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Sleep deprivation and voluntary alcohol consumption in adolescent rats

Sancho N. Sequeira
James Madison University

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Special committee directing the thesis work of Sancho Sequeira

Jeff Dyche Date

Daniel Holt Date

Sonia Jeanne Horst Date

Academic Unit Head or Designee Date

Received by The Graduate School

Date

Sleep Deprivation and Voluntary Alcohol Consumption in Adolescent Rats

Sancho Sequeira

A thesis submitted to the Graduate Faculty of

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Abstract

Alcohol is one of the most common psychoactive drugs, and has been used by humans for thousands of years. Research has focused on the effects of alcohol on sleep, however recent trends in the literature have taken a more bidirectional approach to the relationship between alcohol and sleep. This research investigates the effects of chronic, partial sleep deprivation on alcohol consumption. Twelve adolescent Sprague Dawley rats had free access to two bottles at all times, one containing water and one containing a 7% alcohol and water solution. Sleep deprivation was achieved by using a forced exercise wheel. All rats were sleep restricted for a small (18hrs), medium (20hrs), and large (22hrs) amount every day for 7 days, one week. Additionally, there was a wheel control (WC) condition. Each rat experienced every level of the sleep deprivation condition and one week of the wheel control condition for a total of 4 experimental weeks. There was a significant effect of sleep condition on voluntary alcohol consumption, $F(4, 44) = 9.191, p < .001$, partial $\eta^2 = .455$. Post hoc testing using pairwise comparisons showed that the only group that was significantly different was the control group. Thus the WC condition also showed an increase in consumption, which does not allow us to conclude that there is a causal relationship between sleep deprivation and alcohol. Implications and future directions are discussed.

Sleep Deprivation and Voluntary Alcohol Consumption in Adolescent Rats

Alcohol refers to any organic compound that has a hydroxyl group, one oxygen atom and one hydrogen atom, bonded to its carbon atom. Typically, when people say alcohol they are referring to ethanol ($\text{CH}_3\text{CH}_2\text{OH}$), which is a specific type of alcohol. There are many other alcohols that people consume, but not for the same reason as ethanol and with different outcomes. Other examples of chemicals that are technically alcohols include disparate compounds such as sorbitol (a sweetener) and retinol (a form of Vitamin A). In the present study, the word alcohol will follow its vernacular definition and be used to refer to ethanol.

Alcohol is one of the most common psychoactive drugs, and has been used by humans for thousands of years. In the United States, responsible alcohol use is socially acceptable and legal for people over the age of 21. Excessive alcohol use can be damaging and is annually responsible for approximately 88,000 deaths in the United States, which makes it the 3rd leading cause of death due to preventable, lifestyle factors (Centers for Disease Control and Prevention, 2014). Until recently, there was a differentiation made between alcohol abuse and dependence. Now they are categorized as alcohol use disorder with mild, moderate, and severe sub-classifications (American Psychiatric Association, 2013). Regardless of how it is categorized, alcohol use disorders are a serious problem at the societal level.

Alcohol primarily functions as a central nervous system (CNS) depressant. These effects are due to alcohol being an indirect γ -aminobutyric acid (GABA) agonist, specifically for the GABA_A receptor which is a subtype of the GABA receptor (Davies, 2003). An agonist is a chemical that increases the release of a neurotransmitter, and

GABA is the primary inhibitory neurotransmitter in the CNS. By increasing GABA, alcohol has an overall depressant effect on the CNS. This is why higher doses can lead to loss of motor skills, impaired senses, and eventually unconsciousness.

In small doses (0.125-0.5g/kg), 1-2 drinks in a 150 pound human, alcohol has a mildly euphoric effect. This amount produces a dose-dependent increase in dopamine release in the Ventral Tegmental Area (VTA) (Gessa, Muntoni, Collu, Vargiu, & Mereu, 1985). The VTA plays an important role in the reward pathway, and its activation stimulates dopamine release in the Nucleus Accumbens and the frontal cortex (Gronier, Perry, & Rasmussen, 2000). While small and moderate doses of alcohol provide a feeling of euphoria, higher doses produce depressant effects on the CNS.

Long-term abuse of alcohol has several negative health effects. The metabolism of alcohol is taxing on the liver and can lead to cirrhosis during long-term abuse. Severe alcohol abuse can even lead to other diseases such as Korsakoff's syndrome, which is due to lack of thiamine (B₁) and is characterized by amnesia, confabulation, and lack of motivation. Another issue with long-term abuse is the severe withdrawal symptoms that occur when cessation of alcohol occurs including anxiety, insomnia, hallucinations, seizures, and delirium.

Many of the issues that occur with withdrawal are due to dysregulation of inhibitory and excitatory mechanism in the brain. Specifically, these effects are caused by a combination of decreased GABA_A response to alcohol and *N*-Methyl-D-aspartic acid (NMDA) receptor down-regulation (Bayard, McIntyre, Hill & Woodside, 2004). This is because the GABA_A receptors get overstimulated by the large amounts of GABA released in the CNS by alcohol so it becomes desensitized to its effects. At the same time, NMDA

receptors are inhibited by GABA release which causes the up-regulation of glutamate. Glutamate, the metabolic precursor to GABA, is the most common excitatory neurotransmitter in the CNS. In a long-time heavy user, once alcohol consumption ceases the brain no longer releases large amounts of GABA but it is still up-regulating NMDA and the NMDA receptor is no longer inhibited by GABA. This combination of effects, decreased inhibition and increased excitation, causes hyper-excitability in the CNS and results in many of the long-term symptoms described above.

