



Perineuronal Nets in the Thalamic Reticular Nucleus after Postnatal Alcohol Exposure in a Rodent Model of FASD

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INTRODUCTION

- Fetal alcohol spectrum disorders (FASDs) may result from prenatal exposure to alcohol (AE).
- One of the most prevalent symptoms of FASDs is impaired executive functioning (EF)¹.
 - EF is reliant on communication and connection between the medial prefrontal cortex and hippocampus - circuitry that involves structures in the midline thalamic region².
- Inhibitory action in the thalamus is driven by the **thalamic reticular nucleus** (TRN) - a region rich in inhibitory Parvalbumin-positive (PV+) interneurons.
- The majority of interneurons, including PV+ interneurons, are surrounded by **perineuronal nets** (PNNs) - a component of the extracellular matrix⁶.
- The cortex has a high number of PV+ interneurons, and thus PNNs.
- PNNs are important in maintaining the excitatory/inhibitory balance, as well as synaptic stabilization⁷.
- PNNs are vulnerable to insult during periods of development, such as the brain growth spurt (BGS) - a period of rapid neurodevelopment spanning two postnatal weeks in rats⁸.
 - We use postnatal days (PD) 4 through 9 to examine maximal developmental insult.
- AE disrupts typical development of PNNs in the cortex (**Fig. 7**).

We aim to investigate how AE affects the expression of PNNs in the TRN at different time points in development in a rodent model of the third trimester.

METHODS

Subjects: Long-Evans rats

Single Day Exposure on PD9:

- PD9 Sacrifice
 - AE - 8F, 8M
 - SI - 6F, 7M
- PD15 Sacrifice
 - AE - 7F, 6M
 - SI - 6F, 7M
- PD65 Sacrifice
 - AE - 7F, 8M
 - SI - 7F, 7M

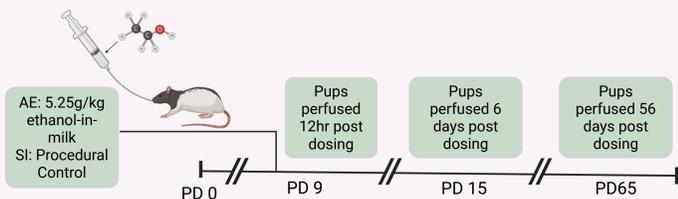
Housing: One cohort each of PD15 and PD30 full dose were exposed to superintervention conditions (aerobic exercise followed by environment complexity [WR/EC]⁹). Remaining rats were socially housed following dosing until perfusing.

Tissue Collection: Rats were anesthetized and intracardially perfused with 4% paraformaldehyde in 0.1M PBS on PD9, PD15, PD30, PD65 or PD80. Whole brains were extracted, cryoprotected, and later sectioned coronally at 40 μ m.

Alcohol Exposure: Pups were given two doses (5.25 g/kg/day) of ethanol in milk substitute 2 hours apart on a single day (PD9) or spanning six days (PD4-9). SI groups received intubation with no liquid.

Immunocytochemical Staining: Tissue was stained with biotinylated Wisteria Floribunda Agglutinin (WFA) for PNNs, followed by nickel enhanced-Diaminobenzidine incubation to visualize PNNs in TRN.

Optical Densitometry: Optical densitometry of cortex or TRN (rostral-caudal extension) will be measured using ImageJ.



RESULTS

- There was a significant effect of Treatment ($F_{1,21} = 6.296, p = 0.020, \eta^2 = 0.231$) wherein PD 9 AE rats had a 32.9% lower average optical density of PNN compared to SI controls. In addition, there was a significant effect of Sex ($F_{1,21} = 8.347, p = 0.009, \eta^2 = 0.284$) where males have a lower density of PNNs on PD 9 irrespective of treatment. Post-hoc analyses revealed a significant difference between AE and SI males on PD 9 ($p = 0.05$) (**Fig. 1**)
- PD 9 male AE rats had significantly decreased PNN density in the TRN compared to PD 15 SI, PD 65 SI and AE male rats ($p = 0.49$) (**Fig. 4**)

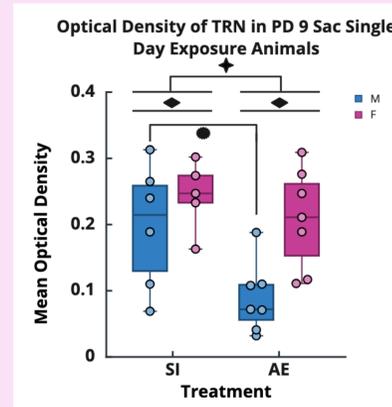


Figure 1. AE pups on PD 9 have 32.9% lower average PNN density compared to SI controls ($p = 0.02$), specifically AE males ($p = 0.05$).

