

Effects of Estrogen on Bladder

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Background

- Interstitial Cystitis(IC) is disease characterized by chronic pain and pressure in bladder/pelvic region
- Women are 5-10x more likely to get IC compared to men
- IC bladders are hyperinnervated. This includes sensory nerves
- Women with IC report greater levels of pain during specific stages in their menstrual cycle especially when levels of estrogen are high

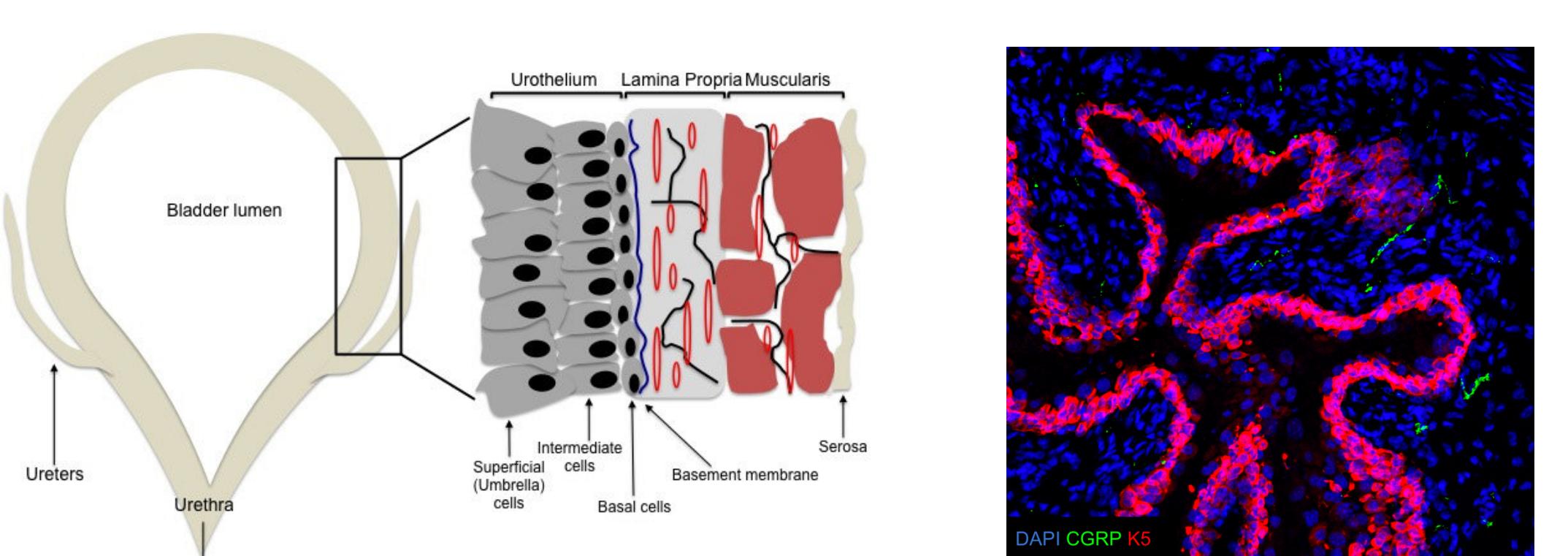
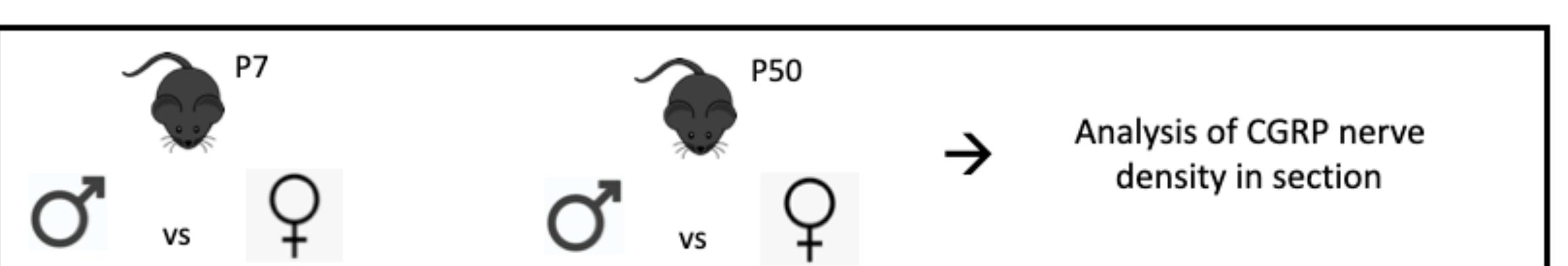