When used in moderation, alcohol does have some positive health benefits. When compared to non-drinkers, people who drank moderate amounts of alcohol have a decreased risk of cardiovascular disease and its various risk factors including coronary heart disease, hypertension, diabetes, and stress (Grønbæk, 2009). This relationship is often referred to as a “J curve”, as alcohol consumption increases there is an initial reduction in risk followed by an increase that goes beyond the initial risk level. However, most of the literature is on adults and moderate use. There is less information on how drinking impacts the physiology and behavior of younger individuals.

Adolescence and Alcohol

There are many neurobiological changes that occur during adolescence that can effect current and future alcohol use. According to Witt (1994), there are not enough studies about how adolescence itself could mediate individual response to alcohol during this time period of development. For example, typical neurobehavioral changes during adolescence include increased risk-taking, seeking of novel stimuli, sensation seeking, and susceptibility to stressors (Spear, 2000). These factors increase the chance of alcohol use during adolescents, which is a particularly vulnerable time because of the increased

sensitivity to the effects of alcohol and the incomplete development of the judgment and reward system (Spear, 2002).

Alcohol use during adolescent has also been used as a predictor of later alcohol abuse; this often referred to as age of onset. People who start drinking younger are more likely to be dependent on or to abuse alcohol. In fact, the odds of dependence decrease on average 14% for every one year increase in the age of onset (Grant & Dawson, 1997). The causality of this finding cannot be determined in humans because of other confounding environmental factors that could influence it such as social acceptance of alcohol use and parent's alcohol use. Additionally, the causal effects of alcohol on adolescents cannot be studied because of the ethics involved in administering alcohol to people under the age of 18. Therefore, any research that attempts to answer causal relationship would need to use alternate methods, such as animal models.

Rats are a useful model for alcohol research because specific strains can be bred with different predilection to alcohol consumption. For example, Eriksson (1968) selectively bred Wistar rats over several generations to develop the AA strain that prefer alcohol over water, and the ANA strain that prefer water over alcohol. These strains are useful for studies that want to target alcoholic and abstinent populations of rats. However, for research questions that are more general in scope, an outbred strain would allow a wider range of alcohol preference. One such outbred strain that has previously been used for adolescent alcohol research in rats is the Sprague-Dawley (SD).

Alcohol research with SD's has found that alcohol differentially affects younger rats. When compared to adult rats, adolescent rats had more damage in the frontal piriform and perirhinal cortices, which are responsible for olfaction and memory (Crews,

Braun, Hoplight, Switzer, & Markwiese, 2000). Adolescent rats that were under the influence of alcohol also showed significant impairment in spatial memory acquisition compared to adult rats in Morris water maze (Markwiese, Acheson, Levin, Wilson, & Swartzwelder, 1998). These studies highlight how there are age related differences in the effect of alcohol on the brain. Thus, age is an important fact to consider when selecting rats.

Directly comparing a human developmental construct like adolescence to the development of rats is difficult. Rats undergo rapid development so the standard measurement for rat age is days as opposed to humans who measure age in years; this is usually noted as post-natal day. According to the literature, the main developmental stages in rat's lives are weaning, puberty, adolescence, adulthood, and aged. For example, Sengupta (2013) compared rat and human development at different stages and found that rats mature more quickly physically, but mature socially more slowly. According to Sengupta, pre-adolescence occurs after weaning, about post-natal day 21 (P21) and sexual maturity is reached for most by P42, however rats are not socially mature until about P180. Therefore, the definition of adulthood (social or sexual maturity) in rats can differ drastically depending on the criteria being used. The definition of adolescence and adulthood should depend on the research question and the type of research being conducted.

In the literature, the Sprague-Dawley rat does not have a specific post-natal day associated with adolescence, but instead a range. Focusing on alcohol research with adolescent Sprague-Dawley's, adolescence begins between P30-P35 and young adulthood begins between P65-P70 (Crews et al., 2002; Markwiese et al., 1998;

Varlinskaya & Spear, 2004). When directly relating this period to humans, rats' development over 10.5 days is equal to human development over 1 year (Sengupta, 2013). This lack of specificity and rapid development makes pinpointing a specific day difficult. A more useful paradigm is to envision adolescence in rats as a window of development over a range of time. Many of the previous studies used "treatments" lasting a single session so precision was critical; research literature could benefit by utilizing a design that has a long-term exposure to alcohol.

Long-term exposure to alcohol also has negative effects on sleep. There is an effect on sleep architecture, rapid eye movement (REM) sleep decreases and non-rapid eye movement sleep increases in a dose-dependent pattern (Mendelson & Hill, 1978). Additionally, chronic alcohol exposure can produce long-term changes in sleep quality in animal models (Ehlers & Slawecki, 2000). Sleep problems are a common complaint among people with alcohol use disorder, with one study finding that 91% of participants had sleep disturbances, as measured by the Pittsburg Sleep Quality Index (Cohn, Foster & Peters, 2003). Finally, GABA plays an important role in the neurochemistry of both sleep and alcohol, so it is not surprising that there is a relationship.

