EFFECTS OF ALCOHOL ABSTINENCE ON CRAVING, STRESS, AND NEUROBIOLOGICAL FUNCTIONING IN MODERATE TO HEAVY ALCOHOL CONSUMERS

BY

RHIANNON ELAINE MAYHUGH

A Dissertation Submitted to the Graduate Faculty of

WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

Neuroscience

May, 2018

Winston-Salem, North Carolina

Approved By:

Paul J. Laurienti, M.D. / Ph.D., Advisor

Sean L. Simpson, Ph.D., Chair

Jonathan H. Burdette, M.D.

Brian McCool, Ph.D.

W. Jack Rejeski, Ph.D.
DEDICATION

This work is dedicated to my family. Thank you for instilling in me the desire for learning, growth, and persistence. You inspired this endeavor.
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my mentor, Dr. Paul J. Laurienti. I am certain that I have never learned so much from one single person. Without your support, guidance, and inspiration this would not have been possible. I would also like to thank my committee members, Drs. Sean L. Simpson, Jonathan H. Burdette, Brian McCool, and W. Jack Rejeski for their support, advice, and encouragement over the last several years. Thank you to all of the members of the Laboratory for Complex Brain Networks for your time and friendship. Finally, thank you to my husband, Jon Mayhugh, for all of your support, patience, and understanding.
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Published 2018 in PLoS ONE doi: 10.1371/journal.pone.0195063

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Submitted February 2018 to PLoS ONE (under review)

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LIST OF ABBREVIATIONS

ACC: Anterior cingulate cortex
ACE: Alcohol craving experience questionnaire
ANS: Autonomic nervous system
ANTs: Advanced normalization tools
AUD: Alcohol use disorder
BAC: Blood alcohol content
BMI: Body mass index
BOLD: Blood-oxygen-level-dependent
CAN: Central autonomic network
CES-D: Center for epidemiological studies depression scale
CIWA-Ar: Clinical institute withdrawal assessment of alcohol
CVT: Cardiac vagal tone
ECG: Electrocardiogram
EI: Elaborated intrusion
EMA: Ecological momentary assessment
fMRI: Functional magnetic resonance imaging
HF-HRV: High frequency heart rate variability
ICC: Intraclass correlation coefficient
PFC: Prefrontal cortex
RSA: Respiratory sinus arrhythmia
RSA-react: Respiratory sinus arrhythmia-during a postural challenge
RSA-rest: Respiratory sinus arrhythmia-at rest
TLFB: Time line follow back
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ABSTRACT

The importance of prevention in alcoholism is underscored by relapse rates as high as 70%. Although further development of addiction treatment remains a high priority, equally important is advancement in prevention. In this regard, attention is warranted on the neural and behavioral markers of vulnerability to transitioning from controlled drinking to AUD. Indeed, the majority of research to date has focused on alcohol dependency. Much less work has focused on a lifestyle including regular alcohol use without a history of AUD. The goal of this dissertation was to provide a step forward in the field of prevention research by studying individuals who exhibit moderate to heavy alcohol consumption (averaging 2.3 servings, 6 days per week). The specific aims were to evaluate the impact of imposed alcohol abstinence and normal drinking routines on stress and craving and to examine whether craving and brain connectivity are moderated by patterns of cardiac vagal tone.

Moderate to heavy alcohol consumers from the local community (n=32, 24-60 years old) were assessed for fluctuations in craving and stress across the day via Ecological Momentary Assessment (EMA) during 3 consecutive days of imposed alcohol abstinence and their normal drinking routine. Cardiac vagal tone (CVT) was assessed during rest and in response to a postural shift. Resting-state functional magnetic resonance imaging data was collected during normal drinking routines and following an imposed abstinence. These data were used to assess changes in whole brain connectivity.

These moderate to heavy drinkers were found to be a heterogeneous group characterized by two phenotypes. At one end of the spectrum were those with lower craving, lower stress, more optimal CVT functioning, and more regionally specific functional brain connectivity. On the other end were those with higher craving, higher stress, dysfunctional features of CVT, and
functional brain connectivity organized for more distributed processing. Although these phenotypes existed on a continuum, they may be characteristics that future research can explore as markers of AUD vulnerability. If future work validates these characteristics, they could be used in conjunction with low risk interventions, such as mindfulness meditation, to guide treatment before addiction takes hold.
CHAPTER I

INTRODUCTION
The difficulty in finding effective interventions for alcohol use disorder (AUD) is demonstrated by relapse rates as high as 70% within 3 months of leaving treatment facilities (Seo, Lacadie et al. 2013). Although addiction treatment development remains critical, the prevention of alcoholism is ideal. Even though prevention of alcoholism may prove as challenging problem as addiction recovery, when the extensive personal and economic cost associated with addiction is considered, it is certainly worth investigation (www.niaaa.nih.gov). The exploration of neural and behavioral markers of vulnerability to transitioning from controlled drinking to AUD is needed if the goal of prevention is to be achieved. Understanding these markers is the first step towards making the goal of prevention a reality. However, the majority of research to date has focused on alcohol dependency. As drinking patterns vary greatly, those diagnosed with an AUD are at the extreme end of the spectrum. Much less work has been done in those with a lifestyle that includes regular alcohol exposure but have not, at least of yet, progressed to AUD.

Although factors that contribute to AUD risk are much more complex than drinking patterns alone, guidelines for low risk alcohol exposure have been established. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) has published that only 2 in 100 people drinking within their “Low Risk for AUD” criteria reported having AUD (www.niaaa.nih.gov). This is defined as women drinking no more than 3 drinks per day and 7 drinks per week or men drinking no more than 4 drinks per day and 14 drinks per week. Those that exceed this limit have an elevated risk of AUD, however more research is needed to understand factors related to this risk. The National Institute on Alcohol Abuse and Alcoholism director, Dr. George Koob, has expressed this concern stating, “The science just hasn’t been done” (Aubrey, 2015).
This dissertation is dedicated to providing a step forward toward the greater goal of prevention by studying moderate to heavy drinkers that consumed alcohol regularly, averaging 2.3 servings 6 days per week. This routine alcohol consumption allowed for the assessment of the impact of alcohol abstinence in those with no history of alcohol use disorder (AUD). Those that consume alcohol in less regular patterns may not be affected by an imposed period of alcohol abstinence in any meaningful way. As alcohol abstinence is a known stressor in those with alcohol dependence and is associated with a variety of neurobiological changes (Koob and Volkow 2010), here we examine the impact of a period of imposed alcohol abstinence on subjective experience, autonomic nervous system (ANS) functioning, and functional brain network connectivity compared to during routine drinking within the same individuals. Understanding the impact of alcohol abstinence in moderate to heavy drinkers may provide important insight, as abstinence is associated with a variety of changes in both subjective experience and neurobiology in addiction.

Considering the routine nature of those drinking in this pattern, some will eventually transition to AUD, however, others will not. This study was not designed to determine ultimate vulnerability to AUD. However, it did aim to investigate heterogeneity within this group by identifying characteristics for further exploration as potential markers of future AUD. In addition, this study also provided a baseline for which to compare how other alcohol consumption patterns might vary in their neural and behavioral responses to alcohol abstinence. The first section of the Introduction provides a review of alcohol craving as it relates to drinking behavior and AUD. The second section describes what is known about stress, a known trigger for alcohol craving, and its
potential role in AUD. The third section discusses Ecological Momentary Assessment (EMA) methodology, the primary approach used here to assess craving and stress, and discusses related findings in the literature. The forth section reviews the literature on functional magnetic resonance imaging (fMRI) research on the brain and addiction. The fifth section details what is known about the role of the autonomic nervous system (ANS) in the experience of craving as well as Respiratory Sinus Arrhythmia (RSA), which was the methodology used here to assess ANS functioning. The sixth section discusses what is known about the functional link between the brain and the ANS. The seventh and final section of the Introduction outline the three experiments designed and conducted to address hypotheses on the effects of alcohol abstinence on behavioral and neurobiological functioning in moderate to heavy alcohol consumers.

1.1 Craving and Addiction

Craving is the term used to describe a *strong desire* or *urge* for a behavior or substance and has become an important marker of alcohol dependence. Evidence for the importance of craving in AUD is supported by research showing that levels of craving improves the ability to make the distinction between normal use versus abuse and abuse versus dependence (de Bruijn, Korzec et al. 2004).

Although craving seems like a fairly simple concept, extensive research has lead to a variety of theories and perspectives ranging from physio-pathological to cognitively focused explanation of craving (Addolorato, Leggio et al. 2005). Taken together, these theories point to the importance of considering the cyclic nature of addiction; involving an interplay between external (i.e. environmental) stimuli and the impact it has on
internal processes that then influence behavior, thereby effecting one’s exposure to these external influences.

Conditioning theories emphasize the importance of “cues” in addiction. Cues are conditioned stimuli in the environment, such as the smell of alcoholic beverages or walking past a restaurant one often drinks at, which through repetitive pairing with alcohol consumption can become a trigger of craving for substances of abuse (Drummond 2000). Internal states, such as emotion and stress, can also become antecedent cues for craving. This is thought to happen with repeated pairing of these cues with rewarding feelings experienced from alcohol consumption. The Elaborated Intrusion theory of desire focuses on the immediate experience of desire and is rooted in the idea that imagery is a key element of craving (May, Andrade et al. 2004, Kavanagh, Andrade et al. 2005). This theory emphasizes that desire is a conscious cognitive experience that is strongly influenced by affect as well as unconscious antecedents of desire. These thoughts can be intrusive, are triggered by external and internal cues, and are accompanied by elaborations about the target (i.e. alcohol) that then drive continued ruminations about the target.

Over time, one can lose the ability to override the desire for alcohol triggered by these cues and alcohol consumption then becomes a more habitually driven behavior. In other words, a shift from goal-directed behavior to habitual alcohol seeking occurs (Barker and Taylor 2014). In controlled, non-habitual alcohol use, one weighs out the pros and cons related to the decision to consume alcohol and makes responsible decisions accordingly. Once one has progressed to more habitual drinking behavior, exposure to a cue for craving can lead to the decision to drink regardless of the potential negative
consequences of doing so. This pattern has been described to occur by a progression through stages of the addiction cycle.

Three main stages in the addiction cycle have been identified and each is associated with a range of neuroadaptations that drive drug-seeking behavior in addiction (summarized in Figure 1) (Koob and Volkow 2010). The binge/intoxication stage is when the individual experiences the feel good, rewarding impact of the drug. This is also the stage when stimulus-response learning occurs and craving cues are formed. This stage involves extensive neurocircuitry as illustrated in the blue section of Figure 1. When the substance, such as alcohol, is consumed dopamine (DA) increases in the ventral striatum which is thought to be fundamental in the biological basis of the behavioral reinforcement of substance use (Koob and Volkow 2016). With repeated exposure, the drinking behavior is increased due to this positive reinforcement. Cue learning takes place by associating co-occurring stimuli with the rewarding experience of drug taking. This transition has been associated with a progression of cellular changes occurring in the dorsal striatum. This brain region has been shown to be important to the development of stimulus-response learning, with automatic/habitual behaviors associated with gradual engagement of dorsal striatal mechanisms (Koob and Volkow 2010).
Figure 1.1: The 3-stage cyclic model of addiction showing neuroadaptations and related brain areas (used with permission from (Koob and Volkow 2010)).

The next stage involves withdrawal from the substance and the associated negative affect. This stage has been related to a variety of negative internal states, such as dysphoria and emotional distress, and is accompanied by a range of biological changes. As depicted in the red section of Figure 1, the extended amygdala is heavily involved in this stage and is thought to integrate brain stress systems with hedonic processing, driving this state of negative affect. Evidence has also shown increased dopamine in the amygdala during craving, suggesting the important role of the amygdala during the Preoccupation/Anticipation stage as well (Koob and Volkow, 2016). Section 1.2 provides further discussion on the role of stress in addiction.

Preoccupation/anticipation is the final stage. This stage is marked by an increase in the experience of craving. There is also enhanced sensitivity to both external (i.e. smell of your favorite drink) and internal (i.e. stress) cues. This drug and cue induced craving has been associated with a range of biological changes as shown in the green section of
Figure 1. For example, changes in amygdala functioning have been related to the conditioned cues that trigger these cravings and prefrontal cortex changes effect executive control over habitual behaviors (Koob and Volkow 2010). Altered insular functioning has also been observed, which has been related to the integration of these sensory experience, bringing the sense of craving into an embodied, conscious awareness (Craig 2011). These changes are not limited to the brain as altered ANS functioning has also been related to internal triggers of craving, such as stress (Sinha, Fox et al. 2011). If the craving for alcohol is strong enough, and the ability to inhibit these urges are insufficient, then the cycle continues and the binge/intoxication stage begins again.

1.2 Stress and Addiction

As was noted in the previous section, stress has been identified as playing an important role in the addiction cycle. Stress has been shown to increase the chance of relapse in recovering abstinent alcoholics and promote drinking escalation (Breese, Chu et al. 2005, Sinha, Fox et al. 2011). One general theme that has been identified in the progression to addiction is the transition from initial use, to impulsive use, to compulsive use (Koob, Buck et al. 2014). All along this continuum, both subjective stress and biological mechanisms related to stress have been noted as important. Initially, drug use is motivated by the pleasurable, positively reinforcing effects of the substance. Repeated drug use over time augments this pleasurable effect, eventually becoming a way to find temporary relief from the negative emotional state (i.e. stress) experienced during withdrawal and craving. This is a primary component of the withdrawal/negative affect stage of the addiction cycle and is driven by a range of alterations to brain circuitry, as
This transition involves a complex relationship between alcohol and stress, with alcohol both increasing and alleviating stress. Alcohol has been shown to have anxiolytic properties, suggesting that the motivation to drink may come, at least partially, from this ability to reduce stress and anxiety (negative reinforcer) (Becker 2017). Acute alcohol exposure also acts as a stressor via activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, a main component of the neuroendocrine stress response (Smith and Vale 2006). Koob and colleagues have suggested that “...an overall conceptual theme …is that drug addiction represents an excessive and prolonged engagement of homeostatic brain regulatory mechanisms that regulate the response of the body to stressors” (Koob, Buck et al. 2014).

1.3 Ecological Momentary Assessment

Considering that craving and stress levels are greatly impacted by the external environment and typically fluctuate across the day (Shiffman, Stone et al. 2008, Wray, Merrill et al. 2014, Swendsen 2016), utilizing methods that can capture these fluctuations is important. Ecological Momentary Assessment (EMA) is one of these methods and is becoming an increasingly prevalent technique for recording participant data. This approach takes advantage of technology, such as smart phones and smart phone applications, to collect data from participants while they are participating in daily activities within the environment in which the behaviors or physiology of interest naturally occurs. This form of assessment allows for measurements in real time so that
participants may provide data reflective of their realities without disrupting their daily
routines (Shiffman, Stone et al. 2008). It also provides a way of avoiding the reliance on
historical recall of past experiences, which is the primary approach used in past research.
Research that focused on triggers of craving, such as substance abuse or desire for food,
is especially positioned to benefit from this methodology as environmental cues are a key
driver of craving (Wray, Merrill et al. 2014).

EMA methodology has already begun to increase our understanding of alcohol
use. For example, craving intensity across the day has been shown to predict greater
number of drinks consumed in both dependent and non-dependent heavy alcohol drinking
adults (Ray, Miranda et al. 2010, Fazzino, Harder et al. 2013). Ray and colleagues also
found that the urge to drink was positively associated with alcohol consumption in the
same drinking episode in non-treatment seeking, heavy drinkers (Ray, Miranda et al.
2010). This relationship also existed among outpatients who were newly seeking
treatment for a variety of substance use disorders (Fatseas, Serre et al. 2015). In addition,
Fatseas and colleagues found a strong association between EMA craving intensity and
substance use, with alcohol addiction demonstrating a stronger concurrent
craving/consumption relationship than other substances. A mediating effect of craving
levels on the association between cue exposure and later substance use was also shown,
again demonstrating the influence of craving on control over consumption behavior.
Craving during the first 3 servings (ascending limb of intoxication) of a drinking episode
in non-treatment seeking heavy drinkers was also associated with greater alcohol
consumption across sessions (Miranda, MacKillop et al. 2016).
EMA assessments of stress have also provided insight to substance use. Although the majority of EMA research on substances of abuse has focused on tobacco, overall, stress has been found to be positively associated with craving levels (Serre, Fatseas et al. 2015). Specific to alcohol use, morning reports of stress were predictive of time until the next drink in frequent alcohol consumers (Epler, Tomko et al. 2014). Many EMA assessments of alcohol use assessed affect or mood and facets of negative affect/mood often included stress. For example, in female college student drinkers with low response inhibition, negative mood before drinking was positively associated with the number of drinks consumed (Dvorak, Pearson et al. 2016). These findings demonstrate the power of EMA and how it may be used to study the biology behind craving.

1.4 Functional Brain Imaging and Alcohol Use Disorder
fMRI studies have extended our knowledge of these neuroadaptations in addiction. These studies have mainly looked at changes in brain functioning during alcohol cue exposure and how these changes relate to risk of relapse and craving. Historically, the majority of these studies measured whether activity changed in certain brain areas during exposure to cues and whether that differed in dependence vs healthy controls (Buhler and Mann 2011). These studies revealed altered mesocorticolimbic activity, a DA projection circuit important for goal directed and reinforcing reward-related behavior in alcohol dependence. The ventral striatum repeatedly showed increased activity during alcohol cue exposure, risk for relapse, and craving. In alcohol dependent individuals, altered brain activity has also been shown in the ventromedial prefrontal cortex, anterior
cingulate cortex, and precuneus (Seo, Lacadie et al. 2013). This altered activity was correlated with alcohol cue and stress induced craving.

These findings point to the broad range of brain areas that are affected in addiction. It is increasingly being acknowledged that these regions often overlap with areas associated with identified functional brain networks (Volkow and Baler 2013). Functional brain network analysis is a methodology used to study the brain as a system, with the focus being on the interactions between regions instead of changes in the regions themselves. To capture these patterns of interactions, different network variables can be used to understand the organization of the network (see Bullmore and Sporns, 2009 for more details on the following descriptions). One commonly used variable that reflects local efficiency of information transfer is the clustering coefficient, which is the number of connections between the nearest neighbors of a node as a proportion of the maximum possible connections. Networks with high clustering support information flow that is regionally organized and less distributed across the entire network. Measures such as global efficiency can be used to understand the ability of information to be distributed across the network. Networks with high global efficiency support information flow that is distributed across the network with limited regional segregation. Global efficiency is the inverse of path length, a measure based on the average minimum number of edges required to travel from one node to another across the network. These two measures together provide a sense of overall network organization; whether it is subdivided into patterns that reflect more neighborhood organization or more evenly distributed connections overall.
Connectivity in those with a high risk for AUD (as assessed by familial density of AUD) revealed decreased clustering and local efficiency compared to healthy low risk males (Holla, Panda et al. 2017). In addition, whole brain networks of alcohol dependent patients exhibited reduced average clustering with more severe alcohol use (Sjoerds, Stufflebeam et al. 2017). These findings point to network organization that may be less segregated or specialized in alcohol dependence. Functional connectivity analyses in alcohol dependence have also shown differences during resting state (absence of any cue or task) in a range of far-reaching brain subnetworks. For example, weaker within network connectivity and expanded outside-network connectivity in the default mode, salience, reward, and executive control networks during resting state was correlated with poorer cognitive performance and mood in sober alcoholics (Muller-Oehring, Jung et al. 2015). Although this work used a different approach to examining brain network connectivity than the current study, it still reflects network organization that is less segregated, or specialized, in alcoholics.

Emotional and motivational processing has been shown to affect brain connectivity by increasing signal communication between regions, most notably between cortical and subcortical regions (Kinnison, Padmala et al. 2012). As network analysis and its interpretation continue to advance, a shift away from the view that certain brain areas are responsible for AUD-related behaviors is occurring. It is already apparent that brain areas and circuits affected in addiction have much in common with that related to emotional processing. For example, Pessoa explains that the cortical-subcortical changes seen in emotional processing should be viewed in context of the larger scale network interactions, with interactions such as cortex-amgydala regional connectivity viewed as a
“momentary circuit” occurring at a given time (Pessoa 2017). In other words, brain regions and circuitry that have been shown to alter in addiction should not be seen as exclusive to addiction. Instead they should be viewed as belonging to multiple networks simultaneously, which can change at any given moment. Addiction also should not be seen as limited to this circuitry, but involving complex interactions spanning the entire brain.