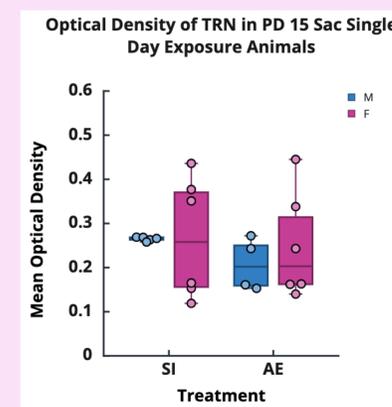


Figure 2. No significant differences in PNN density in TRN between groups on PD 15.

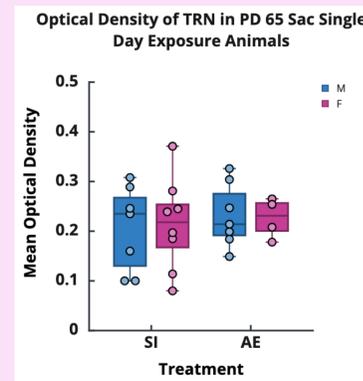


Figure 3. No significant differences in PNN density in TRN between groups on PD 15.

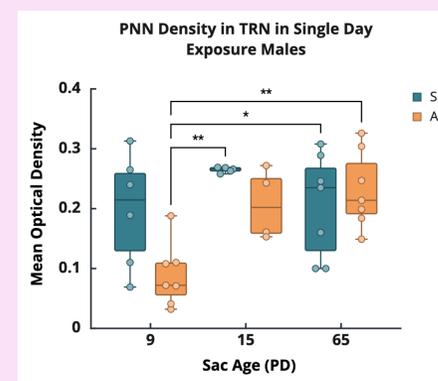


Figure 4. PD 9 AE males have significantly lower PNN density, but this value recovers by adulthood indicating a potential critical period or recovery mechanism.

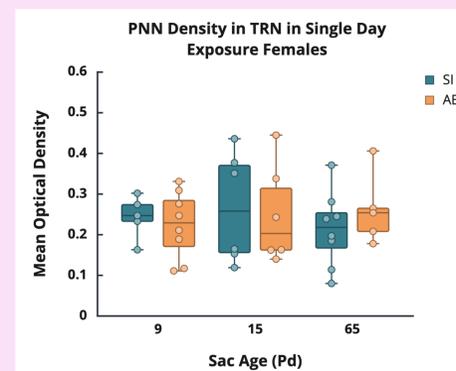


Figure 5. Females across all time points maintain PNN density in the TRN.

PNN IMAGES

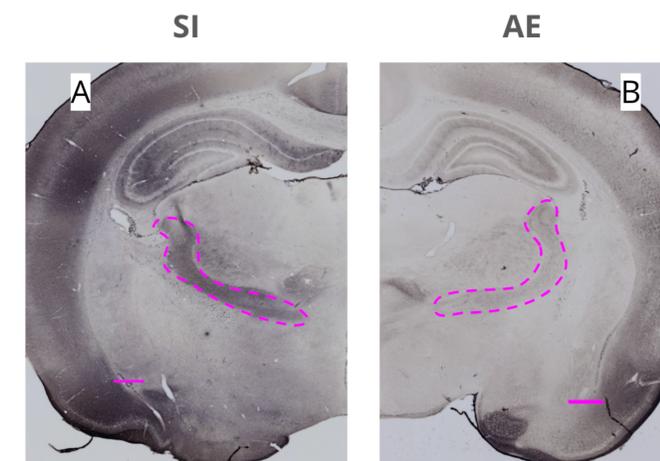


Figure 6. Visibly decreased expression of PNNs in TRN in PD 9 AE animals (B) compared to their SI counterparts (A).

PNN DENSITY IN THE CORTEX

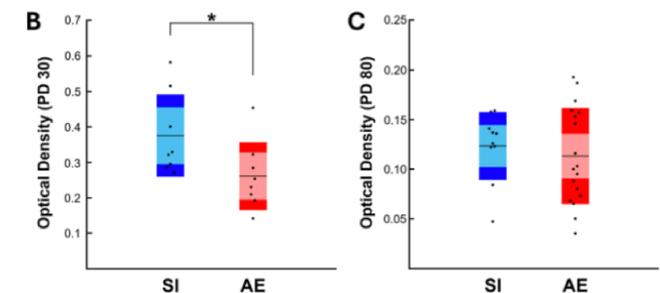


Figure 7. Optical density of PNN in the cortex of PD30 AE animals is significantly reduced compared to SI rats. This difference is no longer detected at PD80. (Data from Williams et al., 2025)

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