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Sleep deprivation and voluntary alcohol consumption in adult rats

Charles M. Cowan II
James Madison University

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Sleep Deprivation and Voluntary Alcohol Consumption in Adult Rats

Charles Cowan

A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

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FACULTY COMMITTEE:

Committee Chair: Dr. Jeff Dyche

Committee Members/ Readers:

Dr. Daniel Holt

Dr. S. Jeanne Horst

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Abstract

Alcohol is a psychoactive drug with a large userbase among adults across the globe. However, alcohol use also reduces the quality of sleep in the user. Historically, research has focused on the effects of alcohol on sleep architecture, but recent research has started to examine the effects of sleep deprivation on alcohol consumption. This research examines the effects of sleep deprivation on voluntary alcohol consumption in adult rats. Twelve Sprague Dawley rats were given ad libitum access to food, alcohol (7% solution), and water for the duration of this study. Subjects were then placed into non-moving forced exercise wheels to acclimate the environment in which they would be sleep deprived. Subjects then experienced 18 and 6 hours of sleep deprivation every day for 7 consecutive days for each condition. Subjects were then experienced the non-moving forced exercise wheel for a final control condition. There was a significant effect of experimental condition on voluntary alcohol consumption. Post-hoc comparisons using a Bonferroni correction showed that during the 18 and 6-hour conditions subjects drank a significantly larger amount of alcohol than in their home cage environment. Subjects also consumed a visibly larger amount of alcohol during the final control condition than the first, which may be due to a conditioned compensatory response.

Sleep Deprivation and Voluntary Alcohol Consumption in Adult Rats

The term alcohol commonly refers to ethanol (C_2H_6O), a specific type of alcohol that is consumable, and sold for human consumption. Alcohol is one of the most common drugs of abuse in the western world, and is the most common drug of abuse in the United States, with a 2015 study conducted by the National Institute of Health finding that 56% of people over the age of 18 had consumed alcohol in the past month, and 70% reporting that they had consumed alcohol during the past year (National Institute on Alcohol Abuse and Alcoholism, 2017a). Though the term alcohol may refer to any compound with a saturated carbon atom bound to a hydroxyl group, the term alcohol will refer solely to the drug ethanol for the purpose of this study.

Alcohol is a central nervous system (CNS) depressant drug that, in moderate doses, reduces sleep latency, the time it takes to fall asleep upon attempting to sleep in some adults (Stone, 1980). However, large doses of alcohol can change sleep architecture, depriving the consumer of rapid eye movement (REM) sleep and causing unwanted nighttime arousals (Landolt & Borbély, 2000). When individuals consume an atypically large amount of alcohol, over 5 standard drinks within two hours for the average adult male, their quality of sleep is reduced (Yules, Lippman, & Freedman, 1967). The effects of alcohol use on sleep architecture have been thoroughly investigated, however the effect of sleep deprivation on alcohol consumption is a largely unexplored area of research that demands further investigation. If sleep deprivation enhances voluntary alcohol consumption, then there are serious implications in individuals who chronically consume large amounts of alcohol. If alcohol causes a form of sleep deprivation, and sleep deprivation enhances voluntary alcohol consumption, then there

may be a reciprocating cycle in individuals who chronically consume large amounts of alcohol. However, in order to understand hypotheses about the effects of sleep deprivation on voluntary alcohol consumption, one must first understand alcohol, sleep, and the effects of alcohol consumption on sleep.

Alcohol

Alcohol is a gamma-aminobutyric acid (GABA) agonist that has indirect action on the dopamine system (Wallner & Olsen, 2008; Boileau et al., 2003). Of recent interests are the GABAergic and dopaminergic aspects of alcohol, as they are the primary mechanisms through which alcohol relates to abuse potential and impacts on sleep (Spanage & Weiss, 1999). GABA is the primary inhibitory neurotransmitter throughout the central nervous system. This is significant in regards to alcohol as it contributes to its anxiolytic (anti-anxiety) and overall depressive effects. The downside of this GABAergic activity is that it can lead to respiratory depression and death in extreme doses. Even in moderate dosages, alcohol can lead to memory loss and reduced fine motor control. Additionally, most GABA agonists come with serious physiological dependency and withdrawal issues. Withdrawal from GABA agonists, including alcohol, can include seizures, parkinsonism-like shaking, anxiety, insomnia, and death (Calixto, 2016; Kosten & O'Connor, 2003). Alcohol acts primary as a CNS depressant due to these GABAergic effects, however, alcohol's indirect dopaminergic action likely causes part of the reinforcing effects of the drug.

Research suggests that most, if not all, drugs of abuse get some of their reinforcing effects from dopaminergic activity in the mesolimbic dopamine system (Pierce & Kumaresan, 2006). Dopamine is a predominantly excitatory neurotransmitter in

the CNS, and is associated with the brain's reward system. This dopaminergic activity is generally thought to be responsible for the reinforcing effects of eating, sexual activity, and psychoactive drugs. Though the anxiolytic effects of alcohol alone have the potential to make the drug desirable, alcohol also precipitates the release of dopamine in the Nucleus Accumbens, the brain's primary reward center (Yoshimoto et al., 1992). Even beyond the anxiolytic and sedating effects of alcohol, alcohol has inherently reinforcing properties. The reinforcing effects of a drug with such significant side effects, both while under the influence of the drug and during withdrawal from the drug, become a public concern given its widespread availability.

Alcohol is legal for human consumption in the United States for adults over the age of 21, and moderate use of alcohol is considered socially acceptable. Moderate consumption of alcohol is associated with health benefits, including the reduction of stress and depression, and even a declined risk of heart attack in individuals who consume an average of one-half drink per day of the week, with that average spread out among several days of the week (Thakker, 1998). However, the social acceptability of alcohol use and the benefits of consuming small quantities of alcohol do not eliminate the dangers associated with excessive use. Between 2006 and 2010, excessive drinking was directly linked to 1 in 10 deaths among adults aged 20 to 64 years in the United States (National Institute on Alcohol Abuse and Alcoholism, 2017). Additionally, long term alcohol use has been associated with high blood pressure, heart disease, stroke, liver diseases, multiple forms of cancer, learning and memory problems, depression, and anxiety (Centers for Disease Control and Prevention, 2016).

Sleep

The sleep-wake cycle is comprised of two primary systems, one that promotes sleep and one that promotes wakefulness. These systems are somewhat mutually exclusive, with the actions of the sleep-on system inhibiting the wake-on system, and the wake-on system inhibiting the sleep-on system. The wake-on system is known as the Ascending Reticular Activating System (ARAS) and is found in the brainstem, however it projects to much of the forebrain (Fuller & Lu, 2009). The ARAS has excitatory effects on much of the forebrain and an inhibitory effect on the ventrolateral preoptic nucleus, which is associated with sleep-on, and is active during non-rapid eye movement sleep; NREM (McCarley & Sinton, 2008). Sleep is often discussed as having five stages, however, the blanket terms rapid eye movement (REM) and non-rapid eye movement (NREM) will be used for the sake of parsimony. As the name implies, REM sleep is characterized by rapid movement of the eyes, in addition to complete muscle atonia, which is attributed to stimulated glycine release during REM sleep (Zeitler, 2009).

