A single dose of alcohol does not meaningfully alter circadian phase advances and phase delays to light in humans

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A single dose of alcohol does not meaningfully alter circadian phase advances and phase delays to light in humans. Am J Physiol Regul Integr Comp Physiol 310: R759–R765, 2016. First published March 2, 2016; doi:10.1152/ajpregu.00001.2016.—Central circadian timing influences mental and physical health. Research in nocturnal rodents has demonstrated that when alcohol is consumed, it reaches the central hypothalamic circadian pacemaker (suprachiasmatic nuclei) and can directly alter circadian phase shifts to light. In two separate studies, we examined, for the first time, the effects of a single dose of alcohol on circadian phase advances and phase delays to light in humans. Two 23-day within-subjects placebo-controlled counterbalanced design studies were conducted. Both studies consisted of 6 days of fixed baseline sleep to stabilize circadian timing, a 2-day laboratory session, a 6-day break, and a repeat of 6 days of fixed sleep and a 2-day laboratory session. In the phase advance study (n = 10 light drinkers, 24–45 yr), the laboratory sessions consisted of a baseline dim light phase assessment, sleep episode, alcohol (0.6 g/kg) or placebo, 2-h morning bright light pulse, and final phase assessment. In the phase delay study (n = 14 light drinkers, 22–44 yr), the laboratory sessions consisted of a baseline phase assessment, alcohol (0.8 g/kg) or placebo, 2-h late night bright light pulse, sleep episode, and final phase assessment. In both studies, alcohol either increased or decreased the observed phase shifts to light (interaction P ≥ 0.46), but the effect of alcohol vs. placebo on phase shifts to light was always on average smaller than 30 min. Thus, no meaningful effects of a single dose of alcohol vs. placebo on circadian phase shifts to light in humans were observed.

alcohol; circadian; human; light; melatonin

The central mammalian circadian clock, which regulates many circadian rhythms, is located in the suprachiasmatic nuclei in the hypothalamus (SCN; Ref. 24). In humans, the central circadian clock has an average endogenous period slightly greater than 24 h (~24.2 h) (9, 14) and, therefore, requires daily adjustment to remain synchronized to the external 24-h day. Much of this daily resetting is accomplished through light exposure captured by retinal photoreceptors and transmitted to the SCN (22). Whether light shifts the timing of the circadian clock earlier or later is critically dependent on when the light is received (39). Light in the evening or first part of the night causes the clock to shift rhythms later (phase delay), and light in the morning causes the clock to shift rhythms earlier (phase advance). Thus, morning light is essential for producing corrective daily phase advances, while evening light can produce phase delays, which often exacerbate the clock’s endogenous tendency to drift later and promotes circadian misalignment. Correct alignment between the timing of the circadian clock and sleep/wake is essential for optimal mental and physical health (e.g., 15, 37).

Interestingly, a delay in circadian timing is associated with an increase in alcohol consumption in humans, even after controlling for factors such as age, sex, socioeconomic status, and educational level. For example, adults with an evening preference (proxy for delayed circadian timing) are more likely to be alcohol drinkers (45, 47), and to consume more alcohol daily [11.9 cc in evening types vs. 5.1 cc in neither types, (1)]. In one study, adults in the top quintile of eveningness drank 3.1 more standard drinks/day than those in the lowest quintile (17). Possible mechanisms that may explain this association include a delay in circadian timing leading to worse mood (19, 31), sleep onset difficulties (30), and/or reduced reward functioning (16), all of which may drive increased drinking (26, 32, 44). It also remains possible that alcohol directly affects the circadian system’s response to light, further contributing to alcohol use disorders. For example, alcohol levels peak in the SCN about 20 min after hamsters consume alcohol (34). Additionally, alcohol can directly alter phase shifts to light in rodents, when alcohol is chronically consumed (34, 38) or when an acute dose of alcohol is injected into the SCN extracellular space (6, 35). This effect of alcohol on phase shifts to light is proposed to be due primarily to alcohol directly altering levels of GABA and glutamate in the SCN (6, 29, 35). However, the effect of alcohol on circadian phase shifts to light in humans remains to be investigated. Thus, we present the first proof-of-concept experiments to examine the effects of alcohol on phase shifts to light in humans, using a single dose, representative of social drinking. We measured circadian phase shifts with the dim light melatonin onset (DLMO), which is a reliable marker of human central circadian timing (20, 21). In line with the association between later circadian timing in humans and increased alcohol consumption, we hypothesized that a single dose of alcohol would increase phase delays to light but decrease phase advances to light.

