Characterizing Rhythms in Circadian Gene Expression Following Chronic Binge-Like Alcohol Drinking in HDID Mice

By
Tanvi Batish

A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Microbiology
(Honors Scholar)

Presented April 27, 2016
Commencement June 2016

AN ABSTRACT OF THE THESIS OF

Tanvi Batish for the degree of Honors Baccalaureate of Science in Microbiology presented on April 27, 2016. Title: Characterizing Rhythms in Circadian Gene Expression Following Chronic Binge-Like Alcohol Drinking in HDID Mice

Abstract approved:

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Indira Rajagopal

Mammals exhibit circadian rhythms in several physiological processes controlled by a core set of circadian genes that make up the so-called molecular clock. Disruptions in these rhythms, and variations in circadian genes, are associated with the development of psychiatric disorders, including addiction. However, the effect of chronic binge-like alcohol drinking on rhythmic expression of circadian genes (such as *Per2*) in different brain regions is unknown. We focus here on two brain regions, the nucleus accumbens (NAc) – an area important for alcohol drinking, and the suprachiasmatic nucleus (SCN) – an area important in initiating and maintaining circadian rhythms. We used mice selectively bred to achieve high blood alcohol levels after a short drinking session (High Drinking in the Dark, HDID-1) and subjected them to an 8 week drinking in the dark (DID) protocol with either water or alcohol. After 8 weeks, the mice were euthanized, brain tissue was extracted at 8 time intervals and frozen to be processed for qPCR to quantify expression of *Per2* in each region. The results show that alcohol disrupts rhythmic expression of circadian genes in these two regions, where the SCN is somewhat more resilient than the NAc to the effects of chronic binge-like drinking.

Key Words: alcohol, circadian, gene, expression

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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INTRODUCTION

Biological clocks are extremely important in regulating daily rhythmic behaviors and activities of organisms. A majority of living organisms from fungi to mammals, utilize daily cycles that enable them to adapt to their environment and respond to various stimuli known as zeitgebers (time givers) to maximize their chances for survival. Such stimuli include food, light, temperature, etc. These timed rhythms are observed in everything from sleep/wake cycles to hormone levels and even in cognition, attention and mood (McClung 2007). These biological clocks (more commonly known as circadian rhythms) consist of genetic circuitry that puts their rhythm at about 24h period.

Previous studies suggest that natural circadian rhythms could have evolved by originating from a two-gene activation/repression network through which they could have acquired more complex interactions *via* evolution (Guillermo Rodrigo 2007). Such molecular elements play an important role in keeping circadian rhythms oscillating. This form of interval timing is coded at a behavioral, physiological, and genetic level (Tucci 2011).

The circadian system in mammals is organized in a hierarchy of oscillating mechanisms. The suprachiasmatic nucleus (SCN), located in the anterior hypothalamus, is at the top of this hierarchy. The SCN is the central rhythm-generating region of the brain. Rhythms are generated and maintained by a "molecular clock", consisting of a network of transcriptional-translational feedback loops that drive rhythmic, 24h core clock expression patterns throughout the

organism (Ko, 2006). Core clock components are genes whose expression is necessary for the generation and regulation of circadian rhythms throughout the organism in individual cells. There are also peripheral oscillators identified outside the SCN brain region which are either independently controlled or can be controlled by the SCN (Ko, 2006). Additionally, the clock mechanism in the SCN and the peripheral oscillators has been shown to be similar at the molecular level (Ko, 2006). Thus, circadian rhythm mechanisms are present in all cell types, throughout the organism.

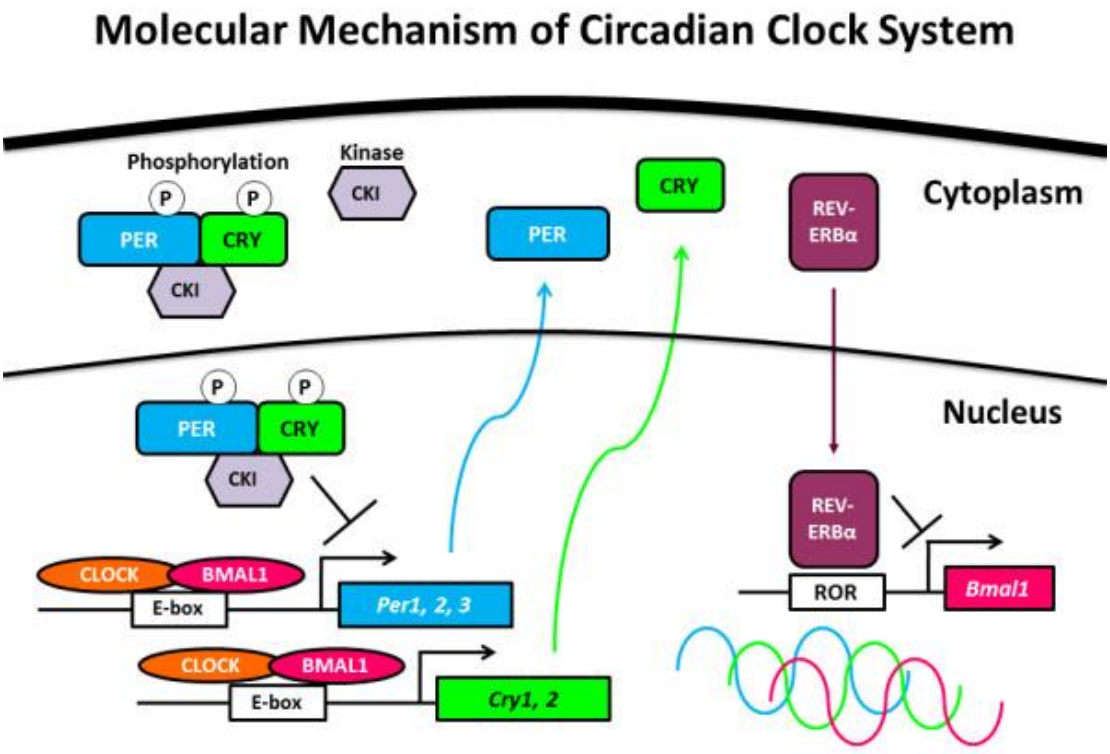

Figure 1: *Visual representation of the molecular mechanism of a circadian clock system(Hida et al. 2012)*

