Determining hippocampal tissue iron status in a transgenic mouse model of hippocampal neuron-specific iron deficiency using immunohistochemistry

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Background and Significance

- Iron deficiency (ID) is the most common nutrient deficiency
- ID affects 40-50% pregnant women and children
- Iron is necessary for metabolic processes in the brain
- ID causes learning and memory deficits
- ID is associated with thyroid dysfunction
- Transgene DnTfR/TTA mice need to be ID confirmed

Results

- Neurons are quantifiable with DAPI (Fig. 3)
- Individual microglia are visible with Iba1 (Fig. 6)
- Individual dendrites are visible with Map2 (Fig. 5)
- Ferritin may have worked (Fig. 1)
- eGFP for DnTfR/TTA brain is inconclusive (Fig 8)

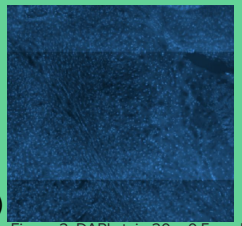

Figure 3: DAPI stain 20x_0.5x

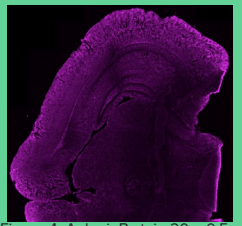

Figure 4: AnkyrinB stain 20x_0.5x

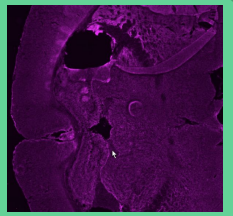

Figure 5: Map2 stain 20x_0.5x

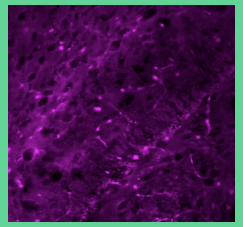

Figure 1: Ferritin 647 25x_0.5x

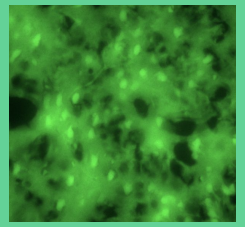

Figure 2: Background 488 stain 50x

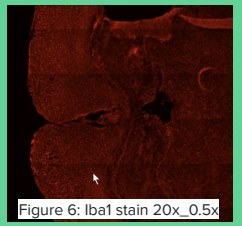

Figure 6: Iba1 stain 20x_0.5x

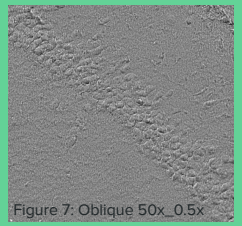

Figure 7: Oblique 50x_0.5x

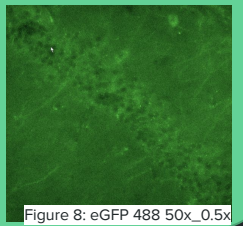

Figure 8: eGFP 488 50x_0.5x

Materials and methods

- CD1 mice were perfused and had their brains extracted
- Mice brains were preserved in OCT medium at -80°C
- Brain tissues were cryostat sectioned in 20um layers and placed onto warm glass slides
  - Hippocampal regions were singled out
- Immunohistochemistry (IHC) was performed on slides of tissue
  - Antibodies DAPI, Map2, Iba1, AnkyrinB, and Ferritin were used
  - IHC steps include blocking, primary antibody, PBS washes, secondary antibody, dehydration, and mounting
  - Slides were imaged in oblique, 488, 568, and 647
- DnTfR/TTA mouse brain was perfused and cryostat sectioned
  - Slides were imaged at 488 to look for eGFP

Limitations

- The cryostat machine produces tears in the tissue
- Tissue gets folded when placed on the glass slide
- Microscope immersion does not always produce clear, high resolution images

Future directions

- Keep troubleshooting the microscope
- Test other slides to see if the slide cover or mounting medium is faulty
- Look at more DnTfR/TTA mouse brains to confirm iron deficiency transgene