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AAV Injection and Tissue Preparation V.2

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Abstract

This protocol provides guidelines for injection of AAVs into the cortex and striatum of young and adult mice. For post-injection tissue processing, the protocol for perfusing mice and preparing brain tissue for sectioning is also included.

Materials

- **U-100 Insulin syringes**, BD, Cat #324702
- **2,2,2 tribromoethanol (Avertin)**, Sigma, Cat# T48402-25G
- **2-methyl-2-butanol, Sigma**, Cat# 152463-250mL
- **PFA 16%**, Electron Microscopy Sciences, Cat# 15710
- **Tissue-Tek O.C.T. Compound**, Sakura Finetek, Cat# 4583

Prepare Avertin according to the following recipe:

1. Dissolve 0.3125 g bromoethanol into 0.625 mL methylbutanol in an Eppendorf tube.
2. Place into a heat block at 40C and shake until well dissolved
3. Add 24.5 mL ddH2O to the 50 mL falcon tube and add the dissolved solution.
4. Wrap in foil, keep in the dark at 4C.

Stable for up to 2 weeks (not past this). Degraded when pH <5

Mouse Retroorbial AAV injection

1 **Procedure:** Retro-orbital injection of adeno-associated virus in P21-P35 mice

2 **- Sterilization**: Surgery area will be regularly cleaned and disinfected with 10% bleach followed by water and 70% ethanol, or other disinfectant.

3 Surgical instruments should be autoclaved prior to starting surgical procedures, and sterilized by bead sterilization between surgeries on the same day. All personnel should wear protective coveralls, bonnet, face mask, gloves and shoe covers to limit exposure and contaminants.

4 **-Pre-operative**: Mouse should be anesthetized with isoflurane (induction at 3-5%). Breathing and withdrawal reflex should be monitored by visual observation and toe pinch during the procedure to ensure homeostasis and check for signs of distress.

5 Once they are in deep anesthesia, as demonstrated by the absence of withdrawal using the toe pinch test, the mouse should be moved to stereotactic stand and the nose inserted into a head holder which provides anesthesia at 1.5-2%.

6 Care is taken from the induction of anesthesia until the time of complete recovery to keep the mice off of cold surfaces, maintain their body temperature through the use of insulated surfaces (snuggle safe/ heating pad or table), and local ambient temperature monitoring.

7 **- Procedural: Viral injection:** Place animal into stereotaxic apparatus and immobilize. Eye ointment is then applied to eyes of the mouse to prevent drying out.

8 Using an insulin syringe with a 28 gauge needle, 0.01mL of non-replicating adeno-associated virus is injected retro-orbitally.

9 The entire procedure will last approximately 3-6 minutes and each animal will be kept under anesthesia for the entire duration.

10 Procedural anesthesia should be sufficient to prevent the animal from feeling pain. Animals will be monitored until they are fully awake.

11 Should animals show any signs of distress after injections, a dose of Meloxicam at 1-5 mg/kg will be administered.

12 The volume of solution injected will not exceed the IACUC maximum injection volume for mice, according to the reference 'A Good Practice Guide to the Administration of Substances and

Removal of Blood, Including Routes and Volumes.' K.H. Diehl, et. al, 2001, Journal of Applied Toxicology.

13 **- Record of procedures and controlled substances:** Surgeries will be recorded and tracked stating the date and time, animal identification, amount of anesthesia administered per animal and post operative observations

Perfusion of Animals and Tissue Processing

14 **A) Setup/Preparation**

15 1. Thaw out 4% PFA at least 30mins prior to perfusion. Estimate 15-25ml/animal (P21 depending on size).

16 2. Prepare TBS/Heparin solution according to the following recipe:
1. 250mL/1000mL of TBS
2. 28.2mg/112.8mg of Heparin

17 **3. Perfusion bench set up:**

18 a. Attach butterfly needles to pump

19 b. Set up carcass bag

20 c. Ensure that there are 8 small pins and 2 large pins on the Styrofoam cover. Large pin is to hold the butterfly needles in place and the small pins are for securing the animal's limbs on the Styrofoam cover.

21 d. Prepare ice box for avertin

22 e. Prepare dissection tools (check to make sure that they are clean). I like to use 1 large scissors, 1 small scissors, 1 curved scissors, 1 forceps and 1 flat (thin) spatula.

23 f. (optional) If perfusing >6 animals, prepare a 6 well plate to store heads on ice. This will help to speed up the process of perfusion

24 **B) Perfusion/Dissection**

25 1. Inject 0.6ml of avertin intraperitoneally to the bottom right quadrant of the mouse. Place the mouse in mouse box (weighing optional) and wait until the mouse no longer reacts to toe pinch.

26 2. Secure the mouse onto the Styrofoam cover, ensuring that the mouse is pretty taut. This will make the Y incision easier

27 3. Start the pump so that TBS/Heparin is flowing at a low rate (<10ml/min)

28 4. Cutting under the skin, make a Y incision using the small scissors and forceps. Remove the skin to expose the ribcage. Holding the sternum with the forceps, cut around the ribcage to expose the heart, lungs, and liver. For best results, the heart should still be pumping

29 5. Stick the butterfly needle through the right ventricle, making sure not to perforate into the left side of the heart/through the heart. If done well, the heart should swell up slightly and the right side of the heart become slightly paler than the left.

30 6. Using the curved scissors, snip the left atrium at the uppermost tip slightly to allow flow.

31 7. Transcardial perfusion with TBS/Heparin for approx. 5-6mins at flow rate of 10-12ml/min. If done correctly, there should be a steady dripping and the liver begin to blanch (become pale). Perfuse for a longer period of time if uncertain

32 8. Switch to 4% PFA and perfuse for 6-7mins. The mouse should stiffen up (check by testing the rigidity of the tail)

33 9. Remove the head using the large scissors and remove the cortex using the curved scissors and forceps

34 10. Store in Bijoux tube (filled with 2-3ml of 4% PFA) – place on ice during perfusion and store at 4C overnight

35 **C) Clean up**

36 1. Pour away the PFA/TBS/Heparin/Blood waste into PFA waste bottle using a funnel

37 2. Wash the tray and Styrofoam cover well and let dry

38 3. Wash and dry all dissection tools, keeping them back into the drawer immediately

39 4. Throw away all non-PFA contaminated material in the regular trash bin. Empty PFA tubes must be thrown in the biohazard bin while needles are to be thrown into the sharps bin.

40 The next day, wash with 3X TBS and switch to 30% sucrose in TBS for 2 O/N before cryopreserving in sucrose: OCT (2 parts sucrose, 1 part OCT). Store in -80C until ready for sectioning