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## Sleep and circadian differences between light and heavy adult alcohol drinkers

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## Abstract

**Background:** Numerous studies have reported that eveningness is associated with increased alcohol consumption. The dim light melatonin onset (DLMO) and circadian photoreceptor responsivity (post-illumination pupil response, PIPR) have rarely been assessed in the context of habitual alcohol consumption. This study aimed to examine sleep, circadian timing and photoreceptor responsivity in adult alcohol drinkers.

**Methods:** Participants (21-45 years) included 28 light and 50 heavy drinkers. The 8-day study consisted of a week of adlib sleep monitored with wrist actigraphy, followed by a 9-hour laboratory session with a photoreceptor responsivity and circadian phase assessment.

**Results:** The heavy drinkers obtained on average 28 more minutes of sleep ( $p=0.002$ ) and reported more eveningness than the light drinkers ( $p=0.029$ ). There was a trend for a shorter DLMO-midsleep interval ( $p=0.059$ ) in the heavy drinkers, reflecting a tendency for them to sleep at an earlier circadian phase. The PIPR in the heavy drinkers was significantly smaller than in the light drinkers ( $p=0.032$ ), suggesting reduced circadian photoreceptor responsivity in the heavy drinkers. A larger PIPR was significantly associated with a later DLMO in the light drinkers ( $r=0.44$ ,  $p=0.019$ ), but this relationship was absent in the heavy drinkers ( $r=-0.01$ ,  $p=0.94$ ).

**Conclusions:** These results are consistent with earlier reports of more eveningness and a shorter DLMO-midsleep interval in association with heavier alcohol drinking. The novel finding of reduced circadian photoreceptor responsivity in the heavy drinkers is consistent with prior rodent studies. Future studies should explore the impact of habitual alcohol consumption on other measures of circadian photoreceptor responsivity.

**Keywords:** alcohol, circadian, light, post-illumination pupil response

## Introduction

Numerous studies have reported that later circadian timing in humans, as reflected in proxy markers of circadian timing such as evening chronotype or later sleep timing, is associated with increased alcohol consumption. For example, adolescents with an evening preference reported almost twice the lifetime drinking occasions as compared to those with intermediate or morning preference (Urban et al., 2011). Other studies have reported that a high evening preference and/or later sleep times in adolescents was significantly associated with more alcohol use (Negriff et al., 2011, Pieters et al., 2010, Gau et al., 2007), significantly higher AUDIT scores (i.e., self-reported alcohol consumption, drinking behaviors, and alcohol-related problems; (Saxvig et al., 2012)) and greater alcohol misuse (Glozier et al., 2014). One longitudinal study of adolescents (12-21 years) found that evening preference was associated with more binge drinking and at-risk alcohol use at baseline, but also predicted binge drinking one year later (Hasler et al., 2017). The reported effects in these studies remained significant even after analyses were adjusted for factors such as pubertal development, age, sex, race/ethnicity, socioeconomic status, educational level and psychopathology. Similar associations have also been observed in adults ( $\geq 18$  years). For example, adults with an evening preference and/or later sleep times were more likely to be alcohol drinkers (Wittmann et al., 2006, Whittier et al., 2014), consumed more alcohol (Tavernier and Willoughby, 2014, Kanerva et al., 2012, Adan, 1994, Van Reen et al., 2016), had higher AUDIT scores (Prat and Adan, 2011, Taylor et al., 2011, Taylor et al., 2020), and higher Alcohol Dependence scores (Hasler et al., 2013). Thus, the relationship between proxy markers of circadian timing and alcohol consumption is consistently reported.

This literature on circadian timing and alcohol consumption is however limited in at least two ways. First, proxy circadian markers, such as questionnaires, have been largely used, instead of biological markers of circadian timing. This is likely because the assessment of such biological markers is a time intensive process. For example, the assessment of the dim light melatonin onset (DLMO) typically requires half-hourly sampling of saliva in dim light (as light suppresses melatonin), in the 6 hours prior to habitual bedtime (Benloucif et al., 2008). Nonetheless, the DLMO is considered the gold standard circadian phase marker in humans (Lewy et al., 1999, Klerman et al., 2002). In the only two studies to date that have assessed the DLMO in the context of habitual alcohol drinking, one found later DLMO timing in emerging adult alcohol drinkers, and this was significantly associated with more drinking on the following weekend (Hasler et al., 2019). In the other study, recently abstinent alcohol-dependent individuals had later DLMOs than healthy controls (Conroy et al., 2012). These results are consistent with the literature, but more studies with larger sample sizes are needed to assess the DLMO in relation to habitual alcohol consumption.

Second, the role of the intrinsically photosensitive retinal ganglion cells (ipRGCs), the primary circadian photoreceptors, in the relationship between alcohol use and circadian timing remains unexplored. IpRGCs transmit the light signal to the circadian pacemaker and therefore play a key role in influencing circadian timing (Berson et al., 2002). IpRGCs express melanopsin, a primary photopigment that can respond directly to light (Provencio et al., 2000). The melanopsin response in ipRGCs can be quantified with chromatic pupillometry, which examines the pupil diameter during a post-illumination period, termed the post-illumination pupil response (PIPR, (Gamlin et al., 2007)). The PIPR reflects ipRGC responses, providing quantification of

individual differences in melanopsin-driven sensitivity to light (Kardon et al., 2009, Zele et al., 2019).

The aim of this study was to compare sleep, circadian timing, and circadian photoreceptor responsivity in two carefully defined and distinct groups of relatively healthy adults who regularly consumed alcohol but had significantly different habitual alcohol use patterns. With this approach, sleep, circadian timing, and circadian photoreceptor responsivity could then be directly compared between light and heavy drinkers. Based on the pre-existing literature, it was hypothesized that relative to light drinkers, heavy drinkers would have later circadian timing as reflected in both proxy markers of circadian timing and in the gold standard DLMO. It was also hypothesized that such later circadian timing in the heavy drinkers would be positively associated with greater circadian photoreceptor responsivity (i.e., greater PIPR) to light in the afternoon/evening, as light exposure at this time of day is associated with phase delays.