REM sleep is generally considered to be a significant contributor to working memory function, and in the consolidation of memory (Dinges & Banks, 2009; Stickgold & Walker 2007). REM sleep is the first stage of sleep to decrease in instances of partial sleep deprivation in humans, as it typically occurs in greater amounts later in the night (Carskadon & Dement, 1980). In cases of extended total sleep deprivation (TSD), individuals experience a rebound of NREM sleep on the first night of recovery sleep, followed by a night with a disproportionately large amount of REM sleep. This was observed in the famous case of Randy Gardner, who currently holds the record for the

longest recorded period of TSD (11 days and 25 minutes) (Nielsen, Dumont, & Montplaisir 1995).

NREM sleep is essential to produce growth hormone (GH), which is primarily secreted during slow wave sleep (SWS), referring to sleep stages 3 and 4 (Leprout, Spiegel, & Cauter, 2009). Cortisol, a hormone primarily associated with being a stress response, shows a significant decline during SWS (Follenius et al., 1992). The secretion of thyroid-stimulating hormone, an important regulatory metabolic hormone, is inhibited during sleep. This regulation of thyroid-stimulating hormone is also attributed primarily to SWS. When individuals are deprived of SWS, glucose tolerance and insulin sensitivity both decrease (Leprout, Spiegel, & Cauter, 2009). It is not a stretch to generalize NREM sleep as being essential for hormonal regulation, while generalizing REM sleep as essential for cognitive processes, such as memory consolidation and decision making.

Rat Models of Alcohol Consumption and Effects

Rats have been used as experimental models in both sleep and pharmacological research for decades, but animal models may not always accurately represent humans. Specifically, in the case of rats, the typical animal spends over 50% of the 24-hour day asleep, with that sleep more dispersed over the 24-hour day than in humans. Rats also cycle through their stages of sleep much more quickly, and thus more often, than humans. The typical 3-month-old rat experiences 29 REM-NREM cycles in a 24-hour period, while humans typically experience 4-6 REM-NREM cycles in the same amount of time (Mendelson & Bergmann, 1999; Jenni & Carskadon, 2009). Compared to humans, rats experience a much larger REM-rebound relative to their NREM-rebound following

extended (> 24 hours) periods of TSD (Rechtschaffen, Bergmann, Gilliland, & Bauer 1999).

Rats are desirable models for sleep research because of their short lifespans, and therefore short time to reach maturity, and, to some extent, their similarity to humans in terms of recovery from sleep deprivation. When sleep depriving rat subjects, one would expect rats to experience a rebound in NREM sleep before REM, at least after short (approximately 24-hour) periods of TSD (Rechtschaffen, Bergmann, Gilliland, & Bauer 1999). In short, rats recover from sleep deprivation in a fashion similar to humans under some conditions, but do not resemble human recovery from sleep deprivation under extended periods of TSD. In addition to these practical advantages, there are ethical benefits to choosing rat models over human participants, as much of sleep research would be unethical to conduct using humans.

Alcohol and Sleep

The effects of alcohol consumption on sleep have been thoroughly investigated in previous research. Though the depressive effects of alcohol can be beneficial to insomniacs in small doses, moderate to large doses of alcohol have well documented detrimental effects on human sleep (Stone, 1980). Alcohol increases the frequency of sleep apneic events in adult men, whom are otherwise asymptomatic of sleep apnea (Carole et al., 1981). Alcohol consumption increases nocturnal sleep disturbances and suppress REM in individuals without Alcohol Use Disorder, and sleep in individuals with Alcohol Use Disorder is characterized by reduced total sleep time, NREM sleep, and increased sleep latency (Landolt & Borbély, 2000; Zarcone et al., 1975). A moderate to large dose of alcohol prior to sleep onset results in sleep that is of lower overall quality

and potentially spotted with interruptions. Essentially, going to sleep after drinking alcohol results in partial sleep deprivation and going to sleep after consuming alcohol on a persistent basis results in chronic partial sleep deprivation.

To investigate the potential of REM sleep deprivation on voluntary alcohol consumption, Aalto and Kiiianmaa (1984a) found that rats consumed a significantly larger amount of alcohol when deprived of REM sleep. The researchers also found that after REM deprivation, during the “REM-rebound” phase, alcohol consumption decreased until it matched the levels of alcohol-naïve rats. This research provided evidence for the effects of REM sleep deprivation on voluntary alcohol consumption, yet it failed to account for the impact of stress. Aalto and Kiiianmaa (1984a) deprived rat subjects of REM via the reverse flowerpot technique modified with an electric grid floor instead of water. The reverse flowerpot REM deprivation technique is known to cause acute stress in subjects exposed to it (Suchecki & Tufik, 2000). The substitution of an electrified grid in place of a pool of water may have even exacerbated the subjects’ stress levels farther than the traditional reverse flowerpot technique. This is a problem for Aalto and Kiiianmaa’s (1984a) study, as stress has been shown to increase voluntary alcohol consumption in some strains of laboratory rat (Vengeliene, et al., 2003). In rats with lesioned suprachiasmatic nuclei, the neurological circadian pacemaker, rats did not drink a significantly larger amount of alcohol post-surgery recovery than before surgery. However, rats with lesioned suprachiasmatic nuclei exhibited increased voluntary alcohol consumption when deprived of REM sleep (Aalto & Kiiianmaa, 1984b). This further supports the theory that their initially observed increase in voluntary alcohol consumption

was due to REM sleep deprivation. Unfortunately, this lesioning study also used the reverse flowerpot technique for REM deprivation.

Behavioral Acclimatization, Sleep, and Stress

Previous unpublished research from this laboratory examined the effects of sleep deprivation on voluntary alcohol consumption in adolescent rats. The study featured a within-groups design in which all rats ($N = 12$) experienced 18, 20, and 22 hours of sleep deprivation per 24-hour day for seven days at each condition (Sequeira, 2015). Rats were given three weeks of recovery time in their home cages, fed ad libitum, before experiencing another consecutive seven-day period in which they would be sleep deprived. The rats were sleep deprived in groups of three, and the order in which each trio of rats was sleep deprived was counterbalanced to account for any potential age effects. Sleep deprivation occurred in slow moving forced exercise wheels, in which all rats had ad libitum access to alcohol, food, and water. During each week of sleep deprivation, one of the other nine rats, which was not being sleep deprived that week, was placed into a stationary wheel away from the forced exercise apparatus. This wheel control condition was used as an additional measure of control, in addition to each rat's home cage, to measure any effects the environment of the wheel may have had on voluntary alcohol consumption.

The results of this previous study were promising, if a bit conflicting. The rats consumed between three and four times the average amount of alcohol per 24-hour day whenever they were in a wheel compared to when they were in their home cages. This includes the wheel control condition, in which the rats were not forcibly sleep deprived, and there were no significant differences between the 18-hour, 20-hour, 22-hour, and

wheel control conditions in terms of average alcohol consumption per kilogram per day. This warrants further investigation to the original research question of whether sleep deprivation increases voluntary alcohol consumption. This previous study demonstrated that rats voluntarily consumed more alcohol while inside the forced-exercise wheels, but it did not demonstrate that this increase in alcohol consumption was due to sleep deprivation. The results of this study left the researchers with multiple possible explanations for their findings. The present study seeks to find support for one of two possible explanations for these findings; that the rats consumed more alcohol in the control wheel condition because the wheel condition is inherently stressful, or that the rats consumed more alcohol because of a Pavlovian association made with the wheel environment. Previous research similar to these face the same limitations inherent in Aalto and Kiianmaa's (1984a; 1984b) research, in that the role of stress is left ambiguous.