The molecular clock is composed of transcriptional – translational feedback loops (TTFLs). Two transcription factors, Circadian Locomotor Output Cycles Kaput CLOCK (or its functional homolog, Neuronal PAS Domain Protein 2, NPAS2) and Brain and Muscle Arnt-like Protein 1 (BMAL1), heterodimerize in the nucleus of the cell and bind to enhancer box (E-box) sequences of clock controlled genes to then regulate their transcription (Figure 1). Core molecular clock genes include *Period* (*Per1, Per2, Per3*) and *Cryptochrome* (*Cry1, Cry2*). Over a 24 hour period, *Per* and *Cry* mRNA are expressed, translocated from the nucleus to the cytoplasm, translated into protein, post-translationally modified (e.g. phosphorylated by casein kinase 1 epsilon, CK1ε). The proteins can then hetero- or homo-dimerize, and translocate back into the nucleus to inhibit the transcriptional activity of CLOCK/BMAL1, which in turn inhibits *Per2/Cry* expression ("The Mammalian Molecular Clock Model | HHMI's BioInteractive," n.d.; Figure 1). Mammalian CK1ε is very important in regulating the period of circadian oscillations. This kinase phosphorylates susceptible PER proteins and makes them less stable which in turn leads to their degradation. It also participates in the degradation of the inhibitory complex formed by PER and CRY dimers in the nucleus ("The Mammalian Molecular Clock Model | HHMI's BioInteractive," n.d.; Figure 1). This degradation allows transcription to begin again, thus completing the negative TTFL. *Per2* expression is the most robustly rhythmic clock gene and is reliably used as a readout of molecular clock period, amplitude, and phase. The CLOCK/BMAL1 complex also regulates the transcription of other genes by binding to E-boxes in their promoter regions. These clock-controlled genes

contribute to neuronal signaling in mesolimbic systems that are involved in reward processing and development of addictive behaviors (Arey et al., 2013; Ozburn et al., 2015; Sidor et al., 2015). The nucleus accumbens (NAc) is one such brain region that plays a significant role in cognitive processing of reward, pleasure, motivation, aversion, and reinforcement. Hence, the NAc has a significant role in addiction and substance abuse, especially alcohol abuse (Ozburn et al., 2015). Elements of the molecular clock are expressed in the NAc and play an important role in mood- and drug- related behaviors (Arey et al., 2014, Falcon et al., 2013, Spencer et al., 2012; Ozburn et al., 2015). It is not entirely clear how alcohol affects rhythmic gene expression of circadian genes in different brain regions. Alcohol has been shown to affect the SCN and NAc, by inhibiting light-induced phase-shifts in the SCN for example, as well as changing the circadian rhythms of sleep/wake cycles (Prosser et al., 2014; Leggio et al., 2009). However, there is no clear evidence thus far as to how alcohol affects the diurnal expression of circadian genes in brain regions important for alcohol drinking (NAc) and circadian rhythms (SCN).

Disruptions in circadian genes can promote alcohol intake (Parekh and Ozburn, et al., 2014). Previous research has shown that disruption (*via* mutation) of circadian genes such as *CLOCK, Per1,* and *Per2* can promote ethanol intake in mice. Mice with a dominant negative mutation in *Clock* exhibit increased alcohol and cocaine intake (Ozburn et al., 2012; Ozburn et al., 2013). *Clock* mutant mice also display reduced anxiety- and depression-like behaviors (McClung et al 2007; Arey et al., 2014). Spanagel et al. (2005) showed that *Per2* mutant mice also drink more

alcohol than do their wild-type littermates. Gamsby, et al. (2013) tested the effect of functional mutations in *Per1* and/or *Per2* genes on ethanol consumption, reinforcement, and metabolism in ethanol-experienced C57BL/6J mice. They discovered that *Per1* mutant mice, as well as *Per2* mutant mice showed increased ethanol intake and this increase seemed to result from a change in ethanol metabolism and a change in rewarding effects of ethanol, but not from a change in sensitivity to ethanol's sedating effects (Gamsby, et al, 2013).

To observe associations between the circadian clock and mood disorders, clock gene polymorphisms in *Per2, CLOCK, and BMAL1* were studied in patients and healthy people. A silent polymorphism in *Per2* was found to be associated with depression vulnerability, and single nucleotide polymorphisms (SNPs) in *CLOCK* and *NPAS2* were found to be associated with seasonal affective disorder (along with polymorphisms in the *BMAL1* and *Per2* genes). SNPs in *CLOCK* were also found to influence moral valence decisions in depressed patients (Albrecht 2010) In fact, single nucleotide polymorphisms (SNPs) in circadian genes including *CLOCK* and *Per2* have been shown to be associated with increased alcohol consumption and alcohol use disorders in humans as well (Brower et al. 2012; Spanagel et al, 2005; Wang et al., 2012). Therefore, there is a definite link between altered circadian gene expression levels (in *CLOCK, Per2, BMAL1*) and mood disorders and drug seeking behaviors, suggesting that they confer some genetic risk for alcohol dependence.