## **Materials and Methods**

### Participants

Participants were 78 alcohol drinking adults recruited through on-line advertisements who met criteria for light or heavy drinker. The screening process consisted of an online survey, followed by a telephone interview and then an in-person screening interview. Alcohol use had to be consistently reported on all three screening occasions for a candidate to be considered for the study with a consistent and predominant drinking pattern for a minimum of the past one year or longer. Based on prior work (Holdstock et al., 2000, King et al., 2002, King et al., 2011), inclusion criteria for heavy drinkers ( $n=50$ ) were consumption of  $\geq 10$  standard alcoholic drinks/week and at least one weekly binge drinking occasion per week as per the NIAAA

definition of  $\geq 5$  drinks/occasion for male participants and  $\geq 4$  drinks/occasion for female participants (NIAAA 2005). Further, over the past year, the majority of heavy drinkers (96%, 48/50) reported the first 5 drinks (4 for women) were consumed within the first two hours of an episode with fairly regular frequency. The inclusion criteria for light drinkers were consumption of 1-5 standard alcoholic drinks/week as the predominant drinking pattern, and no/rare ( $\leq 3$  per year) episodes of binge drinking in the past year and any history of regular heavy drinking was exclusionary. For both drinking groups, participants could not report any past or current significant alcohol withdrawal symptoms (e.g., seizures), nor treatment for alcohol or substance abuse, nor describe any plans to immediately change their drinking pattern.

Other inclusion criteria for both drinking groups were: 1) age between 21-45 years; 2) body mass index between 19-35 kg/m<sup>2</sup>; 3) no significant chronic disease (e.g. heart, lung, gastro-intestinal, vascular, endocrine, autoimmune disease, cancer); 4) no high likelihood of obstructive sleep apnea (Netzer et al., 1999) or restless leg syndrome (Hening and Allen, 2003); 5) no extremely short or long sleepers (defined as  $\leq 5$  hours/night,  $\geq 10$  hours/ night); 6) no reported eye disease or colorblindness (assessed with Ishihara test (Clark, 1924)); 7) no past or present psychotic or bipolar disorders, post-traumatic stress disorder, or obsessive compulsive disorder; 8) no current suicidal ideation or intent; 9) no significant anxiety or depressive symptoms (Beck Depression Inventory II  $\geq 17$  (Beck et al., 1996), State Trait Anxiety Inventory-Trait  $\geq 80$  (Spielberger et al., 1970)); 10) no use of prescription medications (contraceptives, acne medications and inhalers for exercise-induced asthma permitted) or supplemental melatonin; 11) no heavy cigarette smoking or vaping ( $\geq 10$  cigarette equivalents/day); 12) no travel across time zones in the past month; and 13) no shift work currently or in past month. Female participants who were pregnant, breast-feeding, perimenopausal or menopausal were excluded. At the in-person screening interview, all

participants had to have a breathalyzer reading of  $<.000$  g/dL and a negative urine toxicology screen (for cocaine, amphetamine, methamphetamine, marijuana [tetrahydrocannabinol], opiate, phencyclidine, barbiturates and benzodiazepines), and were instructed to be drug free during the study. The overall goal was to generate two distinct and carefully characterized groups of relatively healthy adult alcohol drinkers, to better examine the sleep and circadian differences between them. See Table 1 for sample characteristics.

### Design

The study consisted of an 8-day protocol (Figure 1). On Day 1, participants attended the laboratory and received a wrist actigraphy monitor (30 second epochs, Actiwatch Spectrum, Respironics, Bend, OR) to wear on their non-dominant wrist for the duration of the protocol. They were instructed to press the event marker on the monitor before and after adlib sleep each night and completed daily sleep and event diaries which tracked sleep, caffeine, alcohol and medication use during the protocol. During this visit, participants also participated in a practice PIPR assessment (see below) to become familiar with the procedure (data not analyzed).

On Day 8, participants arrived at the laboratory 9 hours prior to their habitual bedtime. They were instructed not to consume any alcohol or caffeine in the prior 24 hours, and no non-steroidal anti-inflammatory drugs in the prior 72 hours, to avoid confounding melatonin levels (see below). They were breathalyzed to confirm recent alcohol abstinence. Following this, their wrist actigraphy data was downloaded, and they completed questionnaires, and underwent a circadian photoreceptor responsivity assessment (described below). Participants then completed a 6.5-hour circadian phase assessment session, which completed their laboratory session. They were not permitted to drive themselves home and so either travelled home via taxi/rideshare or



with a friend or family member who drove them. Participants started the study protocol and their laboratory session on a weekday (Monday, Tuesday, Wednesday or Thursday) or weekend (Saturday or Sunday). Friday nights were reserved for another study in the laboratory. Data collection occurred at both Rush University Medical Center and the University of Michigan, and the Institutional Review Boards at both institutions approved the study protocol. All participants gave written informed consent prior to participating. Participants were compensated \$520 after they completed the 8-day protocol.

## Measures

### Wrist actigraphy

Objective measures of sleep were derived from the 7 days of wrist actigraphy recordings made just prior to the laboratory session. Data were analyzed with the Actiware 6.0.9 program (medium sensitivity, Respironics, Bend, OR). The setting of nightly rest intervals for analysis was guided by the event markers, sleep diaries, light data, and activity level (Patel et al., 2015). Objective actigraphic estimates of sleep timing (sleep onset time, final wake time), total sleep time (number of minutes scored as sleep in each rest interval) and sleep efficiency (proportion of time between sleep onset and final wake time scored as sleep in each rest interval, expressed as a percentage) were extracted for each study night, and averaged over the 7 days.

### Questionnaires

At the start of the laboratory session, participants completed multiple questionnaires. Sleep quality and insomnia symptoms were assessed with the Pittsburgh Sleep Quality Index (Buysse et al., 1989) and Insomnia Severity Index (Bastien et al., 2001). Circadian preference was assessed with the Morningness-Eveningness Questionnaire (Horne and Ostberg, 1976) and

chronotype and social jet lag were assessed with the Munich ChronoType Questionnaire (Roenneberg et al., 2003). Current depressive and anxiety symptoms were assessed with the Beck Depression Inventory (Beck et al., 1961) and State Trait Anxiety Inventory-State Questionnaire (Spielberger et al., 1970). Attitudes to alcohol were assessed with a three-item version of the Alcohol Purchase Task (Amlung et al., 2015) which asked if drinks were free, how many drinks would the participant consume right now, and what maximum dollar amount they would pay for a single drink and for the drinking session in total. Participants also completed the Anticipated Biphasic Effects of Alcohol Scale (A-BAES) which assessed anticipated levels of stimulation and sedation after imagining drinking 4 alcoholic drinks (Earleywine and Martin, 1993) and the Anticipated Drug Effects Questionnaire (A-DEQ) which asked how much they would feel, like and want more alcohol after consuming 4 alcoholic drinks (Fridberg et al., 2017).

