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We use this protocol and it's working

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Anxa1 Immunostaining

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ABSTRACT

Protocol for perfusion, slicing, and immunostaining for Anxa1 in the striatum.

Perfusion

1 Anesthetize mouse in an anesthesia chamber set to 5% isoflurane, until breathing slows and foot

pinch reflex stops.

2 Restrain the anesthetized mouse with tape such that the chest is facing upwards, and all limbs are secured.

3 Using surgical scissors, cut the skin away to reveal the ribs, and cut through the diaphragm and sides of the rib cage to expose the heart and lungs.

4 Using a Masterflex L/S Easy-Flow peristaltic pump, insert the PBS needle into the left ventricle aimed at the left atrium, and secure the needle in place with forceps.

5 Cut right atrium with surgical scissors, and begin flow of 1x PBS at the max flow rate until the liquid from the chest cavity flows clear.

6 Switch the source from PBS to 4% PFA, and maintain flow until mouse muscle reflexes from fixation ceases.

7 Remove flow needle and sever head with thick surgical scissors.

8 Using fine surgical scissors, remove scalp and cut the dorsal skull posterior to anterior, and pull skull away revealing fixed brain.

9 Remove brain and store in 4% PFA for 24 hours.

10 Transfer brain from 4% PFA to 30% sucrose solution for at least 2 days, or until the brain sinks to the bottom of the sucrose solution.

Slicing

11 Place dry ice on all sides of the microtome, and wait until frost forms on the sides.

12 Create a small frozen 30% sucrose solution platform for the brain to rest on and freeze to

13 Cut cerebellum to create a flat surface on the brain so that the brain can rest on the platform with the olfactory bulbs facing upwards.

14 Wait until the brain has fully frozen before slicing coronal slices 50 microns thick, capturing the striatum with anterior posterior coordinates off bregma from 1.7 mm to -.34 mm.

15 Store coronal slices in 1x PBS

Immunostaining

16 (All steps at room temp) Wash slices in 1x PBS 3 times, transferring from old PBS to new and letting sit for 5 minutes.

Soak slices in block solution (PBS 1x + .3% Triton-X + 5% Normal Goat Serum) for 30 minutes.

17

18 Transfer to block solution with Rabbit-anti-Anxa1 (Invitrogen #71-3400) primary antibodies (1:1000) for 1.5 hours, covered from light and on a rocking platform.

19 Wash in 1x PBS + 0.3% Triton-X 4 times for 5 minutes each.

20 Transfer to block solution with goat-anti rabbit (Invitrogen #A11037) antibodies (1:250) for 2 hours, covered and rocking.

21 Wash in 1x PBS 3 times for 5 minutes each, and store slices in PBS.