The Effect of Apremilast on Binge Drinking and Circadian Gene Expression in Mice

**Abstract:** Chronic binge drinking negatively impacts society and is a leading cause of preventable deaths. The purpose of the experiment is to determine if regulation of circadian rhythms in the ventral tegmental area (VTA) will contribute to less ethanol intake. The VTA plays a major role in addiction. A simple genetic animal model examines iHDID-1 mice and their binge-like drinking intake. The mice undergo Drinking in the Dark (DID) for 6 weeks prior to administration of apremilast or saline treatment at weeks 7 and 8. After the final day of DID the mice are sacrificed at two separate time points (ZT 3 and ZT 15) to account for the peaks and troughs of circadian rhythms. Apremilast treatment has shown evidence to support amelioration of circadian rhythms results in a decrease in ethanol intake. If circadian rhythms are increased in the VTA, then the amount of ethanol consumed will decrease.

I. Introduction.

Alcohol Use Disorders (AUD) negatively impacts individuals, societies, and has a significant economic effect. Binge drinking is the third leading cause of preventable deaths in the United States (Sacks et al., 2015; Witkiewitz et al., 2019). Binge drinking is characterized as reaching a blood alcohol level (BAL) of >80mg% within a 2-hour time period (National Institute on Alcohol Abuse and Alcoholism, 2004). The COVID-19 pandemic has brought further challenges to those with AUD, as a change in alcohol availability and the impact of isolation has caused an increase in alcohol use, all while countries that enacted alcohol restrictions have shown higher rates of fatal alcohol withdrawal (Nadkarni et al, 2020).
Alcoholism causes a disruption in circadian rhythms that continues into sobriety and leads to an increased risk for relapse (Brower, 2001; Fonzi et al, 1994; Kuhlwein et al, 2003; Landolt and Gillin, 2001; Sano et al, 1993). Circadian rhythms operate on a 24-hour time period to regulate the sleep/wake cycle and have known peaks and troughs that fluctuate and are maintained by an internal molecular clock. The ventral tegmental area (VTA) plays an important role in reward, motivation, and addiction. The Ozburn lab found abstinence from ethanol causes a reduced expression of the Circadian Locomotor Output Cycles Kaput (Clock) gene in the VTA of ClockΔ19 mutant mice, a mutant type of BALB/c background (Ozburn et al., 2013).

Harm reduction research is necessary to alleviate the effects of chronic binge drinking. Improving the circadian rhythms, which are affected during and following drinking, may lessen the harmful effects of alcohol. Individuals with disturbed circadian rhythms are more apt to relapse. Remediating the effects of binge drinking on the circadian rhythm suggests potential to decrease alcohol intake while increasing Clock function. In addition, increasing Clock in the VTA may decrease alcohol intake and regulate circadian rhythms.

II. Research Question.

My research question addresses if medications such as apremilast can rescue the effects of chronic drinking by increasing Clock gene expression in the VTA. Another question is if apremilast can increase levels of circadian genes in the VTA in mice with a history of chronic binge drinking. I am interested in evaluating the effects of chronic binge-like drinking in mice and to see if apremilast can assist in regulating circadian rhythms in the VTA, as both circadian rhythms and the VTA region play important roles in addiction.
III. Literature Review.

Ethanol consumption effects Clock gene expression in animal models as shown by the Ozburn lab. Research suggests mice with a mutation in the Clock gene (ClockΔ19) have an increased preference for stimulant rewards and sucrose. Reducing Clock function in the VTA of wild-type mice increased the amount of ethanol consumed, similar to the ClockΔ19 mice. The Ozburn lab found Clock expression in the VTA plays an important role as a negative regulator of ethanol intake (Ozburn et al., 2013).

Another medication that has been researched to rescue the effects of binge drinking is rolipram. Rolipram and apremilast has been shown to increase cyclic adenosine monophosphate (cAMP), both these medications are phosphodiesterase-4 (PDE4) inhibitors. PDE4 is an enzyme responsible for hydrolysis of cAMP. Compounds that increase cAMP can increase circadian gene expression. Rolipram was shown to reduce ethanol intake in mice (Hu et al., 2011). Apremilast is FDA approved for psoriasis and psoriatic arthritis, and in preclinical trials is shown to reduce ethanol intake (Blednov et al., 2018).

IV. Methodology.

In this experiment, mice are used as a genetic simple animal model, adult male and female inbred High Drinking in the Dark (iHDID-1) mice. The iHDID-1 mice were bred to binge drink and show withdrawal following a single drinking session. These mice were chosen as they are genetically diverse allowing for a high-risk model for binge-like ethanol drinking (Crabbe et al., 2017, 2009). If no sex differences are found the data will be collapsed across sex for analyses and compared across treatment and time tissue was collected. This experiment uses a Drinking in the Dark (DID) method. Two time points were accessed to account for the circadian peaks and
troughs of nocturnal drinking patterns. Mice were given access to ethanol or water at 3 hours into their active dark cycle [Zeitgeber time (ZT 15)]. At ZT 15 the mice are active with the lights turned off. Timepoint ZT 3 is a trough in gene expression where circadian genes are least expressed. Whereas ZT 15 is a peak in expression and circadian genes show a higher rate of expression as the mice are active.

For the DID, the mice were given access to water or ethanol 20% at the timepoint ZT 15. The first days of DID, days 1-3, lasted 2 hours, while the final DID session on day 4 lasted 4 hours. The mice underwent DID for 6 weeks to develop a high binge-like drinking average baseline. For weeks 7 and 8, the mice received intraperitoneal injections (IP) of saline or 40 mg/kg apremilast an hour prior to DID. Euthanization of mice was performed one day after the final DID session at either ZT 3 or ZT 15 to account for the peaks and troughs. The VTA tissue is collected using a 0.5 mm punch.

Circadian gene expression is measured using qualitative real-time polymerase chain reaction (qPCR). The VTA mRNA will be isolated and quantified using a method described by (Ozburn et al., 2017). The levels of Clock expression will be measured by synthesis of cDNA using the BioRad iScript Kit. The levels of gene expression will be measured in comparison to 18s, a housekeeping gene that remains unchanged following ethanol exposure or change in time. Data analysis will take 2 weeks for this experiment and will use a 3-way ANOVA (sex x treatment x ZT).
V. Results.

Results expected from this correlational study include an increase in circadian gene expression with the use of apremilast. Treatment is expected to decrease the amount of ethanol voluntarily consumed, while normalizing circadian gene expression. This study will examine the mechanism of action for the use of apremilast treatment to understand the effects on binge-like drinking. A limitation of this study is the use of mice as a simple animal model and the lack of a water treatment group. This is an important first step in determining the effects of treatment, as early data shows apremilast to be useful in nontreatment seeking AUD individuals. Going forward, this pilot project will determine if more control groups and time points will be studied to better understand the effects of apremilast on circadian rhythm within the VTA.