Protocol: Revealing neuron morphology after Ephys recording

1. For patch clamp recording, select that neuron that is at least >50um deep to preserve the dendritic structures.
2. Use an internal that has 0.3% (30mg biocytin to 10ml internal solution). At the end of recording for each cell, pass strong positive current for at least 5 minute. For the series resistance during recording, the smaller the better.
3. After recording for that cell, slow retrace the electrode to better preserve the cell soma staining.
4. Fix the slice in fresh 4% PFA for 20min-1 hour.
5. Wash slice 3X5 min with 0.01M PBS.
6. Endogenous peroxidase activity block: Switch slices to 1% H_2O_2 (330 µl of 30% H_2O_2 for 10ml of PBS), keep at RT for 2 hours.
7. Wash 2X5min with PBS, then switch to 30% sucrose overnight.
8. Cutting with a vibratome into 60um section: using OCT compound and dry ice to make a block, then cut it with sliding microtome to make a flat surface. Put the slice (after 30% sucrose cryoprotection) on a glass slide, lay it flat, then carefully adhere to the flat cutting surface.
9. Using small amount of OCT compound to barely cover the slice on the cutting surface, add a little more dry ice on stage to have it cool down. Lower the stage a little bit (>350um), and start to advance ~20um each time until the blade hit the slice surface. Make sure to obtain optimum temperature of the sample (not too brittle for the slice).
10. Once hit the surface, change it to 60um for each section.
11. Collect all the sections into PBS. Rinse it several times.
12. Prepare PBS-Triton, in 50ml of PBS, add 100ul Triton X 100. Incubate floating sections in PBS Triton for 30min.
13. In 5ml of PBS Triton, add 2 drops of A and 2 drops of B from the Vector ABC Elite kit, let it sit at RT for 30min to form the ABC complex.
14. Switch slices into this ABC complex reagents, let sit in freezer for overnight.
15. Wash it 2X10 min with PBS. Now the slices are ready to develop using chromagen.

Developing:

1. Add 2 drops of buffer stock solution to 5 ml of ddH2O.
2. Add 4 drops of DAB stock solution and mix well.
3. Add 2 drops of hydrogen peroxide sultion and mix well to activate DAB.
4. Optional (if darker color desired): add 2 drops of Nickel solution and mix well.
5. Apply this chromagen solution to the floating section slices to reveal cell morphology. Observe under the stereoscope, after color change is strong enough, switch to PBS to stop the reaction.
6. Wash slices 3 times with PBS.

7. Histology mounting.

    Transfer slices through 70%, 90%, 95% and 100% ethanol gradients, each 5 min.

    Clear slices in Xylene for 2X5min.

    Carefully using a brush to transfer the cleared section to slides.
    Mount with DPX mount.