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The effects of chronic partial sleep deprivation and chronic voluntary alcohol consumption on Δ Fos B accumulation

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The Effects of Chronic Partial Sleep Deprivation and Chronic Voluntary Alcohol
Consumption on Δ Fos B Accumulation

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Abstract

The present study explores the relation between sleep restriction and alcohol use and the neural substrates that result from chronic behaviors. Accumulation of the transcription factors Δ FosB is suggested as a possible outcome of chronic behaviors, such as addiction. Sleep is discussed as possible mediating factor in the relationship between Δ FosB and chronic alcohol consumption. There were four experimental groups in this study: Control (C), Sleep Deprivation only (SD), Alcohol Exposure only (AO), and both sleep deprivation and alcohol exposure (B). Levels of Δ FosB accumulation in the Nucleus Accumbens (NAc) revealed a significant main effect of sleep deprivation, but no effect of alcohol. Chronic partial sleep deprivation increased Δ FosB in the NAc more robustly than alcohol. Because previous research indicates that Δ FosB is involved in epigenetic modification and neuronal signaling pathways of addiction, sleep deprivation may be implicated in addiction.

The Effects of Chronic Partial Sleep Deprivation and Chronic Voluntary Alcohol Consumption on Δ Fos B Accumulation

Every day, 30 people die in drunk driving accidents in the US alone, amounting to a life lost every 51 minutes (Centers for Disease Control and Prevention, 2015). When used in moderation, alcohol can lower the concentrations of cholesterol and triglycerides associated with coronary heart disease (Rimm, Williams, Fosher, Criqui, & Stampfer, 1999). However, chronic use of alcohol can cause long-term brain damage in areas essential for normal functioning (Harper, 1998). Because of its addictive properties, people will continue using alcohol even after experiencing negative side effects, such as emesis and withdrawal.

Chronic sleep deprivation is a problem that is becoming increasingly prevalent (Basner, Rao, Goel, & Dinges, 2013). Sleepy driving caused an annual economic impact of 43-56 billion dollars in 2005 (Drumer, & Dinges, 2005). There has been extensive research on the impact of total sleep deprivation, but less so on the effects of chronic partial sleep deprivation. Chronic partial sleep deprivation is a form of sleep restriction in which the patient obtains a restricted amount of sleep each night. The National Sleep Foundation recommends that healthy adults should obtain seven to nine hours of sleep a night (Womack, Hook, Reyna, & Ramos, 2013). In 2013, 40% of Americans reported getting less than seven hours of sleep, and the national average was only 6.8 hours (Gallup, 2013).

Both chronic sleep deprivation and chronic alcohol consumption are issues that affect the wellbeing of many in the United States. For that reason, the relation between these two variables must be elucidated. Additionally, there may be long-term epigenetic

consequences of these behaviors not only separately, but also additively. The purpose of the current experiment is to identify the biochemical mechanisms behind the epigenetic effect of alcohol use, sleep deprivation, and the combined effects of both.

Alcohol

There are several theories on craving of alcohol: biochemical-receptoral theories, phenomenological theories, conditioning theories, and cognitive theories (Addolorato, Leggio, Abenavoli, & Gasbarrini, 2005). Biochemical-receptoral theories outline changes to specific structures and neurotransmitter functioning as a result of alcohol use. Phenomenological theories focus on craving and drug seeking and as a main symptom of addictive behaviors (Modell, Glaser, Cyr, & Mountz, 1992). Conditioning theories are behavioral and emphasize environmental cues and conditioned stimuli that predict or precipitate substance abuse. Cognitive theories outline the cognitive processes that affect alcohol consumption, including the dual-affect theory which states that craving is induced by both positive stimuli (cues) and negative stimuli (withdrawal) (Baker, Morse, & Sherman, 1987).

Integrating evidence from these aforementioned models attempts to create a comprehensive approach, known as the three-pathway model (Verheul, van den Brink, & Geerlings, 1999). This three-pathway model identifies a dopaminergic reward pathway, a GABA (gamma-aminobutyric acid) relief pathway, and a serotonergic pathway defined by lack of self-control. The present proposal will focus on biochemical theories of alcohol craving, specifically focusing on the dopaminergic reward pathway.

Alcohol and the Brain

Alcohol has a largely inhibitory effect on the central nervous system because of its interaction with the neurotransmitter GABA. Alcohol has a high affinity for the GABA_A receptor subtype, and chloride channel sensitivity at these receptor subtypes decreases with repeated stimulation (Morrow, Montpied, Lingford-Hughes, & Paul, 1990). This decrease in sensitivity could possibly explain tolerance; decoupling the chloride channel would require a higher dose of alcohol to achieve the same effects. Additionally, twenty-five single-nucleotide polymorphisms (SNPs) have been identified on genes that regulate the GABA_A receptor, indicating that genetic factors can influence the susceptibility to alcohol dependence (Edenberg et al., 2004).

The neural reward system common to all drugs of abuse is the mesocorticolimbic dopamine pathway (Pierce & Kumaresan, 2006). This pathway begins in the ventral tegmental area sends efferent dopaminergic projections to the nucleus accumbens (NAc), hippocampus, amygdala, medial prefrontal cortex, and the ventral pallidum. Specifically, alcohol has been found to directly stimulate dopaminergic neurons in the ventral tegmental area, resulting in increased dopamine release in the NAc (Brodie, Pesold, & Appel, 1999). The mesocorticolimbic pathway has been heavily implicated in reward salience and the modification of reward behavior to habit.

