

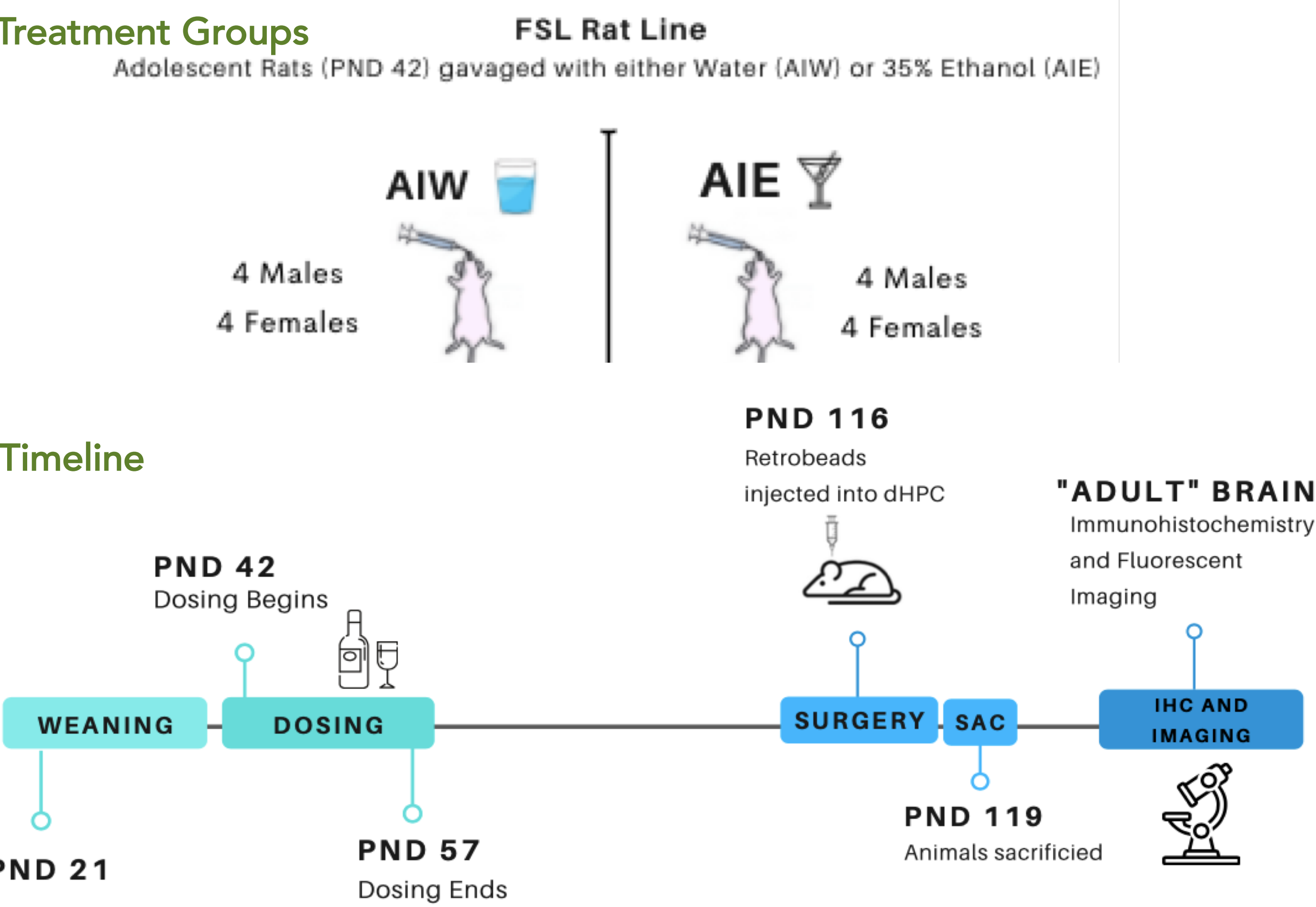
Adolescent alcohol use is a serious public health concern.