1.5 Autonomic Nervous System and Alcohol Use Disorder

The ANS is a component of the peripheral nervous system that provides the brain with sensory information from visceral organs and, in turn, regulates these organs. When the two branches of this system, the sympathetic and parasympathetic, are working properly the body is able to maintain a healthy balance between appropriate reactions to environmental challenge and return to restorative, homeostatic states.

Cardiac vagal tone (CVT) is a measure of ANS functional health and reflects the ability of the ANS to react appropriately to afferent influences by regulation of the heart via efferent pathways of the vagus nerve. As described by the polyvagal theory, mammals have evolved to possess two branches of the vagus nerve, each supporting biological functions and behaviors that allow for adaptive interaction with the environment (Porges 2009). The unmyelinated branch originates in the dorsal motor nucleus of the vagus, is shared with other non-mammalian vertebrates, and is associated with primal survival strategies (i.e. freezing behavior when threatened). The myelinated branch, originating in the nucleus ambiguous, has been shown to foster calm states by
inhibiting the sympathetic nervous system and dampening the HPA axis; thereby allowing for more sophisticated behavioral and affective responses (Porges 2001).

CVT can be estimated by assessing respiratory sinus arrhythmia (RSA). This method is thought to be a more sensitive measure of heart rate variability compared to more descriptive measures, such as beat-to-beat variability, as it reflects neural functioning (vagal efferents to the heart) (Porges 2009). RSA captures heart-rate variability within the respiratory frequency band and reflects the degree to which one is flexible and efficient in their response to fluctuating demands. This measure ultimately reflects the “vagal brake” which, when on, slows heart rate during periods of low threat. When the “vagal brake” is released, heart rate increases to meet the metabolic demands of the threat. It is thought that both resting and phasic vagal (also referred to as vagal reactivity) functioning are important indicators of ANS health and research has shown that both are important to emotional regulation (Balzarotti, Biassoni et al. 2017).

CVT has not only been shown to reflect one’s vulnerability to stress (Porges 1995), but is sensitive to alcohol craving and risk for AUD as well. For example, dysregulated ANS functioning has been shown to play an important role in enhanced sensitivity to negative emotions, such as stress, and alcohol craving (Sinha, Fox et al. 2009). In addition, dysregulated ANS response to stress in youth at risk for substance use disorders reported greater substance use including drinking alcohol more frequently (Evans, Greaves-Lord et al. 2015). Significantly greater changes in heart rate variability in response to emotional and alcohol cue pictures were observed in those considered at high alcohol risk (greater alcohol use and cited disinhibition and negative affect suppression as reasons to drink) compared to the rest of the group (normative) (Mun, von
Eye et al. 2008). In alcohol dependent outpatients, heart rate variability was also found to predict craving, providing further support for the importance of understanding the connection between ANS functioning and risk for addiction (Quintana, Guastella et al. 2013).

1.6 The Functional Link between the Autonomic Nervous System and the Brain

Research on the biological basis of addiction is not limited to brain functioning, but includes changes in the ANS as well. For example, a link between CVT and the brain has been described by the neurovisceral integration model and provides insight to understanding emotional regulation. This model describes emotions as self-regulatory responses that, when appropriate, allows the individual to engage in flexible behavior that is goal-directed and adaptable to the changing environmental demands (Gyurak and Ayduk 2008). This self-regulatory behavior is thought to involve integrated neural feedback mechanisms of the central and autonomic nervous systems. The central autonomic network (CAN) is a functional unit that describes this integration and involves a range of brain areas including, but not limited to, the anterior cingulate cortex, insula, ventral medial prefrontal cortex, and amygdala (Thayer and Lane 2000). Output from the CAN is directly linked to heart rate variability and sensory information from the peripheral nervous system is then relayed back to the CAN. This system provides the mechanisms by which goal-directed behavior and adaptability occur and coordinates cardiac, affective, attentional, and behavioral responses (Thayer and Lane 2000). This relationship is thought to involve a reciprocal inhibitory neural circuit in which subcortical structures involved in stress response related defensive behaviors (i.e. the
amygdala) are under inhibitory control of the prefrontal cortex. These frontal-subcortical circuits play an important role in the modulation of two opposing pathways (Masterman and Cummings 1997). These pathways start off as excitatory projections from the frontal cortex to the basal ganglia, which then diverge as striatal output via direct and indirect pathways. The direct pathway is ultimately excitatory to the frontal cortex and the indirect pathway is inhibitory. Disruption in these feedback circuits has been related to a variety of alterations in affect, cardiac functioning, and motor behavior (Thayer and Lane 2000). Under stress, this tonic prefrontal inhibition would be suppressed resulting in withdrawal of the parasympathetic nervous system (release of the “vagal brake”); allowing for the sympathetic nervous system to activate and metabolically support appropriate stress behaviors (Balzarotti, Biassoni et al. 2017).

The baroreflex, mediated by the autonomic nervous system, is also an important mechanism in this process as it maintains consistent levels of blood pressure. When blood pressure is elevated, heart rate reflexively decreases causing blood pressure to decrease. This decrease in pressure causes baroreflex activation and heart rate then increases to restore the pressure. This reflex is critical for the body to appropriately adjust for the decrease in blood pressure when shifting from a seated to standing position. (Tanaka, Sjoberg, et al., 1995, Duscheck, Werner, et al., 2013). This baroreflex involves baroreceptor neurons, which monitor and inform the brain about changes in blood pressure (Duscheck, Werner, et al., 2013). Afferent vagal feedback from cells, such as the baroreceptors, influences the central nervous system mechanisms involved in RSA regulation (Porges 2007).
Although much of the research described above was developed with a focus on emotional regulation, it is also relevant to addiction. Heavy alcohol exposure has been shown to involve dysregulation of the HPA axis and altered heart rate variability, suggesting reduced inhibitory feedback control (Thayer, Hall et al. 2006). Neural circuits related to control over emotion and cognition, both important processes in addiction, undergo neuroadaptations with chronic alcohol exposure. For example, alcohol related neuroadaptations have been observed in the prefrontal-striatal-limbic circuit, which is known for involvement in modulation of emotion (Buhler and Mann 2011, Seo and Sinha 2015). These areas also play a role in reward (striatum), stress (amygdala), and inhibitory/executive functions (prefrontal cortex); all important in addiction.

1.7 Dissertation Outline

The focus of this dissertation is on examining heterogeneity among moderate to heavy drinkers by assessing the impact of a period of imposed alcohol abstinence on subjective experience, autonomic nervous system (ANS) functioning, and functional brain network connectivity. Chapter II details an experiment designed to determine how alcohol craving, stress, and the relationship between craving and stress vary across the day. The first hypothesis was that craving would increase across the day with higher craving experienced during days of abstaining from alcohol. On normal drinking days, craving before drinking was compared to craving after drinking. It was expected that drinking would substantially reduce EMA craving and that craving would be higher in those with greater Alcohol Craving Experience Questionnaire (ACE) scores. Due to the impact of negative affect on craving, we expected participants with higher ACE scores to report
higher EMA stress across the day and to exhibit a stronger relationship between EMA stress and craving.

Chapter III details an experiment designed to determine how resting CVT, CVT reactivity to a postural challenge, and their interaction influenced craving during imposed alcohol abstinence and usual drinking routine. CVT was hypothesized to be related to craving experienced across the day. More specifically, those with more optimal CVT functioning were expected to have lower craving and those with less optimal CVT functioning were expected to have higher craving.

Chapter IV details an experiment designed to determine whether brain networks were associated with CVT in moderate to heavy alcohol consumers during their normal drinking routine and following an imposed period of alcohol abstinence. The impact of the interaction between RSA-rest and RSA-react on functional brain connectivity was assessed. It was hypothesized that imposed alcohol abstinence would affect brain network organization and that this change would be related to CVT functioning.

Chapter V concludes the dissertation with a summary of the findings and a discussion on how the results fit into our current understanding of addiction. The discussion also addresses what the findings have added to the literature and suggests potential avenues for future research.
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CHAPTER II

DIFFERING PATTERNS OF STRESS AND CRAVING ACROSS THE DAY IN MODERATE-HEAVY ALCOHOL CONSUMERS DURING THEIR TYPICAL DRINKING ROUTINE AND AN IMPOSED PERIOD OF ALCOHOL ABSTINENCE

Rhiannon E. Mayhugh, W. Jack Rejeski, Meredith R. Petrie, Paul J. Laurienti, Lise Gauvin

Published 2018 in PLoS ONE doi: 10.1371/journal.pone.0195063
Abstract

Background: Stress is a known factor related to alcohol use. However, how the relationship between alcohol craving and stress varies across the day is not fully understood. As craving is a consistent predictor of alcohol use disorder (AUD), understanding stress and craving patterns across the day in routine, non-dependent, moderate-heavy alcohol consumers may help in understanding those who may be vulnerable to transitioning into AUD.

Method: Moderate-heavy drinkers were recruited from the local community (n=32) and assessed for fluctuations in craving and stress intensity across the day via Ecological Momentary Assessment (EMA) during 3 consecutive days of imposed alcohol abstinence (abstained trial) and their normal drinking routine (normal trial). A multilevel modeling statistical approach assessed differences in diurnal craving and stress patterns with the Alcohol Craving Experience Questionnaire (ACE) examined as a moderator.

Results: Immediately following alcohol consumption on normal trials, EMA craving levels were significantly reduced compared to pre-drinking levels. Moreover, the post-drinking craving levels were lower than on abstained trials. Higher ACE scores were associated with significantly higher EMA craving across the day and higher peaks at midday. Higher ACE scores were also associated with greater EMA stress across the day. Drinking relieved stress relative to abstained trials, but not in individuals with higher ACE scores. Higher stress was associated with greater EMA craving, which was stronger among those with higher ACE scores.

Conclusion: These findings suggest that ACE scores are important to understanding patterns of stress and craving experienced across the day in routine, non-dependent,
moderate-heavy drinkers and may provide new insights for vulnerability to transitioning into AUD.
2.1 Introduction

The impact of stress on public health has become a major concern. A 2015 national survey showed that one-third of adults reported higher stress levels over the previous year with a significantly higher proportion reporting extreme stress (Babor and Higgins-Biddle 2000). One major concern related to rising stress levels is the elevated risk for Alcohol Use Disorder (AUD) and relapse in those suffering from addiction (Sinha 2008).

Although stress is a known factor in motivation to drink, the alcohol-stress relationship is complex, influenced by diverse environmental and biological factors, and changes along the transition to addiction (Breese, Chu et al. 2005, Becker 2017).

Craving for alcohol has been associated with consumption and is a subjective marker of AUD (NIAAA(a), de Bruijn, Korzec et al. 2004). For example, craving intensity across the day predicted greater number of drinks consumed in both dependent and non-dependent heavy alcohol drinking adults (Ray, Miranda et al. 2010, Fazzino, Harder et al. 2013). The Elaborated Intrusion (EI) Theory of Desire views craving as a cognitive-emotional event consisting of sensory imagery episodes that vary in duration and intensity (Kavanagh, Andrade et al. 2005). EI theory emphasizes negative affect, a common correlate of stress (Dua 1993), as central to triggering intrusive desires for substances such as alcohol. Negative affect also increases awareness of any physiological deficit or feeling of deprivation, motivating people to approach activities, such as alcohol consumption, as a means of rebalancing one’s hedonic state (Kavanagh, Andrade et al. 2005). Indeed Cooney et al. have observed that, in men with alcoholism undergoing inpatient treatment, greater urge to drink when exposed to negative mood imagery and alcohol cues predicted shorter time to relapse after discharge (Cooney, Litt et al. 1997).
The current study investigated the stress-craving relationship throughout the day in routine moderate-heavy alcohol consumers during periods of both drinking in their normal routine (normal trial) and an imposed period of alcohol abstinence (abstained trial). We also examined the acute effects of alcohol consumption by evaluating before drinking (pre-drinking) compared to after consuming alcohol (post-drinking) on normal trial days. Triggers of stress and craving are dependent on contextual, temporal, and environmental factors that vary across the day (Shiffman, Stone et al. 2008, Wray, Merrill et al. 2014, Swendsen 2016). To capture these variations in stress and craving across the day, this study utilized Ecological Momentary Assessment (EMA) methodology. This method involves repeated measurements, typically utilizing portable technology, of a subject’s experience occurring in real time within their natural environment.

To understand how craving and stress across the day relates to an individual’s trait craving characteristics, participants were also assessed with the Alcohol Craving Experience Questionnaire (ACE). The ACE is based on EI Theory of Desire and is an established measure of craving known to effectively discriminate between high and low risk drinkers and clinical versus non-clinical populations (Statham, Connor et al. 2011). This study chose the ACE for this discriminative power and, although we did not expect extremely high scores within this group, it allowed us to classify individuals into lower and higher trait craving.

ACE scores were assessed for associations with EMA stress and craving patterns across the day. Based on EI Theory of Desire’s view that the sensory-imagery experience behind craving is cumulative with additional cue exposure, we hypothesized that craving
would increase across the day on both normal and abstained trials (hypothesis 1). We also expected higher alcohol craving across the day on the abstained trial, as drinking was not an available option to relieve the unfulfilled craving for alcohol (hypothesis 2a). In a more fine-grained analysis of abstinence and craving, we stratified responses on the normal trial as a function of whether they occurred prior to or following a drink. We expected that EMA craving would be substantially higher pre-drinking than post-drinking (hypothesis 2b) and that ratings would be greater across the day among those scoring higher on the ACE (hypothesis 3). Most importantly, due to the impact of negative affect on craving, we expected participants with higher ACE scores to report higher stress across the day (hypothesis 4a) and to exhibit a stronger relationship between EMA stress and craving (hypothesis 4b). Given limited previous research on EMA stress as a function of ACE scores, we did not formulate hypotheses regarding the association of time of day and drinking on stress and as a function of ACE scores.

In summary, the overarching goal of the current study was to investigate the experience of stress and craving across the day in moderate-heavy alcohol consumers during routine drinking behavior and imposed abstinence, and to determine if the relationship between these measures differed as a function of ACE scores. These findings are important, not only in understanding the experience of stress and craving across the day in routine, non-dependent drinkers, but in assessing the impact of drinking (acquisition of the target of craving), or lack thereof (abstinence) on stress and craving; a key manipulation from the perspective of the EI Theory of Desire.
2.2 Materials and Methods

Participants

We recruited thirty-four participants from the greater Winston-Salem, North Carolina area as part of a larger study that included brain imaging, which was approved by the Wake Forest University Health Sciences IRB. We excluded two participants due to missing EMA data, resulting in a final sample of 32 participants (14 men and 18 women). Inclusion criteria required that participants (a) were 24-60 years old, (b) consumed alcohol ≥ 50% of days in the past 3 months, and (c) maintained an average daily alcohol consumption of 1-3 drinks/day for women and 2-4 drinks/day for men for ≥ the past three years. Exclusion criteria included (a) previous or current diagnosis of AUD; (b) binge drinking as defined by the National Institute on Alcohol Abuse and Alcoholism (≥ 4 drinks for females, ≥ 5 drinks for males within 2 hours (NIAAA(b)) more than once a month; (c) > 3 occurrence of consuming alcohol before noon in the past 3 months; (d) currently undergoing treatment for a serious illness (diagnosed depression allowed if treated and stable for at least 2 months); (e) scored > 20 on the Center for Epidemiological Studies Depression Scale (CES-D); (f) a neurological disease diagnosis; (g) consuming ≥ 500 mg/day of caffeine; (h) smoking more than 1.5 packs/day; or (i) a positive urine drug screen (Methamphetamine, Cocaine, Marijuana, Amphetamine, Opiates, & Benzodiazepines). Due to the association between body mass index (BMI) and blood-alcohol content (BAC), BMI was restricted to 18.5 kg/m² to ≤ 35 kg/m² (Wang, Nicholson et al. 1992). Finally, because this study was part of a larger MRI project, participants had to be right-handed, not claustrophobic, or have any other medical condition that would put them at risk during the scanning protocol. The Clinical
Institute Withdrawal Assessment of Alcohol (CIWA-Ar) was used as a screening measure for alcohol withdrawal symptoms on the abstained trial (Sullivan, Sykora et al. 1989), which also served as a check for physical dependence (positive result never occurred). Participants received a total of $350 for study completion.

Procedure

Study Overview

The study protocol consisted of an initial screening visit and two experimental trials: three consecutive days of a normal drinking routine (normal trial), and three consecutive days of abstaining from alcohol (abstained trial). We scheduled both trials (normal and abstained) to ensure participants had no major disruptions (i.e. medical procedures) to their typical drinking routines or events that might impede the three consecutive days of alcohol abstinence, while randomizing participants to the order in which they received the normal and abstained trials. All study trials were scheduled to avoid any major life stressors or atypical life events. EMA data were collected throughout the three days of each experimental trial (Fig 1). Although not the focus of this report, both three consecutive trial days were followed by an MRI scan appointment (4th day). A urine drug test (see the Participants section above for details) was administered at the beginning of each MRI appointment which ensured the absence of drug use during the extent of study participation.
Figure 2.1: Design of the study, stimuli for Ecological Momentary Assessment (EMA) recordings, and compliance.

Study Measures

We employed the Time Line Follow Back (TLFB) (Vakili, Sobell et al. 2008), modified to record time of day (morning, afternoon, and evening) of consumption, to quantify alcohol consumption over the previous three months. This measure was used to verify that individual’s drinking patterns met the eligibility criteria and later to describe the sample’s drinking behavior (Table 1). The EMA protocol utilized a Likert measure to assess intensity of craving and stress asking: “Do you have a craving for alcohol right now?” or “How much stress do you feel right now?” The response scale ranged from 0 – 10, with 0 for “no craving/no stress” and 10 for “extreme craving/extreme stress.” In
addition, participants self-administered a breath test for alcohol content after each Likert survey (see EMA protocol section below). This increased confidence that participants maintained alcohol abstinence during the abstained trial. We administered the ACE questionnaire following both the normal and abstained trials to capture participants’ perceived intensity of craving across the past 3-days of each trial. In addition, we used the ACE strength of craving questionnaire following both trials using a “right now” or state-based response set because our interest was to use data from the strength measure in conjunction with functional magnetic resonance imaging (fMRI) data collected following each 3-day trial. The analyses presented here are restricted to the 11-item ACE frequency measure as a potential moderator of EMA responses collected during the two 3-day trials. Published psychometric data on the ACE supports its validity and reliability (Statham, Connor et al. 2011, May, Andrade et al. 2014). In the current study, we also computed an alpha internal consistency reliability on the scale during both 3-day trials. An internal consistency reliability of 0.94 for the normal trials and 0.89 for the abstained trials support our using a composite total score for the ACE frequency questionnaire. Because there was no statistically significant difference between scores on the ACE frequency questionnaire assessed following either the normal or abstained trials (p = 0.34), we averaged values across the two trials for use in our analyses.

**Participant Screening**

A phone screen served as the initial evaluation of eligibility, followed by an in-person screening visit that included informed consent, a final determination of eligibility, and completion of self-report questionnaires including the ACE Questionnaire (May, Andrade et al. 2014).
The EMA Protocol

EMA data collection occurred with study-issued iPhones that had three smartphone applications. iSurvey by Harvest Your Data (Wellington, New Zealand) allowed participants to complete a single item EMA craving and stress assessment. Audible Alerts (Wake Forest Medical Center Health Sciences) dispatched these random alerts to study participants prompting an EMA assessment. Breathometer brand (www.breathometer.com, Burlingame, CA), then BACtrack Mobile Pro (for improved functionality, www.bactrack.com, San Francisco, CA,) testing devices helped ensure alcohol abstinence.

