The role of Diacylglycerol-lipase-β (DAGLβ) in the development of neuroinflammation in a mouse model of alcohol-induced peripheral neuropathy (AIPN)

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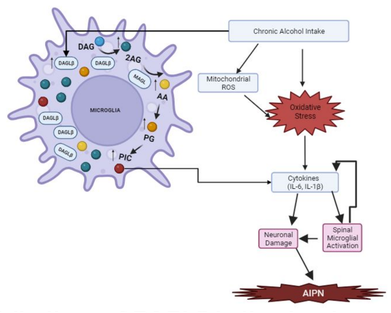
BACKGROUND

- **Alcohol-Induced Peripheral Neuropathy (AIPN) develops in 60% of patients with Alcohol Use Disorder (AUD)[1]**
  - Nerve damage, due to chronic alcohol consumption, that leads to somatosensory symptom in the extremities (i.e., pain, allodynia, numbness, and tingling)
- **Mechanisms underlying AIPN are poorly understood but EtOH consumption is known to increase neuroinflammation, which is implicated in mechanisms underlying AIPN**
  - Alcohol activates immune cells (i.e., macrophages and microglia) that release pro-inflammatory cytokines (PIC) which contribute to mechanisms already associated with neuropathy: oxidative stress, axonal damage, and demyelination[2]
  - To find novel and more efficacious treatment strategies, there is a need to better understand mechanisms, and targets, which contribute to the development of AIPN
- **Endocannabinoid system: regulates several important processes including pain, inflammation, and immune responses[3]**
  - Cannabinoid receptor 1 (CB1) and Cannabinoid Receptor 2 (CB2) → highly expressed and distributed throughout central nervous system (CNS) and peripheral nervous system (PNS)
  - 2 endogenous ligands, Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), bind and activate CB1 and CB2 □ Produced and degraded on-demand by their respective enzymes
    - Ex: Both isoforms of diacylglycerol-lipase (DAGLα and DAGLβ) synthesize 2AG from DAGs, and monoacylglycerol lipase (MAGL) metabolizes 2AG into arachidonic acid (AA)
    - Unlike DAGLα primarily expressed on neurons, the expression of DAGLβ is restricted to macrophages and microglia[4]
- **Pre-clinical models implicate DAGLβ is involved in mechanisms underlying AIPN**
  - Chronic pain: development of hypersensitivity in mice was associated with a dysregulation of DAGLβ activity, and the production of PIC downstream of the metabolism of 2AG to AA, in macrophages and microglia[4,5]
  - Alcohol dependence: DAGLβ and 2AG is upregulated in the liver and specific brain regions of mice following chronic alcohol intake
  - Previous studies from our lab: Four weeks of 5% EtOH intake in mice increases: (1) cold hypersensitivity; (2) expression of DAGLβ in spinal cord, and (3) expression of interleukin-6 (IL-6), IL-1β in *only* female mice



The direct contributions of DAGLβ in the development of AIPN and associated neuro-inflammation has yet to be directly investigated.

HYPOTHESIS

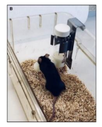
DAGLβ contributes to the development of AIPN in mice through an increase in neuroinflammation

GENERAL METHODS AND MATERIALS

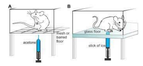
Animals: M/F DAGLβ knockout/wildtype mice were bred in house (9-11 weeks of age on C57BL/6J background)

AIPN Model: Lieber-DeCarli EtOH Liquid Diet

- Food restricted to liquid diet
- Assigned 5% EtOH or control diet (0% EtOH) that were calorically matched
- Body mass (g) and diet consumed (mL) recorded daily to determine average daily EtOH intake weekly (g EtOH/kg body mass)



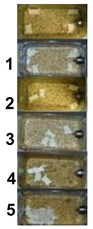
Cold Sensitivity (Acetone Test): 20 µL acetone applied to hind paw and total "PW time (s)" observed for 60s



Nesting: nestlet shredding in 2 hours quantified as "*shred score*" (1-5)

Locomotor Activity: Total beam breaks in 30 minutes

Neuroinflammation (Spinal Cord): qrt-PCR (Tagman):
RNA expression (IL6 and IL-1β) expressed as $2^{\Delta\Delta CT}$ (vs.B2M)



Statistics: If sex not significant factor, collapsed, analyzed via 2-way ANOVA and Tukey's post hoc.

1) Timeline and Experimental Design

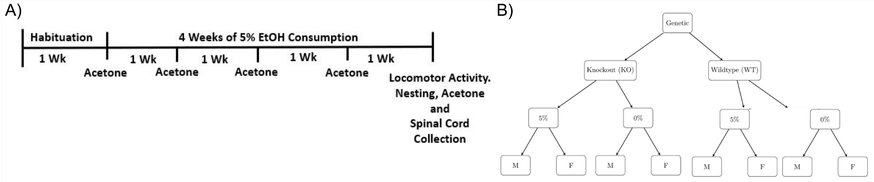


Figure 1: The development of alcohol-induced peripheral neuropathy and spinal neuroinflammation in DAGLβ KO and WT mice following four weeks of 5% EtOH intake. (A) Experimental Timeline (B) Experimental Design and groups.

