Adolescent Binge Ethanol Exposure Accelerates Alzheimer’s Disease Neuropathology in the Basal Forebrain

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Abstract

Cholinergic degeneration and neuroimmune system activation are hallmark features of many disease states, including alcohol use disorder (AUD) and Alzheimer’s disease (AD). Heavy alcohol use is an etiological factor associated with AD, but little is known about the interaction between adolescent binge alcohol exposure and AD pathology. Preclinical studies using the adolescent intermittent ethanol (AIE) paradigm that mimics weekend binge drinking behavior find basal forebrain cholinergic neuron degeneration and increased neuroimmune activation in brain, similar to observed pathology in AD. Using the 5x Familial Alzheimer’s disease (5xFAD) mouse model of AD, we tested the hypothesis that AIE treatment would accelerate onset of AD-associated pathology. We report AIE accelerated the loss of basal forebrain cholinergic neurons and hnRNP expression relative to age-matched 5xFAD CONs in female, but not male, subjects. This was accompanied by accelerated accumulation of amyloid beta as well as upregulation of AD-related genes. In addition, AIE upregulated glial genes (e.g., Gfap and Iba1), increased microglial Iba1+IR, and induced proinflammatory innate immune signaling genes in the basal forebrain, relative to 5xFAD CONs. In post-mortem human basal forebrain samples of individuals with AUD and an adolescent age of drinking onset, we found AUD increased amyloid beta expression in the basal forebrain and in ChAT+ neurons of AUD individuals, and decreased hnRNP expression that was negatively correlated with loss of cholinergic cell markers. These data reveal that adolescent binge ethanol exposure accelerates AD-associated neuropathology in the female adult basal forebrain and suggests that adolescent binge drinking may be an etiological factor contributing to AD neuropathology. Supported by K01AA025713, NADIA of NIAAA, and R01AG072894.

Adolescent Intermittent Ethanol Paradigm, 5xFAD Model

Fig. 1. Methods. Female 5x Familial Alzheimer’s disease (5xFAD) and Non-Transgenic (Non-Tg) mice bred at the University of North Carolina at Chapel Hill were used in this study. On P30, female Non-Tg (N=50) and 5xFAD (N=50) animals were semi-randomly assigned to either AIE or water conditions. From P30 to P55, AIE subjects received a single intragastric administration of ethanol (EtOH; 5.0 g/kg) in the morning on a two days-on-two days-off schedule, as shown in Figure 1A. After AIE, animals aged with no dosing until sacrifice at P100 for basal forebrain collection (ROI shown in Figure 1B).

A. Experimental Timeline

B. Schematic of Basal Forebrain

C. Transgenes in AD Model

Amyloid Precursor Protein

- Swedish (K670N)
- Florida (APP7176V)
- London (Y717I)
- Presenilin I

M146V

L286V

D. 2x2 Experimental Design

Result: AIE Accelerates Amyloid Plaque Deposition

Fig. 2. Amyloid plaque staining in lateral septum. (A) Approximate 20% increase in AD plaque burden in the lateral septum of ethanol dosed 5xFAD subjects relative to water dosed 5xFAD controls. (B) Follow up staining of Thioflavin S showed similar increases of approximately 30%. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01.

Result: AIE Accelerates Loss of ChAT+ and hnRNP+ Cells in 5xFAD mice

Fig. 3. AIE accelerates loss of ChAT+ neurons in adult basal forebrain of 5xFAD mice. Main effect of AIE shows 30% reduction of ChAT+ cells, despite no neuronal loss (data not shown). Data are presented as mean ± SEM. **p < 0.01. t-test: †p < 0.05.

Result: AIE Accelerates AD-associated Neuroimmune Activation in 5xFAD mice

Fig. 4. Accelerated loss of heterogeneous nuclear ribonucleoproteins following AIE. Positive correlation (r=0.55, p<0.05) between hnRNP’s and ChAT expression suggests role in cholinergic expression. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, 5xFAD T-test: †p < 0.01.

Result: AIE Accelerates Induction of Glial Expression in 5xFAD mice

Fig. 5. 5xFAD genotype increases expression of gliogenic genes through RTPCR analysis. Main effect of Genotype: ab p < 0.05, aa,bb p < 0.01. 5xFAD/CON vs 5xFAD/AIE: t-test = †p < 0.05, ††p < 0.01.

Result: Human Postmortem Alcohol Use Disorder Individuals Exhibit Similar Acceleration of AD Pathology Within the Basal Forebrain

Fig. 6. AIE increases microglial activation relative to water dosed 5xFAD and Non-Tg controls in medial and lateral septum. (A) Medial septum and (B) lateral septum Iba1+IR pixel density quantification show main effect of genotype and further increased activation within 5xFAD animals following AIE. (C) Iba1+ cells colocalize with AB+ protein expression. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01. 5xFAD/CON vs 5xFAD/AIE: t-test = †p < 0.05, ††p < 0.01.

Fig. 7. Postmortem AUD individuals express increased Alzheimer’s disease-associated neuropathology relative to age-matched moderate drinking controls. Postmortem AUD individuals, who as adolescent age of drinking onset, show similar increases in amyloid, loss of cholinergic cells, and decreases in hnRNP expression as the 5xFAD mouse model. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01.

Conclusion

- AIE accelerates female, but not male, AD-associated neuropathology in the basal forebrain in the 5xFAD mouse
- AIE causes acceleration of neuroimmune and glial upregulation into adulthood, suggesting the adolescent brain is especially vulnerable to long-term damage following alcohol binges
- Individuals with AUD and adolescent age of drinking onset exhibit accelerated AD-associated neuropathology in the basal forebrain
- These data adolescent alcohol exposure is as an etiological risk factor for development of AD neuropathy