Participants completed EMA assessments upon waking each morning, going to bed each evening, and when randomly prompted (9am-9pm) during both the normal and abstained trials. During normal trials, assessments occurred immediately before the first drink and after the last drink. The waking, going to bed, prior to the initial drink, and following the final drink of a drinking episode on normal trial assessments contributed to data points outside the 9am-9pm window. For each EMA assessment, participants first recorded the reason for the EMA assessment (“I just woke up”, “I received a random alert”, “I am about to start drinking”, “I have just finished drinking”, or “I am going to sleep”). They then completed the assessment by sliding a bar to the appropriate location on the Likert scale to indicate their current level of craving and stress, followed by a BAC breath test.
Statistical Analysis Strategy

EMA reports from all participants were stacked and the number of responses was tallied as a function of whether they occurred on abstained or normal trials. EMA recordings during the normal trials were further stratified as occurring either prior to (pre-drinking) or following (post-drinking) a drink. Time of day was transformed into military time and centered at 15:00 hours (3 PM). EMA reports recorded after midnight, but before retiring for the night, were given values above 24:00 hours to accurately reflect the day on which they occurred (e.g., 25:00 for 1 AM). To account for nonlinear variations in levels of craving associated with time of day, the centered value of military time was squared to operationalize quadratic trends. Within participant variables were created to distinguish the abstained trials from the normal trials (i.e., the main study manipulation). One set of within participant variables was used to contrast EMA responses occurring on abstained vs. normal trials. Another set of within participant variables was used for a more fine-grained analysis of the effects of alcohol consumption on normal trials. The first contrasted occasions on normal trials following the occurrence of the first drink (post-drinking, value of 1) to all other occasions (value of 0). The second contrasted occasions on normal trials after the occurrence of the first drink (pre-drinking occasions, value of 1) to all other occasions. For between participant variables, a categorical variable was used for sex (women = 1, men = 0) and ACE scores were centered at the sample mean.

Descriptive analyses were performed with SPSS (version 19).

The distribution of craving reports was examined for normality and was found to exhibit positive skewness. Attempts to normalize the distribution by performing square
root and logarithmic transformations were unsuccessful. To ensure that the skewness did not meaningfully impact the results, additional analyses were conducted using different operational definitions of the craving variable that were less skewed (for example, modeling the data with a Poisson distribution). The results of these analyses were identical for pre-drinking and post-drinking vs. abstained trials, the effects of time of day, and abstained trials vs. normal trials. The effects of ACE and female sex did however vary somewhat across these analyses possibly due to the relatively small sample size. Given that the main findings were not sensitive to the operational definitions of the craving variable, the findings presented in the current manuscript were limited to the main analysis with the craving score treated as a continuous variable.

Given the nested structure of the data set (i.e., repeated measures of cravings on abstained and normal trials which were nested within respondents) and the interest in ascertaining the influence of both within and between participant variables, the main statistical analysis strategy consisted of growth curve analyses, also known as multilevel modeling (Raudenbush, 2001). Multilevel modeling was performed with HLM (version 7.0, Scientific Software International, Chicago, IL). In a first set of analyses as a manipulation check, EMA responses occurring immediately before the first drink and immediately after the participant indicated that they had finished drinking (last drink) were extracted from the data set. A null multilevel regression model was applied to the extracted data set to estimate the intraclass correlation coefficient (ICC) (proportion of the total variance attributable to between person variation) and to test whether or not the between participant variance in overall craving was statistically significant. Then, the
variable contrasting EMA reports collected immediately before the 1st drink and after the last drink investigated the effect of consuming alcohol on craving.

All subsequent analyses were performed on the dataset from which recordings immediately prior to the first drink and following the last drink were removed from the normal trial (not necessary in the abstained trial). A null model was applied to estimate the ICC and to test the statistical significance of the between participant variance. Then, the variables operationalizing the linear and quadratic trends of time throughout the day were entered into the model to explore within day variations in craving intensity. Then, the variable contrasting EMA reports collected on abstained trials in comparison to normal trials was entered into the model to examine the effects of the study’s main experimental manipulation.

A separate set of analyses, which was similar to the previous, was then conducted using two variables to operationalize the effects of drinking on normal trials. That is, the two variables operationalizing effects of pre-drinking and post-drinking on normal trials in comparison to abstained trials were entered into the model. This allowed for a more fine-grained examination of craving as a function of the study’s experimental manipulation alongside the effects of alcohol consumption. Finally, the between participant variable operationalizing sex and ACE scores were added as a moderator of overall craving, time of day, abstaining, and alcohol consumption. Results were plotted to better illustrate findings.

Next, we investigated stress as an outcome variable in order to examine the effects of time of day, abstaining from drinking, and consumption of alcohol. We
performed multilevel modeling by entering the effects of time of day (linear and quadratic trends), as well as the variables operationalizing alcohol consumption, in comparison to abstained trials and the moderating effects of ACE scores. Again, results were also plotted to illustrate findings. In a final set of models, we entered the effects of time of day, alcohol consumption, and stress while adjusting for the moderating influence of ACE on each of these variables.

2.3 Results

The 34 participants provided a total of 1602 responses to the EMA craving assessment. Data from two participants were removed because one participant did not complete the ACE measure (n = 47 craving responses removed) and the other did not comply with the EMA procedure (n = 4 craving responses removed). The final data set included 1551 EMA responses recorded from 32 included participants. Among the 1551 EMA reports, 1365 were elicited either because the person had just awakened in the morning (n=187), because s/he was about to go to bed (n=166), or because s/he received an alert to complete an assessment (n=1012). Of the 1365 reports, 712 occurred on abstained trials and 653 on normal trials (508 pre-drinking and 145 post-drinking). Another 186 EMA reports were triggered by the decision to drink, with 101 reports occurring immediately before the first drink and 85 after the last drink. Given that some participants experienced more than one drinking episode on normal trials, they provided more of these ancillary reports than requested. Individual participants provided between 34 and 48 EMA responses in response to getting up, going to bed, or random alerts (M = 42.7, SD = 3.5) and between 1 and 13 responses prior to and following drinks and during normal trials (M = 5.8, SD = 2.3).
Compliance with the EMA procedure for each of the different response conditions appears in Fig 1. As can be seen, compliance was high ranging from 82% (random alerts on normal trials) to 98.9% (waking up on normal trials). Abstinence from alcohol consumption on the abstained trials was confirmed both verbally and by random breath testing for all participants. On the three occasions when the breath test device malfunctioned, participants contacted the study coordinator and device troubleshooting was conducted. To encourage honesty, the participant could reschedule the abstained trial days without penalty in the event that a drink was consumed (this was never necessary). Although alcohol abstinence could not be confirmed with 100% certainty, we believe that the breath test protocol and communication with the study coordinator served to greatly enhance the confidence that the abstained state was maintained. For the entire sample, there were only three days on which participants reported consuming no alcohol during the normal trial. These occasions occurred in three separate individuals (all participants drank at least 2/3 of the drinking days) increasing confidence that the 3-day abstinence period disrupted normal drinking routines. Although the recruitment criteria required that alcohol be consumed on at least 50% of days on the TLFB, the actual sample reported drinking on 80.8% of days. Table 1 provides participant characteristics including age, BMI, race, and alcohol use. It should be noted that the sample included slightly more women (56.6%) and averaged ACE scores ranged from 0 to 4.23 and varied substantially (M = 1.14, SD = 1.02, 95% CI [0.73, 1.50]).
**Table 2.1: Sample Characteristics.** Males and females did not differ significantly in any of the above categories (alpha = 0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall (n = 32)</th>
<th>Male (n = 14)</th>
<th>Female (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38.28 (10.8)</td>
<td>37.29 (6.8)</td>
<td>39.06 (13.2)</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25.02 (3.9)</td>
<td>26.03 (3.7)</td>
<td>24.23 (4.0)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black or African American</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>White</td>
<td>28</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Alcohol Use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Years Drinking</td>
<td>18.22 (10.8)</td>
<td>17.86 (7.2)</td>
<td>18.50 (13.2)</td>
</tr>
<tr>
<td>Years Maintained Current Drinking Pattern</td>
<td>8.06 (5.9)</td>
<td>9.29 (5.4)</td>
<td>7.11 (6.2)</td>
</tr>
<tr>
<td>Previous 3 Months (Time Line Follow Back)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of days that were drinking days</td>
<td>80.79 (15.6)</td>
<td>79.53 (16.1)</td>
<td>81.77 (15.6)</td>
</tr>
<tr>
<td>Average drinks consumed on drinking days</td>
<td>2.26 (0.7)</td>
<td>2.37 (0.3)</td>
<td>2.17 (0.9)</td>
</tr>
</tbody>
</table>

In the first set of analyses, the null model applied to the EMA data set immediately before the 1st drink and immediately after the last drink (n=186 reports nested within 32 participants) revealed statistically significant between participant variance in the craving intensity ($X^2(31) = 130.72$, p<0.001). The ICC was estimated to be $\rho = 0.33$ indicating that 67% of the variance in craving intensity was within person variation whereas 33% was between participant variation. The intensity of cravings were significantly higher (p<0.001) pre-drinking in comparison to post-drinking (predicted values=4.45 vs. 1.06) supporting the well-known effects of alcohol in reducing craving.

The null model applied to the data set involving the remaining 1365 EMA reports also showed statistically significant between participant variance craving ($X^2(31) =$
The ICC was estimated to be $\rho = 0.37$ again suggesting that most of the craving was within person variation. Modeling of the effects of time showed that craving increased as the day progressed but then dropped off later in the day (linear effects: $b=0.14$, SE=0.01, $p<0.001$; quadratic effects $b=-0.022$, SE=0.002, $p<0.001$), supporting hypothesis 1. Further, modeling showed that craving was significantly higher on abstained trials in comparison to normal trials ($b=0.20$, SE=0.10, $p<0.045$), also in support of hypothesis 2a.

To further elucidate these findings, the next set of analyses jointly examined the influence that the experimental manipulation (alcohol abstinence) and alcohol consumption had on cravings. Once the effects of the time of day were included in the model, we observed that pre-drinking EMA reports of craving (on normal trials) were not different ($p>0.05$) from EMA reports recorded at comparable times on abstained trials. However, post-drinking EMA craving reports were significantly lower ($b=-1.08$, SE=0.19, $p<0.001$) than both pre-drinking and abstained trial EMA reports, supporting hypothesis 2b. These findings are illustrated in Fig 2, Panel A. For hypothesis 3, we observed that individuals with higher ACE scores showed unique patterns of diurnal variations in craving throughout the day (Fig 2, Panel B). That is, these individuals reported higher EMA craving throughout the day ($b=1.57$, SE=0.18, $p<0.001$) and reached higher levels at midday ($b=0.02$, SE=0.002, $p<0.001$). ACE scores had no effect on craving reported on abstained versus normal trials; also, regardless of ACE scores, participants reported similar decreases in craving as a result of drinking (see Fig 2, Panels A & B).
Examination of within-day patterns of stress and the effects of alcohol consumption while adjusting for the moderating influence of ACE scores revealed unique findings (see Fig 3). The ICC for stress was substantially higher than for craving with $\rho = 0.50$ indicating that about 50% of variability in stress was between participant variability with the remaining variance being within person. For craving, the ICC was lower at about $\rho = 0.37$. Stress showed within-day variations that were different from those observed for craving. That is, stress levels were lowest early and late in the day and thus showed an inverted-U pattern throughout the day (linear trend: $p>0.05$; quadratic
trend: \( b = -0.017, \ SE = 0.002, \ p < 0.001 \). More interesting, participants’ ACE scores moderated the pattern of variation in stress throughout the day and as a function of alcohol consumption. That is, as illustrated in Fig 3, participants with higher ACE scores reported significantly greater stress throughout the day (moderating effect of ACE on the intercept: \( b = 1.04, \ SE = 0.26, \ p < 0.001 \) along with a more accentuated inverted-U pattern (hypothesis 4a) (moderating effects of ACE on quadratic trends: \( b = -0.009, \ SE = 0.001, \ p < 0.001 \)). In addition, those with higher ACE scores reported elevated stress levels pre-drinking in comparison to comparable times on abstained trials (moderating effect of ACE: \( 0.22, \ SE = 0.10, \ p < 0.04 \)). Interestingly though, individuals with average ACE scores experienced a decrease in stress post-drinking compared to abstained trials (\( b = -0.38, \ SE = 0.17, \ p < 0.024 \); see Fig 3A) but the moderating influence of ACE nullified this effect (\( b = 0.34, \ SE = 0.14, \ p < 0.018 \)) such that individuals with higher ACE scores did not show this significant decrease post-drinking in comparison to abstained trials (see Fig 3, Panel B).
Figure 2.3: EMA stress in average and higher ACE scores. Predicted stress scores as a function of time of day, normal or abstained trials, and alcohol consumption across individuals with average (Panel A) and higher ACE scores (Panel B).

The final set of analyses that combined time of day, alcohol consumption, and stress as predictors of craving while accounting for the moderating influence of ACE scores replicated and extended the findings observed in prior analyses. That is, individuals with higher ACE scores reported higher craving throughout the day (b=1.07, SE=0.17, p<0.001) and reached higher levels at midday (moderating effect of linear trend: b=0.03, SE=0.01, p<0.02); moderating effect of the quadratic trend: -0.01, SE=0.002, p<0.001), and all participants regardless of ACE scores reported similar
decreases in craving \( \text{b} = -1.11, \text{SE} = 0.18, p < 0.001 \) as a result of drinking \( \text{b} = -0.03, \text{SE} = 0.15, p = 0.84 \). However, although higher stress was associated with greater craving \( \text{b} = 0.22, \text{SE} = 0.03, p < 0.001 \), this association was stronger among those with higher ACE scores \( \text{b} = 0.11, \text{SE} = 0.02, p < 0.001 \); see Fig 4, Panels A and B).
Figure 2.4: Normal trial (A&B). EMA craving in higher and lower stress in average and higher ACE scores. Predicted craving scores during the normal trial as a function of time of day, higher and lower EMA stress, and alcohol consumption for individuals reporting average (Panels A) and higher (Panel B) ACE scores. Higher ACE scores exaggerated the relationship between stress and craving. Abstained trial (C & D). EMA craving in higher and lower stress in average and higher ACE scores. Predicted craving scores during the abstained trial as a function of time of day and higher and lower EMA craving for individuals reporting average (Panel C) and higher (Panel D) ACE scores. Higher ACE scores exaggerated the relationship between stress and craving.
2.4 Discussion

The current study showed that, after accounting for time of day and the impact of alcohol consumption on cravings, moderate-heavy drinkers’ responses were similar during both the normal and abstained trials. During the normal trial, craving intensity increased throughout the day (pre-drinking) followed by alcohol consumption that significantly lowered craving intensity (Fig 2). These results are consistent with the EI Theory of Desire which posits that substance related cues have an additive effect on craving (Kavanagh, Andrade et al. 2005). In other words, as moderate-heavy drinkers move throughout the day, they are likely exposed to a greater number of cues that increase the probability of triggering intrusive, craving related thoughts that lead to rumination about the target of desire (alcohol). The imposed period of abstinence did not enhance craving, a finding that was contrary to our original expectation. However, according to the EI Theory of Desire, when individuals expect consumption to be delayed, they engage in strategies to reduce the discomfort associated with craving (Kavanagh, Andrade et al. 2005). Hence, one interpretation of our finding is that, because participants knew that the abstinence period was only 3-days, they suppressed cravings during this period.

It is important to emphasize that individuals scoring higher on the ACE became more distinct from the rest of the group when considering EMA stress responses (Fig 3). As expected, as a group, moderate-heavy drinkers exhibited an inverted-U pattern of stress throughout the day. However, individuals with higher ACE scores reported significantly higher levels of stress and a steeper increase in stress at midday compared to ratings for the overall group. Interestingly, compared to abstained days, those with higher ACE scores reported higher stress on days they anticipated having a drink, but did not
enjoy the same relief from craving post-drink as those with average ACE scores. The fact that individuals with higher ACE scores exhibited an elevated pre-drinking stress response on the normal as compared to the abstinence trial is interesting considering that the anticipation of consumption alone can trigger craving (Juliano and Brandon 1998, Kavanagh, Andrade et al. 2005). In addition, the EI Theory of Desire posits that craving can enhance negative emotions that accompany stress due to increased attention on the state of deprivation, which may explain the elevated pre-drinking stress levels observed during abstinence in those with higher ACE scores. Also of note, Fig 3 illustrates that post-drinking stress levels in those with higher ACE scores remained similar to levels during abstained trials in the overall group.

Most importantly, higher ACE scores were associated with a significantly stronger stress-craving relationship in EMA response patterns (Fig 4). The EI Theory of Desire describes negative affect as a crucial component of the experience of craving, with stronger craving elicited in the context of negative emotion and cognitive elaboration more likely when the intrusive, craving related thoughts have stronger ties to negative affect (Kavanagh, Andrade et al. 2005). Negative affect can increase the motivation for behaviors aimed at returning to a positive hedonic state and thus increase drinking behavior (Simons, Dvorak et al. 2010, Epler, Tomko et al. 2014, Dvorak, Pearson et al. 2016). Stress often precipitates and can intensify negative affect and has been found to increase craving in alcohol dependent individuals, but not in light-moderate drinkers (Sinha, Fox et al. 2009). This suggests that craving in the presence of stress may be an important indicator of AUD risk, particularly for individuals with higher ACE scores. Greater stress related increases in craving were also found to be associated with
decreased treatment outcomes in recovering alcohol dependent individuals (Higley, Crane et al. 2011), further illustrating the importance of understanding the stress-craving relationship. Although previous work has also shown conflicting results for the role of stress in alcohol craving and consumption (Wray, Merrill et al. 2014), the current findings suggest that momentary stress may be more closely linked to craving in moderate-heavy drinkers who regularly experience frequent craving (higher ACE scores), providing insight to one potential path to AUD.

Strengths of this study including contrasting stress and craving during normal and abstained trials using random EMA surveys taken across the day, as well as directly before the 1st drink and after the final drink of the day during the normal drinking trial. However, there are some limitations to our study design. As cue exposure has been associated with craving (Miranda, Ray et al. 2014, Wray, Merrill et al. 2014, Serre, Fatseas et al. 2015), assessing these variables during the time of craving ratings may have provided additional insight into our findings. Additionally, the random alerts only occurred between the hours of 9am – 9pm to avoid disruption of participants’ sleep. On some occasions, this may have led to a lapse in data sampling depending on the participant’s sleep schedule. However, the participant triggered alerts (i.e. “I am going to sleep”) served to provide additional data outside the random alert schedule. In addition, this study’s somewhat selective recruitment criteria are a consideration when generalizing these findings to the population. To our knowledge, this study design (imposed period of alcohol abstinence in contrast to normal drinking routine) is new to the alcohol literature. The three consecutive days of imposed abstinence was used as a starting point, but craving across the day may have increased with additional consecutive days of alcohol
abstinence. It would be interesting for future work to build on these findings by extending the abstinence period. A longitudinal study including reassessment of craving/stress and drinking behavior over time would help confirm our findings and the implications they may have for risk of AUD. It is also important to note that the use of the labels “high” or “higher” ACE scores in this sample refers to scores in a sample of this demographic and should not be taken to represent high scores on the ACE when considering the entire range of possible scores. Finally, future research should assess negative affect in conjunction with craving responses to better understand how negative affect tracks with increases in craving, albeit Kavanagh and colleagues (Kavanagh, Andrade et al. 2005) view craving as a negative emotion.

In summary, EMA stress and craving throughout the day varied within this sample of moderate-heavy drinkers. Those with higher ACE scores reported a steeper increase in craving and stress throughout the day, elevated stress pre-drinking, a return to abstained levels of stress post-drinking, and a stronger stress-craving relationship compared to the rest of the group. These findings suggest that the frequency with which moderate-heavy drinkers experience craving is important to understanding patterns of stress and craving experienced across the day and may provide new insights to the vulnerability of transitioning to AUD. They also provide a baseline for which to compare how other alcohol consumption patterns may vary in their experience of stress and craving.
References


responses following stress and cue exposure in alcohol dependent individuals."

Neuropsychopharmacology 34(5): 1198-1208.


CHAPTER III

CARDIAC VAGAL DYSFUNCTION MODERATES PATTERNS OF CRAVING ACROSS THE DAY IN MODERATE TO HEAVY CONSUMERS OF ALCOHOL

Rhiannon E. Mayhugh, Paul J. Laurienti MD, PhD, Jason Fanning, PhD, Lise Gauvin, PhD, Keri J. Heilman, PhD, Stephen W. Porges, PhD, W. Jack Rejeski, PhD

Submitted February 2018 to PLoS ONE (under review)
Abstract

Background: Alcohol craving, an indicator of vulnerability to Alcohol Use Disorder (AUD), has been found to be inversely related to cardiac vagal tone (CVT). Here we examined how resting CVT, CVT reactivity to a postural challenge, and their interaction influence craving during imposed alcohol abstinence and their usual drinking among moderate to heavy drinkers.