Alcohol can also disrupt circadian rhythms (Parekh and Ozburn, et al., 2014). Chronic alcohol use or alcohol dependence can alter expression of circadian genes.

Huang et al. (2010) examined mRNA levels of circadian genes in blood from patients with alcohol dependence undergoing alcohol-withdrawal treatment. They found that mRNA levels of *CLOCK* were significantly lower in patients with alcohol dependence at admission to withdrawal treatment and lasted one week into withdrawal. Furthermore, another study examined the effects of alcohol dependence on *Per2* expression patterns in peripheral tissues. Skin fibroblasts from alcohol dependent and control subjects were collected for use in a bioluminescent reporter gene assay to measure circadian rhythms *via* gene expression of *Per2* (McCarthy et al., 2013). They found that the *Per2* period was inversely correlated with illness severity (defined as the number of alcohol dependence criteria met). This means that *Per2* period decreased as the illness severity of the subjects increased and thus suggests that increasing alcohol dependence induces significant changes in rhythmic expression of *Per2*.

However, there are no studies reporting the effect of ethanol intake on diurnal circadian gene expression in multiple brain regions. Thus, the present study will delve into measuring whether chronic binge-like ethanol consumption will alter *Per2* gene expression and whether these ethanol-induced changes in gene expression are similar in different regions of the brain, specifically the NAc and the SCN. This will be done by subjecting selectively bred High Drinking in the Dark (HDID-1) mice to an 8 week Drinking in the Dark (DID) paradigm with either ethanol or water and then examining circadian gene expression of *Per2* at various time points. HDID mice were selective by Dr. John Crabbe using the founder line of HS/Npt mice

(a hybrid cross of 8 different standard inbred strains of lab mice; Hitzemann et al., 1994). HDID-1 mice have been selectively bred to achieve a high blood ethanol concentration (BEC) after a 4h DID session. In other words, after a 4h DID session, individual BECs were assessed and the mice with highest BECs were selected to breed further (Crabbe et al., 2009 and Crabbe et al., 2014). There is no doubt that these animals are drinking to pharmacologically meaningful (i.e., behaviorally intoxicating) levels of EtOH (Crabbe et al., 2009). Their drinking is focused (in some respects binge-like), and exceeds the NIAAA criterion for binge-like drinking [BAC > 80 mg%; NIAAA Newsletter, (2004)]. Since we know from this selective breeding process that HDID-1 mice drink to intoxication in the dark (binge-drink), they serve as an excellent animal model to assess effects of alcohol use disorders. Here, we determine whether chronic ethanol consumption changes rhythmic circadian gene expression in brain regions known to be important for circadian rhythms and ethanol intake. Also, it may help provide insight into future treatments that can target such circadian genes effected in order to reduce chronic ethanol consumption in individuals with alcohol abuse disorders.

METHODS

Animals

High Drinking in the Dark (HDID-1) mice were selectively bred by Dr. John Crabbe at the Portland VA Medical Center to achieve high blood ethanol concentrations after controlled exposure to ethanol (Crabbe et al., 2014). Adult female HDID-1 (S29.G31) mice were acquired from Dr. Crabbe. Mice were bred, weaned, and group housed in a reverse 12 hr light/12 hr dark cycle (lights on at 8:30 pm, lights off at 8:30 am) with food and water available at all times. Mice were habituated to a one-hour phase shift (lights off at 7:30am, on at 7:30pm), individual housing in a new behavioral testing room, and new sipper tubes one week prior to starting the experiment (n=6-7/treatment/time point). All animal experiments were approved by the Portland VA Medical Center Institutional Animal Care and Use Committees.

Experimental Design – Chronic Binge-like Alcohol Drinking

Mice were subjected to a drinking in the dark (DID) schedule for 8 weeks, where one bottle of 20% EtOH (v/v in tap water) or tap water was available to the mice 4 days a week (Mondays, Tuesdays, Wednesdays) for 2 hours (10:30 am-12:30 pm) and for 4 hours (10:30 am-2:30 pm) on Thursdays. Fluid intake was measured daily during DID and mice were weighed once weekly.

Data Collection

All solutions were dispensed through plastic sipper bottles. Each tube was weighed before and after administering to the mice and the weights were recorded daily

(Ozburn et al., 2013). The difference in tube weights was used to calculate the total fluid intake by each mouse.

At the end of 8 weeks of DID, 21 hours after the last DID session, mice were euthanized by cervical dislocation and rapid decapitation at 8 time points (3 hours apart over a 24 hour time period). Brain tissue was removed, frozen on dry ice, and sectioned using a cryostat. Fresh frozen tissue punches were collected from NAc and SCN regions of each brain collected.

RNA Isolation and cDNA Preparation

RNA was isolated from mechanically homogenized tissue in PureZol (BioRad, Hercules, CA, USA). RNA isolation from frozen tissue punches was conducted using the Bio-Rad Aurum Total RNA Fatty and Fibrous Tissue for RNA Isolation kit protocol. The RNA was processed for cDNA using the BioRad iScript cDNA synthesis kit.

qRT PCR and Data Analysis

Quantitative real-time PCR was performed using Bio-Rad SsoAdvanced Universal SYBR kit with primers specific for *Per2* and *18S* (*Per2* forward: 5'-ACCGCAGCTAGGAATAATGGA-3', *Per2* reverse: 5'-GCCTCAGTTCCGAAAACCA-3'; *18s* forward: 5'-ACCGCAGCTAGGAATAATGGA-3', *18S* reverse: 5'GCCTCAGTTCCGAAAACCA-3'). Each PCR reaction was performed in duplicate using a CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA).