Genetically inherited dysfunctions of dopamine receptors are one possible mechanism for the transgenerational heritability of addictive behaviors. Of the five dopamine receptor subtypes, the subtype most connected to alcohol dependence is the D₂ receptor (D2R). Genetic dysfunctions related to D2R are associated with alcohol abuse (Bowirrat & Oscar-Berman, 2005). Allele genotype has an effect on alcohol sensitivity

and reward pathway behavior (Lawford et al., 1995). Reduced dopamine receptor binding affinity created by the D2R TaqI A1 allele has been implicated in drug cravings following alcohol cessation. The TaqI A1 allele is thought to increase dopamine receptor activation following alcohol consumption. However, there are several variables that could affect addiction phenotypes, including other genomic differences and epigenetic changes.

Recent research has identified transcription factors as a possible contributor to the heritability of dependence. Transcription factors are proteins that bind to DNA and influence the rate at which genes are transcribed into proteins. Transcription factors bind to DNA in several different ways, and transcription factors are often classified by how they accomplish this binding (Latchman, 1997). Genome area responsible for regulating how the transcription factor binds to the DNA is called the DNA-binding domain (DBD). The activation domain is the portion of the protein that regulates the transcription. Transcription factors can have either an activating or repressing effect on gene transcription.

Activation of transcription is accomplished either by stimulation of complex assembly, facilitating the transcription of DNA by RNA polymerase, or it can be stimulated by increasing protein activity after assembly (Latchman, 1997). Activating and repressing transcription factors may be mutually inhibitive, making the quantity of these proteins a strong determinate in the transcription of a select genomic region.

Sleep

Sleep is an important physiologically driven behavior that probably all animals experience in various lengths. Sleep is an active process and is generally defined as a reversible behavioral state characterized by perceptual disengagement and

unresponsiveness from the environment (Carskadon & Dement, 2000). Sleep is characterized by cycles of rapid eye movement (REM) sleep and non-REM (NREM) sleep that last 90 minutes in humans. NREM sleep is broken up into Stage 1 sleep, Stage 2 sleep, and Slow Wave Sleep (Stages 3+4). REM sleep is characterized by wake like EEG measurements, but complete muscle atonia below the neck.

There are several neurobiological systems that are involved with sleep regulation. The wake-on system begins in the reticular formation and projects upwards sending excitatory projections to the cortical areas via the ascending reticular activating system (ARAS), while the sleep-off system starts in the ventromedial lateral pre-optic area (VLPO) and mutually inhibits excitatory ARAS projections (Saper, Scammell & Lu, 2005). More specifically, the reticular formation (wake-on) projects to several areas activating numerous neurotransmitters. The waking system recruits acetylcholine from the laterodorsal tegmental nuclei and the pedunculopontine tegmental nuclei, norepinephrine from the locus coeruleus, dopamine from the periaqueductal grey matter, serotonin from the raphe nuclei, and histamine from the tuberomammillary nucleus (Saper, Chou, & Scammell, 2001). The sleep-off system projects to all of the same areas from the VLPO, however it projects GABA, which inhibits wakefulness.

Stimulation of GABA receptors is common to both sleep and alcohol exposure, however alcohol has interesting effects on sleep physiology. Alcohol consumption results in shorter sleep onset (Roehrs & Roth, 2001). Alcohol results in increased stage 2 sleep and a decrease in REM sleep, and results in greater SWS during the first half of the night and greater Stage 1 sleep during the second half of the night (Roehrs, Yoon, & Roth, 1991). Upon cessation of alcohol, chronic alcohol consumers report sleep fragmentation

immediately, improvement over the first year of abstinence, and continued abnormal sleep for up to two years after cessation (Drummond, Gillin, Smith, & DeModena, 1998). Sleep problems at 5 months following cessation were predicative of relapses at 14 months. This suggests a bi-directional relationship between sleep and alcohol, and thus possibly transcription factors.

Δ FosB

Δ FosB is an activating transcription factor heavily implicated in addiction. FosB is a member of the Fos family that links to, or dimerizes with, Jun family proteins to create the activator protein (AP-1) element (Abate, Luk, Gentz, Rauscher, & Curran, 1990). AP-1 is thought to regulate target genes associated with cell proliferation, and may play role in brain plasticity (Karin, Liu, & Zandi, 1997). FosB is synthesized as an inactive protein when dopamine D1 type neurons are stimulated and must be phosphorylated in order to function (Schuermann, Joos, & Muller, 1991). Specific biological events such as drug use are thought to increase phosphorylation of FosB, but when chronically stimulated, the protein becomes maladaptive in function due to its prolonged half-life.

Δ FosB is a spliced version of the FosB protein that occurs after chronic stimulation of dopaminergic receptors; the gene coding for Δ FosB and FosB is the same, but the proteins can serve different functions. Δ FosB lacks the C-terminus which contains the degron domain (Ruffle, 2014). The degron is the point of degradation for a protein, and the lack of this domain allows Δ FosB to be more stable than FosB. There are currently no methods for staining directly for Δ FosB, however the rapid degradation of FosB allows stains for FosB to be used as long as they do not target the C terminus.

Previous studies have waited 18-24 hours after the final drug administration so only Δ FosB remains (Perrotti et al., 2008). The exact degradation time of Δ FosB is currently unknown.

