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Alcohol Craving: Clinical, Assessment, and Genetic Considerations

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Alcohol Craving: Clinical, Assessment, and Genetic Considerations

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in Psychology

by

Emily Hartwell

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ABSTRACT OF THE DISSERTATION

Alcohol Craving: Clinical, Assessment, and Genetic Considerations

by

Emily Hartwell

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2018

Professor Lara Allison Ray, Chair

Though alcohol craving is a long recognized phenomenon, there has been growing recognition of the clinical salience and utility over the past generation, particularly in the domains of diagnosis and intervention. Broadly defined, craving is a strong desire or urge to use a substance. However, much remains to be explored in this critical phenotype as theories regarding development and methodologies regarding assessment are highly variable.

Assessment of alcohol craving has long been an area of debate, which is investigated in Study 1. A number of measures have been developed to assess alcohol craving, which are frequently used interchangeably. Measures of craving are designed to either assess longer-term, unprovoked *tonic* or in the moment, provoked *phasic* craving. Little is known about the relationship between these types of craving. Thus, this study fills a gap in the existing literature

to examine the association of tonic and phasic craving when alcohol craving is provoked by alcohol administration in a sample of individuals with an alcohol use disorder. Results indicated that tonic craving is predictive of phasic craving in the laboratory, particularly when alcohol is ingested, and that different measures of tonic craving may be capturing unique aspects of the craving experience.

In Study 2, the factor structure and diagnostic conversion of alcohol use disorders (AUD) to the Diagnostic and Statistical Manual-5 (DSM-5) from DSM-IV is examined. The DSM-5 included two major modifications: the legal criterion was dropped in favor of the addition of craving and the distinct syndromes of abuse and dependence were replaced with a single dimensional syndrome with severity specifiers. Non-treatment seeking alcohol users completed a structured clinical interview and the Penn Alcohol Craving Scale (PACS). PACS scores were used as a stand-in for the craving criterion with scores greater than 20 were considered to meet diagnostic criteria for craving. Overall, few participants (16.2%) were met the criterion using this cut-off score. Despite the low endorsement, craving loaded well onto the existing symptoms and supported the structural change of creating a unidimensional syndrome for AUD. Though prevalence did slightly increase in the sample when converted to DSM-5, this was due to the structural change as opposed to the addition of craving. Implications for a non-treatment seeking sample are discussed.

Study 3 is an exploratory examination of the role the alpha-synuclein (*SNCA*) gene plays in predicting alcohol craving. Using previous literature, two single nucleotide polymorphisms (SNP) rs356219 and rs356221 and their haplotype were identified to investigate as predictive of alcohol craving. The sample was Caucasian and Hispanic problem alcohol users from the community. Despite the theory driven approach to identify the genotype of interest, hypotheses

were not supported. Neither the haplotype nor either SNP predicted alcohol craving as assessed by the PACS, OCDS, or either subscale, save for a trend level effect of one SNP predicting the Obsessive subscale of the OCDS. Additionally, the haplotype did not predict DSM-IV alcohol dependence. It is possible this study did not replicate prior work due to the heterogeneity of alcohol users and general low levels of craving endorsed.

Together, these studies met the aim of this dissertation to better characterize and explore the phenotype of alcohol craving. These studies inform the literature by examining clinical, diagnostic, assessment and genetic components. Ultimately, improving characterization and assessment of this phenotype is critical to advance understanding of craving in order to improve diagnosis and intervention.

The dissertation of Emily Hartwell is approved.

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2018

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Hartwell, E.E., & Ray, L.A. (2015) Gender differences in craving and internalizing symptoms in methamphetamine dependence. College on Problems of Drug Dependence meeting, Phoenix, AZ.

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DISSERTATION INTRODUCTION

Alcohol use disorders (AUD) are a highly prevalent and costly problem. Recent estimates place past year prevalence of AUD at 13.9% (Grant et al., 2015) and carry an average cost per year of $180 billion (Rehm et al., 2009). One facet of alcohol use that has long been recognized is craving (Drummond, 2001; Jellinek et al., 1955), though acknowledgment of the clinical importance and utility of craving has increased over the past generation. Broadly defined as a strong urge or desire to use a substance, craving has been implicated in the diagnosis, prognosis, intervention focus, and outcome of AUD (Tiffany & Wray, 2012). However, there is no unified theory regarding the development and perpetuation of craving with theories ranging from phenomenological to conditioning to conditioning concepts (Drummond, 2001). Further, the genetic underpinnings of the development of craving continue to be poorly understood and explained (Ehlers & Wilhelmsen, 2005).

Part of the difficulty of coalescing around a unified theory of craving is the extreme heterogeneity of craving experiences, both between and within alcohol using individuals. Craving volatility and variability is due to a host of factors including tolerance, withdrawal symptomatology, severity of alcohol use, salience of consumption, and subjective attention paid to the urge to drink (Haass-Koffler, Leggio, & Kenna, 2014). Within individuals, craving may be intensified during times of stress, in situations previously associated with consumption, or when experiencing withdrawal (Drummond, 2001; Haass-Koffler et al., 2014).

The increased salience of craving to the field of alcohol research is also due to the recent addition of craving as a diagnostic symptom for alcohol use disorders (Hasin, Fenton, Beseler, Park, & Wall, 2012; Keyes, Krueger, Grant, & Hasin, 2011) in the latest iteration of the Diagnostic and Statistical Manual (DSM-5), published in 2013 (American Psychiatric

Association, 2013). The other major change in DSM-5 was to eliminate the separate syndromes of abuse and dependence in favor of a unidimensional syndrome with a severity specifier. Though these changes were primarily met with support, criticism exists. Some have argued that these changes will result in higher AUD prevalence and that craving represents a more severe symptom that will be infrequently endorsed thus not improving validity.

The lack of a unified theory, and furthering the debate over craving as a diagnostic criterion, has perhaps added to the varying methodologies for assessing craving. Various self-report measures and clinical interview questionnaires have been developed over the years. Though frequently used interchangeably, available measures assess different aspects of craving and gather variable amounts of information. For example, some measures focus on the cognitive aspects of craving whereas others focus on anticipated effects of alcohol use or inability to resist use. Many measures attempt to capture multiple facets of craving to produce "composite" craving scores. The ability to capture real time, provoked craving responses in the laboratory has improved with the development of cue reactivity paradigms that frequently capture both subjective craving (e.g. questionnaires), as well as objective reactivity (e.g. physiological response of cardiovascular system or brain activation) and behavioral indices (e.g. alcohol consumption). Such paradigms have been utilized to characterize the experience of craving in order to inform treatment development.

Despite these ongoing debates regarding the development, assessment, and diagnosis of alcohol craving, it is clear from preclinical, clinical, and laboratory studies that craving represents an important phenomenon in the perpetuation of AUD and a key target for intervention. Though a number of specialized psychosocial and pharmacological treatments have been developed to address and attenuate craving, success has been highly variable (Conklin &

Tiffany, 2002; Drummond, 2001; Drummond, Cooper, & Glautier, 1990; Haass-Koffler et al., 2014; Havermans & Jansen, 2003; O'Brien, 2005; Witkiewitz, Bowen, Douglas, & Hsu, 2013). Therefore, the overarching goal of this dissertation is to advance the study of the phenotype of alcohol craving by examining the assessment, clinical diagnosis, and genetic underpinnings in three studies.

Study 1: Relationship between Tonic and Phasic Craving for Alcohol

Measures of craving are designed to assess either tonic (i.e. long-term, stable levels) or phasic (i.e. in the moment, provoked) craving. However, these measures are often used interchangeably and administered in paradigms that lack external validity. This study seeks to address a gap in the literature by examining the association between tonic alcohol craving and phasic craving for alcohol that is provoked by alcohol administration. To test this hypothesis, the Penn Alcohol Craving Scale (PACS) and Obsessive Compulsive Drinking Scale (OCDS) were administered to non-treatment seeking, dependent alcohol users from the greater Los Angeles community at the initial screening visit. Subsequently, participants completed two laboratory visits in which they were either an alcohol infusion (designed to reach breath alcohol concentration of 0.06% g/dL) or saline control, administered in counterbalanced and randomized fashion. Phasic craving, as captured by the Alcohol Urge Questionnaire (AUQ), was administered at several time points during both infusion sessions. Analyses examined if the measures of tonic craving predicted phasic craving in response to alcohol administration. It was hypothesized that tonic craving would predict phasic craving in response to alcohol administration, but not during the saline control administration.

Study 2: Craving as a DSM-5 Symptom of Alcohol Use Disorder in Non-treatment Seekers

Published research in various nationally representative samples has provided support for the changes implemented in DSM-5 (Agrawal, Heath, & Lynskey, 2011; Hasin et al., 2013; Keyes et al., 2011; Mewton, Slade, McBride, Grove, & Teesson, 2011). Specifically, previous factor analyses have shown that AUD are better represented by a unidimensional syndrome over the previously separated diagnoses of abuse and dependence. Perhaps more controversial was the addition of craving as a diagnostic criterion. Though craving has been shown to load well onto the ten retained symptoms from the previous DSM iteration (Casey, Adamson, Shevlin, & McKinney, 2012), it is less well understood who will endorse this symptom and if this addition will improve validity. In a study of treatment seeking alcohol users, the Penn Alcohol Craving Scale (PACS) was used as a stand-in for the craving criterion (Murphy, Stojek, Few, Rothbaum, & MacKillop, 2014). In that sample, nearly half the sample met the predetermined threshold to meet criteria the symptom and the sample loaded strongly onto the retained symptoms. However, recent literature has shown that there are clinically relevant differences between treatment seeking alcohol users and non-treatment seekers (Ray, Bujarski, Yardley, Roche, & Hartwell, 2017; Rohn et al., 2017), who are traditionally enrolled in laboratory studies and intervention trials. One of these differences is that craving is thought to represent a more severe symptom that is less likely to be endorsed by non-treatment seekers (Anton & Drobes, 1998; Ray et al., 2017).

Therefore, the goal of this study was to examine the impact of the structural and diagnostic changes of DSM-5 on prevalence and factor structure in a sample of non-treatment seeking alcohol users. To accomplish this goal, non-treatment seeking problem alcohol users completed a clinical interview and battery of self-report questionnaires. The PACS was used to determine craving status. Analyses included determination of diagnostic conversion from DSM-

IV to DSM-5 and an exploratory factor analysis of retained symptoms and the new craving symptom. It was hypothesized that craving would load well onto extant symptoms and that prevalence of AUD would increase.