Given the smaller than home cage size of the forced exercise wheels and the large period each subject will spend inside the wheels, concerns have been raised that these wheels may be considered prolonged restraint, and thus inherently stress inducing. However, a previous study indicated that this may not be the case. Rats placed inside functional magnetic resonance imaging (fMRI) devices must be tightly restrained to collect data, and this process is inherently stressful to the rat. However, rats placed in a mock-fMRI restraining device for 8 days of daily 90-minute trials showed no significant difference from baseline in terms of respiratory rate, heart rate, and corticosterone levels by the final day of the acclimatization period (King et al., 2005). Additionally, rats placed in a mock-fMRI restraint device over 5 days of daily 60-minute trials showed a decreased stress response to restraint in terms of ultrasonic vocalizations (Reed, Pira, & Febo,

2013). These examples clearly demonstrate that rats will not only acclimate to prolonged restraint, but will do so in environments far more restrictive than the currently used forced exercise wheels.

Acclimatization is defined, in a general sense, as denoting the adjustment of any organism to its environment, typically a new or novel environment. It is often used interchangeably with adaptation, however there is often a tendency to associate adaptation with genetic “adaptedness” in a Darwinian sense (Mazess, 1975). For this study, acclimatization will be used in a behavioral sense, referring to an adjustment in behavior following extended exposure to a novel environment. Ideally, this adjustment in behavior would represent behavior typical of a subject in a non-novel or home cage environment.

The present study is an extension of our previous research on sleep deprivation and voluntary alcohol consumption that utilized a systematic replication design to account for the effect of wheel environment. We examined the same Sprague Dawley strain of rat with the same sample size ($N = 12$), exposing all subjects to a seven-day week period of 24 hours per day in non-moving forced exercise wheels. This period was the experimental control period and was predicted not to be statistically significantly different from the subjects’ home cage in terms of voluntary alcohol consumption. Researchers exposed subjects to 18 and 6-hour per day sleep deprivation conditions. We introduced a 6-hour sleep deprivation condition, as due to the large portion of the 24-hour day rats spend sleeping it may be better representation of chronic partial sleep deprivation than the three previous conditions (Sequeira, 2015). We predicted that while rats are in this 6-hour condition they would consume significantly more alcohol than in either their

home cages or the wheel control condition. The 18-hour condition was chosen out of the original three conditions as there were no significant differences between the 22, 20, and the 18-hour condition, and the 18-hour condition is assumed to be the least stressful, as it is the shortest of the three original conditions. The researchers also wanted to further investigate the possibility of a dose-dependent relationship between sleep deprivation and alcohol consumption and so it was hypothesized that subjects would consume a significantly larger amount of alcohol under the 18-hour condition than in any of the other experimental conditions. Finally, a final wheel control condition was conducted in which all subjects will experience a second seven-day 24-hour per day period inside of the non-moving sleep control wheels. This final condition was an additional experimental control to examine for any changes in response to the wheel environment as a result of the previous exposure to the wheel during sleep deprivation. This final wheel control condition was not predicted to be significantly different from the first wheel control condition. For a table detailing each of the experimental conditions, please see Table 1 in the appendix.

Method

Subjects

Subjects were 12 Sprague-Dawley rats from Envigo (formerly Harlan). The rats were housed in cages that are 40.64 cm long, 22.86 cm wide, and 20.32 cm tall for the duration of the study. Subjects were housed solitarily, fed ad libitum, and kept on a 12-hour light-dark cycle throughout the study. The rats lived in an environment that is approximately 22 degrees Celsius and between 40% and 60% humidity for the duration of the study.

Apparatus

The subjects had free access to two bottles during the study. One bottle contained water and was available throughout the study, and one bottle contained a 7% alcohol and water solution that was available starting when the rats reached four weeks of age, for the remainder of the study. Previous unpublished research within our laboratory has shown that rats will voluntarily consume a solution of this strength.

Acclimation and sleep deprivation occurred in three forced exercise wheels from Lafayette Instrument Company. The forced exercise wheels consisted of aluminum rings and polycarbonate sides. The internal width of each wheel was 11.18 cm, with an internal diameter of 33.88 cm. Each identical wheel had 82 rungs .48 cm in diameter spaced 1.34 cm apart from each other. Each moving wheel was driven by a motor at approximately 1.5 meters per minute, or slightly over one full rotation per minute. In the center of each wheel, on both sides of the wheel, there was a small hole through which a water bottle mouthpiece fit through. Food could also be placed inside the wheel so that the subjects could continue to have ad libitum access to food. Each wheel also contained a small ramp that followed the direction of wheel rotation. These ramps were added to the wheels following observations during previous studies in the lab in which a rat was able to attain sleep for short periods of time by sleeping on the moving wave of food pellets. Once the ramps were added, subjects were forced to step over one of the ramps each rotation of the wheel, or the ramp would drop the subject approximately 3cm, waking the subject. The present study sought to maintain total sleep deprivation in subjects while the wheel was moving, so the placement of the ramp was needed.

Procedure

Alcohol Exposure

Animals were obtained on postpartum day 21 (P21), and were handled daily, with ad libitum access to food and water, until P28. Beginning on P28, the rats were given free access to the 7% alcohol solution, in addition to water. The placement of the water and 7% alcohol solution bottles was counterbalanced in their placement on cages in attempt to control for side bias.

Wheel Acclimation

Starting on P42, after seven days of access to the alcohol and water solution in addition to ad libitum access to food and water, the first three rats were placed in the three (non-moving) forced exercise wheels. Each subject lived in a non-moving forced exercise wheel for seven consecutive 24-hour days. This rotation occurred for 28 days, giving all 12 subjects a full week inside the non-moving wheels in order to acclimate to the wheel environment, because a previous unpublished study within our laboratory found that the environment in which the sleep deprivation occurred, the exercise wheel, is potentially linked to stress (Sequeira, 2015).

Sleep Deprivation

During the 8 weeks following the acclimation condition, all rats experienced a week each of 18-hour and 6-hours daily sleep deprivation. Since the facility possessed only three forced-exercise wheels, three rats were exposed to the same condition per week. At the start of the following week, the three rats that were previously inside the forced-exercise wheels were returned to their home cages, and the next three rats would be placed inside of the forced-exercise wheels. Each week the sleep deprivation condition

changed from the week before, alternating between the 18-hour and 6-hour conditions to counterbalance for any age and/or order effects. For example, if the first three rats experienced 6 hours of sleep deprivation, then the next three rats would experience 18 hours, and four weeks following the end of the first three rats' 6-hour week, those same rats would experience 18 hours of sleep deprivation.

After all 12 rats had experienced both sleep deprivation conditions over 8 weeks, the rats repeated the wheel control condition from the first 4 weeks, in which they spent 24 hours per day inside of the non-moving forced-exercise wheels for 7 consecutive days. The post sleep deprivation wheel control (WC-2) condition was introduced in order to test the effect of the acclimation period in reducing stress, or any other confounds, associated with the wheel environment. This study had a within subject design, so every rat experienced all four levels of the intervention (WC, 18 hours, 6 hours, WC-2).

