatula
Isofluorane box

Troubleshooting

Safety warnings

4% paraformadelyde is toxic and flammable.

Ethics statement

Perfusions must be done in a fume hood.

PROCEDURE

1 Pick the mouse up by its tail, place it into the isofluorane box, wait ~5 min. Assess if the mouse has reached a surgical plane of anesthesia by loss of response to toe pinch. Do not wait too long, since the heart may stop, and you need the heart beating to get a good perfusion.

2 Fill one beaker with PBS, add heparin to it (10 units per 100mL of PBS) and mix. Fill a second beaker with PFA solution.

3 From this step on, please operate in the fume hood. Place the mouse on its back. Firmly pin its four paws to a Styrofoam surgical station so that nothing moves during the perfusion procedure.

4 Connect the transfusion needle to a 20 ml syringe filled with PBS. Flush the tubing and needle to expel any air.

5 Perform transcardiac perfusion with PBS. Grip the skin on the chest with forceps and make an incision using tissue scissors to expose the abdominal cavity.

6 Grip the top of the skin with ophthalmic forceps and make lateral incisions beneath the ribcage using tissue scissors to expose the diaphragm and liver.

7 Carefully make incisions in the diaphragm along the entire length of the rib cage.

8 Make two cuts through both sides of the rib cage up to the collarbone using tissue scissors. Reflect the sternum up over the head of the mouse to fully expose the heart and lung

9 Carefully tear off pericardial sac and any other tissues covering the heart using dissecting forceps to provide a clear view of the heart and vessels.

10 Secure the heart with dissecting forceps at a steady position. Insert the needle from the tip of the left ventricle at an angle approximately parallel to the midline of the heart. Do not push too hard and mind the angle to make sure the needle does not advance into the left atrium or right ventricle. The heart should be beating and one may observe backflow of blood into the needle before perfusion starts.

11 Make a small incision on the right atrium using scissors. Dark venous blood should flow out immediately. Start the PBS perfusion at once at a constant speed by manfully pushing the syringe. As PBS flushes out the blood, the liver should gradually turn pale.

12 Stop perfusion when the fluid flowing out is clear of blood. For adult mice, it takes ~10-15 ml saline.

13 Switch from saline to 4% PFA. Make sure no bubble gets into the perfusion system during the switch.

14 Steadily perfuse 4% PFA at a constant speed. As PFA goes into circulation, one can observe signs including body twitching, tail flicking and head moving. The tail standing up is a good sighn of a good perfusion.

15 Perfuse ~10-15 ml 4% PFA for adult mouse. The whole mouse body should be stiff. Do not forget to flush your line with PBS before going to the next animal.

16 Unpin the mouse from the surgical station.

17 Decapitate the mouse with tissue dissecting scissors.

18 Remove the skin by first making an incision along the midline from the neck to the nose and then reflect the two flaps of skin rostrally and laterally to expose the skull.

19 Hold the head tightly with one hand. Trim off bones at the caudal end with dissecting scissors. Clear off any residual muscle on the skull with forceps or scissors. If the 4% PFA was done properly, the brain will shrink a little bit and there will be some space between the brain and the bones forming the foramen magnum.

20 Slide one blade of the fine scissors into the foramen magnum (underneath the brain) with the sharp side facing the bone on one side. Make a lateral cut. Repeat the cut on the contralateral side. Carefully remove the skull covering the cerebellum with ophthalmic forceps.

21 At the caudal end of the mid-sagittal suture, insert the tip of the fine tweezers between the foramen magnum and the brain. Make sure to disconnect the jaw bones from the skull as well. Rapidly lift skull away from the brain. Carefully remove the skull until the whole brain is exposed. An additional lifts may be necessary to expose the olfactory bulb.

22 Using a micro spatula to sever the nerve bundle on the ventral surface of the brain and scoop the brain out.