Impact of adolescent intermittent ethanol exposure on interneurons and their surrounding perineuronal nets in adulthood

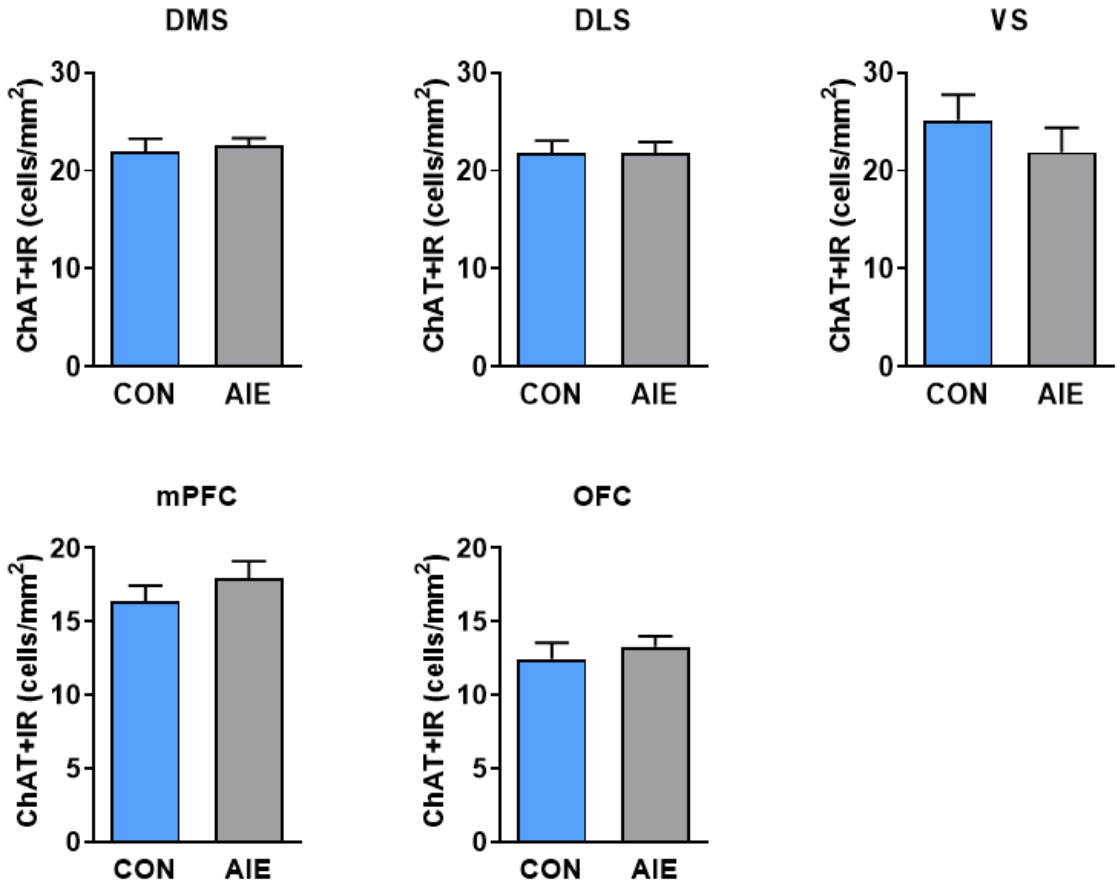
Supplemental Material

Authors: Carol A. Dannenhoffer, PhD[1] *, Alexander Gómez-A, PhD[1] *, Victoria A. Macht, PhD[1], Rayyanoor Jawad, BS[1], E. Blake Sutherland[1], Ryan P. Vetreno, PhD [1,5], Fulton T. Crews, PhD [1,5,6], Charlotte A. Boettiger, PhD [1,2,3,4], Donita L. Robinson, PhD [1,4,5]
*co-first authors

Author affiliations:
[1]Bowles Center for Alcohol Studies, School of Medicine, University of North Carolina at Chapel Hill
[2]Department of Psychology and Neuroscience, University of North Carolina at Chapel Hill
[3]Biomedical Research Imaging Center, University of North Carolina at Chapel Hill
[4]Neuroscience Curriculum, University of North Carolina at Chapel Hill
[5]Department of Psychiatry, School of Medicine, University of North Carolina at Chapel Hill
[6]Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill

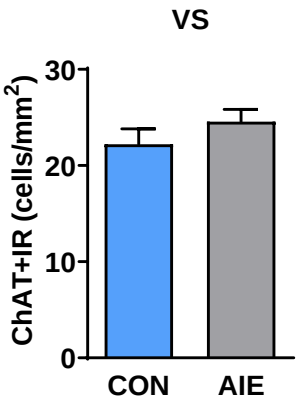
Corresponding author:
Donita L. Robinson, PhD.
Bowles Center for Alcohol Studies, CB 7178
University of North Carolina at Chapel Hill, NC, 27599-7178, USA
Telephone: 919-966-9178
Fax: 919-966-5679
E-mail: dlr@unc.edu

Supplemental Figure 1.