The relatively long stability of Δ FosB is one of the reasons it has been implicated as a mechanism for alcohol and drug abuse. Bachtell, Wang, Freeman, Risinger, and Ryabinin (1999) were among the first to show that alcohol consumption can affect transcription factors. While this study stained exclusively for the non-truncated FosB, findings revealed an increase in accumulation of FosB after alcohol exposure. More recently, high concentrations of Δ FosB have been found to form monodimers, without Jun family proteins, which means that with enough alcohol exposure, the over expression of Δ FosB could form AP-1 and initiate transcription on its own (Jorissen et al., 2007). Given that Δ FosB increases due to chronic exposure to ethanol (Perrotti et al., 2008), the existence of monodimers strengthens the argument that Δ FosB could be the mechanism responsible for the long-term changes associated with alcohol dependence.

While Δ FosB is relatively stable its over expression alone cannot fully explain the long-term behavioral changes following drug abuse. There are currently two proposed mechanisms as to how Δ FosB mediates changes in addictive behavior; the first is Δ FosB mediates long-term changes in synaptic structure specifically increasing the dendritic spines density, or by causing more permanent change in gene expression through the modification of chromatin (Nestler, Barrot, & Self, 2001). Changes in dendritic formation in the hippocampus and NAc may lead to sensitized responses to drug use and the environmental cues related to it. The second theory assesses how Δ FosB can alter the chromatin remodeling through histone modification (Nestler, 2008). Δ FosB can exert an

epigenetic effect on the expression of target genes directly, by affecting histone modification (via methylation and phosphorylation). These proposed changes are more permanent and provide a possible mechanism for how Δ FosB can initiate long-term changes after overexpression.

Specific to alcohol use, studies have identified the regions in which Δ FosB is most highly expressed. Voluntary alcohol consumption has been found to increase Δ FosB in the NAc core, dorsolateral striatum, and orbitofrontal cortex (Li et al., 2010). Naltrexone, a drug used to treat alcohol dependence, has been found to decrease Δ FosB expression in the NAc, dorsolateral striatum, and the dorsomedial striatum. Ethanol increases Delta Fos B accumulation in the NAc core and the Caudate, an area of the dorsal striatum, more than cocaine, THC, and morphine (Perotti et al., 2008). This further implicates these specific regions in the mesolimbic pathway as integral for addiction and substance dependence.

The role of Δ FosB in sleep regulation has not been well researched. A published abstract found a link between chronic sleep deprivation and Δ FosB overexpression in sleep related brain areas including paraventricular thalamic nucleus, lateral region of the medial preoptic area, and the ventrolateral preoptic nucleus (Hall, Deurveilher, Burns, & Semba, 2013). Gene mapping has found FosB overexpression in response to sleep deprivation in mice (Thompson et al., 2010). Additionally, the levels of FosB significantly increased in the basal forebrain, cerebral cortex, thalamus and cerebellum (Terao, Greco, Davis, Heller, & Kilduff, 2003). Although FosB is the precursor to the spliced variant, it does not cause the same effects on transcription rate. There is a significant gap in the literature regarding the relationship between Δ FosB and sleep,

particularly with respect to chronic partial sleep deprivation, the most common type of sleep restriction.

A possible limitation of previous research on transcription factors and sleep is the confounding effect of stress. Δ FosB expression has been found to increase in response to chronic stress induced by restraint (Perrotti et al., 2004). Considering the stressful nature of certain sleep deprivation methods it is conceivable that stress is an intervening variable that could explain an increase in transcription factors during sleep deprivation. However, it has been found that Hypothalamic-Pituitary-Adrenal response to restraint stress is actually decreased after eight days of sleep restriction (Meerlo, Koehl, van der Broght, & Turek, 2002). Conversely, corticosterone levels remained elevated. Despite contradictory findings on stress and sleep, transcription factors may play a modulating role in the stress response during sleep deprivation.

Additionally, non-drug activities have been found to increase Δ FosB expression. For example, wheel running has been found to increase expression of Δ FosB in the NAc (Werme et al., 2002). Δ FosB may be responsible for both naturally occurring rewards (exercise) and drug-induced rewards (alcohol). Rats that received sugar as a dietary component showed increased levels of Δ FosB in the basolateral amygdala, central nucleus of the amygdala, and the NAc core upon cessation (Christiansen, DeKloet, Ulrich-Lai, & Herman, 2011). Additional research is necessary, but previous experiments suggest that Δ FosB may be a common mechanism for many types of pathological reward behaviors lacking a pharmacological component, such as exercise addiction, gambling, and eating disorders.

The goal of the current study is to begin to elucidate the relationship between Δ FosB, alcohol exposure, and sleep deprivation. Researchers aimed to examine the Nucleus Accumbens (NAc) and the Caudate for reward related areas because Δ FosB has been shown to accumulate in these areas in response to alcohol (Perrotti et al., 2008). Specifically, the present experiment predicted that rats that only experience sleep deprivation will increase Δ FosB expression in the thalamus and the preoptic area (PoA), but not the Nucleus Accumbens (NAc), or the Caudate. Researchers predicted that rats only consuming alcohol will increase Δ FosB expression in a dose-response manner in the NAc, Caudate, but not the thalamus and PoA. Lastly, rats experiencing both sleep deprivation and access to alcohol may have interaction such that they will have increased Δ FosB expression in the thalamus, POA, NAc, Caudate in a summative manner, as both alcohol and sleep deprivation are chronic behaviors thought to induce Δ FosB.