Relative gene expression was calculated by first normalizing the CTs (cycle thresholds) for *Per2* to *18S* and then calculating the fold change using the ddCt method, and then relative expression was calculated as described in Falcon et al., 2013 (Haimes and Kelley, 2006; Falcon et al., 2013).

Statistical Analysis

Data are presented as mean +/- the standard error of the mean. Gene expression data for *Per2* expression were analyzed by two-way analysis of variance (ANOVA). In all experiments, $p < 0.05$ is considered significant. Regression analyses (Pearson's R) for fluid intake and *Per2* gene expression levels were carried out separately for the NAc and SCN. Data analysis was performed using GraphPad Prism version 5.00 (GraphPad Software, San Diego, California USA).

RESULTS

Chronic Water or Binge-like Alcohol Intake in HDID-1 Mice

The figures below show water (Fig. 1a) and ethanol (Fig. 1b) intake for mice in all groups (by time point). On days 1, 2, 3 of each week (under the DID protocol), we measured fluid intake for 2 hours. On day 4, we measured fluid intake for 4 hours. These figures display that mice in different time point groups (ZT) were drinking similar amounts of fluid during each week.

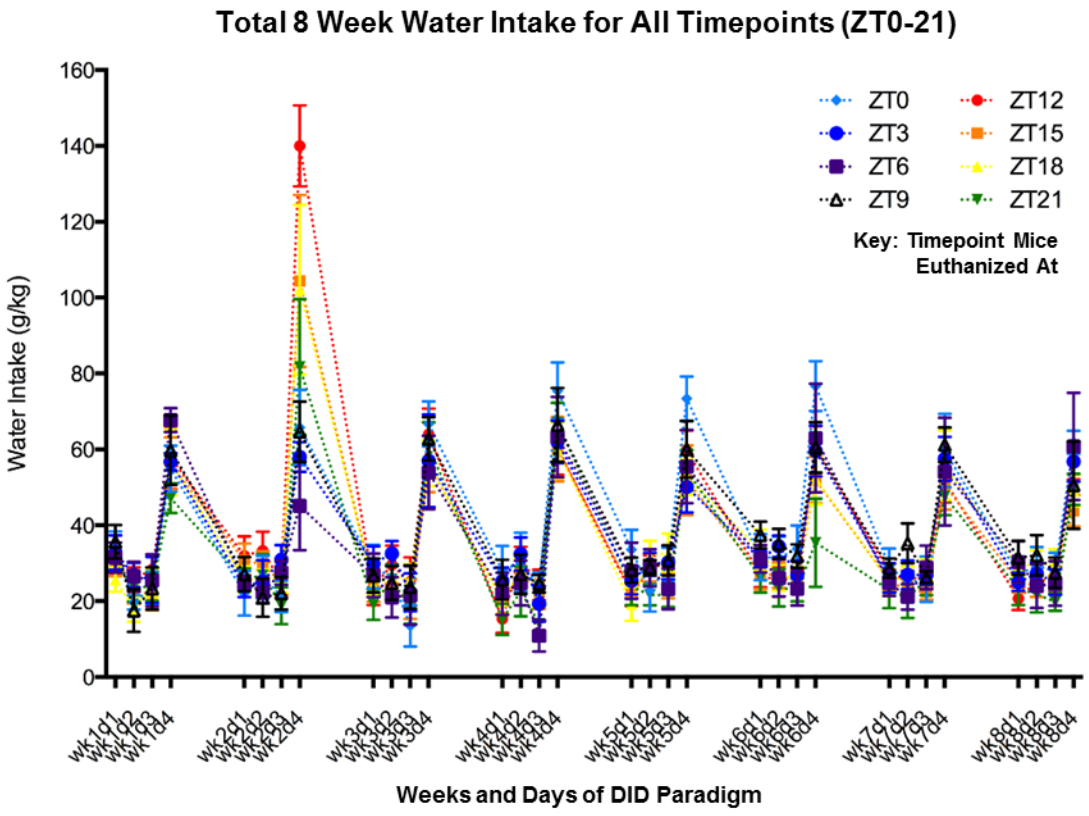

Figure 2a: *Average water intake for mice in all groups (by time point) from week 8 of the DID protocol. Days 1-3 water intake was measured for two hours, each hour and day 4 intake was measured for 4 hours every hour.*

