Title

The convergence of neurotranslational and laboratory paradigms in predicting alcohol consumption and pharmacotherapy response

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Publication Date

2021

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

The convergence of neurotranslational and laboratory paradigms in predicting alcohol consumption and pharmacotherapy response

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Psychology

by

Aaron Changjo Lim

2021

ABSTRACT OF THE DISSERTATION

The convergence of neurotranslational and laboratory paradigms in predicting alcohol consumption and pharmacotherapy response

by

Aaron Changjo Lim

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2021

Professor Lara A. Ray, Chair

Smoking and alcohol use problems contribute to over 250 million disability-adjusted life-years worldwide, with an estimated 1 in 5 adults engaging in recent heavy alcohol use and 1 in 7 reporting daily tobacco use. Effective pharmacotherapies for smoking and drinking are needed to test these effects among treatment-resistant populations who report significant cessation difficulties, particularly among those who co-use tobacco and alcohol. Converging evidence indicates that neuroimaging methods can be used to elucidate mechanisms of action and potentially, treatment outcomes, for addiction pharmacotherapies; these include varenicline and naltrexone, which are effective smoking cessation and drinking reduction aids, respectively. The proposed dissertation study therefore aims to: 1) examine pharmacotherapeutic effects of naltrexone and varenicline on neuroimaging paradigms of translational value (i.e. substance cue-induced neural activation), in an understudied population of East Asian heavy drinkers; 2)

elucidate the relationship between response to alcohol cues in neuroimaging cue paradigms and responses in gold standard human laboratory paradigms (i.e. oral self-administration of alcohol); 3) explore whether smoking cue-induced neural responses predict smoking cessation outcomes in a comparison pharmacotherapy clinical trial. Such work is critical to understand the role of neuroimaging in medications and substance use research more broadly, and can support the prioritization of neuroimaging paradigms as indicated for treatment development pipelines.

The dissertation of Aaron Changjo Lim is approved.

Adriana Galván

Christine E. Grella

Steve S. Lee

Lara A. Ray, Committee Chair

University of California, Los Angeles

2021

This dissertation is dedicated to my wife Sarah for her love and encouragement throughout college and graduate school. It is also dedicated to my sisters Sonia and Sharon, and to my parents, whose sacrifices always remind me of how much more they have deserved. Finally, to my friends Lyric and ReJoyce, who supported and grounded me through all the time I've known them.

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Curriculum Vitae

Aaron Changjo Lim

Education

2015-2016	Master of Arts, Clinical Psychology University of California Los Angeles Thesis Title: *The effects of alcohol dependence severity on neural correlates of delay discounting* Advisor: Lara Ray, Ph.D.
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2017-2019	UCLA Letter of Clinical Excellence
2016-2019	Student Merit Award, Research Society on Alcoholism
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2016	UCLA Graduate Summer Research Mentorship - $6,000
2016	NSF Graduate Research Fellowship Honorable Mention
2015-2016	UCLA Distinguished University Fellowship - $21,000

Peer Reviewed Publications (selected from 26)

1. **Lim, A.C.**, Grodin, E.N., Green, R., Venegas A., Meredith, L.R., Donato, S., Burnette, E., & Ray, L.A. (in press). Alcohol cue-induced ventral striatum activity predicts subsequent alcohol self-administration. *Alcoholism: Clinical and Experimental Research*.
2. **Lim, A.C.**, Grodin, E.N., Green, R., Venegas, A, Meredith, L., Courtney, K.E., Moallem, N.R., Sayegh, P., London, E., & Ray, L.A. (in press). Executive function moderates effects of naltrexone on methamphetamine-induced craving. *American Journal of Drug and Alcohol Abuse*.
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7. **Lim, A.C.**, Roche, D.J.O., & Ray, L.A. (2018). Distress tolerance and craving for cigarettes among heavy drinking smokers. *Journal of Studies on Alcohol and Drugs, 79,* 918-928.
8. **Lim, A.C.**, Moallem, N.R., Courtney, K.E., Allen, V.C., Leventhal, A.M., & Ray, L.A. (2018). A brief smoking cessation intervention for heavy drinking smokers: Treatment development, feasibility, and preliminary results. *Frontiers in Psychiatry, 9,* 362.
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10. **Lim, A.C.**, Cservenka, A., & Ray, L. (2017). Effects of alcohol dependence severity and *OPRM1* on neural correlates of delay discounting. *Alcohol and Alcoholism, 52,* 506-515.
11. Borges, A., Dahne, J., **Lim, A.C.**, & MacPherson, L. (2017). Negative affect mediates the relation between trait urgency and behavioral distress tolerance. *Emotion, 17,* 707-716.
12. Collado, L., **Lim, A.C.**, & MacPherson, L. (2016). A systematic review of depression psychotherapies among Latinos. *Clinical Psychology Review, 45,* 193-209.

Grant Funding

04/2019-04/2020	Predoctoral Fellowship Award - $45,058 Agency: Tobacco Related Disease Research Program Grant No.: T29DT0371 – Dynamic functional connectivity among heavy drinking smokers (PI: Aaron Lim)
10/2018-10/2019	Translational Neuroscience of Drug Abuse Program - $34,942 Agency: National Institute on Drug Abuse Grant No.: T32 DA02463-10 (PI: Edythe London)
10/2016-10/2018	Integrated Programs of Substance Abuse - $68,342 Agency: National Institute on Drug Abuse Grant No.: 4T32 DA007272-25 (PI: Christine Grella)

Ad Hoc Reviewer for:

Addictive Behaviors
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GENERAL INTRODUCTION

Tobacco smoking and alcohol consumption continue to produce significant health, psychological, and economic damage to US adults. While tobacco use has declined within the past several decades, 14% of US adults (34.3 million) were current cigarette smokers in 2017, and approximately 480,000 adults in the US die from cigarette smoking and secondhand smoke exposure annually (Wang et al., 2018). Over 140 million US individuals aged 12 or older reported any alcohol use within the last month in 2017, and approximately 1 in 4 people aged 12 or older reported at least one alcohol binge within the last month, defined as an occasion of 4 or more drinks in one occasion for women, and 5 or more drinks for men (*2017 National survey on drug use and health: Detailed Tables.*, 2018).

Treatments

In light of the recurringly large number of individuals consuming cigarettes and alcohol, there are large-scale efforts to develop effective and efficacious treatments for alcohol and smoking cessation. Multiple behavioral smoking and alcohol cessation therapies have demonstrated success, and meta-analyses have found that therapies such as Cognitive Behavioral Therapy for alcohol use disorder and smoking cessation significantly increase treatment success through one year follow up (Lancaster & Stead, 2017; Magill & Ray, 2009).

Studies have also sought to identify effective pharmacotherapies for smoking and alcohol cessation. One of the most widely studied alcohol medications is naltrexone (NTX), a mu-opioid receptor antagonist. The endogenous opioid system is deeply involved in addiction to multiple substances; alcohol, in turn, induces Beta-endorphin and associated dopamine release in the nucleus accumbens, as well as inhibits GABAergic interneurons in the ventral tegmental area

(Johnson, 2008); naltrexone has been shown to block both of these alcohol-induced mechanisms in animals, implicating a modulation of reward and substance cue-related processing (Johnson, 2008; Zalewska-Kaszubska, Gorska, Dyr, & Czarnecka, 2006). In humans, early clinical trials demonstrated that naltrexone in combination with counseling decreased subsequent alcohol drinking days, as well as prevented the risk of additional alcohol binges (O'Malley et al., 1992; Volpicelli, Alterman, Hayashida, & O'Brien, 1992). Subsequent experimental studies indicated that naltrexone blunts the subjectively rewarding effects of alcohol consumption (e.g. feelings of stimulation and a "high") (O'Malley, Jaffe, Rode, & Rounsaville, 1996; Volpicelli, Watson, King, Sherman, & O'Brien, 1995). Additional studies have replicated these analyses and/or found that naltrexone increased subjective sedative feelings of alcohol and/or reduces alcohol cravings (Davidson & Amit, 1997; Swift, Whelihan, Kuznetsov, Buongiorno, & Hsuing, 1994), particularly among individuals with family history and/or genetic risk for alcohol use disorder (A. C. King, Volpicelli, Frazer, & O'Brien, 1997; Ray & Hutchison, 2007). Meta-analyses of naltrexone clinical trials have consistently yielded small to moderate effects relative to placebo on alcohol abstinence, binge drinking, and craving (Maisel, Blodgett, Wilbourne, Humphreys, & Finney, 2013; Rösner et al., 2010; Srisurapanont & Jarusuraisin, 2005).

For smoking cessation, one of the most effective pharmacotherapies is varenicline (VAR), a partial agonist of alpha4-beta2 nicotinic acetylcholine receptor. Nicotine binding to nicotinic acetylcholine receptors' (nAChR) a4b2 sites occurs as a result of cigarette smoking, activating mesolimbic and mesocortical circuits to produce dopamine and condition tobacco consumption (Subramaniyan & Dani, 2015; Tapper et al., 2004). Varenicline's binding to the receptor causes approximately half the release of dopamine as nicotine and thereby reduces nicotine-seeking behavior. It also serves to block the receptor from additional binding of nicotine

(Rollema et al., 2007). Initial phase II clinical trials found that varenicline reduced baseline levels of cigarette craving and withdrawal, in addition to cue-induced cigarette craving and stimulatory and pleasurable effects of cigarettes through 12 weeks of treatment (Nides et al., 2006; Oncken et al., 2006). Subsequent phase III trials corroborated varenicline's long-term benefits for smoking. After a 12 week open-label titration to varenicline, individuals were titrated to an additional 12 weeks of varenicline or placebo; 44% and 37% were abstinent (as determined by bioverified 7-day point prevalence) for those titrated to varenicline and placebo, respectively. Meta-analyses have indicated that varenicline is superior to other nicotine replacement therapy products (NRT) for smoking cessation, yielding moderate to large effect sizes. Varenicline, however, was not found to be more effective than combination NRT or pharmacotherapies (Cahill, Stevens, Perera, & Lancaster, 2013; Eisenberg et al., 2008).

Overall, studies on pharmacotherapy indicate efficacy of medications such as naltrexone and varenicline in addressing reduction and cessation of alcohol and smoking behaviors. There are, however, continued gaps in the medication literature given the modest effect sizes and relatively elevated rates of relapse after quit attempts across a range of pharmacotherapies.

Heavy-Drinking Smokers

One critical understudied area in the pharmacotherapy literature is the high levels of alcohol and cigarette co-use among US adults. Approximately 20-25% of regular smokers report heavy drinking (Dawson, 2000; Jiang, Lee, & Ling, 2014; Toll et al., 2012), and abstinent smokers are five times as likely to experience a smoking lapse during drinking episodes (Kahler, Spillane, & Metrik, 2010). While the literature has suggested that greater alcohol use is associated with a greater likelihood of a failed smoking cessation attempt (Augustson et al.,

2008; Dollar, Homish, Kozlowski, & Leonard, 2009), the frequency of heavy drinking (defined as >3 drinks on any day or ≥7 drinks per week for women and >4 drinks on any day or ≥14 drinks per week for men, according to the U.S. National Institute of Alcohol Abuse and Alcoholism) in particular appears to be more prognosticative of poor cessation outcomes than frequency of drinking more generally across the overall continuum of drinking levels (Dawson, 2000; Kahler et al., 2010). Laboratory studies have shown that even smokers who drink at moderate levels are less able to resist smoking a cigarette after consuming alcohol, relative to a placebo beverage (McKee et al., 2006). Therefore, efforts to address smoking cessation among heavy drinking smokers may be more successful by addressing both alcohol and smoking within the same intervention.

In this vein, **there is initial evidence from our laboratory that a combination regimen of varenicline and naltrexone (VAR+NTX) may be effective in smoking cessation efforts among heavy-drinking smokers.** Relative to VAR alone and NTX alone, VAR+NTX more strongly reduced basal cigarette craving and subjective reports of cigarette and alcohol "high" during the medication titration period (Ray et al., 2014). Participants also underwent a functional magnetic resonance imaging (fMRI) smoke cue paradigm, and VAR+NTX titration was associated with reduced activation of the anterior cingulate cortex when viewing cigarette versus neutral cues; such results raised the possibility that VAR+NTX reduce neural activation associated with appetitive smoking behavior (Ray et al., 2015). In the same study, VAR+NTX relative to VAR and NTX monotherapies decreased smoking topography indicators (i.e. puff volume, inter-puff interval) (Roche, Bujarski, Hartwell, Green, & Ray, 2015). Additionally, naltrexone has been used as an adjunct to nicotine replacement therapy with some, although not

uniform, benefit (Epstein & King, 2004; Krishnan-Sarin, Meandzija, & O'Malley, 2003; O'Malley et al., 2006; Toll et al., 2008; Walsh, Epstein, Munisamy, & King, 2008).

VAR+NTX may thus be a potentially effective therapy among heavy drinking smokers, a subgroup especially vulnerable to relapse during their cessation attempts. A current limitation of the existing data on VAR+NTX is that the majority of the studies have focused on experimental paradigms to examine the efficacy of this combination regimen and thus few clinical trials have been conducted to date. This gap will be addressed by a current and ongoing R01 by Dr. Ray's laboratory testing the clinical efficacy of VAR + NTX, compared to VAR alone, for smoking cessation among heavy drinking smokers. Proposed study 3 from this dissertation will leverage resources and data from this NIDA-funded R01.

Experimental Paradigms in Medication Development

One commonality among studies examining the efficacy of addiction pharmacotherapies is the use of laboratory experimental paradigms. Within clinical psychology, such gold-standard paradigms have been developed to study experiential effects of drug use, characterize phenomenology in the development and reinforcement of an addiction syndrome (e.g. withdrawal, craving), and capture behavior in standardized settings that could predict future substance use behavior (Plebani et al., 2012). The use of these laboratory paradigms has therefore been critical in ethically testing interventions intended to alter substance use behaviors. Within pharmacotherapy development pipelines, understanding medication effects on laboratory paradigms is necessary for any drug to receive approval from the US Food and Drug Administration for treating a specific drug addiction (Ray et al., 2010; Yardley & Ray,

2017; Van Norman, 2016). Three classes of these experimental paradigms and existing gaps are summarized below.

Subjective response paradigms assess for an individuals' subjective experiences when systematically titrated to the drug at different concentrations, including feelings of stimulation and sedation, as well as reported craving for the drug (Vocci, Acri, & Elkashef, 2005). Numerous addiction studies, particularly within the alcohol literature, have indicated that these dimensions of subjective response are risk factors for the development of a substance use disorder (King et al., 2002; Ray, Mackillop, & Monti, 2010; Wardle, Marcus, & de Wit, 2015; Zhang et al., 2007). Specifically, sensitivity to acute drug effects, such as the stimulatory effects of cigarette smoking and alcohol consumption as breath alcohol concentration (BrAC) increases, are theorized to be one important mechanism through which individuals are incentivized to maintain substance use and thereafter transition into more severe addiction (Koob & Le Moal, 2001; Koob & Schulkin, 2018; Robinson & Berridge, 2008). In the context of abstinence, it is hypothesized that reduced stimulation from a single drinking episode decreases the likelihood of a full relapse (Ray, Bujarski, & Roche, 2016). Therefore, efforts to develop effective addiction medications have targeted brain receptors involved in blunting the rewarding effects of substances, such as the mu-opioid and alpha4beta2 nicotinic receptor targets of naltrexone and varenicline, respectively.