Method

Subjects

The subjects included 16 Sprague Dawley Rats from Harlan Laboratories. Animals were 35 days old at the beginning of the study. The rats were individually housed in plexiglass cages (16in x 9in x 8in), with a metal lid, and cardboard bedding. The rats experienced a 12-hour reverse light dark cycle with lights off at 8am and lights on at 8pm. Rats had access to food, water, and alcohol ad libitum throughout the study, depending on group. There were four experimental groups in this study: Control (C), Sleep Deprivation only (SD), Alcohol Exposure only (AO), and both sleep deprivation and alcohol exposure (B). There were three rats in the C and AO groups, and five rats in the SD and B groups. This inequality in group size was due to the reallocation of rats

from a previous experiment from the same laboratory on sleep deprivation and alcohol consumption.

Apparatus

All subjects, regardless of group, had access to one bottle, which contained tap water at all times. The subjects in the AO and B groups also had access to a second bottle containing the alcohol and water solution. The alcohol was grain alcohol, specifically 151 proof Everclear produced by Luxco, diluted to 7% alcohol. Previous studies have used up to 10% alcohol dilutions without negative effects (Aalto & Kiianmaa, 1984). Researchers added no flavoring component to the alcohol dilution because of the tendency of sucrose to affect Δ FosB (Christiansen et al, 2011).

Sleep deprivation occurred for the SD and B groups in a forced exercise wheel from Lafayette Instrument Company. The sleep deprivation wheel consists of aluminum rungs and polycarbonate sides. The internal width is 4.4in with an internal diameter of 13.38 inches. There are 82 rungs, which are 0.188 inches in diameter with 0.526 inch spacing. The wheel is driven by a motor at 1.5 m/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. Food can be placed inside the wheel. Additionally, each wheel contains a ramp that follows the direction of the wheel rotation. Previous, anecdotal observations in the lab found that minorities of rats were able to attain some sleep by lying on their backs in the wheel without having to perform the forced locomotion. Addition of a ramp ensures that rats are required to stay in constant locomotion.

Procedure

Alcohol Exposure

Beginning on P35, rats in the AO and B groups received free access to both water and alcohol for 3 weeks, previous research observed stability of alcohol consumption at three weeks (Aalto & Khanmaa, 1984). Alcohol and water bottles were counterbalanced in their placement on the cages to control for possible side bias. Rats had variability in their drinking habits during baseline ranging from 1.08 to 4.63 grams per kilogram of body weight ($M = 2.33$, $SD = 1.72$) Sleep deprivation increased alcohol consumption, but a range of consumption values from as low as .46 g/kg to as high as 6.16 g/kg existed between rats ($M = 3.54$, $SD = 1.97$).

Sleep Deprivation

After the 3 weeks of baseline, the sleep deprivation portion of the study began for rats in the SD and B groups. All rats in the SD and B groups were sleep restricted up to 22 hours a week following a previous procedure out of the same laboratory (Sequeira, 2015). Animals in the B and SD groups also experienced a wheel control condition, being a non-moving wheel in which the rats could achieve sleep voluntarily. This attempted to eliminate a possible confound, if any, due to the wheels themselves.

Immunohistochemistry

Upon completion of the sleep deprivation portion, alcohol was removed from AO and B rats. Approximately 18 to 24 hours after the removal of alcohol and cessation of the final sleep deprivation trial, the rats were euthanized via CO₂ inhalation and rapid decapitation. The 18 to 24 hour delay was necessary for the degradation of FosB, leaving only Δ FosB. Brains were extracted and placed in paraformaldehyde for fixation and

stored at 4 degrees Celsius. Researchers sliced the brains on a Leica Cryostat at 50 μ m. Sections of the thalamus, POA, NAc, thalamus and Caudate were washed, and further incubated in a free floating manner with the primary antibody (rabbit polyclonal, 1:500; Sc-48, Santa Cruz Biotechnology, CA, USA) in PBS. The sections were incubated for 2 hours and then incubated in the secondary antibody (Anti-rabbit biotinylated goat, Sc-2053, Santa Cruz Biotechnology, CA, USA) for 30 minutes. Sections were incubated for up to 20 minutes in dye (DAB chromogen). Slices were mounted with DPX. All sections were photographed with a Leica Microscope. Slides were analyzed with the Image-J software in order to quantify the concentration of positively stained cells for each slide.

Results

A 2x2 Factorial ANOVA revealed a significant main effect of sleep, $F(1, 15) = 10.81, p=.006, \eta^2=.47$, such that accumulation of Δ FosB in the NAc was significantly higher during conditions involving sleep deprivation ($M = 227, SD = 55.69$) than for conditions where sleep was not restricted. ($M = 150.17, SD = 38.90$). The main effect of alcohol was non-significant, $F(1, 15) = 1.03, p=.33$. An interaction between sleep and alcohol was non-significant, $F(1, 15) = 2.92, p=.11, \eta^2=.20$ (See Figure 1).

A 2x2 Factorial ANOVA revealed a non-significant main effect of sleep, $F(1, 15) = .451, p=.515$, such that accumulation of Δ FosB in the Caudate did not differ by sleep condition. The main effect of alcohol was non-significant, $F(1, 15) = 1.60, p=.23$. An interaction between sleep and alcohol was non-significant, $F(1, 15) = 1.51, p=.24$ (See Figure 2).