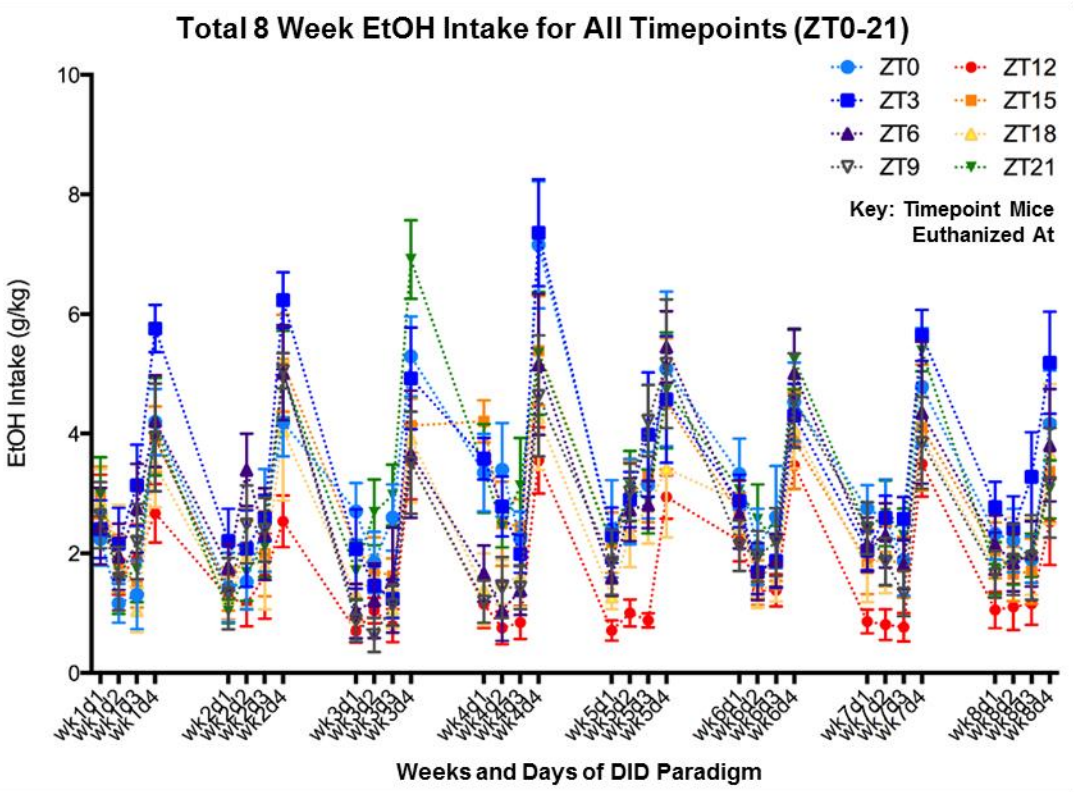

Figure 2b: *Average ethanol intake for mice in all groups (by time point) from week 8 of the DID protocol. Days 1-3 ethanol intake was measured for two hours, each hour and day 4 intake was measured for 4 hours every hour.*

Chronic Ethanol Consumption Reduced *Per2* Expression in NAc

We hypothesized that due to chronic ethanol consumption, altered levels of *Per2* would be observed in the NAc. To determine if *Per2* expression was modulated by ethanol consumption, HDID-1 mice voluntarily consumed ethanol in a DID paradigm for 8 weeks, and were subsequently euthanized at eight time points after the last DID day. *Per2* mRNA expression in NAc was quantified *via* qRT-PCR. *Per2* expression levels in NAc were significantly lower in ethanol-experienced mice in a ZT-dependent manner; *Per2* expression increased as the zeitgeber time the mice were euthanized at progressed (Figure 2a). A two-way ANOVA revealed a significant group x ZT

interaction (F (7, 80) =2.76, p<0.05) with a main effect of ZT (F (7, 80) =28.63, p<0.0001), and a main effect of group (F (1, 80) =6.40, p<0.05). Bonferroni post-hoc testing revealed a significant effect of group at ZT15 and ZT18 (p<0.05 for both time points).

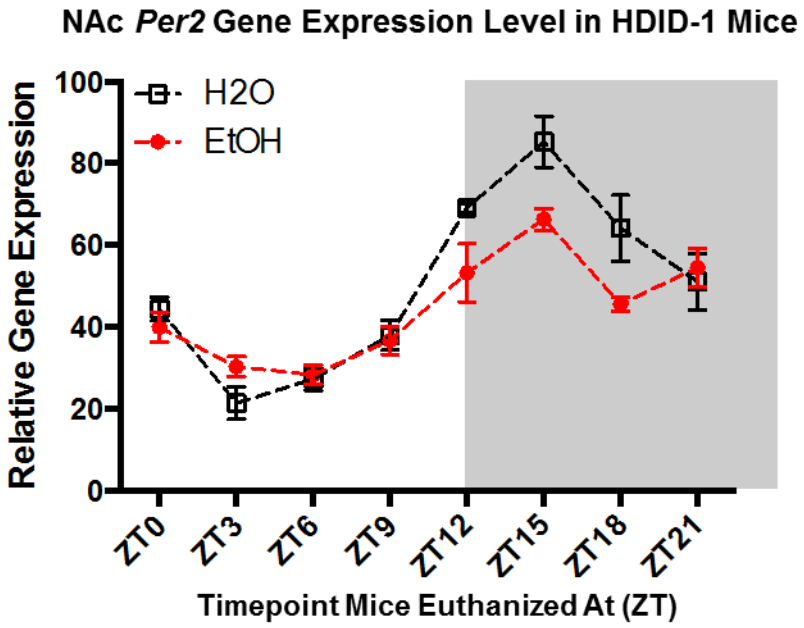

Figure 3a: *Chronic ethanol intake results in significantly reduced Per2 expression in a ZT-dependent manner. Per2 expression in the NAc was normalized to 18S, fold change was calculated using the ddCT method, and relative expression was calculated as described in Falcon et al., 2013. *p<0.0001 relative to control (H_2O drinking mice) after post-hoc testing; ZT, Zeitgeber time. The white background area indicates when the lights were on and the gray background area indicates lights off.*

Chronic Ethanol Consumption Reduced *Per2* Gene Expression in SCN

We hypothesized that after chronic ethanol consumption, *Per2* expression levels would be altered in the SCN. HDID-1 mice were subjected to an 8 week DID paradigm as described previously. After mRNA analysis from qRT-PCR, we observed that *Per2* expression levels significantly decreased in ethanol-experienced mice

(Figure 2b). A two-way ANOVA revealed a main effect of ZT ($F(7, 76) = 5.40$, $p<0.0001$), and a significant main effect of group ($F(1, 80) + 5.78$, $p<0.05$).