MATERIALS AND METHODS

Subjects. Thirteen healthy people were enrolled in the Phase Advance study, although two did not complete the protocol: one due to a death in the family and the other due to an illness that developed before the first laboratory session. Fifteen healthy people were enrolled in the Phase Delay study, although one did not complete the protocol as a result of accepting a full-time job offer. Thus, 11 subjects completed the Advance protocol (5 males, 6 females, age range: 24–45 yr) and 14 subjects completed the Delay study (7 males, 7 females, age range: 22–44 yr). All subjects were medication-free, with a body mass index between 17.9–31.0 kg/m². On the basis of an
in-person interview and their responses to screening questionnaires, all subjects had no medical (41), psychiatric (4, 11, 42), or sleep disorders (2, 3, 27), and reported good sleep quality (13). All subjects passed a urine drug screen for nicotine and other common drugs for abuse. Subjects consumed only moderate doses of caffeine (<300 mg/day). Nonsteroidal anti-inflammatory drugs were not permitted throughout the study, as they suppress melatonin (25). All subjects had not worked any night shifts or traveled across more than three time zones in the month preceding the study. All subjects had no personal or family history of drug/alcohol abuse and were instructed not to consume alcohol during the study apart from what they were given as part of the protocol. All females were also required to pass a urine pregnancy test at the start of each laboratory session and were informed of their responsibility to use effective birth control during the study. None of the females reported breastfeeding. All subjects gave written informed consent prior to participation. The study was approved by the Rush University Medical Center Institutional Review Board.

Protocols. Both studies were 23 days long and consisted of a within-subjects, placebo-controlled counterbalanced design with two parts (Fig. 1). Each part consisted of 6 days of fixed sleep at home to stabilize circadian phase followed by a 2-day laboratory session. There was a 6-day study break between the two parts, during which subjects returned to their baseline sleep times ± 30 min. During the laboratory sessions, alcohol or placebo was administered prior to a bright light pulse (more details below), with the order of drink type (alcohol or placebo) counterbalanced across subjects. During each laboratory session in the Advance study, there was a baseline circadian phase assessment, a 5-h sleep episode, a 30-min drink window (alcohol, placebo), and a 2-h bright light pulse, followed by a final circadian phase assessment. Five subjects received alcohol in the first laboratory session, followed by placebo in the second laboratory session, and six subjects received placebo in the first laboratory session, followed by alcohol in the second laboratory session. During the laboratory session in the Delay study, there was a baseline circadian phase assessment, a 30-min drink window (alcohol, placebo), a 2-h bright light pulse, and an 8-h sleep episode, followed by a final circadian phase assessment. Seven subjects received alcohol in the first laboratory session, followed by placebo in the second laboratory session, and seven subjects received placebo in the first laboratory session, followed by alcohol in the second laboratory session. The timing of each protocol was tailored to each individual’s habitual sleep times collected in the week before the study start with sleep diaries, which, on average, was 23:43 ± 0:50 (SD) – 7:32 ± 0:52 (SD) h.

Sleep at home. All subjects slept at home except during the 2-day laboratory sessions. During their scheduled 8-h sleep episodes at home, subjects were instructed to lie in bed and try to sleep. Subjects were not permitted to read, use any electronic devices, or talk at this time. Daytime naps were not permitted. To ensure compliance with the sleep schedule, all subjects were required to call the laboratory voice mail (time and date of call was recorded) before turning off their lights at night and at their wake time each morning. Subjects also

Fig. 1. A sample protocol for the Phase Advance study (top) and the Phase Delay study (bottom) for a subject who typically slept from 2300 to 0700. Both the Phase Advance and Phase Delay protocols consisted of 6 days of fixed sleep at home to stabilize circadian phase, a 2-day laboratory session, a 6-day study break, another 6 days of fixed sleep at home followed by another 2-day laboratory session. During the laboratory session in the Phase Advance study there was a baseline circadian phase assessment, a sleep episode, a 30-min drink window (alcohol, placebo), a 2-h bright light pulse, followed by a final circadian phase assessment. During the laboratory session in the Phase Delay study, there was a baseline circadian phase assessment, a 30-min drink window (alcohol, placebo), a 2-h bright light pulse, a sleep episode, followed by a final circadian phase assessment. The black rectangles represent scheduled sleep times. The dots represent the first and last saliva sample during the dim light circadian phase assessments. The D represents the 30-min drinking window. The L represents the 2-h bright light pulse. Square brackets indicate approximate arrival and departure times from the laboratory.
completed daily sleep logs, noting bedtime, estimated sleep onset time, any awakenings during the night, and time of final awakening. Each subject also wore a waterproof actigraphy monitor with photometrics (Actiwatch Spectrum, Philips Respironics, Bend, OR; 30-s epochs) on their nondominant wrist throughout the protocol. Subjects came to the laboratory every fourth day of the fixed-at-home sleep episodes to have their sleep logs and activity data inspected in their presence to ensure compliance to the sleep schedule.