2) EtOH intake and body mass were the same in DAGLβ KO and WT mice

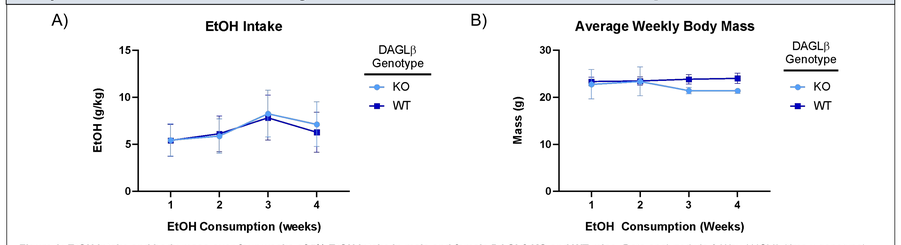


Figure 2: EtOH intake and body mass over four weeks of 5% EtOH intake in male and female DAGLβ KO and WT mice. Data analyzed via 2-Way ANOVA (time x genotype). (n=5-6/sex/group). (A) DAGLβ did not impact EtOH intake. (B) DAGLβ did not impact body mass.

3) The development of AIPN related behaviors was prevented in DAGLβ KO mice

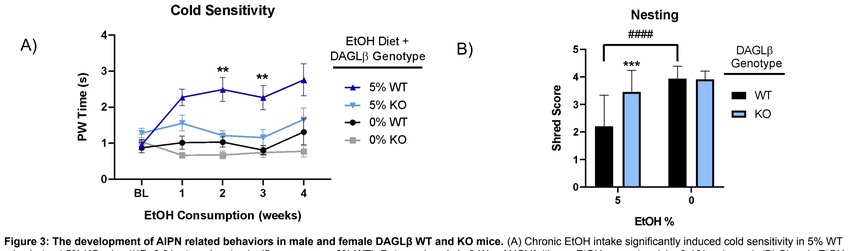


Figure 3: The development of AIPN related behaviors in male and female DAGLβ WT and KO mice. (A) Chronic EtOH intake significantly induced cold sensitivity in 5% WT mice but not 5% KO mice (**P<0.01; stars denote significance versus 0% WT). Data analyzed via 3-Way ANOVA (time x EtOH x genotype) (n=8-10/sex/group). (B) Chronic EtOH intake significantly decreased nesting behavior in 5% WT mice, but not 5% KO mice (####P<0.0001 0% WT vs 5% WT; ***P<0.001 5% WT vs 5% KO). Data analyzed via 2-Way ANOVA (EtOH x genotype) (n=8-10/sex/group).

4) DAGLβ KO did not impact the development of AIPN associated neuroinflammation in female mice

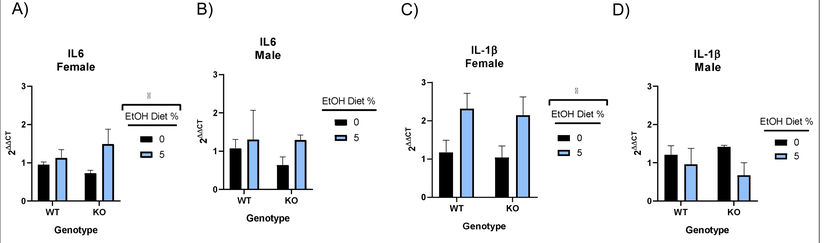


Figure 6: **The impact of four weeks 5% EtOH intake on the RNA expression of pro-inflammatory cytokines in the spinal cords of male and female DAGLβ WT and KO. Data analyzed via 2-Way ANOVA (EtOH x genotype) followed by Tukey's post hoc.** (A) Chronic EtOH increased RNA expression of IL-6 in female DAGLβ WT and KO mice (Main Effect of EtOH *P<0.05; n=6-7/group). (B) Chronic EtOH did not impact RNA expression of IL-6 in male DAGLβ WT and KO mice. (n=3-6/group). (C) Chronic EtOH increased RNA expression of IL-1β in female DAGLβ WT and KO mice. (B) Chronic EtOH did not impact RNA expression of IL-1β in male DAGLβ WT and KO mice.

CONCLUSIONS

- **Replicated results from previous studies using four week of 5% EtOH in the AIPN model in WT mice --> important to show reproducibility of mouse models**
  - Development of cold sensitivity and nesting deficits in WT mice
  - Increased IL-6 and IL-1β expression in spinal cords of only female WT mice
  - Although male and female mice may manifest similar disease severity, underlying neuroinflammatory mechanisms may be distinct
- **DAGLβ plays an important but complicated role in the development of AIPN in mice**
  - DAGLβ KO and WT mice did not differ in ETOH intake □ any differences between genotypes are not due to differences in EtOH levels consumed
  - DAGLβ KO only prevented the development of AIPN-associated behavioral changes but not inflammation

FUTURE DIRECTIONS

- **Evaluate the development and potential prevention of AIPN-associated neuroinflammation in a peripheral tissue associated with mechanisms of neuropathic pain (i.e., dorsal root ganglia)**
- **Determine if AIPN-associated changes in IENF density are prevented in DAGLβ KO mice.**

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REFERENCES

1) *Papantoniou et al., 2024*
2) *Waldschmidt et al., 2006*
3) *Donvito et al., 2018*
4) *Hsu et al., 2012*
5) *Wilkerson et al., 2016*
6) *Wolfe et al,, 2022*