A one-way ANOVA with four levels of condition (C,AO,SD, and B) revealed a significant difference between conditions in Δ FosB accumulation in the NAc, $F(3,12) =$

5.32, $p=.015$, $\eta^2=.57$. A Tukey HSD post hoc analysis revealed that the SD condition had significantly higher accumulation than the C ($p=.019$) and AO ($p=.044$) groups. A similar one-way ANOVA revealed no significant differences in accumulations in the Caudate, $F(3,12) = .982$, $p=.434$.

A Pearson's correlation coefficient revealed a statistically significant positive relationship between Δ FosB accumulation in the NAc and the Caudate in rats that experienced sleep deprivation, $r = .647$, $p = .033$. Rats that experienced no sleep deprivation had no correlation between accumulations in these areas.

A Pearson's correlation coefficient revealed a non-significant relationship between Δ FosB accumulation in the NAc and the Caudate in rats that experienced alcohol conditions, $r = .689$, $p = .059$. Rats that experienced no alcohol also had no correlation between accumulations in these areas.

A Pearson's correlation coefficient revealed a non-significant relationship between Δ FosB accumulation in the NAc and amount of alcohol consumed after exposure to sleep deprivation, $r = .606$, $p = .202$. Thirty six percent of the variance in Δ FosB accumulation in the NAc can be accounted for by the amount of alcohol consumed during sleep deprivation.

Discussion

The primary hypothesis that there would be an additive effect of Δ FosB in the sleep deprivation and alcohol condition was partially supported, there was a significant main effect of sleep such that sleep deprivation increased Δ FosB accumulation in the NAc. However, in an unanticipated finding, the SD group had the highest Δ FosB

accumulation in the NAc. It appears that there was an increase in Δ FosB accumulation between the AO and B conditions, but not in a significant manner. Trends from both non-significant interactions show a clear cross of experimental conditions. It is possible that with an increased sample size and decreased variance, interactions may be present. Accumulation in the NAc and the Caudate were correlated, but only in certain conditions, leading to the conclusion that this relationship may be strengthened or weakened by environmental factors. Alcohol consumption was non-significantly correlated with Δ FosB accumulation in the NAc, however a large effect size indicates that, while not significant, alcohol consumption specifically during sleep deprivation may share a positive relation with Δ FosB accumulation. No staining was observed in the Thalamus or POA in any condition, therefore that data is not presented.

The current research is not supported by previous literature, as there was no statistical difference between Δ FosB accumulation between the C and AO groups. Previous literature has indicated that alcohol significantly increases Δ FosB accumulation (Perrotti et al., 2008; Li et al., 2010) however this trend was not evident in the present experiment. Previous research has also indicated accumulation in sleep related areas (Hall, Deurveilher, Burns, & Semba, 2013), which was not present in the current experiment. However, lack of research on Δ FosB and sleep deprivation may explain the disparity in findings. This is the first study to examine the effects of specifically chronic partial sleep deprivation, which may induce a different biochemical response in the brain. The unanticipated robust accumulation during sleep deprivation may be due to the ARAS. Prolonged activation of the dopaminergic system to maintain wakefulness during sleep deprivation may have been responsible for the significant accumulation.

Another possible explanation for the obtained results may be stress. Because Δ FosB is known to accumulate during stress (Perrotti et al., 2004), it is possible that the animals in the sleep deprivation condition experienced stress that caused the robust accumulation. Alcohol, because of its anxiolytic properties (Barbaccia et al., 1999), may have ameliorated some of the stress of being in the sleep deprivation wheel. It is possible that the SD group, without these effects of alcohol were more stressed in the sleep deprivation wheels. Because Δ FosB has also been shown to accumulate during wheel running exercise (Werme et al., 2002), it is possible that the method of sleep deprivation influenced the findings of the current study. However, the differential expression seen in the Caudate indicates that the wheel conditions (SD and B) were not significantly higher in all areas for Δ FosB accumulation.

Future studies should include an acclimation time in the wheel in an attempt to control for stress. Because there were a small number of animals, the group variance was large. Future studies should incorporate a larger, more balanced sample size. Additionally, because the histological stain had recently been validated in our lab, there may be some experimenter error in performing the stain. Overall, more research is necessary to validate results. However, considering the scarcity of sleep deprivation research, the current findings successfully propose a neurobiological profile of one area of sleep deprivation that affects several members of the working population.

The findings of the current study suggest that exposure to sleep deprivation significantly increases Δ FosB accumulation in the NAc. Findings also suggest a differential relationship of sleep and alcohol in different areas of the reward system that may elucidate their specific roles in long-term addictive behavior. Because previous

research indicates that Δ FosB is involved in epigenetic modification and neuronal signaling pathways of addiction (Nestler, 2008), sleep deprivation may be implicated in addiction. Drug intervention and prevention programs may want to incorporate a strictly regulated sleep hygiene regimen into their treatment programs in order to prevent an increased risk of drug initiation and drug relapse behaviors.