#### *Circadian Photoreceptor Responsivity*

The post-illumination pupil response (PIPR) was then assessed with a laboratory-made pupillometer, which consisted of blue (488 nm) and red (632 nm) LEDs. The light from the LEDs traveled through optic fibers before being combined in a spatial homogenizer and a diffuser, producing a highly uniform field. A field lens with a 2-mm artificial pupil was used to create a Maxwellian view (Kelbsch et al., 2019). The LED light outputs were digitally controlled by software developed in Objective-C on a Mac computer.

For the PIPR assessment, participants entered a dark room, sat in a height adjustable chair, and rested their chin on a chin rest, which was adjusted to position their right eye in front of the artificial pupil to view the LED light. Light pulses (500msec) of red light (2000 Troland, which

quantified retinal illuminance based on light luminance in  $\text{cd}/\text{m}^2$  and pupil size in mm) or blue light (2000 Td) were applied to the right eye, in a  $30^\circ$  circular field, with the central  $10.5^\circ$  blocked to minimize the effect of macular pigment's selective absorption. The diameter of the left pupil was recorded with an infrared camera from an EyeLink II eyetracker (SR Research, Canada) with a sampling rate of 250 Hz and spatial resolution of 0.1% of baseline pupil size. The test started with 3 trials of red LED light followed by 3 trials of blue LED light. Each trial lasted 35.5 sec (2 sec before light pulse, 0.5 sec light pulse, and 25 sec after lights off). The obtained pupil response curve for each trial was normalized to percentage of baseline pupil size (which was measured over 2 sec before light pulse). Then the normalized pupil response curve was averaged first for red LED and blue LED lights. Overall circadian photoreceptor responsivity for each participant was calculated as the difference between the red light (average of 3 trials) and blue light (average of 3 trials) pupil response curves at 6 seconds after each light pulse (i.e., net blue minus red, percent of baseline (Adhikari et al., 2016, Kelbsch et al., 2019)).

#### Circadian Phase Assessment

After the pupil responsivity testing, at 6.5 hours prior to habitual bedtime, participants entered a room in the laboratory specifically designed for circadian phase assessments. Participants remained awake and seated in dim lit ( $<5$  lux, at level of eyes, in direction of gaze, measured every 2 hours, Extech EA33 light meter, Nashua, NH) and were continuously monitored by staff. After 30 minutes in the dim light, subjects gave a saliva sample every 30 minutes using Salivettes (Sarstedt, Newton, NC). Toothpaste or mouthwash were not allowed during the phase assessments. Small snacks and fluids were permitted, except in the 10 minutes before each sample, and subjects were required to brush their teeth and rinse with water while remaining seated 10 minutes before each sample if they had consumed food or drink. The samples were

centrifuged immediately upon collection and frozen. The samples were later shipped in dry ice to Solidphase (Portland, ME), which radioimmunoassayed the samples for melatonin using the Buhlmann RIA assay, which is the most accurate assay for salivary melatonin (Kennaway, 2019). The assay sensitivity was 0.5 pg/ml. Intra-assay and inter-assay coefficients of variation for low levels of salivary melatonin are 20.1%, and 16.7%, respectively. A dim light melatonin onset (DLMO) was calculated for each participant, as the clock time (with linear interpolation) when the melatonin concentration exceeded the mean of three low, consecutive, daytime values plus twice the standard deviation of these points (Voultsios et al., 1997, Benloucif et al., 2008). This low threshold more closely tracks the initial rise of melatonin (Molina and Burgess, 2011). Like prior related studies (Hasler et al., 2019, Conroy et al., 2012) the alignment between sleep and circadian timing was also calculated, as the interval between DLMO and midsleep (halfway point between sleep onset and final wake time).

### Statistical Analysis

All analyses were conducted using Stata 15.1 (StataCorp, College State, Texas). Independent samples t-tests were used to compare continuous variables between the two drinking groups and Chi-square tests were used for categorical variables. Group differences and the relationships between variables were explored using linear regression, which adjusted for variables known to impact sleep and circadian variables: age, sex, photoperiod (daylength) on the day of the laboratory session, and day of week of laboratory assessment (weekday or weekend). Statistical significance was determined at  $p < 0.05$ .

## Results

### Sample Characteristics

The final sample consisted of 28 light drinkers and 50 heavy drinkers who participated from February 2017 - February 2020. Their demographics and baseline characteristics are shown in Table 1. There were plans to enroll more light drinkers, but the study ended abruptly with a laboratory shutdown due to the COVID-19 pandemic. The average age of the sample was in the late twenties. There were significantly more male participants in the heavy drinking group. There were no significant group differences in race or ethnicity, and both groups showed minimal depressive and anxiety symptoms, as expected from the screening criteria. There was also no group difference in the photoperiod (daylength), season, or day of the week of the laboratory session. The clock time of the laboratory session was not different between groups – the first saliva sample (first dot in Figure 1) occurred at  $18:05 \pm 1.23$  in light drinkers and at  $18:10 \pm 1.31$  in the heavy drinkers ( $p=0.82$ ). The circadian time of the laboratory session was derived *post-hoc* as the interval from the first saliva sample in the phase assessment (first dot in Figure 1) to the later calculated DLMO. There was a significant group difference in the circadian time of testing such that the heavy drinkers were assessed on average 36 minutes later relative to their DLMO than the light drinkers (Table 1).

### Alcohol-Related Variables

As expected, the heavy drinkers reported consuming more alcohol drinks per week, and having more alcohol binges per week, on an online 30 day timeline follow back questionnaire (Sobell and Sobell, 1995) collected at the in-person screening interview (Table 2). Similarly, as reported on the daily diaries during the baseline week, the heavy drinkers continued to drink more than the light drinkers, even though drinking was limited with the instruction to abstain from alcohol

in the 24 hours prior to the laboratory session. The heavy drinkers had a significantly higher AUDIT score than the light drinkers, which reflected potentially harmful or hazardous alcohol consumption (Babor et al., 1989). Most of the heavy drinkers did not meet criteria for an alcohol use disorder (AUD), with only 3 of them (6%) meeting criteria for mild AUD 2 or 3 symptoms according to the DSM-5 criteria. As expected on the Alcohol Purchase Task, heavy drinkers proposed drinking significantly more and paying significantly more for a drinking session than did light drinkers. Both groups reported similar levels of anticipated stimulation from 4 standard alcohol drinks, and the heavy drinkers anticipated significantly higher liking and wanting with lower levels of sedation and feeling alcohol effects, generally consistent with subjective responses measured in alcohol challenge research (King et al., 2011).

