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Anxa1 Immunostaining

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Protocol status: Working

We use this protocol and it's working



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Abstract

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

Troubleshooting

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