Methods: Participants were recruited from the local community (final n=29) and assessed for CVT functioning via respiratory sinus arrhythmia (RSA) at rest (RSA-rest) and during a postural challenge (RSA-react). Craving intensity was assessed throughout the day during 3-day periods of imposed alcohol abstinence (abstained days) and drinking as usual (normal days) via Ecological Momentary Assessment (EMA). Multilevel statistical modeling assessed relationships between patterns of CVT and diurnal craving. The primary hypothesis of interest was that the interaction of RSA-rest with RSA-react would be significantly associated with increased craving across the day.

Results: Overall, craving increased through the day and significantly decreased after drinking (p < 0.001). There was a significant interaction between RSA-rest and RSA-react with plots revealing that this effect was driven by an aberrant craving pattern among participants with higher RSA-rest and a sluggish vagal brake in response to a postural shift—atypical RSA-react.

Conclusion: Although additional research is needed to corroborate these findings, our results suggest that moderate to heavy drinkers characterized by higher RSA-rest and atypical RSA-react exhibit aberrant patterns of craving across the day that may represent a risk factor for AUD.
3.1 Introduction

During the past decade of investigation into alcohol use disorders (AUDs) and the potential health benefits of moderate alcohol consumption, considerable attention has been devoted to the autonomic nervous system (ANS). The ANS is a component of the peripheral nervous system that provides the brain with sensory information from the body and, in turn, regulates bodily functions. When the two branches of this system, the sympathetic and parasympathetic, are working properly the body is able to maintain a healthy balance between appropriate reactions to environmental challenge and return to restorative, homeostatic states. This balance is thought to be affected by frequent alcohol consumption and play an important role in addiction (Seo and Sinha 2015).

Cardiac vagal tone (CVT) reflects the influence of the myelinated branch of the vagus nerve, which originates in the nucleus ambiguus, on the heart. CVT is commonly quantified by high frequency heart rate variability (HF-HRV) or respiratory sinus arrhythmia (RSA), and has been applied as a general index of ANS functional health (Porges 2007). Specifically, CVT captures the functional relationship between the heart and brainstem. In a healthy system, high CVT acts as a “vagal brake,” inhibiting the sympathetic nervous system’s influence on cardiac output. Consistent with Polyvagal Theory (Porges 1995, Porges 2007), upon exposure to a stressor, CVT is reduced, lifting the “vagal brake”; thus enabling cardiac output and metabolic activity to increase to meet situational demands (Porges, Doussard-Roosevelt et al. 1996). Dysfunctional CVT has implications for impaired psychological and physical health (Thayer, Ahs et al. 2012, Geisler, Kubiak et al. 2013).
CVT has been found to be sensitive to alcohol exposure and addiction. Recent systematic reviews have concluded that alcohol has a J-shaped effect on CVT, with moderate alcohol consumption increasing CVT and heavy consumption decreasing it (Quintana, Guastella et al. 2013, Karpyak, Romanowicz et al. 2014). Altered CVT functioning has also been associated with craving. For instance, higher craving has been related to lower resting state CVT in alcohol dependence (Quintana, Guastella et al. 2013). This altered CVT-craving relationship is important considering that higher levels of alcohol craving are thought to place one at increased risk for AUD (de Bruijn, Korzec et al. 2004). For example, craving intensity across the day has predicted greater alcohol consumption in both dependent and non-dependent heavy alcohol consumers, as well as relapse in AUD recovery (Moos and Moos 2006, Ray, Miranda et al. 2010, Fazzino, Harder et al. 2013).

Whereas early investigations of CVT targeted resting state measures (RSA-rest) and led to the conclusion that higher RSA-rest is often found to be related to more flexible coping and more favorable regulation of emotional responses to stressful encounters, it was not long before researchers focused on reactivity during physical and psychosocial challenge (RSA-react; see reviews by Beauchaine and Thayer 2015, Balzarotti, Biassoni et al. 2017). As specified by Polyvagal Theory (Porges, Doussard-Roosevelt et al. 1996), investigators have recognized the theoretical importance of the “braking function” of the myelinated vagal pathway as an important contribution to effective self-regulation. For example, research on tobacco has observed that smokers with blunted vagal reactivity to a psychological threat reported higher craving and were
more prone to use smoking as a means of coping with stress than those with more normative responses to challenge (Ashare, Sinha et al. 2012).

At this junction we want to emphasize that with the exception of the work by Ashare and colleagues and a few other examples that we will discuss below, in the study of RSA-react, most research has focused on either how RSA-rest influences RSA-react to threat or challenge (see review by Balzarotti, Biassoni et al., 2017), or investigating RSA in response to appetitive cues (Rajan, Murthy et al. 1998, Culbertson, Nicolas et al. 2010, Garland, Franken et al. 2012). Less attention has been given to studying whether different patterns of RSA-react to challenge might have prognostic value in understanding downstream emotional and/or behavioral responses. Moreover, although the current direction of research is to study RSA-rest and RSA-react as main effects, there is ample evidence that investigators should consider the interactive effects of these two metrics. First, there is strong evidence that the optimal phenotype for CVT is higher RSA-rest combined with vagal withdrawal under challenge, a typical RSA-react response (Hinnant and El-Sheikh 2009, Kreibig 2010, Cribbet, Williams et al. 2011); however, data have shown that higher RSA-rest combined with a sluggish vagal brake in response to threat, an atypical RSA-react response, is a risk factor for both emotional (Yaroslavsky, Bylsma et al. 2013) and behavior dysfunction (Heilman, Connolly et al. 2012). Second, individuals with lower RSA-rest who manifest further withdrawal in RSA in response to challenge, a response in RSA-react that is atypical in light of the lack of vagal tone during resting state, have been shown to be at high risk for psychopathology (Beauchaine, Gatzke-Kopp et al. 2007), although Yaroslavsky and colleagues
(Yaroslavsky, Bylsma et al. 2013) found that augmentation of RSA to challenge is a risk factor for depression at any level of RSA-rest.

In a novel exploration into potential phenotypes of AUD vulnerability, the current study examined whether resting CVT (operationalized as RSA-rest), CVT reactivity measured as the change in RSA from a seated to standing posture (operationalized as RSA-reat), or their interaction moderated alcohol craving across the day. Ecological Momentary Assessment (EMA) methodology (Shiffman, Stone et al. 2008) was used to assess craving during a 3-day period of normal patterns of drinking (normal days) and during 3-days of imposed alcohol abstinence (abstained days). On normal days, the impact of alcohol consumption on craving was also assessed. Given the substantial evidence for the relation between CVT and emotion (Balzarotti, Biassoni et al. 2017) and that craving has been characterized as an emotional state (Kavanagh, Andrade et al. 2005), we hypothesized the presence of a significant interaction between RSA-rest and RSA-reat with this effect potentially being driven by two phenotypes: higher RSA-rest and an atypical RSA-reat response to challenge (a blunted vagal response) and/or lower RSA and an atypical response to challenge (higher levels of vagal withdrawal). The hypothesized interaction was tested using continuous variables, with exploratory plotting of interaction effects (Aiken and West, 1991).

3.2. Materials and Methods

Participants

Thirty-four moderate to heavy drinkers were recruited from the Winston-Salem area via study advertisements placed throughout the community, mass mailing, and business
cards. The final sample included twenty-nine participants (13 men, 16 women) after excluding for missing data (n = 5). Enrollment criteria included those aged 24-60 years, alcohol consumption ≥ 50% of days in the past 3 months, average daily alcohol consumption of 1-3 drinks/day for women and 2-4 drinks/day for men for ≥ the past three years. Exclusion criteria included AUD diagnosis, binge drinking (NIAAA definition of ≥ 4 drinks for females, ≥ 5 drinks for males within 2 hours > once/month (NIAAA), consuming alcohol before noon > 3 times/month, active severe medical conditions, a score of > 20 on the Center for Epidemiological Studies Depression Scale (CES-D), history of neurological disease diagnosis, > 500 mg/day of caffeine, smoked > 1.5 packs/day, or positive urine drug test (methamphetamine, cocaine, marijuana, amphetamine, opiates, & benzodiazepines). Due to the association between body mass index (BMI) and blood-alcohol content (BAC), BMI was restricted to between 18.5 kg/m² and 35 kg/m² (Wang, Nicholson et al. 1992). The Time Line Follow Back (TLFB), modified to record time of day, was administered during screening (Vakili, Sobell et al. 2008). The Clinical Institute Withdrawal Assessment of Alcohol (CIWA-Ar) was used as a safety screening for alcohol withdrawal symptoms (Sullivan, Sykora et al. 1989).

3.2.2 Study Overview

Both phone and in-person screens were conducted to ensure eligibility, complete informed consent, administer self-report questionnaires, and collect cardiac functioning data. The research visits occurred directly following the 3 consecutive normal and abstained days and included an fMRI scan and additional self-report questionnaires. The order of research visits was randomly assigned (3-day minimum wash out period). This
report focuses on the RSA data collected during the screening visit and the EMA data collected throughout the 3 days prior to each research visit (normal and abstained days).

**Data Processing for RSA Calculation**

During the screening visit, an electrocardiogram (ECG) was collected via a Biopac MP150 by Biopac Systems, Inc. ([www.biopac.com](http://www.biopac.com), Goleta, CA). Three electrodes (one on each collar bone and one on the lower right rib for grounding) and a pulse oximeter (right index finger) were placed on the participant. The participant sat quietly for 5 minutes prior to data collection. Data were first collected in the seated position (5 minutes), then in standing position (2 minutes). Heart rate data were visually inspected and edited off-line with CardioEdit software (Brain-Body Center, University of Illinois at Chicago, 2007).

Heart rate and RSA were calculated from the ECG data using CardioBatch Plus software (Brain-Body Center for Psychophysiology and Bioengineering, University of North Carolina at Chapel Hill, 2016) consistent with procedures developed by (Porges 1985). CardioBatch Plus quantified the amplitude of RSA using age-specific parameters that are sensitive to the maturational shifts in the frequency of spontaneous breathing. The method includes the following steps: (1) timing sequential R-R intervals to the nearest millisecond; (2) producing time-base data by resampling the sequential R-R intervals into 500 msec intervals; (3) detrending the time-based series with a 21-point cubic moving polynomial that is stepped through the data to create a smoothed template then the template is subtracted from the original time-based series to generate a detrended residual series; (4) bandpass filtering the detrended time series to extract the variance in the heart period pattern associated with spontaneous breathing in adults (.12 - .40 Hz);
and (5) transforming the variance estimates with a natural logarithm to normalize the
distribution of RSA estimates (Riniolo and Porges 1997). These procedures are
statistically equivalent to frequency domain methods (i.e., spectral analysis) for the
calculation of the amplitude of RSA when heart period data are stationary (Porges and
Byrne 1992). RSA was quantified during each sequential 30-sec epoch and the averages
within each condition were used in the data analyses. To calculate a RSA-react score, we
subtracted the average 5-min RSA-rest value from the standing 2-min RSA value and
then controlled for the the 5-min RSA-rest values creating a residualized score.

EMA Protocol

Participants completed daily EMAs on study-issued iPhones. The iSurvey App
(Harvest Your Data; Wellington, New Zealand) was utilized to assess craving intensity
on an 11-point Likert scale, asking “Do you have a craving for alcohol right now?” The
response scale was from 0 (“no craving”) to 10 (“extreme craving”). Likert assessments
of stress and anxiety were also taken, but were not included in this analysis. To increase
confidence that no alcohol was consumed during the abstained days, an alcohol content
breath test was self-administered after each Likert survey. Breath tests were completed on
Breathometer brand (www.breathometer.com, Burlingame, CA) testing devices and then
BACtrack Mobile Pro (www.bactrack.com, San Francisco, CA,) for improved
functionality.

EMA craving assessments were completed upon waking each morning, going to bed
each evening, and when randomly prompted (total of 6 prompts sent between 9am & 9pm
via the Audible Alerts application) on both normal and abstained days. The waking and
going to bed assessments provided data points outside of the 9am-9pm window. During
normal days, participants also recorded craving immediately prior to the initial drink and following the final drink of a drinking episode. These assessments established drinking commencement time and allowed for creation of dummy variables used to distinguish EMA reports occurring before and after drinking (see below). For each EMA assessment, participants recorded the “reason for completing the survey” (“I just woke up”, “I received a random alert”, “I am about to start drinking”, “I have just finished drinking”, or “I am going to sleep”). Upon enrollment, each participant received one-on-one training with the study coordinator (also available via text for assistance during the entire data collection period).

**EMA Data Processing and Statistical Analyses**

The EMA reports resulting from random alerts, upon waking, or going to bed were stacked. A set of continuous and dummy variables were created to account for diurnal variations in cravings and to distinguish recordings occurring on abstained days versus normal days prior to and following the first drink. That is, the hour values of the timestamps of recordings were transformed into military time and the minute values were converted to fractions (e.g., 7:30 PM to 19.50, 9:45 PM to 21.75). Resulting times were centered at 15.00 (i.e., 3PM). EMA reports recorded after midnight but before going to bed were given values above 24:00 hours to accurately reflect the day on which they occurred (e.g., 25.25 for 1:15 AM, 26.00 for 2 AM, etc). In addition, EMA recordings occurring on abstained and normal days were stratified and then EMA recordings on normal days were further stratified as occurring either prior to (pre-drinking) or following (post-drinking) a drink with dummy variables. A first dummy variable contrasted EMA recordings occurring on normal days prior to the occurrence of the first drink (pre-
drinking, value of 1) to all other occasions (value of 0). The second contrasted occasions on normal days after the occurrence of the first drink (post-drinking occasions, value of 1) to all other occasions. The reference category was therefore an abstained day.

Using SPSS, version 19, the distribution of craving reports was plotted and found to exhibit substantial positive skewness and could not be normalized using various mathematical transformations. To safeguard against erroneous conclusions from the raw data, the main analyses were replicated using other modeling approaches, for example, modeling for Poisson distribution. As determined by conducting Shapiro-Wilk tests, both RSA-rest and residualized RSA-react were normally distributed.

Since the data included repeated EMA reports of cravings that were nested within participants, the main statistical analysis strategy consisted of growth curve analyses, also known as multilevel modeling (Raudenbush 2001) and was performed with Hierarchical Linear and nonlinear Modeling software (HLM, version 7.0, Scientific Software International, Chicago, IL). First, a null multilevel regression model was applied to estimate the intraclass correlation (proportion of the total variance [including within day and between participant variance] attributable to between person variation) and to test whether or not the between participant variance in overall craving was statistically significant. Then, the variables capturing both linear and quadratic trends of time throughout the day were entered into the model along with the dummy variables contrasting EMA reports collected prior to and following drinking on normal days in comparison to abstained days. This allowed us to account for both diurnal variations in craving and the effects of drinking on craving in comparison to the 3-day period of abstinence. Finally, the centered values of the between participant variables of RSA-rest
and residualized RSA-react, as well as their interaction, were entered into the model as continuous variables. Following recommendations of Aiken and West (1991), significant interactions were further explored by plotting model-based estimates of craving as a function of four hypothetical cases: 1) RSA-rest set at 1 SD above the mean and residualized RSA-react set at 1 SD below than mean (higher RSA-rest/typical RSA-react); 2) RSA-rest set at 1 SD above the mean and residualized RSA-react set at 1 SD above than mean (higher RSA-rest/atypical RSA-react); 3) RSA-rest set at 1 SD below the mean and residualized RSA-react set at 1 SD above the mean (lower RSA-rest/typical RSA-react); and 4) RSA-rest set at 1 SD below the mean and residualized RSA-react set at 1 SD below than mean (lower RSA-rest/atypical RSA-react). A model was also run adjusting for age and sex.

### 3.3 Results

**Daily Patterns of Craving**

Among the 34 recruited participants, 2 did not comply with EMA prompting and 3 were missing RSA data. The remaining 29 participants provided a total of 1242 responses to the EMA survey with 650 occurring on abstained days, 462 before drinking, and 150 after drinking (592 total on normal days). Among the 1242 EMA reports, 170 were elicited upon waking in the morning, 130 because they were about to go to bed, and 922 because they received a random alert to complete an assessment. Individual participants provided a total of between 34 to 48 EMA responses combined across these categories (M = 42.8, SD = 3.5). EMA procedure compliance was above 80% and thus well above the 75% threshold expected for EMA studies. See Table 1 for participant characteristics.
The mean and (SD) for RSA-rest was 5.76 (1.69), whereas it was -0.83 (0.72) for RSA-react (residualized RSA-react = -0.03 (0.69)).

**Table 3.1. Sample Characteristics: Mean (SD) or Frequency**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall: EMA</th>
<th>Male (n= 13)</th>
<th>Female (n = 16 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38.8 (10.9)</td>
<td>36.6 (6.6)</td>
<td>40.5 (13.3)</td>
</tr>
<tr>
<td>BMI</td>
<td>24.8 (3.8)</td>
<td>25.6 (3.5)</td>
<td>24.2 (4.0)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black or African American</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>White</td>
<td>26</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Alcohol Use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Years Drinking</td>
<td>18.9 (10.8)</td>
<td>17.3 (7.2)</td>
<td>20.1 (13.1)</td>
</tr>
<tr>
<td>Years Maintained Current Drinking Pattern</td>
<td>8.3 (6.0)</td>
<td>9.2 (5.6)</td>
<td>7.6 (6.4)</td>
</tr>
<tr>
<td>Previous 3 Months (Time Line Follow Back)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of days that were drinking days</td>
<td>81.2 (16.0)</td>
<td>78.6 (16.4)</td>
<td>83.4 (15.8)</td>
</tr>
<tr>
<td>Average drinks consumed on drinking days</td>
<td>2.3 (0.73)</td>
<td>2.4 (0.26)</td>
<td>2.3 (0.96)</td>
</tr>
</tbody>
</table>
**Legend:** Males and females did not differ significantly in any of the above categories (alpha = 0.05).

In the first set of analyses, between participant variance in craving was statistically significant ($X^2(28) = 826.8$, $p<0.001$). The intraclass correlation coefficient was 0.40 suggesting that most of the variance in craving was within person. Time effect modeling showed craving increased as the day progressed, but then dropped off later in the day depicting an inverted J-shape with linear and quadratic effects both statistically significant, $p < 0.001$. Further modeling revealed that craving was significantly lower following drinking on normal days ($p < 0.001$) in comparison to levels during abstained days. Craving was not different prior to drinking during the 3 normal days as compared to similar times during the 3 abstained days. Table 2 outlines results of this model and Figure 1 depicts predicted craving as a function of time of day and drinking using a drink consumed at 6 PM to illustrate the effect that drinking had on craving. Using alternative scaling for the craving variable with Poisson or dichotomized outcomes yielded identical results.
Table 3.2 Effect of time of day and having a drink on craving.
Results of growth curve analyses examining the effects of time of day (linear & quadratic trends) and having a drink on reported craving for alcohol

<table>
<thead>
<tr>
<th>Effect in multilevel model</th>
<th>Unstandardized Coefficient (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall level of craving</td>
<td>2.21 (0.32) ***</td>
</tr>
<tr>
<td>Linear effect of time of day (centered at 3PM)</td>
<td>0.17 (0.01) ***</td>
</tr>
<tr>
<td>Quadratic effect of time of day (centered at 3PM)</td>
<td>-0.02 (0.002) ***</td>
</tr>
<tr>
<td>Pre-Drinking (ref: abstaining)</td>
<td>0.11 (0.11)</td>
</tr>
<tr>
<td>Post-Drinking (ref: abstaining)</td>
<td>-0.96 (0.20) ***</td>
</tr>
</tbody>
</table>

* p < 0.05  
** p < 0.01  
*** p < 0.001
To determine the effects of CVT on craving, the influence of RSA-rest, residualized RSA-react, and their interaction on all effects of the previous model were assessed as continuous variables. Results (see Table 3) revealed that the linear and quadratic effects of time (diurnal patterns) and drinking effects were statistically significant (as in the first model). More importantly, the interaction of RSA-rest and residualized RSA-react significantly moderated the overall pattern of craving (p < 0.001) as well as the linear and quadratic effects of time (p values < 0.001).
Table 3.3 Moderating effect of RSA on time of day and having a drink on craving.
Results of growth curve analyses examining the moderating effects of RSA-rest, RSA-react, and their interaction on the effects of time of day (linear & quadratic trends) and having a drinking on reported craving for alcohol.