Sleep and Alcohol

Sleep is comprised of two main systems, sleep-promoting and wake-promoting. These separate systems were discovered by von Economo (1926) when he found that there are two separate areas in the brain for sleep and wakefulness. The wake-promoting system is located in the brain stem and is called the Ascending Reticular Activating System (ARAS). The ARAS projects to several parts of the forebrain and has an

excitatory effects, but its effect on the Ventrolateral Preoptic nucleus (VLPO) is inhibitory (McCarley & Sinton, 2008). Projection refers to one part of the brain “communicating” with another part by sending specific neurotransmitters. The VLPO is the center of the sleep-promoting system and it projects to many of the same areas as the ARAS including the tuberomammillary nucleus (TMN), Raphe nuclei, Locus coeruleus (LC) and the pedunculopontine/laterodorsal tegmental nucleus (LDT/PPT), however the VLPO has an inhibitory effect on these areas (Saper, Chou, & Scammell, 2001). Simply put, the sleep-promoting system works by inhibiting the wake-promoting system.

The sleep patterns of rats are different than humans in a number of ways. Rats require much more sleep than humans and on average rats of all ages tend to spend slightly over 50% of their day asleep (Mendelson & Bergmann, 1999). The same study found that younger rats had average sleep bouts of 31.22 minutes and an average of 29 REM-NREM cycles in a 24 hour period, much of that time during the daylight hours. This differs considerably from the human who sleep in one long bout lasting several hours, go through 4-6 REM-NREM cycles in a 24 hour period, and consolidate most of their sleep at night. Finally, all rats that undergo long term total sleep deprivation (TSD) eventually die, within approximately 3 weeks (Everson, Bergmann, & Rechtschaffen, 1989). This cannot be empirically tested in humans, however sufferers of Fatal Familial Insomnia routinely go months without sleep before falling into a coma and dying (Gallasi et al., 1996). It is not known if the sleep loss is simply a symptom of the disease, which manifests in numerous other neurological symptoms, or if sleep itself causes death.

Rats are a commonly used animal model for sleep research and are useful for research involving recovery from sleep deprivation due to the similar effects on sleep

architecture in both humans and rats. Sleep deprived rats and humans both show the same initial increases in slow-wave sleep (SWS) followed by an increase in REM during the second night after recovery (Borbély & Neuhaus, 1979). Rats are also useful for pharmacology research and previous research has used rats to study the role of drugs that effect adenosine, dopamine, and serotonin on sleep (Bagetta, Sarro, Priolo, & Nisticò, 1988; Ticho & Radulovacki, 1991; Thomas et al., 2003). There are also ethical reasons for using rats as much of the research conducted with rats would be unethical to conduct with human participants. Although they are not perfect, rats are a valid and commonly used animal model for sleep research.

Similar to humans, rats experience age related changes in sleep over their lifetime. Although not all changes are the same cross species, both humans and rats experience decreasing sleep bout length, an increase in the number of sleep bouts, and a reduction in REM sleep (Mendelson & Bergman, 1999). Taken together, these results show a reduction in sleep quality during the aging process. Interestingly, rats showed no age-related changes in total sleep time in a 24 hour period, so the changes are due to other factors that influence sleep. Mendelson and Bergman (1999) also showed that age related changes are already apparent in rats that were a year old, corresponding to humans midlife. The age of the rats being used does play an important factor in sleep quality, therefore any research targeting a specific developmental period should be mindful of these changes.

Traditionally, research has focused on the effects of alcohol on sleep, however recent trends in the literature have taken a more bidirectional approach to the relationship between alcohol and sleep. When alcohol is administered during free running conditions

with unchanging light levels, the circadian rhythm is disrupted and can become either longer or shorter (Rosenwasser, Fecteau, & Logan, 2005). Interestingly, the same study found shorter circadian rhythms during baseline predicted longer than normal rhythms during alcohol exposure. Other studies with humans show that increased alcohol consumption co-varies with shorter durations of nighttime sleep (Chaput et al., 2012). Additionally, the PER2 gene, which follows a circadian pattern of expression, has been shown to modulate alcohol consumption (Spanagel et al., 2005). Gene expression refers to the process by which genes are transcribed into proteins, and a circadian pattern of expression means this expression follows a 24 hour pattern. If it is the case that the relationship between sleep and alcohol is bi-directional, there is a possibility that there is a vicious cycle between disrupted sleep patterns and increased alcohol intake. Specifically, if alcohol consumption is disrupting sleep and disrupted sleep increases alcohol consumption, then these two mechanisms could be propagating each other.

The assertion that sleep deprivation can affect alcohol consumption is not well covered in the literature. Aalto and Kiianmaa (1984b) looked at the effects of REM sleep deprivation (by use of inverted flower pot technique) on voluntary alcohol consumption in rats. Their results suggested that there was an increase in alcohol consumption following REM sleep deprivation compared to baseline levels of alcohol consumption. After REM sleep deprivation was discontinued, a decrease in alcohol consumption was observed. Aalto and Kiianmaa (1984a) looked at the differential effects of REM sleep deprivation and lesioning of the Suprachiasmatic Nucleus (SCN), a fundamental biological regulator of circadian rhythms, on voluntary alcohol consumption. Their results showed that REM sleep deprivation alone caused an increase in alcohol

consumption, whereas lesioning of the SCN did not, suggesting that the effects of increased alcohol consumption observed are due to some aspect of REM sleep deprivation, and not solely the regulation of circadian rhythms.