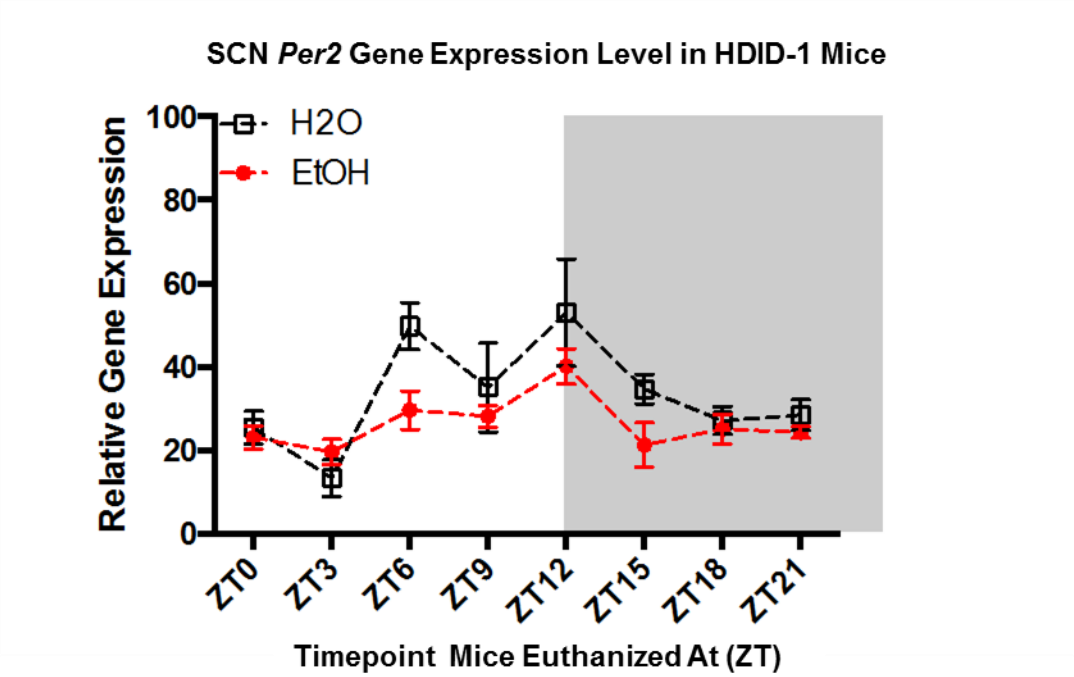

Figure 3b: *Chronic ethanol intake results in significantly reduced Per2 expression in a ZT-dependent manner. Per2 expression in the SCN was normalized to 18S, fold change was calculated using the ddCT method, and relative expression was calculated as described in Falcon et al., 2013. The white background area indicates when the lights were on and the gray background area indicates lights off.*

Amplitude of *Per2* Gene Expression in the NAc, but not the SCN, is reduced by chronic binge-like drinking

The amplitude of the *Per2* gene expression rhythms was calculated for each fluid group (ethanol or water) in each brain region (SCN and NAc), separately. We used a peak to peak measurement of amplitude (where A (amplitude) = maximum - minimum relative gene expression value). The amplitude of the *Per2* rhythm is indicative of the strength of the molecular clock, where a higher amplitude indicates a more stable and strong molecular clock rhythm. Values for NAc *Per2* amplitude

were found to be 63.81 +/-7.39 for the water group and 37.97 +/-3.52 for the ethanol group (Figure 3a). A two-tailed Student's t-test revealed significant differences in amplitude when comparing the amplitude of NAc *Per2* rhythms for water and ethanol group of HDID-1 mice ($t=3.156$, $df=7.163$, $p < 0.05$). Values for SCN *Per2* amplitude were found to be 39.72 +/- 13.57 for the water group and 20.49 +/- 5.19 for the ethanol group (Figure 3b). A two-tailed Student's t-test did not reveal significant differences in amplitude when comparing the amplitude of SCN *Per2* rhythms for water and ethanol groups of HDID-1 mice ($p = 0.23$). Taken together, this indicates that the SCN is more resilient to the effects of chronic ethanol drinking than the NAc.

***Figure 4a**: Amplitude for Per2 expression was calculated for each fluid group (water and EtOH) by using a peak to peak measurement. Values for NAc Per2 amplitude were 63.81 +/- 7.39 for the water group and 37.97 +/- 3.52 for the ethanol group. A two-tailed Student's t-test revealed significant differences in amplitude when comparing NAc Per2 rhythm amplitudes for the water and ethanol group of HDID-1 mice ($t=3.156$, $df=7.163$, $p<0.05$) Amplitude of Per2 expression seemed to decrease with chronic alcohol binge-drinking in the NAc.*

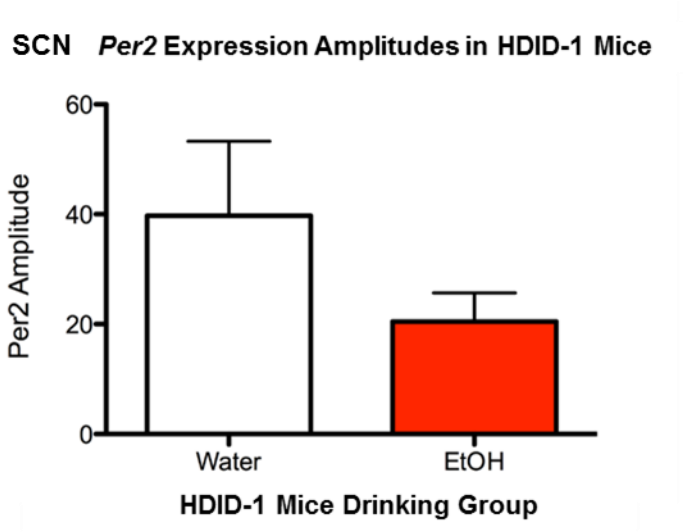

Figure 4b: *Amplitude for Per2 expression was calculated for each fluid group (water and EtOH) by using a peak to peak measurement. Values for SCN Per2 amplitude were 39.72 +/- 13.57 for the water group and 20.49 +/- 5.19 for the ethanol group. A two-tailed Student's t-test revealed no significant differences in amplitude when comparing SCN Per2 rhythm amplitudes for the water and ethanol group of HDID-1 mice (p=0.23) The SCN seems to be more resilient to the effects of chronic ethanol drinking than the NAc.*