Another important experimental paradigm involves acute drug craving inductions via cue-exposure in the laboratory. Such craving inductions are distinct from tonic levels of craving that tend to be stable and may be more distal in motivating drug use; rather, acute cravings are elicited by environmental or internal cues (e.g. stress, drug paraphernalia, substance-related scents), and are consistently and proximally predictive of relapse during cessation attempts (Monti & Ray, 2012; Sayette & Tiffany, 2013; Serre, Fatseas, Swendsen, &

Auriacombe, 2015; Sinha et al., 2011). The types of substance-related cues that elicit craving can vary across individuals and may even include, for example, friends associated with drug use (Conklin, Salkeld, Perkins, & Robin, 2013). In experimental and ecological momentary assessment studies, images, videos, and imaginal instructions related to drug use/paraphernalia are used to elicit self-reported cravings (Serre et al., 2015). Such cue-induced cravings have been found to shift attentional processing resources to attune to drug-related relative to unrelated cues, and insodoing increase likelihood of relapse during a quit attempt (Sayette, 2016). Both pharmacological and behavioral addiction treatments thus seek to reduce cue-induced cravings due to their causal and temporal effects on relapse. Notably, some authors have questioned whether cue-induced cravings can be altered (Perkins et al., 2009), and others have found that such cravings can be incubated and only modified after long periods of time (Pickens et al., 2011).

Finally, one paradigm in the alcohol literature may be critical to modeling actual alcohol consumption behaviors in the real world: alcohol/drug administration. In contrast to standardized administrations of titrated drug use (i.e. subjective response paradigms) or standardized drug cues (i.e. cue-induced craving paradigms), alcohol/drug administration paradigms allow individuals to consume alcohol with parameters that closely mimic real-world conditions. One paradigm used in Dr. Ray's laboratory, for instance, allows individuals to consume alcohol and alcohol mixes of their choice up to a ceiling breath alcohol concentration while watching a movie in the lab (O'Malley et al., 2007; Ray et al., 2018). Other sophisticated paradigms recreate a bar-like environment in the laboratory; such "bar labs" replicate environments in which drinking behaviors naturally occur, and are hypothesized to therefore increase external validity of laboratory paradigms (Fridberg et al., 2017); indeed, there is

evidence that alcohol consumption in free-choice paradigms more closely approximate real world drinking relative to standardized paradigms (Moss et al., 2015). Medication studies have increasingly included such paradigms to examine potential pharmacotherapy effects (Hendershot et al., 2017; Ray et al., 2018).

Though these experimental paradigms have been critical to understanding how pharmacotherapy may alter individuals' experiences of and associations with a given substance, one current gap in the literature is the transition of studying mechanisms of action from animals to humans. That is, while mechanisms of action for pharmacotherapies, such as naltrexone and varenicline, are established early in drug development in molecular and preclinical trials in animals (Van Norman, 2016), it is more difficult to corroborate such mechanisms of action in human and clinical trials; experimental lab studies assessing pharmacotherapy effects on subjective response or cue-induced craving are most frequently conducted separately from phase II and III clinical trials that examine real substance use cessation attempts. The few clinical trials that have examined cue-induced craving have not consistently identified pharmacotherapy effects on cessation (e.g. Wray et al., 2013). There is therefore a growing need to corroborate links between laboratory paradigms and real-world consumption to improve medication development pipelines in order to establish whether such reductions in cue-induced cravings and subjective responses directly correspond to clinical outcomes (Sayette, 2016). This research pipeline that comprises the historical majority of addictions pharmacology work can be simplified in the figure below.

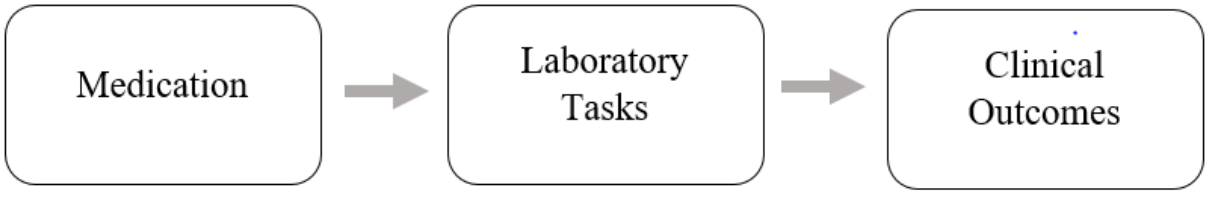

Figure 1. Existing addictions medications development pipeline

The Role of Neuroimaging

Neuroimaging paradigms may be useful to capture brain-based pharmacotherapy-induced changes that could, in theory, lead to behavioral change (Litten et al., 2016). Indeed, modern theories of addiction have built upon neurobiological changes underlying progression of substance use and related dysfunction (Koob & Schulkin, 2018). Functional magnetic resonance imaging (fMRI) has been the most widely utilized modality to study addiction due to its relatively low cost and high availability (Fowler, Volkow, Kassed, & Chang, 2007). fMRI machines detect changes in a given local magnetic field that are caused by shifts in the ratio of oxygenated to deoxygenated hemoglobin in blood vessels throughout the brain. Oxygenated blood is needed for increased cellular activity. These differences in oxygen content are illustrated by variations in color during an fMRI scan; these signals are termed blood oxygen level dependent (Herron et al. 2010) contrast (Parvaz, Alia-Klein, Woicik, Volkow, & Goldstein, 2011). Early fMRI studies found that exposure to drug cues, including cocaine, alcohol, and cigarettes, produced BOLD activation in regions subsequently theorized to reward processing pathways, including the ventral striatum/nucleus accumbens, anterior cingulate, and orbitofrontal cortex (Breiter et al., 1997; Knutson & Cooper, 2005; Kufahl et al., 2005). Severity of chronic substance use and subjective reports of craving (i.e. strong urges to use or acquire the substance) during these cue exposure tasks also correlate with these localized activations (Dager et al., 2014; Lim, Cservenka, & Ray, 2017; Parvaz et al., 2011). A recent meta-analysis identified moderate effect sizes for these craving-related neural activation across substances and behavioral

addictions, though there are several substance-specific activation patterns based on neural molecular targets of substances (Starcke et al., 2018).

Given the strong basis for neural correlates of craving and as neuroimaging technology has advanced, there have been concerted efforts to identify pharmacotherapy effects on brain activation relevant for cessation. The most commonly utilized experimental neuroimaging paradigm is neural activation in response to cue-induced craving; these tasks naturally extend preclinical and experimental paradigms used to test efficacy of pharmacotherapies (Schacht, Anton, & Myrick, 2013). Additionally, fMRI paradigms are less impacted by the limitations of self-report inherent in subjective response and laboratory cue-exposure tasks (Schact et al., 2017). Neuroimaging modalities such as fMRI and Positron Emission Tomography (PET) more directly capture purported neural mechanisms of action for addiction pharmacotherapies, and may therefore be suited to testing whether such potential effects translate into clinical outcomes.

A more thorough review of the naltrexone neuroimaging literature is presented in Study 1; in summary, there is a mixed literature suggesting that naltrexone attenuates alcohol cue-induced neural activation in mesolimbic regions in both treatment and non-treatment seeking individuals, with some variation based on the type of substance cue in the task (e.g. smell, taste, picture) (Schacht et al., 2013). A noted weakness among naltrexone studies is the frequent use of prospectively genotyped, primarily Caucasian samples that may not generalize to other racial groups; this sampling procedure has been utilized to examine pharmacogenetic effects of naltrexone and genes that encode mu-opioid receptor binding potential (Lim et al., 2019; Ray et al., 2018).

Varenicline, in turn, has been shown to reduce activation in the ventral striatum and medial orbitofrontal cortex when viewing smoking-related pictures in non-treatment seeking

samples (Franklin et al., 2011; Schacht et al., 2014). Within our group, varenicline and a combination of varenicline and naltrexone separately attenuated activation in left ventral striatum in response to visual smoking cues (Ray et al., 2015). One study identified that varenicline reduced alcohol cue-elicited orbitofrontal cortex activation but not ventral striatum in a sample of non-treatment seeking alcohol-dependent individuals, while two studies have not corroborated an effect of varenicline on cue-induced neural response (Hartwell et al., 2013; Versace et al., 2017). This mixed set of results corroborates the need for additional studies, particularly as nearly all of these studies were conducted with non-treatment seeking populations.

Beyond these medications, several recent reviews have underscored the potential importance of cue-induced neural activation as a potential predictor of pharmacotherapy response (Courtney et al., 2016; Schacht et al., 2017). In light of the potential utility of neuroimaging paradigms as important to elucidating mechanisms of action in humans, additional research is needed to understand the associations among these neuroimaging paradigms, existing experimental laboratory paradigms that have been used to inform pharmacotherapy research, and operant consumption behavior.

A Translational Framework

The integration of pharmacology, experimental psychopathology, and neuroimaging provide powerful tools to advance our understanding of addiction pathophysiology and to provide targeted treatments. Study of the interrelationships among these constructs are increasingly warranted to clarify 1) the relationship among laboratory and neuroimaging paradigms utilized in medications development, particularly those paradigms putatively proximal to real consumption behaviors; 2) the translational validity of neuroimaging response in

predicting treatment outcomes; and 3) the external validity of these approaches in diverse populations. These interrelated efforts expand upon existing medications development research frameworks to clarify the role of neuroimaging in addictions pharmacotherapy research, simplified below. This approach is consistent with the science of behavior change (SOBC) recommendations for translational science by testing potential methods to increase efficiency of pharmacologically relevant laboratory paradigms in medications development (Litten et al., 2012; 2016).

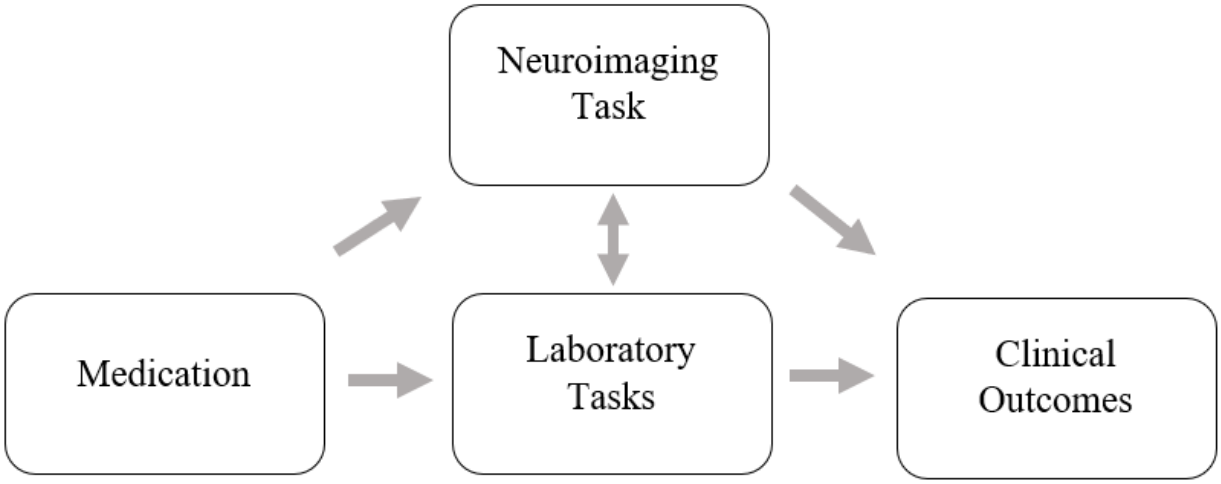

Figure 2. Expanded medications development framework

Overview of Studies

Each dissertation study will examine aspects of the above framework. Study 1 expands on two aforementioned dearths in this subfield of neuroimaging as it relates to pharmacotherapy. First, the majority of naltrexone studies have been conducted in majority or exclusively Caucasian samples that are prospectively genotyped to reduce potential genetic mitigating factors that could impact naltrexone response (Hulse, 2013). This potentially reduces external validity of such studies not only to other racial groups, but also because recruited samples gene

allele frequencies do not match those of target populations of heavy alcohol drinkers when samples are intentionally recruited for genotype rather than as an incidental factor (Schacht et al., 2017). Therefore, study 1 examines medication and pharmacogenetics effects of naltrexone on neural cue-induced craving in a sample of heavy drinkers of East Asian descent (*N*=41). Beyond expanding the diversity of participant pools for testing naltrexone, gene allele frequencies for the most commonly tested genotype, OPRM1, are more evenly distributed amongst East Asians and therefore preclude the need to prospectively genotype the sample.

Study 2 examines the relationships among alcohol cue-induced neural activation, subjective response paradigms, and real alcohol consumption. While several novel studies have examined the relationships between fMRI paradigms and subjective response (Courtney et al., 2014; Grodin et al., 2018), and between fMRI paradigms and alcohol cessation outcomes (Schact et al., 2017), no studies have examined the relationship between neural response and laboratory proxies for real-world drinking (i.e. oral self-administration). In large part, such associations are unknown because of ethical concerns in administering substances to treatment-seeking populations (National Institute on Alcohol Abuse and Alcoholism, 2005). To address these gaps in the literature in light of these ethical concerns, study 2 examines these relationships within a non-treatment seeking sample. Specifically, this study will utilize the identical sample as study 1, as the 41 non-treatment seeking heavy drinkers of East Asian descent completed all three lab paradigms: 1) fMRI alcohol taste cue paradigm; 2) subjective response to an intravenous alcohol infusion; and 3) oral alcohol self-administration paradigm. Negative binomial and cox regression models will be utilized to examine the relationships of responses to these different paradigms. Based on previous studies (Courtney et al., 2014), we hypothesize that ventral striatum activation

specifically will be associated with oral self-administration outcomes, including total number of drinks consumed and latency to first drink.

Study 3 expands the previous studies' focus on laboratory and neuroimaging paradigms to examine their applicability to real world cessation outcomes. Study 3 examines this applicability in the context of the need for developing and understanding mechanisms of action for effective medications in treating nicotine dependence. That is, while there is promising initial evidence that VAR+NTX is a powerful treatment for heavy-drinking smokers, no studies to date have expanded beyond experimental lab studies. Study 3 utilizes data from a recently completed Phase II randomized, double-blind clinical trial in Dr. Ray's lab comparing VAR alone (1 mg twice daily) to the combination of VAR (1 mg twice daily) + NTX (50 mg once daily) for smoking cessation in a sample of heavy-drinking daily smokers. As part of Dr. Ray's R01, treatment-seeking heavy drinking smokers were randomized to: (1) VAR only vs. (2) VAR + NTX. Smoking abstinence is being measured at 2, 8, 12, 16, and 26 weeks post quit date. Participants also completed a neuroimaging session from days 9-13 of medication titration; during this session, participants complete a smoking cue-induced craving task. Based on posited mechanisms of action of naltrexone and varenicline on stimulatory effects of alcohol and smoking, Study 3 examines the predictive relationship between neural activation in regions involved in reward processing and smoking abstinence outcomes at 26-week post-quit follow-ups. Given the exploratory nature of this study, we hypothesize that smoking cue-induced ventral striatum, anterior cingulate cortex, anterior insula, and/or orbitofrontal cortex, regardless of medication condition, will predict lower rates of 7-day point prevalence abstinence at follow-up.

In sum, these three studies will expand the scope of neuroimaging cue paradigms for the purpose of: 1) increasing diversity of existing pharmacotherapy trials 2) exploring potential

relationships among existing gold standard medications development paradigms, emerging neuroimaging tasks, and alcohol consumption behavior closely associated with real drinking outcomes; and 3) testing the translational link between neural smoking cue-induced craving and smoking cessation outcomes among heavy-drinking smokers. Such work is crucial to not only improve upon literatures for existing and upcoming pharmacotherapies, but also in increasing the efficiency of the medications development pipeline for novel addiction pharmacotherapies.

Study 1.