Research on sleep deprivation and alcohol consumption in adolescent animals is even scarcer in the literature with most research looking at the effects of alcohol on sleep and not sleep on alcohol. Additionally much of the research pertains to the difference between adolescent and adult rats or focuses solely on adult rats. The research that has been done suggests that there are differences between adolescent and adult rats sleep when administered alcohol as measured by EGG (Pian, Criado, Walker, & Ehlers, 2008). The timing of alcohol consumption also differs, with adolescent rats' drinking more alcohol during the light phase compared to adult rats (Walker, Walker, & Ehlers, 2008). Interestingly, alcohol exposure during adolescence has long-term changes that persist, into adulthood including reduced Slow wave sleep (SWS) duration and shorter SWS episodes compared to controls (Criado, Wills, Walker, & Ehlers, 2008). Alcohol clearly differentially impacts adolescent development in the animal literature, but a limitation to many of these studies is the alcohol consumption is involuntary.

The present study aims to extend the results of previous research on sleep and alcohol in adolescent rats to voluntary alcohol consumption. If the results are supported in voluntary consumption it would make a stronger case for this effect to be generalizable to humans. Another goal for this research is to investigate the effects of chronic, partial sleep deprivation on alcohol consumption. The term chronic refers to sleep deprivation occurring over several days, and partial meaning that rats are allowed to sleep for a portion of every 24 hour period. Again, this would help with generalizability because

most humans sleep deprivation is chronic and partial. It is hypothesized that alcohol consumption will increase during weeks that rats are sleep deprived compared to weeks where they are not.

Method

Subjects

The subjects were 12 Sprague Dawley rats from Harlan. Throughout the study, rats were housed in cages that are 16 inches long, 9 inches wide and 8 inches tall. Rats were fed ad libitum. Animals were individually housed and kept on a 12 hour light-dark cycle throughout the study.

Apparatus

The subjects had free access to two bottles at all times, one containing water and one containing a 7% alcohol and water solution. Previous studies (Aalto & Khanmaa, 1984) have used up to a 10% alcohol solutions without negative effects. However, emesis is not possible in rats and other studies have used solutions that have an alcohol content as low as 5% (Walker, Walker, & Ehlers, 2008). Intoxication can still be attained with a 7% solution, but the slightly lower mixture ensured that rats did not overdose.

Sleep deprivation occurred in a forced exercise wheel from Lafayette Instrument Company. The sleep deprivation wheel consisted of aluminum rungs and polycarbonate sides. The internal width was 4.4in with an internal diameter of 13.38in. There were 82 rungs 0.188 inches in diameter with 0.526in spacing. The wheel was driven by a motor at 1.5 meter/min, or a little more than one full rotation per minute. There is a small hole on both sides of the wheel for the mouthpiece of the water bottles to fit through. Additionally, food can be placed inside the wheel, so they always had access to food. A

ramp was also added to the wheel to ensure that rats could not attain sleep in the wheel. This was done because during a previous study it was observed that rats could temporarily attain sleep by lying on the pellets. The goals of the present study were total sleep deprivation, so the ramp was necessary to make sure rats were totally sleep deprived while in the wheels and not undergoing sleep fragmentation.

Procedure

Animals were obtained on P28, and allowed a week to acclimate to the facilities with free access to water and food. Beginning on P35, the rats were given free access to both water and alcohol for three weeks, previous research observed stability of alcohol consumption at three weeks (Aalto & Khanmaa, 1984). As previously discussed, adolescence in rats is a window. Previous research targeting adolescence with Sprague Dawley's began on P35, therefore the present study sought to target the same window (Crews et al., 2002; Markwieste et al., 1998; Varlinskaya & Spear, 2004). In addition, alcohol and water bottles were counterbalanced in their placement on the cages to control for any side bias.

After 3 weeks the sleep deprivation portion of the study began. All rats were sleep restricted for a small (18hrs), medium (20hrs), and large (22hrs) amount every day for 7 days. Additionally, there was a wheel control (WC) condition. Every rat spent one week in a wheel; however, the wheel did not rotate so that the rat can still attain sleep. This was done to ensure that any changes in alcohol consumption could be attributed to the sleep deprivation and not other factors associated with being in the wheel.

This was a within subject design and every rat experienced every level of the sleep deprivation condition and one week of the wheel control condition for a total of 4

experimental weeks. The animal research facility had access to 3 sleep deprivation wheels, so sleep deprivation cannot occur all at once and was counterbalanced across subjects. Half of the rats experienced the largest amount of sleep deprivation first and the other half experienced the least amount of sleep deprivation first. The dependent measure was the amount of alcohol consumed. Alcohol consumption was measured by weighing the alcohol bottles every 24 hours, and calculating the change in weight from the previous weighing. The difference between each weighing (in grams) represented daily alcohol consumption. This was done to avoid losing liquid by transferring it to another receptacle for measuring. Every rat had at least one week between experimental and wheel control conditions, so that full recovery from sleep deprivation could occur. The study ended once all animals had experienced all experimental conditions, which took 12 weeks, not including the 3 weeks of baseline.