Data Analysis

The results were analyzed through a repeated measures ANOVA and through visual analysis. Before beginning alcohol consumption, nearly every rat weighed under 300 grams, and by completion of the study, all rats weighed over 400 grams. Since the effects of alcohol are dependent on the weight of the consumer, our dependent variable was transformed from grams of alcohol consumed to grams of alcohol consumed per gram of body weight. Due to the relatively small sample size ($N = 12$), visual analysis was used as a secondary measure of data analysis. The experimental conditions were home cage, wheel control (pre-movement exposure), wheel control (post-movement exposure), 18 hours of sleep deprivation, and 6 hours of sleep deprivation. We anticipated

a dose-dependent relationship between sleep deprivation (18 and 6-hour conditions) and alcohol consumption.

Results

A repeated measures ANOVA was conducted for voluntary alcohol consumption (g alcohol solution / rat weight in kg) for the five different conditions, for descriptives see table 2 in the appendix. Mauchley's test of sphericity was significant, thus sphericity was not assumed $\chi^2(9) = 22.658, p = .008$. There was a significant effect of condition on voluntary alcohol consumption with a Greenhouse-Geisser correction applied, $F(2, 23) = 5.90, p = .008, \text{partial } \eta^2 = .349$, see table 3. The partial eta squared showed a noteworthy effect, with 34.9% of the variance in alcohol consumption explained by condition. The post hoc tests were conducted using a pairwise comparisons with a Bonferroni correction. There was no significant difference between the home cage condition ($M = 12.58, SD = 7.41$) and the first wheel control condition ($M = 15.74, SD = 8.58, p > .99, d = .395$), but there was a significant difference between the home cage condition and the 6-hour condition ($M = 30.53, SD = 19.40, p = .019, d = 1.22$) and the 18-hour condition ($M = 32.27, SD = 23.62, p = .045, d = 1.12$). The 6-hour and 18-hour conditions were not significantly different from each other ($p > .99, d = .08$). The first wheel control condition was not significantly different from the 6-hour condition ($p = .119, d = .98$), the 18-hour condition ($p = .091, d = .92$), or the second wheel control condition ($p = .746, d = .60$). The second wheel control condition ($M = 26.63, SD = 24.13$) did not significantly differ from either the 18-hour ($p > .99, d = .23$) or the 6-hour ($p = .969, d = .17$). For more information, please see table 4 in the appendix.

Discussion

Although alcohol consumption was significantly higher during both of the sleep deprivation conditions, the hypotheses were not fully supported. Though the first wheel control condition did not significantly differ from the original home cage condition, it was expected that this condition would significantly differ from both of the sleep deprivation conditions. Additionally, a dose-dependent relationship was hypothesized between sleep deprivation and alcohol consumption, however there was not a significant difference between the 6-hour and 18-hour sleep deprivation conditions. Though technically the hypothesis that the final wheel control condition would not differ from the first wheel control condition was supported, this is not entirely the case as these conditions were expected to differ from the sleep deprivation conditions.

During the original iteration of this study, the means were either so far apart or so close together that significance and non-significance were visually obvious (Sequeira, 2015). This caused the researcher to believe that power would not be an issue despite the small sample size ($N = 12$). Moving forward, this may not have been the case. The home cage ($M = 12.58$, $SD = 7.41$) and the first wheel control ($M = 15.74$, $SD = 8.58$) conditions are clearly quite similar in terms of alcohol consumption considering the means of the other conditions. That being said, the first wheel control condition was not significantly different from the 6-hour ($M = 30.53$, $SD = 19.40$) and 18-hour ($M = 32.27$, $SD = 23.62$) conditions, despite the fact that the mean of the first wheel control condition was under half the mean of the 18-hour condition. These two conditions had very large standard deviations. Further examination led researchers to believe that the large amount of variability between individual rats may have caused the non-significance of the

difference between the first wheel control condition and the two deprivation conditions. A post-hoc power analysis revealed that (holding all else constant) having a slightly larger sample size ($N = 15$) would have made the difference between the first wheel control condition and both sleep deprivation conditions statistically significant. Finally, the mean of the 18-hour condition was slightly higher than the 6-hour condition, however with such a tiny effect size ($d = .08$), the researcher believes this difference is likely due to chance.

The present study supports the findings of the previous study in this lab, that sleep deprivation increases voluntary alcohol consumption in rats, with a dose-dependent relationship being too small to make any concrete attributions to. It also generalizes the findings of the previous study from adolescent rats to adult rats. The original study in our lab also found that it contracted a major criticism of Aalto and Kiianmaa (1984a), that the reverse flowerpot technique with an electrified grid stressed the rats and thus the increased alcohol consumption could be attributed to a stress response rather than sleep deprivation alone (Sequeira, 2015). This may not be entirely true, as the current study sought to elucidate the relationship between forced exercise sleep deprivation wheels and stress. Though there was a slight, though non-significant, increase in voluntary alcohol consumption in the non-moving wheels, it seems apparent that the wheel environment does not increase voluntary alcohol consumption on its own. Additionally, since the original study in our laboratory some concerns were raised that alcohol consumption increased merely as a product of dehydration from the forced exercise (as the alcohol solution was only 7% ethanol, with the remainder of the solution being water). Visual analysis of the means of water consumption at every condition showed an inverse

relationship with alcohol consumption. In conditions where rats consumed more alcohol, rats consumed less water (see table 2). In the home cage and first wheel control conditions rats drank the least alcohol and the most water, while in the 6-hour and 18-hour conditions rats drank the most alcohol and the least water.

Perhaps the most interesting condition is the final wheel control condition ($M = 26.63$, $SD = 24.13$), with a mean sitting midway between the first wheel control condition and the two sleep deprivation conditions, and a standard deviation roughly three times as large as the original wheel control condition. This result may be better explained via a behavioral explanation rather than a pharmacological one. Duncan, Alici, and Woodward (2000) found even greater evidence that Pavlovian conditioning, more specifically, a conditioned compensatory response, is involved with drug tolerance via measuring spontaneous motor activity (SMA) in adult rats. These rats were injected with either saline or ethanol paired with two stimuli. Removing these paired stimuli from the ethanol-injected rats resulted in these rats experiencing greater behavioral depression when injected with ethanol. Essentially, the rats in this study experienced a greater tolerance to alcohol, and thus needed a greater dose of alcohol to achieve the same effect when these conditioned stimuli were present (Duncan et al., 2000). In the current study, it is quite likely that the same phenomenon is being observed. The subjects likely consumed more alcohol in the final wheel control condition in order to achieve the same effect of the drug, due to an increased tolerance caused by the conditioned stimulus of the wheel environment.

The current study also seems to support the original study's claim that the relationship between sleep deprivation and voluntary alcohol consumption is bi-

directional (Sequeira, 2015). Alcohol consumption leads to reduced quality of sleep and thus a form of sleep deprivation, and sleep deprivation appears to enhance voluntary alcohol consumption, at least to some degree. However, this bi-directional relationship is likely quite different in terms of a dose-response curve. Larger doses of alcohol reduce the overall quality of sleep, and thus cause greater amounts of sleep deprivation, via greater REM suppression and increased nocturnal arousals (Landolt & Borbély, 2000; Zarcone et al., 1975). However, larger amounts of sleep deprivation do not seem to cause greater amounts of voluntary alcohol consumption. The original iteration of this study attempted to find a dose-dependent relationship with 18, 20, and 22 hours of sleep deprivation in rats with no success (Sequeira, 2015). The current study attempted to find a dose-dependent relationship through much greater separation of the sleep deprivation conditions by using 6-hour and 18-hour sleep deprivation conditions, also with limited success. The original unpublished study from our lab suggested that a curvilinear relationship may exist between sleep deprivation and voluntary alcohol consumption, and though that may be the case, further examination is needed. The point at which a much smaller amount of sleep deprivation (< 6 hours) roughly equates to a much larger amount of sleep deprivation (> 18 hours) in terms of its effects on voluntary alcohol consumption is still unclear.

