

Expression of Perineuronal Nets and Cannabinoid Type 1 Receptors Across Development

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Introduction

- It has been widely recognized that the brain undergoes major changes throughout development.
 - This project looked specifically at the prevalence of **Perineuronal Nets (PNNs)** in the **retrosplenial cortex (RSC)** and **Cannabinoid Type 1 Receptors (CB1Rs)** in the **prelimbic frontal cortex (PL)** in adolescent, juvenile, and adult mice.
- PNN maturation marks the end of a sensitive window in adolescence but little is known of their expression in the RSC across developing age groups.
- CB1R prevalence in the PL fluctuates over time, peaking in early adolescence and plateauing in adulthood.
- Stress is known to cause neurological changes; if the onset is during adolescence, these effects can last throughout adulthood.
- Less research has been done on adolescents and juveniles, so it is crucial to understand the neural underpinnings of these periods.
- Through this project, we aim to **establish benchmark levels** for PNNs and CB1Rs across age groups in order to better understand developmental brain changes.
- We also wanted to **pilot and refine immunohistochemistry (IHC)** protocols for future application in the lab.

Methods

Subjects:

- 3 Juveniles (PND23; 2F, 1M)
- 3 Adolescents (PND35; 3M)
- 3 Adults (PND70; 2F, 1M)

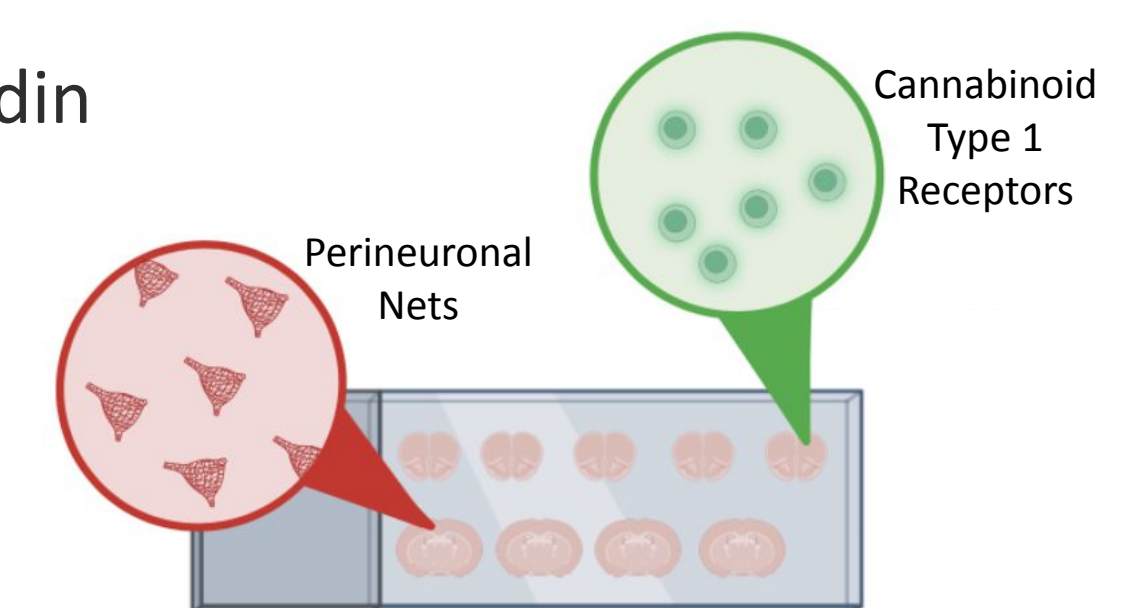
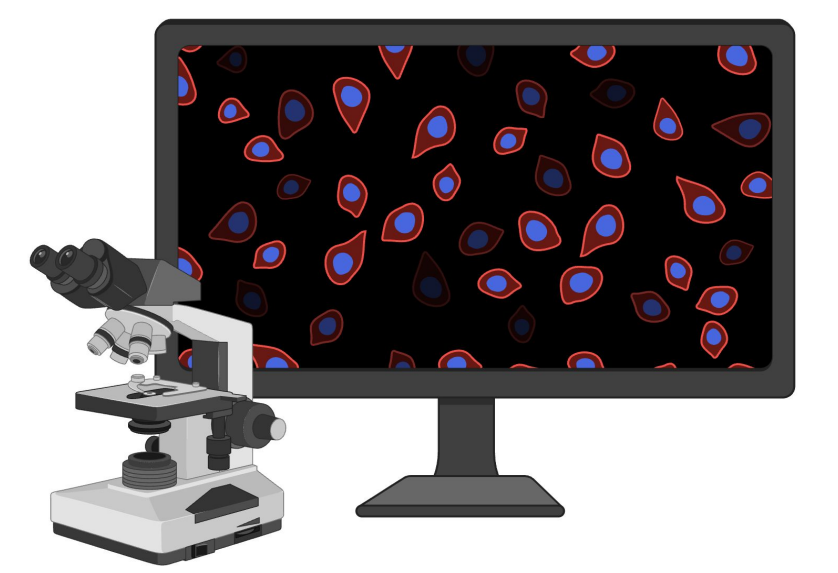
*For CB1R, the PL of one mouse per age group was stained

