Summer 6-28-2022

Bakner and Coste Faculty-Student Summer Collaborative Research Grant: Grant Activities Evaluation Post Grant

Sarah Coste

Bakner and Coste Faculty-Student Summer Collaborative Research Grant
Grant Activities Evaluation Post Grant
Summer'21: June 7th – August 13th, 2021 (Experiment phases 2-4, see below)
Faculty: Sarah Coste & Lee Bakner
Student Collaborator: Madison Rodriguez (S'22)

Project Title: Ethanol consumption using the "Drinking In the Dark" (DID) procedure in Sprague Dawley and Long Evans rat lines: Strain comparisons, blood alcohol levels and the impact of naltrexone on alcohol intake

1. Concise Abstract of the Results of the Work.

Our research attempted to understand mechanisms underlying alcohol use and factors that may contribute to alcohol use disorder (AUD). More specifically, the present series of experiments, phases 1 through 4, expand on rodent models research to study binge-like ethanol consumption using the "Drinking In the Dark" (DID) procedure (see Thiele & Navarro, 2014 for a review). In DID, ethanol (EtOH) is provided in the home cage for 3 hours, beginning 3 hours after the start of the dark phase of the light:dark cycle when nocturnal rodents are most active (adapted from Rhodes et al., 2005). Few studies have explored the use of DID to study ethanol consumption in genetically heterogenous outbred rats.

Phase 1 of the research attempted to determine whether binge-like ethanol consumption (20% EtOH, 28 days) may be observed in two genetically heterogenous rat lines used in alcohol drinking research (i.e., Sprague-Dawley - SD, and Long Evans - LE). Additionally, the impact of naltrexone (1 mg/kg), an opiate antagonist, on ethanol drinking was assessed across 6 additional days at the end of phase 1. Phase 2 evaluated the impact of an abstinence period, inserted after completion of phase 1, on subsequent ethanol consumption and provided an opportunity to evaluate abstinence induced drinking across the strains (see Simms et al., 2008). This was accomplished by allowing animals to consume 20% EtOH for 5 consecutive days after a 60-day abstinence period. Phase 3 commenced after a 2-day abstinence period and animals consumed 10% EtOH for 5 consecutive days following phase 2. Prospectively, positive behavioral contrast may be observed, and animals may consume greater quantities of 10% EtOH having consumed less palatable 20% EtOH. Finally, phase 4 attempted to explore whether strain differences may be observed in consumption of 10% EtOH sweetened with "supersac" solution. Super sac (3% glucose w/v, 0.125% saccharine w/v) added to 10% EtOH may encourage binge-like drinking in outbred rats and may be a valid animal model of ethanol consumption given humans typically consume sweetened ethanol beverages (Ji et al, 2008). Phase 4 began after a 23-day abstinence period and followed the same DID procedure used in phases 1-3. The last phase continued for 10 days with 2 separate brief abstinence periods inserted between drinking days. The first was a 2-day abstinence period between days 4 and 5, and the second was a 4-day abstinence period inserted between day 9 and 10.

Phase 1 outcomes indicated that LE strain achieved higher doses of EtOH compared to SD strain but did not attain binge-like criteria (1.5 g/kg EtOH) within the initial 30-minute drinking period. Importantly, naltrexone did suppress consumption of EtOH leading to lower doses attained especially so in the LE strain. Phase 2 results showed no initial increase in consumption following an abstinence period and LE achieved higher doses of EtOH. During phase 3, abstinence induced drinking was not observed although LE animals achieved higher doses of EtOH consumed. Interestingly, phase 4 analyses indicated that both strains consumed ethanol in a manner to achieve higher doses especially so in the LE strain. Within phase 4, abstinence induced drinking was observed in the LE strain following the initial 2-day abstinence period, and similar abstinence induced drinking was observed especially so in the LE strain following a 4-day abstinence period inserted between days 9 and 10 of phase 4. Overall, the DID paradigm produced binge drinking in outbred rats, most notably LE strain, when 10% ethanol solution

was sweetened with supersac, especially after a brief period of abstinence from drinking. Additionally, naltrexone significantly decreased ethanol consumption, supporting its continued use as a treatment for AUD. Across the 4 Phases, LE rats consistently consumed higher amounts of ethanol, suggesting that they serve as a better outbred rat model for studying ethanol consumption and binge drinking using DID procedures.