Study 3: Genetic Markers of alpha-Synuclein and their Relationship with Alcohol Craving

Though it is known that AUD are highly heritable (Verhulst, Neale, & Kendler, 2015), exact mechanisms at play remain to be thoroughly explained. For example, there is evidence of a genetic underpinning to the experience of alcohol craving (Ehlers & Wilhelmsen, 2005), yet much work remains to elucidate the genes that have been implicated. This is at least partially due to the complex pathophysiology of alcohol, which impacts a host of systems and neurotransmitters (Köhnke, 2008), and due to the intricate interplay of the multitude of genes that are required to produce a behavior (Plomin, DeFries, Knopik, & Neiderhiser, 2016). Despite these complexities, one candidate gene that has received some attention is alpha-synuclein (*SNCA*). *SNCA* has previously been shown to be related to alcohol dependence and alcohol craving (Foroud et al., 2007; Janeczek, Brooker, Dodd, & Lewohl, 2015). This study leveraged data from a well characterized sample of community alcohol users to investigate the role of two single nucleotide polymorphisms and their haplotype in alcohol craving. In order to explore this aim, the sample completed measures of craving and a diagnostic interview in addition to providing a saliva sample for genotyping. It was hypothesized that risk alleles and the risk haplotype would predict alcohol craving.

Overarching Goal

For craving research to advance, greater understanding of the experience of craving and the subsequent impact on diagnosis and treatment is needed. Together these three studies

enhance our knowledge about the clinical phenotype of alcohol craving. Given the importance of craving as a marker of diagnosis, prognosis, and intervention, better characterization and assessment is key.

STUDY 1: Relationship between Tonic and Phasic Craving for Alcohol

Abstract

Background: Multiple measures are utilized to assess alcohol craving, often interchangeably. Little is known about the relationship between tonic and phasic craving. This study fills this gap in the literature by examining the association between tonic levels of alcohol craving and phasic craving for alcohol that is provoked by alcohol administration.

Methods: Forty-three non-treatment seeking problem drinkers underwent an initial interview and two laboratory testing sessions, with either alcohol or a saline placebo administered intravenously. Tonic craving was assessed via the Penn Alcohol Craving Scale (PACS) and Obsessive Compulsive Drinking Scale (OCDS) at the initial interview. Phasic craving was assessed during the laboratory sessions (i.e., alcohol and saline administrations, single blinded) at baseline and at 3 subsequent breath alcohol concentrations (0.02, 0.04, and 0.06 g/dl).

Results: There was a main effect of PACS in predicting phasic craving across both saline and alcohol administration conditions ($p < .05$). The OCDS was predictive of phasic craving when alcohol, but not saline, was administered ($p = 0.057$); the obsessive subscale ($p = 0.01$), but not the compulsive, predicted phasic craving during alcohol, as compared to saline administration.

Conclusions: In this study, tonic craving was predictive of phasic craving, particularly when alcohol was administered. Implications for the utilization of the PACS and OCDS as well as assessments of tonic and phasic craving in alcoholism research are discussed.

Keywords: Alcohol, Craving, Assessment

Introduction

The phenomenon of craving for substances of abuse has been long recognized (Drummond, 2001; Jellinek et al., 1955), however, understanding of the clinical utility of craving has grown increasingly over the past generation. Though definitions vary, craving has broadly been defined as a desire or strong urge to use a substance (Flannery et al., 2001). Craving has been implicated in multiple substance use disorder domains, including prognosis, intervention target, clinical outcome (Tiffany & Wray, 2012), and notably has been included as a diagnostic criterion in the latest iteration of the Diagnostic and Statistical Manual of Mental Disorders (Hasin et al., 2013). However, the experience of craving varies widely both between and within individuals. This volatility is due to a host of factors including severity of alcohol use, environmental factors, and subjective attention paid to craving (Haass-Koffler, Leggio, & Kenna, 2014). Within individuals, craving may be intensified during times of stress, in situations associated with consumption, or when experiencing withdrawal (Drummond, 2001; Haass-Koffler et al., 2014). Also of note, craving is often assessed in laboratory settings, where it could be dampened due to lack of external cues and inability to consume alcohol (Wertz & Sayette, 2001).

Various methods of assessing alcohol craving have been developed. Self-report measures of subjective craving capture either longer-term, *tonic* craving or in the moment, provoked, *phasic* craving (Ray, Courtney, Bacio, & MacKillop, 2013). Tonic measures of craving are, by nature, retrospective and capture a general subjective experience of craving over a prescribed time period when craving has not been provoked (Ray et al., 2013). Tonic craving has been predictive of drinking and treatment outcomes (Bottlender & Soyka, 2004; Flannery, Poole, Gallop, & Volpicelli, 2003; Oslin, Cary, Slaymaker, Colleran, & Blow, 2009). Two widely used

measures are the Penn Alcohol Craving Scale (PACS) and the Obsessive Compulsive Drinking Scale (OCDS). The PACS is a 5-item measure assessing frequency and severity of craving over the previous week (Flannery, Volpicelli, & Pettinati, 1999). This measure has good reliability and moderate validity with other craving measures (Flannery et al., 1999). The PACS benefits from asking specifically about duration and frequency of craving, whereas most other measures assess intensity of craving alone, producing a "composite" craving score (Tiffany & Wray, 2012). Further, the PACS was designed to assess characteristics of craving, instead of providing an exact definition of craving as either an aversive or appetitive state (Flannery et al., 1999).

Alternatively, the OCDS is a 14-item measure of alcohol related urges and thoughts that produces two subscales, obsessive and compulsive (Anton, Moak, & Latham, 1995). The OCDS is based on the notion that alcohol use disorders (AUD) are akin to obsessive compulsive disorders and thus assesses severity of alcohol-related urges, obsessive thoughts, and compulsive alcohol use over a specified timeframe. The OCDS has high reliability and convergent validity with other measures of craving, alcohol use disorder, and alcohol consumption (Bohn, Barton, & Barron, 1996; Connor, Jack, Feeney, & Young, 2008; Kranzler, Mulgrew, Modesto-Lowe, & Burleson, 1999; Moak, Anton, & Latham, 1998; Ray et al., 2013). The OCDS may be particularly effective at differentiating between non-problematic users and those who will likely meet for an AUD (Ray et al., 2013).

Phasic measures of alcohol craving, on the other hand, assess in vivo, current, state-levels of subjective craving for alcohol. Phasic craving is often the result of provocation, for example during laboratory cue-exposure and alcohol administration paradigms, and has been shown to predict drinking outcomes (Drummond & Glautier, 1994; Litt, Cooney, & Morse, 2000). This dynamic state of craving may fluctuate based on a number of factors, such as the presence of

alcohol related cues or alcohol itself (Ray et al., 2013). The 8-item Alcohol Urge Questionnaire (AUQ; Bohn, Krahn, & Staehler, 1995) assesses an individual's severity of craving at the given moment and is frequently used in laboratory based paradigms that include a craving provocation (e.g. O'Malley, Krishnan-Sarin, Farren, Sinha, & Kreek, 2002; Ray & Hutchison, 2007). The AUQ has demonstrated high reliability and validity, showing positive correlations with the OCDS and amount of alcohol consumption (Bohn et al., 1995; Drummond & Phillips, 2002; MacKillop, 2006). Moreover, the utility of the AUQ to capture real time craving fluctuations has been shown in alcohol cue exposure studies (e.g. MacKillop, 2006).

The relationship between tonic and phasic levels of craving for alcohol, within the individual, remain poorly understood. This study seeks to advance the literature on alcohol craving by comparing tonic (i.e., PACS and OCDS at screening) and phasic (i.e., craving during controlled alcohol and saline administration in the laboratory) craving for alcohol in a sample of non-treatment seeking problem drinkers. Specifically, I hypothesize that tonic craving will predict phasic craving in the laboratory in response to alcohol administration but not the saline control condition. This relationship may inform interventions as targeting tonic and phasic craving for alcohol remains a high priority area.

Methods

Participants

A total of 295 problem drinkers from the greater Los Angeles community completed the in-person screening visit. The inclusion criteria were: (1) 21-65 years of age; (2) endorse problems related to alcohol use; (3) report drinking ≥ 48 drinks per month; (4) meet DSM-IV criteria for alcohol dependence (current, defined as past year). Exclusion criteria were: (1) currently in or seeking treatment for alcohol problems; (2) report no alcohol use in past three

weeks; (3) history of major psychiatric disorder (e.g. psychosis); (4) Clinical Institute Withdrawal Assessment (CIWA; Sullivan, Sykora, Schneiderman, Naranjo, & Sellers, 1989) score ≥ 10.

Procedures

Participants responded to online and print advertising by calling the laboratory to complete a telephone interview. Eligible participants were invited to an in-person assessment where they provided written informed consent and completed individual difference measures. Participants then completed a physical examination. Forty-three participants were invited to complete two infusion visits, saline and alcohol, which were completed in randomized, blind, counterbalanced order at least one week apart (Ray et al., 2013).

Upon arrival for infusion sessions, participants were breathalyzed to confirm a breath alcohol concentration (BrAC) of 0.00 g/dl and regular smokers were allowed to have a cigarette. In order to mitigate variability in blood alcohol concentration observed between individuals, a 5% ethanol solution was administered intravenously using a formula accounting for sex and weight (Ray et al., 2013). Upon reaching each target BrAC, 0.02, 0.04, and 0.06 g/dl, the infusion rate was reduced in half to maintain constant BrAC level while participants completed a series of measures. During the saline infusion visit, measures were administered at 0, 18, 43, and 75 minutes during the saline infusion, to mirror the approximate time points at which target BrACs were reached in the alcohol administration session. When participants reached a BrAC ≤ 0.02 g/dl they were permitted to leave (0.00 g/dl if driving).

Measures

At the screening visit, a master's level graduate student, supervised by a licensed clinical psychologist, administered the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon,

& Williams, 1995), the Timeline Follow-back (Sobell, Sobell, Klajner, Pavan, & Basian, 1986), and the CIWA (Sullivan et al., 1989). Self-report measures included: a demographics questionnaire, the Beck Depression Inventory-II (BDI; Beck, Steer, & Brown, 1993), the Beck Anxiety Inventory (BAI; Beck & Steer, 1993), and the Fagerström Test of Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991).To assess tonic craving, the PACS (Flannery et al., 1999) and the OCDS (Anton et al., 1995) were completed. During the infusion visits, the AUQ (Bohn et al., 1995) was completed as each target BrAC was reached.

Data Analysis Plan

Means and frequencies were determined for demographic variables. Analyses were conducted in SAS using PROC Mixed. Linear regression models were formulated, first for the PACS and secondly the OCDS and the two subscales, where the dependent measure was mean phasic craving, as assessed by the AUQ. All models were designed with individual intercepts where the within subject variables, BrAC and Alcohol condition, were Level 1 variables and tonic craving was a Level 2 variable. Covariates tested in all models include smoking status, BDI, BAI and sex. The models examined *BrAC*, which was used as 4-level, within subject indicator of *time* (baseline was time-point zero, BrAC = 0.02 g/dl was considered time-point 1, etc), *alcohol condition* (alcohol versus saline), and *tonic craving* (PACS, OCDS total score and subscales), and their *interactions*.