Per2 Gene Expression Does Not Correlate with Fluid Intake

Under the 8 week DID paradigm laid out above, we measured ethanol and water intake by calculating the difference in weights of sipper tubes with the respective fluids in them. To determine whether *Per2* expression was ethanol responsive, we investigated whether fluid intake and *Per2* expression levels were correlated. We focused on the total intake of the last week of the 8 week DID protocol prior to measuring gene expression. The average weekly fluid intake for week 8 is 132.9 +/- 4.9 g/kg for the water drinking mice and 9.41 +/- 0.77 g/kg for the ethanol-drinking mice. Thus, we carried out regression analyses (Pearson's R) for week 8 fluid intake (ethanol or water) and all ZT *Per2* gene expression levels separately for NAc and SCN

(Figure 3a-d). The R squared values obtained for the NAc were 0.00009 for the ethanol group (Fig. 3a) and 0.051 for the water group (Fig. 3b). The R squared values obtained for the SCN were 0.024 for ethanol (Fig. 3c) and 0.006 for water (Fig 3d). We found no significant correlation between fluid intake and gene expression levels.

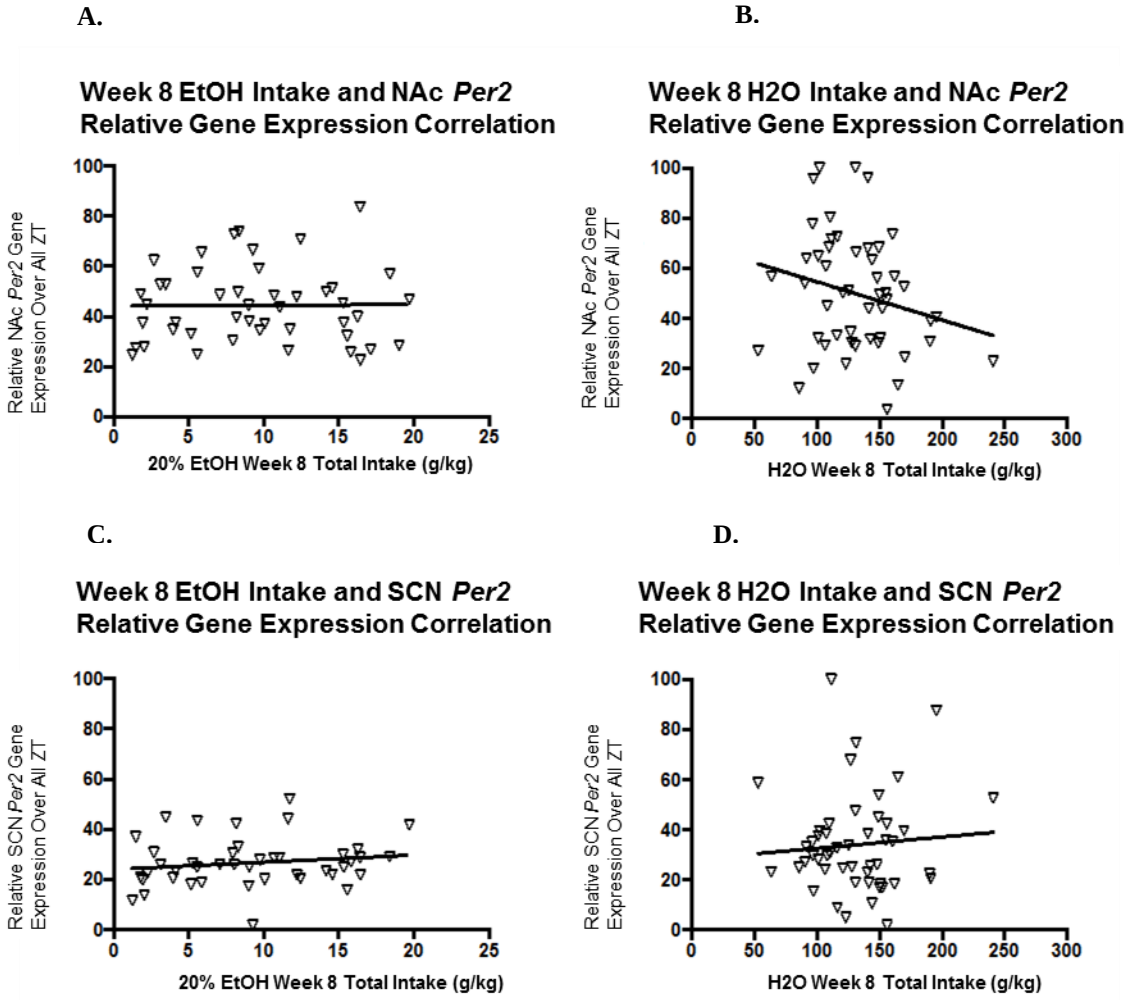

Figure 5a-d: *Pearson's R regression analyses carried out for total fluid intake (EtOH or H_2O) in Week 8 of the DID paradigm and total Per2 expression levels over all ZTs separately for the NAc and SCN. No significant correlation between fluid intake (g/kg) and Per2 expression levels was found. Note: ethanol intake is based on measurements from the 20% EtOH solution and indicates amt. of 20% EtOH consumed in g/kg.*