#### Sleep and Circadian Variables

On average, the heavy drinkers obtained 28 more minutes of sleep per night than the light drinkers (Table 3). This was mostly due to a later wake time in the heavy drinkers. The heavy drinkers reported more insomnia symptoms, but on average both groups did not show clinically significant insomnia symptoms (average Insomnia Severity Index score  $\leq 7$ ). Likewise, both groups had good sleep quality and good sleep efficiency. A significantly greater tendency to eveningness was observed in the heavy drinkers. A later average chronotype was also observed in the heavy drinkers, but this was not statistically significant. There was a trend for the heavy drinkers to have more social jet lag, although both groups on average had social jet lag of less than 2 hours. The DLMO occurred on average 32 minutes later in the heavy drinkers, although this was not statistically significant. There was a trend for a shorter DLMO to midsleep interval in the heavy drinkers, reflecting that they slept at an earlier circadian phase. There were seven participants with missing DLMOs – all were heavy drinkers. Two participants may have had

earlier DLMOs which could have been determined if the phase assessment had started earlier, two participants may have had later DLMOs which could have been determined if the phase assessment had ended later, and three participants showed a very erratic rise in melatonin such that a DLMO was not readily discernible. Lastly, the net difference between red and blue pupil response curves 6 seconds after the light pulse in the heavy drinkers was significantly smaller than in the light drinkers, suggesting reduced circadian photoreceptor responsivity in the heavy drinkers (Table 3, Figure 2).

#### Associations Between Circadian Timing and Circadian Photoreceptor Responsivity

In contrast to expectations, while heavy drinkers had a later average DLMO (but not statistically significantly so), they also had a significantly reduced PIPR, reflecting *reduced* circadian photoreceptor responsivity (Table 3, Figure 2). As the PIPR was assessed in the afternoon/evening hours, it was expected that *more* circadian photoreceptor responsivity, which should amplify phase-delaying effects of light in the evening, would associate with a later DLMO. To further explore this, the relationship between DLMO and PIPR were separately explored in each drinking group (Figure 3). In the light drinkers, greater circadian photoreceptor responsivity (larger PIPR) was significantly associated with a later DLMO as expected ( $r=0.44$ ,  $p=0.019$ ). However, in the heavy drinkers the correlation was neither significant nor meaningful ( $r=-0.01$ ,  $p=0.94$ ). These associations between DLMO and PIPR in each group were still observed after adjusting the analyses for sex, age, photoperiod, day of week of laboratory and even circadian time of testing (light drinkers,  $r=0.44$ ,  $p=0.033$ ; heavy drinkers  $r=-0.04$ ,  $p=0.80$ ). Circadian time of testing was derived *post-hoc* as the time interval from the first saliva sample to the DLMO. Thus, heavy alcohol consumption was associated with a smaller PIPR and the relationship between the DLMO and PIPR seen in the light drinkers was not present in the heavy

drinkers.

## **Discussion**

In this study, two distinct and carefully characterized groups of relatively healthy adult alcohol drinkers were examined: light and heavy drinkers. Compared with the light drinkers, the heavy drinkers engaged in more alcohol drinking and bingeing behavior, both at screening and in the baseline week prior to the laboratory session. The heavy drinkers also reported more hazardous alcohol consumption on the AUDIT, were willing to pay more for a drinking session, and anticipated that after consuming 4 standard alcoholic drinks that they would feel less sedation, but like and want more alcohol, as compared with the light drinkers. The main sleep and circadian differences between the groups (after adjusting for age, sex, photoperiod, and day of week of laboratory assessment) were that the heavy drinkers woke about 30 minutes later and endorsed more eveningness. There was no group difference in the DLMO, but a trend towards a shorter DLMO to midsleep interval in the heavy drinkers. The heavy drinkers also exhibited a smaller PIPR, suggesting reduced circadian photoreceptor responsivity to light.

This study is only the third study in the literature to examine the DLMO, the gold standard circadian phase marker in humans, in the context of habitual alcohol drinking. The finding of more eveningness in the heavy drinkers is consistent with the broader literature (Negriff et al., 2011, Pieters et al., 2010, Gau et al., 2007, Tavernier and Willoughby, 2014, Kanerva et al., 2012, Adan, 1994, Van Reen et al., 2016). However, this greater eveningness was not reflected in a significantly later DLMO. Instead, there was a trend observed for a shorter DLMO-midsleep interval in association with heavy alcohol consumption which is consistent with the other DLMO and alcohol consumption studies (Hasler et al., 2019, Conroy et al., 2012). Indeed, the shorter



DLMO-sleep midpoint of about 25 minutes observed in the heavy versus light drinkers, matches the difference in the DLMO-sleep midpoint observed between recently abstinent alcohol dependent individuals and healthy controls (Conroy et al., 2012). Thus, heavy drinkers do not necessarily have later circadian timing per se, but instead have later circadian timing relative to the timing of their sleep, potentially reflecting greater circadian misalignment (Hasler et al., 2019). Interestingly, the light and heavy drinkers did not meaningfully differ in their mood symptoms, nor in their sleep quality or sleep efficiency, and the heavy drinking was not associated with poor mood or sleep disturbance. In fact, the heavy drinkers actually obtained more sleep per night than the light drinkers. Thus, the heavy drinking was not associated with later bedtimes, and not simply due to the heavy drinkers having more evening hours available to them. One possibility is that circadian misalignment characterized by the shorter DLMO-sleep midpoint interval (phase angle) was associated with altered reward functioning and impaired impulse control (Hasler et al., 2021), leading in turn to more alcohol consumption (Hasler and Clark, 2013). Indeed, eveningness has been repeatedly linked to greater global impulsivity (e.g., Russo et al., 2012, Kang et al., 2015), and was recently found to associate with multiple subdimensions of impulsivity measured at the state-level over and above the effects of actual sleep timing or duration (Hasler et al., 2022). Importantly, eveningness was associated with greater urgency (positive and negative), which has been particularly linked to alcohol use and related problems (Littlefield et al., 2014). This suggests that the clinical treatment of patients seeking treatment for heavy drinking may be enhanced by assessing circadian preference, as an eveningness tendency might indicate the utility of more attention to monitoring the role of impulsivity in the patients' alcohol use, as well as consideration of their sleep schedules. Preliminary evidence suggests that imposing circadian misalignment impairs the neural underpinnings of impulse control (Hasler et al., 2021) while correcting it may reduce impulsivity

(Fargason et al., 2017) Lastly, there is evidence that later sleep/circadian timing may be associated with later timing of peak alcohol craving (Hisler et al., 2021), which could be informative for heavy drinking evening types.