Background

- What is adolescent binge drinking, or adolescent intermittent ethanol (AIE) exposure?
- **Adolescence:** begins with the onset of puberty, spans the teen years, and proceeds well into the 20's
 - Critical for an individual's ability to function effectively in the adult world
 - In both humans and rodents, adolescents and young adults respond differently to acute ethanol exposure
 - Become more vulnerable to enduring negative effects of repeated ethanol exposure that persist into adulthood, including impaired cognitive function and memory deficits (Crews et al, 2014)
 - AIE: A rodent model for human adolescent alcohol binge drinking

What We Know	What We Don't Know
• Memory-related hippocampal functioning is driven by cholinergic inputs from the Medial septum and diagonal band of Broca (Ch1-2) to the dorsal hippocampus (dHPC) (Madison et al, 1987) • Choline Acetyltransferase (ChAT), a cholinergic marker, is reduced in Ch 1-2 following AIE exposure (Crews et al, 2014)	Does AIE exposure reduce neuronal density in Ch 1-2 in general? Of any surviving neurons, what fraction are cholinergic projections in the Ch 1-2 → dHPC circuit, thus influencing hippocampal memory function? Speculated Mechanism Reduced cholinergic projections from Ch 1-2 → dHPC following AIE exposure → impaired memory effects in adulthood

Methods



Immunohistochemistry

As of now, a pilot IHC has been completed on practice tissue following Retrobead surgery to...

- Optimize primary antibody (goat anti-Choline acetyltransferase)
- Perform IHC pilot at varying dilutions (1:100 and 1:200) of primary antibody (goat anti-ChAT, ChAT=cholinergic marker)
- Image using Axio Imager Microscope: look in GFP and Cy5 channels for Retrobeads and ChAT-positive neurons, respectively