DISCUSSION

Results of this study establish that chronic ethanol intake alters circadian gene expression of *Per2* gene in the SCN and NAc brain region. HDID-1 mice undergoing the 8 week DID paradigm with ethanol exhibit significantly altered *Per2* gene expression in the NAc and SCN brain regions when compared with the control HDID-1 mice. *Per2* gene expression was altered in a group and time dependent manner. Chronic binge-like drinking resulted in a reduced amplitude of the rhythmic expression of *Per2* in the NAc, but not SCN. This suggests that the SCN may be more resilient to the effects of chronic ethanol drinking than the NAc. Regression analyses conducted led us to conclude that there was no significant correlation between fluid intake and gene expression levels, although this is likely due to the fact that the gene expression varies by time of day. These findings demonstrate the impact of chronic ethanol consumption on rhythmic *Per2* levels, an important gene in circadian rhythm function and affirm the hypothesis.

Years of research support the theory that there is a bidirectional relationship between chronic ethanol intake and circadian rhythm disruptions. A previous study has shown ethanol can disrupt expression of the circadian gene, *Clock*, and that disruption of *Clock* expression can in turn promote ethanol intake in mice (Ozburn et al., 2014). In a study conducted with rats, circadian expression of *Period* genes (including *Per1* and *Per2*) in the arcuate nucleus was significantly altered upon chronic ethanol consumption, as well as the circadian rhythms of *Per2* and *Per3*

levels in the SCN. Further, genetic variations in *Clock, Per2,* and *Per3* genes have been associated with alcoholism in humans (Huang et al., 2010). Also, mice and rats that are selectively bred for high ethanol consumption have been shown to have altered circadian phenotypes (Hofstetter et al., 2003; McCulley et al., 2013; Rosenwasser et al., 2005). These findings suggest that there is a genetic linkage between alcohol-related behaviors and circadian gene expression.

Altered circadian rhythms and disruptions in such rhythms can persist through extended periods of ethanol abstinence which may then promote relapses to drinking (Brower, 2003; Drummond et al., 1998; Gillin et al., 1994; Kuhlwein et al., 2003; Landolt and Gillin, 2001). This effect is noted with other substance abuse drugs such as cocaine as well (Kosobud et al., 2007). Other research has shown that 6 weeks of chronic DID drinking also increases anxiety- and depression-like behavior in mice (Lee et al., 2014). C57BL/6 mice undergoing withdrawal after being subjected to 6 weeks of the DID procedure, presented with higher indices of anxiety in various behavioral tests (Lee et al., 2014). Also, the mice showed a higher prevalence of behavioral despair and impaired emotional memory, suggesting that these behaviors emerge very early into withdrawal and persist throughout. This study suggests that a history of heavy binge drinking can produce lasting neuroadaptations within the brain circuitry dealing with emotional arousal. (Lee et al., 2014)

Other abused drugs such as cocaine, have also been shown to disrupt circadian rhythms. Cocaine exposure specifically can alter the rhythms of autonomic,

immune and sleep mechanisms (Falcon and McClung, 2009). Also, there is a diurnal variation in the sensitivity to almost all of the drugs of abuse. In fact, retrospective studies have revealed that most of the drug overdose patients in the emergency room of more urban hospitals presented at 6:30 p.m. compared to other times of the day (Falcon and McClung, 2009). This implies a diurnal effect however, there may be other environmental and societal factors that could affect the results as well. Furthermore, a compromised circadian function has been correlated with more prevalent rates of addiction in such individuals suggesting a bidirectional relationship.

There have been some pharmacological treatments proposed to combat alcohol abuse and dependence. One such therapy has been to administer CK1ε inhibitor drugs which have been shown to prevent relapse-like drinking in rat models (Perreau-Lenz et al., 2012). In this study, long-term alcohol-drinking rats were either given a CK1ε inhibitor or vehicle injections at 0, 10, and 30mg/kg dosages and alcohol intake was recorded upon re-exposure to alcohol after deprivation using a four-bottle free-choice paradigm with water and various concentrations of ethanol solutions. CK1ε inhibition reduced the high daytime alcohol intake typically observed upon re-exposure and induced a phase shift of locomotor activity toward (Perreau-Lenz et al., 2012). This is done molecularly since inhibiting the binding of CK1ε to the *Per2/Cry* dimers would lead to less translocation of the dimers and less degradation of the dimers thus, leading to more prolonged expression of these genes which could aid in prolonging the circadian rhythm and normalizing it. This

indicates that pharmacological treatments targeting circadian mechanisms such as the CK1ε protein are useful and may prove to be beneficial for alcoholism and alcohol abuse disorders.

In conclusion, our data suggest that chronic binge-like ethanol drinking does, in fact, alter circadian expression of *Per2* in both the NAc and SCN regions of the brain in a similar fashion in selectively bred HDID-1 mice. The amplitudes of rhythmic *Per2* expression were decreased in both the NAc and the SCN, however more robustly so in the NAc implicating that the SCN is more resistant to circadian rhythm changes as it is the pacemaker of circadian clocks in mammalian organisms. These results suggest that chronic ethanol intake alter circadian gene expression in multiple brain regions and that this disruption has a bidirectional relationship as well. Thus, various therapeutics geared towards normalizing circadian rhythm function in these regions of the brain look promising in alcoholism and alcohol abuse disorder treatment.

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