In the light drinkers, the anticipated relationship between circadian photoreceptor responsivity (PIPR) and DLMO was observed – namely that higher circadian photoreceptor responsivity was associated with a later DLMO. However, this relationship was not observed in the heavy drinkers who displayed a significantly reduced circadian photoreceptor responsivity (reduced PIPR). The reduced circadian photoreceptor responsivity in the habitual heavy drinkers may indicate impaired photoentrainment, leading to a disrupted relationship between light input and circadian timing. While acute alcohol intake does not alter pupil diameter with a steady light exposure (Brown et al., 1977), acute alcohol exposure has been reported to modulate the sensitivity of retinal neurons to various neurotransmitters such as gamma-aminobutyric acid (GABA) (Yeh and Kolb, 1997). The effects of an acute alcohol dose on PIPR have not yet been assessed. The possibility that a history of heavy alcohol use modulates circadian photoreceptor responsivity to light should be further explored with other methods, such as melatonin suppression to light (Phillips et al., 2019). While a single dose of alcohol was not shown to systematically alter circadian phase shifts to light in healthy humans (Burgess et al., 2016), robust evidence in rodents suggests chronic alcohol consumption can reduce photic inputs into the clock, disrupt circadian entrainment, and alter circadian period (Ruby et al., 2009, Brager et al., 2010). Circadian phase shifts to light remain to be studied in habitual heavy drinkers, and future research should try to tease apart the effects of heavy alcohol use from the effects of circadian misalignment.

This study has several strengths and several limitations. In terms of strengths, this is the largest study to date to examine habitual alcohol drinking and the DLMO, and it corroborates the pre-existing literature that suggests an association between eveningness, a shorter DLMO-midsleep interval, and greater alcohol consumption. In addition, this is the first study to examine circadian photoreceptor responsivity in the context of habitual alcohol drinking, and it has revealed the possibility that heavy alcohol drinking may suppress melanopsin-driven pupil responsivity. Important factors known a priori to impact sleep and circadian variables, such as age, sex, photoperiod (daylength), season, and day of week of laboratory assessment (weekday/weekend), were included in the analyses. Further, the study protocol timing was anchored to habitual bedtime, which led to participants being assessed around the same circadian time (average group difference of 36 mins), thus likely reducing variance in the data. Finally, calculating the PIPR as % of baseline reduces error due to individual differences in baseline pupil size, and using equal 2,000 Td illuminance at the retina for red and blue light allowed for the adjustment of the response to blue light for non-specific effects on the PIPR as measured by the response to red light (i.e., autonomic influence on the PLR (Kelbsch et al., 2019)). In terms of limitations, this study may be underpowered as the recruitment of light drinkers was cut short due to the COVID-19 pandemic. Additionally, this is a cross-sectional study, and causation of the reported group differences and associations cannot be determined. In terms of the PIPR, the absence of eye disease was only determined from participant self-report and ophthalmological exams were not conducted to confirm this, although prior studies with similar sample sizes found no previously unreported retinal health conditions (Roecklein et al., 2021). Furthermore, PIPR was only assessed in the evening, and given the circadian rhythm in PIPR (Zelevansky et al., 2011), group differences in the PIPR in the morning may or may not have been different to what was observed in the evening. Finally, the heavy alcohol drinkers were screened to be relatively healthy and

therefore may not represent less healthy heavy alcohol drinkers who engage in other drug use such as cannabis or experience comorbid psychiatric disorders. Future research should compare these sleep and circadian variables between a healthy control group and patients meeting a diagnosis of AUD according to DSM-5 criteria.

In summary, this study found that in generally healthy humans, heavy alcohol drinking was associated with more eveningness, a shorter DLMO-midsleep interval, and reduced circadian photoreceptor responsivity, as compared to light drinkers. Heavy drinking in this sample was not associated with poor mood or sleep disturbance. Future research should interrogate whether heavy habitual alcohol consumption is either a consequence or cause of reduced melanopsin-driven retinal responsivity.

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## Figure Legends

**Figure 1.** A representation of the 8-day study protocol, for a participant with an average bedtime of midnight. On Day 1, participants attended the laboratory and received a wrist actigraphy monitor and were instructed on how to complete daily diaries while sleeping adlib at home (participants were not required to follow a fixed 8-hour sleep schedule). On Day 8, participants arrived for the laboratory session 9 hours before their habitual bedtime. They completed questionnaires (Q), completed a circadian photoreceptor responsivity assessment (P) and then began a circadian phase assessment (dots represent timing of first and last saliva sample). Time of arrival and departure from the laboratory on Day 8 is represented by square brackets.

**Figure 2.** The averaged pupil response curves after red and blue LED lights in the light (top) and heavy alcohol drinkers (middle). The left y-axis shows the pupil size normalized to baseline in %, and the right y-axis shows the average corresponding pupil size in mm. The bottom figure shows the net red-blue difference in each drinking group (LD (lighter line)= light drinkers, HD (heavier line)=heavy drinkers), and highlights the reduced difference in the heavy versus light drinkers at 6 seconds after the light pulses.

**Figure 3.** Scatterplots of the timing of the dim light melatonin onset (DLMO) versus the circadian photoreceptor responsivity (PIPR, which was calculated as the net difference at 6 seconds from pupil response curves between the red and blue LED lights) in the light and heavy alcohol drinkers. The unadjusted correlations are shown.

**Table 1.** Sample characteristics.

	Light Drinkers ( <i>n</i> =28)	Heavy Drinkers ( <i>n</i> =50)	<i>p</i> -value
Age (mean, SD)	27.9 (5.9)	27.3 (5.1)	0.66
Sex assigned at birth (%)			<b>0.04</b>
Male	32%	56%	
Female	68%	44%	
Race (%)			0.10
Asian	18%	16%	
Black	21%	4%	
Other	4%	4%	
White	57%	76%	
Ethnicity (%)			0.50
Hispanic/Latinx	7%	12%	
Beck Depression Inventory (mean, SD)	1.26 (2.01)	1.66 (2.36)	0.46
State Trait Anxiety Inventory-State (mean, SD)	40.93 (6.27)	40.10 (7.01)	0.61
Laboratory session day of week (%)			0.92
Weekday	42.9	40.0	
Weekend	57.2	56.0	
Photoperiod on day of lab session (mean, SD)	12.39 (2.24)	12.60 (1.96)	0.68
Season on day of lab session			0.84
Winter	18%	14%	
Spring	32%	42%	
Summer	25%	24%	
Fall	25%	20%	
Circadian time of testing (mean, SD)*	2.50 (1.16)	3.10 (1.21)	<b>0.049</b>

The mood questionnaires were collected at the start of the laboratory session.