Ji, D., Gilpin, N. W., Richardson, H. N., Rivier, C. L., & Koob, G. F. (2008). Effects of naltrexone, duloxetine, and a corticotropin-releasing factor type 1 receptor antagonist on binge-like alcohol drinking in rats. *Behavioral Pharmacology,* 19(1), 1-12.

Rhodes, J. S., Best, K., Belknap, J. K., Finn, D. A., & Crabbe, J. C. (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiology & Behavior*, 84, 53-63.

Simms, J.A., Steensland, P., Medina, B., Abernathy, K.E., Chandler, L.J., Wise, R., & Bartlett, S.E. (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcoholism: Clinical and Experimental Research*, 32(10), 1816-1823.

Thiele, T.E., & Navarro, M. (2014). "Drinking in the dark" (DID) procedures: A model of binge-like ethanol drinking in non-dependent mice. *Alcohol*, 48, 235-241.

2. Response to Questions.

a. How well were the objectives of the project met?

We believe the objectives of the project were well met. Madison did excellent work collecting the data throughout the summer and her most conscientious efforts met with fascinating outcomes. Her findings clearly show that the LE strain may serve as a better rodent model to explore factors that impact AUD. Importantly, her work shows that binge-like consumption of ethanol may be observed in outbred rats, a rare finding, when using the DID procedure to study consumption of EtOH sweetened with supersac. These techniques also provide an opportunity to examine abstinence induced drinking and contributions to binge-like consumption of EtOH. Madison's work clearly sets the stage for additional investigations to ask important questions.

Madison presented her work at the May 2022 Linfield University Scholarly Symposium and successfully defended an Honors Thesis in Psychology, December 2021, graduating with Honors in Psychology, May 2022.

b. What impact will this project have on an advancement of knowledge in a discipline or interdisciplinary area, the applicant's professional development, the education Linfield students, and/or the Linfield community?

Outcomes from our findings, we believe, will make important contributions to the development of outbred rat models of EtOH drinking, using DID, and set the stage for additional questions to help us understand factors that contribute to AUD and treatment of AUD.

Findings will be submitted for presentation at the June 2023 meeting of the Research Society on Alcoholism (abstracts submitted December 2022/January 2023). All submissions are peer reviewed by the membership of this professional organization. Only original, non-published, research may be presented. We will begin preparing a manuscript for publication and incorporate feedback from colleagues after presenting at the RSA meeting.

c. Any additional comments, such as: How could this committee or the various Linfield University offices be of more assistance in implementing supported projects in the future?

No comments at this time.

IV. Project Implementation Description

1. Detailed timeline of project events.

June 14 - July 23, '21: Data Collection DID Phase (5 days per week, 5 hours per day)

- Completed

July 23, '21: Blood samples collected following 30 day drinking period and assayed over the next 2 weeks

- Completed and assay outcomes were not interpretable
- Will process samples with different assay Summer 2022

July 26 – August 6, '21: Reestablish DID phase 10 day drinking period

- Completed and added super sac + EtOH Phase
- Completed and added more abstinence induced drinking periods

August 9 – August 14, '21: Naltrexone, Opiate Antagonism phase

- Did not complete this phase given pivot to supersac + EtOH phase with abstinence periods

Fall '21 – Analyze data using SPSS and inferential factorial analyses as well as correlational analyses

- Completed Fall 2021

Fall '21 – Prepare abstract for conference submission

- Symposium abstract submitted May 22
- Will submit abstract for peer review and potential presentation at RSA Fall 22 and Spring 23
- Summer '22 – Summer '23 – pending feedback, prepare manuscript