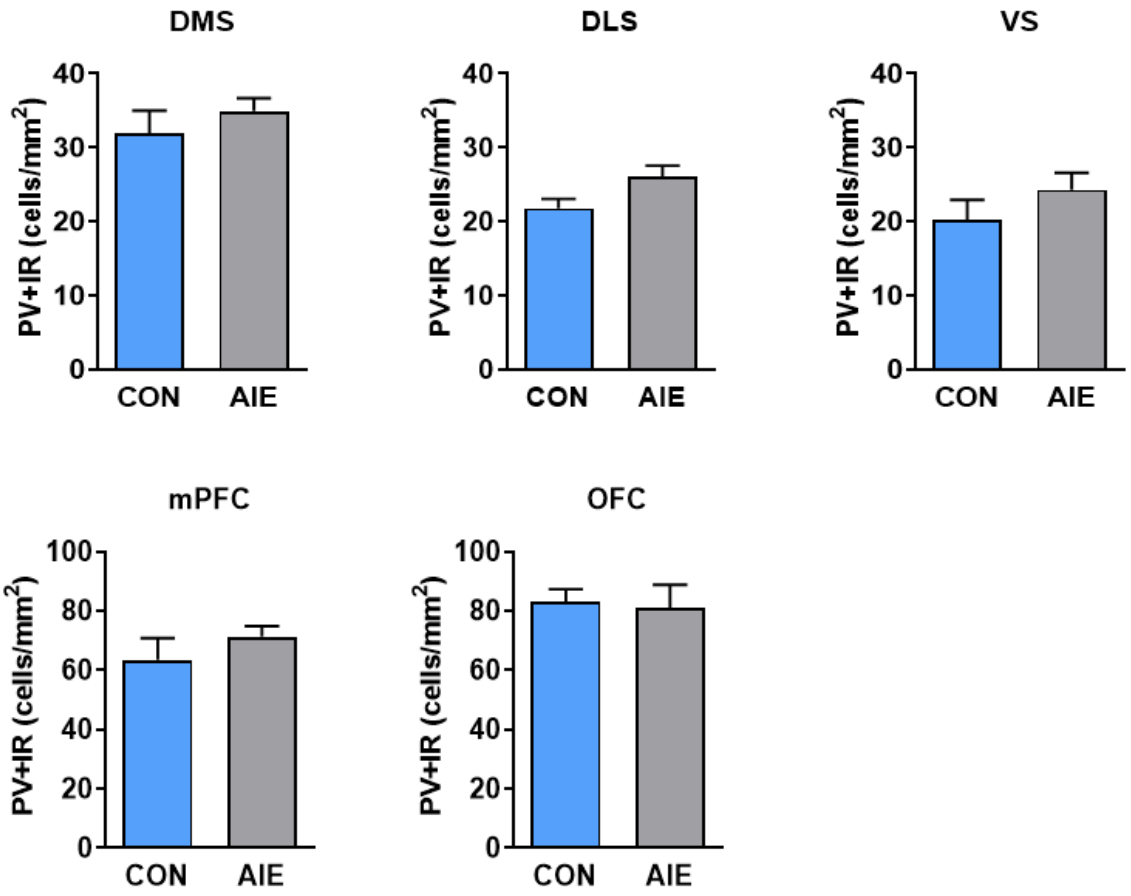
Supplemental Figure 1. Adolescent intermittent ethanol (AIE) exposure did not alter striatal or prefrontal choline acetyltransferase (ChAT) expression. T-tests were used to assess group differences within subregion; however, α levels were adjusted to correct for multiple comparisons (striatum: α=0.0167; PFC: α=0.025). Top panel: The striatum was subdivided into dorsomedial (DMS), dorsolateral (DLS), and ventral (VS) regions. ChAT immunoreactivity (ChAT+IR) was not significantly different between AIE and control (CON) subjects in the DMS (p=0.677), DLS (p=0.957), or VS (p=0.387). Bottom panel: The prefrontal cortex was subdivided into medial (mPFC) and orbitofrontal (OFC) regions. Neither of these subregions had differences in expression of ChAT (mPFC: p=0.335; OFC: p=0.515). Data are presented as mean ± SEM.

Supplemental Figure 2.