NEUROIMAGING FINDINGS FROM AN EXPERIMENTAL PHARMACOLOGY TRIAL OF NALTREXONE IN HEAVY DRINKERS OF EAST ASIAN DESCENT

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Manuscript published in Drug and Alcohol Dependence. Full citation is:

Lim, A.C., Ghahremani, D.G., Grodin, E.N., Greem, R., Bujarski, S., Hartwell, E.E., Courtney, K.E., Hutchison, K.E., Miotto, K., & Ray, L.A. (2019). Neuroimaging findings from an experimental pharmacology trial of naltrexone in heavy drinkers of East Asian descent. Drug and Alcohol Dependence, 200, 181-190.

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Abstract

Background: Despite known genetic variation across races, studies examining pharmacogenetics of a single nucleotide polymorphism (SNP) of the mu-opioid receptor gene (OPRM1) on clinical response to naltrexone have been conducted in predominantly Caucasian samples. Evidence is mixed for pharmacogenetic OPRM1 and naltrexone effects on neural responses to alcohol cues. The current study tests the pharmacogenetic effects of naltrexone and OPRM1 on neural responses to alcohol taste cues in heavy drinkers of East Asian descent.

Methods: Participants (N = 41) completed two double-blinded and counterbalanced functional magnetic resonance imaging (fMRI) sessions: one after taking naltrexone (50 mg/day) for four days and one after taking placebo for four days. Following titration, participants completed an fMRI alcohol taste-cues task. Analyses tested effects of naltrexone, OPRM1, and their interaction in whole-brain and region of interest (ROI) analyses of functional activation and functional connectivity in response to alcohol versus water taste cues.

Results: We found no effects of naltrexone orOPRM1 on neural activation in whole-brain and ROI analyses, which included left and right ventral striatum (VS), anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC). Naltrexone increased functional connectivity between left VS and clusters in medial prefrontal cortex, posterior cingulate gyrus, as well as right VS and occipital cortex, compared to placebo.

Conclusions: Naltrexone treatment enhanced functional connectivity in a key reinforcement-related pathway during alcohol versus water taste cues, corroborating neuroimaging work with other substances. Null medication and pharmacogenetics effects on functional activation add to a mixed naltrexone literature and may underscore the modest size of these effects in East Asians.

1. INTRODUCTION

Endogenous opioid transmission mediates acute hedonic and subjective rewarding effects of alcohol consumption. Naltrexone, which functions predominately as an opioid receptor antagonist, attenuates endogenous opioid activity to reduce these motivationally salient effects of alcohol (Donoghue et al., 2015). Naltrexone reduces alcohol administration within the laboratory (O'Malley et al., 2002), neural responses to alcohol consumption and craving (Myrick et al., 2008; Schacht et al., 2017b) and drinking behavior in real-world settings (Anton et al., 2006). Meta-analyses of naltrexone, however, have identified relatively modest effect sizes for relapse rates in treating alcohol use disorder (AUD), with variability in its effectiveness across individuals (Donoghue et al., 2015; Jonas et al., 2014). For this reason, efforts to identify potential moderators of naltrexone treatment response are underway to individualize and improve naltrexone pharmacotherapy.

Genetic contributions to variability in endogenous opioid transmission may be one moderator of naltrexone pharmacotherapy response (Krishnan-Sarin et al.,, 2007; Ray et al., 2012; Rubio et al., 2005). Given naltrexone's high affinity for the mu-opioid receptor, studies have focused on a single nucleotide polymorphism (SNP) that encodes the binding affinity of this receptor (*OPRM1*; rs1799971). Individuals with at least one Asp40 allele (Asp40 carriers) exhibit up to three times greater binding affinity for beta endorphins compared to Asn40 homozygotes, and are posited to be more responsive to and experience better clinical outcomes when treated with naltrexone. However, evidence for this pharmacogenetic effect is mixed; meta-analyses of retrospective pharmacogenetic trials have found that the Asp40 allele may be associated with reduction in heavy drinking related to naltrexone pharmacotherapy (Chamorro et al., 2012; Jonas et al., 2014), though multiple laboratory studies (Anton et al., 2012; Ehlers, Lind,

& Wilhelmsen, 2008; McGeary et al., 2006; Ziauddeen et al., 2016) and prospective pharmacogenetic trials have failed to replicate these effects (Oslin et al., 2015; Schacht et al., 2017b).

The inconsistency of *OPRM1* and naltrexone pharmacogenetic findings may be attributable to multiple causes, including heterogeneity in phenomenology of AUD, and the likely overall small effect size of *OPRM1* on naltrexone treatment response (Donoghue et al., 2015). Relatedly, most studies examining pharmacogenetic effects have been limited to Caucasian samples due to concerns about population stratification effects. The *OPRM1* Asp40 allele frequency varies across ethnicities, such that the minor allele frequency is approximately 20% in Caucasians, 5% in individuals of African ancestry, and up to 50% among individuals of East Asian descent (i.e., Chinese, Korean, or Japanese; Arias, Feinn, & Kranzler, 2006). In light of mixed findings regarding the Asn40Asp SNP in predominantly Caucasian samples with AUD, further study is needed to examine the role of *OPRM1* variation in naltrexone-related outcomes within ethnically diverse populations.

Despite the high prevalence of the Asp40 allele in East Asian populations, only three studies have examined naltrexone pharmacogenetics in East Asian individuals. A small clinical trial in 32 Korean alcohol dependent patients found that Asp40 carriers who were medication-compliant had a longer time to relapse than Asn40 homozygotes (Kim, 2009). In a randomized, crossover laboratory pilot study from our group, 35 heavy drinkers of East Asian descent completed an intravenous alcohol (up to 0.06 g/dl) administration session after taking naltrexone or placebo for four days. Asp40 carriers, relative to Asn40 homozygotes, reported greater alcohol-induced sedation and subjective intoxication, and lower alcohol craving on naltrexone compared with placebo (Ray et al., 2012). However, a follow-up to that pilot study which

included 77 heavy drinkers of East Asian descent found no pharmacogenetic effects for alcohol-induced stimulation, sedation, craving for alcohol, or alcohol self-administration in the laboratory. Asp40 carriers exhibited a longer latency to first drink and consumed fewer total drinks relative to Asn40 homozygotes across medication conditions (Ray et al., 2018). Further exploration of neural modulators of the pharmacogenetic effects of naltrexone in this population may help to elucidate the cause of this variability observed across studies.

Neuroimaging methods have been used to study neural substrates of Asn40Asp SNP effects on alcohol phenotypes, as evidence indicates that cue-induced neural activation may be an important predictor of treatment response Courtney et al., 2016; Schacht et al., 2017b). In a seminal study, Filbey and colleagues employed an fMRI-based alcohol taste-cue paradigm to activate the mesocorticolimbic circuitry underlying craving among heavy drinkers (Filbey et al., 2008a; 2008b). This study found that among individuals homozygous for the short allele of the DRD4 exon 3 VNTR, Asp40 carriers had greater blood oxygenation level dependent (BOLD) response in mesocorticolimbic areas before and after a priming dose of alcohol, relative to control cues, compared to Asn40 homozygotes (Filbey et al., 2008b). Notably, however, a limitation of this study was the small sample of Asp40 carriers (n=11). A separate translational study combined intravenous alcohol administration with positron emission tomography (PET) to examine striatal dopamine (DA) response to alcohol in social-drinking men (Ramchandani et al., 2011); Asp40 carriers displayed greater striatal DA release in response to alcohol, compared to Asn40 homozygotes.

With respect to naltrexone neuroimaging studies, there is evidence that naltrexone attenuates alcohol cue-elicited activation of VS, anterior cingulate cortex (ACC), medial prefrontal cortex, and orbitofrontal cortex (OFC) - brain regions implicated in reward processing,

decision making, and selective attention (Myrick et al., 2008; Schacht et al., 2013; 2017b). Some studies, however, have either not found injectable, extended-release naltrexone effects (XR-NTX) on cue-elicited VS activation (Lukas et al., 2013), or found that naltrexone increased VS activation (Spagnolo et al., 2014) in response to alcohol. Lukas and colleagues (2013), however, did find that XR-NTX reduced cue-elicited activation of the orbitofrontal and medial prefrontal cortex. Fewer studies have examined naltrexone's effects on functional connectivity measures. One study of methamphetamine users found that naltrexone decreased functional connectivity between precuneus and sensorimontor regions and increased functional connectivity between dorsal striatum and precuneus with frontal regions, suggesting that naltrexone may alter communication between brain reward regions and those involved in executive function and effortful decision making (Courtney et al., 2016).

Results from neuroimaging studies of naltrexone and *OPRM1* pharmacogenetic effects remain relatively mixed. Some studies have found that *OPRM1* does not moderate the effects of naltrexone on alcohol infusion- and cue-elicited activation of VS among both alcohol-dependent treatment seekers (Spagnolo et al., 2014) and non-treatment seekers (Schacht et al., 2013; Ziauddeen et al., 2016). In contrast, one study found that relative to Asn40 homozygotes, Asp40 carriers exhibited less OFC activation in response to alcohol cues (Schacht et al., 2013), and that Asp40 carriers more quickly escalated to heavy drinking after discontinuing naltrexone (Schacht et al., 2017b). Overall, these mixed results suggest a potential *OPRM1* pharmacogenetic effect, but imply that mechanisms underlying this effect, particularly for localized functional activation, are less reliably replicated.

In light of the mixed literature on naltrexone and *OPRM1* pharmacogenetics and the need to extend these findings to diverse populations, this study examined the pharmacogenetic effects

of naltrexone on neural responses to alcohol taste cues in a sample of heavy drinking individuals of East Asian descent. The present study is an extension of our previous trial (Ray et al., 2018), whereby a subset of participants from our laboratory study completed a task involving the presentation of alcohol and water taste cues during fMRI. Specifically, we examined the pharmacogenetic effects on functional activation using both whole-brain and regions of interest (ROI) analyses, using *a priori*-defined anatomical ROIs (VS, ACC, OFC) that have been shown to be attenuated by naltrexone during alcohol craving (Mann et al., 2014; Schacht et al., 2013; 2017b). We also examined the pharmacogenetic effects on functional connectivity during alcohol taste cue presentation, using the left and right VS as seed regions that correspond to reward-related neural circuitry. Based on previous studies, we hypothesized that naltrexone, compared with placebo, would attenuate neural response to alcohol relative to water taste cues in the mesocorticolimbic pathway, and that naltrexone would do so to a greater extent in Asp40 carriers relative to Asn40 homozygotes. For functional connectivity, we anticipated that naltrexone would decrease VS connectivity with sensorimotor regions, and increase connectivity with precuneus and/or prefrontal cortex (Courtney et al., 2016); though largely exploratory, we hypothesized that naltrexone would produce greater such functional connectivity changes in Asp40 carriers relative to Asn40 homozygotes.

2. MATERIALS AND METHODS

2.1 Participants & Screening Procedures

Participants were recruited between July 2013 and December 2016 from the community through fliers, advertisements, and social media. Inclusion criteria were: 1. Alcohol-Use Disorders Identification Test (AUDIT; Allen et al., 1997a) score $\geq$ 8; 2. East Asian ethnicity

(i.e., self-identified as Chinese, Korean, Japanese, or Taiwanese); and 3. age 21-55 years old. Exclusion criteria were: 1. history of depression with suicidal ideation; 2. lifetime psychotic disorder; 3. current non-alcohol substance use disorder (except cannabis); 4. $\geq$10 on the Clinical Institute Withdrawal Assessment-revised (CIWA-R) (Sullivan et al., 1989); 5. currently seeking AUD treatment; 6. history of epilepsy, seizures, or severe head trauma; 7. non-removable ferromagnetic objects in body; 8. claustrophobia; and 9. pregnancy. All participants were required to have a breath alcohol concentration (BrAC) of 0.00 g/dL before each neuroimaging session. The study was approved by the University of California, Los Angeles Institutional Review Board.

Initial assessment of the eligibility criteria was conducted through a telephone interview. Eligible participants were invited to the laboratory for additional screening. Upon arrival, participants completed informed consent procedures and provided a saliva sample for DNA analyses (see supplementary materials). Participants then completed a series of measures and interviews, including the 30-day Timeline Follow-back (TLFB; Sobellet al., 1986). All participants were required to test negative on a 10-panel urine drug test (except for marijuana). This panel assesses for amphetamines, methadone, tetrahydrocannabinol (marijuana), benzodiazepines, barbiturates, methamphetamine, phencyclidine, cocaine, opiates, and oxycodone. Prospective genotyping was not utilized in this study due to the anticipated allele frequency of nearly 50% and the previously successful utilization of this approach by our group (Ray et al., 2012). Eligible participants completed a physical examination at the UCLA Clinical and Translational Research Center (CTRC) to determine medical eligibility. A total of 199 participants were screened in the laboratory, and 106 completed the physical exam, 5 of whom were ineligible for medical reasons and 14 of whom declined participation in the parent

laboratory study. Of the 87 individuals randomized to the parent study, 7 individuals reported MRI contraindications and 6 declined to participate in the neuroimaging study. The study scanner was upgraded during the end of the study; due to concerns related to changes in scanner parameters and image quality, scanning data were not collected for 12 MRI-eligible participants at the end of the study. Therefore, 62 participants were randomized for the current study, 48 of whom completed both neuroimaging sessions. Of these 48 participants, we excluded 7 participants due to excessive motion (>2 mm translation) and/or poor registration. The final analyzed sample consisted of 41 participants. See **Figure 1** for a CONSORT Diagram for this trial.

2.2. Medication Procedures

Participants were assigned to a medication sequence based on randomization pattern of ABBA. Participants completed one fMRI session after taking naltrexone for 4 days (25 mg for days 1-2, 50 mg for days 3-4) and one fMRI session after taking a matched placebo for 4 days (minimum 7-day wash-out between conditions). Active medication and placebo were delivered in a counterbalanced and double-blinded fashion. Participants were asked to report any side effects to the study physician. A series of non-parametric Fisher's exact tests, accounting for small cell sizes (Fisher, 1922), were conducted to examine 24 possible side effects from the medication (Levine & Schooler, 1986). Five participants dropped out of the study as a result of anticipated medication side effects. Active medication and placebo capsules were packaged with 50mg of riboflavin allowing for medication compliance to be visually examined via urine samples collected prior to each lab visit. As analyzed under ultraviolet light (Del Boca et al., 1996), all samples tested positive for riboflavin content.

2.3. fMRI Scanning Visit

At the start of the scanning visit, participants were required to have a BrAC of 0.00 g/dL, a negative urine toxicology screen for all drugs (excluding marijuana), and a negative pregnancy screen for female participants. Participants who smoked cigarettes were allowed to smoke 30 minutes prior to the scan to prevent cigarette craving. To assess for pre-scan alcohol craving, participants completed the Alcohol Urges Questionnaire (AUQ) immediately before entering the scanner (Bohn et al., 1995).

2.4. fMRI Task

The taste cues task employed was a modification of the Alcohol Taste Cues Task (Filbey et al., 2008a; 2008b), which has been previously used in our laboratory (Courtney et al., 2014; 2015; Ray et al., 2014). Each trial began with the presentation of a visual cue such that the words *Alcohol* or *Water* were visually presented to participants (2 second duration). This was followed by a fixation cross (duration jittered using an exponential distribution with a mean of 3 seconds and a range of 0.5 to 6 seconds), presentation of the word *Taste* upon which corresponding liquid was delivered (2 mL alcohol or water; 5 seconds), and a second fixation cross (duration jittered as above). All visual cues corresponded with the delivered liquid for that trial. Alcohol and water tastes were delivered through Teflon tubing using a computer-controlled delivery system (Infinity Controller) as described by Filbey and colleagues (Filbey et al., 2008a). Participants were instructed to press a button on a response box to indicate the point at which the bolus of liquid was swallowed. Alcohol tastes consisted of participants' preferred wine (either red or white), which has been effective in eliciting alcohol-cue related activation in previous studies

from our group (Ray et al., 2014). Beer could not be administered due to incompatibility of the alcohol administration device with carbonated liquids. A total of 16 participants from the final analyzed sample chose white wine and 25 participants chose red wine, and 5 total participants overall reported wine as their preferred alcohol. Visual stimuli and response collection were programmed using MATLAB (Mathworks, Natick, MA) and the Psychtoolbox (www.psychtoolbox.org), and visual stimuli were presented using MRI-compatible goggles (Resonance Technologies, Van Nuys, CA). The taste cues task was administered over the course of two runs with 50 trials per run.