Data Analysis

The data were analyzed using a repeated measures ANOVA and visual analysis. The rats weighed approximately 120g at the beginning of the study and many weighed over 400g by the end of the study. The effects of alcohol are dependent on size, therefore to correct for the drastic change in weight the grams of alcohol consumed, dependent variable, was converted to g/ethanol per kilogram of body weight. The small sample size ($N = 12$) is not ideal for inferential statistics, so results were also examined using visual analysis. The different experimental conditions were home cage (control), WC, 18hrs, 20hrs, and 22hrs. It was also anticipated that sleep deprivation will have a dose-response relationship with alcohol consumption such that more sleep deprivation will result in more alcohol consumption.

Results

A repeated measures ANOVA was conducted for voluntary alcohol consumption across 5 different sleep conditions, for descriptives see Table 1. Mauchley's test of sphericity was not significant, therefore sphericity was assumed and no correction was applied to the interpretation of the repeated measures ANOVA, $\chi^2(9) = 15.534$, $p = .081$. There was a significant effect of sleep condition on voluntary alcohol consumption, $F(4, 44) = 9.191$, $p < .001$, partial $\eta^2 = .455$, see Table 2. The partial eta squared showed a large effect, 45.5% of the variance in alcohol consumption could be explained by sleep condition. The post hoc tests were conducted by running pairwise comparisons with a Bonferroni correction. There was a significant difference between the home cage consumption ($M = 1.02$, $SD = .61$) and every other condition including WC ($M = 3.53$, $SD = 2.16$), 18hrs ($M = 3.96$, $SD = 2.24$), 20hrs ($M = 3.16$, $SD = 1.87$), and 22hrs ($M = 3.53$, $SD = 1.74$), such that rats consumed more alcohol during wheel weeks compared to home cage weeks (all $p \leq .007$). There were no significant differences between any of the wheel conditions including the WC condition. Additionally, 95% confidence intervals were calculated for repeated measures, see Figure 1. The data was also plotted on the individual level and a majority of the rat's individual trends matched the overall trend, see Figure 2.

Discussion

Although alcohol consumption was higher during sleep deprivation compared to the home cage, the hypothesis was not fully supported because there was no difference in alcohol consumption between the WC condition and the sleep deprivation conditions. This was unanticipated because the rats were able to attain as much sleep as they wanted

in the WC condition. If there was a causal relationship between sleep deprivation and alcohol consumption, then alcohol consumption in the WC condition should have been similar to the home cage. Instead, alcohol consumption in the WC condition was similar to the sleep deprivation conditions, which suggests that there could be a confounding variable responsible for the increase in alcohol consumption. Additionally, a dose-response relationship was not found between sleep deprivation level and alcohol consumption, and there were no difference between sleep deprivation conditions. If there had been a dose-response relationship, then being more sleep deprived would have resulted in higher alcohol consumption compared to being less sleep deprived. The present study partially supports previous research because alcohol consumption increased during sleep deprivation conditions, but the increase in alcohol consumption during the WC condition brings into question the cause of this increase.

The current study extends the finding by Aalto and Kiianmaa (1984b), which found that REM sleep deprivation increases voluntary alcohol consumption. This study shows that other forms of sleep deprivation could increase alcohol consumption. Specifically, this study suggests that different methodologies for sleep deprivation, reverse flower pot extend and slowly rotating wheel, can increase alcohol consumption. In addition, sleep deprivation that targets all types of sleep equally can increase consumption, and the phenomenon is not just constrained to REM sleep deprivation. It also extends the previous finding to adolescent rats, the present study showed a similar increase in alcohol consumption that had been found in older rats. Finally, it contradicts one potential criticism to Aalto and Kiianmaa. Namely, that stress induced by the electrified grid floor used to sleep deprive rats could have been responsible for the

increased alcohol consumption. The present study shows that alcohol consumption increases even when using sleep deprivation methods (slowly rotating wheel), which do not significantly increase stress (Meerlo, Koehl, Van der Borght, & Turek, 2002). While stress is a potential factor, it was not measured in this study so it is possible that animals were still stress in the wheel conditions, despite previous research to the contrary.

The present study also contributes to previous literature suggesting that sleep deprivation increases alcohol consumption, which strengthens the argument that the relationship between these variables is bi-directional. To clarify, even though we cannot establish a causal relationship, the present study demonstrates that there is a relation between sleep deprivation and alcohol consumption. As previously stated, most of the prior research was unidirectional and only investigated the effect of alcohol consumption on sleep (Chaput et al., 2012; Rosenwasser, Fecteau, & Logan, 2005). The present study supports the theory that the relationship between sleep and alcohol consumption is bi-directional. This has implications for treatments of both alcohol and sleep disorders, because attempting to treat one issue while ignoring the other could result in poor treatment outcomes for patients. Future research could further examine this relationship as bi-directional, and new therapies could test the efficacy of treating both problems concurrently.