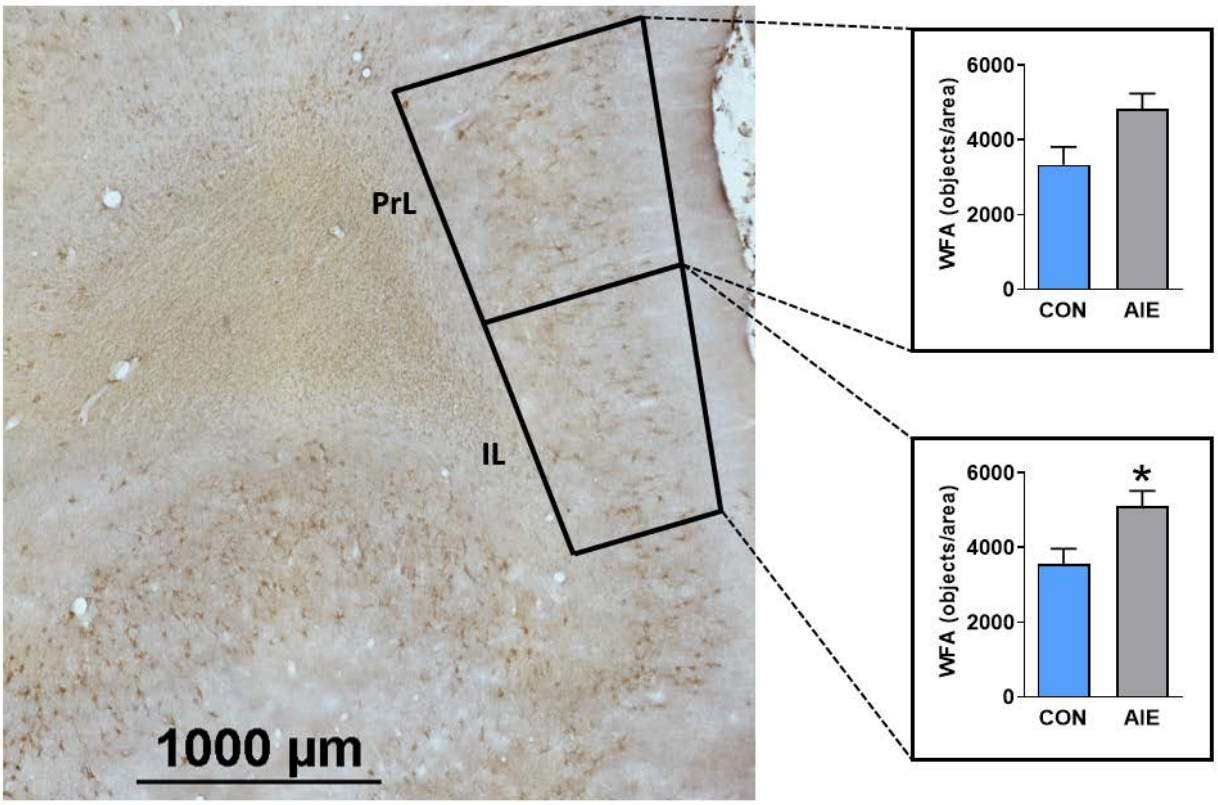
Supplemental Figure 2. Archived tissue from a previous study (CON n=8; AIE n=8) was used for follow-up analyses; that study used male Sprague-Dawley rats that underwent identical alcohol and water exposures. This follow-up analysis confirmed no change (p=0.255) in ventral striatal ChAT expression following AIE exposure. Data are presented as mean ± SEM.

Supplemental Figure 3.