Another possible explanation for the increased alcohol consumption during all sleep deprivation conditions in the present study is that there is no relationship between sleep deprivation and voluntary alcohol consumption. The major difference in hours of sleep deprivation between the two sleep deprivation conditions did not significantly affect the amount of alcohol consumed by rats. Stress is often a culprit in sleep

deprivation research that extraneous results are attributed to (e.g., McEwen, 2006), but even if the sleep deprivation conditions were inherently stressful, an increase in voluntary alcohol consumption between the 6-hour and 18-hour conditions should be expected. If time spent inside of the moving forced exercise wheel is stressful enough to cause enhanced voluntary alcohol consumption, then a dose-dependent relationship would be expected between 6 hours and 18 hours of exposure.

The present study's findings lead the researchers to speculate that the similarity in the means of voluntary alcohol consumption of the 6-hour and 18-hour sleep deprivation conditions may be due to a conditioned compensatory response. This speculation is based on the thought that experiencing 6-hours and 18-hours of daily sleep deprivation is more similar than anticipated, at least in terms of being an environmental stimulus. This is concerning as it brings conclusions about a potential bi-directional relationship between voluntary alcohol consumption and sleep deprivation into question. However, researchers observed that there was an inverse relationship between water consumption and alcohol consumption throughout the study. In the experimental conditions in which subjects consumed the most alcohol, they consumed the smallest amounts of water (also in g/kg). This demonstrates that, at a minimum, the rats were not consuming more alcohol based on the exercise alone.

During the present study, we found that sleep deprivation did enhance voluntary alcohol consumption, however there did not seem to be a dose-dependent relationship. Future research should consider further separating the amount of daily sleep deprivation between conditions as, even if no dose-dependent relationship exists, the threshold at which sleep deprivation causes an increase in voluntary alcohol consumption is still

obscured. Additionally, contrary to our hypotheses, we found that after rats had experienced all sleep deprivation conditions, when moved into the non-moving wheels for the final control condition, the rats consumed an observably larger amount of alcohol than before, despite this increase not being statistically significant. Thus, future research must be done to better understand the relationship between sleep deprivation and voluntary alcohol consumption. In near direct contrast to the initial run of this study, sample size may be a direct limitation of this study's findings (Sequeira, 2015). There were instances in which the mean of one condition was nearly 50% higher than the mean of another condition, yet this difference was not significant due to the large standard error of the sample. Having a much larger sample size could either grant this study the power to demonstrate that its findings were consistent enough to be statistically significant, or raise additional questions if the differences between means were smaller.

The theory behind the present study was strongly grounded in the results of the previously mentioned unpublished research in our laboratory (Sequeira, 2015). The present study attempted to better elucidate the relationship between voluntary alcohol consumption and sleep deprivation by using behavioral acclimatization to reduce the confound of stress. This study succeeded in that it found that the wheel environment was not inherently stressful, at least in terms of voluntary alcohol consumption, but it falls short in giving conclusive evidence for a dose-dependent relationship between alcohol consumption and sleep deprivation. The results of this study give greater insight to using forced exercise wheels as a tool for sleep deprivation research, and, considering the results of the original study, may even be conclusive in terms of this research question; the wheel environment alone does not cause increased alcohol consumption in rat

subjects. Future research should employ different methodologies, specifically in terms of sleep deprivation apparatus used and hours of sleep deprivation. For example, gentle handling techniques and new devices that simulate this process could reduce the confound of stress further, and potentially reduce the effect of the conditioned compensatory response. Additionally, if our 6-hour and 18-hour conditions are equivalent in terms of voluntary alcohol consumption, future research should seek to make the 6-hour condition even smaller. Another valuable change may be to setup the wheel control conditions in a way that the rat is able to move the wheels enough to achieve exercise without forcing continuous movement in the rat. A lickometer, a device that records when subjects lick the end of a water or alcohol bottle, may also be useful to determine when a rat consumes alcohol, and to control for any leakage in either the alcohol or water bottle. Finally, consumption of both water and alcohol was only documented by researchers once per 24-hour day. In the future, it would be useful to document alcohol and water consumption both before and after leaving the exercise wheels, to determine consumption while in the wheel. Though our results suggest the contrary, if exercise is a factor in voluntary alcohol consumption, then a different sleep deprivation technique may also find different results.

The present study further supports that there is a bi-directional relationship between sleep deprivation and alcohol consumption. If sleep deprivation does cause an increase in an individual's voluntary alcohol consumption, there are likely significant implications for clinical interventions. It could theoretically double the number of variables for a clinician to manipulate in the treatment of alcohol addiction. Future research should utilize different tools and sleep deprivation conditions and will,

hopefully, better elucidate the link between voluntary alcohol consumption and sleep deprivation.

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Appendix

Table 1

Chronological Listing of the Experimental Conditions

	Weeks	Hours	Moving?
First Wheel Control	1-4	24	No
18-Hour Deprivation	5-12	18	Yes
6-Hour Deprivation	5-12	6	Yes
Final Wheel Control	13-16	24	No

*Please note that "Hours" refers to the number of hours inside of the wheel per 24-hour day

Table 2

Means and Standard Deviations for the Five Conditions on Consumption

Variable	<i>Alcohol</i>		<i>Water</i>	
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>
Home Cage	12.58	7.40	93.23	29.06
Wheel Control 1	15.75	8.58	112.15	34.51
6 Hour	30.53	19.40	68.92	28.06
18 Hour	32.27	23.62	70.71	44.48
Wheel Control 2	26.63	24.13	61.10	25.07

Table 3

One-Way Analysis of Variance for the Effects of Sleep Deprivation on Alcohol Consumption

Source	df	SS	MS	F	<i>p</i>	η^2
Between-group	2.10	3785.42	1802.58	5.90	.008*	.349
Within-group	23.12	7057.89	305.27			
Total	25.22	10843.31				

*Denotes significance at .05

Table 4

Significance Values, Standard Errors, Cohen's d Values, Mean Differences, and Confidence Intervals of Post-hoc Comparisons

Comparison	Mean Difference (g/kg alcohol)	Std. Error	<i>p</i>	95% CI of Mean Difference		<i>d</i>
				Lower Bound	Upper Bound	
HC – WC1	-3.17	1.99	.999	-10.12	3.79	.395
HC – 6-hour	-17.95	4.44	.019*	-33.46	-2.44	1.22
HC – 18-hour	-19.69	5.55	.045*	-39.08	-.30	1.12
HC – WC2	-14.05	5.27	.219	-32.48	4.38	.78
6-hour – WC1	14.78	4.91	.119	-2.40	31.96	.98
6-hour – 18- hour	-1.74	7.00	.999	-26.20	22.72	.08
6-hour – WC2	3.90	6.68	.999	-19.45	27.25	.17
18-hour – WC1	16.52	5.23	.091	-1.76	34.81	.92
18-hour – WC2	5.64	3.11	.969	-5.22	16.50	.23
WC1 – WC2	-10.88	5.53	.746	-30.21	8.44	.6