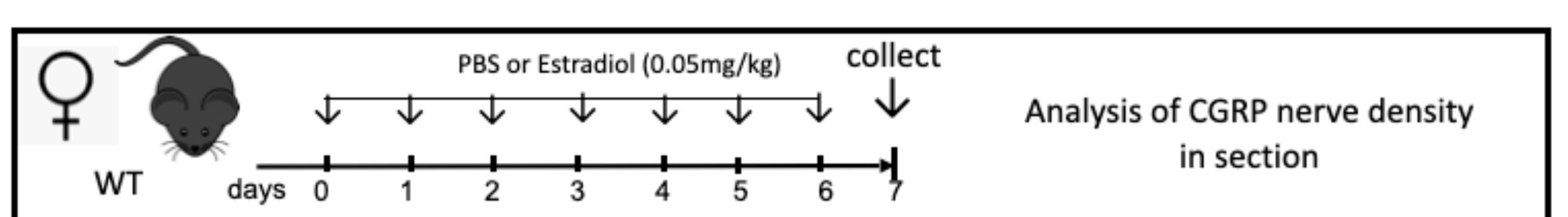
Figure 1. Chan et. al (2017). "The Current Use of Stem Cells in Bladder Tissue Regeneration and Bioengineering. Biomedicines," 5. 4. 10.3390/biomedicines5010004.

Methods

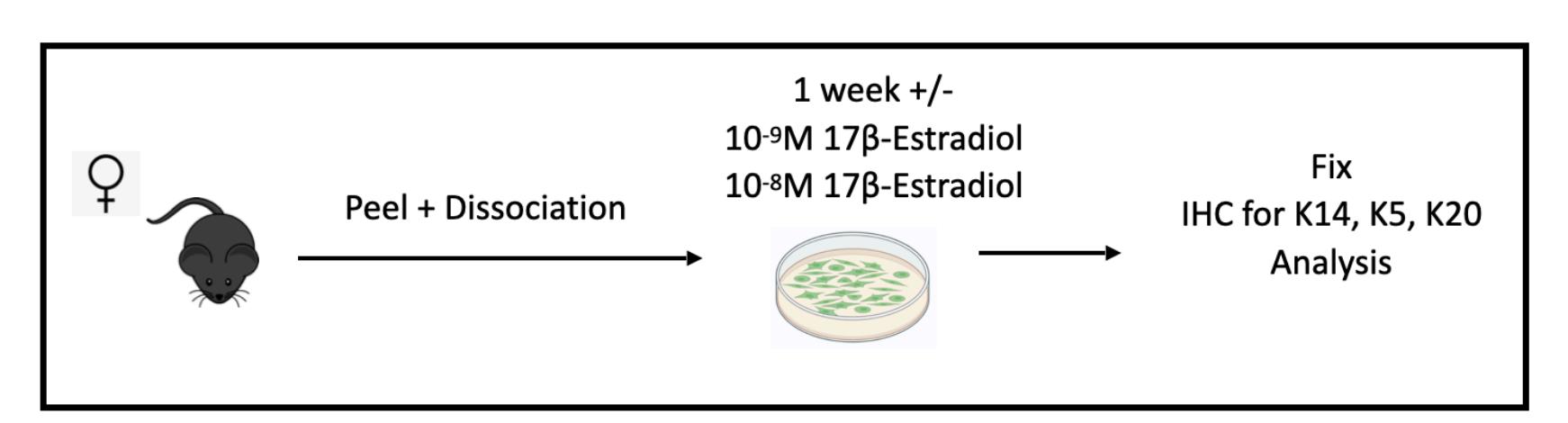
Experiment 1:



Experiment 2:



Experiment 3:



Physiological serum [17 β -Estradiol] (pre-menopause) = 1.0×10^{-10} M to 1.47×10^{-9} M

Hypothesis/Aims

Hypothesis: We hypothesis that estrogen will impact bladder sensory innervation and urothelial homeostasis

Aims:

- To develop a Neurite Tracing Tool to quantify bladder sensory innervation
- To determine the effect of endogenous estrogen on bladder sensory innervation over time
- To determine the effect of estrogen supplementation on bladder sensory innervation
- To determine the effect of exogenous estrogen on urothelial organoid number, size, and composition

Results

Experiment 1:

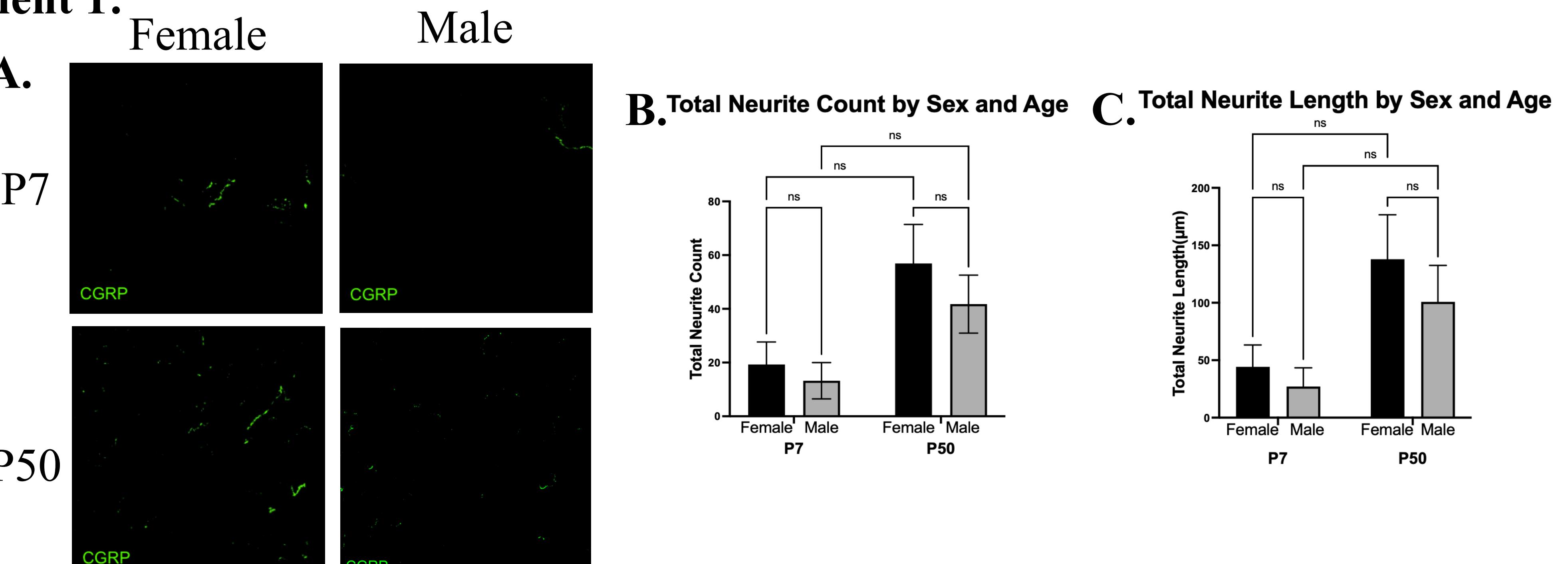


Figure 3. (A) Immunostaining of CGRP nerves in female and male bladders at P7 and P50. Sections are 12 μ m thick. Scale bar is 100 μ m. (B) Quantification of neurites in (A) found by the Neurite Tracing Tool. Although not significant, bladders appear more innervated with age (P50>P7) and female bladders appear more innervated than male bladders. (n = 6-10 for each condition). (C) Quantification of total neurite length in (A). Total green pixel number was divided by the average neurite width. Results are consistent with total neurite count in (B).