<table>
<thead>
<tr>
<th>Effect in multilevel model</th>
<th>Unstandardized Coefficient (SE)</th>
<th>Unstandardized Coefficient (SE) adjusting for sex and age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall level of craving</td>
<td>2.33 (0.27) ***</td>
<td>2.20 (0.45) ***</td>
</tr>
<tr>
<td>Moderating effect RSA-rest</td>
<td>0.10 (0.19)</td>
<td>-0.08 (0.24)</td>
</tr>
<tr>
<td>Moderating effect of RSA-react</td>
<td>1.03 (0.48) *</td>
<td>0.80 (0.54)</td>
</tr>
<tr>
<td>Moderating effect of interaction of RSA-rest &amp; RSA-react</td>
<td>1.08 (0.23) ***</td>
<td>0.94 (0.26) **</td>
</tr>
<tr>
<td>Moderating effect of female sex</td>
<td>-</td>
<td>0.25 (0.62)</td>
</tr>
<tr>
<td>Moderating effect of age</td>
<td>-</td>
<td>-0.04 (0.03)</td>
</tr>
<tr>
<td>Linear Effect of time of day (centered at 3PM)</td>
<td>0.16 (0.01) ***</td>
<td>0.21 (0.02) ***</td>
</tr>
<tr>
<td>Moderating effect RSA-rest</td>
<td>0.05 (0.01) ***</td>
<td>0.05 (0.01) ***</td>
</tr>
<tr>
<td>Moderating effect of RSA-react</td>
<td>0.07 (0.02) ***</td>
<td>0.09 (0.02) ***</td>
</tr>
<tr>
<td>Moderating effect of interaction of RSA-rest &amp; RSA-react</td>
<td>0.04 (0.01) ***</td>
<td>0.05 (0.01) ***</td>
</tr>
<tr>
<td>Moderating effect of female sex</td>
<td>-</td>
<td>-0.08 (0.02) ***</td>
</tr>
<tr>
<td>Moderating effect of age</td>
<td>-</td>
<td>0.0008 (0.001)</td>
</tr>
<tr>
<td>Quadratic effect of time of day (centered at 3PM)</td>
<td>-0.02 (0.002) ***</td>
<td>-0.02 (0.003) ***</td>
</tr>
<tr>
<td>Moderating effect RSA-rest</td>
<td>0.002 (0.001)</td>
<td>0.005 (0.002) ***</td>
</tr>
<tr>
<td>Moderating effect of RSA-react</td>
<td>-0.009 (0.004) *</td>
<td>-0.007 (0.004)</td>
</tr>
<tr>
<td>Moderating effect of interaction of RSA-rest &amp; RSA-react</td>
<td>-0.014 (0.002) ***</td>
<td>-0.011 (0.002) ***</td>
</tr>
<tr>
<td>Model-based estimates for four hypothetical RSA phenotypes: 1) higher RSA-rest/typical RSA-react; 2) higher RSA-rest/atypical RSA-react; 3) lower RSA-rest/typical RSA-react); and 4) lower RSA-rest/atypical RSA-react. Results showed that individuals with a higher RSA-</td>
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<tr>
<td>Moderating effect of female sex</td>
<td>-</td>
<td>0.004 (0.005)</td>
</tr>
<tr>
<td>Moderating effect of age</td>
<td>-</td>
<td>0.0006 (0.0002) *</td>
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<tr>
<td>Pre-drinking (ref: abstaining)</td>
<td>0.09 (0.11)</td>
<td>0.27 (0.17)</td>
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<tr>
<td>Moderating effect RSA-rest</td>
<td>0.11 (0.08)</td>
<td>0.16 (0.09)</td>
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<tr>
<td>Moderating effect of RSA-react</td>
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<td>0.28 (0.21)</td>
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<tr>
<td>Moderating effect of interaction of RSA-rest &amp; RSA-react</td>
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<td>0.15 (0.11)</td>
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<tr>
<td>Moderating effect of female sex</td>
<td>-</td>
<td>-0.32 (0.23)</td>
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<tr>
<td>Moderating effect of age</td>
<td>-</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>Post-drinking (ref: abstaining)</td>
<td>-0.97 (0.19) ***</td>
<td>-1.37 (0.34) ***</td>
</tr>
<tr>
<td>Moderating effect RSA-rest</td>
<td>-0.26 (0.12) *</td>
<td>-0.21 (0.16) *</td>
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<td>Moderating effect of RSA-react</td>
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<td>-0.64 (0.37)</td>
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<tr>
<td>Moderating effect of interaction of RSA-rest &amp; RSA-react</td>
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<td>-0.08 (0.17)</td>
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<tr>
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<tr>
<td>Moderating effect of age</td>
<td>-</td>
<td>0.01 (0.02)</td>
</tr>
<tr>
<td>* p &lt; 0.05</td>
<td>** p &lt; 0.01</td>
<td>*** p &lt; 0.001</td>
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rest and atypical RSA-react phenotype had a very unique pattern in their craving responses. That is, as shown in Figure 2, Panel B, they had a steeper increase in craving as the day progressed in comparison to those with a higher RSA-rest/typical RSA-react score (Panel A). The two other phenotypes, those with lower RSA-rest and either typical or atypical RSA-react showed both moderate increases in craving throughout the day and decreases in craving upon taking a drink. In our data set, older age was associated with lower RSA-rest ($r=-0.50$, $p<0.006$) and women had lower RSA-rest than men ($M=5.56$, $SD=2.13$ vs. $M=6.00$, $SD=0.93$), but there were no associations with residualized RSA-react. To examine possible confounding of the effects of RSA-rest, residualized RSA-react, and their interaction by age and sex, we reran the analyses while adjusting for age and sex. As shown in Table 3, the interaction remained statistically significant despite controlling for age and sex.
Figure 3.2 Plot of model-based estimates of craving across the 3-days of abstinence and before and after taking a drink during the 3-day normal period for four hypothetical cases: 1) RSA-rest set at 1 SD above the mean and residualized RSA-react set at 1 SD below the mean (higher RSA-rest/typical residualized RSA-react; Panel A); 2) RSA-rest set at 1 SD above the mean and residualized RSA-react set at 1 SD above than mean (higher RSA-rest/atypical residualized RSA-react; Panel B); 3) RSA-rest set at 1 SD below the mean and residualized RSA-react set at 1 SD above the mean (lower RSA-rest/typical residualized RSA-react, Panel C); and 4) RSA-rest set at 1 SD below the mean and residualized RSA-react set at 1 SD below than mean (lower RSA-rest/atypical residualized RSA-react; Panel D).
3.4 Discussion

As shown in Figure 1, craving responses for this sample of moderate to heavy drinkers across the day during a 3-day period in which alcohol was consumed as usual and during a 3-day period of imposed abstinence were modest with a peak average value of just below 3 on a visual analogue scale that ranged from 0-10. Yet, even at these levels, it was clear that drinking effectively reduced cravings during days of normal consumption. Moreover, consistent with our primary hypothesis, there were important between-group differences based on the statistically significant interaction effect between RSA-rest and RSA-react.

As supported by the data in Table 3 and the plots in Figure 2, the driving influence behind the significant interaction term was the aberrant craving pattern of individuals with a phenotype characterized by higher RSA-rest in combination with an atypical RSA-react response (Figure 2, Panel B). Specifically, a case with an RSA-rest score 1SD above the mean and a RSA-react score 1SD above the mean—a sluggish vagal brake in response to a postural challenge—had a dramatic increase in craving as the day progressed. This effect becomes most apparent when contrasting the craving pattern of this phenotype with that of someone with a higher RSA-rest and a typical vagal withdrawal to challenge (Figure 2, Panel A), reinforcing the adaptive role that this latter phenotype has on emotional and behavioral functioning (Hinnant and El-Sheikh 2009, Kreibig 2010, Cribbet, Williams et al. 2011). The aberrant pattern of craving observed for those with a higher RSA-rest and atypical RSA-react response is consistent with data published by Heilman and colleagues (Heilman, Connolly et al. 2012) concerning the
dysfunctional social behavior of children with selective mutism and data demonstrating that this phenotype is at an increased risk for depression (Yaroslavsky et al., 2013).

These data are particularly interesting considering that high RSA-rest is often associated with enhanced self-regulation and better psychological health (Porges, Doussard-Roosevelt et al. 1996, Thayer, Ahs et al. 2012). In fact, in the alcohol literature, craving has been reported to be inversely related to RSA assessed during a resting state (Quintana, Guastella et al. 2013). What the current data suggest is that, although main effects for RSA-rest do occur, the conceptual significance of RSA-rest values need to be interpreted in light of RSA-react. Considering that craving has been characterized as an emotional state (Kavanagh, Andrade et al. 2005), individuals with higher RSA-rest/atypical RSA-react may be experiencing more intense craving across the day in a manner consistent with the flexibility hypothesis proposed by Balzarotti and colleagues (Balzarotti, Biassoni et al. 2017); that is, they have a propensity to experience more intense emotional responses—positive or negative. Although lower RSA-rest (typical or atypical RSA-react) was not associated with elevated cravings for alcohol, there was a continuum of craving with changes in RSA-rest and RSA-react. As shown in Figure 2, higher RSA-rest/typical RSA-react had the lowest pre-drink craving. Craving then increases as the CVT profiles move from lower RSA-rest/typical RSA-react, lower RSA-rest/atypical RSA-react, and then to higher RSA-rest/atypical RSA-react. These results also do not rule out the possibility that those with lower RSA-rest may well be using drinking to counter dysphoric mood (Yaroslavsky, Rottenberg et al. 2014). This hypothesis deserves to be tested in future research (Balzarotti, Biassoni et al. 2017).
We did not have a sufficient numbers of participants in various subgroupings of RSA-rest and RSA-react to explore how changes in RSA during challenge within various subgroups may be related to change in heart rate responses. Such analyses are warranted in future research since Porges and colleagues have observed that an uncoupling of change in RSA with change in heart rate during periods of challenge is consistently related to dysfunctional social behavior (Porges, Doussard-Roosevelt et al. 1996, Umhau, George et al. 2002, Dale, O'Hara et al. 2011, Heilman, Harden et al. 2011) and may well be important in understanding alcohol craving. Needless to say, the patterns observed in the EMA craving responses for a phenotype with higher RSA-rest and an atypical RSA-react to postural challenge are disconcerting, and perhaps highlights one pathway by which craving is linked to AUD (de Bruijn, Korzec et al. 2004, Moos and Moos 2006, Fazzino, Harder et al. 2013).

Due to our exclusionary criteria for those diagnosed with AUD or alcohol dependence, the drinking behavior of moderate to heavy drinkers is likely predominately goal-directed reward seeking (e.g., a means to increased social connection) and not driven by automatic processes. Goal-directed reward seeking occurs when the decision to consume is driven by a conscious evaluation of the hedonic value of alcohol outweighing competing priorities, such as negative consequences related to use (Berridge and Robinson 2003, Everitt and Robbins 2005). This is in contrast to automatic drinking behavior seen in addiction that is unconscious, stimulus dependent, and driven by dopaminergic reward pathways (Naqvi, Gaznick et al. 2014). Considering the relationship between craving and abnormal CVT functioning (Quintana, Guastella et al. 2013, Quintana, Guastella et al. 2013), the higher overall craving responses observed in
the moderate to heavy drinkers with a higher RSA-rest/atypical RSA-react phenotype suggests that they may represent a subpopulation with greater vulnerability to drinking escalation and future AUD. As the craving response of this phenotype may be due to the higher hedonic value of alcohol in this subgroup, likely involving unconscious processes, a hypothesis exploring functional brain network studies may provide further insight. Within this theoretical framework, those with higher hedonic value of alcohol would be more likely to prioritize drinking over competing interests or avoidance of negative consequences related to drinking, again putting them at greater risk for AUD.

A final point that we would like to mention is the complexity underlying the concept of RSA reactivity. CVT functioning, specifically the myelinated vagal pathways originating in nucleus ambiguus, has shown to be important in self-regulation; however, emotional and/or behavioral responses can be either approach or avoidance related (Beauchaine, Gatzke-Kopp et al. 2007). This distinction is important to consider as a number of papers have demonstrated relationships between cue reactivity, HRV, and craving (Rajan, Murthy et al. 1998, Culbertson, Nicolas et al. 2010). We infer from these HRV studies, since there are strong correlations between overall heart rate variability and RSA, although quantifying RSA provides a metric that is more tightly linked to neural mechanism (Lewis, Furman et al. 2012). For example, Garland and colleagues (Garland, Franken et al. 2012) found higher HRV reactivity with greater attentional bias to drinking-related cues in alcohol-dependent individuals. Specifically, relapsing alcohol-dependent patients had higher HF-HRV reactivity to stress-primed alcohol cues. Within the current study design, we intentionally chose to examine patterns of RSA-react to a postural challenge—transitioning from a seated to standing position—because a postural
shift elicits a reflex and reflexes should be minimally influenced by contextual and other psychological variables. We then used this information to characterize how aberrant tonic and phasic RSA responses may interact to create physiological phenotypes that might explain downstream differences in craving. This approach parallels some extant work by Porges and colleagues (e.g., Heilman et al., 2012).

Although this study has a number of strengths it is not without limitations. First, although we did not observe that sex has a significant impact on the results of this study, in fairness, we were not powered to detect such effects and future work should examine this possibility. Second, differences in craving during drinking have been related to amount consumed and differ between dependent and non-dependent drinkers (Bujarski and Ray 2014, Miranda, MacKillop et al. 2016). This study only assessed craving after their final drink (upon going to bed) which minimized disruption of participants typical drinking, reducing chances of the craving assessment itself influencing ratings. And third, the random alerts only occurred between 9am – 9pm to avoid disruptions in sleep. On some occasions, this may have led to a lapse in data sampling. However, the participant-triggered alerts (i.e. “I am going to sleep”) provided additional data outside the random alert schedule.

In summary, to our knowledge this is the first investigation in the alcohol literature to examine how abnormal CVT reactivity influences alcohol craving both during periods of drinking as usual and abstinence in moderate to heavy drinkers. Individuals with a higher RSA-rest in combination with an atypical RSA-react phenotype were observed to exhibit higher cravings across the days than their peers with different phenotypes. In fact, the data suggested that unlike their peers, individuals with a higher RSA-rest and atypical
RSA-react phenotype experienced ongoing and substantial increases in cravings throughout the day. Future research is needed to replicate these findings and to investigate whether the self-regulatory dysfunction of CVT in persons who are moderate to heavy drinkers places them at increased risk for AUD.
References


CHAPTER IV

RELATIONSHIP BETWEEN CARDIAC VAGAL TONE AND FUNCTIONAL BRAIN CONNECTIVITY IN MODERATE TO HEAVY ALCOHOL CONSUMERS

R. Mayhugh, W.J. Rejeski, M. Bahrami, S.L. Simpson, K. Heilman, S. W. Porges, P.J. Laurienti

Submitted March 2018 to Addiction Biology (under review)
Abstract

Background: Functional brain networks are beginning to uncover the complex interactions involved in addiction neurocircuitry; however, the relationship between brain networks and autonomic nervous system (ANS) functioning remains relatively unexplored. Cardiac vagal tone (CVT), an indicator of ANS health, has been linked to alcohol craving. Here we investigated whether brain networks were associated with CVT in moderate-heavy alcohol consumers during their normal drinking routine and following an imposed alcohol abstinence period.

Method: Participants (from the local community, n=29, 24-60 years old) were assessed for CVT functioning via respiratory sinus arrhythmia (RSA) at rest (RSA-rest), and a postural shift (RSA-react). In both normal and abstinence states, resting-state functional magnetic resonance imaging data was used to assess whole brain connectivity. A mixed effects statistical model assessed relationships between brain connectivity and CVT.

Results: In abstinence, the RSA-rest/RSA-react relationship significantly interacted with network properties (clustering, p<0.05 & global efficiency, p<0.05). As RSA-rest increased and RSA-react became more typical, stronger brain network connections related to higher clustering and lower global efficiency. This brain connectivity profile reflected more regionally efficient but less globally distributive processing in more “optimal” CVT functioning (higher RSA-rest/typical RSA-react). There were no significant relationships during normal drinking.

Conclusion: Previous results from this study population indicated that participants with this optimal CVT profile exhibited the lowest alcohol craving patterns across the day.
Together, these findings suggest potential neural and physiological mechanisms underlying alcohol craving, a common marker for AUD vulnerability.
4.1 Introduction

Alcohol Use Disorder (AUD) relapse rates as high as 70% underscore the importance of AUD prevention (Seo, Lacadie et al. 2013). The exploration of neural mechanisms underlying vulnerability to transitioning from controlled drinking to addiction is critical if the goal of prevention is to be achieved. These mechanisms are increasingly being shown to be a dynamically interconnected system involving various neurobiological elements, the interactions of which are not fully understood (Seo and Sinha 2015).

Brain imaging studies have revealed widespread changes in brain functioning with addiction, pointing to the importance of studying these changes using brain network analyses that capture the interdependence between different regions of the brain. For example, the prefrontal cortex (PFC) is strongly implicated in AUD but almost always in association with another brain region. In recovering abstinent alcoholics, increased ventromedial PFC and anterior cingulate cortex (ACC) activity during a neutral cue was correlated with high craving during exposure to alcohol and stress cues. Increased activity in these areas also predicted alcohol relapse (Seo, Lacadie et al. 2013). Decreased activity in PFC and striatal regions have also been implicated in impaired self-control in addiction (Tang, Posner et al. 2015). The precuneus and PFC have demonstrated co-occurring changes in addiction (Seo, Jia et al. 2011, Seo, Lacadie et al. 2013) and it has been suggested that studies should be focused on subnetworks, such as the default mode network, instead of individual regions (Volkow and Baler 2013).

Functional brain connectivity studies are beginning to assess network organization associated with AUD (Chanraud, Pitel et al. 2011, Camchong, Stenger et al. 2013). In comparison to healthy controls, sober alcoholics exhibit differing connectivity
in a range of subnetworks including the default mode, salience, executive control, and subcortical reward networks with weaker within-network connections and expanded connectivity to regions outside these networks (Muller-Oehring, Jung et al. 2015). These authors suggest that alcoholics may have a reduced capacity to coordinate neural coherence within known networks and are activating areas that are outside of systems typically observed in healthy populations. These patterns are reflected in measures such as clustering and global efficiency. For example, clustering reflects connectivity that is more segregated or within known networks and global efficiency reflects connectivity that is more distributed across the brain (beyond known networks). Whole brain measures of network connectivity in those with a high risk for AUD (as assessed by familial density of AUD) revealed decreased clustering and local efficiency compared to healthy low risk males (Holla, Panda et al. 2017). In addition, whole brain networks of alcohol dependent patients exhibit reduced average clustering with more severe alcohol use (Sjoerds, Stufflebeam et al. 2017). For a review of network measures see (Bullmore and Sporns 2009). Together, this work suggests a decrease in regional specificity in AUD and emphasizes the importance of assessing changes in patterns of whole-brain networks for further understanding of addiction.

The autonomic nervous system (ANS) is likely a critical component of the addiction process as it provides a link through which the brain receives sensory information from visceral organs and in turn the brain regulates these organs. When the peripheral and sympathetic nervous system (branches of the ANS) are optimally regulated, the body is able to appropriately adjust to changing environmental challenges. Not only is ANS regulation adversely affected by alcohol consumption and addiction, but
it is also associated with alcohol craving (Quintana, Guastella et al. 2013, Karpyak, Romanowicz et al. 2014, Seo and Sinha 2015). As specified by Polyvagal Theory, cardiac vagal tone (CVT), an indicator of ANS health, captures the functional relationship between the heart and brainstem and is commonly quantified by the high frequency heart rate variability (HF-HRV) observed in respiratory sinus arrhythmia (RSA). For example, high CVT at rest reflects a “vagal brake” that inhibits the sympathetic nervous system’s influence on cardiac output (Porges 2007). In order to meet situational demands, such as an environmental stressor requiring an immediate motor behavior, this “vagal brake” is released allowing cardiac output to increase (Porges, Doussard-Roosevelt et al. 1996). Thus, in addition to examining CVT during a resting state additional research has examined vagal reactivity to a stressor or challenge (Porges, Doussard-Roosevelt et al. 1996, Heilman, Connolly et al. 2012, Yaroslavsky, Bylsma et al. 2013, Balzarotti, Biassoni et al. 2017). Nevertheless, the majority of research has focused on either the main effects of CVT at rest or during reactivity to a challenge. However, there is evidence that considering the interactive effects of the two metrics provides new insight. For example, previous research has shown that optimal functioning involves higher RSA at rest due to a strong vagal brake combined with greater RSA changes in response to a challenge indicating an efficient release of the vagal brake (Hinnant and El-Sheikh 2009, Cribbet, Williams et al. 2011).