Results

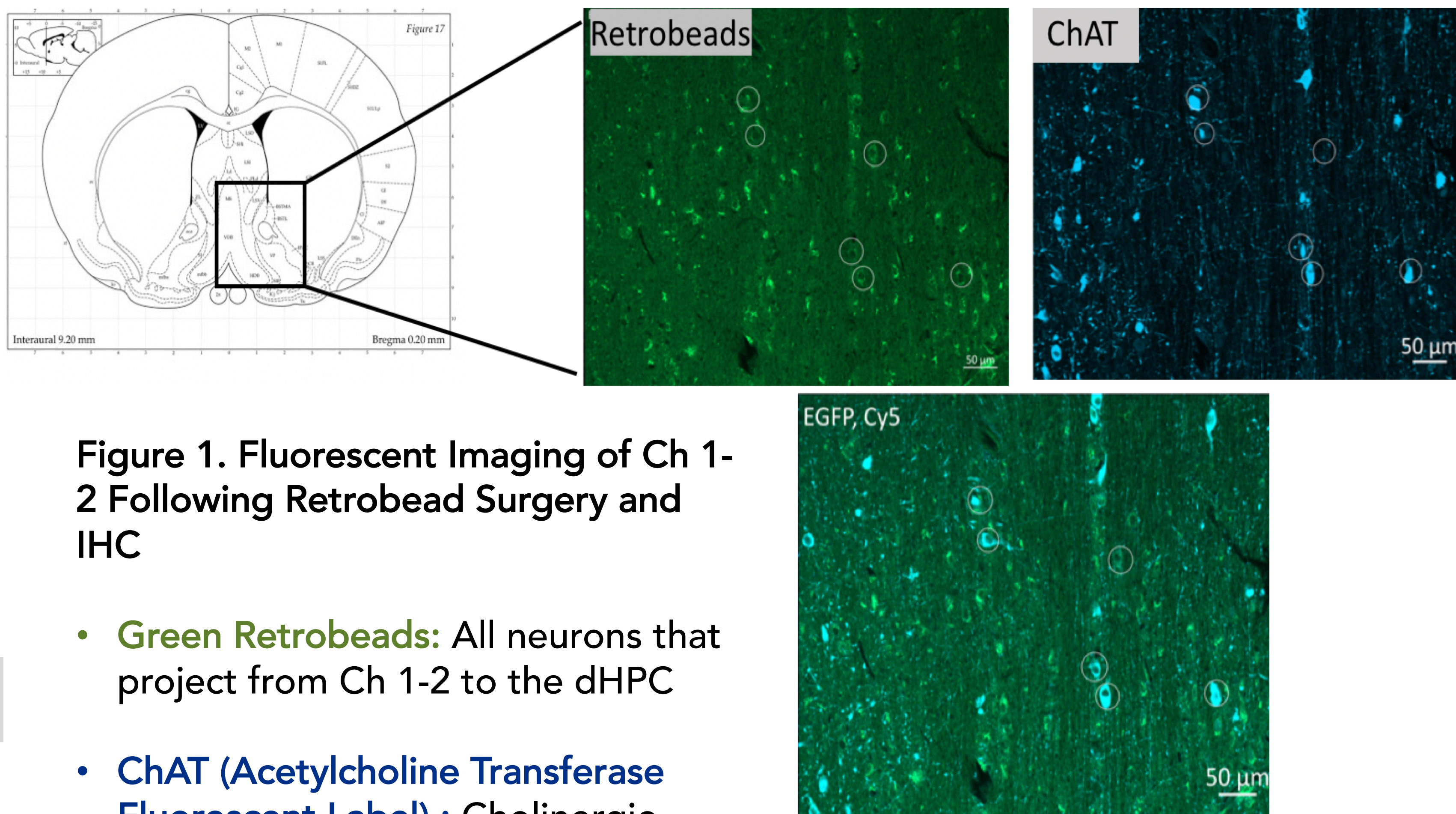


Figure 1. Fluorescent Imaging of Ch 1-2 Following Retrobead Surgery and IHC

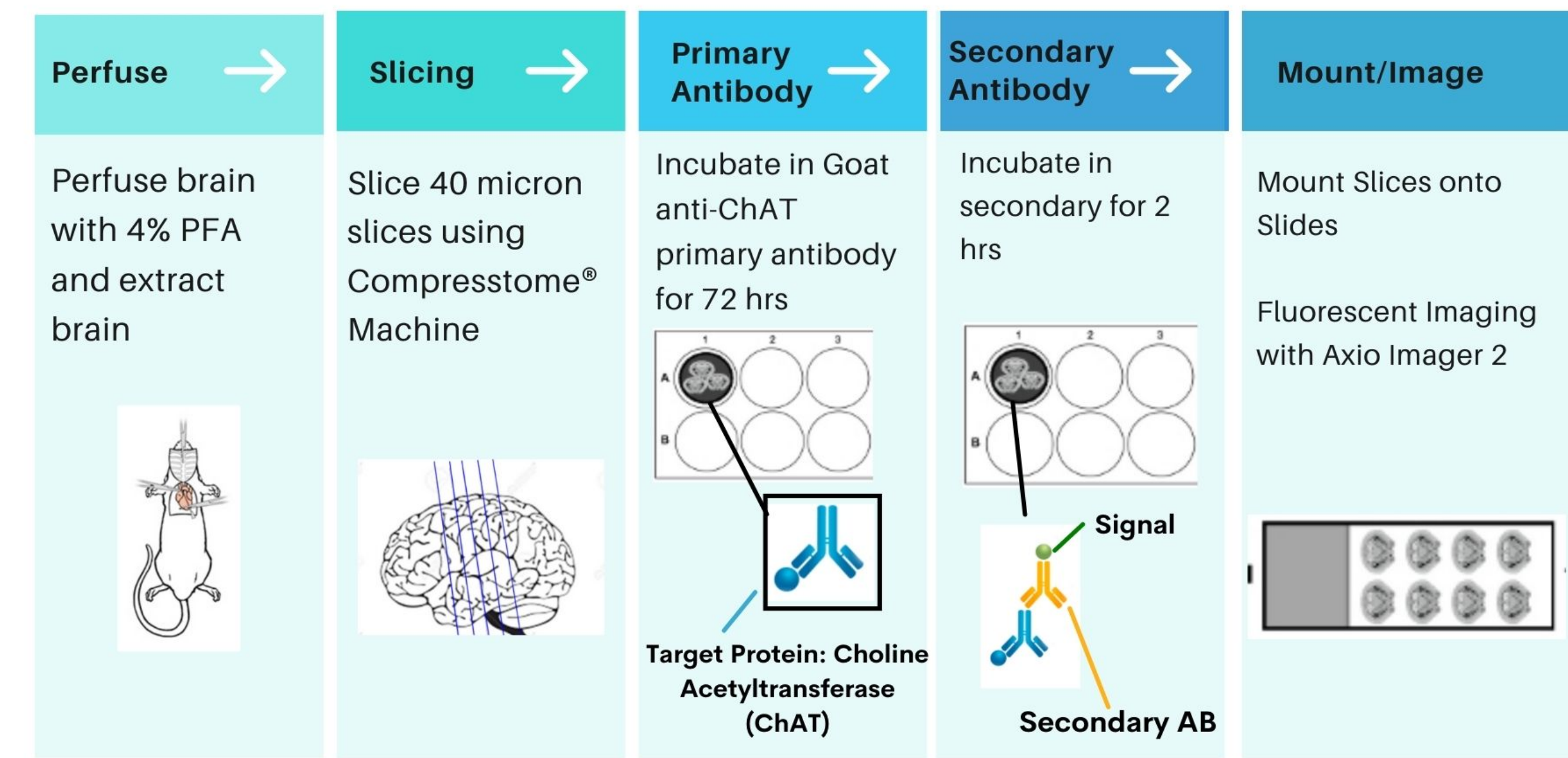
- **Green Retrobeads:** All neurons that project from Ch 1-2 to the dHPC
- **ChAT (Acetylcholine Transferase Fluorescent Label) :** Cholinergic Neurons
- **Colocalization:** Cholinergic neurons in Ch 1-2 → dHPC circuit

