The Effect of Binge-like Ethanol Consumption on Orexin Receptors' mRNA Expression in Lateral Hypothalamus and Amygdala of Male and Female C57BL/6J Mice

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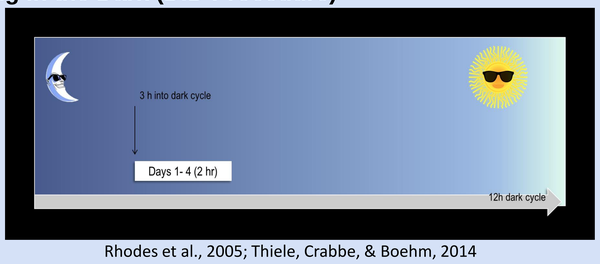
Introduction

- **Goal:** To understand how binge-like ethanol drinking behavior affects orexin receptor mRNA expression in the lateral hypothalamus (LH) and amygdala (Amg).
- **Prior Literature:**
  - Site-directed infusion of orexin receptor antagonist into the central amygdala and ventral tegmental area blunts binge-like ethanol drinking in mice (Olney et al., 2017)
  - A history of binge-like ethanol drinking reduces orexin protein expression in the lateral hypothalamus of mice (Olney et al., 2015)
- **How:**
  - Quantify orexin-1 and orexin-2 receptor mRNA expression in the LH and Amg using qPCR
- **Hypothesis:** Binge-like ethanol drinking behavior will decrease the amount of orexin mRNA expression in the LH and Amg.

Materials and Methods

Subjects: 50 C57BL/6J Mice (25 male, 25 female)

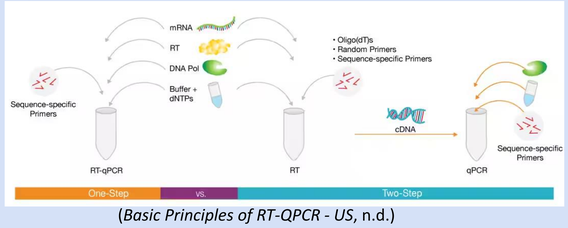
Drinking in the Dark (DID Procedure)



Rhodes et al., 2005; Thiele, Crabbe, & Boehm, 2014

RT-qPCR & Gene Assays

- Beta Actin (control), ORX1R, ORX2R Gene Assays (ThermoFisher Scientific)



(*Basic Principles of RT-QPCR - US*, n.d.)

Results

How the Variables Were Assessed

Color	Significance
X	No
X	Yes
X	Trend

	Ethanol Consumption	ORX1R mRNA Change in LH	ORX2R mRNA Change in LH	ORX1R mRNA Change in Amg	ORX2R mRNA Change in Amg
Group	X	X	X	X	X
Sex	X	X	X	X	X
Group/Sex	X	X	X	X	X

Weekly Ethanol Consumption

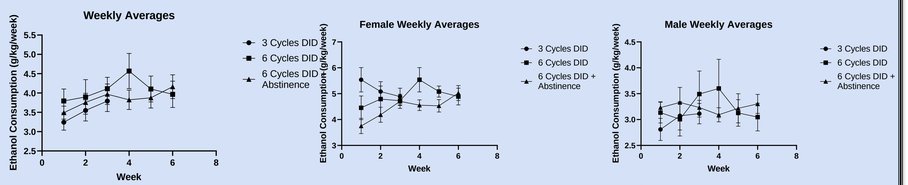


Figure 1. A) Weekly ethanol consumption averages by DID cohort group. In the factorial ANOVA across the 6 weeks, there were significant group (F (1,120) = 5.062, p = 0.026) and sex differences (F (1,120) = 95.596, p <.001), but no effect of week (F (5,120) = 1.120, p = 0.354There were no significant group differences in the last week (F (2,31) = 0.732, p = 0.489). B-C) Weekly ethanol consumption averages by DID cohort group for females (B) and males (C). Females drank significantly more than males across group and week.

Orexin-1 and Orexin-2 in LH

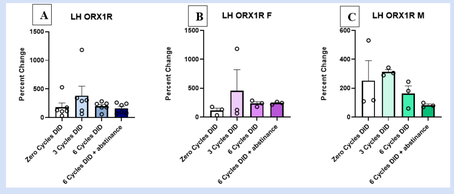


Figure 2. The effect of the DID procedure across the different experimental groups on the percentage change in mRNA production as measured by A) ORX1R in the lateral hypothalamus in C57/BL6J mice, B) ORX1R in the lateral hypothalamus in C57/BL6J female mice, and C) ORX1R in the lateral hypothalamus in C57/BL6J male mice. There were no main effects of group (F (3,16) =9.65, p=.433) or, sex (F (1,16) =1.34, p =7.19) on orexin receptor 1 mRNA expression in the lateral hypothalamus, nor were there group by sex interactions (F (3,16) =1.803, p=.187).