The lack of a dose-response relationship between sleep deprivation and alcohol consumption is noteworthy. Given previous research, it would be expected that more sleep deprivation leads to higher risk taking and more drinking. One possible explanation is the sleep deprivation amounts were only 2 hours apart from each other and had a total range of 4 hours, so a larger range of sleep deprivation amounts could be used. However,

even the observed trend did not follow this pattern, with the most alcohol being consumed during the 18hrs condition. It could be that the sleep deprivation amounts were all extreme, given that rats typically sleep 12 hours a night (Mendelson & Bergmann, 1999). Future studies could investigate voluntary alcohol consumption during mild, partial, and chronic sleep deprivation to see if drinking is increased. For example, future studies might induce sleep deprivation for 18, 12, and 6 hours a day, with the smaller deprivation amounts targeted during the sleep portion of the cycle.

The results of the current research suggest that there could be a curvilinear relationship between sleep deprivation and alcohol consumption. This means that alcohol consumption initially increases as sleep deprivation increase then eventually levels out and then decreases when deprivation becomes too extreme. This is one possible explanation as to why the 18hrs condition had the highest amount of alcohol consumption in the present study. In addition, one thing the current study did not measure is the 24 hour pattern of drinking. In this study alcohol consumption was measured once a day, but future research could use lick-o-meters to establish 24 hour profiles of alcohol consumption. By obtaining a 24 hour pattern of drinking, it could make clear how sleep effects factors such as binge drinking and timing of consumption. Such research could investigate which environment the animals are drinking in, specifically to see if there is a difference between drinking in the wheel versus the home cage.

The most interesting and unexpected finding is that alcohol consumption increased during the WC condition relative to the home cage week, but the WC week did not differ from the weeks when the wheel was moving. This indicates that sleep deprivation itself might not be causing the increase in alcohol consumption because the

rats were able to sleep in the WC condition. If sleep deprivation wasn't responsible for the increased alcohol consumption, then it was most likely a confounding variable, such as being removed from the home cage which caused the increase. Three alternative explanations for the increase in drinking during the WC condition relative to the home cage condition are adjunctive behavior, stress derived from removal from home cage, and no relationship.

One explanation for the increase in drinking during the WC weeks is adjunctive behavior. Adjunctive behavior is when a behavior is occasioned by a stimulus, but the stimulus is irrelevant to the outcome associated with that behavior. One of the first studies to research this phenomenon was Falk (1961), the study found that when rats were food restricted and placed on a Variable Interval (VI) schedule with water concurrently available, they drastically increased their water consumption even though they were never water restricted. In this case, drinking water (adjunctive behavior) was occasioned by food delivery (stimulus), but the food delivery is irrelevant to the availability of water. An example in humans is alcohol consumption (adjunctive behavior) being occasioned by a bar environment, though the bar environment is irrelevant to the availability of alcohol. Thus, an increase in drinking at bars could be occasioned by merely being in a bar. Applying this to the present study, drinking alcohol (adjunctive behavior) might have been occasioned by being placed in the wheel (stimulus), but being placed in the wheel is irrelevant to the availability of alcohol. This usually occurs during schedule-induced reinforcement; however, in the present study the "schedule" was not a traditional operant schedule, but instead a change in environment that may have resulted in adjunctive drinking behavior. Therefore, adjunctive behavior could explain why

drinking was increased in the WC condition despite the lack of sleep deprivation. Future studies could eliminate this as an issue by keeping animals in the wheels throughout the experiment, or by sleep depriving animals in the home cage. However, in the present study the animals were in the wheels for an entire week, so they should have acclimated to the environment.

Another possible explanation for the alcohol consumption during WC weeks is the stress associated with unfamiliar environments. This study reduced stress caused by using a sleep deprivation method that has reported no significant increases in plasma corticosterone (Tobler, Murison, Ursin, Ursin, & Borbely, 1983), but the wheel is still an unfamiliar environment so it could have caused an increase in stress in our animals. Hennessy, Heyback, Vernikos, and Levine (1979), found significantly increased corticosterone levels in Sprague-Dawley rats when they were moved from their home cage to an unfamiliar environment. Thus, an increase in stress hormones due to homecage removal could have increased drinking behavior in all wheel conditions, including WC, relative to the home cage. However, while this appears plausible, Meerlo et al. (2002) found that adrenocorticotrophic hormone (another stress hormone) levels were significantly higher in wheels that moved compared to wheels that did not move. Unfortunately, Meerlo et al. only compared the effects of sleep deprivation to a WC condition and did not include a home cage condition in his study. Therefore, it is possible that stress could be significantly lower in the home cage compared to the wheel conditions, which could explain why drinking increased in the WC condition. Future sleep deprivation research utilizing forced locomotion wheels could benefit from including both a WC condition and a home cage condition in conjunction with

experimental conditions to ensure that effects are solely due to the intervention and not other confounding variables.

A final explanation for the alcohol consumption during the WC condition is sleep deprivation might not have any meaningful effects on alcohol consumption. The WC condition was included in the experiment for the explicit purpose of ensuring that any changes in alcohol consumption are due to sleep deprivation and not due to any other variables. The results suggest that there could be a confounding variable responsible. If this is the case then it is plausible that previous research into sleep deprivation and alcohol consumption could be explained by this confound. It could also be the case that sleep deprivation has some effect on alcohol consumption, but another confounding variable has a much larger effect in comparison. This finding disagrees with previous findings and introduces the possibility that the relationship between sleep and alcohol consumption is not causal.