*Denotes significance at .05

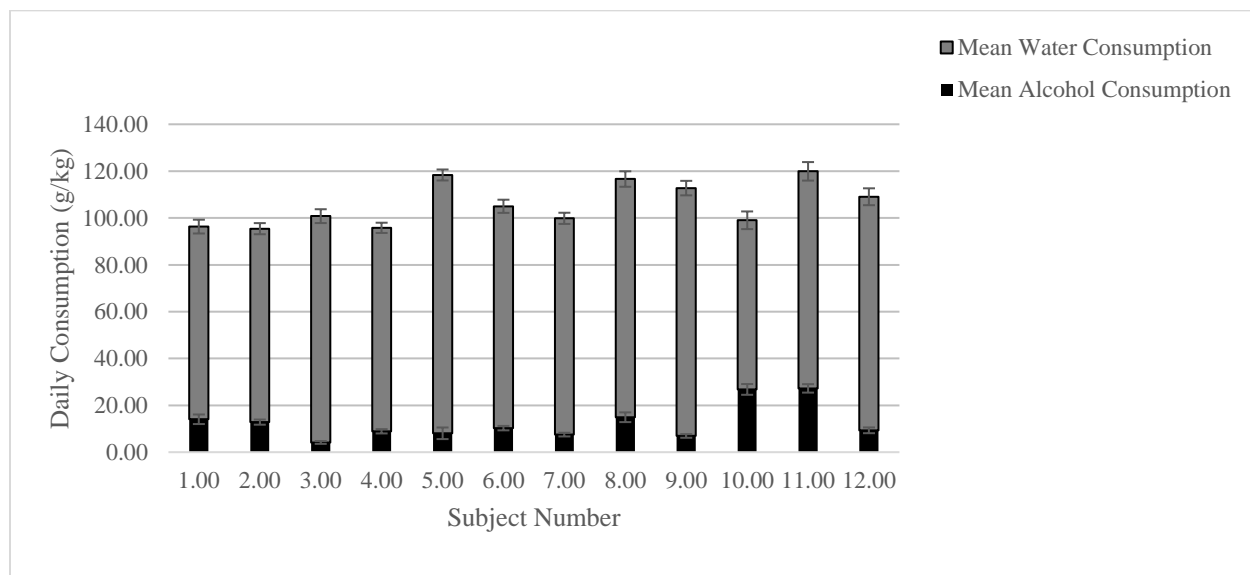


Figure 1. Mean Daily Water and Alcohol Consumption in the Home Cage Condition, by Subject

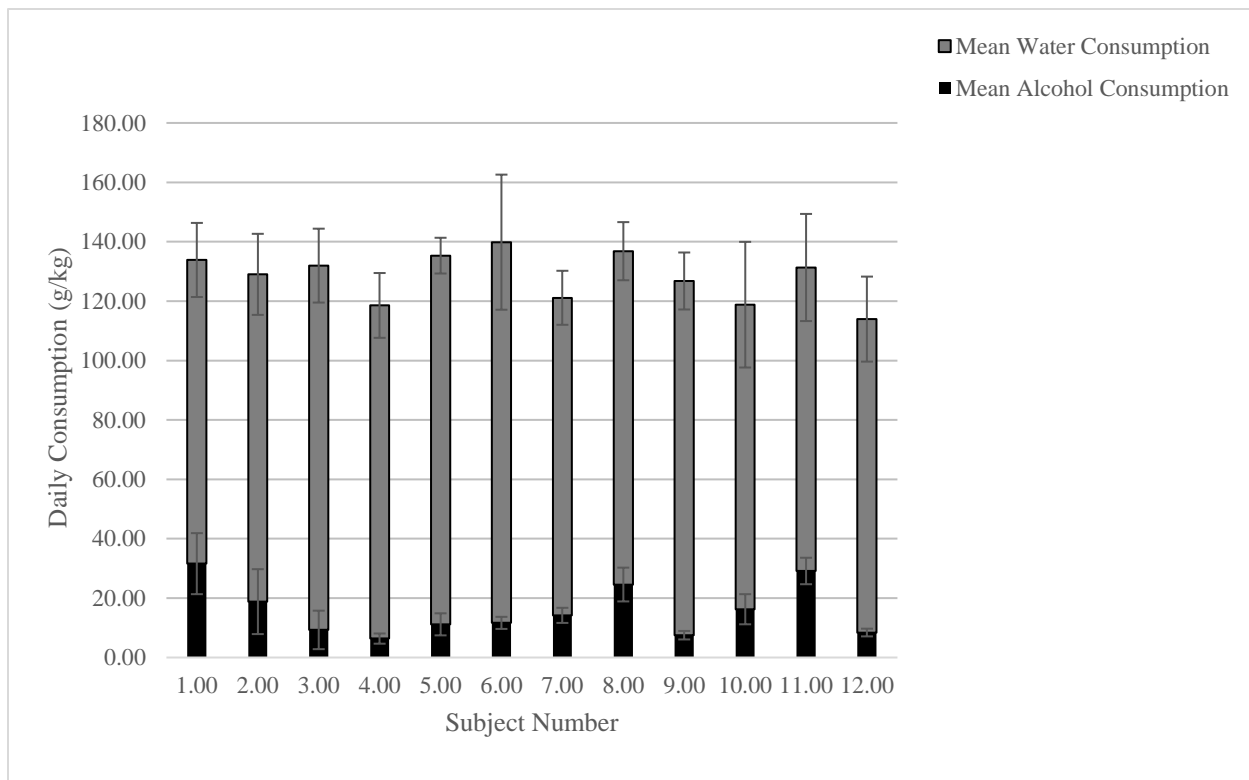


Figure 2. Mean Daily Water and Alcohol Consumption in the First Wheel Control Condition, by Subject