2.5. Analytic Plan

Information regarding image acquisition parameters and preprocessing steps are available in Supplementary Materials. The main contrast of interest was difference in activation corresponding to alcohol taste delivery relative to water delivery, across the two task runs (Alcohol > Water); however, all variations of this contrast were modeled (i.e., Water > baseline, Alcohol > baseline, Water > Alcohol), as well as time periods corresponding with the visual text prior to taste delivery. These analyses were conducted for each within-subject medication condition. Group-level analyses utilized FSL's FLAME 1 (Woolrich et al., 2004) with outlier deweighting (M. Woolrich, 2008); Z-statistic images were thresholded with cluster-based corrections for multiple comparisons based on the theory of Gaussian Random Fields with a cluster-forming threshold of $Z > 2.3$ and a cluster-probability threshold of $p < 0.05$ (Worsley, 2001).

Pre-test comparisons were conducted to determine whether *OPRM1* groups differed on demographic and drinking variables using t-tests and chi-square tests with a significance

threshold of $p < 0.05$. To ensure that activation from the main contrast of Alcohol > Water was not broadly driven by genetic differences in neural activation, *OPRM1* effects were examined for Alcohol Taste and Water Taste separately. Multilevel mixed models were used to test group level aims, specifically to assess the effects of medication, *OPRM1* genotype, medication × *OPRM1* genotype interaction on task-related activation for whole-brain and ROI analyses. The primary dependent variable was the contrast of Alcohol > Water. Medication was a two-level within-subjects factor [naltrexone (NTX) and placebo (PLAC)] and *OPRM1* genotype was a two-level between-subjects factor (Asp40 carriers and Asn40Asn). A 3-level genotype analysis (Asp40Asp, Asp40Asn, Asn40Asn) was not conducted due to small cell sizes. Pre-scan AUQ scores, AUDIT total scores TLFB number of drinking days and days since last drink, gender, age, and cigarette and marijuana use status were examined as potential covariates in separate whole brain and ROI functional activation analyses. To further validate that medication effects were not impacted by alcohol metabolizing genes, all analyses examined ALDH2 (rs671) and ADH1B (rs1229984) markers as potential covariates, but these genotypes were ultimately not significantly associated with activation for any of the primary analyses.

2.6. ROI Analyses

Based on previous studies examining alcohol and cue-induced craving (Aalto et al., 2015; Ray et al., 2015; Schacht et al., 2013; 2017b), four anatomically-defined *a priori* regions of interest were utilized to examine pharmacogenetic effects on functional activation, including left and right VS, bilateral ACC, and bilateral OFC. ROIs were anatomically defined using the Harvard-Oxford atlas (in standard MNI space) and transformed into individual participants' native space using FSL's FLIRT (see **Figure S1**). Mean contrast estimate values from the Alcohol > Water

contrast were extracted from these regions for each subject and submitted to mixed models for group-level analyses.

2.7. PPI Analyses

Functional connectivity analyses were conducted in FSL 5.0 using psychophysiological interaction (PPI) analyses which examines the interaction of task conditions and functional connectivity between the time course of activation for specific seed regions with the rest of the brain (O'Reilly et al., 2012). Based on previous work that utilized anatomically-defined left and right VS as primary regions of interest (Schacht et al., 2017b), PPI analyses were conducted to examine the interaction of the Alcohol > Water contrast and the left and right VS seed regions for the comparisons: NTX > PLAC and PLAC > NTX. The first-level PPI models included four regressors: 1) Alcohol - Water; 2) Alcohol + Water; 3) "physiological" regressor modeling the seed time course; and 4) interaction regressor (regressor 1 multiplied by regressor 3). Whole-brain contrast images were generated separately for the left and right VS seed regions, with cluster-forming thresholds of Z>2.3 and cluster-probability thresholds of $p<0.05$.

3. RESULTS

3.1. Baseline and Demographic Comparisons

The pre-test comparisons on demographic and drinking variables revealed no significant *OPRM1* genotype group differences across demographic variables (*p's* ≥ 0.12; see **Table 1**). Results revealed a trend for a genotype difference in drinking days and days since last drink over the past 30 (*p's* = 0.06-0.07), although no other alcohol or substance use variables approached significance (*p's* ≥ 0.15). There were no significant differences in pre-scan craving or reported

side effects between conditions (*p*'s > 0.18). There were also no significant differences in dropout or reported side effects by genotype (*p*'s > 0.46).

3.2 Main Effect of Task (Alcohol > Water Contrast)

Within the placebo condition, alcohol taste cues, compared to water taste cues, elicited eight clusters of activation at the whole-brain level, including the thalamus, precuneus, occipital cortex, parietal operculum cortex, and temporal and angular gyri, and central opercular cortex (see **Figure 2** and **Table 2**). Whole brain activation clusters did not differ as a function of medication condition.

3.3 Naltrexone and Genotype Effects: Whole Brain Analyses

There were no significant effects of medication condition on whole-brain activation for the Alcohol > Water contrast. Activation related to the Alcohol > Water contrast was also not found to significantly differ by *OPRM1* genotype. Finally, there was no pharmacogenetic effect (*OPRM1* x Medication) on Alcohol > Water activation. Controlling for age, sex, pre-scan AUQ, AUDIT, number of drinking days and days since last drink, cigarette smoking and marijuana status, and ALDH2 and ADH1B genotypes did not alter these results. Of note, there were also no significant differences between medication conditions or *OPRM1* genotype groups on activation in response to the alcohol taste or water taste alone relative to baseline. Uncorrected medication effects for the Alcohol > Water contrast and by OPRM1 genotype are depicted in **Figures S2 and S3.**

3.4 ROI Analyses

For left VS, there was no significant medication effect [F(1,39) = .05, p = 0.82] or medication by *OPRM1* genotype interaction [F(1,39) = .12, p = 0.73]. There was, however, a significant main effect of *OPRM1* genotype [F(1,39) = 4.26, p = 0.05, η_p^2 = .10], such that Asp40 carriers exhibited higher left VS activation than Asn homozygotes (parameter estimate M(SD) = 4.11(15.49) and -1.66(13.15), respectively). For the right VS, there was no significant medication effect [F(1,39) = 1.20, p = 0.28], *OPRM1* effect [F(1,39) = .67, p = 0.42], or pharmacogenetic effect by *OPRM1* genotype [F(1,39) = 1.02, p = 0.32].

For ACC, there was no significant medication effect [F(1,38) = .45, p = 0.51] or medication by *OPRM1* genotype interaction [F(1,34) = .10, p = 0.75]. There was, however a significant *OPRM1* effect [F(1,38) = 5.82, p = 0.02, η_p^2 = .13], such that Asp40 carriers exhibited higher ACC activation than Asn40 homozygotes (parameter estimate M(SD) = 8.27(13.40) and 4.24(16.00), respectively). Significant covariates included 30-day TLFB drinks per drinking day [F(1,38) = 4.20, p = 0.04].

For OFC, there was no significant medication effect [F(1,37) =.07, p = 0.79] or medication by *OPRM1* genotype interaction [F(1,37) = 2.13, p = 0.15]. There was, however, a significant *OPRM1* effect [F(1,37) = 6.20, p = 0.02, η_p^2 = .14], such that Asp40 carriers exhibited higher OFC activation than Asn40 homozygotes (parameter estimate M(SD) = 4.00(8.32) and 0.29(9.60), respectively). Drinks per drinking day in the last 30 days (as measured by TLFB) [F(1,37) = 5.99, p = 0.02] showed a significant relationship with OFC activation, and there was a trending effect of sex [F(1,37) = 3.36, p = 0.08].

3.5 PPI Analyses

For the left VS seed, PPI analyses indicated that, relative to placebo, naltrexone elicited stronger connectivity with the frontal pole and cingulate gyrus within the Alcohol > Water contrast (see **Figure 3A** and **Table 3**). For the right VS seed, PPI results indicated that naltrexone relative to placebo elicited stronger connectivity with the clusters in the lateral occipital cortex within the Alcohol > Water contrast (see **Figure 3B** and **Table 3**). There were no differences in functional connectivity or the "physiological" regressor maps by *OPRM1* genotype, nor was there a pharmacogenetic effect of naltrexone and *OPRM1* on functional connectivity for either the right or left VS. Controlling for age, sex, pre-scan AUQ, AUDIT, number of drinking days and days since last drink, cigarette smoking and marijuana status, and ALDH2 and ADH1B genotypes did not alter these results.

4. DISCUSSION

In light of the mixed literature on naltrexone and *OPRM1* pharmacogenetic effects, the current study examined neural pharmacogenetic effects of naltrexone and *OPRM1* within a sample of heavy drinkers of East Asian ancestry. Relative to Asn40 homozygotes, Asp40 carriers exhibited increased activation in VS, ACC, and OFC during alcohol versus water taste cues. Overall, we did not find a significant medication or pharmacogenetic effect on functional activation during alcohol taste cues in this sample. Naltrexone did, however, increase functional connectivity between left VS and posterior cingulate cortex and medial prefrontal cortex, as well as increase functional connectivity between right VS and occipital cortex. Similar to the localized functional activation results, there was no pharmacogenetic effect on functional connectivity.

These results replicate previous studies that have found that *OPRM1* Asp40 carriers exhibit greater VS, vmPFC, and OFC activation in response to alcohol taste cues among heavy drinkers (Filbey et al., 2008b; Ray et al., 2014); this study corroborates that the *OPRM1* effect is likely small, as it has primarily been observed in ROI rather than whole-brain voxel-wise results, and this result has not been replicated in visual alcohol cue studies with alcohol dependent individuals (Schacht et al., 2013). The results of the current study also suggest that these *OPRM1* effects may be localized to reward processing regions, without significantly impacting functional interactions between VS and other brain regions. Notably, the lack of genotype differences in functional connectivity for left and right VS contrast earlier findings that, relative to Asn40 homozygotes, Asp40 carriers exhibit reduced cue-induced connectivity between VS and insula, frontal medial cortex, thalamus, putamen, and paracingulate gyrus (Ray et al., 2014). These differing results may in part be due to the higher average AUD severity in this previous study. As alcohol dependence severity is associated with weakened frontostriatal connectivity and dysregulated activity during effortful decision making (Courtney et al., 2013), these results suggest that Asp40 carriers with more severe AUD require increased recruitment of frontal systems to regulate striatal reward processing regions.

The present results suggest that naltrexone may affect communication between brain regions to a greater degree during alcohol relative to water tastes than localized region activation specifically, as there were no significant effects of naltrexone relative to placebo on localized functional activation during consumption of alcohol relative to water taste cues. Notably, nonsignificant naltrexone effects were found both at the whole-brain voxel-wise level and in ROI analyses of reward processing regions (namely, VS, ACC, and OFC) that have previously been shown to be attenuate with naltrexone during alcohol consumption and cue paradigms (Mann et

al., 2014; Myrick et al., 2008; Schacht et al., 2013; 2017b),. These null findings do, however, corroborate and extend previous studies that have failed to observe significant naltrexone-induced changes in VS and in response to alcohol cues (Lukas et al., 2013) or during a monetary incentive delay task (Nestor et al., 2017).

Despite null localized functional activation results, naltrexone increased functional connectivity between left ventral striatum and medial prefrontal cortex (mPFC) and posterior cingulate cortex (PCC), regions implicated in coordinating attentional focus, decision making, and other executive functions (Hayden et al., 2009; Mashhoon et al., 2014). Intrinsic connectivity distribution analyses have indicated that individuals with AUD exhibit blunted cingulate connectivity with frontal regions, thalamus, and precuneus in response to both alcohol and stress cues, and PCC connectivity with frontoparietal regions specifically predicted a longer time to relapse in an AUD treatment study (Zakiniaeiz et al., 2017). With respect to mPFC, nucleus accumbens-mPFC connectivity during a monetary reward task has been shown to be negatively associated with drinking frequency and family history of AUD (Forbes et al., 2014). Altogether, these results suggest that connectivity among VS, mPFC, and PCC could be potential pathways of action for naltrexone.

The few naltrexone studies that have examined functional connectivity vary in analysis parameters, populations of interest, and study designs. These studies have shown that naltrexone modulates connectivity between ACC and hippocampus as a function of childhood adversity during an emotional priming task among alcohol-dependent individuals (Savulich et al., 2017), and that naltrexone improves local network efficiency in alcohol dependent individuals, reaching that of healthy controls (Morris et al., 2018). Most notably, in a study of methamphetamine users, naltrexone decreased connectivity between precuneus and sensorimontor regions and

increased connectivity between dorsal striatum and precuneus with frontal regions (Courtney et al., 2016). This study's results, therefore, go against our hypotheses and do not replicate these previous results regarding sensorimotor connectivity; future studies with both alcohol and methamphetamine-using populations are warranted to determine the reliability of such connectivity results. This study's results do, however, corroborate a potential common effect of naltrexone across alcohol and methamphetamine through strengthened connections between frontal systems and reward processing regions. This result, in theory, may indicate greater activation of self-control networks in the brain over reward signals, following naltrexone treatment, and as compared to placebo.

Naltrexone also increased connectivity between right VS and occipital cortex. This is an unexpected finding, as most studies have either not observed or not examined an impact of naltrexone on this functional connectivity pattern or on occipital cortex activation (Mann et al., 2014; Schacht et al., 2013; 2017b). However, most visual alcohol cue studies find significant cue-elicited activation in occipital cortex (Hanlon et al., 2014), and one study found that naltrexone attenuates occipital cortex activation, thereby reducing salience of visual substance-related cues (Lukas et al., 2013). Interactive occipital cortex functional activation during cue and taste paradigms are not well-understood, and future functional connectivity studies may help to elucidate the significance and replicability of this particular finding.

There is a growing literature on the predictive value of cue-induced neural activation for real-world clinical outcomes in drug cessation (Courtney et al., 2016; Schachtet al., 2017b; Zakiniaeiz et al., 2017). Incorporating underrepresented groups in pharmacogenetics studies is critical for addressing health disparities in the context of personalized medicine (Cservenka et al., 2017). This study provides initial evidence that pharmacogenetic effects of naltrexone and

OPRM1 are not supported in non-treatment seeking heavy drinkers of East Asian descent, with respect to alcohol taste-elicited neural activation. It is plausible that a robust effect in tightly controlled preclinical and experimental medicine models "fades" in the context of complex, real world clinical application and with heterogeneity of AUD. (Ray et al., 2012; Roche & Ray, 2015).

Importantly, these results should be interpreted in light of the human laboratory arm of the study, which found no support for pharmacogenetic effects of *OPRM1* and naltrexone among individuals of East Asian descent (Ray et al., 2018) for alcohol-induced stimulation, sedation, craving for alcohol, or alcohol self-administration. There were no main effects of medication on those phenotypes, and the main effect of genetics on alcohol self-administration suggested that the Asp40 allele was protective for alcohol self-administration. In the context of significant naltrexone effects on functional connectivity in the absence of pharmacogenetic effects, these findings in East Asians add to the rather mixed literature on naltrexone pharmacogenetics in predominantly Caucasian samples and highlight the complexity of these effects and their overall limited replicability.