Results

Demographics

Sample demographics are presented in **Table 1**. Participants were drinking on nineteen days of the previous month and were drinking seven drinks per occasion. Of the total possible eleven symptoms of a DSM-IV alcohol use disorder, participants met for an average of 6.5

symptoms (SD = 2.3). Participants met for few clinically relevant withdrawal symptoms. Nearly twenty-four percent categorized themselves as daily smokers.

Relationship between Tonic and Phasic Craving

Overall, AUQ scores were greater when participants received alcohol compared to placebo, supporting a main effect of alcohol administration ($p < 0.05$; Ray et al., 2013). Results of models testing the association between tonic craving (indexed by the PACS or the OCDS) and phasic craving (during alcohol vs. saline conditions) are reported in **Table 2**.

Results for the PACS indicated that tonic craving by the PACS had a significant simple effect ($\beta = 0.09$, SE = 0.04, $p = 0.02$), such that regardless of alcohol condition higher PACS scores predict higher AUQ scores. There was also a simple effect of alcohol condition such that AUQ scores were higher when alcohol was administered compared to saline. Including covariates did not significantly impact results.

Models using the OCDS as an indicator of tonic craving revealed a trend level interaction of OCDS × condition ($F = 3.63$, $p = 0.057$). To further investigate this effect, the model was tested in each condition (i.e. alcohol administration and saline administrations). Results indicate OCDS is predictive of phasic craving during the alcohol administration ($\beta = -0.05$, SE = 0.02, $p = 0.03$), but not during saline ($\beta = -0.02$, SE = 0.02, $p > 0.10$). In addition, probing for the subscales of the OCDS, results indicated that the obsessive subscale is driving these effects such that there was a significant interaction of obsessive subscale with condition ($F = 6.17$, $p = 0.01$) such that craving was predictive of phasic craving during alcohol administration ($\beta = 0.11$, SE = 0.05, $p = 0.02$) but not during saline ($\beta = 0.04$, SE = 0.05, $p > 0.10$). These effects were not observed for the compulsive subscale of the OCDS ($F = 0.75$, $p > 0.10$).

Discussion

Despite the long recognition of craving as a critical phenomenon in AUD, little is known about the relationship between tonic and phasic craving for alcohol. Results from this study indicated that the PACS did not predict phasic craving in response to alcohol administration but instead, there was a main effect of PACS such that higher tonic craving (measured by the PACS) was predictive of higher phasic craving, regardless of whether alcohol or saline was administered. On the other hand, the OCDS was predictive of phasic craving when alcohol was administered, but not during the saline administration; similarly, the Obsessive subscale, but not the Compulsive subscale, was predictive of phasic craving when alcohol was administered. Thus, tonic craving, as assessed by the OCDS, may be predictive of phasic craving that is provoked by alcohol administration (but not saline), whereas the PACS may more generally predict increased phasic craving, regardless of presence of alcohol, suggesting that these measures may function differently.

Overall, the OCDS is based on the theory that addictive disorders are similar to obsessive-compulsive disorders and focuses on alcohol related cognitions and urges. Such cognitions, measured by the obsessive subscale, may be heightened when alcohol is present. However, data are mixed regarding the concurrent validity of the OCDS. One study of alcohol dependent patients did not find any relation of the OCDS to other measures of alcohol use (Connor et al., 2008). Additionally, Kranzler et al. (1999) questioned the predictive validity of the OCDS as it did not strongly predict drinking after completion of a pharmacotherapy trial. In contrast, the PACS has shown unique prognostic utility in predicting number of standard drinks after treatment, above the effects of the AUQ (Flannery et al., 2003). In this study, higher PACS score generally predicted higher phasic response but was not different based on alcohol

condition, perhaps speaking to the general ability of this measure to capture a broader dimension of craving. This study provides further characterization of the relationship between tonic and phasic alcohol craving, such that higher tonic craving predicted phasic craving, particularly when alcohol was ingested. This relationship is clinically relevant as both types of craving have shown to predict drinking behaviors. Interventions targeting tonic craving may in turn dampen phasic response to alcohol administration or cues, thus assessing craving in multidimensional fashion and accounting for combined phasic and tonic effects appears warranted. To that end, a recent human laboratory study found that a novel neuroimmune medication (ibudilast) reduced tonic craving compared to placebo (captured by the PACS) yet there were no medication effects on alcohol- or cue-induced phasic craving (Ray et al., 2017). This serves to illustrate the complex clinical interplay between tonic and phasic craving and its treatment implications.

Results should be interpreted in light of study strengths and limitations. Strengths include the experimental manipulation where participants completed both alcohol and placebo administration sessions. Limitations include the small sample size and the fact that craving could have been dampened during the infusion due to lack of other cues (e.g. taste, visual). The study also relied on self-report measures of craving which may be subject to recall bias. Future studies should examine relationships between tonic and phasic craving using other craving provocations, such as cue and stress exposure paradigms and include objective indicators of alcohol craving (e.g. physiological arousal, activation of the stress response system, behavioral markers of alcohol-related activities; Flannery et al., 2001).

Table 1

Demographic, substance, and mood variables of the sample (n=43).

Demographics	
Age (SD)	29.3 (9.5)
% Male (N)	74.4 (32)
% Caucasian (N)	69.8 (30)
Substance Use Variables	
DPDD (SD)	7.1 (2.9)
Drinking days (SD)	19.2 (7.5)
Total number DSM-IV AUD symptoms (SD)	6.5 (2.3)
AUD age of onset (SD)	23.1 (6.7)
CIWA (SD)	5.6 (4.4)
% Daily smokers (N)	32.56 (14)
FTND (SD)	2.2 (2.8)
Alcohol Craving	
PACS	15.0 (6.2)
OCDS	20.6 (9.2)
OCDS-Obsessive	8.8 (5.2)
OCDS-Compulsive	11.8 (4.7)
Mood Variables	
BDI (SD)	18.9 (12.8)
BAI (SD)	15.7 (12.5)

Table 2

Alcohol administration models

	F	*p*
Alcohol	**12.14**	<0.01
Time	0.67	0.57
PACS	**4.37**	0.04
Alcohol *Time	0.43	0.73
PACS* Alcohol	1.66	0.20
PACS*Time	1.14	0.33
PACS* Alcohol *Time	0.11	0.95
Alcohol	0.62	0.43
Time	0.88	0.45
OCDS	1.27	0.27
Alcohol *Time	0.06	0.98
OCDS * Alcohol	**3.63**	0.057
OCDS *Time	1.41	0.24
OCDS* Alcohol *Time	0.49	0.69

Note: Bolded items signify $p < 0.05$. Alcohol refers to alcohol or placebo administration. Time refers to BrAC levels (0.00, 0.02, 0.04, 0.06 g/dl). Tonic Craving is assessed via the PACS or OCDS.

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STUDY 2: Craving as a DSM-5 Symptom of Alcohol Use Disorder in Non-treatment Seekers

Abstract

Aims: The DSM-5 has added craving as a new criterion and changed the diagnostic structure of alcohol use disorders (AUD). Though craving has long been a target of interventions, less is known about the impact this addition will have on prevalence and factor structure of AUD, particularly in non-treatment seeking alcohol users.

Methods: Non-treatment seeking alcohol users (N = 296) completed a structured clinical interview and the Penn Alcohol Craving Scale (PACS). PACS scores greater than 20 were considered to meet diagnostic criteria for craving. This secondary analysis examined DSM-IV to DSM-5 diagnostic conversion and performed an exploratory factor analysis to test the factor structure of the retained symptoms and the new craving symptom.

Results: The sample was predominately male, young, and Caucasian. They reported frequent and heavy alcohol consumption (18.3 drinking days in past month, 7.2 drinks per occasion). The mean PACS score was 13.1 and craving was strongly correlated with other measures of alcohol use. Using the proposed cut-off score, 46 participants (16.2%) met the craving criterion. Craving loaded moderately (.47) onto the retained DSM symptoms and produced a unidimensional factor structure. The majority of participants who met for a DSM-IV AUD also met for a DSM-5 AUD (98.8%).

Conclusions: Craving prevalence using the PACS was relatively low compared to the retained 10 DSM symptoms, possibly due to the non-treatment seeking status of the participants. Conversion of DSM-IV to DSM-5 in this sample did lead to a small increase in overall AUD prevalence. Craving loaded well onto a single diagnostic factor.

Introduction

Subjective craving for substances of abuse has become an increasingly salient point for the diagnosis and treatment of substance use disorders. In fact this salience has impacted the latest iteration of the Diagnostic and Statistical Manual (DSM-5; American Psychiatric Association, 2013). Published in its fifth iteration in 2013, the update has made two critical updates to the alcohol use disorder (AUD) and substance use disorder (SUD) section (Hasin et al., 2013) since the previous edition, which was published in 1994 (DSM-IV). First, there was a diagnostic structure change that replaced the separate diagnoses of "dependence" and "abuse" with a unidimensional diagnostic structure that qualifies a SUD by severity based on the number of symptoms endorsed (i.e. "Mild," "Moderate," or "Severe"; Hasin et al., 2013; Ray, Kahler, Young, Chelminski, & Zimmerman, 2008). Secondly, the legal criterion of abuse was dropped, due to infrequent endorsement and poor discriminant validity (Agrawal, Heath, & Lynskey, 2011), in favor of the addition of craving as a criterion (Hasin, Fenton, Beseler, Park, & Wall, 2012; Keyes, Krueger, Grant, & Hasin, 2011). Though the structural change was viewed favorably, debate continues regarding the optimum threshold for these new diagnostic categories, the impact on prevalence, and whether craving will aid in the discrimination or validity of AUD.

There is debate regarding whether the structural and criteria updates to the DSM will impact prevalence. For example, epidemiological studies suggest the prevalence of AUD may increase under DSM-5 criteria due to the diagnostic structure change (Bartoli, Carrà, Crocamo, & Clerici, 2015; Mewton, Slade, McBride, Grove, & Teesson, 2011). However, prevalence is not expected to be significantly impacted by the criteria change (Agrawal et al., 2011; Cherpitel et al., 2010). The stability in AUD prevalence between DSM editions has been viewed as a positive as a sudden increase in prevalence of AUDs due to criteria changes would be questionable

(Tiffany & Wray, 2012). Alternatively, using the third wave of the National Epidemiological Survey on Alcohol and Related Conditions (NESARC) data, prevalence of lifetime DSM-5 AUD was lower than that of DSM-IV (29.1% versus 43.6%; Grant et al., 2015). Another impact of the proposed changes would be the elimination of diagnostic orphans (Agrawal et al., 2011; Hasin & Paykin, 1998). These individuals meet for one to two symptoms of dependence and thus may be experiencing clinically relevant impairment. However, under DSM-IV such individuals did not meet the criteria for AUD diagnosis which could limit access to care. With the new DSM-5 structure, such individuals will likely convert to an AUD diagnosis, potentially increasing AUD prevalence.