The neurovisceral integration model suggests that specific brain circuitry results in the heart rate variability observed in CVT and can be used to understand emotional regulation. Specifically, this model emphasizes the importance of inhibitory circuitry from the prefrontal cortex to the amygdala in self-regulation and goal-directed behavior, both
key aspects of the addiction process (Thayer and Lane 2000). A meta-analysis of studies measuring the relationship between cerebral blood flow and heart rate variability found a number of brain regions with significant associations and suggested that this is a means of understanding flexible control over behavior and response to stress (Thayer, Ahs et al. 2012). One of the few functional connectivity studies assessing brain/CVT relationships showed a positive association between HRV and medial PFC, dorsal and perigenual ACC, and anterior insula connectivity in healthy midlife adults (Jennings, Sheu et al. 2016). Another study showed that resting state connectivity from the dorsal ACC and amygdala to other brain areas was enhanced during elevated HRV (Chang, Metzger et al. 2013).

Taken together this work provides a strong impetus to conduct studies evaluating the relationship between alcohol use, CVT, and functional brain networks. The analyses presented here were designed to evaluate brain functioning at a systems level. This is not only ideal for capturing the widespread interactions of brain regions in addiction, but expands the understanding of brain/CVT functional relationships beyond circuitry between specific brain areas to whole brain patterns of functional connectivity. Here we investigated whether brain networks were associated with CVT in moderate-heavy alcohol consumers during their normal drinking routine (normal days) and following an imposed period of alcohol abstinence (abstained days). Importantly, we evaluated the impact of the interaction between RSA assessed in a sitting positon (RSA-rest) and the reactivity of RSA measured while transitioning from a seated to a standing position (RSA-react) on functional brain connectivity. This approach is novel to ANS/brain functioning research and certainly to the field of alcohol addiction. We hypothesized that
imposed alcohol abstinence would affect brain network organization and that this change would be related to CVT functioning. Considering that previous evidence has shown higher RSA-rest combined with greater vagal withdrawal under challenge (typical RSA-react) to be optimal CVT functioning (Hinnant and El-Sheikh 2009, Kreibig 2010, Cribbet, Williams et al. 2011) and that reduced regional specificity of brain network organization has been reported in AUD, we expected that higher RSA-rest with typical RSA-react would be related to greater regional specificity of brain connectivity.

4.2 Methods

Study Overview

The study protocol consisted of a baseline visit and two magnetic resonance imaging (MRI) scanning visits. After passing an initial phone screen, a baseline visit was conducted to complete informed consent, insure eligibility, administer self-report questionnaires, and collect cardiac functioning data. The scanning visits included self-report questionnaire administration and the functional MRI (fMRI) scan and occurred directly following three consecutive days of normal drinking and three days of abstinence. The scanning visit order was randomly assigned (3-day minimum wash out period between sessions). The focus of this report is on the relationships between the RSA data collected during the baseline visit and the fMRI data collected during the normal and abstained day scanning visits.

Participants

Thirty-four moderate to heavy drinkers were recruited from the Winston-Salem, North Carolina area. The final sample included twenty-nine participants (13 men and 16
women) after excluding for missing data (four participants missing RSA data and one missing functional brain network data). Enrollment criteria included an age range between 24 & 60 years old, alcohol consumption \( \geq 50\% \) of days in the past 3 months, average daily alcohol consumption of 1-3 drinks/day for women and 2-4 drinks/day for men for at least the past three years. The Time Line Follow Back (TLFB) was administered during the baseline visit to quantify participant’s drinking over the previous three months and establish eligibility. The TLFB is a well-established retrospective measure of alcohol consumption (Vakili, Sobell et al. 2008) and was modified to allow for time of day (morning, afternoon, and evening) of consumption to be collected. Exclusion criteria included AUD diagnosis, binge drinking (National Institute on Alcohol Abuse and Alcoholism definition of \( \geq 4 \) drinks for females, \( \geq 5 \) drinks for males within 2 hours (NIAAA(b)) more than once a month, consuming alcohol before noon \( > 3 \) times/month, a history of severe medical conditions stable for < 2 months, a score of > 20 on the Center for Epidemiological Studies Depression Scale (CES-D), history of neurological disease diagnosis, > 500 mg per day of caffeine, smoked > 1.5 packs per day, or positive urine drug screen test (Methamphetamine, Cocaine, Marijuana, Amphetamine, Opiates, & Benzodiazepines). Due to the association between body mass index (BMI) and blood-alcohol content (BAC), BMI was restricted to a range from 18.5 kg/m\(^2\) to \( \leq 35 \) kg/m\(^2\) (Wang, Nicholson et al. 1992). Due to the MRI portion of the protocol, participants had to be right-handed, not claustrophobic, or have any other medical condition that would put them at risk during the scanning protocol. The Clinical Institute Withdrawal Assessment of Alcohol (CIWA-Ar) was used as a safety screening for alcohol withdrawal symptoms (Sullivan, Sykora et al. 1989).
Heart Rate Assessment and Data Processing for RSA Calculation

An electrocardiogram (ECG) was collected via a Biopac MP150 by Biopac Systems, Inc. (www.biopac.com, Goleta, CA) during the baseline visit. Three electrodes (one on each collar bone and one on the lower right rib) and a pulse oximeter (right index finger) were placed on the participant. The data collection protocol was as follows: participants first sat quietly for 5 minutes prior to collection, resting data was then collected in the seated position (5 minutes), reactivity data was collected following a postural shift (sit to stand, 2 minutes). CardioEdit software (Brain-Body Center, University of Illinois at Chicago, 2007) was used to visually inspect and edit off-line heart rate data.

CardioBatch Plus software (Brain-Body Center for Psychophysiology and Bioengineering, University of North Carolina at Chapel Hill, 2016) was used to calculate heart rate and RSA from the ECG data consistent with procedures developed by (Porges 1985). CardioBatch Plus quantified the amplitude of RSA using age-specific parameters that are sensitive to the maturational shifts in the frequency of spontaneous breathing. The method when applied to adults includes the following steps: (1) timing sequential R-R intervals to the nearest millisecond; (2) producing time-base data by resampling the sequential R-R intervals into 500 msec intervals; (3) detrending the time-based series with a 21-point cubic moving polynomial that is stepped through the data to create a smoothed template then the template is subtracted from the original time-based series to generate a detrended residual series; (4) bandpass filtering the detrended time series to extract the variance in the heart period pattern associated with spontaneous breathing in adults (.12 - .40 Hz); and (5) transforming the variance estimates with a natural logarithm to normalize the distribution of RSA estimates (Riniolo and Porges 1997). These
procedures are statistically equivalent to frequency domain methods (i.e., spectral analysis) for the calculation of the amplitude of RSA when heart period data are stationary (Porges and Byrne 1992). RSA was quantified during each sequential 30-sec epoch and the averages within each condition were used in the data analyses. To calculate RSA reactivity (RSA-react), we subtracted the average 5-min RSA value while sitting (RSA-rest) from the standing 2-min RSA value.

**Brain Imaging and Functional Brain Network Analysis**

**Image Collection**

The imaging protocol consisted of a T1-weighted structural scan followed by blood-oxygen-level-dependent (BOLD)-weighted scans in the following order: resting state (6 minutes), non-stressful personalized guided imagery task (6 minutes), stressful personalized guided imagery task (6 minutes), “stress recovery” scan (10 minutes), and final resting state (6 minutes). The first resting state scan was the focus of this analysis. During the resting state scans, participants were told to keep their eyes-open and to focus on a fixation cross projected onto a screen.

MRI data were obtained on a 3T Siemens Skyra equipped with a 32-channel head coil, a rear projection screen, pulse oximeter, and MRI compatible audio headphones. High-resolution (1.0 x 1.0 x 1.0 mm) T1-weighted structural scans were acquired in the sagittal plane using a single-shot 3D MPRAGE GRAPPA2 sequence (Repetition Time (TR) = 2.3 seconds, Echo Time (TE) = 2.99 ms, 192 slices). The resting state BOLD-weighted image sequence were acquired in the transverse plane using an echo-planar imaging sequence (resolution = 3.5 x 3.5 x 5.0 mm acquisition time = 6 minutes, TR =
2.0 seconds, TE = 25 ms, flip angle = 75˚, 35 slices per volume, 177 volumes). The first 20 seconds (10 image volumes) were discarded to allow signal to achieve equilibrium.

**Image Preprocessing & Network Generation**

Image preprocessing was performed using SPM12 software ([www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)). The functional images were slice time corrected and realigned to the first image of the series. Preprocessing for the structural image consisted of skull removal (skull-stripped) with the remaining image segmented into gray matter, white matter, and cerebrospinal fluid maps using a unified segmentation algorithm (Ashburner and Friston 2005).

The skull stripped structural image was warped to the Colin template (Holmes, Hoge et al. 1998) using Advanced Normalization Tools (ANTs) ([http://stnava.github.io/ANTs/](http://stnava.github.io/ANTs/)). The resulting inverse warp deformation map was applied to a functional atlas specifically designed for brain network analyses (Shen, Tokoglu et al. 2013), warping the atlas to each subject’s original (native) space anatomical image. The atlas was then co-registered and resliced to match functional data.

Physiological noise and low frequency drift were reduced by regressing out the mean signals for whole-brain, white matter, and cerebrospinal fluid and applying band-pass filtering (0.009–0.08 Hz). Motion correction was performed to eliminate scan volumes with excessive frame-wise displacement and BOLD signal change (Power, Barnes et al. 2012). Voxel wise time series data was converted to the 268 node functional atlas by averaging functional signals within atlas regions. The functional atlas time series data was then used generating functional brain networks by performing a node-by-node Pearson’s correlation analysis using the WFU_MNNET toolbox (Bahrami, Laurienti et
Statistical analyses focused on unthresholded positive correlation matrices. The typical procedure for brain network analyses of setting negative values to zero was used because most graph analysis algorithms that yield topological network properties cannot accommodate negative edges [for discussion see (Fraiman, Balenzuela et al. 2009, Schwarz and McGonigle 2011, Telesford, Simpson et al. 2011)].

**Statistical Analysis and Mixed-effects Modeling Framework**

A mixed effects regression framework was chosen for its ability to account for correlations between network connections within the individual participants (repeated measures), as a functional connection between two brain regions within an individual is not independent of the other connections. This mixed effects model assessed relationships between whole brain network connectivity and continuous measures of CVT (RSA-rest and RSA-react) and confounding variables (total years drinking, % of days drank in last 3 months, average drinks on drinking days, age, sex, and BMI). For a detailed report on this mixed effects modeling framework methodology and previous applications see (Simpson and Laurienti 2015, Bahrami, Laurienti et al. 2017).

This statistical model was run using fMRI data collected following a 3-day period of their normal drinking routine, and then again following a 3-day period of imposed alcohol abstinence. The model allowed for the analysis of both network and non-network variables as well as the assessment of interaction covariates. The methodology uses a two-part model to examine the probability of network connections (presence/absence) as well as the strength of connections if they exist. That is, this multivariate regression framework quantifies the relationships between brain connections (probability and strength) as the outcome (dependent) variables, and network and non-network covariates.
(including interactions between the two sets) as the independent variables. All covariates in this analysis were analyzed as continuous measures, maximizing the statistical power of these results. Analyses were conducted using Matlab (R2016b) and SAS v9.4 software.

**Covariates**

**Cardiac Vagal Tone:** Our main covariate of interest was RSA-react, which captured the phasic response of RSA to a postural shift. RSA-rest was also included in the model and assessed for interactions with RSA-react and network metrics (see Network Covariates below). Table 1 reports descriptive statistics on RSA-rest and RSA-react scores.

**Network covariates:** The network topology from each individual participant was summarized with standard graph theory variables (Newman 2003, Rubinov and Sporns 2010) computed for each node pair including average clustering coefficient (local specialization), average global efficiency (global integration), and difference in degree between each nodal pair. Overall network modularity, the extent to which the network subdivides into densely interconnected communities that are sparsely connected to the rest of the network (Newman and Girvan 2004), was also calculated. Each of these summary variables was included as a covariate in the analyses.

**Confounding Covariates:** Measures that captured variance in drinking behavior and alcohol exposure were included in the model. These included the average number of drinks per day consumed on days they chose to drink alcohol, the percent of days that they reported drinking at least one serving of alcohol over the last three months (via the TLFB), and the total years they had been consuming alcohol. Age, sex, and body mass
index (BMI) were included due to previous associations with alcohol use and/or cardiac vagal function (Wang, Nicholson et al. 1992, Demeersman 1993, Seo, Jia et al. 2011). See Table 1 for sample demographics and descriptive statistics of these confounding covariates. The spatial and square of spatial Euclidean distance between network nodes (brain regions) was also included (Friedman, Landsberg et al. 2014).

<table>
<thead>
<tr>
<th>Table 4.1. Sample Characteristics: Mean (SD) or Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>Black or African American</td>
</tr>
<tr>
<td>Asian</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Alcohol Use</td>
</tr>
<tr>
<td>Total Years Drinking</td>
</tr>
<tr>
<td>Previous 3 Months (Time Line Follow Back)</td>
</tr>
<tr>
<td>% of days that were drinking days</td>
</tr>
<tr>
<td>Average drinks consumed on drinking days</td>
</tr>
<tr>
<td>Cardiac Vagal Tone</td>
</tr>
<tr>
<td>RSA-react</td>
</tr>
<tr>
<td>RSA-rest</td>
</tr>
</tbody>
</table>
4.3 Results

Normal Drinking Days – Model Results

Table 2 provides detailed results from the two-part model for probability and strength of connections for normal drinking days. The table includes the parameter estimates, standard error, and p-values. The main effect for each variable describes how changes in that variable are related to changes in the probability and strength of functional brain connections. The specified interactions examine 2-way and 3-way interactions between brain connectivity probability/strength, CVT, and network topology.

Network Variables: With the exception of modularity, network variables were significantly associated with the probability of connections. Global efficiency increased as the probability of connections increased, however clustering and degree difference decreased. Network metrics were also significantly associated with the strength of connections. Clustering and global efficiency increased with strength of connections, but degree difference and modularity decreased.

Confounding Variables: There was a marginal positive relationship between total years drinking and probability of connections. However, as total years drinking and average drinks consumed on drinking days increased, strength of connection significantly decreased. The percent of days participants drank in the last 3 months was not associated with probability or strength of connections. Age only approached a significant relationship with probability and strength of connections. BMI and sex were related to neither connection probability nor strength. The spatial and square of spatial Euclidean distance between network nodes (brain regions) significantly contributed to both probability and strength of connections. Including these variables in the analyses allowed
for relationships between CVT and brain network properties to be captured while controlling for these significant confounding relationships.

**CVT:** The probability of connections had no relationship with RSA-react or RSA-rest. There were no significant interactions between probability of connections, RSA-react, and network metrics nor RSA-react, RSA-rest, and network metrics. Connection strength was not significantly associated with RSA-react; however there was a trend towards a negative relationship between RSA-rest and strength of connections. The interaction between RSA-react, RSA-rest, and connection strength also approached significance. There were no significant interactions between connection strength, RSA-react, RSA-rest, and network metrics.
Table 4.2. Mixed Method Model Results - Normal Days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Probability of Connections</th>
<th>Strength of Connections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Standard Error</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.1872</td>
<td>0.03567</td>
</tr>
<tr>
<td>Clustering Coefficient</td>
<td>-0.3515</td>
<td>0.0372</td>
</tr>
<tr>
<td>Global Efficiency</td>
<td>0.3247</td>
<td>0.03047</td>
</tr>
<tr>
<td>Degree</td>
<td>-0.1551</td>
<td>0.02095</td>
</tr>
<tr>
<td>Modularity</td>
<td>0.03836</td>
<td>0.02667</td>
</tr>
<tr>
<td>Total Years Drinking</td>
<td>0.1269</td>
<td>0.0656</td>
</tr>
<tr>
<td>% of Days Drank in Last 3 Months</td>
<td>0.00845</td>
<td>0.02169</td>
</tr>
<tr>
<td>Average Drinks On Drinking Days</td>
<td>-0.03223</td>
<td>0.0273</td>
</tr>
<tr>
<td>Age</td>
<td>-0.1249</td>
<td>0.06972</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.05562</td>
<td>0.05425</td>
</tr>
<tr>
<td>BMI</td>
<td>0.04814</td>
<td>0.03106</td>
</tr>
<tr>
<td>Spatial Distance</td>
<td>-0.3581</td>
<td>0.02362</td>
</tr>
<tr>
<td>Spatial Distance Squared</td>
<td>0.1347</td>
<td>0.0092</td>
</tr>
<tr>
<td>RSA-react (COI)</td>
<td>-0.00152</td>
<td>0.02986</td>
</tr>
<tr>
<td>RSA-rest</td>
<td>-0.02777</td>
<td>0.02804</td>
</tr>
<tr>
<td>RSA-react (COI)*RSA-rest</td>
<td>-0.01394</td>
<td>0.02329</td>
</tr>
<tr>
<td>RSA-react (COI)*Clustering Coefficient</td>
<td>0.03693</td>
<td>0.0441</td>
</tr>
<tr>
<td>RSA-react (COI)*Global Efficiency</td>
<td>-0.04068</td>
<td>0.03608</td>
</tr>
<tr>
<td>RSA-react (COI)*Degree</td>
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<td>0.02482</td>
</tr>
<tr>
<td>RSA-react (COI)*Modularity</td>
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<td>0.04851</td>
</tr>
<tr>
<td>RSA-react (COI)<em>RSA-rest</em>Clustering Coefficient</td>
<td>0.02767</td>
<td>0.02369</td>
</tr>
<tr>
<td>RSA-react (COI)<em>RSA-rest</em>Global Efficiency</td>
<td>-0.02307</td>
<td>0.0194</td>
</tr>
<tr>
<td>RSA-react (COI)<em>RSA-rest</em>Degree</td>
<td>0.00465</td>
<td>0.01331</td>
</tr>
<tr>
<td>RSA-react (COI)<em>RSA-rest</em>Modularity</td>
<td>-0.05137</td>
<td>0.06343</td>
</tr>
</tbody>
</table>
**Abstained Days – Model Results:**

Table 3 provides detailed results from the two-part model for probability and strength of connections for abstained days. The table includes the parameter estimates, standard error, and p-values. The main effect for each variable describes the relationships between that covariate and the probability and strength of functional brain connections. The specified interactions examine 2-way and 3-way interactions between brain connectivity probability/strength, CVT, and network topology.

**Network Variables:** There were significant statistical association between network variables and probability of connection. As found on normal drinking days, global efficiency increased as the probability of connections increased, however clustering and degree difference decreased. Modularity was not significantly associated with probability of connections. Also as found on normal drinking days, increased connection strength was associated with increased clustering and global efficiency but decreased degree difference and modularity.

**Confounding Variables:** Spatial distance and spatial distance squared were the only confounding covariates that were significantly related to the probability and strength of connections.

**CVT:** There were no significant relationships between connection probability and RSA-react or RSA-rest. Connection probability and RSA-react did not have any significant interactions with RSA-rest or any of the network metrics. Connection strength did not significantly interact with RSA-react or RSA-rest directly. However, global efficiency significantly interacted with RSA-react and RSA-rest to positively predict connection strength. Clustering also interacted with RSA-react and RSA-rest to negatively predict
connection strength. This interaction was not observed in degree difference or modularity.

The estimates generated from the mixed effects model were used with chosen RSA-rest and RSA-react scores to visually depict the significant 3-way interactions described above. The minimum and maximum RSA scores from our sample collected during rest were chosen to depict “lower” and “higher” RSA-rest, respectively. The larger RSA-react value is considered “typical” reactivity in higher RSA-rest and “atypical” reactivity in lower RSA-rest. The smaller RSA-react is the score is considered “typical” reactivity in lower RSA-rest and “atypical” in higher RSA-rest.

