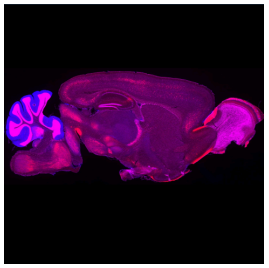
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Mapping and Counting High-Intensity Perineuronal Nets in the Somatosensory Cortex V.1



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We use this protocol and it's working

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Abstract

The purpose of this protocol is to outline the process by which we count high-intensity perineuronal nets (PNNs) in whole brain analysis. Specifically, high-intensity PNNs within the primary somatosensory cortex of adult mouse brain sections cut in a sagittal orientation. This protocol accompanies the paper “Lateralized expression of cortical perineuronal nets during maternal experience is dependent on MECP2” (https://doi.org/10.1101/787267).

First, we align a digital map from the Franklin & Paxinos Mouse Brain Atlasonto individual brain sections. We then explain the process by which the S1 and its subregions areoutlined based on this atlas overlay. Finally, we outline the process of counting high-intensity PNNs within these regions.

High-intensity PNNs are thought to be more mature structures due to a larger accumulation of the proteoglycans that make-up the structures[1–3]. We look at PNNs as a marker for changes in experience-dependent plasticity within the brain, where it can inhibit plasticity or solidify the changes that occurred in response to an experience.

The video tutorial accompanying this protocol can be found here: **Mapping and Counting High-Intensity Perineuronal Nets in the Somatosensory Cortex Tutorial**

Different regions of the mouse brain have differing densities of PNNs. Adult somatosensory cortex has the highest density based on what we have observed. This protocol can also be used to count all PNNs, just skip the contrast step (7.2) in PNN analysis.

Citations
1.Slaker, M. L., Harkness, J. H. & Sorg, B. A. A standardized and automated method of perineuronal net analysis using Wisteria floribunda agglutinin staining intensity.*IBRO reports* **1**, 54–60 (2016).
2.Carulli, D.*et al.*Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components.*The Journal of Comparative Neurology* **494**, 559–577 (2006).
3.Foscarin, S.*et al.*Experience-Dependent Plasticity and Modulation of Growth Regulatory Molecules at Central Synapses.*PLoS ONE* **6**, e16666 (2011).

Guidelines

Caution:
1) Make sure you calibrate ImageJ correctly every time. If you do not, this can drastically change the results of the test.
2) Make sure you ROTATE the image before PNN counting!!!!

Materials

ImageJ/FIJI Computer Program (NIH)

Digital Version of the Atlas that you are using (we use Paxinos and Franklin Mouse Atlas, Fourth Edition)

It helps to have two monitors and a mouse, this is difficult on a track pad.

Mapping and Counting High-Intensity Perineuronal Nets in the Somatosensory Cortex Tutorial

Preparation

1 Download ImageJ from the NIH

Have 10x whole somatosensory cortex images containing the dentate gyrus and striatum (these are sagittal directions)
-- Know measured equivalent of a pixel from the microscope using 10x objective

Have the images that you will be analyzing downloaded to the computer

Have a digital copy of your mouse atlas

2 **Calibrate ImageJ to the scale from the objective of the microscope**

2.1 Open image in ImageJ
Analyze→ Set Scale→Insert your measurement values from the microscope→ check Global→
Click out of image

> Note
>
> Every time you open another image, make sure to uncheck "deactivate global calibration", otherwise you will have to reset the parameters every time you open a new image.

Mapping

3 **Align map to fluorescent image**

3.1 Open corresponding map image in ImageJ

3.2 Overlay map onto fluorescent image
Image→ Overlay→Add Image→ select appropriate map
Image→ Overlay→ To ROI Manager

Note

When adding the image, make sure you make it ~20% opaque so you are able to see the image beneath it

3.3 Make the map fit to the cortex of the fluorescent image.

To rotate the fluorescent image to the map, deselect the map then go to Image→ Rotate... and insert how many degrees you would like to rotate this image. Reselect the map and drag to fit again.