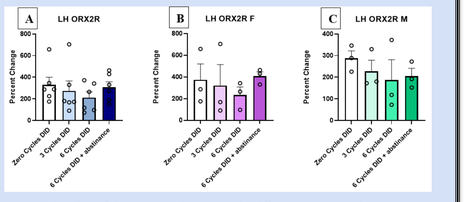


Figure 3. The effect of the DID procedure across the different experimental groups on the percentage change in mRNA production as measured by A) ORX2R in the lateral hypothalamus in C57/BL6J mice, B) ORX2R in the lateral hypothalamus in C57/BL6J female mice, and C) ORX2R in the lateral hypothalamus in C57/BL6J male mice. There were no main effects of group (F (3,16) = 1.017, p = .411) or, sex (F (1,16) =1.415, p = 2.52) on orexin receptor 2 mRNA expression in the lateral hypothalamus, nor were there group by sex interactions (F (3,16) = .365, p =.779).

Orexin-1 and Orexin-2 in Amg

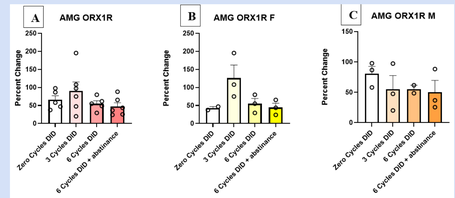


Figure 5. The effect of the DID procedure across the different experimental groups on the percentage change in mRNA production as measured by A) ORX1R in the amygdala in C57/BL6J mice, B) ORX1R in the amygdala in C57/BL6J female mice, and C) ORX1R in the amygdala in C57/BL6J male mice. There were no main effects of group (F (3,14) =1.125, p=.373) or, sex (F (1,14) =.038, p=.848) on orexin 1 mRNA expression in the amygdala, nor were there group by sex interactions (F (3,14) =2.233, p=.130).

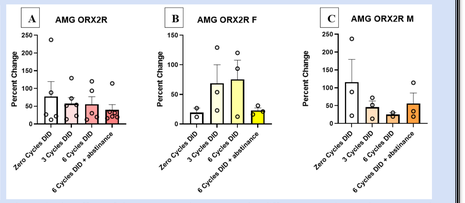


Figure 6. The effect of the DID procedure across the different experimental groups on the percentage change in mRNA production as measured by A) ORX2R in the amygdala in C57/BL6J mice, B) ORX2R in the amygdala in C57/BL6J female mice, and C) ORX2R in the amygdala in C57/BL6J male mice. There were no main effects of group (F (3,14) =.181, p=.908) or, sex (F (1,14) =.399, p=.538), nor were there group by sex interactions (F (3,14) =1.587, p=.237).

Discussion

- No novel evidence that ORX1R or ORX2R mRNA expression in LH and Amg is decreased following binge-like ethanol consumption
- A larger sample size may be needed to reveal significant group differences
- mRNA expression not affected in the same manner as protein expression in other studies
  - Sterling et al. (2016) used a chronic model of alcohol use compared to a binge-like ethanol drinking model → could account for significance

Future Directions

- Increase sample size
  - Draw out statistical significance from trends
- Different model of alcohol use closer to alcohol dependence
  - Ethanol delivered through swimming in water for Zebrafish (Sterling et al., 2016)
  - Every 8 hours for 4 days given gastric intubation of alcohol (Sharma et al., 2020)
- Conduct longer DID procedure → 12 weeks
- Need to explore VTA (Sterling et al., 2016)
- Consider plasticity
  - Assess changes orexin ligand mRNA for downstream regulation

Citations

Olney, J. J., Navarro, M., & Thiele, T. E. (2015). Binge-like consumption of ethanol and other salient reinforcers is blocked by orexin-1 receptor inhibition and leads to a reduction of hypothalamic orexin immunoreactivity. *Alcoholism, clinical and experimental research*, *39*(1), 21–29. https://doi.org/10.1111/acer.12591

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Sterling, M. E., Chang, G.-Q., Karatayev, O., Chang, S. Y., & Leibowitz, S. F. (2016). Effects of embryonic ethanol exposure at low doses on neuronal development, voluntary ethanol consumption and related behaviors in larval and adult zebrafish: Role of hypothalamic orexigenic peptides. *Behavioural Brain Research*, *304*, 125–138. https://doi.org/10.1016/j.bbr.2016.01.013