Experiment 2:

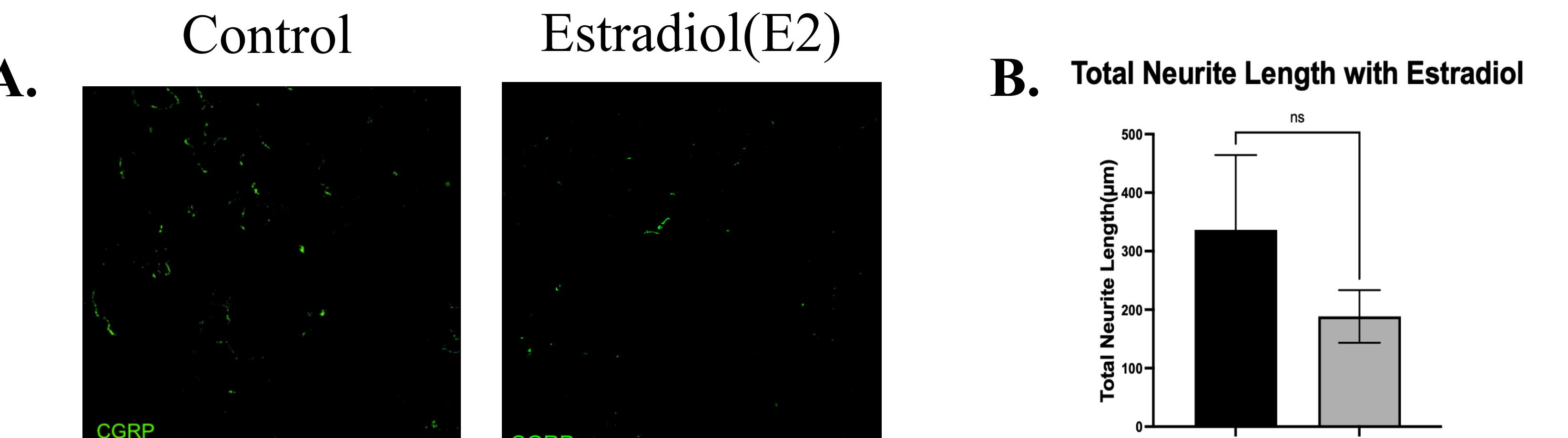


Figure 4. (A) Immunostaining of CGRP nerves in control and estradiol-treated female mice. Sections are 12 μ m. Scale bar is 100 μ m. (B) Quantification of neurites found by the Neurite Tracing Tool. Total green pixel number was divided by the average neurite width to achieve total neurite length in an image. Results are consistent with total neurite count (data not shown) (n = 5 for each condition). We did not find a significant effect of supplemental estrogen on bladder sensory innervation. In fact, too much exogenous estrogen may impair sensory neurites in the bladder.

Experiment 3:

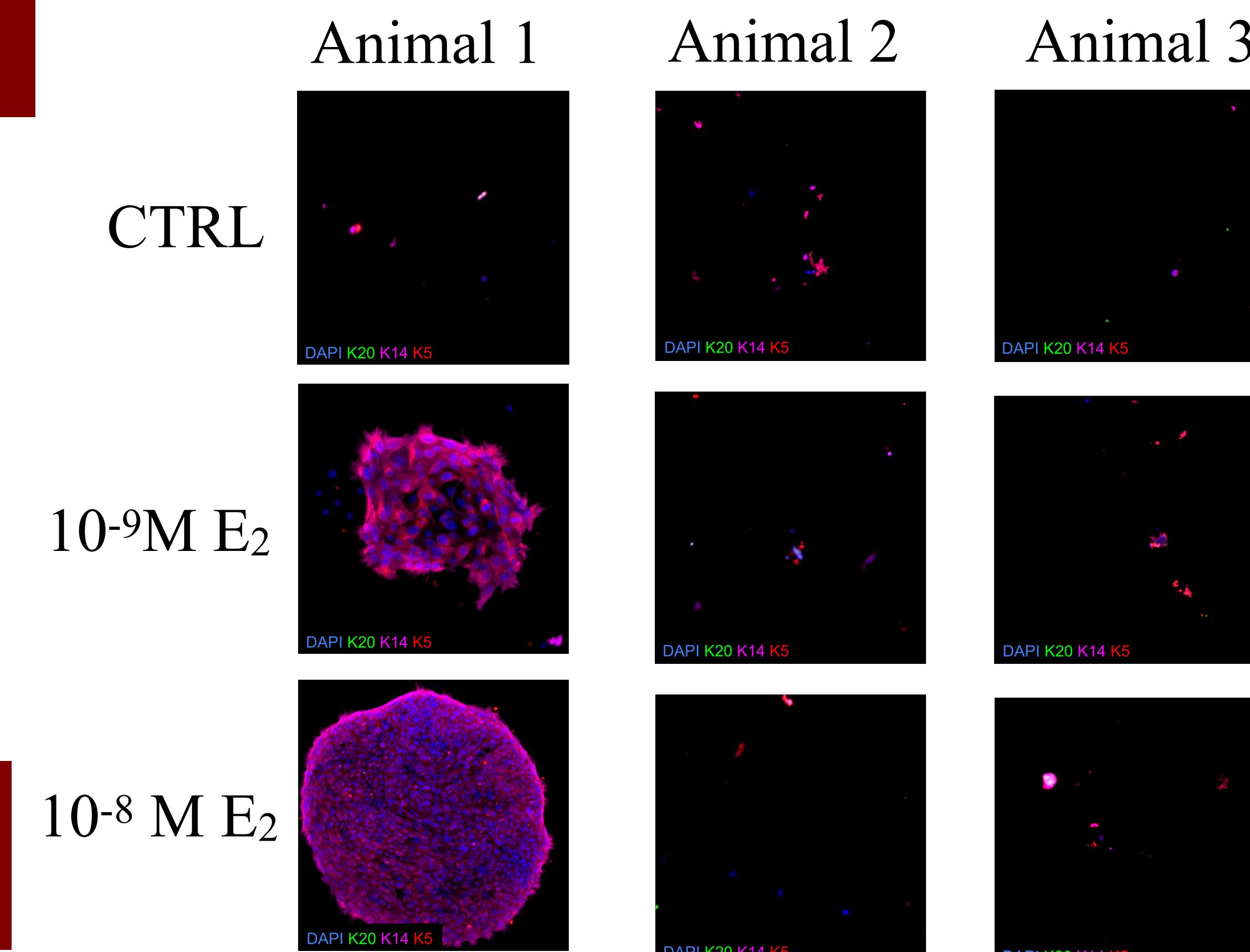


Figure 5. Immunostaining of organoids grown in control, low estradiol (10^{-9} M E₂), and high estradiol (10^{-8} M E₂) conditions for 7 days. K20 marks umbrella cells, K5 marks basal cells, and K14 marks bladder stem cells. Organoids were only formed in Animal 1 for both estradiol dose-dependent conditions. Organoid size was greatest in the high estradiol condition. Additionally, we found more organoids in the high estradiol condition, than in the low estradiol and ctrl conditions (23 vs 7 vs 0). Scale is 100 μ m.

Conclusions

- Although not significant, bladder sensory innervation increases overtime and female bladders appear more innervated than male bladders.
- Estradiol supplementation did not appear to increase bladder sensory innervation
- In 1/3 animals, estrogen appeared to increase urothelial organoid size and number in dose-dependent manner

Future Directions

- Quantify the width of neurites within the Neurite Tracing Tool
- Connect the broken neurite segments to count as a singular neurite in the Neurite Tracing Tool
- Repeat urothelial organoid experiment with estradiol gradient
- Determine whether ovariectomy reduces bladder sensory innervation

Acknowledgment

- This research was funded by Stanford Diversity and Inclusion, Packard Foundation, Stanford Office of Scientific Outreach - Kyle Cole, Weissman Family
- Dr. Phil Beachy for allow me to work in the Beachy Lab
- Mallory Laboulaye for guiding me through these experiments