The interaction between connection strength, clustering, RSA-rest, and RSA-react is depicted in Figure 1. There was an overall increase in strength as clustering increased regardless of RSA scores. With lower RSA-rest scores (A), there was no meaningful difference in the relationship between the typical and atypical react scores. However, with higher RSA-rest scores (B), there was a divergence between typical and atypical react scores. Note that the increase in the slope of the higher RSA-rest/typical react was the main driver of this interaction. Thus, the relationship between clustering and connection strength was enhanced in the higher RSA-rest/typical react relative to other RSA score combinations. Figure 2 depicts this relationship using a simplified cartoon model.
Figure 4.1: Graphs using RSA-rest and RSA-react scores to visually depict the significant interaction between connection strength, RSA-rest, RSA-react, and clustering. In lower RSA-rest scores, connections strength and clustering relationships were only slightly impacted by RSA-react scores (panel A). As RSA-rest scores increased, the relationship between connection strength and network topology differed by RSA-react scores (panel B). As shown in panel B, for any given clustering value (example value represented by solid black line), connection strength (dotted lines) was higher in Higher RSA-rest/typical RSA-react compared to Higher RSA-rest/atypical RSA-react. This was especially true as clustering increased.
Figure 4.2: Depicts the relationship between connection strength and clustering using a simplified cartoon model. The left side of the figure depicts more “optimal CVT phenotype” (higher RSA-rest/typical RSA-react) and the right side, all “other CVT phenotypes” (higher RSA-rest/atypical RSA-react, lower RSA-rest/typical RSA-react, and lower RSA-rest/atypical RSA-react). Larger network nodes (spheres) have greater clustering and thicker edges (lines) depict stronger connection strength. At lower clustering, connections were weaker regardless of CVT phenotype. As clustering increased in the optimal CVT phenotype, connections increased in strength more severely than the other CVT phenotypes.

The interaction between connection strength, global efficiency, RSA-rest, and RSA-react is depicted in Figure 3. With lower RSA-rest scores (A), there was a slight overall positive relationship between global efficiency and connection strength. There was no meaningful difference in this relationship between the typical and atypical react scores. With higher RSA-rest scores (B), the relationship between global efficiency and strength was dramatically changed. With higher RSA-rest/atypical RSA-react scores the slight positive relationship observed with lower RSA-rest scores was preserved.
However, the slope of the higher RSA-rest/typical react was reversed such that higher global efficiency was associated with lower connection strength. Thus, the relationship between global efficiency and connection strength was reversed in the higher RSA-rest/typical react relative to other RSA score combinations. Figure 4 depicts this relationship using a simplified cartoon model.

**Figure 4.3**: Graphs using RSA-rest and RSA-react scores to visually depict the significant interaction between connection strength, RSA-rest, RSA-react, and global efficiency. In lower RSA-rest scores, connections strength and global efficiency relationships were only slightly impacted by RSA-react scores (panel A). As RSA-rest scores increased, the relationship between connection strength and global efficiency differed by RSA-react scores (panel B). As shown in panel B, for any given global efficiency value (example value represented by solid black line), connection strength (dotted lines) was lower in Higher RSA-rest/typical RSA-react compared to Higher RSA-rest/atypical RSA-react. This relationship became more extreme as global efficiency decreased. This was especially true as global efficiency increased.
Figure 4.4: Depicts the relationship between connection strength and global efficiency using a simplified cartoon model. The left side of the figure depicts more “optimal CVT phenotype” (higher RSA-rest/typical RSA-react) and the right side, all “other CVT phenotypes” (higher RSA-rest/atypical RSA-react, lower RSA-rest/typical RSA-react, and lower RSA-rest/atypical RSA-react). Larger network nodes (spheres) have higher global efficiency and thicker edges (lines) depict stronger connection strength. As global efficiency increased, connection strength in the optimal CVT phenotype decreased. However, connection strength did not meaningfully change as global efficiency increased in the other CVT phenotypes.
Table 4.3. Mixed Method Model Results - Abstained Days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Probability of Connections</th>
<th></th>
<th></th>
<th>Strength of Connections</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Standard Error</td>
<td>p-value</td>
<td>Estimate</td>
<td>Standard Error</td>
<td>p-value</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.1696</td>
<td>0.0443</td>
<td>0.0001</td>
<td>0.224</td>
<td>0.0057</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Clustering Coefficient</td>
<td>-0.2442</td>
<td>0.02343</td>
<td>&lt;.0001</td>
<td>0.06374</td>
<td>0.00212</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Global Efficiency</td>
<td>0.2438</td>
<td>0.02417</td>
<td>&lt;.0001</td>
<td>0.02321</td>
<td>0.00186</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Degree</td>
<td>-0.1615</td>
<td>0.01847</td>
<td>&lt;.0001</td>
<td>-0.04266</td>
<td>0.00109</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Modularity</td>
<td>0.04914</td>
<td>0.03108</td>
<td>0.1139</td>
<td>-0.01206</td>
<td>0.00399</td>
<td>0.0025</td>
</tr>
<tr>
<td>Total Years Drinking</td>
<td>0.04893</td>
<td>0.07837</td>
<td>0.5324</td>
<td>0.00398</td>
<td>0.01011</td>
<td>0.6936</td>
</tr>
<tr>
<td>% of Days Drank in Last 3 Months</td>
<td>0.03221</td>
<td>0.02974</td>
<td>0.2789</td>
<td>-0.0055</td>
<td>0.00382</td>
<td>0.15</td>
</tr>
<tr>
<td>Average Drinks On Drinking Days</td>
<td>-0.02008</td>
<td>0.03285</td>
<td>0.5411</td>
<td>-0.00465</td>
<td>0.00424</td>
<td>0.2724</td>
</tr>
<tr>
<td>Spatial Distance</td>
<td>-0.361</td>
<td>0.02142</td>
<td>&lt;.0001</td>
<td>-0.05172</td>
<td>0.00164</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Spatial Distance Squared</td>
<td>0.1345</td>
<td>0.00885</td>
<td>&lt;.0001</td>
<td>0.02988</td>
<td>0.00075</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.03871</td>
<td>0.08055</td>
<td>0.6308</td>
<td>-0.00514</td>
<td>0.01038</td>
<td>0.6208</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.06749</td>
<td>0.06712</td>
<td>0.3147</td>
<td>-0.00062</td>
<td>0.00863</td>
<td>0.9429</td>
</tr>
<tr>
<td>BMI</td>
<td>0.03397</td>
<td>0.04098</td>
<td>0.4072</td>
<td>0.00474</td>
<td>0.00528</td>
<td>0.3688</td>
</tr>
<tr>
<td>RSA-react (COI)</td>
<td>-0.00374</td>
<td>0.04076</td>
<td>0.9269</td>
<td>0.00053</td>
<td>0.00524</td>
<td>0.9194</td>
</tr>
<tr>
<td>RSA-rest</td>
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<td>0.5814</td>
<td>0.00367</td>
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<td>0.4843</td>
</tr>
<tr>
<td>RSA-react (COI)*sit_RSA30</td>
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<td>0.04573</td>
<td>0.5964</td>
<td>-0.00083</td>
<td>0.0059</td>
<td>0.8882</td>
</tr>
<tr>
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<td>0.00568</td>
<td>0.02782</td>
<td>0.8383</td>
<td>-0.0017</td>
<td>0.00252</td>
<td>0.5005</td>
</tr>
<tr>
<td>RSA-react (COI)*Global Efficiency</td>
<td>-0.00458</td>
<td>0.02865</td>
<td>0.8731</td>
<td>0.00352</td>
<td>0.0022</td>
<td>0.1104</td>
</tr>
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<td>RSA-react (COI)*Degree</td>
<td>0.00732</td>
<td>0.02189</td>
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<td>0.00129</td>
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</tr>
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<td>RSA-react (COI)*Modularity</td>
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<td>0.8727</td>
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<td>0.4757</td>
</tr>
<tr>
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<td>0.02405</td>
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</tr>
<tr>
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<td>-0.00749</td>
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<tr>
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<td>0.01541</td>
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<td>0.0012</td>
<td>0.0215</td>
</tr>
<tr>
<td>Modularity*RSA-react (COI)*RSA-rest</td>
<td>-0.01113</td>
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<td>0.8018</td>
<td>-0.00609</td>
<td>0.00573</td>
<td>0.2879</td>
</tr>
</tbody>
</table>
4.4 Discussion:

Consistent with the study hypothesis, the interaction between RSA-rest and RSA-react was associated with functional brain network connectivity, an effect that was observed during the abstinence trial but not during the normal drinking trial. Specifically, RSA modulated the relationship between the strength of brain network connections as they related to both clustering and global efficiency. This relationship was driven by the fact that as RSA-rest scores increased, RSA-react became a significant factor in predicting brain network connectivity.

As RSA-rest scores increased and RSA-react responses became more typical (larger RSA-react in those with higher RSA-rest) stronger brain network connections were related to higher clustering and lower global efficiency. Comparatively, during the period of abstinence, RSA-react had virtually no impact on brain connectivity in those individuals with lower RSA-rest (Figure 1A and 3A). This relationship between connection strength with both clustering and global efficiency in higher RSA-rest/typical RSA-react deviated markedly from the other RSA phenotypes. That is, other phenotypes (higher RSA-rest/atypical RSA-react, lower RSA-rest/typical RSA-react, and lower RSA-rest/atypical RSA-react) were all similar and demonstrated smaller changes in connection strength as clustering and global efficiency changed compared to higher RSA-rest/typical RSA-react (Figure 1 and 3).

The relationships between RSA and the brain network variables during abstinence are important given the previous work demonstrating associations between brain network variables and brain health (Bassett and Bullmore 2009). For example, reduced clustering was observed in those diagnosed with schizophrenia compared to healthy controls.
Clustering is also related to addiction, with more severe alcohol use and longer duration of alcohol dependence associated with reduced clustering (Sjoerds, Stufflebeam et al. 2017). Global efficiency of brain network connectivity should also be considered as it has been related to brain diseases and alcohol use. For example, increased global efficiency has been observed in major depressive disorders (Zhang, Wang et al. 2011). In moderate-heavy alcohol consumers, older adults had significantly greater global efficiency of whole brain functional connectivity than younger adults during rest (Mayhugh, Moussa et al. 2016). These findings point to the importance of assessing measures that reflect both regional and global organization of brain connectivity. The existing evidence suggests that higher and/or dysfunctional alcohol use may be associated with a shift toward less regional and more distributed brain network organization (greater random connectivity).

Here we show that as RSA rest and react scores approach what is considered to be “optimal” CVT functioning (higher RSA-rest/typical RSA-react), functional brain connectivity reflects that which has more regionally efficient but less globally distributive processing (less random network). It should be noted that this shift to more regionally specific processing in more optimal CVT functioning (higher RSA-rest/typical RSA-react) was not statistically significant under normal drinking conditions. This is interesting considering that both polyvagal and neurovisceral integration models view CVT as a measure of autonomic system flexibility to environmental and contextual demands (Porges, Doussard-Roosevelt et al. 1996, Thayer and Lane 2000, Yaroslavsky, Bylsma et al. 2013) allowing for appropriate regulation of emotion. This relationship
between brain network organization and RSA functioning may reflect differing biological responses brought on by the stress to the system induced by imposed alcohol abstinence.

This difference in brain functioning observed at the systems level in those with higher RSA-rest/typical RSA-react versus higher RSA-rest/atypical RSA-react may reflect changes in interactions within the extensive circuitry that has been previously identified in the neurobiology of addiction (see (Koob and Volkow 2010) for a detailed review of addiction cycle stages and neurocircuitry). This circuitry has been shown to change as one moves through the stages of the addiction cycle, which are comprised of binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation “craving”. These last two stages occur during the absence of acute alcohol consumption and are associated with negative feelings such as anxiety and stress, which can lead to heightened craving. Previous work has identified extensive interacting brain circuitry associated with these experiences. For example, the amygdala has been identified as a key driver of anxiety and stress experienced during alcohol abstinence and craving. Polyvagal Theory describes activation of circuits from the amygdala to the nucleus ambiguus as releasing the vagal brake, resulting in decreased RSA (Porges 2001, Porges 2003). Amygdala activity also activates the HPA axis, resulting in increased cortisol levels (van Stegeren, Wolf et al. 2007). These actions result in a stressed bodily state, which is communicated to the brain via sensory afferents. This input eventually converges in the insula, a brain area known for its association with conscious awareness of body states, such as craving (Craig 2011). Acting to relieve this craving and anxiety likely requires involvement of extensive brain subnetworks, such as the executive, motor, and visual. Clearly, to move through the addiction cycle requires extensive distributive processing across the brain.
Given that craving can be viewed as an emotion, as described by EI theory of desire (Kavanagh, Andrade et al. 2005), it is especially interesting to consider that behavioral results from the current study revealed that the lowest craving for alcohol across the day was in those with higher RSA-rest/typical RSA-react scores and the highest craving in higher RSA-rest/atypical RSA-react scores (Mayhugh, Laurienti et al. under review). These two phenotypes also reflected the greatest difference in brain network organization. This might suggest that a retraction in whole brain functional connectivity to a more regionally specific organization, in combination with optimal ANS functioning, may be ideal for effective regulation of alcohol craving. Our findings show that stronger connections are more clustered and less globally efficient in the optimal CVT/lowest craving group, possibly suggesting strong connections predominate regionally, leaving weaker connections to adjoin these regions. Further, the functional brain connectivity in higher RSA-rest/typical RSA-react may be reflecting a decrease in the influence of areas associated with craving and addictive behaviors, such as the amygdala (Yin and Knowlton 2006, Everitt and Robbins 2013) on the rest of the brain.

As described above, this lack of distributive processing may reflect a disruption in the neurocircuitry driving the addiction cycle. In contrast, the higher RSA-rest/atypical RSA-react (highest cravers) had greater distributed processing, allowing for a more fully engaged addiction cycle. As craving for alcohol is a known indicator of AUD, these findings may provide insight to physiological mechanism behind resilience against future addiction.

One of the strengths of this study is that the mixed effects statistical model allowed for all variables to be included as continuous measures, including potentially
confounding variables. Variability of alcohol exposure is a key factor to consider when studying addiction vulnerability (Caetano, Tam et al. 1997). To control for this variability, here we included the total amount of years participants had been drinking, average drinks consumed on days they drank alcohol, and the percent of days they chose to consume alcohol over the last 3 months in the statistical model as confounding covariates. Total years drinking and average drinks per day had a significant negative relationship to connection strength during normal days. These results are in line with previous work showing disrupted brain connectivity in aging (Onoda, Ishihara et al. 2012, Tsvetanov, Henson et al. 2016), suggesting that greater duration and amount of alcohol consumption may have a negative impact on brain functioning mirroring the effects of aging. Although it was surprising that these measures did not significantly impact results during abstained days, it may be that the influence of the significant topological organization patterns observed in abstinence accounted for the majority of the variability in this state. The lack of sex differences was also surprising as males and females have been shown to differ in behavioral and biological mechanism behind addiction (Becker 2016). However, as noted below, this study had a modest sample size and was likely not powered to detect differences by sex. Future research is needed to clarify the unexpected results.

Weaknesses include the lack of ability to determine how specific subnetworks contributed to the observed relationship between brain connectivity and CVT. Future development of the mixed model methodology (Simpson and Laurienti 2015) to include regional covariates would be needed for such analyses. It should also be noted that, although these results are discussed referencing theories focused on specific circuitry,
such as the neurovisceral integration theory, the current analysis takes a systems level approach. This level of analysis is ideal for understanding large-scale organization of information processing, but is not intended to address functioning of smaller scale circuits in isolated brain structures. The functional atlas that was used to produce the brain networks has been extensively evaluated (Shen, Tokoglu et al. 2013, Finn, Shen et al. 2015, Rosenberg, Finn et al. 2016), nevertheless, the method requires averaging the time series from of all the voxels in each of the 268 functional regions. The assumption that the voxels within these a priori brain regions fluctuate in unison may not hold in all populations or all conditions. The overarching goal of this study was to increase our understanding of the underlying biological mechanisms leading to potential transition to AUD. However, the interpretive value of these results are limited without longitudinal data assessing variables such as increases in alcohol consumption over time and corresponding changes in CVT and brain connectivity. Finally, one should also keep in mind the relatively small sample size used for this study limits generalizability and calls for further replication to confirm these findings.

Together, this study suggests that the neurobiological mechanisms behind vulnerability to the transition from controlled drinking to AUD may involve a dynamic relationship between CVT and brain functioning, which is apparent in those drinking at a moderate-heavy level. Further, it suggests that the pattern of ANS/brain network connectivity in those with higher RSA-rest/typical RSA-react scores may demonstrate that which is optimal and potentially adaptive in response to a period of alcohol abstinence.
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CHAPTER V

DISCUSSION
The overarching goal of this dissertation was to identify the impact of alcohol abstinence (a central manipulation throughout these analyses) on craving, stress, and neurobiological functioning in moderate to heavy routine alcohol consumers. Heterogeneity within this group of moderate to heavy routine alcohol consumers was explored by examining characteristics known to be altered in addiction. The regular exposure to alcohol in this group of routine drinkers allowed for the impact of abstinence to be assessed in those with no history of alcohol use disorder (AUD). Specifically, a research study was designed to assess patterns of craving and stress ratings across the day, cardiac vagal tone, and functional brain network connectivity in routine moderate to heavy alcohol consumers. Craving was used as an estimator of risk for AUD. The work done as part of this dissertation has generated novel findings consisting of potential phenotypes to be further explored as markers for risk of transition to AUD.

This study showed that stress and craving throughout the day varied within this sample of moderate to heavy drinkers (Chapter II). Those with higher trait craving reported a steeper increase in real time craving and stress throughout the day, elevated stress pre-drinking, and a stronger relationship between stress and craving compared to the rest of the group. When the interaction of tonic and phasic CVT was evaluated, differing levels of craving across the day were also revealed (Chapter III). Individuals with higher RSA at rest, in combination with atypical RSA reactivity (sluggish vagal brake), exhibited higher cravings than their peers with different RSA phenotypes. In contrast, the most “optimal” CVT functioning (higher RSA-rest/typical RSA-react) experienced the lowest craving across the day. Although stress ratings were not specifically analyzed for associations with CVT, taken together, these findings suggest
that moderate to heavy drinkers that experience higher stress and craving across the day had less optimal CVT functioning and those with lower stress and craving had more optimal CVT functioning.

Considering the discussion in Chapter I regarding the role of CVT in appropriate stress response, and that craving is influenced by stress, here we suggest that CVT may be a useful measure to consider when investigating potential markers of AUD vulnerability. Tonic and phasic CVT also showed relationships with functional brain connectivity (Chapter IV). Interestingly, this relationship between CVT and brain networks was observed following abstinence but not following normal drinking. Specifically, as RSA rest and react scores approach what is considered to be “optimal” CVT functioning (higher RSA-rest/typical RSA-react), stronger functional brain network connections became more regionally efficient but less globally distributive. As discussed in Chapter IV, this difference in brain functioning observed at the systems level in those with higher RSA-rest/typical RSA-react versus higher RSA-rest/atypical RSA-react may reflect changes in interactions within the extensive circuitry that drives the addiction cycle. For example, the phases of the addiction cycle that occur during alcohol abstinence (when the significant association between CVT and brain functioning was found) are associated with the experience of craving and stress. How reduced distributed processing might be compensatory in those with the lesser experience of stress and craving within this group can be thought of as follows: the amygdala triggers the body’s stress response by signaling to the nucleus ambiguous to release the vagal brake as well as to the HPA axis to increase cortisol levels (van Stegeren, Wolf et al. 2007, Porges 2001). The body’s stress response, and all of the resulting sensory afferents, is ultimately conveyed to the
insula where conscious awareness of body state, including craving, is processed (Craig, 2011). This information is then communicated with higher levels of brain functioning, such as attention and motor networks, which are likely important for behaviors involved in the planning of actions to alleviate stress and craving, such as acquisition of alcohol.

Overall, these data suggest heterogeneity within this population of moderate to heavy drinkers with alcohol abstinence related to varied affects among the utilized measures. A potential characteristic of resilience to AUD worth exploring may involve a dynamic relationship between CVT and brain functioning with higher RSA-rest/typical RSA-react demonstrating an optimal and potentially adaptive response to a period of alcohol abstinence.