Takeaways:

- **Retrograde transport of Green Retrobeads (Lumafluor) successful:** Green Retrobeads injected into dHPC found in upstream Ch 1-2 targets
- **Colocalization between Retrobeads and ChAT:** Some projections from Ch 1-2 → dHPC have cholinergic specificity
- **1:200 Primary Antibody dilution** sufficient for ChAT protein labeling of cholinergic neurons
- **Cy5 Secondary Antibodies for IHC:** Cy5 fluorescent label allows for successful visualization of cholinergic neurons in Ch 1-2

IMMUNOHISTOCHEMISTRY:

Cholinergic neuron labeling in Ch1/2 after AIE vs AIW



Next Steps

On "Real" Experimental Tissue:

Perform IHC, imaging, and statistical analysis to assess differences in the Ch 1/2 → dHPC circuit of interest in AIE vs. AIW FSL rats

Hypothesized Results Based on Previous Work (Vetreno et al, 2014)

- Diminished Green Retrobead fluorescent intensity in the Ch 1/2 regions in the AIE treatment group compared to AIW
- AIE- induced attenuation in ChAT-positive neurons in Ch 1/2
- Reduced colocalization of Retrobead and ChAT-positive fluorescent signals

References

Crews, F. T., Vetreno, R. P., Broadwater, M. A., & Robinson, D. L. (2016). Adolescent Alcohol Exposure Persistently Impacts Adult Neurobiology and Behavior. *Pharmacological reviews*, 68(4), 1074–1109. <https://doi.org/10.1124/pr.115.012138>

Madison, D. V., Lancaster, B., & Nicoll, R. A. (1987). Voltage clamp analysis of cholinergic action in the hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 7(3), 733–741. <https://doi.org/10.1523/JNEUROSCI.07-03-00733.198>

Vetreno, R. P., Broadwater, M., Liu, W., Spear, L. P., & Crews, F. T. (2014). Adolescent, but not adult, binge ethanol exposure leads to persistent global reductions of choline acetyltransferase expressing neurons in brain. *PLoS one*, 9(11), e113421. <https://doi.org/10.1371/journal.pone.0113421>