In addition to the debate regarding the impact on prevalence, deliberation about the inclusion of craving as a criterion persists. Craving has previously been included in the International Classification of Diseases (ICD; World Health Organization, 2004) diagnostic system, which provides comparable prevalence rates of AUD and may have better reliability over the DSM-IV (Hasin, Hatzenbuehler, Keyes, & Ogburn, 2006). Further supporting the inclusion of craving is that craving for alcohol has been predictive of alcohol consumption (McHugh, Fitzmaurice, Griffin, Anton, & Weiss, 2016; Schneekloth et al., 2012) and, with treatment, this predictive relationship can decrease over time (McHugh et al., 2016). Craving is also associated with relapse (Schneekloth et al., 2012) and thus has become a target for interventions, both psychosocial and pharmacological (Addolorato, Abenavoli, Leggio, & Gasbarrini, 2005; Anton et al., 1999; Witkiewitz, Bowen, Douglas, & Hsu, 2013).

On the other hand, despite the approval of the DSM-5, criticism of the changes exists. For example, there is no unified theory on the development of craving (Drummond, 2001) and debate exists regarding whether the root is biological, psychobiological, or psychosocial (Monti,

Rohsenow, & Hutchison, 2000). Another pertinent criticism is that there is no consensus regarding the best method of assessing craving and that cultural differences could make assessment difficult (Cherpitel et al., 2010). The lowered threshold of number of criteria needed to meet for an AUD has also been raised as a concern due to the potential for increasing prevalence, perhaps due to diagnostic recategorization of "diagnostic orphans" (Mewton et al., 2011).

Additionally, craving may represent a more severe symptom that may not be commonly endorsed, thus limiting incremental validity. Craving is thought to escalate depending on treatment and severity of alcohol use. Anton and Drobes (1998) showed that craving scores, as assessed by the Obsessive Compulsive Drinking Scale (OCDS), are lowest in non-alcohol dependent individuals, then become successively higher in non-treatment seekers, those receiving outpatient therapy, and highest in inpatient populations (Anton & Drobes, 1998). Though factor analyses have shown that craving loads strongly onto the other AUD criteria to form a unidimensional structure (Casey, Adamson, Shevlin, & McKinney, 2012; Cherpitel et al., 2010; Keyes et al., 2011; Mewton et al., 2011), supporting the argument that craving is related to the other symptoms, questions have arisen regarding the differences between treatment seeking and non-treatment seeking populations. Non-treatment seekers are often used in clinical research to test safety and efficacy of new medications and in human laboratory studies that inform clinical trials. Evidence has arisen that treatment seeking status is likely impacting results of such studies (Perkins et al., 2008) and that there are significant clinical differences in presentation between these two groups (Ray, Bujarski, Yardley, Roche, & Hartwell, 2017; Rohn et al., 2017). Rohn and colleagues (2017) found that non-treatment seeking alcohol users, compared to treatment seeking alcohol users, reported fewer AUD symptoms, lower alcohol consumption,

lesser mood and anxiety symptomatology, and were less impulsive. Thus understanding craving as a symptom of AUD in non-treatment seekers is warranted.

In treatment-seeking heavy alcohol users, recent work by Murphy and colleagues (2014) showed that the eleven DSM-5 symptoms were a unidimensional system. The group capitalized on the Penn Alcohol Craving Scale (PACS; Flannery, Volpicelli, & Pettinati, 1999), a widely used and well validated measure of tonic craving, as a stand in for the craving criterion. The PACS has the advantage over single item assessment in querying duration, frequency, ability to resist, and intensity of craving over the given time period. Participants with total scores of greater than 20 were considered to meet the craving symptom criteria, indicative of strong urges and great difficulty in resisting alcohol use. Accordingly, forty-seven percent of the sample met the symptom of craving based on the PACS cut-off score. It is not known whether these results translate to non-treatment seekers, a group frequently enrolled in clinical trials and behavioral studies of addiction.

Strong evidence from preclinical, clinical, and laboratory studies support the importance of craving in the phenomenology of AUD and as a treatment target. However, better understanding of the diagnostic function of craving is warranted and much is yet to be understood regarding the manifestation of craving in non-treatment seeking alcohol users. Thus, the present study seeks to test: (a) how the addition of craving as an AUD symptom will alter AUD prevalence estimates in non-treatment seeking heavy alcohol users and (b) how the new criterion will load onto the remaining criteria, particularly in a community sample of non-treatment seeking alcohol users.

Methods

Participants and Procedures

Non-treatment seekers problem drinkers were recruited from the greater Los Angeles area to participate in a laboratory study examining the impact of genotype on subjective effects of acute alcohol administration (Ray et al., 2013) with approval from the UCLA Institutional Review Board. When participants arrived for the screening visit, they provided written informed consent and completed a battery of self-report measures and clinician-administered interviews to determine eligibility. Inclusion criteria were: (1) between ages 21-65; (2) report alcohol related problems; and (3) endorse drinking ≥ 48 drinks per month. Exclusion criteria were: (1) seeking treatment or currently in alcohol treatment; (2) alcohol abstinence for the previous three weeks; (3) self-reported lifetime history of bipolar disorder or psychotic disorder; and (4) lack of endorsement of DSM-IV AUD symptoms. Two hundred ninety-six individuals completed the initial in-person assessment; however, twelve individuals did not endorse symptoms of an AUD and were excluded from this secondary analysis.

Measures

After providing informed consent, participants completed a battery of individual difference measures, including: (1) demographic questionnaire querying age, gender, ethnicity, and other variables; (2) the Alcohol Dependence Scale (ADS; Skinner & Horn, 1984) to assess severity of current alcohol use problems; and (3) the Drinker's Inventory of Consequences (DRINC; Miller, Tonigan, & Longabaugh, 1995) which ascertained the severity of alcohol related consequences.

The Structured Clinical Interview for DSM-IV (SCID; First, Spitzer, Gibbon, & Williams, 1995), the Clinical Institute Withdrawal Assessment (CIWA; Sullivan, Sykora,

Schneiderman, Naranjo, & Sellers, 1989) and Timeline Follow-back (TLFB; Sobell, Sobell, Klajner, Pavan, & Basian, 1986) assessed for current alcohol use disorders, presence and degree of withdrawal symptomatology, and past 30-day alcohol consumption, respectively. SCID symptoms are rated on a scale of 1 to 3 where 1 is indicative that the symptom is absent, 2 is considered subthreshold, and 3 indicates the symptom is present. From the TLFB, total number of drinking days and drinks per drinking day (DPDD).

Data were collected prior to the publication of DSM-5 and utilized the DSM-IV based SCID. Thus, the Penn Alcohol Craving Scale (PACS; Flannery et al., 1999) will be used to formulate the craving symptom. This psychometrically reliable and valid measure is composed of five items that capture craving over the previous week. Participants rate each item on a scale of 0 to 6 for which the sum of the scores are indicative of severity of craving. Using the approach utilized by Murphy et al. (2014), total score greater than 20 will be categorized as meeting for the craving symptom. This total score would indicate that the average score on each item is at least a "4," indicating that the individual is experiencing a "strong urge" and that it is "very difficult" to resist craving. Scores between 15 and 20 will be considered subthreshold and scores less than 15 will consider the symptom absent.

Data Analysis Plan

First, demographic and substance use variables were calculated. In order to meet the first study aim, participants were placed into the correct diagnostic categories: abuse (without dependence), dependence, any DSM-IV diagnosis (abuse and/or dependence), diagnostic orphans (i.e. participants who met 1-2 dependence criteria and no symptoms of abuse) using the DSM-IV SCID data. Next, the total number of SCID symptoms, with the subtraction of the legal question and the addition of craving, was calculated. Participants were then placed into the

appropriate DSM-5 categories (i.e. no, mild, moderate, or severe AUD) based on the number of symptoms reported. Cross-tabulations were used to compare diagnostic conversion. Correlations between all possible diagnostic symptoms and indicators of alcohol use, namely DRINC, ADS, and alcohol use in the past month as calculated from the TLFB, were computed.

To meet the second study aim, exploratory factor analysis (EFA) was conducted. First, the ten retained symptoms from the DSM-IV were examined and, secondly, craving will be added in order to resemble the DSM-5 structure. The EFA approach utilized principle axis factoring (PAF). PAF alters the correlation matrix to represent communalities between each set of variables. This method additionally allows each variable to be influenced by unique error. Eigenvalues and Scree plots were examined to determine the number of optimal number of factors to retain. Items loadings of .30 or greater were considered significant.

Results

Demographics

Participants were predominantly young, male and Caucasian (**Table 1**). Participants endorsed drinking for a mean of 18.3 days in the previous month and reported 7.2 drinks per drinking day (DPDD). Withdrawal was minimal (CIWA mean = 5.7), whereas indicators of alcohol use severity were elevated (ADS mean = 15.8, DRINC = 44.7). Of the eleven total possible AUD symptoms, participants met for an average of 5.5 symptoms.

Craving Endorsement

Craving, as determined by the PACS cut-off score, was met by 16.2% of the sample (n = 46), and 21.8% were considered subthreshold (**Table 1**). Craving was significantly correlated with other measures of alcohol use, specifically the DRINC, ADS, number of drinking days, and DPDD (**Table 2**); further, craving had the strongest correlation with the first three of these

measures as compared to the other AUD symptoms. However, like the legal criterion, craving was the least frequently endorsed symptom compared to the other 10 retained symptoms.

DSM-IV versus DSM-5 AUD Prevalence

Per DSM-IV criteria, the 75.3% of the sample would have met for dependence, 12.3% met for abuse without dependence, and 12.3% were diagnostic orphans. According to DSM-5 criteria, 5.6% of the sample would not meet for an AUD, 21.1% would meet for mild, 24.7% moderate and 48.6% severe. **Table 3** shows the conversion of participants from DSM-IV to DSM-5 diagnostic structure. Of those meeting for DSM-IV abuse without dependence, the majority went on to meet for mild or moderate abuse (91.4%). All subjects who met for DSM-IV dependence also met for an AUD in DSM-5. Of the 249 participants who met for either abuse or dependence, only 3 did not convert to a DSM-5 diagnosis meaning 98.8% continue to meet for a diagnosis. Further, the majority of those meeting for any DSM-IV diagnosis were moderate (28.1%) or severe (55.4%) when converted to DSM-5 structure. Of the 35 diagnostic orphans in the sample, only 37.1% of participants remained undiagnosed whereas the rest converted to a mild AUD in DSM-5.