Regardless of explanations for the WC condition findings, the results suggest that there could be confounding factors between sleep and alcohol consumption that we are currently not measuring. For example, previous studies have found that sleep deprivation increases physiological stress (McEwen, 2006). Thus, regardless of sleep deprivation method the lack of sleep could increase stress and in turn increase drinking. Another plausible alternative are behavioral explanations, such as effects due to the removal from the home cage. Thus, the change in environment could increase drinking due to unfamiliarity or because of adjunctive behavior. It is also possible that another confounding factor that has not been considered is responsible for the increasing in drinking that is not sleep deprivation.

The present study obtained results that both support and contradict the hypothesis that sleep deprivation increases alcohol consumption. Therefore, future research must be done to further elucidate the relationship between sleep and alcohol. Specifically, new methodologies need to be established that allow sleep deprivation to be performed in the home cage. Alternatively, animals could undergo an acclimation period in which they spend a week in the wheel before undergoing any sleep deprivation. Both of these interventions would rule out adjunctive behavior as the confounding variable. Additionally, by keeping them in the same place for the duration of the study, environmentally-induced stress could be tested as an explanation. At a minimum, researchers should always utilize both a wheel control and a home cage condition if their aim is to demonstrate causal relationships. However, the present study supports components of previous research as alcohol consumption increased during sleep deprivation.

The present study speculates the cause of the increase in alcohol consumption during the WC condition, but previous research does not provide a definitive answer. However, the results were favorable as alcohol consumption did increase during sleep deprivation weeks. Thus, even if the link is indirect and the exact mechanism is unknown, there is still a need to define the variables that are mediating the relationship between sleep deprivation and alcohol consumption. The present study does not definitely support the theory that alcohol and sleep restriction have a bi-directional relationship. If it is later found that this relationship is bi-directional, it will be necessary for clinical interventions in humans to target both sleep and alcohol issues at the same

time. Further research can help clarify this link and hopefully new methodologies can be used to control or measure the confounding variables implicated in the present study.

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Table 1

*Means and Standard Deviations for 5 Sleep
Deprivation Conditions on Alcohol Consumption*

Variable	<i>M</i>	<i>SD</i>
Home cage (No SD)	1.02	0.61
Wheel Control (No SD)	3.53	2.16
18 hour (SD)	3.96	2.23
20 hour (SD)	3.16	1.87
22 hour (SD)	3.53	1.74

Table 2

Repeated Measures Analysis of Variance for the Effects of Sleep Deprivation on Alcohol Consumption

Source	df	SS	MS	F	<i>p</i>	η^2
Between-group	4	65.15	16.28	9.191	<.001	0.455
Within-group	44	77.98	1.772			
Total	48	143.13				