Phase assessments. Each subject participated in four dim-light phase assessments in the laboratory to determine their endogenous melatonin profiles (Fig. 1). Subjects were required to be caffeine-free for the 6 days prior to each laboratory session and were breathalyzed for alcohol at the start of each laboratory session. During the phase assessments, subjects remained awake and seated in dim light (<5 lux, at the level of the subjects’ eyes, in the direction of gaze, measured every 2 h, Extech 403125 light meter, Nashua, NH) and were continuously monitored by staff. After 30 min in the dim light, subjects gave a saliva sample every 30 min 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-min before each sample, and subjects were required to rinse and brush their teeth with water while remaining seated 10 min 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 commercially available kits (ALPCO, Salem, NH). The assay sensitivity was 0.5 pg/ml. Intra-assay and inter-assay coefficients of variation for low levels of salivary melatonin were 20.1%, and 16.7%, respectively. A dim-light melatonin onset (DLMO) was calculated for each phase assessment, 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 (5, 43). This low threshold more closely tracks the initial rise of melatonin (23). The phase shift during each 2-day laboratory stay was calculated as the difference between the baseline DLMO and the final DLMO.

Alcohol and placebo drinks. The alcohol and placebo drinks were administered to seated subjects in a private, temperature-controlled, bedroom. In the Advance study, in which subjects were required to drink in the early morning, males received 0.60 ± 0.06 g/kg of alcohol, and females received 0.51 ± 0.05 g/kg, after a 5-h fast during the scheduled sleep episode. Given the slower metabolism of alcohol in the morning (46), females received lower doses of alcohol in the morning to reduce the risk of adverse events. In the Delay study, where subjects were required to drink late at night, both males and females received 0.80 ± 0.08 g/kg, after a 2.5-h fast. The drinks were prepared about 1 h before administration, out of sight of the subjects. The alcohol drinks consisted of a 1:4 mix of vodka (80 proof) and tonic water, with a splash of lime juice to mask the taste (33, 36). The placebo drink consisted of the same volume of liquid, but was primarily tonic water with the same splash of lime juice and three drops of vodka dropped on the top of the drink with an eye dropper (33, 36). The total volume of the alcohol or placebo drink was divided into three cups, and each cup was consumed in a 10-min window, for a total drinking window of 30 min. Subjects were breathalyzed (Alco-sensor III, Intoximeters, Saint Louis, MO) every 30 min after the first drink to the end of the bright light exposure, but were kept blinded from the breath alcohol concentration (BrAC) readings. Thus, the study was single-blind, with subjects not staff blind to condition, as staff had to record the BrAC readings. On average, the BrAC readings recorded after the third drink were 0.053 ± 0.034 g/210 l (SD) in the Advance study and 0.063 ± 0.047 g/210 l (SD) in the Delay study. In the alcohol condition, the BrAC readings always remained above zero for the duration of the light pulse in both the Advance and Delay studies. In the placebo condition, all BrAC readings were 0.00 g/210 l as expected. Subjects were brethalyzed at the start of each phase assessment to ensure a BrAC of zero prior to subsequent saliva collection.

Bright light. During each laboratory stay, subjects were exposed to a continuous 2-h bright light pulse [mean intensity 4,763 ± 689 (SD) lux, measured periodically at angle of gaze with Minolta TL-1 light meter, Ramsey, NJ]. The bright light was produced by a single light box (61 × 61 × 10 cm, Enviro-Med, Vancouver, WA) placed on a desk about 45 cm in front of the subject’s eyes. Each light box had a diffuser screen and contained four 54-cm long 40-W fluorescent horizontal tubes (Philips PL-L40W/41/RS/IS, 4100K). At this distance, subjects received 5.1 × 1015 photons·cm−2·s−1, and specifically 1.1 × 1015 photons·cm−2·s−1 in the blue range (400–490 nm), with an irradiance of 1,741 μW/cm2 (400–750 nm). Subjects were permitted to read materials flat on the desk in front of the light box. Subjects were monitored continuously by staff either in person or via video cameras to ensure they were not closing their eyes, looking away, or falling asleep during the bright light exposure.