Appendix A

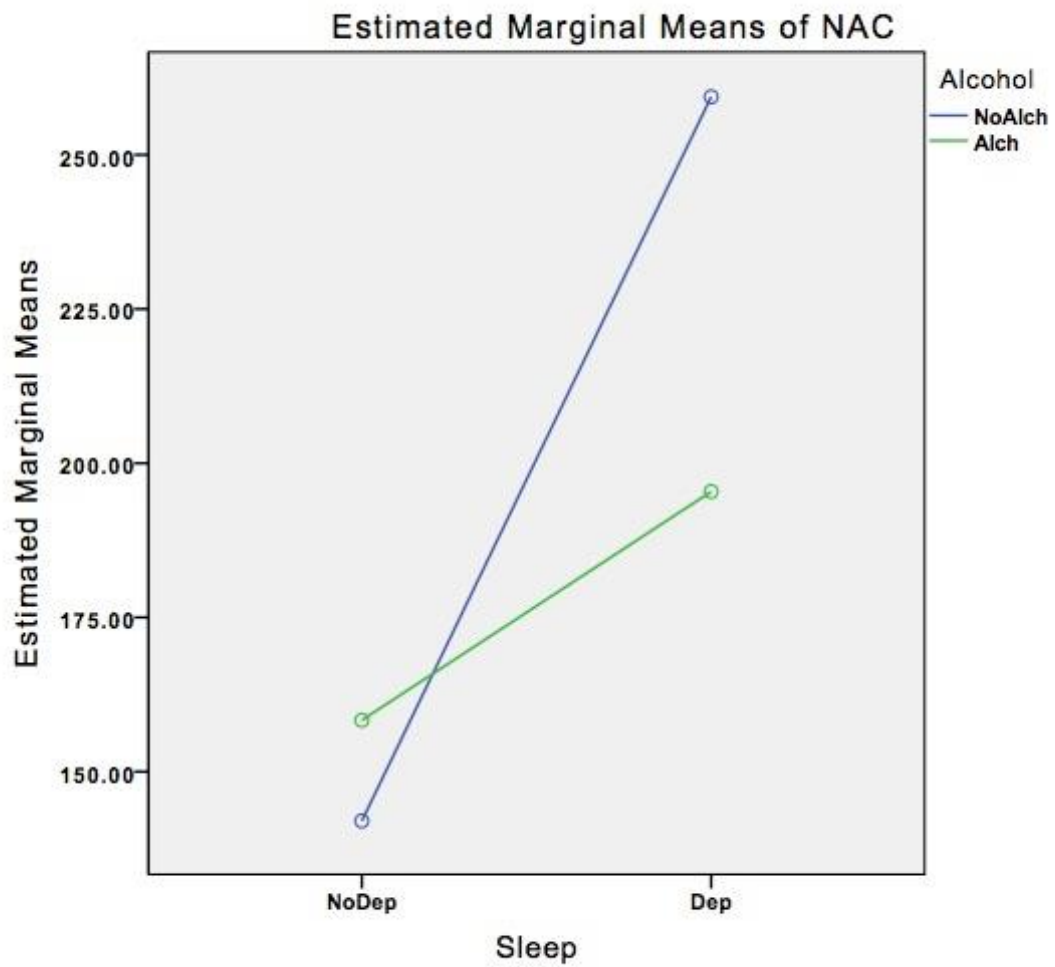


Figure 1. A significant main effect of sleep condition occurs such that sleep deprivation increases Delta Fos B accumulation.