*P* values <0.05 are bolded.

\* circadian time of testing was the time interval from the first saliva sample to the later determined DLMO.

**Table 2.** Alcohol-related variables.

	Light Drinkers ( <i>n</i> =28) (mean, SD, range)	Heavy Drinkers ( <i>n</i> =50) (mean, SD, range)	Adjusted <i>p</i> -value
Alcohol drinks/week from TLFB	2.57 (1.09, 0.75-4.75)	17.89 (6.84, 9.5-46.75)	<b>&lt;0.001</b>
Alcohol binges/week from TLFB	0.02 (0.07, 0-0.25)	2.0 (0.8, 0.75-5)	<b>&lt;0.001</b>
Alcohol drinks in baseline week	3.50 (3.23, 0-12)	14.06 (11.86, 0-56.5)	<b>&lt;0.001</b>
Alcohol binges in baseline week	0.11 (0.31, 0-1)	1.44 (1.33, 0-5)	<b>&lt;0.001</b>
AUDIT	3.0 (1.2, 0-6)	11.4 (4.2, 5-21)	<b>&lt;0.001</b>
Alcohol Purchase Task			
Number of drinks	2.48 (1.50, 0-6)	5.89 (2.72, 2-14)	<b>&lt;0.001</b>
Maximum \$ on single drink	8.16 (3.83, 0-15)	8.16 (3.29, 2-16)	0.65
Maximum \$ on total drinks	18.48 (9.93, 0-40)	27.08 (9.93, 8-40)	<b>&lt;0.001</b>
Anticipated Biphasic Alcohol Effects			
Stimulation	39.25 (16.98, 0-64)	43.40 (10.07, 15-65)	0.13
Sedation	30.93 (15.74, 3-70)	11.88 (9.15, 0-36)	<b>&lt;0.001</b>
Anticipated Drug Effects			
Feel effects of alcohol	80.3 (23.0, 0-100)	54.5 (21.8, 0-99.5)	<b>&lt;0.001</b>
Like effects of alcohol	61.3 (21.8, 0-100)	75.4 (18.1, 10.5-100)	<b>0.002</b>
Want more alcohol	24.7 (24.1, 0-77)	67.6 (19.9, 14-100)	<b>&lt;0.001</b>

TLFB = 30-day timeline follow back questionnaire administered during the in-person screening interview. The AUDIT questionnaire was completed during the in-person screening interview. *P*-values were adjusted for age, sex, photoperiod, and lab assessment day (weekend/weekday). *P* values <0.05 are bolded.