There were several notable study strengths, including a within-group, double-blind, randomized design, pharmacogenetic testing in a population that has a balanced *OPRM1* allele frequency distribution, and consideration of multiple genetic and individual difference covariates. There were also several important study limitations. While the taste cues paradigm is based upon validated fMRI paradigms, the iteration utilized in this study increased the number of trials administered at the expense of reducing the duration of each individual trial. Future replication studies may be needed to further validate this taste paradigm, particularly as the main contrast of interest (Alcohol > Water) did not yield significant clusters of activation in the VS in

the whole-brain analysis, and a post-scan AUQ was not conducted. Though this contrasts with other fMRI and PET alcohol taste studies (Oberlin et al., 2016; 2013; Schacht et al., 2013), this lack of activation has been replicated in alcohol infusion studies with alcohol dependent treatment-seeking patients (Spagnolo et al., 2014) and alcohol olfactory cues studies (Lukas et al., 2013). It is possible that longer trial durations may be required to reliably recruit VS activation, though it is notable that naltrexone modulated functional connectivity despite this potential limitation. Drink choice was also limited to red or white wine, and these results warrant replication with other types of alcohol preference, particularly as only a minority of the sample reported wine as their preferred alcohol and this could potentially impact neural activation in response to a taste cue. Larger samples may also be required to identify effects of specific individual characteristics such as sex and cigarette smoking status that have been shown to moderate naltrexone response (Fridberg et al., 2014; King et al., 2012). Similarly, while pharmacogenetics effects are theoretically testable in absence of a main medication effect, it is possible that decreased variability and/or power of naltrexone-induced as well as general task-induced neural activation may have made it difficult to detect a pharmacogenetic effect; one potential explanation for a nonsignificant main effect may have been the relatively short duration of naltrexone treatment in the current study (4 days) relative to longer durations (7-14 days) reported in other studies (Lukas et al., 2013; Myrick et al., 2008; Schacht et al., 2017a). Relatedly, riboflavin testing was conducted via visual inspection rather than quantitative testing; as riboflavin concentrations of 900 ng/mL have been established to visually classify positive samples 2 to 24 hours after ingestion (Herron et al., 2013), it is possible that the 100% adherence rate may refer to these more immediate periods rather than full compliance over the titration period. Additionally, while the sample consisted of heavy drinkers, approximately half of the

sample did not meet criteria for an alcohol use disorder; future studies may benefit from examining these pharmacogenetic effects in individuals with more severe drinking, as higher alcohol dependence severity may be predictive of cue reactivity (Sjoerds et al., 2014).

In sum, this study does not support a pharmacogenetic effect for naltrexone and *OPRM1* on alcohol taste-induced neural activation in individuals of East Asian descent. There was no medication effect on localized functional activation, yet naltrexone increased functional connectivity during alcohol taste between regions involved in reward processing and frontal regions critical to executive function. On balance, these results add to a mixed naltrexone literature that has primarily been conducted in Caucasian individuals, and corroborate a potential common effect of naltrexone on functional connectivity across substances.

Table 1. Pretest Differences Between Genotype Groups

Variable[a]	Asn40Asn (n=18)	Asn40Asp/Asp40Asp (n=23)	Test for Difference
Gender			χ^2 (1) = .146, p = 0.702
Female (%)	6 (33%)	9 (39%)	
Male (%)	12 (67%)	14(61%)	
Ethnicity			Fisher's exact test, p = 0.20
Chinese (%)	8 (44%)	7 (30%)	
Japanese (%)	0 (0%)	3 (13%)	
Korean (%)	7 (39%)	12 (52%)	
Taiwanese (%)	3 (17%)	1 (4%)	
Age[c]	30.17 (8.61)	26.78 (5.00)	t(39) = 1.58, p = 0.12
AUD[d]			Fisher's exact test, p = 0.57
None	9 (50%)	9 (39%)	
Mild	5 (28%)	11 (48%)	
Moderate	2 (11%)	2 (9%)	
Severe	2 (11%)	1 (4%)	
AUDIT[e]	15.39 (4.89)	13.74 (5.41)	t(39) = 1.01, p = 0.32
Drinking Days[f]	15.78 (7.49)	12.00 (5.33)	t(39) = 1.89, p = 0.07
Drinks/Drinking Day[f]	5.38 (2.78)	4.32 (1.75)	t(39) = 1.48, p=0.15
Marijuana Days	1.50 (2.64)	2.17 (4.91)	t(39) = -0.53, p = 0.60
PLAC Days since Drink	1.83 (1.10)	2.78 (1.78)	t(39) = -1.98, p = 0.06
NTX Days since Drink	2.33 (1.53)	3.26 (1.66)	t(39) = -1.84, p = 0.07
PLAC pre-scan AUQ	8.11 (6.06)	7.53 (5.39)	t(39) = 0.33, p = 0.77
NTX pre-scan AUQ	5.94 (6.46)	5.53 (5.64)	t(39) = 0.20, p = 0.84

[a] Standard deviations appear within parentheses for continuous variables.

[b] *I/*I = GG, *I/*2= AG, *2/*2 = AA.

[c] Assumption of homogeneity of variance not met, adjusted degrees of freedom, t-statistic, and significance level accounted for within table.

[d] Current (past 3 months) Alcohol Use Disorder (AUD) assessed by the Structure Clinical Interview for Alcohol Use Disorder (DSM-5).

[e] Alcohol Use Disorder Identification Test (AUDIT) score $\geq$ 8 indicates hazardous drinking pattern; possible range of scale: 0 – 40.

[f] Assessed by Timeline Follow Back (TLFB) interview for the past 30 days

Table 2. Alcohol > Water contrast cluster peaks.

	Peak MNI coordinates					
Cluster region	**X**	**Y**	**Z**	**# Voxels**	**Max-Z**	***p*-value**
Left thalamus	0	-20	-4	560	21.9	1.79E-07
Right Parietal operculum	62	-20	12	479	17.6	1.13E-06
Right Inferior temporal gyrus	44	-68	-18	396	18.1	8.17E-06
Precuneus	6	-78	44	222	19.7	0.0009
Left Middle temporal gyrus	-48	-60	8	155	10	0.0078
Precentral gyrus	0	-26	46	154	15.2	0.0081
Right Angular gyrus	64	-52	18	147	13.1	0.0103
Left Central opercular cortex	-58	-20	12	138	13.5	0.0141

Table 3. Significant clusters for psychophysiological interaction analyses using the Alcohol > Water contrast

	Peak coordinates					
Cluster region	**X**	**Y**	**Z**	**# Voxels**	**Max-Z**	***p*-value**
Left Ventral Striatum PPI						
Frontal pole	-2	58	20	971	3.28	0.006
Cingulate gyrus	-2	-36	24	662	3.33	0.045
Right Ventral Striatum PPI						
Right Lateral occipital cortex	42	-74	32	865	4.07	0.02
Left Lateral occipital cortex	-34	-84	32	704	3.67	0.04

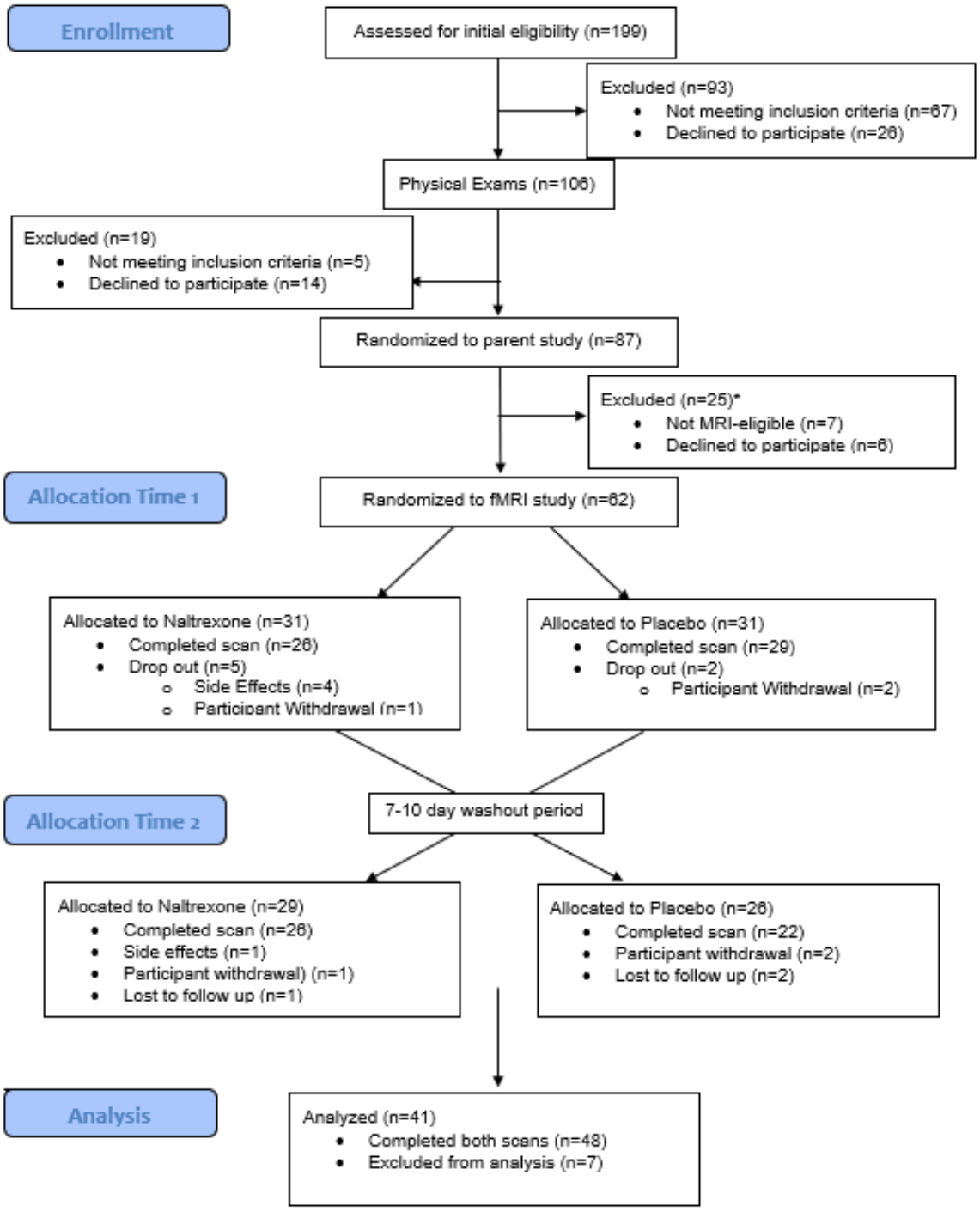


Figure 1. CONSORT Diagram

*The scanner utilized for the study was upgraded towards the end of the study. Due to parameter compatibility concerns, scanning data was not collected from 12 MRI-eligible participants.

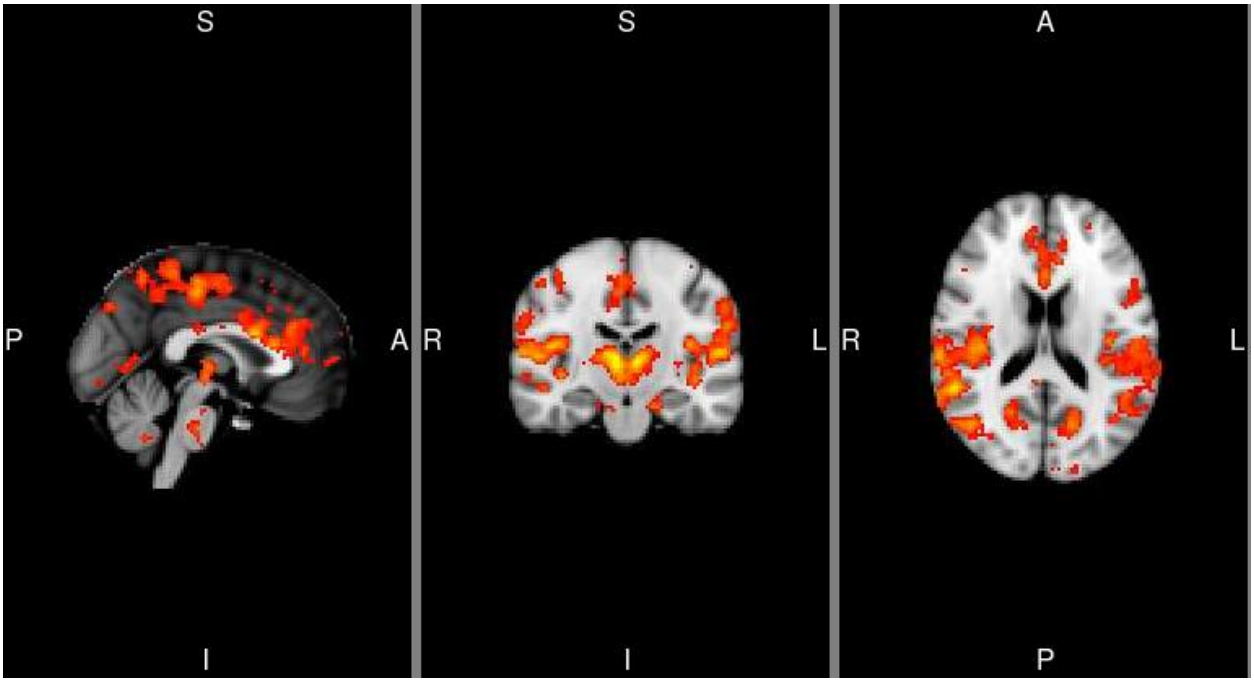
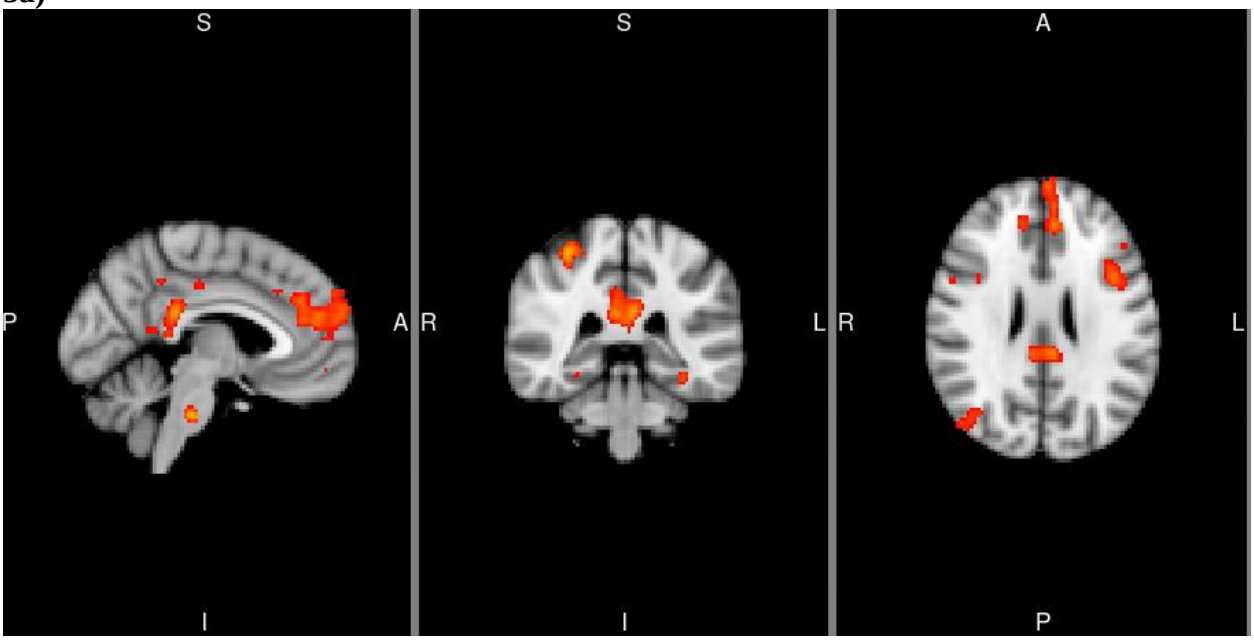