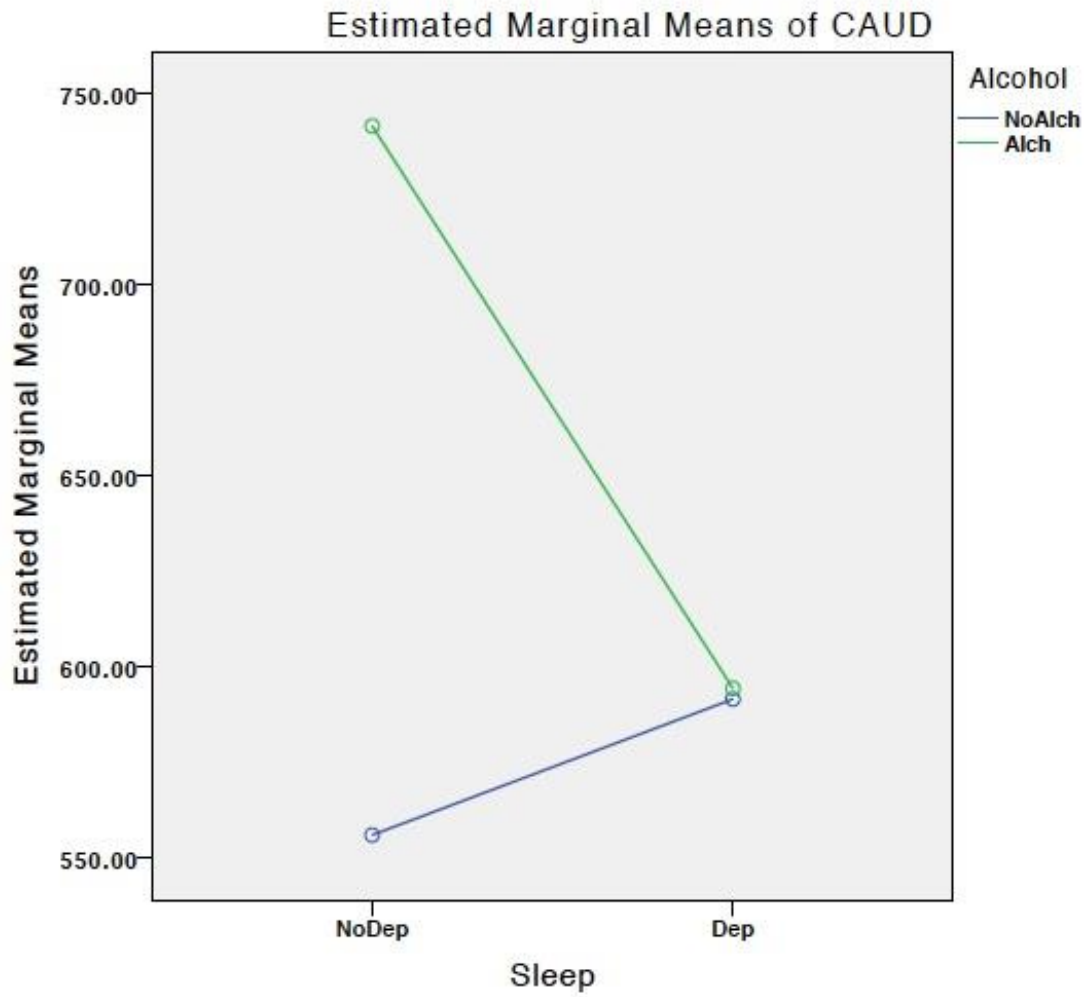


Figure 2. A non-significant interaction between sleep and alcohol conditions on accumulation in the Caudate.

References

- Aalto, J., & Kiianmaa, K. (1984). Increased voluntary alcohol drinking concurrent with REM sleep deprivation. *Alcohol, 1*(1), 77-79.
- Abate, C., Luk, D., Gentz, R., Rauscher, F., & Curran, T. (1990). Expression and purification of the leucine zipper and DNA-binding domains of Fos and Jun: Both Fos and Jun contact DNA directly. *Proceedings of the National Academy of Sciences, 87*(3), 1032-1036.
- Addolorato, G., Leggio, L., Abenavoli, L., & Gasbarrini, G. (2005). Neurobiochemical and clinical aspects of craving in alcohol addiction: A review. *Addictive Behaviors, 30*(6), 1209-1224.
- Aiken, L., & West, S. (1991). *Multiple regression: Testing and interpreting Interactions*. Thousand Oaks, CA: Sage.
- Bachtell, R., Wang, Y., Freeman, P., Risinger, F., & Ryabinin, A. (1999). Alcohol drinking produces brain region-selective changes in expression of inducible transcription factors. *Brain Research, 847*(2), 157-165.
- Baker, T. B., Morse, E., & Sherman, J. E. (1987). The motivation to use drugs: A psychobiological analysis of urges. In P. C. Rivers (Ed.), *The Nebraska Symposium on Motivation: Alcohol Use and Abuse* (pp. 257 – 323).
- Barbaccia, M. L., Affricano, D., Trabucchi, M., Purdy, R. H., Colombo, G., Agabio, R., & Gessa, G. L. (1999). Ethanol markedly increases “GABAergic” neurosteroids in alcohol preferring rats. *European journal of pharmacology, 384*(2), R1-R2.
- Basner, M., Rao, H., Goel, N., & Dinges, D. F. (2013). Sleep deprivation and neurobehavioral dynamics. *Current opinion in neurobiology, 23*(5), 854-863.

- Brodie, M. S., Pesold, C., & Appel, S. B. (1999). Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcoholism: Clinical and Experimental Research*, 23(11), 1848-1852.
- Carskadon, M., & Dement, W. (2000). Normal human sleep: An overview. *Principles and Practice of Sleep Medicine*, 4, 13-23.
- Centers for Disease Control and Prevention. (2015). Injury prevention and control: motor vehicle safety. Retrieved from http://www.cdc.gov/motorvehiclesafety/Impaired_Driving/index.html.
- Christiansen, A., Dekloet, A., Ulrich-Lai, Y., & Herman, J. (2011). "Snacking" causes long term attenuation of HPA axis stress responses and enhancement of brain FosB/deltaFosB expression in rats. *Physiology & Behavior*, 103(1), 111-116.
- Durmer, J. S., & Dinges, D. F. (2005). Neurocognitive consequences of sleep deprivation. *Seminars in neurology*, 25 (1), 17-129.
- Hall, S., Deurveilher, S., Burns, J., & Semba, K. (2013). Neuronal responses to chronic sleep restriction studied using FosB/ Δ FosB immunoreactivity in rats. *Sleep*, 36, 100. Abstract retrieved from Sleep Abstract Supplement. (Accession No. 0278).
- Harper, C. (1998). The neuropathology of alcohol-specific brain damage, or does alcohol damage the brain?. *Journal of Neuropathology & Experimental Neurology*, 57(2), 101-110.
- Edenberg, H., Dick, D., Xuei, X., Tian, H., Almasy, L., Bauer, L., Crowe, R., Goate, A., Hesselbrock, V., Jones, K., Kwon, J., Li, T., Nurnberger, J., O'Connor, S., Reich, T., Rice, J., Schuckit, M., Porjesz, B., Foroud, T., & Begleiter, H. (2004). Variations in *GABRA2*, encoding the α 2 subunit of the GABA_A receptor, are