Statistical analysis. In each study, the baseline DLMOS measured in the baseline phase assessment in each part of the study were compared with a post hoc paired samples t-test to ensure subjects received the alcohol or placebo drink and bright light at approximately the same circadian phase. This verification was important, as circadian phase is an important predictor of subsequent phase shifts to light (39).

For each study, the DLMOS were initially analyzed with a three-way repeated-measures ANOVA with within-subjects factor “drink” (alcohol vs. placebo), within-subjects factor “time” (before vs. after light), and between-subjects factor “order” (alcohol condition first vs. second). However, in all cases, the between-subjects factor “order” was not significant (all P ≥ 0.15), so this factor was subsequently removed from the analysis. The drink × time interaction for each study was of most interest, as it would indicate if the magnitude of the phase shifts to bright light differed between conditions. Statistical significance for all analyses was determined with two-tailed tests at P < 0.05. Results are reported as means ± SD.

RESULTS

The distributions of the DLMOS in both studies were normal (Kolmogorov-Smirnov test P ≥ 0.20).

Advance study. Of the 11 subjects who completed the Advance study, one subject displayed an initial rise in melatonin, a decrease, and then a second rise in melatonin, at baseline, in the placebo condition. As the DLMO could not be reliably calculated, this subject was removed from subsequent analyses. In the remaining 10 subjects, the average absolute difference between the baseline DLMOS was 0.27 ± 0.16 (SD) h, and the baseline DLMOS were not significantly different from each other (P = 0.28). In the Advance study, there were individual cases where the phase advances to bright light were larger with alcohol than placebo (Fig. 2). However, we also observed the opposite effect in other subjects (Fig. 2). Overall, in the Advance study, five subjects showed larger phase advances to light with alcohol (mean increase in phase advance 0.39 h), four subjects showed smaller phases advances to light with alcohol (mean decrease in phase advance 0.26 h), and one subject phase delayed with alcohol (by 0.42 h, Fig. 4). All these changes were smaller than 0.5 h, and less than the 30-min saliva sampling rate. In addition to these changes not being meaningful in size (d = 0.08), the drink × time interaction was not significant (P = 0.74), indicating no statistical effect of alcohol on phase advances to light.

Delay study. In the Delay study, the average absolute difference between the baseline DLMOS was 0.44 ± 0.41 (SD) h, and the baseline DLMOS were not significantly
different from each other ($P = 0.90$). In the Delay study, there were individual cases in which the phase delays to bright light were larger with alcohol than placebo (Fig. 3). However, we also observed the opposite effect in other subjects (Fig. 3). Overall, in the Delay study seven subjects showed larger phase delays to light with alcohol (mean increase in phase delay 0.33 h), five subjects showed smaller phase delays to light with alcohol (mean decrease in phase delay 0.42 h), and two subjects showed phase advances to light with alcohol (by less than 0.38 h, Fig. 4). All of these changes were smaller than 0.5 h, and less than the 30-min saliva sampling rate. In addition to these changes not being meaningful in size ($d = 0.20$), the drink × time interaction was not significant ($P = 0.46$), indicating no statistical effect of alcohol on phase delays to light. One subject in the Delay study had baseline DLMOs that differed by 1.5 h, due to him sleeping 25 min past his scheduled wake time on the day of the first baseline phase assessment in the placebo condition. When the analysis was repeated after removing this subject, the drink × time interaction remained nonsignificant ($P = 0.60$). Overall, a power analysis revealed low statistical power due to the small sample size in each study ($\text{power} \approx 0.20$). However, given the small effect sizes, a sample size of at least $n = 120$ in each study would have been required to detect any statistical significance associated with these small effects.
The results of the two studies reported here are the first investigations of the effects of alcohol on phase shifts to light in humans. A single high dose of alcohol was administered late at night, and a single moderate dose of alcohol was administered in the morning to account for time-of-day differences in the metabolism of alcohol (46). In both studies, the alcohol or placebo was administered to light drinkers immediately before exposure to a 2-h bright light pulse. In both studies, the average changes in phase shifts to light observed with a single dose of alcohol vs. placebo were consistently of small magnitude, less than 30 min in size, and no consistent effects were observed.