Histology:

- PNN:
 - Primary antibody: biotinylated Wisteria floribunda agglutinin (WFA; 1:500 dilution, Vector Labs, #B1355)
 - Secondary antibody: Alexa Fluor 647 conjugated to streptavidin (1:500, Abcam, ab272189)
- CB1:
 - Primary antibody: gifted to us by Ken Mackie (#22)
 - Secondary antibody: Alexa Fluor 488 (1:500, Signaling)
- DAPI staining for nuclei

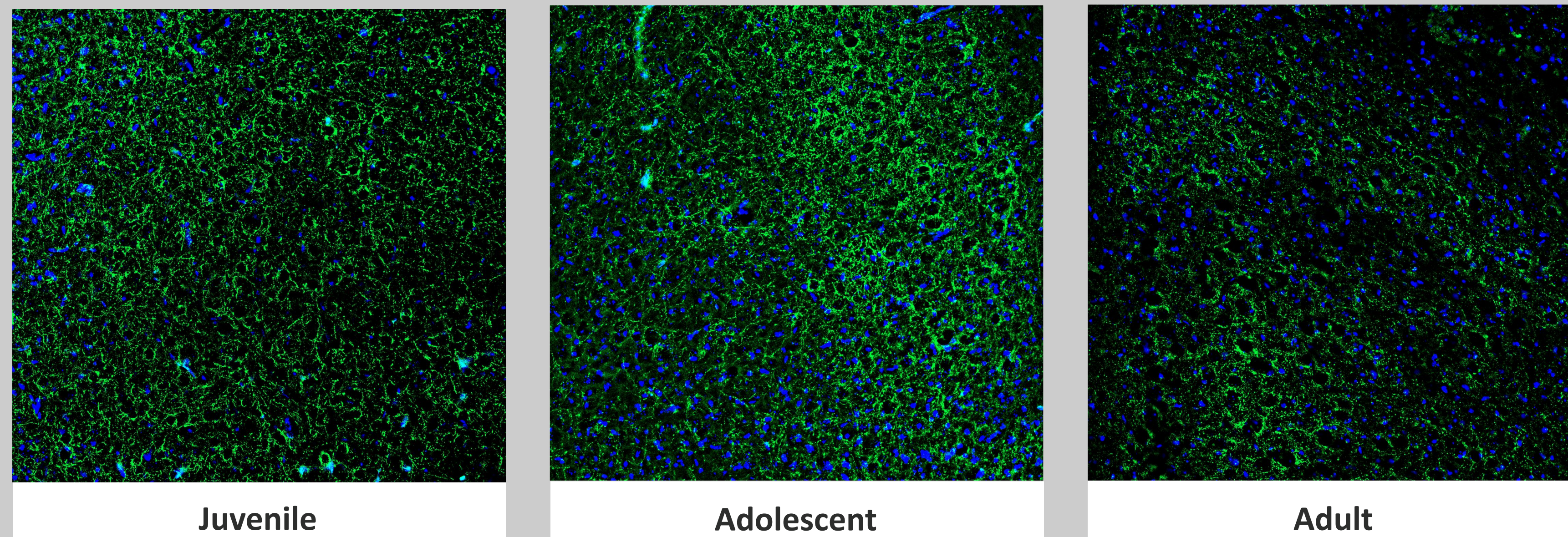
Imaging:

- THUNDER Imager Tissue microscope** (Leica Microsystems) used to visualize the RSC and PL at 20x magnification
- AIVIA** (machine learning model for cell counting) used to quantify PNN expression across age groups in the RSC.
- ImageJ** (image processing program) was used to measure CB1R intensity levels, a marker for CB1R prevalence, in the PL

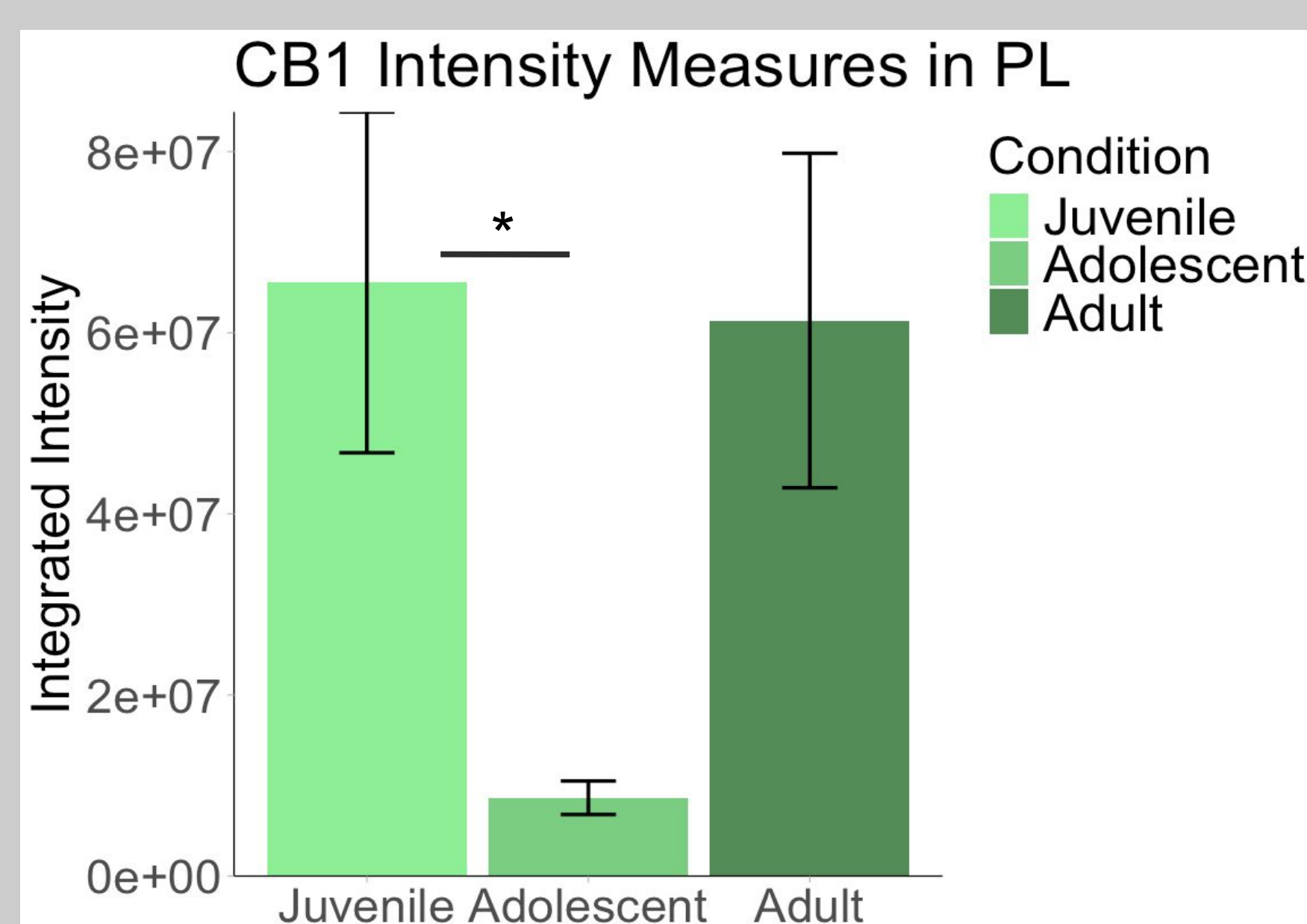
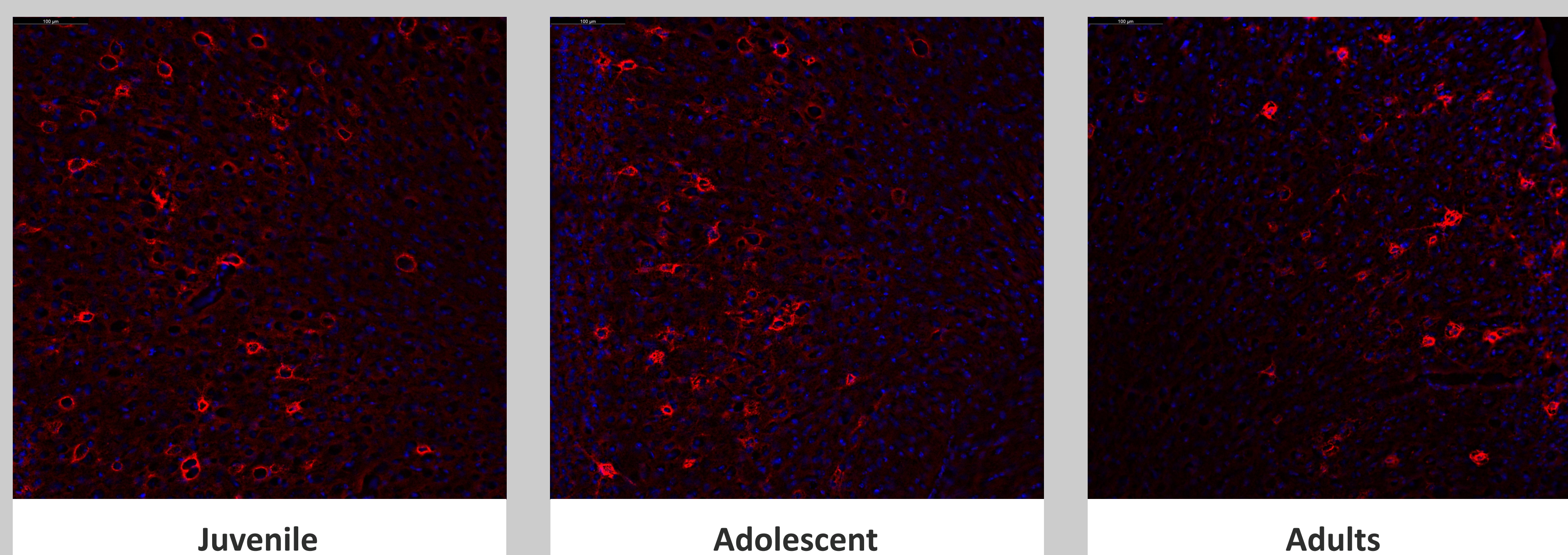