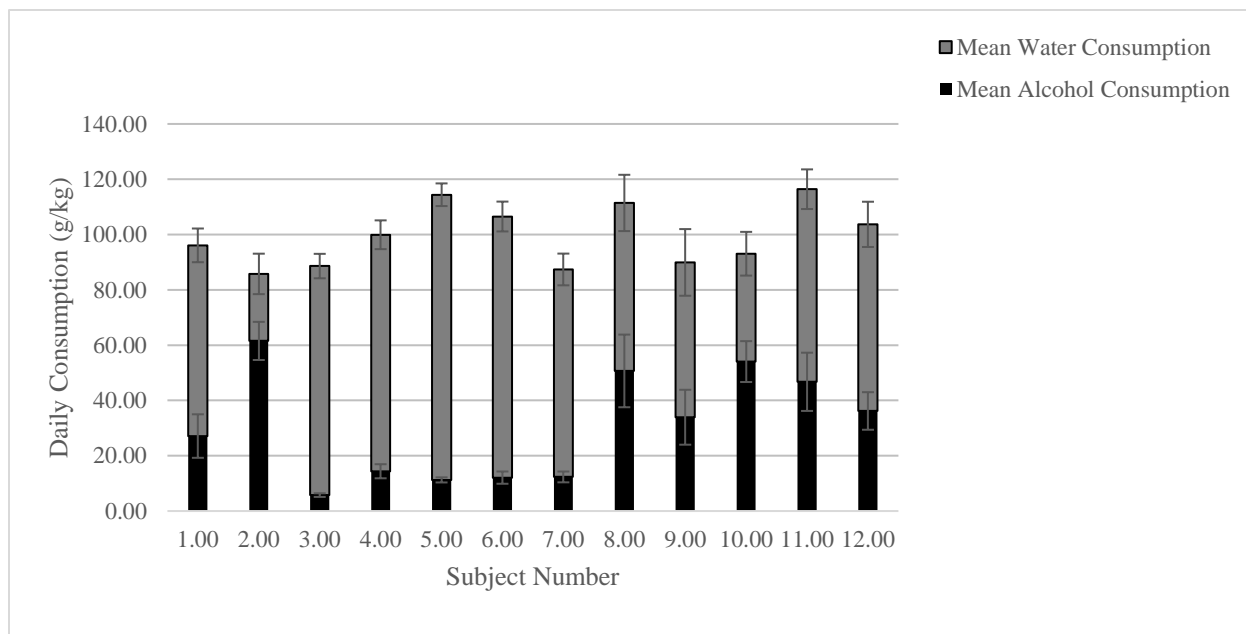


Figure 3. Mean Daily Water and Alcohol Consumption in the 6-Hour Deprivation Condition, by Subject

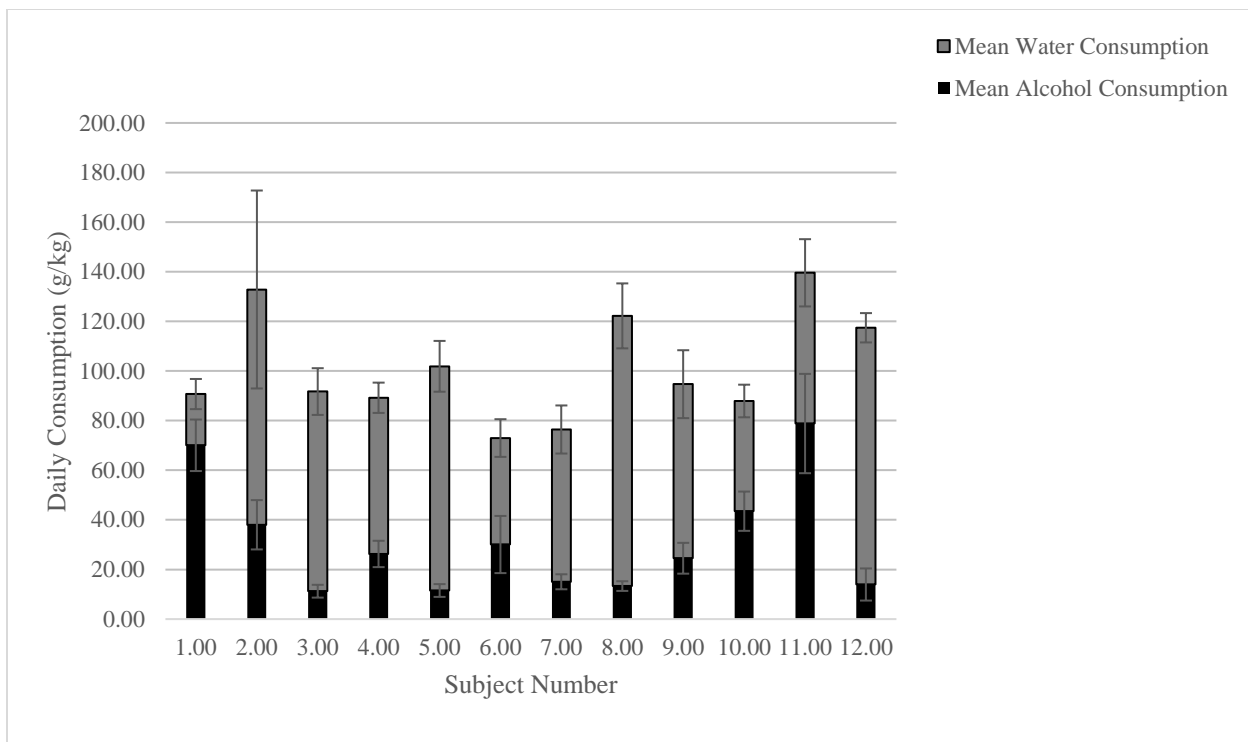


Figure 4. Mean Daily Water and Alcohol Consumption in the 18-Hour Deprivation Condition, by Subject

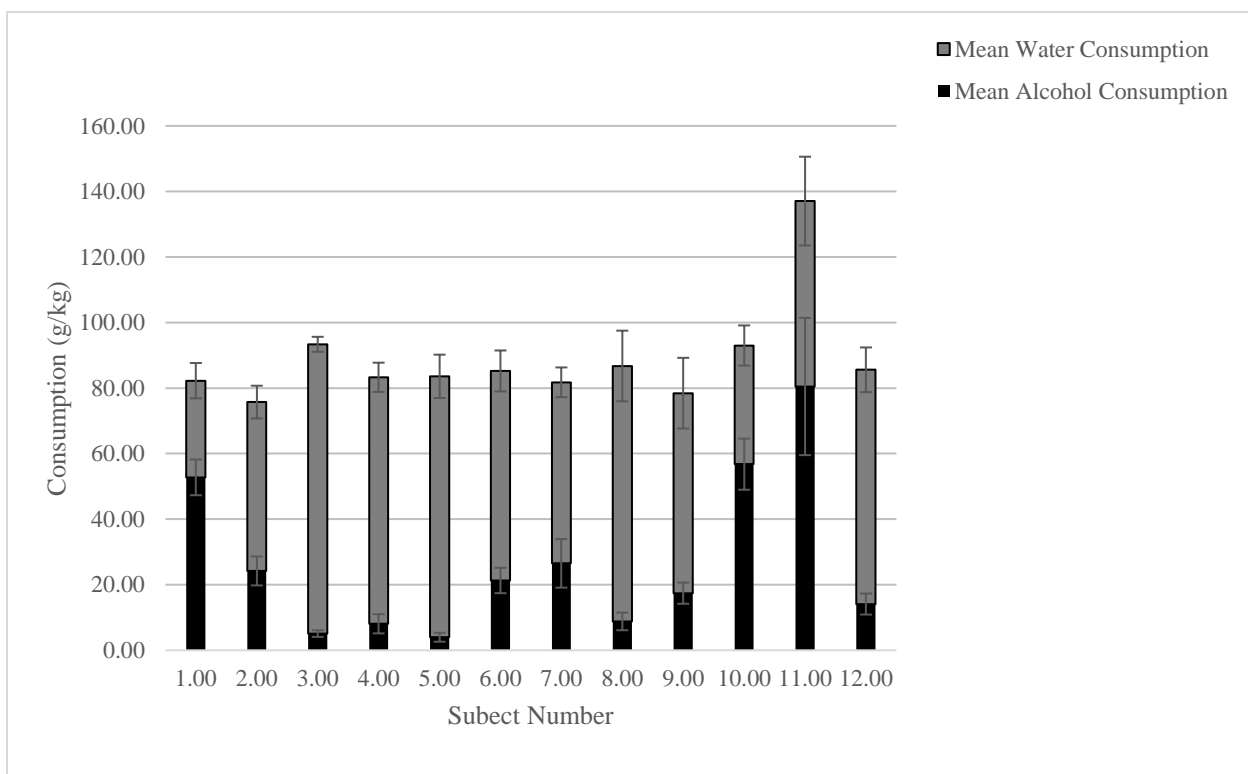


Figure 5. Mean Daily Water and Alcohol Consumption in the Second Wheel Control Condition, by Subject