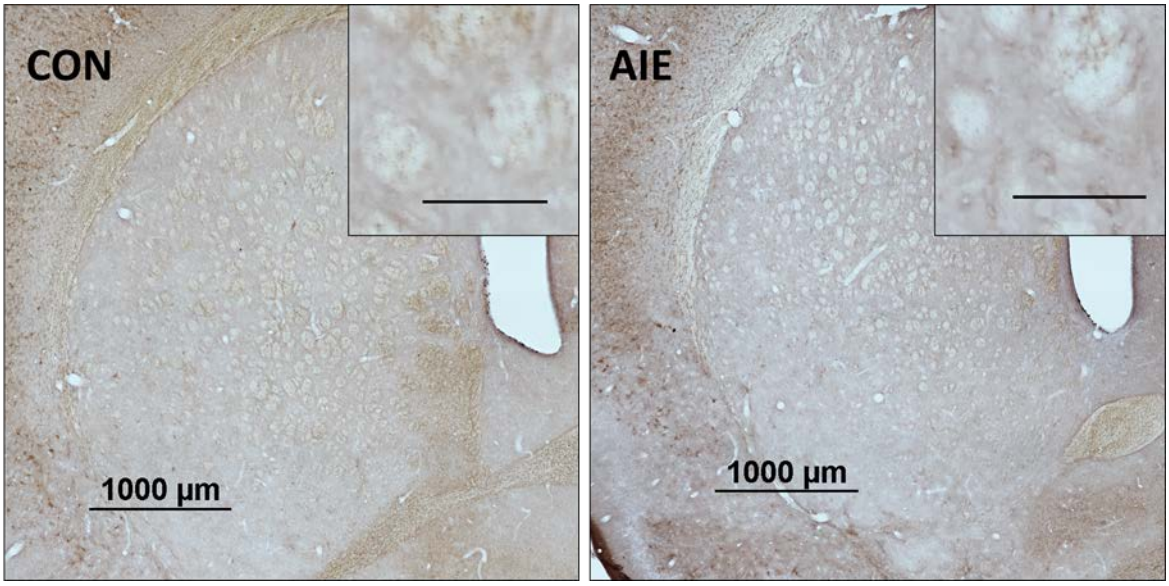
Supplemental Figure 3. Parvalbumin (PV) expression is unaltered following AIE exposure. T-tests were used to assess group differences within subregion; however, α levels were adjusted to correct for multiple comparisons (striatum: α=0.0167; PFC: α=0.025). Top panel: The striatum was subdivided into dorsomedial (DMS), dorsolateral (DLS), and ventral (VS). PV immunoreactivity (PV+IR) was not significantly different between AIE and CON subjects in the DMS (p=0.387), DLS (p=0.051), or VS (p=0.267). Bottom panel: The PFC was subdivided into mPFC and OFC; however, no difference in PV expression was detected in either subregion (mPFC: p=0.317; OFC: p=0.816). Data are presented as mean ± SEM.

Supplemental Figure 4.



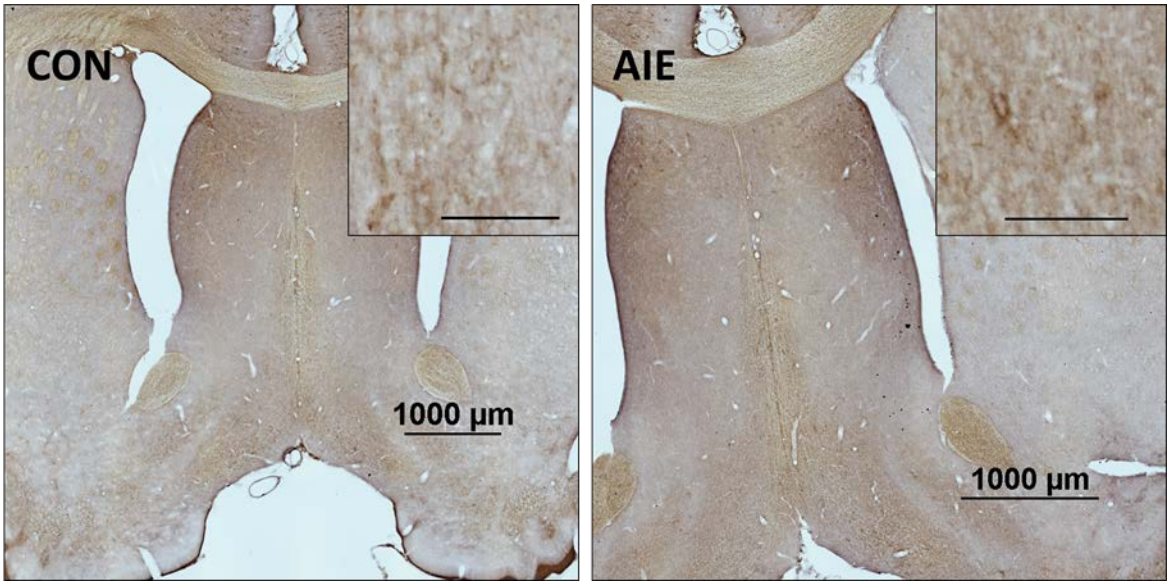
Supplemental Figure 4. We observed an increase in overall PNN density in the mPFC; hence we further subdivided this region into prelimbic (PrL) and infralimbic (IL) and adjusted α to correct for multiple comparisons (α=0.025). PNN density was significantly higher within the IL of AIE rats (t (17) =2.63; p=0.02) and a trend was observed within the PrL (p=0.0255). Data are presented as mean ± SEM. * indicates main effect of exposure.

Supplemental Figure 5.



Supplemental Figure 5. We observed no PNNs within the striatum of rats; therefore, data analysis was not conducted on this area. Scale bar in small inset image represents 100 µm.

Supplemental Figure 6.



Supplemental Figure 6. We observed no PNNs within the basal forebrain of rats; therefore, data analysis was not conducted on this area. Scale bar in small inset image represents 100 µm.