Factor Analysis of Retained DSM-IV Criteria and Craving

Table 4 shows the results of the exploratory factor analysis for the ten retained DSM-IV symptoms and with the inclusion of the PACS craving symptom. Both models had a Kaiser's measure of sampling adequacy above 0.80, indicating sufficient correlation matrices. Eigenvalues and scree plots indicated a unidimensional factor structure. For the model of retained DSM-IV symptoms, the eigenvalue of the single extracted factor was 2.32, accounting for 73% of the variance. Including craving did not significantly alter factor structure and results showed that it loads moderately well (.47) onto existing symptoms. The eigenvalue of the eleven

DSM-5 symptoms was 2.53 and accounted for 72% of the variance. For both models, loadings were similar and all positively loaded onto the single factor; however, two symptoms fell below the predetermined significance level: tolerance and drinking more than intended.

Discussion

The current study sought to examine the impact of the addition of craving on prevalence and the factor structure of DSM-5 AUD diagnosis in a community sample of non-treatment seeking heavy alcohol users. Converting DSM-IV diagnostic status to DSM-5 showed an increase in overall prevalence of AUD from 87.7% to 94.3%. Similar to Mewton et al. (2011), all individuals who endorsed DSM-IV dependence converted to a DSM-5 AUD diagnosis, predominantly moderate or severe. Of those meeting for either abuse or dependence, only 1.2% did not receive a DSM-5 diagnosis. Further, nearly two-thirds of diagnostic orphans converted to a mild AUD diagnosis, a rate considerably higher than what Mewton et al. (2011) observed.

Bartoli and colleagues (2015) concluded that DSM-5 would likely increase prevalence of AUD based off their review of twelve epidemiological studies. Moreover, they posited this increase is primarily due to non-clinical populations and the conversion of diagnostic orphans to diagnosis. This aligns with findings presented herein where a non-clinical sample demonstrated increased prevalence of AUD with DSM-5 structure and a higher rate of orphan conversion. Due to this increased rate of diagnosis, the threshold of two symptoms to meet for an AUD may be too low, potentially leading to over pathologizing alcohol use problems as the prevalence rate of AUD will be artificially inflated.

Craving, here assessed via the PACS, was significantly correlated with all measures of alcohol use (i.e. DRINC, ADS, and TLFB indications), which were stronger than the legal symptom correlations. When examining factor structure of the retained DSM-IV symptoms and

the added symptom of craving, the unidimensional factor structure was demonstrated in both models with the single factor accounting for a majority of the variance. Akin to Casey et al. (2012), who utilized NESARC data, factor analysis showed that craving fit in well to the unidimensional structure proposed by DSM-5. These findings are also consistent with Murphy et al. (2014) who also found a similar unidimensional structure in a treatment seeking sample by utilizing the PACS as a stand in for the craving criterion and, furthermore, extend results to a non-treatment seeking population. Though this utilization of the PACS in this manner is an atypical approach, this measure is widely used and a psychometrically sound assessment of tonic craving. As noted by Murphy and colleagues, the DSM is categorical in nature and a certain threshold of severity must be met for a symptom to become clinically relevant and considered impairing. Though the study should be interpreted with the caveat of this unusual approach to craving diagnosis, the PACS cut-off did correlate strongly with other measures of alcohol consumption and problematic use lending support to the validity of this approach.

Despite these findings, the low endorsement of craving must be noted; 46 participants (16.2%) met for craving using the cut-off score. This level of endorsement is in contrast to Murphy et al. (2014) where nearly half of the sample endorsed clinically significant craving using the PACS. As earlier noted, craving is thought to be a more severe symptom of AUD. For example, in nationally representative samples, craving has been demonstrated to be a moderate to severe symptom as compared to the other 10 symptoms of AUD (Casey et al., 2012; Keyes et al., 2011), thus corroborating the relatively low endorsement in this sample. This discrepancy may relate to the difference in treatment status between the samples. Anton and Drobes' (1998) observed that craving increased as a function of treatment seeking status. Recent research has also suggested that treatment seekers likely represent a more severe group of alcohol users who

have been shown to endorse a greater number of AUD symptoms and higher craving scores (Ray et al., 2017; Rohn et al., 2017). Alternatively, craving is also a heterogeneous experience both within and between alcohol using individuals. Craving volatility is due to a host of factors including tolerance, withdrawal symptomatology, severity of alcohol use, salience of consumption, and subjective attention paid to the urge to drink (Haass-Koffler, Leggio, & Kenna, 2014). Within individuals, craving may be intensified during times of stress, in situations previously associated with consumption, or when experiencing withdrawal (Drummond, 2001; Haass-Koffler et al., 2014). The PACS administered in this study assessed past week craving, which may not have fully captured individual's experience or the predetermined cut-off was too high for this non-treatment seeking sample.

Strengths of the study include the large, diverse sample of community alcohol users who are reflective of the individuals typically recruited for clinical laboratory research. This study also utilized the PACS to assess craving, a widely used, reliable, well validated assessment. The multi-question structure yields a composite score of craving that may be advantageous over single-item assessment (Ray, Courtney, Bacio, & MacKillop, 2013). Limitations of the study include the modest sample size for factor analysis and use of the PACS to replace a criterion typically assessed via structured interview. Future studies should consider the relationship between self-report and interview assessment of craving and the role treatment status plays in this relationship.

In conclusion, this study in non-treatment seeking individuals found support of the structural change to collapse the DSM-IV AUD categories of abuse and dependence into the single unidimensional syndrome. Craving loaded well onto existing symptoms, despite being a less frequently endorsed symptom. Although prevalence did increase in this sample, this was

primarily due to the diagnostic switching of diagnostic orphans who came to meet criteria for a mild AUD, thus capturing a group of individuals who may be ripe targets of early intervention and perhaps give a previously unidentified group greater access to care. Further exploration of the assessment and development of craving as individuals progress in AUD is warranted.

Table 1

Demographic and substance use variables.

	Mean (SD) or %(N)
Demographics	
Age, M(SD)	30.9 (10.4)
Male, %(N)	73.1 (204)
Ethnicity	
% Caucasian (N)	55.0 (153)
% African American	24.5 (68)
% Native	6.5 (18)
% Latino	22.7 (63)
% Asian	9.4 (26)
Substance Use Variables	
PACS, M(SD)	13.1 (6.5)
DPDD, M(SD)	7.2 (4.7)
Drinking days, M(SD)	18.3 (7.2)
Total number DSM-IV AUD symptoms, M(SD)	5.5 (2.7)
Age of AUD onset, M(SD)	24.1 (8.7)
CIWA, M(SD)	5.7 (7.0)
DRINC, M(SD)	44.7 (22.7)
ADS, M(SD)	15.8 (7.4)
Endorsed daily nicotine use, %(N)	24.1 (71)
FTND of daily smokers, M(SD)	3.7 (2.6)
Alcohol craving	
PACS >20, %(N)	16.2 (46)
PACS 15-20, %(N)	21.8 (62)
PACS <15, %(N)	62.0 (176)

Table 2

Percentage of endorsement and frequency for each symptom of AUD. Correlations of symptoms with other indices of alcohol use.

		% Endorse-ment (Frequency)	Correlations			
			DRINC	ADS	Drinking days	DPDD
Abuse	Inability to fulfill major roles	44.4 (126)	0.36***	0.31***	0.12	0.04
	Hazardous use	44.7 (127)	0.17**	0.11†	0.14*	0.003
	Legal issues	18.0 (51)	0.28***	0.2***	0.15*	0.22***
	Social & interpersonal problems	46.8 (133)	0.37***	0.31***	0.12*	0.21***
Dependence	Drinking more than intended	80.3 (228)	0.07	0.14*	-0.02	0.003
	Inability/persistent desire to cut down	50.0 (141)	0.29***	0.22***	0.12*	0.17**
	Time spent obtaining/ recovering	51.8 (147)	0.35***	0.36***	0.22***	0.27***
	Activities reduced	37.7 (107)	0.46***	0.32***	0.24***	0.19**
	Psychological/physical problems	63.4 (180)	0.28***	0.35***	0.07	0.10†
	Tolerance	81.0 (230)	0.19**	0.39***	0.27***	0.12*
	Withdrawal	32.8 (93)	0.44***	0.39***	0.27***	0.12*
New	Craving	16.2 (46)	0.49***	0.46***	0.32***	0.20***

Significance indicated: ***p<0.001, **p<0.01, *p < 0.05, †p<0.10

Table 3

Transition from DSM-IV to DSM-5 AUD diagnoses (%) for the full sample (n=284).

	DSM-5			
	No AUD (n=16)	Mild AUD (2-3 symptoms) (n=60)	Moderate AUD (4-5 symptoms) (n=70)	Severe AUD (6+ symptoms) (n=138)
DSM-IV abuse (without dep) (n = 35)	8.6	71.4	20.0	0
DSM-IV dependence (n = 214)	0	6.1	29.4	64.5
DSM-IV abuse/dependence (n =249)	1.2	15.3	28.1	55.4
DSM-IV diagnostic orphans (n = 35)	37.1	62.9	0	0

Table 4

Exploratory factor analysis of AUD symptoms in DSM-IV and DSM-5.

	Retained DSM-IV	DSM-5
Activities reduced	0.62	0.64
Social & interpersonal problems	0.58	0.58
Withdrawal	0.55	0.57
Psychological/physical problems	0.55	0.54
Time spent obtaining/recovering	0.53	0.53
Inability to fulfill major roles	0.53	0.52
Craving	—	0.47
Inability/persistent desire to cut down	0.45	0.44
Hazardous use	0.30	0.30
Tolerance	0.27	0.26
Drinking more than intended	0.25	0.25

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STUDY 3: Genetic Markers of alpha-Synuclein and their Relationship with Alcohol Craving

Abstract

Background: The alpha-synuclein (*SNCA*) gene has been implicated in various genetic studies as a top candidate gene for alcohol use disorders (AUD). *SNCA* is thought to mediate neurotransmission of dopamine at all points, from synthesis, storage, release, to reuptake and dopamine is thought to play a major role in alcohol craving. Further, *SNCA* has been associated with alcohol craving and dependence in previous studies.