5.1 Including assessments of stress across the day, in combination with craving, provides additional insight to heterogeneity within moderate to heavy drinkers than craving alone

Craving is a key marker of addiction and can be used as an estimate of risk for AUD (de Bruijn, Korzec et al. 2004). As chapter II demonstrates, craving across the day did differ within this group of moderate to heavy drinkers. Some had a much steeper increase in craving across the day with higher peak levels and all experienced a significant decrease in craving post-drinking. The three consecutive days of imposed abstinence did not enhance craving. However, pre-drinking craving levels on normal drinking days were higher than equivalent times on abstained days. This may reflect previous work showing that when the opportunity to drink is absent, cue-reactivity does not have the same salience as when alcohol is available (Meyer 2000, Addolorato, Leggio et al. 2005).
Interesting differences emerged when stress experienced across the day in higher, compared to average, cravers was assessed. Those with average craving experienced about the same stress on normal drinking days prior to having a drink compared to abstained days. However, stress levels significantly dropped after drinking. This drop in stress was not observed during equivalent hours on abstained days. The post-drinking stress relief, without increased pre-drinking stress, suggests that those with average craving levels may not have been as motivated to drink by the anxiolytic effects of alcohol (Becker 2017).

An initially unexpected finding was that on normal drinking days, those with higher craving had significantly higher stress levels before consuming alcohol compared to equivalent times during abstained days. This is interesting considering that the Elaborated Intrusion Theory of Desire posits that craving can enhance negative emotions that correlate with stress due to increased attention on the state of deprivation (Kavanagh, Andrade et al. 2005) and that anticipation of drinking alone can trigger craving (Juliano and Brandon 1998). This also agrees with previous EMA work in addiction demonstrating a positive association between stress and craving (Serre, Fatseas et al. 2015). This may suggests that alcohol use in these higher cravers has shifted from pleasurable to a means of finding temporary relief from the negative emotional state (stress) experienced during withdrawal and craving (Koob and Volkow 2010). This elevated pre-drinking stress in higher cravers may prove an important marker for future investigation as previous research has demonstrated morning reports of stress to be predictive of time until the next drink in frequent alcohol consumers (Epler, Tomko et al. 2014). If higher cravers are more ruminative of their state of negative emotion or stress,
they may also be more likely to increase their alcohol consumption. Dvorak and colleagues supported this by showing that negative mood before drinking was positively associated with the number of drinks consumed (Dvorak, Pearson et al. 2016). Elevated stress as a precursor to drinking is also an important part of the addiction cycle (Figure 1, red section) and may suggest that these higher cravers have altered stress and reward neurocircuitry more similar to compulsive, habitual alcohol use (Uhart and Wand 2009, Koob, Buck et al. 2014).

Post-drinking stress in higher cravers only dropped to levels experienced during the abstained trial, which was still above peak levels reported by average cravers. If anything, these higher cravers experienced alcohol as a stress inducer instead of a stress reliever as experienced in average cravers. As stress is known to enhance sensitivity to cues for craving and promote relapse in alcohol addiction, this elevated stress post drinking suggests that these higher cravers are still in a more primed state for further alcohol consumption and may increase their drinking in the future (Breese, Chu et al. 2005). These findings are inline with previous work that demonstrated a blunted reduction in cortisol after alcohol consumption in high risk, compared to low risk, drinkers (Nakajima, Kumar et al. 2013). In the current study, EMA measures of craving and stress across the day were more strongly associated in individuals with higher trait craving. This provided further evidence that the higher cravers may have already progressed further along the continuum from drinking for pleasurable effects to avoidance of stress, a motivator associated with compulsive drinking.

In summary, these findings demonstrate that moderate to heavy drinkers with higher craving scores also had higher stress in the anticipation of drinking (which
continued to build until a drink was consumed), but still remained fairly elevated post-drinking. As overall craving ratings did not range greatly within this sample, as expected in individuals recruited for non-AUD status, including stress in assessments may provide a better measure of heterogeneity within the group than craving levels alone. Although EMA stress has been assessed in the past, they are often combined with measures of negative affect (Dvorak, Pearson et al. 2016). Here it is demonstrated that stress is not only worth continued exploration, but may provide more sensitive insight to characteristics that put one at more risk of future AUD than assessing craving alone. These findings add to the literature as they demonstrate the changing patterns of craving and stress across the day during normal drinking routine compared to an imposed abstinence period in moderate to heavy drinkers.

**5.2 Combining diverse methodologies to study alcohol abstinence in moderate to heavy drinkers**

One of the central questions of this dissertation was to gain insight into heterogeneity of response to a period of imposed alcohol abstinence within this sample of routine moderate to heavy drinkers. This level of regular alcohol exposure (averaging 2.3 servings 6 days per week), put them at potential risk for AUD (NIAAA). However, the broader questions are also interesting. How bound are they to their drinking routine? How likely are they to increase their drinking in the future? If their stress levels increase due to a major life event, will their drinking also increase? It is expected that these answers may be more difficult to come by in those that have no history of substance abuse problems and currently have a seemingly controlled relationship with alcohol. This is compounded
by the fact that the majority of research has focused on addiction level alcohol use (Buhler and Mann 2011). This study was able to identify variability in craving within moderate to heavy drinkers and related neurobiological functioning during times that could affect their future drinking behavior, such as pre-drinking. For example, craving intensity across the day has been shown to predict greater number of drinks consumed in both dependent and non-dependent heavy alcohol drinking adults (Ray, Miranda et al. 2010, Fazzino, Harder et al. 2013). This provides potential phenotypes to be explored for insight to the potential for future drinking escalation. It also assessed the impact of consecutive days of abstinence, which provides insight to the difficulty moderate to heavy drinkers may have if they wished to reduce their drinking routine.

The work presented here demonstrates that imposed abstinence, as well as the daily abstinence that occurred prior to drinking each day, revealed different effects depending on the variable being assessed. For example, although imposed abstinence did not elevate craving across the day compared to equivalent pre-drinking times (chapter II), stress was actually lower during abstinence compared to pre-drinking levels in higher cravers. When the relationship between craving and CVT functioning was considered (chapter III), the impact of imposed abstinence still did not reveal increased craving compared to equivalent pre-drinking times. However, craving remained much higher in those with higher RSA-rest/atypical RSA-react on abstained days compared to post-drinking levels on normal drinking days. The alcohol consumption related relief from craving may reflect the lack of ability of those with this dysfunctional CVT profile to self regulate. This reflects previous work showing that vagal functioning at rest and reactivity to a stressor are important indicators of emotional regulation (Balzarotti, Biassoni et al.}
This lack of alcohol consumption related craving relief during abstained days in those with higher RSA-rest/atypical RSA-react also demonstrates the difficulty this CVT profile might have in resisting alcohol consumption, exacerbated by higher peak levels pre-drinking. This is supported by previous work demonstrating dysregulated autonomic nervous system functioning in enhanced sensitivity to negative emotions such as craving and stress (Sinha, Fox et al. 2009) and that heart rate variability has been found to predict craving in those recovering from alcohol dependence (Quintana, Guastella et al. 2013). Although this general effect occurred across all CVT profiles, the craving observed post-drinking was still higher then any of the other CVT profiles at their peak craving. The consideration of CVT functioning in combination with EMA craving ratings may prove a more sensitive measure of heterogeneity within moderate to heavy alcohol consumers than considering craving ratings alone.

The impact of imposed abstinence in moderate to heavy drinkers became most apparent when CVT functioning was analyzed for interactions with functional brain network organization (chapter IV). As RSA scores became more optimal, functional brain connectivity shifted such that strong connections collectively moved from more distributed connections to more highly clustered connection. The effect was not significant until they were in the imposed abstinence state. This finding revealed that, in imposed abstinence, these individual’s functional brain network organization became less globally integrated; potentially reflecting less engaged addiction neurocircuitry. For example, this may reflect decreased engagement of the brain circuitry associated with withdrawal (i.e. amygdala) with those related to preoccupation/anticipation (craving, i.e. insula) (Koob and Volkow 2010). Previous findings in alcohol dependent individuals...
showed altered co-occurring activity in widespread brain regions including the ventromedial prefrontal cortex, anterior cingulate cortex, and precuneus; suggesting the role of interactions between far reaching areas in addiction (Seo, Lacadie et al. 2013). Weaker within network connectivity and expanded outside-network connectivity in the default mode, salience, reward, and executive control networks during resting state was correlated with poorer cognitive performance and mood in sober alcoholics, again suggesting reduced regional specialization in addiction (Muller-Oehring, Jung et al. 2015). This circuitry is thought to change as one moves through the stages of the addiction cycle, which are comprised of binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation “craving”. These last two stages occur during the absence of acute alcohol consumption and are associated with negative feelings such as anxiety and stress, which can lead to heightened craving. The current study found that the relationship between brain connectivity and CVT functioning was significant only in alcohol abstinence and reflected less distributive processing in lower cravers. The rest of the group, experiencing comparatively higher craving, may also reflect previous work showing enhanced communication in emotional processing. For example, emotional and motivational processing has been shown to affect brain connectivity by increasing signal communication between regions, most notably between cortical and subcortical regions (Kinnison, Padmala et al. 2012).

Taken together, the experiments conducted in the current study showed that different methodological approaches were able to reveal different aspects of the imposed abstinence manipulation and was able to detect heterogeneity of this effect within this group of moderate to heavy drinkers. This study demonstrated large variability within
this selected drinking pattern, with some potentially more at risk than others. This work will be enhanced by future studies assessing different patterns of alcohol consumption and incorporating additional methodologies within the same individuals.

5.3 Current drinking behavior does not imply homogeneity of response to alcohol abstinence

Risk factors for AUD, such as family history of alcoholism and the age at which one started consuming alcohol, have been widely acknowledged (Grant 1998). However, the main criteria provided as a warning to the public for risk of alcohol use relies on levels of current drinking behavior (www.niaaa.nih.gov). Much research also relies on this criterion comparing subjective, behavioral, and biological changes between drinking levels. For example, many study designs compare a group in recovery for alcohol dependence to healthy controls (often light or moderate alcohol consumers) (Boschloo, Vogelzangs et al. 2011, Seo, Lacadie et al. 2013, Sullivan, Muller-Oehring et al. 2013). Current drinking levels obviously provide a simple and effective estimate of current risk.

However, the results presented in this dissertation provide evidence for further investigation into variability of measures that are known to be affected by the addiction cycle within a given level of drinking behavior. Although these moderate to heavy drinkers all drank within a similar pattern, had no previous history of substance abuse, no mental or physical illnesses that are known to effects the assessed measures, and had maintained this drinking pattern for at least 3 years, differences in their subjective experience, autonomic nervous systems functioning, and functional brain connectivity were revealed. Research into subjective and biological variance within different drinking
patterns could provide insight into what causes some people to transition from one pattern to another as well as how some can maintain regular exposure to alcohol without ever escalating. With the current debate over whether alcohol consumption is ok, if not good, in moderation, this insight could help provide information for appropriate guidance for alcohol consumers (Aubrey 2014, Aubrey 2015). One person’s moderate drinking may be another person’s road to eventual alcohol addiction.

5.4 The potential impact of sex differences

This section provides a brief overview of what is known about sex differences in alcohol addiction with a focus on how this may relate to the current study’s findings and future research. Sex differences have been shown in assessments of both alcohol craving and sex. One study assessing sex differences in stress and alcohol cue related craving in social drinkers found that craving was correlated with greater negative emotion in men, but not women (Chaplin, Hong et al. 2008). However, they did not find significant overall sex differences in alcohol craving in response to stress or alcohol cue. This work shows that assessing sex differences related to alcohol craving may provide important insight, but may need to be targeted in its approach to capture differences. Heart rate variability has also shown sex differences, however these differences have been shown to be smaller than the effects of age (variability decreases with age) and vary depending on the measure used (Jensen-Urstad K., Storck N., et al. 1997, Umetani K., Singer D.H., et al. 1998). Sex differences in brain functioning have been reported as well. For example, Seo et al. investigated sex differences in the neural response (measured via fMRI) to stress and alcohol cues in healthy social drinkers (Seo, Jia, et al., 2011). Their work
revealed a range of brain regions exhibiting activity changes, with some sex differences in brain activity identified. These included greater stress-related activity increases in the medial prefrontal cortex, anterior cingulate cortex, posterior insula, amygdala, and hippocampus in men than women. Also, alcohol craving was significantly related to striatum functioning in men, but not women. These findings suggest that spatial information in brain functioning may be important to identifying sex differences and future work building on the current study’s findings should consider this.

Overall, these findings point to the importance of considering sex when studying alcohol craving and should certainly be considered when assessing characteristics of potential risk for AUD. Although the current study showed relationships between craving, CVT, and brain functioning while controlling for the effects of sex in the analyses, future research may want to consider contrasting sex specifically.

Including information regarding menstrual cycle phase in female participants would also be important for future studies. Epstein and colleagues used a repeated measures design to investigate whether phases of the menstrual cycle affected alcohol craving and consumption in alcohol-dependent females (Epstein, E.E, Rhines K.C., et al. 2009). They found that during the first three months of treatment, lower average number of cravings and higher drinking frequency was reported during the premenstrual phase. They also reported the premenstrual phase as a cue for drinking. As the current study included assessments of craving across days, with comparisons between normal drinking routine and alcohol abstinence occurring on different days, these assessments were collected during different phases of the menstrual cycle, which could have influenced these results.
5.5 Limitations and Weaknesses

There are a few points that should be noted when considering these findings and planning future studies. First, this study’s somewhat selective recruitment criteria should be considered when generalizing these findings to the population. For example, severe depression was controlled for by excluding those with a score of > 20 on the Center for Epidemiological Studies Depression Scale (CES-D) (Haringsma, Engels et al. 2004). This helped ensure that our sample reflected the general, healthy population and that findings were not confounded by mental illness. However, depression is a risk factor for AUD and a commonly co-occurring disorder in addiction (Regier, Farmer et al. 1990). Future work examining moderate to heavy alcohol consumption might include those with current mental health disorders for comparison to a healthy population.

Although sex was included as a confounding variable in these analyses, a larger sample size would have allowed for a more thorough investigation into sex differences. As discussed in section 5.4 above, this would be important for future research to consider due to the known sex differences in alcohol addiction (Kessler, McGonagle et al. 1994, Mann, Ackermann et al. 2005, Seo, Jia et al. 2011).

It should also be noted that the fMRI scans did not occur during peak craving as shown in the real time (EMA) results described in Chapter II. Considering that these moderate to heavy drinkers most often consumed their daily alcohol in the evening (as reported on the Time Line Follow Back questionnaire (Vakili, Sobell et al. 2008)), scheduling fMRI scans during this time may have been more ideal for capturing the impact of the imposed alcohol abstinence period. Of course the drawback to this would be that scheduling the fMRI scan in the evening during the normal drinking days might
have inadvertently disrupted their normal drinking routine, which was intentionally avoided during this study visit. Regarding the EMA analyses, the random alerts only occurred between the hours of 9am – 9pm to avoid disruption of participants’ sleep. Although an EMA survey was also taken when the participant was “about to go to sleep”, this lack of nighttime survey prompts may have resulted in a lower amount of data points occurring during common drinking hours than would be ideal. Also, the EMA questions were phrased to ask “Do you have a craving for alcohol right now?”. As these moderate to heavy drinkers had no history of AUD, the word “crave” may have not been the best word to capture their experience. A less loaded term, such as “desire” may have been more reflective of their experience and resulted in a more sensitive measure of variability within the group. The word “urge” might also be considered in future work with moderate to heavy drinkers as it is a term used in retrospective measures of craving (Statham, Connor et al. 2011).

5.6 Future Directions

Considering that the overarching goal of this work was to identify markers of vulnerability to AUD, the results presented here provide groundwork for future longitudinal investigation to confirm these characteristics by associating with increased alcohol consumption and AUD prevalence. Despite the smaller sample size of the current study, the CVT profiles identified in this study were able to be identified due to the analyses of continuous measures of both CVT and craving scores. However, future work should recruit for larger numbers of each of these CVT profiles specifically. As emotion and stress have been shown to be related to CVT reactivity and vary by CVT functioning,
an analysis of relationships between stress, negative, and positive affect across the day with CVT profiles would also increase understanding of variability of craving and impact of alcohol abstinence in these drinkers (Wray, Merrill et al. 2014, Balzarotti, Biassoni et al. 2017). As cue exposure has been associated with craving (Miranda, Ray et al. 2014, Wray, Merrill et al. 2014, Serre, Fatseas et al. 2015), assessing these variables during the time of the momentary (EMA) craving ratings may also provide additional insight to the current findings.

The current work presented changes in functional brain network connectivity during imposed abstained compared to normal drinking using fMRI data collected during resting state. This provided a baseline for which future work can build upon and has been previously shown to capture functional brain connectivity changes in addiction (Muller-Oehring, Jung et al. 2015). However, extensive work has demonstrated changes in brain activation during alcohol and stress cues in both social drinkers and recovering alcoholics (Seo, Jia et al. 2011, Seo, Lacadie et al. 2013). Future work should investigate whether the patterns of functional brain connectivity presented here differ during presentation of cues for stress and alcohol consumption.

Considering that alcohol exposure has been shown to be an important factor in the progression of the addiction cycle (Koob and Volkow 2010). It would also be interesting to investigate how the current findings relate to different patterns of alcohol consumption, such as binge drinkers, and whether lower levels of routine alcohol consumption still illicit the response observed here.

In conclusion, the work presented in this dissertation provides groundwork for an understudied population of alcohol consumers. The hope is that this will inspire future
work to build upon these findings and provide meaningful insight to the variability of impact alcohol consumption and imposed abstinence can have in what seems, at the surface, a homogenous group of alcohol consumers. The work presented in this dissertation was able to identify a continuum within these moderate to heavy drinkers. On one extreme, lower cravers were associated with lower stress, more optimal CVT functioning, and more regionally specific functional brain connectivity. On the other extreme, higher cravers were associated with higher stress, more dysfunctional CVT, and functional brain connectivity organized for more distributed processing. Although these phenotypes (at the extremes) exist on a continuum, they may serve as characteristics to be explored by future research as makers of AUD vulnerability. If future work validates these characteristics, they could be utilized in conjunction with low risk interventions, such as mindfulness meditation, to guide treatment aimed at addiction prevention.
References


CURRICULUM VITAE

NAME
Rhiannon Mayhugh

ACADEMIC TITLE
Ph.D. Candidate, \textit{expected graduation May 2018}

ADDRESS
Laboratory for Complex Brain Networks
Neuroscience Program
Wake Forest School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157-1088
Telephone: (336) 716-1019 (Office)
Email: rmayhugh@wakehealth.edu

EDUCATION

College:
University of Central Florida
Orlando, FL
Bachelors of Science (Psychology)

Graduate:
Florida Gulf Coast University
Fort Myers, FL
Coursework in Clinical Mental Health Counseling

Wake Forest School of Medicine
Neuroscience Graduate Program
Winston Salem, NC
PhD, \textit{expected graduation May 2018}

Dissertation Title:
Effects of Alcohol Abstinence on Craving, Stress, and Neurobiological Functioning in Moderate to Heavy Alcohol Consumers
Paul Laurienti, MD, PhD (Academic advisor)

PROFESSIONAL APPOINTMENTS AND ACTIVITIES

Research:
Research Laboratory Technician
Dept. of Physiology & Pharmacology
Wake Forest School of Medicine

Graduate Student
Neuroscience Graduate Program

2012 - 2013
2013-present
EXTRAMURAL APPOINTMENTS AND SERVICE

Editorial: Ad hoc Reviewer:

Drug and Alcohol Dependence
Neuro-Psychopharmacology & Biological Psychiatry
Progress in Neuropsychopharmacology & Biological Psychiatry

Book article reviewer:
CONNECTOMICS: Methods, Mathematical Models And Applications. In Press Elsevier

PROFESSIONAL MEMBERSHIPS

Society for Neuroscience 2014 -present
Western North Carolina Society for Neuroscience 2014 -present
Organization for Human Brain Mapping 2015 -present
Research Society on Alcoholism 2016 -present

HONORS AND AWARDS

T-32 Pre-doctoral Traineeship: Multi-Disciplinary Training in the Biology of Alcoholism 2014 -present

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Abstracts/Scientific Exhibits/Presentations at Meetings:


   Poster presentations:
   3. *Neuroscience Program, Wake Forest School of Medicine, Winston Salem, NC* (2015)

   Oral presentations:


   Poster presentations:
   2. *Graduate School Research Day – Wake Forest University and Wake Forest School of Medicine, Winston-Salem, NC* (2016)


   Poster presentations:
   2. *Graduate School Research Day – Wake Forest University and Wake Forest School of Medicine, Winston-Salem, NC* (2017)
   3. *Neuroscience Student Research Day, Wake Forest University,*

    *Poster presentation:*