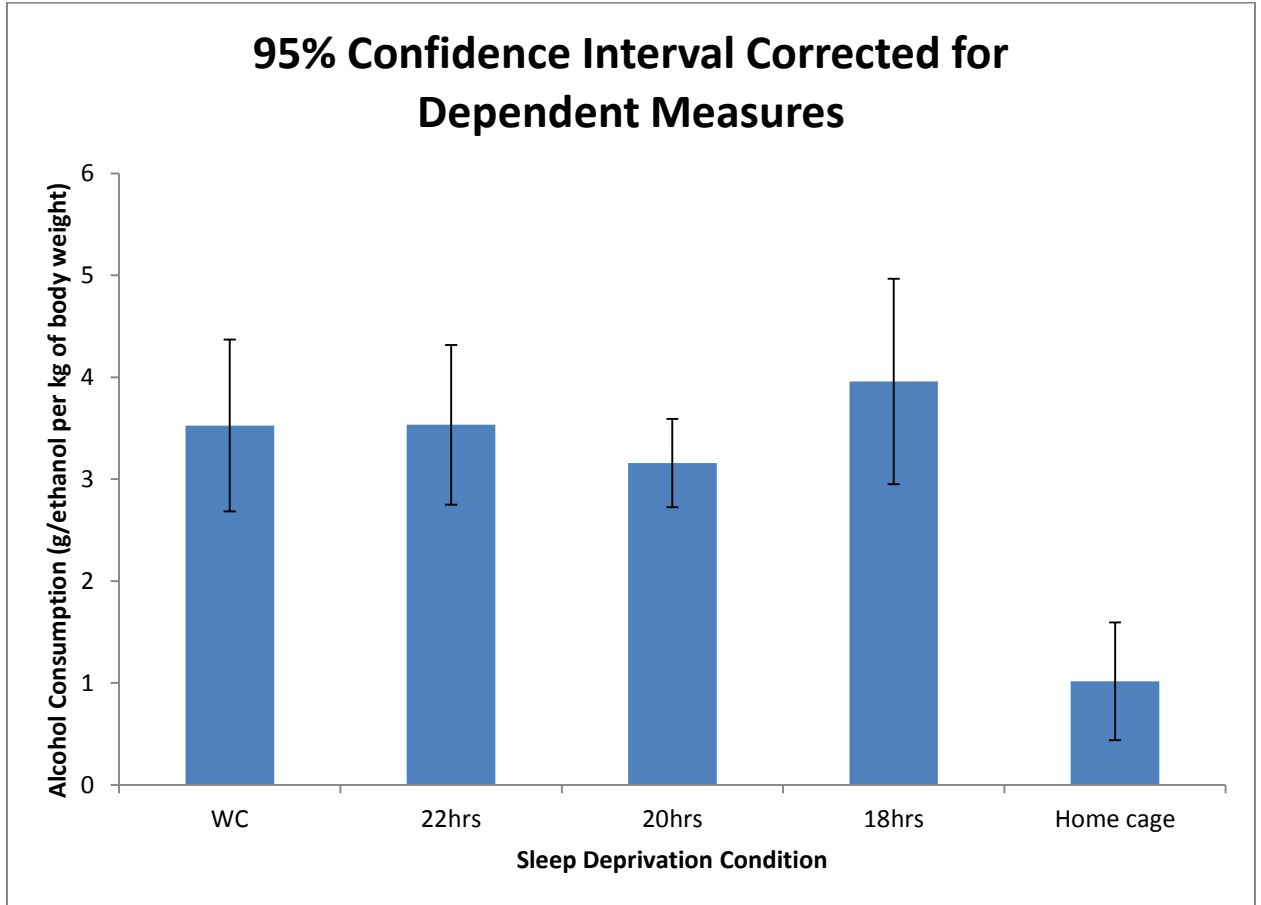


Figure 1. 95% CI across conditions, corrected for dependent measures.

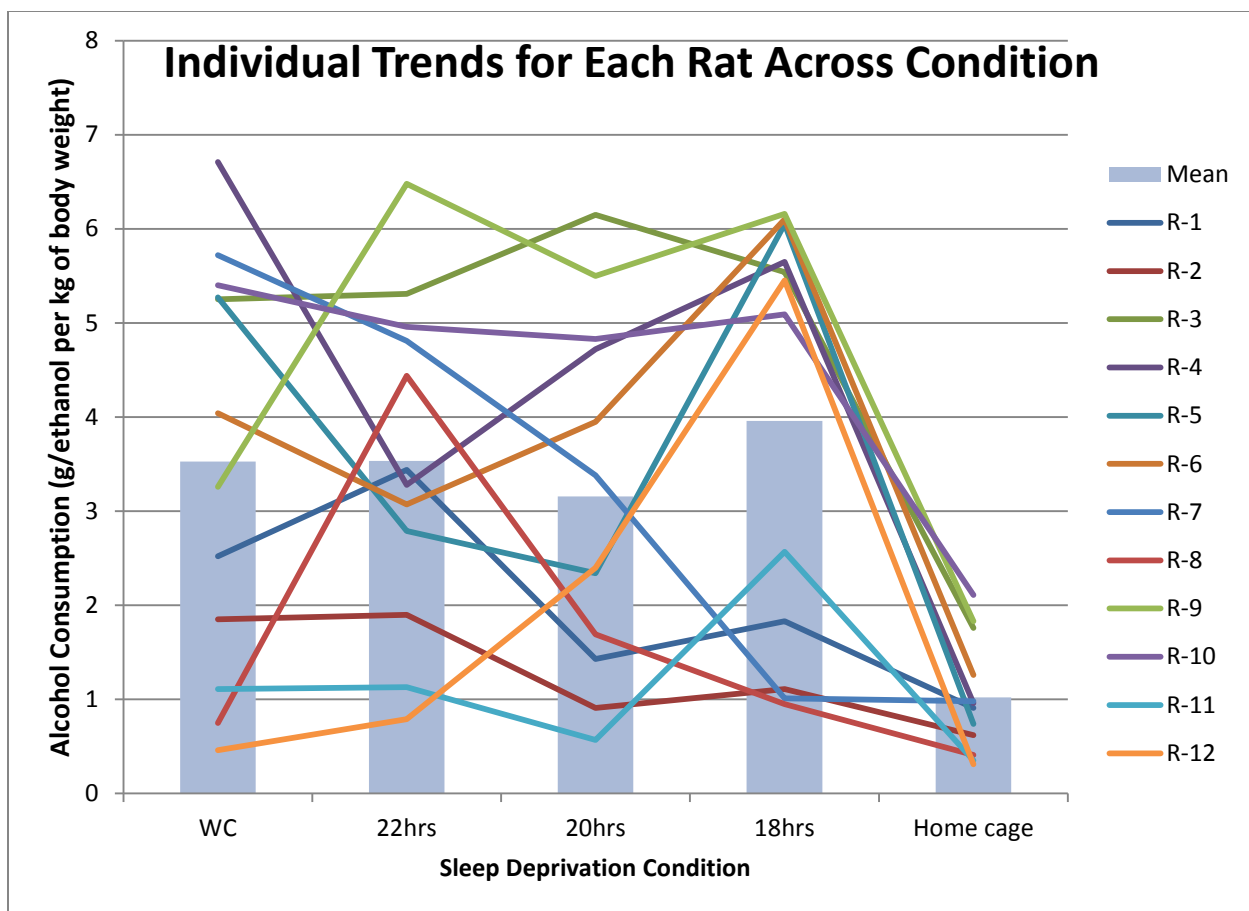


Figure 2. Individual data for each rat across conditions.