Methods: To further investigate this relationship, 209 Caucasian and Hispanic problem alcohol users from the community were genotyped for two single nucleotide polymorphisms (SNPs; rs356221 and rs356219) of the *SNCA* gene. These SNPs were selected a-priori due to previous work implicating them in association with alcohol craving and dependence. Participants completed the Penn Alcohol Craving Scale (PACS) and the Obsessive Compulsive Drinking Scale (OCDS) to assess alcohol craving and were administered the Structured Clinical Interview for DSM-IV for the diagnostic assessment of AUD.

Results: The haplotype was not predictive of either the PACS ($p = 0.8$) or OCDS ($p = 0.94$). Neither individual SNP was predictive of craving measures ($p > 0.2$), save for a trend of risk allele carriers of rs356221 predicating a higher OCDS-Obsessive subscale score ($p = 0.06$), consistent with previous literature.

Conclusions: In sum, this study did not find support of these markers of the SNCA gene having a relationship with two alcohol use phenotypes, namely craving and dependence. Further research is needed to elucidate the relationship of *SNCA* and alcohol use, particularly as craving is not a ubiquitous experience amongst alcohol users.

Introduction

Substance use disorders (SUD) are highly heritable (Han, McGue, & Iacono, 1999), with estimates from family, twin and adoption studies typically reaching approximately 50% for alcohol use disorders (AUD; Verhulst, Neale, & Kendler, 2015). Promising results have been found for a number of candidate genes in relation to AUD, including genes in the dopamine, GABA, glutamate, and opioid systems (Ait-Daoud et al., 2009; Ait-Daoud et al., 2012; Dick et al., 2007; Finckh et al., 1997; Köhnke, 2008; Ray, 2011; Samochowiec et al., 2006; Zhang et al., 2006). Though data are mixed (Enoch, 2013; Heath et al., 2011; Köhnke, 2008), one gene that has received consistent attention is α-synuclein (*SNCA*). Alpha-synuclein, a presynaptic protein, has long been connected to the development of neurodegenerative disorders, including Parkinson's disease, yet the exact role is yet unknown (Janeczek & Lewohl, 2013). Multiple studies have implicated α-synuclein in mediating the neurotransmission of dopamine at all levels, from synthesis, storage, release, to reuptake and it is widely expressed, predominately in presynaptic terminals (Janeczek & Lewohl, 2013). Based on expression levels, α-synuclein is responsible for regulation of dopamine uptake; under abnormal conditions, such as when α-synuclein is suppressed, dopamine transport is lessened which can result in cell death (Janeczek & Lewohl, 2013). Changes in expression are thought to alter dopamine regulated signaling, thus mediating craving and other alcohol related pathways (Janeczek & Lewohl, 2013; Self & Nestler, 1998). Additionally, α-synuclein is part of neuroprotection and neurotoxicity, whereas cytotoxicity occurs at high or low levels of expression (Janeczek & Lewohl, 2013).

In a genomewide association study (GWAS) that included animal and human genetic data, *SNCA* was named a top candidate gene for AUD (Levey et al., 2014). The study analyzed German samples, alcohol dependent individuals (n=1,151) and matched controls (n=2,168), for

the initial discovery and samples from the United States, both familial and non-related alcohol dependent individuals as well as controls, to replicate findings (n=3,368 AUD; n=1,261 controls). Utilizing a convergent functional genomics approach, authors showed that *SNCA* was the most promising gene examined. This approach also used previously published human and animal model evidence and gene expression data, to arrive at a combined rating of each gene considered. Moreover, variations in *SNCA* were able to differentiate those with alcohol abuse or dependence from control participants (Levey et al., 2014). However, the aforementioned study is limited by the inclusion of only Caucasians in all samples, and the initial GWAS cohort was comprised exclusively of men.

As dopamine plays a role in alcohol addiction, specifically craving, withdrawal, and reinforcement (Self & Nestler, 1998), alterations in *SNCA* expression may impact dopamine-mediated neuron signaling, dopamine homeostasis, and the dopamine reward pathway (Janeczek & Lewohl, 2013). In animal studies, mice without the gene showed greater sensitization of the brain reward system, implying that individuals with low *SNCA* may be at greater risk for developing SUD (Oksman, Tanila, & Yavich, 2006). Alcohol-preferring rats expressed greater α-synuclein protein levels than non-preferring rats in key brain 29 regions, indicating that differences in *SNCA* gene expression may be contributing to alcohol-seeking (Liang et al., 2003).

Several genetic studies have examined the role in *SNCA* in AUDs and craving. One theory of craving defines it as a hypersensitivity to the rewarding effects of alcohol. Neurobiologically, this theory is rooted in the dopaminergic system (Verheul, van den Brink, & Geerlings, 1999), thus making SNCA an attractive candidate gene of interest in relation to craving. From the Collaborative Study on Genetics of Alcoholism (COGA) study, utilizing data from more than 200 alcoholic families, thirty SNPs of *SNCA* were genotyped and studied in

relation to alcohol dependence and craving (Foroud et al., 2007). Interestingly, this study did not find an association of *SNCA* and alcohol dependence; however, eight SNPs were associated with alcohol craving. Authors posited that this indicative that *SNCA* does not consistently impact all individuals with an AUD, rather it impacts a subset of such individuals. As craving is less likely to be endorsed by those who did not meet for dependence (2% versus 42% who did meet dependence criteria in this study), this explanation appears plausible. Additionally, one haplotype of three *SNCA* SNPs, that included rs356221 and rs356219, was found to be significantly associated with craving (Foroud et al., 2007). Agrawal and colleagues conducted a study using the Study of Addiction: Genes and Environment (SAGE; N = 3,976) dataset to investigate the genetic role of craving (Agrawal et al., 2013). The SAGE sample was comprised of non-related individuals who met DSM-IV criteria for alcohol dependence versus alcohol exposed controls and also showed that several single nucleotide polymorphisms (SNPs) of *SNCA* were associated with craving; however, these were different SNPs than those implicated by Foroud et al. (2007).

Additional evidence of the role of *SNCA* came from Bönsch and colleagues who completed two studies examining *SNCA* messenger RNA (mRNA; Bönsch et al., 2004) and protein expression (Bönsch et al., 2005). Males with AUD and nondrinking controls were compared such that *SNCA* mRNA and protein expression were significantly greater in those with AUD (Bönsch et al., 2005; Bönsch et al., 2004). Moreover, OCDS total score and both subscales were associated with *SNCA* protein levels (Bönsch et al., 2005) and higher mRNA expression was predictive of higher OCDS total score and Obsessive subscale (Bönsch et al., 2004). The Obsessive subscale aims to gauge severity of alcohol related thoughts or preoccupation and ability to resist those thoughts (Anton, Moak, & Latham, 1995). In fMRI research, several SNPs

of *SNCA* were associated with BOLD response in brain regions implicated in craving during an alcohol taste cue (Wilcox, Claus, Blaine, Morgan, & Hutchison, 2013).

Janeczek and colleagues (2015) completed a study examining the expression of three *SNCA* variants in alcohol users with cirrhosis compared to controls. They found that two of the variants had lower expression in the prefrontal cortex in the brains of the alcohol users and the third had higher expression (Janeczek, Brooker, Dodd, & Lewohl, 2015). This study also examined the eight significant SNPs from Foroud et al.'s (2007) study. Authors found that allele frequencies did vary significantly for three of the SNPs between alcohol misusers and controls. Two SNPs, rs356219 and rs356221, were found to have strong linkage disequilibrium and different haplotypes were more frequently expressed in controls versus alcohol misusers. Authors determined A as the risk allele for rs356219 and T for rs356221 by comparing expression rates in alcohol misusers versus controls. Though expression of either of the risk alleles was not found to be more likely in the alcohol misusers, the G-A haplotype, or protective allele haplotype, was significantly more likely to be expressed in the control group.

Taken together, these studies support the association of *SNCA* with alcohol craving and dependence, particularly given the role of *SNCA* in the dopaminergic system which impacts withdrawal, craving, and reinforcement. Despite this compelling evidence, behavioral studies examining *SNCA* are limited. Thus, this study seeks to examine the relationship between two key SNPs of *SNCA*, selected a-priori based on previous evidence of a relationship between them and alcohol related phenotypes, and alcohol craving in a well characterized sample of problem alcohol users. I hypothesize that the risk alleles (i.e. A carriers for rs356219 and T carriers for rs356221) and risk haplotype will predict greater alcohol craving and alcohol dependence than not carrying either risk allele.

Methods

Participants

Participants were recruited from the greater Los Angeles community through print and online advertising for a study investigating subjective effects of alcohol (Ray et al., 2013). Participants were first screened over the phone for exclusion criteria. Those eligible were invited to in person behavioral screening visit. A total of 295 participants completed the behavioral visit which included providing a saliva sample for genotyping analysis; the 209 participants who identified as Hispanic and Caucasian are included in this analysis.

Inclusion criteria comprised of: (1) age 21-65; (2) identify as Caucasian or Hispanic; (3) self-reported problems with alcohol; and (4) endorse drinking ≥48 drinks per month. Exclusion criteria encompassed: (1) currently treatment-seeking or history of alcohol use treatment within the past 30 days; (2) self-reported current use of other substances (save nicotine and marijuana); and (3) self-reported lifetime diagnosis of major psychiatric disorders (e.g. bipolar or psychotic disorders).

Procedures

Participants invited to the in-person screening after telephone interview provided written informed consent. Subsequently, participants supplied a saliva sample using Oragene kits for DNA analysis and completed a battery of self-report measures. The *SNCA* SNPs was processed at the UCLA Genotyping ad Sequencing (GenoSeq) Core. An ABI7500 real time PCR instrument was used to conduct 5'-nuclease (TaqMan) assays of SNPs which relies on allele-specific hybridization of oligonucleotide probes. Each assay was run in duplicate for each candidate gene, with inconsistencies being resolved with a third run. Allele calling software automatically scored genotypes which were verified by visual inspection.

Measures

Master's level clinicians administered the Structured Clinical Interview for the Diagnostic and Statistical Manual (SCID; First, Spitzer, Gibbon, & Williams, 1995), the Clinical Institute Withdrawal Assessment (CIWA; Sullivan, Sykora, Schneiderman, Naranjo, & Sellers, 1989) and the Timeline Follow-Back (TLFB; Sobell, Sobell, Klajner, Pavan, & Basian, 1986). The SCID assessed for alcohol abuse and dependence according to DSM-IV criteria from which a total symptom count can be summed. The CIWA is a 10-item assessment of current withdrawal symptomatology. From the 30-day TLFB data, assessing participants' alcohol consumption for each day, total number of drinking days and drinks per drinking day (DPDD) were calculated.