Figure 2. Alcohol > Water Taste task-related activation. MNI coordinates for depicted slices are X = 0, Y = -18, Z = 18. Color bar represents z-values. L=left, R=right, S=superior, I=inferior, A=anterior, P=posterior

3a)



3b)

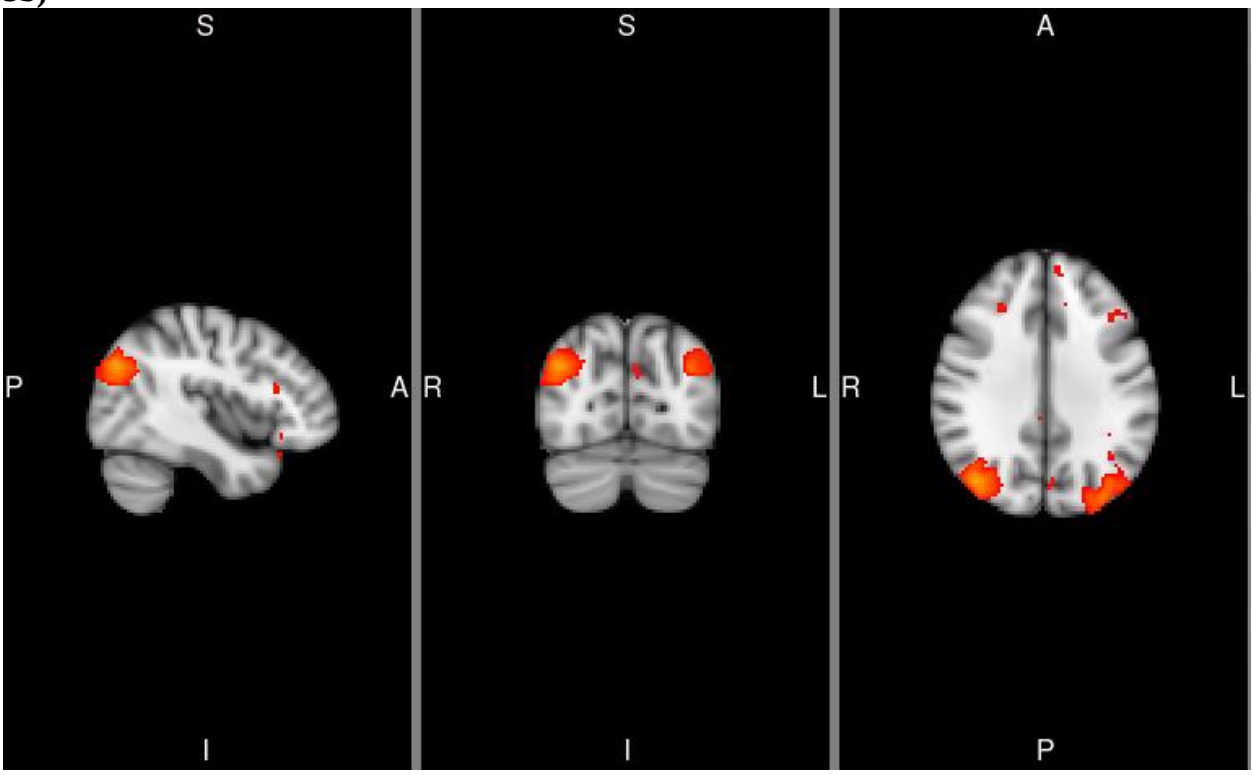


Figure 3. PPI analyses indicating functional connectivity of left **(3a)** and right **(3b)** ventral striatum during alcohol cue presentations. MNI coordinates for depicted slices are X = -4 (left), Y = -36 (middle), Z = 24 (right) in **3a** and X = 42 (left), Y = -74 (middle), Z = 32 (right) in **3b**. Color bar represents z-values. L=left, R=right, S=superior, I=inferior, A=anterior, P=posterior

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Study 2:

ALCOHOL CUE-INDUCED VENTRAL STRIATUM ACTIVITY PREDICTS SUBSEQUENT ALCOHOL SELF-ADMINISTRATION

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[In Press at *Alcoholism: Clinical and Experimental Research*]

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Acknowledgements

This work was supported by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) (R01AA021744) and the UCLA Clinical and Translational Science Institute (CTSI) (grants UL1RR033176 and UL1TR000124). This work is also supported by the Tobacco Related

Disease Research Program Grants T29DT0371 &T30DT0950; the National Institute on Alcohol Abuse and Alcoholism Grants F32AA027699, 3R01AA026190-02S1 & K24AA025704. LAR has received study medication from Pfizer and Medicinova and consulted for GSK. The authors report no other conflict of interest.

Abstract

Background: Human laboratory paradigms are a pillar in medications development for alcohol use disorders (AUD). Neuroimaging paradigms, in which individuals are exposed to cues that elicit neural correlates of alcohol craving (e.g. mesocorticolimbic activation), are increasingly utilized to test the effects of AUD medications. Elucidation of the translational effects of these neuroimaging paradigms on human laboratory paradigms, such as self-administration, are warranted. The current study is a secondary analysis examining whether alcohol cue-induced activation in the ventral striatum is predictive of subsequent alcohol self-administration in the laboratory.

Methods: Non-treatment-seeking heavy drinkers of East Asian descent (n = 41) completed a randomized, placebo-controlled, double-blind, crossover experiment on the effects of naltrexone on neuroimaging and human laboratory paradigms. Participants completed 5 days of study medication (or placebo); on day 4, they completed a neuroimaging alcohol taste cue reactivity task. On the following day (day 5), participants completed a 60-minute alcohol self-administration paradigm.

Results: Multilevel cox regressions indicated a significant effect of taste cue-elicited ventral striatum activation on latency to first drink, Wald $\chi 2 = 2.88$, $p = 0.05$, such that those with higher ventral striatum activation exhibited shorter latencies to consume their first drink. Similarly, ventral striatum activation was positively associated with total number of drinks consumed, $F(1, 38) = 5.90$, $p = .02$. These effects were significant after controlling for alcohol use severity, OPRM1 genotype, and medication.

Conclusions: Neuroimaging alcohol taste cue paradigms may be predictive of laboratory paradigms such as self-administration. Elucidation of the relationships among different

paradigms will inform how these paradigms may be used synergistically in experimental medicine and medications development.

Keywords: neuroimaging, human laboratory, alcohol self-administration, ventral striatum, cue-induced craving

INTRODUCTION

Development of effective treatments for alcohol use disorder (AUD) remain a high priority area which involves screening compounds in the laboratory before proceeding to clinical trials (Grodin & Ray, 2019; Ray, Bujarski, Roche, & Magill, 2018). Within this process, there is a need to develop and understand relationships among human laboratory paradigms to assess the potential efficacy of novel AUD treatments in early-stage clinical trials. To date, reviews of the human laboratory literature in AUD pharmacotherapy development indicate significant outcome variability based on experimental paradigm parameters, population of interest, and sample size, and suggest that these myriad variables contribute to the disconnect between laboratory effect sizes and treatment outcomes (Witkiewitz, Litten, & Leggio, 2019; Yardley & Ray, 2017).

Amidst the efforts to develop translational experimental paradigms, neuroimaging tasks are increasingly used to explore potential pharmacotherapy effects on neural correlates of alcohol-induced craving (Grodin & Ray, 2019). Alcohol consumption produces neuroadaptations in multiple circuits, including GABA-ergic regulation of traditional reward circuitry; alcohol craving is mediated by cortico-striatal-limbic activation, heightens relapse risk (Heinz, Beck, Grusser, Grace, & Wrase, 2009), and can be triggered through internal and external stimuli associated with alcohol consumption (Seo & Sinha, 2014) . For this reason, neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), have been used to explore these circuits as potential medication targets. Recent qualitative reviews and meta-analyses suggested that while such fMRI tasks vary in sensory experiences (e.g. taste vs visual cues) and scan parameters, mesocorticolimbic areas consistently exhibit task-based neural activity and may be viable tools in understanding mechanisms of AUD pharmacotherapy (Grodin & Ray, 2019; Schacht, Anton, & Myrick, 2013).

Based on this emerging literature, there is growing evidence that neural responses to alcohol cues and associated contexts are predictive of real-world consumption behavior and, potentially, clinical outcomes. For instance, among college students, alcohol cue-elicited blood oxygen level-dependent (BOLD) response in caudate, frontal cortex, and left insula predicted escalation to heavy drinking over a 1-year period (Dager et al., 2014). Further, insula and frontal gyrus activation in response to an emotion face recognition task similarly predicted alcohol-related problems five years later in young adults (Schuckit et al., 2016). Regarding treatment outcomes, increased ventral striatum activation in response to alcohol cues was associated with a faster time to relapse in a sample of abstinent AUD individuals (Reinhard et al., 2015). Comparisons of AUD treatment completers and non-completers in a community sample indicated that non-completers showed stronger associations between reported alcohol craving intensity and resting state functional connectivity between striatum and insula, relative to completers (Kohno, Dennis, McCready, & Hoffman, 2017). Of note, one study had contradicting results by reporting that relapsers, compared to successful alcohol abstainers and healthy controls, exhibited reduced alcohol cue-elicited activation in ventral striatum and midbrain (Beck et al., 2012).

Several studies have examined whether AUD pharmacotherapies alter neural responses to contexts that elicit alcohol craving, including alcohol cues, exposure to reward and emotional faces, and stress exposure. While significant variability exists in sample populations, examined tasks, modified areas of activation, and molecular targets of treatments, there is some consistent evidence that AUD pharmacotherapies may reduce reward-related activation in regions such as the ventral striatum, precuneus, and anterior cingulate (Grodin & Ray, 2019). Importantly, in one study of naltrexone, magnitude of reduction in alcohol cue-induced ventral striatum activation

was associated with fewer instances of subsequent heavy drinking (Schacht et al., 2017a). In support, Mann and colleagues (2014) have found that individuals with high ventral striatum cue reactivity demonstrate lower relapse rates when treated with naltrexone than those with low VS reactivity. Bach and colleagues (2019) have also identified that individuals with high alcohol cue-reactivity in the left putamen exhibit longer time to relapse when treated with naltrexone, compared to those with low reactivity. Together, these studies underscore reward circuitry (e.g. VS) as a key area in the translation of neural responses to clinical outcomes in AUD medication development (Nielsen et al., 2018).

Alcohol self-administration tasks in the laboratory are thought to capture alcohol use behavior in controlled settings that approximate consumption in real world settings. Studies have tested multiple variants of self-administration paradigms, including tasks that require participants to orally consume alcohol at the cost of monetary rewards per drink (McKee et al., 2009), and intravenous methods that can closely control breath alcohol concentration levels (e.g. computer-assisted self-infusion of ethanol (CASE); (Zimmermann, O'Connor, & Ramchandani, 2013). Studies have used self-administration methods to test genetic, physiological, and psychological risk factors for heavy drinking (Gowin, Sloan, Stangl, Vatsalya, & Ramchandani, 2017; Green et al., 2019; Wardell, Le Foll, & Hendershot, 2018). Self-administration tasks have also been used extensively in developing effective AUD pharmacotherapies (Hendershot, Wardell, Samokhvalov, & Rehm, 2017; McKee et al., 2009). While both fMRI cue-reactivity tasks and alcohol self-administration tasks are widely used in alcohol research, the extent to which cue-reactivity predicts self-administration in the laboratory remains unknown.

In light of the emerging role of functional neuroimaging in predicting drinking behavior and AUD treatment outcomes, a remaining question is the nature of the relationship between

neuroimaging task-induced neural activation and widely utilized laboratory paradigms considered proximal to real-world consumption, including self-administration tasks. To date, several studies have examined relationships of response across different laboratory paradigms (i.e. subjective response and self-administration) and have consistently identified that alcohol craving during intravenous alcohol administration mediates the relationship between alcohol-induced stimulatory effects and subsequent oral alcohol consumption (Bujarski et al., 2018; Green et al., 2019; Wardell, Ramchandani, & Hendershot, 2015). While relationships across human laboratory paradigms are recently delineated, no studies have yet investigated whether alcohol cue-induced BOLD response is predictive of responses within laboratory self-administration paradigms.

To address this gap in the literature and to further integrate neuroimaging and human laboratory paradigms for AUD, the current study examines whether alcohol taste cue-induced ventral striatum activation predicts subsequent oral alcohol self-administration in the laboratory. These secondary analyses are conducted in a within-subjects design whereby the same participants completed an fMRI cue-reactivity task followed by an alcohol-self administration task (one day later). As striatal activation is thought to underlie craving responses (Ray & Roche, 2018), we hypothesized that those with greater ventral striatum activation would consume their first drink faster than those with lower activation. Similarly, as previous research has demonstrated that mesolimbic activity predicts real-world heavy drinking, we hypothesized that ventral striatum activation would also be positively associated with the total number of drinks consumed during the self-administration paradigm.

MATERIALS AND METHODS

Participants

Participants for this secondary analysis of an experimental laboratory study on naltrexone (Lim et al., 2019; Ray et al., 2018) were adult heavy drinkers of East Asian descent recruited from the Los Angeles metropolitan area through community fliers and online and print advertisements. Inclusion criteria were: 1) a score of 8 or higher on the Alcohol Use Disorders Identification Test (AUDIT; (Allen, Litten, Fertig, & Babor, 1997b); 2) self-identification of East Asian ethnicity (i.e. Chinese, Japanese, Korean, or Taiwanese); and 3) between 21-55 years old. Exclusion criteria were: 1) history of Major Depressive Disorder with suicidal ideation; 2) lifetime psychotic disorder; 3) lifetime non-alcohol substance use disorder (with the exception of cannabis); 4) clinically significant levels of alcohol withdrawal (indicated by a score of 10 or higher on the Clinical Institute Withdrawal Assessment-Revised (CIWA-AR (Sullivan et al., 1989); 5) currently seeking AUD treatment; 6) history of epilepsy, seizures, or severe head trauma; 7) non-removable ferromagnetic objects in body; 8) claustrophobia; and 9) for women, pregnancy. The study was approved by the University of California Los Angeles Institutional Review Board.

Procedures

Recruitment

Interested individuals completed an in-person laboratory screening visit to learn about the study, provide written informed consent, and to assess for inclusion and exclusion criteria. Of note, this study collected information on genotypes encoding endogenous opioid receptors thought to mediate the stimulating effects of alcohol (OPRM1), as well as those associated with metabolism of alcohol (ADH1B, ALDH2). Participants provided a saliva sample for DNA

analyses and completed a medical screening that included a physical examination. Detailed information on recruitment procedures are available in the primary manuscripts from which the current study is based (Lim et al., 2019; Green, et al., 2018). Detailed information on genotyping is available in Supplementary Materials. A study procedure flowchart can be seen in **Figure 1**.

