

Neuron counting protocol

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A. Preparation of mouse brain sections for stereological neuron counting

1. Sacrify a mouse and perfuse it with ~40 ml PBS
2. Dissect the whole brain. For saggital sections cut the brain (right and left hemispheres)
3. Fix both hemispheres in 10% formalin, 1:30 h, RT.
4. Remove formalin and incubate in PBS at 4°C before using the alcohol-paraffin pretreatment machine (Cindys lab)
5. Put the brains in the cassettes and place them in the alcohol-paraffin pretreatment machine overnight
6. Next morning embed brain hemispheres from different mice in paraffin together in the same cassette (use the paraffin embedding machine from Cindy´s lab). I use to embed 4-5 brain hemispheres from different mice in the same cassette.
7. Start sectioning from the first to the last section (it is possible that you loose some sections at the beginning but should be ok). I use to make 10 µm sections. 12 µm sections are also fine. Once, you choose the µm you should always keep it for all groups (control, KO etc...)
8. You should keep most of the sections because you will count each section every 40 sections (example if you start from a random number from 1 to 9 lets say 2, you will count section 2, 42, 82, 122, 162, 202, 242, 282). If you loose any of the above sections you always can use a similar one. For example if you loose section 202 use 201, 204 or 200, 205...)
9. Next day bake sections at 60 °C for 2h in Cindy´s lab oven.
10. Cool down sections before proceeding to next step. At this point you can use you sections for immunohistochemistry or Nissl staining.

B. Stereological neuron counting procedures

1. Switch on Power for camera adaptor. Switch on Power for microscopy. Switch on Power for MS-2000, controller. Finally, turn on PC (shen123).
2. Run BQNOVA.

3. In **System Tools**: click **Held Image**. Make section name in a folder to store files.
4. In **Manual**: check both **Keyboard termination** and **Live measures**.
5. In **Parameters**: check Save **Topo**.
6. In **Select Arrays**: double click **A1 Sampling Area** (select)
7. In **Stereology Tools** : File: check 1st **Load Stereology Defaults**; click Yes.
8. In **Set Landmarks**: click Yes. Go to image window, the mouse becomes **red arrows**. Use the arrow to click on the corner of the image.
9. Click **measure** in the **Manual** window.
10. Use **red arrows** to click the designated areas (image) for neuron counting. Use **space keyboard** to finish the last line. Once selected, right click on the image.
11. Write down the **A1 value** (such as 96622.87).
12. Skip the Trace Contour.
13. Click Grid Wizard. Keep D2 for Select Grid Array. Check the Pick contour, and then click the selected area in the image window. Click Next, go to Next; Keep XY Grid-Lines for (Grid type) and select Blue color. Then click Next.
14. For Grid parameters: keep 100 for both X Interval and Y Interval. Keep the Random.
15. Write down the Total Intersections.
16. Check All for the Mark Intersection Points. Choose any color (yellow) for Point Color and Keep D3 for the Point Array. Click Apply. Click Finish.
17. Click Overview Setup, select all Ds into the right window. Then click OK.
18. Click Disector Setup. Use 50 for both X and Y dissectors. Click Apply. Close.
19. In the Parameters window, change 4X to X100. Now need to change to 100X for the microscopy. Use the light filter to add oil onto the slide.
20. Go to Topo menu, choose Motorized stay controls.
21. In the Motorized stay controls window, choose the symbol for the starting sector. Use the Focus Control to get image focused. In the Select Arrays window, choose D4 Count per Disector (double-click).

22. Click Count Cells. Use Red Arrow to click on each neuron. After counting for each per disector, right click on the Red Arrow, the total number of neurons for this disector will be shown on the List Current Array (D4 column).
23. Click the symbol for Next Disector, click Count cells again. Use Red Arrow to count total neurons. The number of neurons will be added into the List Current Array (D4 column) again. Repeat these steps for the rest of dissectors. Write down the number for the List Current Array and get total number for this section. (If the nucleus of neuron is outside of the left and bottom Red Line, do not count it. If the nucleus of neuron is within the top and right Green Line, count it.) Be careful with the movement controller. If needed to use the micro-manipulator, switch the MS2000 to the Disengaged. If needed to use the MS2000, switch it back to Engaged position.