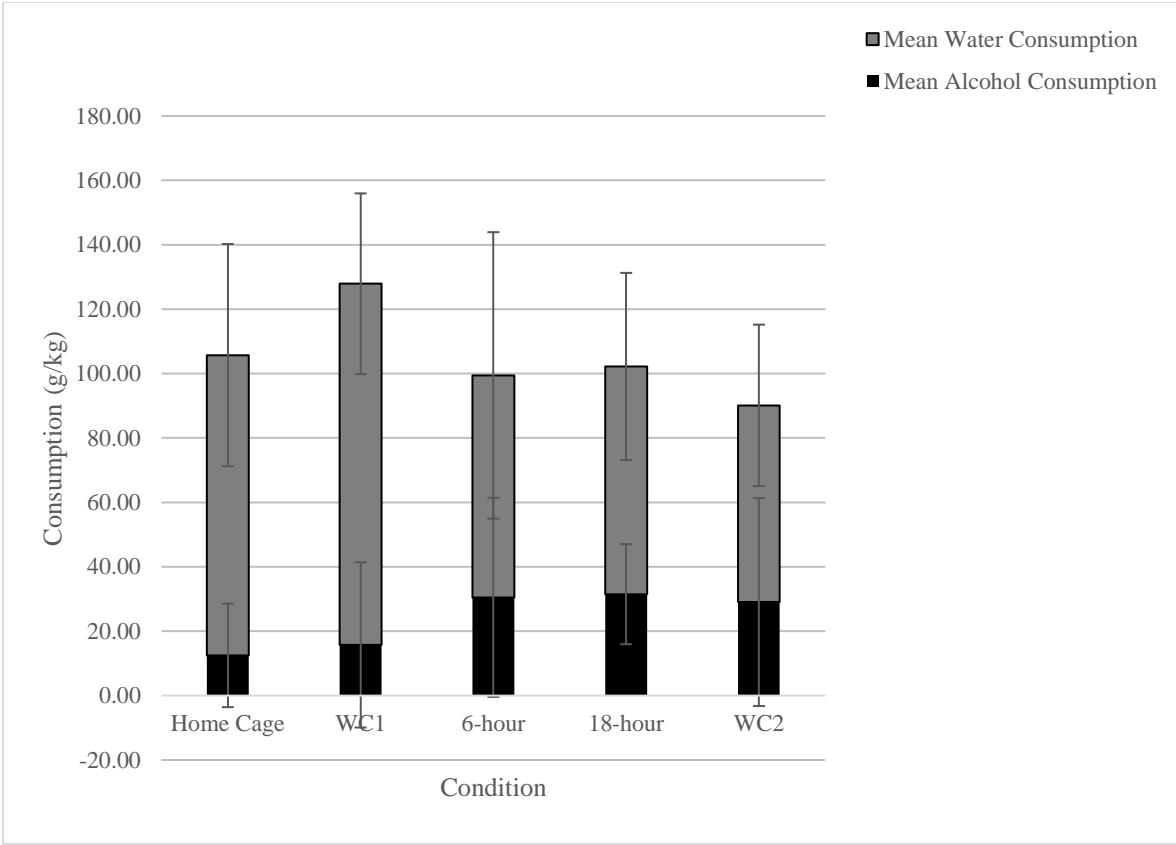


Figure 6. Mean Daily Water and Alcohol Consumption in all Conditions

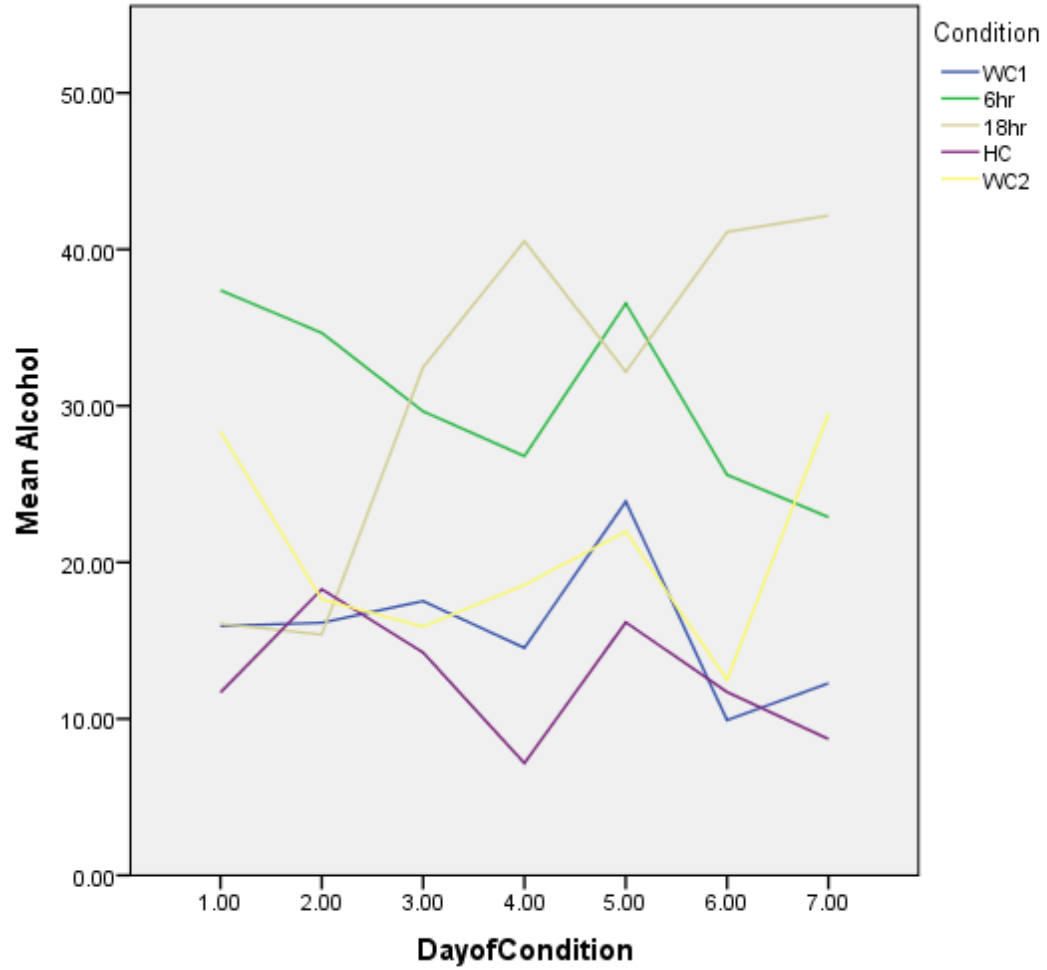


Figure 7. Mean Alcohol Consumption for Each Day of Each Condition Across all Conditions