Medication Procedures

Study procedures followed a double-blind, randomized, placebo-controlled and counterbalanced design. Participants were assigned a medication sequence (placebo, naltrexone) based on a randomization pattern of ABBA. Within each medication condition, participants were titrated to the medication (or matched placebo) for 5 days (for naltrexone, 25 mg for days 1-2, 50 mg for days 3-5). Participants completed an fMRI scan on day 4 and an alcohol self-administration session on day 5 of the medication regimen. At the start of each experimental session, participants completed a urine toxicology screening; all participants tested negative for exclusionary substances during these screening periods. There was a minimum wash-out period between medication conditions of 7 days, with a range of 7-10 days. Regarding medication adherence, naltrexone and placebo capsules were packaged with 50mg of riboflavin. A visual inspection of riboflavin content under ultraviolet light indicated that all urine samples tested positive for riboflavin content.

fMRI Scanning Procedures

At the start of the scanning session (medication day 4), participants were required to have a BrAC of 0.00 g/dL, negative urine toxicology screen for all substances except cannabis, and

negative pregnancy screen. Participants who smoked cigarettes (n = 12, 29% of the sample) were allowed to smoke 30 minutes prior to the scan to prevent acute nicotine withdrawal and craving.

Participants completed a modified version of the Alcohol Taste Cues Task in the scanner (Filbey et al., 2008). Within each task trial, participants initially viewed a visual cue (the words "Alcohol" or "Water") for 2 seconds, followed by a fixation cross (jittered with a mean of 3 seconds and range of 0.5 to 6 seconds). The word "Taste" then appeared, corresponding to oral delivery of the indicated liquid at the start of the trial (2mL alcohol or water; 5 second duration). Participants were also instructed to press a button on a button box to indicate the point at which the bolus of liquid was swallowed and this information was used to model motion associated with swallowing. There were two runs of this task, with 50 trials per run. Alcohol and water were delivered through Teflon tubing using a computer-controlled delivery system. Red or white wine, based on participant preference, was used as the alcohol stimulus; previous work from our group has demonstrated that this paradigm has been used to effectively elicit alcohol-related neural activation (Ray et al., 2014). Carbonated alcohol, such as beer, could not be systematically administered with the paradigm apparatus and was not offered as a drink option to participants. Visual stimuli and response collection were programmed using MATLAB (Mathworks, Natick, MA) and Psychtoolbox (www.psychtoolbox.org), and visual stimuli were presented using MRI-compatible goggles.

Self-Administration Procedures

Participants completed an oral alcohol self-administration paradigm on day 5 of medication titration. At the start of this session, participants were required to test negative for substance use (except cannabis) and to have a BrAC of 0.00 g/dl. Female participants were also

required to test negative on a pregnancy test. Participants fasted for two hours prior to the session and were given a standardized meal before the alcohol administration. Participants initially completed an intravenous alcohol administration discussed in the primary manuscript (Ray, Green, et al., 2018). After completing the alcohol infusion paradigm and reaching a target BrAC of 0.06 g/dl, the IV was removed and, after a standardized period of five minutes, participants subsequently began an oral self-administration session at the testing center. Notably, the alcohol dose of 0.06 g/dl prior to the self-administration period was higher than the typical 0.03 g/dl priming dose implemented in self-administration tasks (McKee et al., 2009; 2006). During the self-administration period, participants were provided 4 mini-drinks of their preferred alcoholic beverage and allowed to watch a movie over a 1-hour period. The 4 mini-drinks allowed participants to consume up to 0.04 g/dl alcohol in total, and were individualized by participant gender, weight, height, and alcohol content. Participants were also told that they would receive 1 dollar for each drink remaining at the end of the session. At the end of the session, participants were provided a meal and required to stay at the testing center until their BrAC dropped below 0.02 g/dl or to 0.00 g/dl if driving.

Data Analytic Plan

For the taste cues paradigm, information regarding image acquisition parameters and preprocessing steps are available in Supplementary Materials and are derived from the primary manuscript (Lim et al., 2019; Ray et al., 2018). The main contrast of interest was the difference in activation corresponding to alcohol taste delivery and water delivery across the two task runs (Alcohol > Water), for each within-subject medication condition. Consistent with previous studies examining relationships among ventral striatum activity, subjective response to alcohol,

and drinking behavior (Morales et al., 2018; Nikolovaet al., 2016; Weafer et al., 2018), an anatomical bilateral ventral striatum region of interest was defined using the Harvard-Oxford atlas in standard MNI space and was transformed into participants' respective native space using FSL's FLIRT (see **Figure 2**). This ROI was selected because ventral striatum is most consistently elicited in alcohol cue and taste reactivity paradigms, as well as most frequently associated with behavioral measures and treatment response (Claus et al., 2011; Oberlin et al., 2016; Schacht et al., 2013). ROI selection was limited to one due to insufficient power to detect incremental model improvement with multiple ROIs. The mean contrast estimate values were extracted from this region for each subject and used in mixed models for group-level analysis (described below).

The self-administration paradigm yielded two outcome measures: (a) latency to first drink (in seconds, from the beginning of the session), and (b) total number of drinks consumed during the session (0-4 mini-drinks). To examine the relationship between alcohol taste-induced neural activation and self-administration, multilevel mixed poisson and cox (i.e. frailty) proportional hazard models were the primary analyses for total number of drinks and latency to first drink, respectively. Frailty models were fitted using a penalized partial likelihood approach available in SAS 9.4 (SAS Institute, Cary, NC). Primary analyses examined effects of variables of interest, including medication condition (naltrexone, placebo), alcohol consumption (30-day TLFB drinks per drinking day), and OPRM1. Due to concerns of overparameterization given the limited sample size, additional covariates of interest (medication randomization order, gender, alcohol abstinence days prior to scan, smoking status, consumption of preferred alcohol choice in scanner (yes/no)) were individually included in separate models to determine whether main effects of ventral striatum would be altered. Alpha corrections were not utilized in this

exploratory study due to limited sample size and constrained power. Tests of proportional hazards are included in Supplementary Materials and **Figures S1a-S1d.** Survival plots for latency to first drink, controlling for covariates within the final model (drinks per drinking day, medication condition, and OPRM1), were generated to further explore ventral striatum activation in predicting latency to first drink. Of note, a dichotomous median-split ventral striatum variable was created for ease of visualization of these relationships, but ventral striatum activation was included as a continuous variable in all models.

RESULTS

Characteristics for the final sample of 41 participants who completed both fMRI and self-administration tasks are presented in **Table 1**. Study participants were, on average, younger adult heavy drinkers of Chinese or Korean descent, and a minority reported recent cigarette smoking and/or cannabis use.

Fisher's exact tests tested the association between medication condition and 24 possible side effects as indicated by the SAFTEE checklist (Levine & Schooler, 1986). These tests indicated a significant association between medication and nausea ($p < .01$), such that 20% of individuals on naltrexone and 0% of individuals on placebo reported experiencing nausea. Similarly, there was a significant association between medication and fatigue ($p < .01$), such that 25% of individuals on naltrexone and 0% of individuals on placebo reported experiencing fatigue. There were no other significant associations among the remaining 22 side effects and medication.

Ventral striatum activation and self-administration outcomes are also presented in **Table 1** by medication condition. Of note, the two primary manuscripts from which this data is derived

did not identify significant effects of naltrexone on ventral striatum activation or self-administration outcomes (total number of drinks and latency to first drink) (Lim et al., 2019; Ray et al., 2018). Ventral striatum activation demonstrated moderate reliability (ICC = .47) and are consistent with other studies examining striatum in fMRI (Peters & Crone, 2017; Vetter et al., 2017) . Ventral striatum activation was also not significantly associated with any of the covariate variables used in the following analyses (*p*s = .11-.86).

Latency to First Drink

The distribution of latencies to first drink was non-normal. Across medication conditions, 52% of individuals refrained from drinking throughout the paradigm, 29% consumed a drink within the first three minutes of the paradigm, and 19% of individuals consumed their first drink at some point during the remainder of the session. Cox regressions for latency to first drink indicated a significant effect of ventral striatum activation, Wald $\chi 2 = 2.88$, $p = 0.05$, such that those with lower ventral striatum activation exhibited longer latencies to first drink (see **Figure 3**). Significant covariates included medication condition, Wald $\chi 2 = 5.99$, $p = 0.01$, such that naltrexone was associated with longer latency to first drink. OPRM1 was also significant, Wald $\chi 2 = 3.31$, $p = 0.03$, such that Asn40Asn homozygotes exhibited shorter latency to first drink. Other covariates of interest (e.g. medication randomization order, gender, medication side effects) were not associated with latency to first drink (*p*s=.15-.98). There were also no interactions of medication X gender on self-administration outcomes.

Total Number of Drinks

Multilevel Poisson analyses for total number of consumed drinks indicated a significant effect of ventral striatum activation, F(1, 38) = 5.90, p = .02. Significant covariates included medication, F(1, 38) = 7.93, p = .01, with naltrexone yielding lower consumption (B(SE) = -.60(.21). OPRM1 genotype was also significant, F(1, 38) = 5.37, p = .03, such that Asn40Asn homozygotes consumed a greater number of drinks. Drinks per drinking day were not associated with consumption, F(1, 38) = 3.58, p = .07. Other covariates of interest (e.g. medication randomization order, gender, medication side effects) were also not associated with total number of drinks, ps=.13-.54.

DISCUSSION

This study examined the relationship between alcohol cue-induced ventral striatum activation and alcohol self-administration in the laboratory. Results from this heavy-drinking sample of East Asians indicated that higher ventral striatum activation was associated with a shorter latency to first self-administered drink. Similarly, ventral striatum activation was positively associated with the total number of drinks consumed during the self-administration paradigm in this sample. These results remained significant after controlling for severity of drinking patterns, OPRM1, and medication condition.

Overall, this is the first study to examine whether neuroimaging outcomes of interest can predict responses within laboratory paradigms commonly used in the alcohol literature. This foundational work adds important validity to the hypothesized interplay between neural bases of alcohol craving and behavioral measures of alcohol seeking, namely alcohol self-administration in the human laboratory. These associations contribute to a growing literature on the translational value of neuroimaging paradigms in alcohol treatment, particularly in elucidating potential

mechanisms through which self-administration paradigms in AUD research are related to real world alcohol consumption (Grodin & Ray, 2019; Hendershot et al., 2017) . Such work is aligned with current efforts in behavioral treatments utilizing neuroimaging to study mechanisms of behavior change for substance use disorders; identifying those individuals with severe orbitofrontal cortex deficits, for instance, may be useful in guiding them away from treatments focused on increasing the salience of future negative consequences of substance use (Morgenstern et al., 2013). In a similar fashion, adjunctive fMRI has been used to train individuals with substance use disorders through resonance-based breathing to reduce visual processing of drug cues and increase activation in areas implicated in internally directed cognition (Bates et al., 2019). Elucidating the translational value of these various experimental paradigms is strongly indicated, as AUD medications can exhibit differential results based on the utilized paradigm (e.g. alcohol challenge or self-administration; (Chukwueke & Le Foll, 2019)) and such variability may in turn inform precision medicine efforts. Expanding the study of inter-experimental paradigms may also shed light on aspects of alcohol consumption unique to individual paradigms. For instance, a greater understanding of individuals' experiences in the transition between the first and subsequent drinks may be an important point of clinical interventions when discussing naltrexone use.

While the primary aim of this study was not focused on genetic determinants of self-administration, it is notable that genotypes encoding the binding potential of mu-opioid receptors (OPRM1) were associated with self-administration outcomes. While it is theorized that individuals with at least one copy of the G-allele for OPRM1 exhibit greater vulnerability to developing AUD, meta-analyses have been mixed, with findings that such an association may not be reliable (Kong et al., 2017; Sloan et al., 2018), are population specific (Chen et al., 2012),

or that G-allele confers a modest protective effect on general substance dependence in European-ancestry cohorts (Schwantes-An et al., 2016). In this study, G-allele carriers of OPRM1 exhibited lower total consumption relative to A-allele carriers at a statistical trend level, as well as slower latency to first drink. This finding is consistent with the primary analyses for this data (Ray et al., 2018), which indicated that G-allele carriers of OPRM1 also reported less severe drinking history and lower AUDIT scores compared to Asn40 homozygotes and may, in turn, help to explain these findings. In sum, we accounted for genetic factors in these analyses given their theoretical and practical salience (Hart & Kranzler, 2015), particularly in this population (Cservenka et al., 2017). And while the genetic findings are notable and largely consistent with the literature, the primary focus on the study is on the fMRI to human laboratory association. This is the area in which the present analyses make a substantive contribution to the literature by supporting a long hypothesized, yet rarely tested, association between brain and behavior.

Finally, this study identified significant effects of naltrexone in increasing latency to first drink and decreasing total alcohol consumption. Notably, while these contrast the primary study (*N*=77) results from which the data are derived (Ray et al., 2018) the current study is a secondary analysis of a subsample of participants (*N*=41) who had completed both neuroimaging sessions. While inclusion of VS activation may have helped to improve model fit, the primary study had greater power in order to test pharmacogenetic effects. For these reasons, while it is possible that consideration of neuroimaging outcomes help elucidate AUD pharmacotherapy effects, replication using larger samples is warranted.

On balance, this study should be interpreted in light of its strengths and limitations. Strengths included assessment of multiple experimental procedures used in the medication development literature and consideration of multiple psychiatric and genetic predictors of self-

administration in the statistical analyses. Another strength is the test of hypothesis at the within-subjects level of analysis. As argued by Curran and Bauer (2011), several psychological processes which are inherently within-person processes, such as the relationship between how one's brain processes alcohol cues and how much s/he wants to drink in the future, are presumed to be explained in between-subjects models, when in fact, within-subject analyses provide a more representative test of the process at hand (Curran & Bauer, 2011). Thus, a within-subjects approach represents a more robust, and methodologically adequate, test of the association between brain and behavior. One of the most important limitations of the current study is a constrained sample and power; given the exploratory nature of this study, alpha corrections were not implemented. A limitation of the taste cues fMRI paradigm used in this study is that it was modified to reduce trial duration in order to increase the number of trials for analysis; in contrast to the original task (Filbey et al., 2008), a whole-brain analysis of the task did not elicit significant clusters of mesocorticolimbic, including ventral striatum, activation. Therefore, replication using other tasks that more strongly elicit ventral striatum activation are needed, both to induce significant enough variability to test medication effects and also to translate such effects into another subsequent experimental modality. Variations of the Monetary Incentive Delay task that administer beer may be particularly useful in disentangling whether anticipation, relative to receipt, of alcohol taste are differently discriminant in predicting self-administration (Groefsema et al., 2019) Relatedly, the taste cues paradigm was limited to the choice of red or white wine, which did not always correspond with participants' drink of choice; while this correspondence was not a significant covariate in self-administration outcomes, administering drink of choice may increase external validity of the imaging task. Another potential weakness is that medication effects from the primary manuscripts were null; future studies are needed to

corroborate that medication effects are consistent across paradigms, particularly in identifying significant such effects. An additional warranted question is whether such consistency of medication effects in laboratory studies would translate directly to clinical outcomes and treatment-seeking populations. Lastly, the "priming dose" that preceded the self-administration period was higher than the usual 0.03 g/dl reported in the literature. While the higher priming dose of alcohol in this study did not suppress alcohol self-administration, it may be interpreted differently in that participants were seeking to self-administer to reach high levels of BrAC, perhaps binge-like levels. If that was the case, results would remain highly relevant and consistent with recent efforts to phenotype binge-drinking in the human laboratory (Gowin et al., 2017).

Limitations notwithstanding, the present findings provide proof-of-concept that neuroimaging and laboratory paradigms may be closely linked. Further, neuroimaging may be a useful tool to explore in greater detail how different paradigms are related to real world consumption behavior. Future studies are warranted to replicate the current results and to identify, refine, and implement translational paradigms in AUD research.