The Penn Alcohol Craving Scale (PACS; Flannery, Volpicelli, & Pettinati, 1999) is a 5-item measure assessing severity of craving during the previous week. Each item is rated on a scale of "0" to "6" and then summed to create a total score. Greater total scores are indicative of greater craving. The Obsessive Compulsive Drinking Scale (OCDS; Anton et al., 1995) is a 14-item measure which assesses urges, craving, alcohol-related thoughts, and ability to resist to drinking. Two subscales, the Obsessive and compulsive, are derived from the measure. The Obsessive subscale intends to capture craving related cognitions whereas the compulsive subscale assesses drive to consume alcohol and ability to control that drive (Anton et al., 1995). Both measures have been widely used in alcohol using populations and shown to be reliable and valid (Kavanagh et al., 2013). Participants also completed the following self-report measures to gauge alcohol use: the Alcohol Dependence Scale (ADS; Skinner & Horn, 1984) and the Drinkers Inventory of Consequences (DRINC; Miller, Tonigan, & Longabaugh, 1995).

Statistical Analyses

The following analyses were planned to address the aims of this study. The Hardy-Weinberg Equilibrium was calculated for each SNP. T-test and chi-square tests for demographic and alcohol use variables were conducted for individuals carrying a risk allele versus those who do not ("A" for rs356219, "T" for rs356221; see Janeczek et al., 2015). To compute the haplotype, participants carrying a copy of either risk allele were assigned a value of 1, others were assigned a value of 0, in accordance with the approach of Janeczek et al. (2015); individual SNPs were tested as a three level variable (no risk allele carriers, single risk allele carriers and double risk carriers). A series of Proc GLM models were run in SAS 9.3 (Cary, NC). First, the PACS score was entered as the dependent variable and subsequently the OCDS and the Obsessive and Compulsive subscales. The haplotype and individual SNPs, with appropriate covariates, were predictors in each model with each craving outcome variable. All models included number of drinking days and DRINC total score as covariates. As this study is exploratory in nature, results were considered significant when $p < 0.05$.

Results

Sample Characteristics

Table 1 presents sample characteristics for the total sample and by risk allele status. Overall, the participants were young and the majority met for DSM-IV alcohol dependence (73.8%) and endorsed low levels of withdrawal (CIWA M = 5.6, SD = 7.2; see **Table 1**). Participants were drinking an average of 17.6 (SD = 6.9) days a month and were consuming 7.2 (SD = 4.3) standard drinks per drinking day. On average, participants met for 5.3 symptoms (SD = 2.8) of the total possible 11 DSM-IV symptoms. Approximately one fifth of the sample

considered themselves daily smokers and endorsed low nicotine dependence (FTND mean 3.1). Mean PACS score for the sample was 13.1 (SD = 6.3) and mean OCDS was 26.6 (SD = 11.5).

SNCA and Alcohol Phenotypes

Allele frequencies (shown in **Table 2**) were found to be in conformity with the Hardy Weinberg Equilibrium for rs356221 ($\chi^2 = 0.03$, $p > 0.05$) and rs356219 ($\chi^2 = 0.62$, $p > 0.05$). First, the haplotype of both SNPs was tested as a predictor of each craving score (see **Table 3**). The haplotypes was not predictive of PACS ($\beta = -0.23$, SE = 0.90, $t = -0.26$, $p = 0.80$) or OCDS ($\beta = -0.14$, SE = 1.71, $t = -0.08$, $p = 0.94$). Haplotype was not predictive of either OCDS subscales (Compulsive $\beta = -0.76$, SE = 1.00, $t = -0.76$, $p = 0.45$; Obsessive $\beta = 0.63$, SE = 0.83, $t = 0.76$, $p = 0.45$). Secondly, rs356221 was tested as a predictor of the PACS ($\beta = 0.08$, SE = 0.52, $t = 0.15$, $p = 0.88$) and OCDS ($\beta = 1.21$, SE = 0.98, $t = 1.24$, $p = 0.22$). Though this SNP was not predictive of the Compulsive subscale ($\beta = 0.31$, SE = 0.58, $t = 0.54$, $p = 0.59$), there was an observed trend with the Obsessive subscale ($\beta = 0.90$, SE = 0.48, $t = 1.90$, $p = 0.06$; see **Figure 1**) such that carriers of both risk alleles reported greater craving on the OCDS-Obsessive subscale. When rs356219 was entered into the models no effect was observed for PACS ($\beta = 0.20$, SE = 0.51, $t = 0.39$, $p = 0.69$) or OCDS ($\beta = 0.13$, SE = 0.98, $t = 0.13$, $p = 0.90$). Neither subscale was predicted by this SNP (Compulsive $\beta = -0.28$, SE = 0.57, $t = -0.50$, $p = 0.62$; Obsessive $\beta = 0.41$, SE = 0.47, $t = 0.87$, $p = 0.39$). Finally, craving was removed from the model and DSM-IV dependence was added. The haplotype did not predict symptom count ($\beta = -0.11$, SE = 0.07, $t = -1.54$, $p = 0.12$).

Discussion

The purpose of this study was to investigate the relationship between the previously implicated rs356221 and rs356219 *SNCA* haplotype and alcohol craving and dependence in a

well characterized sample of problem alcohol users from the community, all of whom were Caucasian to reduce the threat of population stratification. We did not observe a relationship between the haplotype and either the PACS or the OCDS. Further, there were no observable relationships between rs356219 and measures of craving. Consistent with previous literature (Bönsch et al., 2005), there was a trend level relationship between rs356221 and the OCDS Obsessive subscale, where dual risk allele carriers exhibited the highest craving; however, no other associations were found between this SNP and the other indicators of alcohol craving.

The minimal evidence for the association of *SNCA*, as assessed by the haplotype of rs356219 and rs356221, and craving is in contrast to previous findings. For example, Bönsch and colleagues (2004; 2005) found that SNCA mRNA expression and protein levels were elevated in men with AUD compared to controls and that elevation was predictive of increased OCDS. Their work also implicated the OCDS Obsessive subscale, for which a trend level effect was observed for one SNP. However, both of Bönsch's studies only included men with an AUD diagnosis, potentially limiting replicability. Additionally, Foroud's study (2007), utilizing the COGA study sample, observed that a haplotype containing rs356219 and rs356221 was associated with craving, as assessed via a single item clinical interview. Craving is not a ubiquitous experience among alcohol users and is thought to represent a more severe symptom that develops over time with greater use. In Foroud's study, 42% of alcohol dependent individuals also endorsed craving versus 2% of those who did not meet for dependence, thus leading authors to posit that the effect of *SNCA* is only present for a subset of alcohol users. As with other complex phenotypes, it is likely that craving is influenced by a host of genetic markers each having a small effect size (Plomin, DeFries, Knopik, & Neiderhiser, 2016).

The sample presented herein, though all endorsing alcohol related problems and a threshold of 48 alcoholic drinks/month, represent a heterogeneous group of alcohol users. For example, the entirety of the present sample did not met DSM-IV criteria for an AUD, possibly influencing results as previous work enrolled dependent and cirrhotic alcohol users. Further, this sample is community based who were not seeking treatment. Previous work by Anton and Drobes (1998) suggested that community based individuals with AUD would endorse lower levels of craving, compared to those in outpatient or inpatient treatment. More recent work has highlighted the influential differences between treatment versus non-treatment seeking alcohol users, including that craving is higher in treatment seekers (Ray, Bujarski, Yardley, Roche, & Hartwell, 2017; Rohn et al., 2017). In sum, results may have been impacted by the nature of this sample whereas previous work was conducted in severe alcohol users.

Akin to Foroud et al. (2007) findings, there was not support for an association of this haplotype of *SNCA* and alcohol dependence. Contrastingly, Janeczek and colleagues (2015) found that the protective allele haplotype was significantly less common in those with the alcohol misuse phenotype whereas the risk allele haplotype was more common in the alcohol misusers. Though logistic regression did not show increased odds of alcohol misuse when carrying the risk allele haplotype, carriers of the protective haplotype were less likely to be alcohol misusers (Janeczek et al., 2015). The divergence in findings may be partially due to the difference in the characterization of the phenotype. Janeczek (2015) defined alcohol misuse as consumption of >80g ethanol/day whereas the present study utilized DSM-IV dependence. Further, we lack a control group (i.e., no AUD group) in order to carry out comparable analyses regarding the protective phenotype.

The study should be interpreted in context of the strengths and limitations. Strengths of the study include the multi-item assessment of craving using well validated and reliable measures. Additionally, the majority of existing studies included participants based on the amount of alcohol consumed or lacked thorough characterization and assessment of the sample. Further, a hypothesis driven approach was used to identify the candidate genes of interest. Limitations include the small sample size and lack of a control group. Craving was also assessed via self-report which is subject to recall bias and is typically assessed via semi-structured interview.

In sum, thus study found limited support for an association of rs356219 and rs356221 of the *SNCA* gene and alcohol craving or alcohol dependence, though there was a small signal for a relationship with the Obsessive subscale of the OCDS. These findings are in contrast with previous work, though the samples were divergent and this study extends the investigation of *SNCA* into a community sample of alcohol users. Ultimately, small effects take many genes working together to produce a complex phenotype such as alcohol craving (Plomin et al., 2016) and further work is needed to elucidate the role of *SNCA* in alcohol use, particularly craving.

Table 1

Sample characteristics for total sample and by genotype.

	Total Sample	No Risk Allele (n=42)	Risk Allele Carrier (n=160)	*p*-value
Sex (# male)	162 (77.5%)	31 (73.8%)	125 (78.1%)	0.55
Age	29.3 (9.5)	30.9 (10.4)	29 (9.4)	0.26
Daily Smoker	41 (19.6%)	5 (11.9%)	35 (21.9%)	0.35
FTND	3.1 (2.4)	1.6 (2.7)	1.5 (2.0)	0.78
DSM-IV Dependence	148 (74.0%)	35 (83.3%)	113 (71.5%)	0.20
DSM-IV Age of AUD onset	23.1 (7.4)	22.9 (6.8)	22.6 (8.3)	0.80
DSM-IV SX count	5.3 (2.8)	5.8 (3.0)	5.2 (2.7)	0.18
CIWA	5.6 (7.2)	6.6 (7.1)	5.3 (7.2)	0.32
DRINC	45.2 (20.4)	48.3 (23.2)	44.6 (19.7)	0.30
ADS	15.7 (7.1)	16.2 (8.3)	15.6 (6.8)	0.63
DPDD	7.2 (4.3)	7.3 (3.9)	7.2 (4.4)	0.93
Drinking days	17.6 (6.9)	16.2 (7.5)	18.0 (6.8)	0.14
PACS	13.1 (6.3)	13.3 (6.1)	12.9 (6.5)	0.69
OCDS	26.6 (11.5)	26.8 (10.7)	26.4 (11.6)	0.83
OCDS Obsessive Subscale	8.9 (5.5)	8.5 (5.1)	9.0 (5.6)	0.64
OCDS Compulsive Subscale	17.7 (6.6)	18.3 (6.2)	17.5 (6.7)	0.44

*Note: FTND=Fagerström Test for Nicotine Dependence; CIWA=Clinical Institute Withdrawal Assessment; DRINC=Drinker's Inventory of Consequences; ADS=Alcohol Dependence Scale; DPDD is drinks per drinking day; PACS=Penn Alcohol Craving Scale; OCDS=Obsessive Compulsive Drinking Scale

Table 2

Allele frequencies for each SNP and the haplotype.