Results

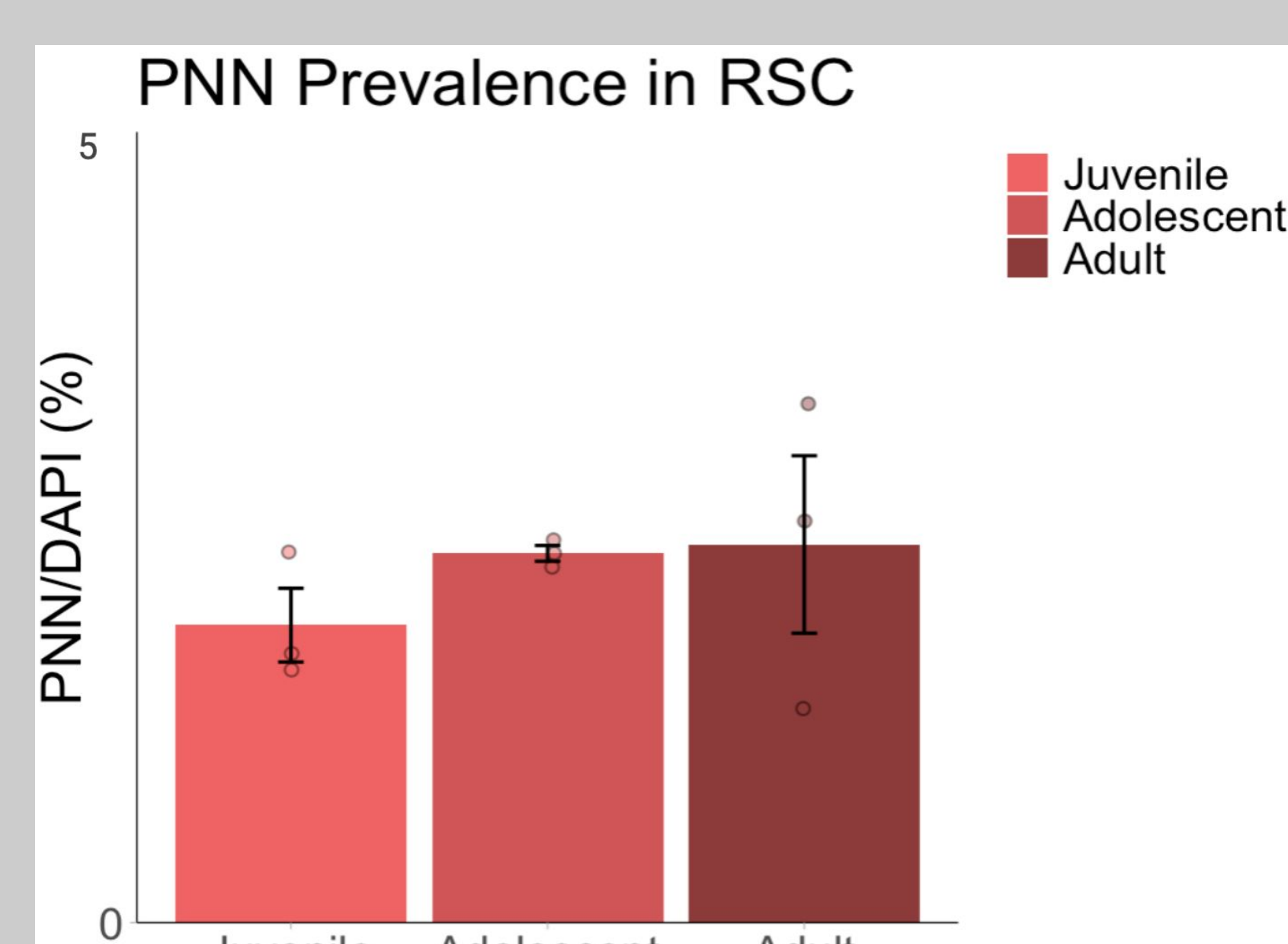
CB1Rs (Green) and Cell Nuclei (Blue) Across Age Groups



PNNs (Red) and Cell Nuclei (Blue) Across Age Groups



There was a **main effect of age ($p=0.033$)** in CB1R prevalence across juvenile, adolescent, and adult data. Following a TukeyHSD test, there is a **significant difference between adolescent and juveniles ($p=0.046$)**, but not across other age groups.



There was **not a main effect of age ($p=0.564$)** across PNN to DAPI cell counts in our juvenile, adolescent, and adult data. Cutoff: PNN Cutoff: >6 or <90 and DAPI Cutoff: >1000

Discussion

Imaging Results:

- PNN quantities were **relatively steady** across all three age groups
 - PNNs in the RSC may already be refined at an earlier age (such as before PND 23, the starting age for our juveniles)
- CB1R intensity levels were **significantly lower in adolescent** mice compared to juvenile and adult groups
 - In the PL, there may be other compensatory regulatory systems that decrease the need for CB1Rs throughout adolescence

Future Work:

- Moving forward, we would want to **include more brains** in the sample to see greater differences across age groups
- Using better blocking solutions, such as donkey serum instead of goat, can help to minimize background noise
- With more refined protocols, we could look for **overlap between PNNs and CB1Rs** in the same region, highlighting potential interactions that may change across development

Relevance:

- We want to better understand how anxiety disorder treatments can be modified to accommodate to the needs of various age groups
- We also included female mice to account for the historical use of primarily adult male mice in studies.

References

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