- associated with alcohol dependence and with brain oscillations. *The American Journal of Human Genetics*, 74(4), 705-714.
- Karin, M., Liu, Z., & Zandi, E. (1997). AP-1 function and regulation. *Current Opinion in Cell Biology*, 9(2), 240-246.
- Latchman, D. (1997). Transcription factors: An overview. *The International Journal of Biochemistry & Cell Biology*, 29(12), 1305-1312.
- Lawford, B., Young, R., Rowell, J., Qualicheckski, J., Fletcher, B., Syndulko, K., Ritchie, T., & Noble, E. (1995). Bromocriptine in the treatment of alcoholics with the D2 dopamine receptor A1 allele. *Nature Medicine*, 1(4), 337-341.
- Li, J., Cheng, Y., Bian, W., Liu, X., Zhang, C., & Ye, J. (2010). Region-specific induction of FosB/ Δ FosB by voluntary alcohol intake: Effects of naltrexone. *Alcoholism: Clinical and Experimental Research*, 34(10), 1742-1750.
- Meerlo, P., Koehl, M., Van der Borght, K., & Turek, F. (2002). Sleep Restriction Alters the Hypothalamic-Pituitary-Adrenal Response to Stress. *Journal of Neuroendocrinology*, 14(5), 397-402.
- Modell, J., Glaser, F., Cyr, L., & Mountz, J. (1992). Obsessive and compulsive characteristics of craving for alcohol in alcohol abuse and dependence. *Alcoholism: Clinical and Experimental Research*, 16(2), 272-274.
- Morrow, A., Montpied, P., Lingford-Hughes, A., & Paul, S. (1990). Chronic ethanol and pentobarbital administration in the rat: Effects on GABA_A receptor function and expression in brain. *Alcohol*, 7(3), 237-244.

- Nestler, E., Barrot, M., & Self, D. (2001). Δ FosB: A sustained molecular switch for addiction. *Proceedings of the National Academy of Sciences*, 98(20), 11042-11046.
- Nestler, E. (2008). Transcriptional mechanisms of addiction: Role of Δ FosB. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1507), 3245-3255.
- Perrotti, L., Hadeishi, Y., Ulery, P., Barrot, M., Monteggia, L., Duman, R., & Nestler, E. (2004). Induction of Δ FosB in reward-related brain structures after chronic stress. *The Journal of Neuroscience*, 24(47), 10594-10602.
- Perrotti, L., Weaver, R., Robison, B., Renthal, W., Maze, I., Yazdani, S., Elmore, R., Knapp, D., Selley, D., Martin, B., Sim-Selley, L., Bachtell, R., Self, D., & Nestler, E. (2008). Distinct patterns of Δ FosB induction in brain by drugs of abuse. *Synapse*, 62(5), 358-369.
- Pierce, R. C., & Kumaresan, V. (2006). The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse?. *Neuroscience & Biobehavioral Reviews*, 30(2), 215-238.
- Rimm, E., Williams, P., Fosher, K., Criqui, M., & Stampfer, M. (1999). Moderate alcohol intake and lower risk of coronary heart disease: Meta-analysis of effects on lipids and haemostatic factors. *BMJ*, 319, 1523-1528.
- Roehrs, T., & Roth, T. (2001). Sleep, sleepiness, and alcohol use. *Alcohol Research and Health*, 25(2), 101-109.

- Roehrs, T., Yoon, J., & Roth, T. (1991). Nocturnal and next-day effects of ethanol and basal level of sleepiness. *Human Psychopharmacology: Clinical and Experimental*, 6(4), 307-311.
- Ruffle, J. (2014). Molecular neurobiology of addiction: What's all the (Δ) FosB about?. *The American Journal of Drug and Alcohol Abuse*, 40(6), 428-437.
- Saper, C., Chou, T., & Scammell, T. (2001). The sleep switch: Hypothalamic control of sleep and wakefulness. *Trends in Neurosciences*, 24(12), 726-731.
- Saper, C., Scammell, T., & Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, 437(7063), 1257-1263.
- Schuermann, M., Jooss, K., & Müller, R. (1991). FosB is a transforming gene encoding a transcriptional activator. *Oncogene*, 6(4), 567-576.
- Sequeira, S.N. (2015). The Effects of Chronic, Partial Sleep Deprivation Voluntary Alcohol Consumption. Unpublished work.
- Terao, A., Greco, M., Davis, R., Heller, H., & Kilduff, T. S. (2003). Region-specific changes in immediate early gene expression in response to sleep deprivation and recovery sleep in the mouse brain. *Neuroscience*, 120 (4), 1115-1124.
- Thompson, C., Wisor, J., Lee, C., Pathak, S., Gerashchenko, D., Smith, K., Fischer, S., Kaun, C., Sunkin, S., Ng, L., Lau, C., Hawrylycs, M., Jones, A., Kilduff, T., & Lein, E. S. (2010). Molecular and anatomical signatures of sleep deprivation in the mouse brain. *Frontiers in Neuroscience*, 4 (165), 1-20.
- Ulery, P., Rudenko, G., & Nestler, E. (2006). Regulation of Δ FosB stability by phosphorylation. *The Journal of Neuroscience*, 26(19), 5131-5142.

- Verheul, R., van den Brink, W., & Geerlings, P. (1999). A three-pathway psychobiological model of craving for alcohol. *Alcohol and Alcoholism*, *34*(2), 197-222.
- Walker, B., Walker, J., & Ehlers, C. (2008). Dissociable effects of ethanol consumption during the light and dark phase in adolescent and adult Wistar rats. *Alcohol*, *42*(2), 83-89.
- Werme, M., Messer, C., Olson, L., Gilden, L., Thorén, P., Nestler, E., & Brené, S. (2002). Δ FosB regulates wheel running. *The Journal of Neuroscience*, *22*(18), 8133-8138.
- Womack, S. D., Hook, J. N., Reyna, S. H., & Ramos, M. (2013). Sleep loss and risk-taking behavior: A review of the literature. *Behavioral sleep medicine*, *11*(5), 343-359.