SNP	Genotype	N (%)
rs356219	GG	45 (23%)
	AG	92 (46.9%)
	AA	59 (30.1%)
rs356221	AA	55 (27.2%)
	AT	102 (50.5%)
	TT	45 (22.3%)
Haplotype	No risk carrier (GG carriers for rs356219 and AA for rs356221)	42 (20.8%)
	Risk carrier (carriers of at least one A allele for rs356219 or T for rs356221)	160 (79.2%)

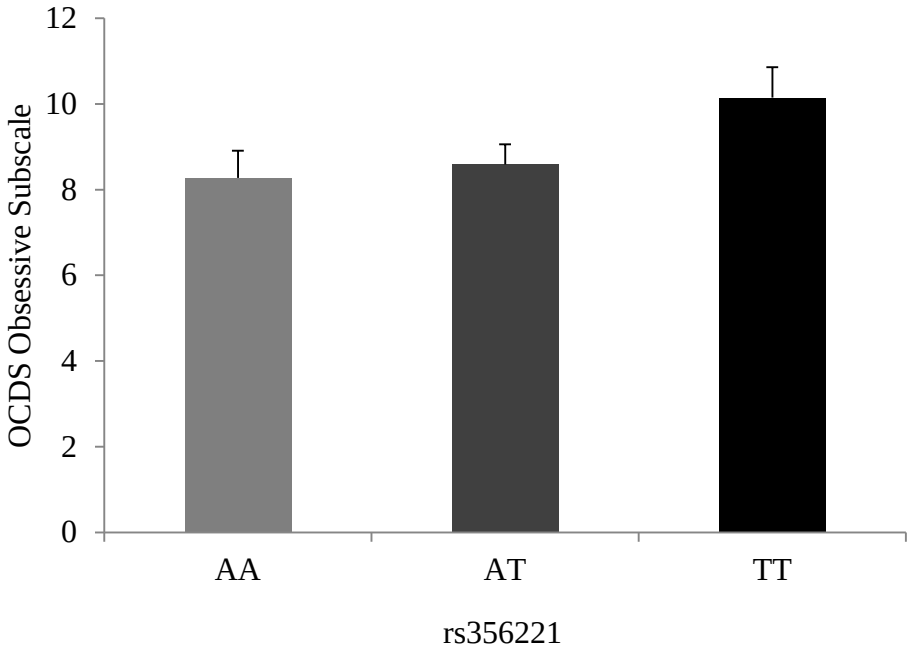
Table 3

Results for predicting each craving measure by haplotype and each SNP of interest.

			F	p
Haplotype	OCDS	Haplotype	0.01	0.94
		Drink days	10.2	0.002
		DRINC	50.9	<0.001
	OCDS-Compulsive	Haplotype	0.6	0.45
		Drink days	10.8	0.001
		DRINC	41.9	<0.001
	OCDS-Obsessive	Haplotype	0.6	0.45
		Drink days	6.8	0.01
		DRINC	48.1	<0.001
	PACS	Haplotype	0.1	0.8
		Drink days	18.2	<0.001
		DRINC	65.1	<0.001
rs356221	OCDS	rs356221	1.54	0.22
		Drink days	10.1	0.002
		DRINC	52	<0.001
	OCDS-Compulsive	rs356221	0.3	0.59
		Drink days	10.1	0.002
		DRINC	42.5	<0.001
	OCDS-Obsessive	rs356221	**3.6**	**0.06**
		Drink days	7.1	0.009
		DRINC	48.2	<0.001
	PACS	rs356221	0.02	0.88
		Drink days	18.1	<0.001
		DRINC	66.3	<0.001
rs356219	OCDS	rs356219	0.02	0.9
		Drink days	9.9	0.002
		DRINC	50.9	<0.001
	OCDS-Compulsive	rs356219	0.3	0.62
		Drink days	10.3	0.002
		DRINC	41.5	<0.001
	OCDS-Obsessive	rs356219	0.8	0.39
		Drink days	6.9	0.01
		DRINC	47.8	<0.001
	PACS	rs356219	0.2	0.7
		Drink days	17.5	<0.001
		DRINC	64.6	<0.001

Figure 1

Obsessive subscale of the OCDS by rs356221 status.



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DISSERTATION CONCLUSIONS

The overarching aim of this dissertation was to advance the understanding of the phenotype of alcohol craving. Specifically, a series of studies were conducted to examine assessment considerations, diagnostic structure, and genetic component of craving.

In Study 1, the relationship between tonic and phasic measures of alcohol craving were examined during alcohol administration in a carefully controlled laboratory setting. This study found that the Penn Alcohol Craving Scale (Flannery, Volpicelli, & Pettinati, 1999) was predictive of phasic craving in the laboratory, regardless of alcohol administration condition. The Obsessive Compulsive Drinking Scale (Anton, Moak, & Latham, 1995) was predictive of phasic craving, but only when alcohol was administered. These results indicate that measures of tonic craving may function differently and capture unique aspects of craving. The PACS may more generally capture a broader dimension of craving whereas the OCDS, and particularly the Obsessive subscale, may uniquely encapsulate the cognitive aspect of craving which in turn is heightened in the presence of alcohol. Together, the findings that tonic craving as measured by the OCDS and PACS is predictive of phasic craving, particularly when alcohol is ingested, is clinically relevant as both forms of craving have been shown to predict drinking behaviors. Thus, interventions targeting tonic craving may in turn dampen phasic response in response to alcohol ingestion and cues. Thorough assessment of the distinctive components of craving is warranted.

Study 2 utilized data culled from a large sample of non-treatment seeking, community alcohol users to examine the diagnostic structure of DSM-5. Existing literature has largely supported the changes to the latest iteration of the DSM (Agrawal et al., 2011; D. Hasin, et al., 2013; Keyes et al., 2011; Mewton et al., 2011), however, it remained unknown how these changes would impact non-treatment seeking alcohol users who have a differential clinical

profile from treatment seekers (Ray et al., 2017; Rohn et al., 2017). Alcohol users who endorsed problematic alcohol use completed a battery of self-report measures and a clinical interview. Using a novel approach (Murphy et al., 2014), the PACS was used to assess craving and determine endorsement of the new craving criterion. As anticipated, prevalence of AUD using DSM-5 criteria and structure did increase as compared to DSM-IV. However, this was due to the diagnostic structural change as opposed to the change in criteria. Further, craving was strongly correlated with other measures of alcohol use and loaded well onto extant symptoms during factor analysis, corroborating its inclusion as a diagnostic criterion. Of note, there was a low endorsement of craving, as assessed by the PACS, in this sample. This perhaps speaks to the non-treatment seeking nature of the sample, where high craving would not be expected (Anton & Drobes, 1998) as well as the heterogeneous nature of the experience of craving (Colin Drummond, 2001).

Finally, Study 3 examined two single nucleotide polymorphisms (SNP) and their haplotype in relation to alcohol craving in a sample of alcohol users from the community. Previous work has consistently implicated the alpha-synuclein (*SNCA*) gene with alcohol phenotypes (Agrawal et al., 2013; Bönsch et al., 2004; Janeczek & Lewohl, 2013; Levey et al., 2014). However, in these data, there was limited evidence of an association with either alcohol craving or dependence. Neither measure of alcohol craving was predicted by risk allele haplotype status. Further, there was no support for either SNP's risk allele status to predict craving save for one trend level effect with the OCDS Obsessive subscale which is consistent with previous literature (Bonsch et al., 2005). Results may in part be due to the heterogeneous nature of the sample that represents a wide array of alcohol users who do not necessarily endorse high craving. Moreover, it is likely that future work will show that the experience of craving is

explained by a host of factors that work together to produce such an experience (Plomin et al., 2016). Further work in large samples is warranted to fully explore the role of *SNCA* with alcohol use phenotypes.

This dissertation study should be interpreted in light of the strengths and limitations. Strengths of Study 1 include the experimental manipulation where participants completed both alcohol and saline administration sessions in a randomized, counterbalanced manner. However, the small sample size, potential for dampening of craving response, and reliance on self-report measures should be noted as limitations. Strengths of Study 2 include the large, diverse sample of alcohol users from the community who are reflective of the individuals frequently enrolled in clinical research. The use of the multi-item, reliable PACS to assess craving is another asset. On the other hand, a large sample for the factor analysis would be advantageous as well as assessment of craving in an interview format as it is typically assessed for the purposes of a clinical diagnosis. Lastly, Study 3 benefited from the use of a well characterized sample of alcohol users who completed multi-item assessments and a clinical interview. Candidate genes examined were identified using a hypothesis driven approach. However, the study was limited by sample size and lack of a control group.

In conclusion, this dissertation met the aim of further exploration and characterization of the craving phenotype. Consistent with previous research, these studies support that craving is a heterogeneous experience that is highly volatile both between and within individuals. Study 1 showed that tonic and phasic craving deserve careful assessment and may be heightened during alcohol ingestion, thus proving a worthy target of intervention. Study 2 highlighted the need to carefully assess and characterize alcohol using samples in research and the impact the DSM changes will have on diagnostic assessment in non-treatment seekers. Study 3 demonstrated the

complexity of genetic relationships with alcohol use phenotypes such as craving and provided some indication in support of a risk allele at the predicting Obsessive craving.

Further work to elucidate the development and maintenance of craving is warranted as craving represents a critical phenotype that is used in various domains. For example, craving is used in early pharmacological development, as a diagnostic criterion, and as a translational phenotype in neuroimaging. Though there continues to be a lack of a unified theory of craving (van Lier et al., 2017), it is generally accepted that there are multiple components to the craving experience and that it is cause for significant distress in substance use treatment seeking patients (Skinner & Aubin, 2010). Future studies should seek to integrate the assessment of craving and consider the best methodologies to do so. Longitudinal studies would enable researchers to determine the progression of the experience of craving in the progression of AUD, which is thought to worsen with severity and length of alcohol use. Better understanding of the contribution of craving to AUD development and maintenance will aid researchers and clinicians to lessen the burden of disease by providing a translational phenotype which in turn can serve as a treatment target and ultimately promote personalized medicine through the development of effective anti-craving agents.

References for Introduction and Conclusion

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