End goal: The curve of the striatum and the front edge of the hippocampus will line up. The front of the tissue should line up with the map as best as possible.

3.4 Flatten this overlay into one picture: Image→Overlay→Flatten.

To Save: File→Save As (tiff)→ select the folder with the OG image → name this image differently
(we suggest adding "flatten (degree of rotation)" to the end of it)

Keep this flattened image open in ImageJ

4 **Outline whole primary somatosensory cortex (SS1)**

4.1 Increase image brightness so you can see the border of the striatum and hippocampus

Image→ adjust→ brightness/contrast (slide the contrast tool)

4.2 Select the polygon tool and outline the whole somatosensory cortex.

Double click on the final point to finish and connect the outline.

4.3 Press 't' on the keyboard to save the outline to the ROI Manager

4.4 Rename the ROI: In ROI manager, select ROI and then the 'Rename' function. Name SS1 Area.

4.5 To acquire the area: In ROI Manager, select Measure and record the area value

4.6 To Save: in ROI Manager, go to 'More'→ 'Save'→ then save in the same folder that you have for the original and flattened image

5 **Outline primary somatosensory subregions**

(the subregions will change depending on what the section is)

5.1 Increase image brightness so you can see the border of the striatum and hippocampus

Image→ adjust→ brightness/contrast (slide the contrast tool)

5.2 Select the polygon tool and outline ONE subregion at a time

Double click on the final point to finish and connect the outline.

5.3 Press 't' on the keyboard to save the outline to the ROI Manager

5.4 Rename the ROI: In ROI manager, select ROI and then the 'Rename' function. Name (Subregion) Area.

Note

Do this for all subregions in the image. Make sure you rename them as you go.

Advice: Make sure to check 'show all' in ROI manager to make sure the borders of the subregions do not overlap. This will help with consistency within one image.

5.5 Save all of the ROIs as individual files:

In ROI Manager, Select ONE ROI file, then go to 'More'→ 'Save'→ then save in the same folder that you have for the original and flattened image

Repeat this until all areas are saved

5.6 To acquire the area: In ROI Manager, select Measure and record the area values

high-intensity PNN counting

6 **Set-up:**

Make sure to calibrate ImageJ!

Open original fluorescent image in ImageJ and rotate image per rotation degree in the flattened name
(Image→ Transform→ Rotate...)

6.1 Open original fluorescent image in ImageJ and rotate image per rotation degree in the flattened name
(Image→ Transform→ Rotate...)

6.2 Drag subregion area file into ImageJ, press 't' to open this in ROI Manager

6.3 Make sure 'show all' is checked in the ROI Manager

7 **Count High-Intensity PNN**

7.1 Select 'point' tool (looks like cross hairs)

7.2 Maximize the contrast using the brightness/contrast tool

Image→ Adjust→ Brightness/Contrast

Note

Make sure you know what the numbers are on the Brightness/Contrast tool are when you first maximize the contrast. The more you slide the tool back and forth, those values will change.

Advice: It is best to hit 'reset' every couple adjustments in order to keep the contrast accurate.

7.3 Count all PNNs that are visible (look at criteria below) by clicking on it (point tool) and pressing 't' on the keyboard to save it to the ROI Manager.

Note

High-Intensity PNN Criteria:
PNN must be at least 80% complete* (you can tell by looking at the unaltered photo)

*these may be filled-in circles or donut shaped. Both are PNNs, it just depends on sectioning planes

7.4 To Save PNN Counts:
In ROI Manager press 'deselect' → More→ Save (make file name "subregion PNN") make sure this is saved to the same folder as the original image

Note

Make sure to press 'deselect' before saving. If you have not done this, none of the counts will be saved.

7.5 How many PNNs did you count?

In ROI Manager: 'deselect'→ Measure
Record the amount of points (that is your count)

7.6 Repeat for every subregion