The 6 days of fixed sleep prior to each laboratory session were included in the study design to increase the likelihood that the bright light would occur at approximately the same circadian phase in both conditions. This is important because the phase shifting effect of light is dependent on the circadian time at which light is received (39). This approach, which has been used in other within-subjects design circadian studies (7, 8), was successful in that the baseline DLMOs between conditions in both studies were not significantly different. Indeed, only one subject had baseline DLMOs that were more than 1 h apart, and this was likely due to him not following the fixed sleep schedule on the morning of the first baseline phase assessment. Nonetheless, the results remained statistically nonsignificant, even after excluding this subject from the analyses.

The finding that a single dose of alcohol did not systematically alter circadian phase shifts to light in humans is in contrast to results reported in studies that tested a single dose of alcohol in nocturnal rodents. For example, in Syrian hamsters, a single dose of alcohol reduced phase advances to light, but did not significantly alter phase delays to light (35). In mice, a single dose of alcohol reduced phase delays to light but did not significantly change phase advances to light (6). Notably, the alcohol in these studies was injected directly into the SCN extracellular fluid compartment, rather than consumed by the rodents, probably reducing the variability in the bioavailability of alcohol that normally occurs after oral consumption. Indeed, chronic intake of alcohol in rodents can alter phase shifts to light (34, 38), suggesting multiple doses of alcohol could meaningfully alter phase shifts to light in humans.

Similarly, we have previously reported that a single dose of alcohol (0.8 g/kg) does not increase intestinal permeability in healthy humans (lactulose/mannitol ratio; Ref. 18), whereas chronic exposure to alcohol (0.4 g/kg or 1–3 glasses of red wine, per night for 1 wk), does increase intestinal permeability in healthy humans by more than 50% (40). Therefore, despite our negative results associated with a single dose of alcohol, it remains quite possible that chronic exposure to alcohol may meaningfully alter circadian phase shifts to light in humans. Given the association of later circadian timing with drinking more alcohol (1, 17, 45, 47), one might predict larger phase delays to light with alcohol, especially as humans most typically consume alcohol in the evening. However, to date, alcohol has only ever reduced phase shifts to light in nocturnal rodents, or produced no change (29). Therefore, how chronic exposure to alcohol influences circadian phase shifts to light in diurnal humans remains an important gap in our knowledge that should be addressed by future research.

There were limitations to this study. Both studies consisted of a within-subjects design, with a study break (wash out) in between the conditions, which led to relatively long 23-day studies. We chose a within-subjects design to ensure that the alcohol and placebo drinks were given at approximately the same circadian phase, and to reduce the high between-subjects variability often observed in phase shifts to light. Indeed, even when light is administered at the same circadian phase in humans, phase shifts in response to a 1-h pulse of bright light can vary by ~1–1.5 h (39). Our within-subjects design led to smaller sample sizes of 10 subjects in the Phase Advance study and 14 subjects in the Phase Delay study, sample sizes that are commonly seen in human circadian research that report positive findings [e.g., n = 5 per group (10), n = 6–7 per group (12)]. Despite the small sample sizes, we noted that, on average, the change in phase shifts with alcohol were not more than 30 min different to those observed with placebo, and, therefore, were not meaningful. We also chose the initial step of testing a single dose of alcohol on phase shifts to light, as per the rodent literature (34). Other studies have observed a significant effect of a single dose of caffeine, for example, on human circadian timing in similarly small sample sizes [n = 5 per group (10)]. Thus, while our sample sizes were small, they...
were adequate to show that a single episode of social drinking does not meaningfully change human circadian phase shifts to light.

**Perspectives and Significance**

Circadian timing has a significant influence on mental and physical health in humans. Light is the strongest environmental influence on circadian timing. Previous research in rodents reveals that alcohol can directly alter the response of the central circadian clock to light. Here, we examined for the first time, the effect of a single moderate to high dose of alcohol on phase shifts to evening light and morning light in humans. No notable changes were observed, demonstrating that a single social drinking episode does not meaningfully change the circadian system’s response to light in humans. The effect of chronic alcohol exposure or multiple doses of alcohol on the circadian response to light in humans remains to be tested.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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