**Table 3.** Sleep and circadian variables.

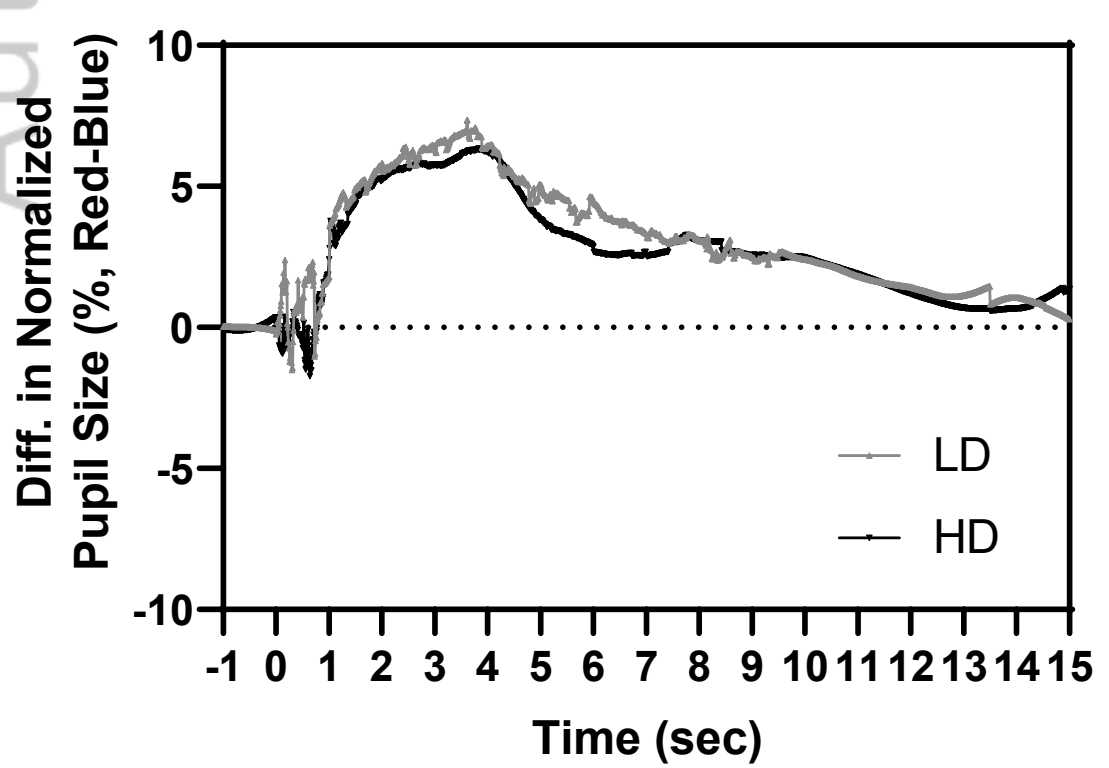
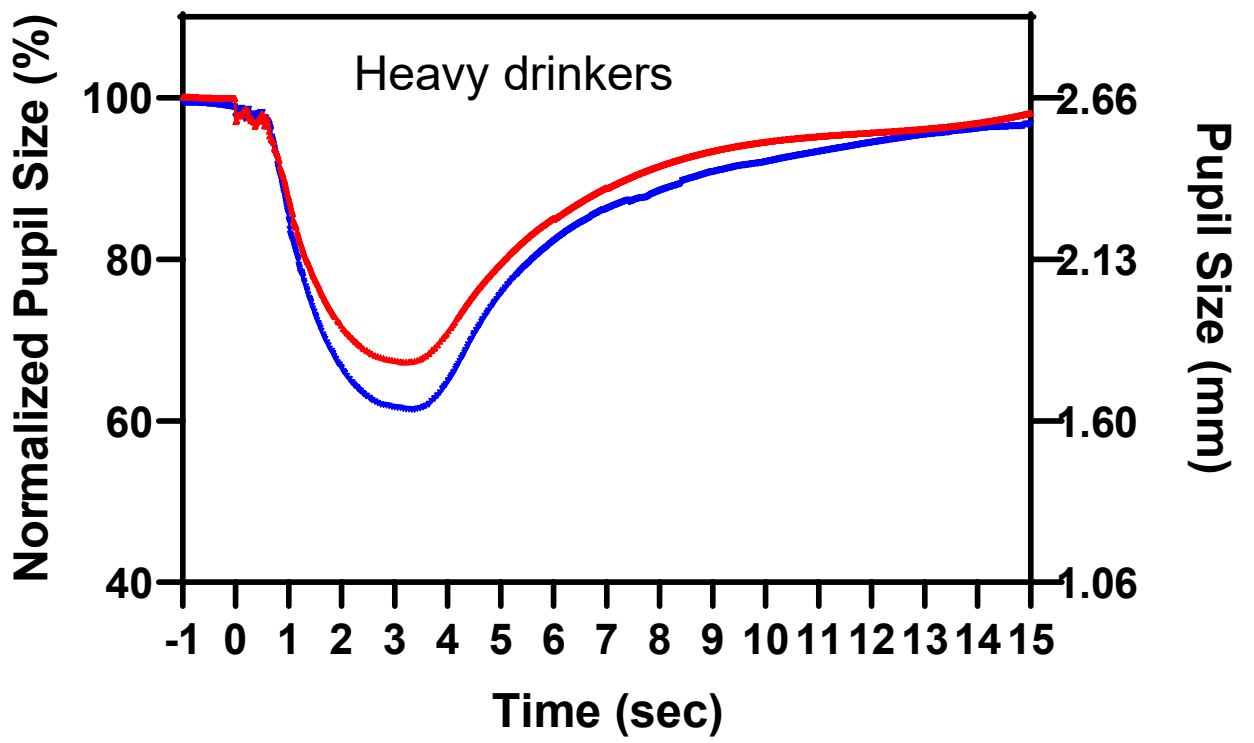
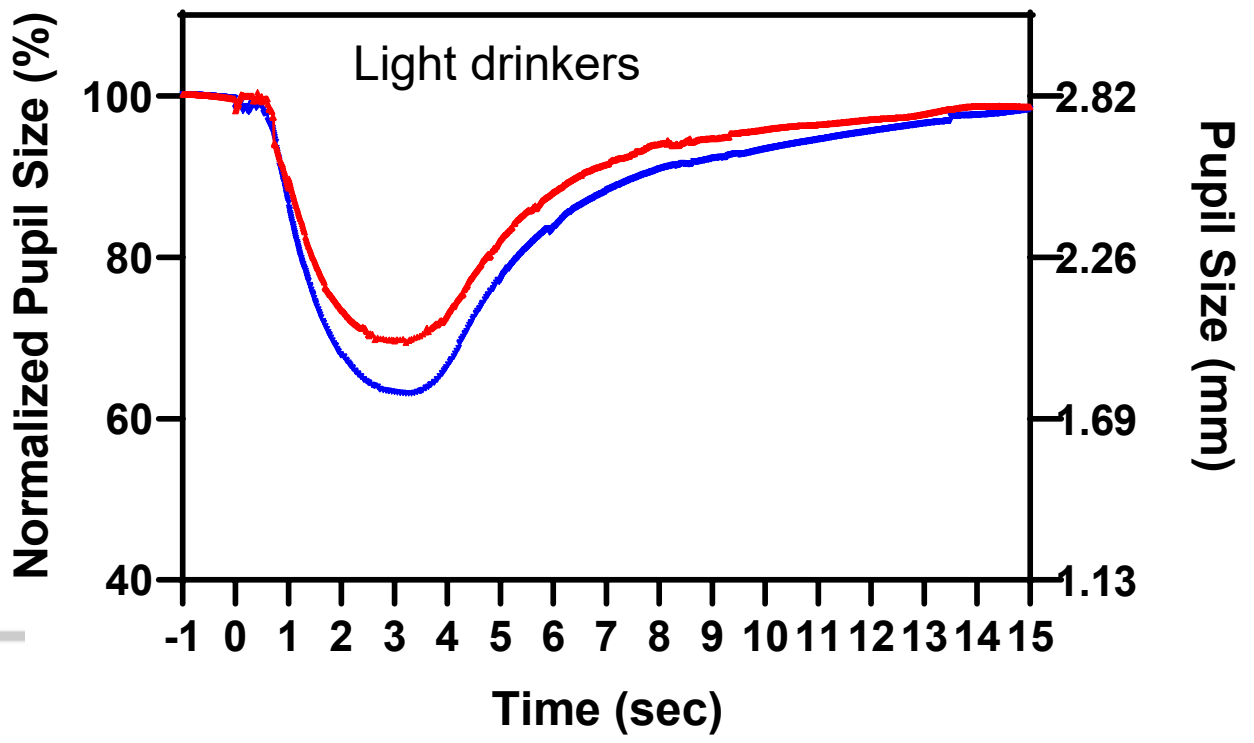
	Light Drinkers (n=28)	Heavy Drinkers (n=50)	Adjusted <i>p</i> -value
Sleep onset time (mean, SD)	00:23 (1.3)	00:15 (1.1)	0.308
Final wake time (mean, SD)	07:40 (1.4)	08:08 (1.3)	0.218
Total sleep time (hours; mean, SD)	6.71 (0.73)	7.18 (0.77)	<b>0.002</b>
Sleep efficiency (%; mean, SD)	92.22 (2.91)	91.34 (3.19)	0.483
Pittsburgh Sleep Quality Index (mean, SD)	3.00 (1.76)	3.60 (1.94)	0.205
Insomnia Severity Index (mean, SD)	2.43 (1.81)	4.10 (3.38)	<b>0.009</b>
Morningness-eveningness (mean, SD)	52.71 (9.97)	47.63 (7.80)	<b>0.029</b>
Munich chronotype (h; mean, SD)	04:39 (1.41)	05:15 (1.17)	0.118
Munich social jet lag (h; mean, SD)	1.29 (1.28)	1.75 (0.88)	0.055
Dim light melatonin onset (mean, SD)	20:35 (1.5)	21:07 (1.5)	0.254
Dim light melatonin onset to midsleep interval (h; mean, SD)	7.44 (0.76)	7.03 (1.0)	0.059
Post-illumination pupil response (net difference, % baseline, 6 secs, mean, SD)*	4.49 (2.55)	2.87 (3.13)	<b>0.032</b>

*P* values were adjusted for age, sex, photoperiod, and day of week of laboratory assessment (weekend/weekday).

