**Brain Processing / Histology Overview**

The following gives an overview of all the immunohistochemistry steps involved with processing a brain that has been injected with a tracer/injection site marker.

1. **Brain removal (~1 hour)**
   1. Brain removal is the process of removing a brain from a skull.
   2. After perfusion, the brain should sink in paraformaldehyde (PFA) overnight. It doesn’t really matter whether the brain has been removed from the head or not as long as the brain is completely penetrated by PFA.
   3. Using the microscope and stereotax, remove the brain by cracking and peeling away the three layers of the skull (pia, arachnoid, and dura mater), severing the spinal cord and optic nerves, and then carefully rolling the brain out of the remaining skull casing.
   4. Store the brain in a falcon tube with 20mL of PFA at 4°C (in the fridge).
2. **Dura Removal (<1 hour)**
   1. Dura removal is the process of removing any remaining dura mater from a brain. This step is crucial as it allows the tissue to stick to the gelatin properly during sectioning.
   2. Using the dissecting scope, carefully peel away the dura with tweezers, making sure to remove it from all the crevices of the brain.
   3. Store the brain in a falcon tube with 30% sucrose solution at 4°C.
   4. Sucrose solution is a cryoprotectant and will protect the brain from cold. The brain must sink to the bottom of the falcon tube before it can be gel-blocked; it takes a few hours to do this so it is best to leave it overnight.
3. **Gel-blocking (>4 hours)**
   1. Gel-blocking is the process of embedding a brain in a block of gelatin in order to be sectioned.
   2. Create a gelatin base in an ice-cube tray, place the brain on the base, and then pour gelatin over top fully envelope the brain. After hardening, trim the gelatin into a small cube and, with the rostral end facing away, cut the left dorsal side.
   3. Store the gel-blocked brain in PFA (to harden the gelatin) at 4°C.
   4. After 2-5 hours, transfer the gel-blocked brain to 30% sucrose solution. It must sit in the sucrose solution for at least 2 hours.
4. **Sectioning (~2 hours)**
   1. Sectioning is the process of slicing the brain tissue into thin sections in order to be viewed under a microscope.
   2. Using the microtome, freeze the gel-blocked brain with dry ice and section at 40μm (microns). Place sections in trays with 1x PBS or 1x PBS-Azide (an antimicrobial) if the sections will be left overnight or longer.
   3. Store trays at 4°C.
5. **Visualizing the BDA** 
   1. This step is necessary for being able to visualize the injection if the brain was injected with BDA during the experiment.
   2. Subject the sections to a series of washes in 1x PBS (30 mins), bleach (20 mins), incubation in the chosen reagent (60 mins), then washes again (30 mins). If using fluorescent staining, cover the sections to reduce light exposure after incubation.
   3. Store the sections in 1x PBS, or 1x PBS-Azide if the sections will be left for longer than 24 hours, at 4°C.
6. **Mounting** 
   1. Mounting is the process of mounting brain sections on slides.
   2. Pour 1x PBS and a splash of PBS-Tx 0.4% into a petri dish and use a paintbrush to carefully guide the sections onto the slides.
   3. Cover the slides with tinfoil and allow them to dry for 15 mins. Store in slide boxes at 4°C.