\* the group comparison of PIPR was also adjusted for the circadian time of assessment (the time interval between first saliva sample shortly after the PIPR and the DLMO, calculated *post-hoc*).

*P* values <0.05 are bolded.





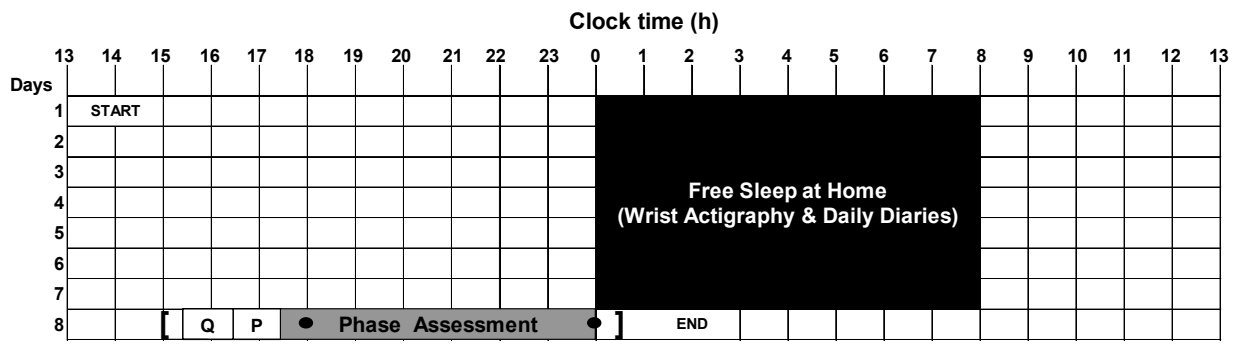


Figure 1

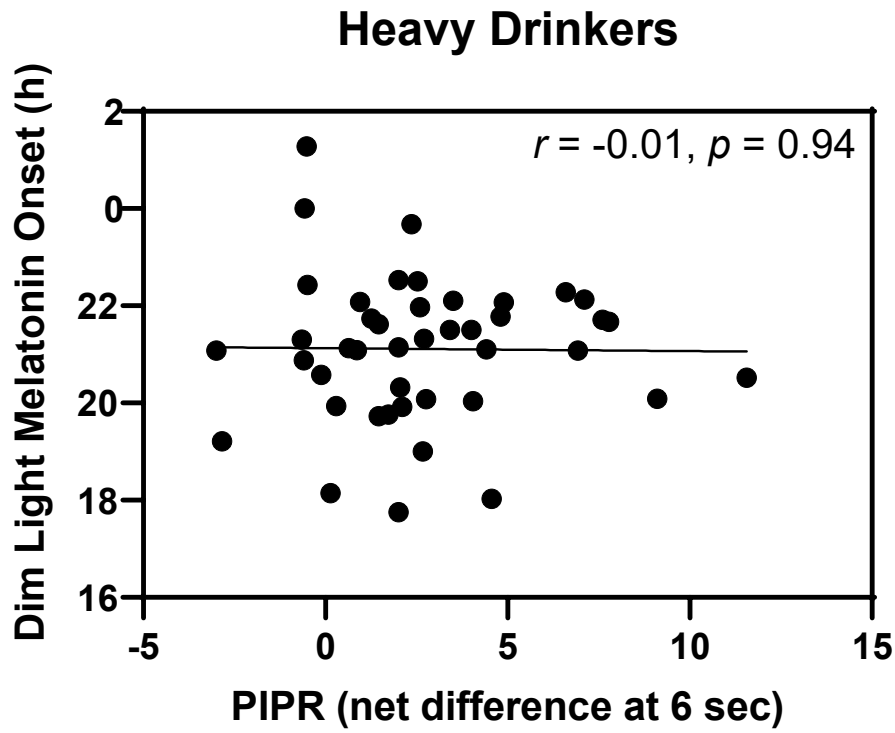
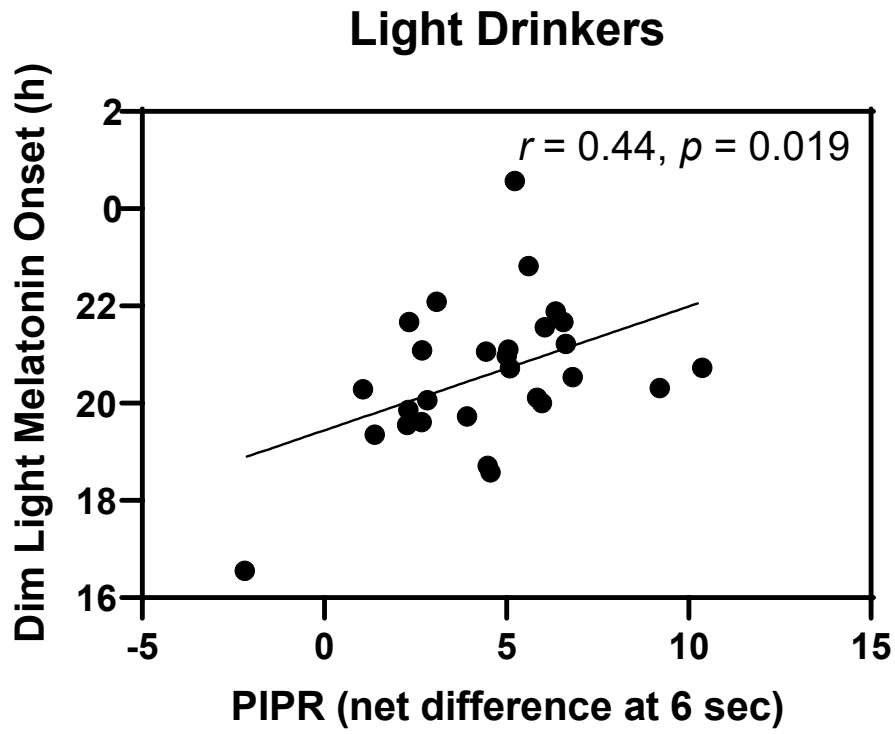


Figure 3