Table 1. Sample Characteristics (*N*=41)

Variable	Statistic (M(SD))
Age	28.27 (6.94)
Sex (% Female)	37%
Ethnicity (n(%))	
Chinese	17 (41.5%)
Japanese	3 (7.3%)
Korean	19 (46.3%)
Taiwanese	2 (8%)
AUDIT Total	14.46 (5.19)
30-Day TLFB Drinking Days	13.66 (6.56)
30-Day TLFB Drinks Per Drinking Day	4.79 (2.29)
Cigarette Smokers (n(%))	12 (29%)
30-Day TLFB Cigarettes Per Day	4.00 (4.89)
Cannabis Users (n(%))	4 (10%)
ADH1B (AA/AG/GG)	5/7/19
ALDH2 (AA/AG/GG)	0/6/35
OPRM1 (AA/AG/GG)	18/17/6
Placebo Self-Administration % who drank (n(%))	39 (53%)
Placebo Self-Administration Latency to First Drink (median)	180 s
Naltrexone Self-Administration % who drank (n(%))	31 (41%)
Naltrexone Self-Administration Latency to First Drink (median)	180 s
Placebo TLFB Pre-scan Days since Last Drink	2.39 (2.20)
Placebo Alcohol > Water Ventral Striatum Activation	1.44 (7.42)
Naltrexone TLFB Pre-scan Days since Last Drink	2.85 (1.65)
	2.83 (9.08)
Naltrexone Alcohol > Water Ventral Striatum Activation	4.86 (3.01)
Washout Period TLFB Drinks Per Drinking Day	3.80 (4.23)
Washout Period TLFB Cigarettes Per Day	

Note. AUDIT = Alcohol Use Disorders Identification Test. TLFB = Timeline Follow-Back. Ventral Striatum contrast estimate units of measure are arbitrary units; higher values correspond to greater activation.

Table 2. Outcomes for latency to first drink and total number of drinks

Outcome: Latency to First Drink

Variable	**Wald Chi-Square**	**Adjusted *p*-Value**
Ventral Striatum	2.88	.05
Medication	5.99	.01
OPRM1	3.31	.03
TLFB Drinks Per Drinking Day	6.39	.003

Outcome: Total Number of Drinks

Variable	**Estimate (SE)**	***p*-Value**
Ventral Striatum	.03(.01)	.02
Medication	-.60(.21)	.01
OPRM1	.78(.34)	.03
TLFB Drinks Per Drinking Day	.13(.07)	.07

Note. TLFB = Timeline Follow-Back. Latency to first drink outcomes generated from cox frailty models that produce adjusted degrees of freedom and p-values. Total number of drinks outcomes generated from multilevel poisson models.

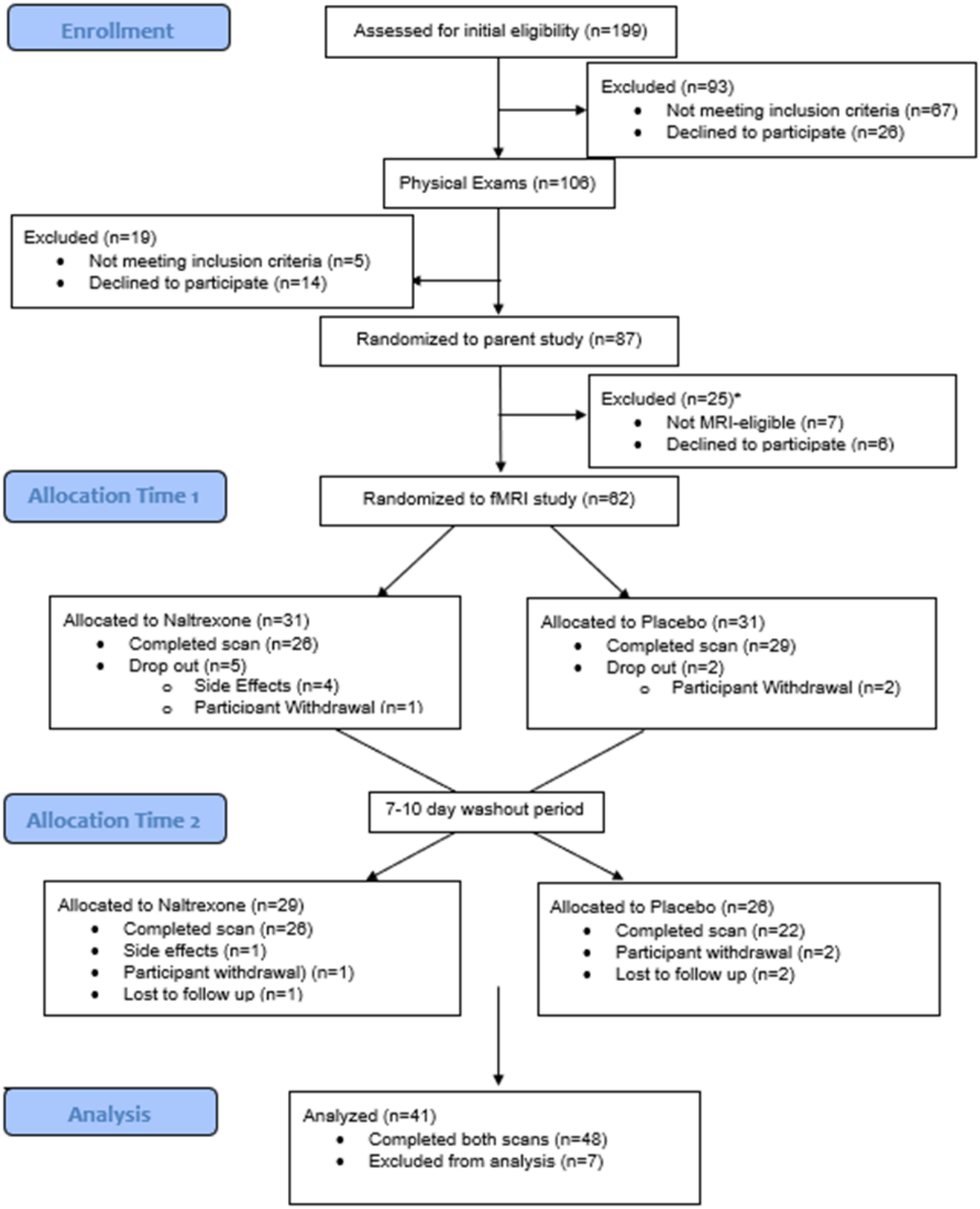

Figure 1. CONSORT Diagram

*The scanner utilized for the study was upgraded towards the end of the study. Due to parameter compatibility concerns, scanning data was not collected from 12 MRI-eligible participants.

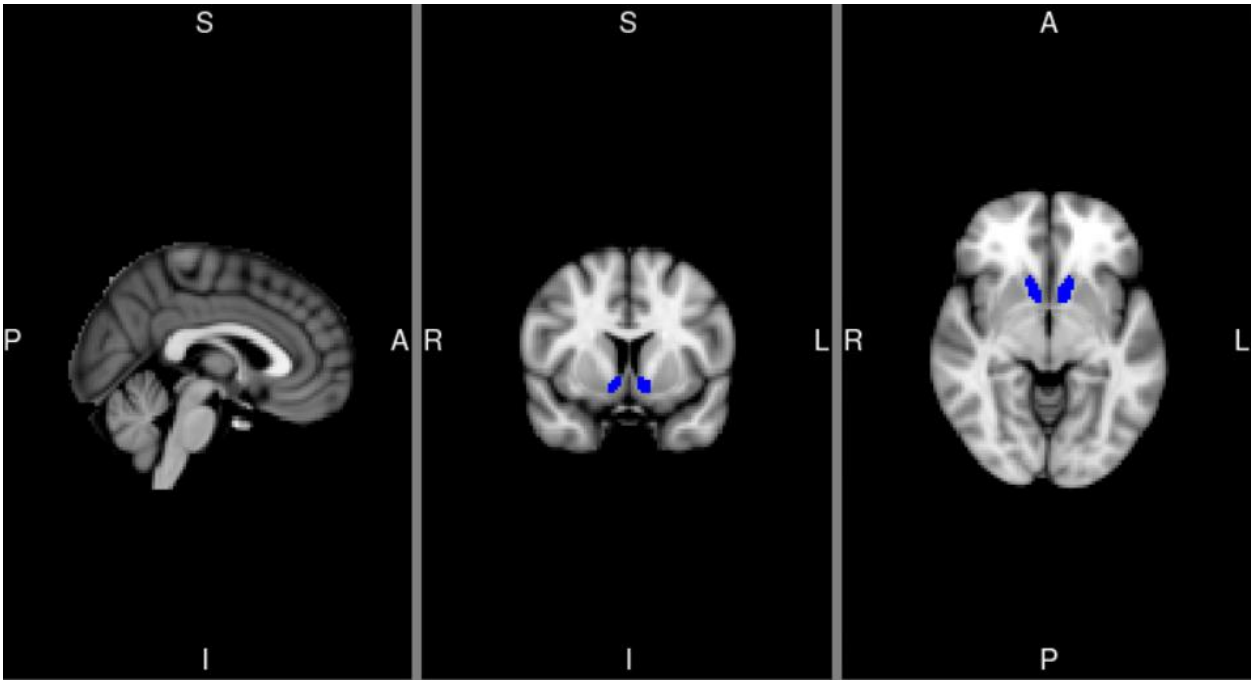

Figure 2. Anatomical region of interest mask for ventral striatum (left and right: 108 and 86 voxels, respectively). ROI extracted from the Harvard Oxford atlas thresholded at 25% based on the maximum probability labels. MNI coordinates for depicted slices are X=2 (left), Y=8 (middle), Z=-6 (right). L=Left, R=right, S=superior, I=inferior, A=anterior, P=posterior.

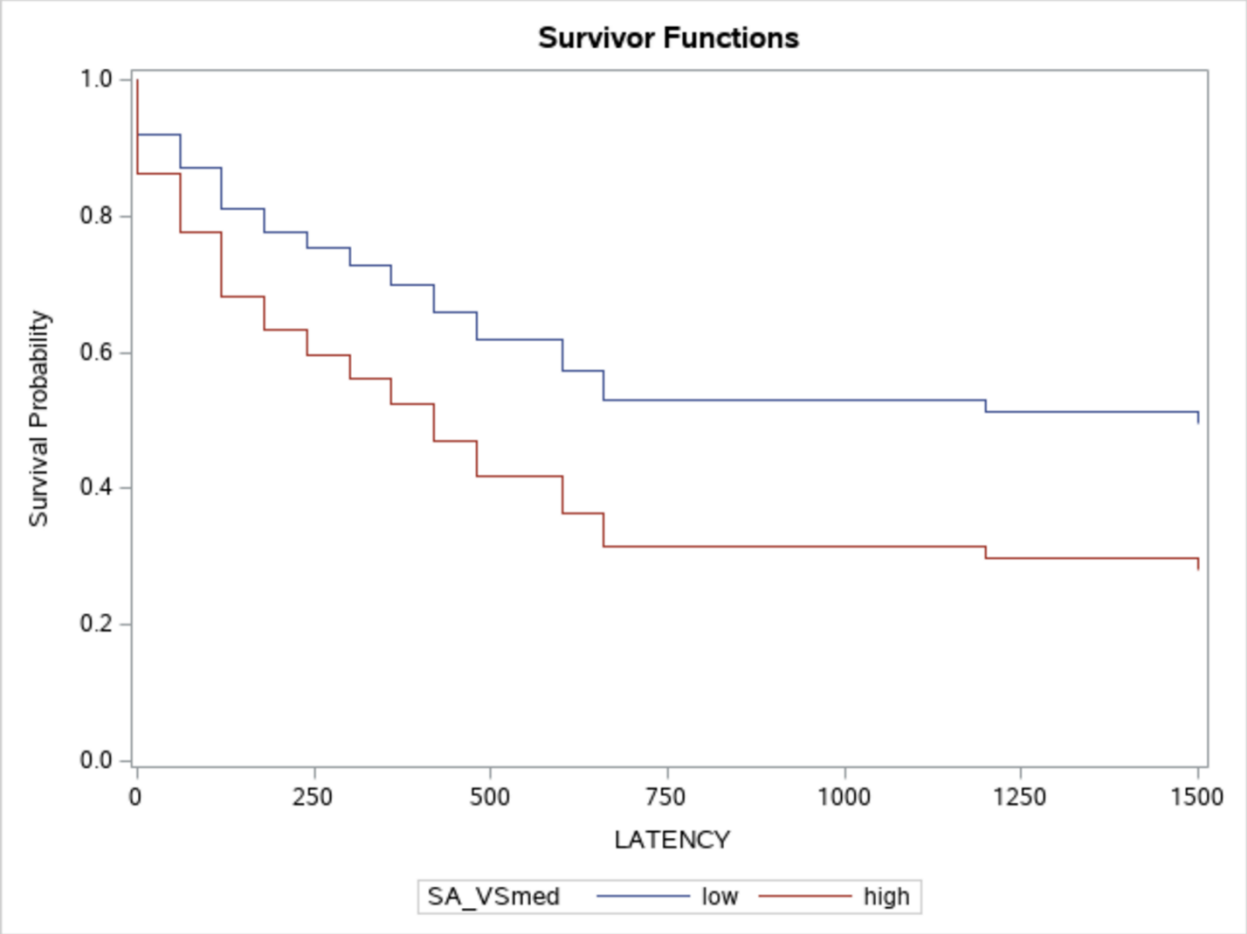

Figure 3. Multilevel cox regressions depicting the relationship between alcohol-elicited ventral striatum activation and subsequent latency to first drink (seconds), controlling for medication, OPRM1, and Timeline Follow-Back drinks per drinking day. Ventral striatum median-split activation (SA_VSmed; 0 = below median, 1 = above median) is for visualization purposes only.

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Supplementary Materials

Genotyping

Oragene saliva kits were used to collect samples for DNA analysis. The UCLA Genotyping and Sequencing (GenoSeq) Core assayed *OPRM1* (rs1799971), alcohol dehydrogenase gene (*ADH1B*, rs1229984), and aldehyde dehydrogenase gene (*ALDH2*, rs671). Polymerase chain reaction (PCR) primers were labeled with fluorescent dye (6-FAM, VIC, or NED), and PCR was performed on Applied Biosystems dual block PCR thermal cyclers. An AB 7900HT Fast Real-Time PCR System ran the SNP sequencing and analyzed data using the Sequence Detection Systems software version 2.3. Each run included two positive control samples. Allele calling software automatically scored the genotypes, which was verified by visual inspection. The average call, reproducibility, and concordance rates are 96%, 99.7%, and 99.8%, respectively, at the UCLA GenoSeq Core.

Image Acquisition

Scanning took place at the UCLA Staglin Center for Cognitive Neuroscience on a 3.0T Siemens Trio scanner. A T2-weighted, high resolution matched-bandwidth (MBW) anatomical scan (Time to Repetition (TR) = 5,000 ms, time to echo (TE) = 34 ms, flip angle = 90 degrees, voxel size: 1.5 mm x 1.5 x 4 mm, field of view (FOV) = 192 mm^2, 34 slices, ~1.5 minutes) and a T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2,530 ms, TE = 1.74 ms, Time to Inversion (TI) = 1,260 ms, flip angle = 7 degrees, voxel size: $1mm^3$, FOV = 256 mm^2, ~6.2 minutes) were acquired for co-registration to the functional data. Two runs of a T2*-weighted echo planar imaging scan (TR = 2,000 ms, TE = 30 ms, flip angle = 90 degrees, voxel size: 3 mm x 3 mm x 4 mm, FOV = 192 mm^2, 325 TRs, ~10.83 minutes/run) were

acquired to examine the BOLD signal during the Alcohol Taste Cues Task (total time: ~22 minutes). The first six TRs were discarded to allow for steady-state longitudinal magnetization to be reached.

Image Preprocessing

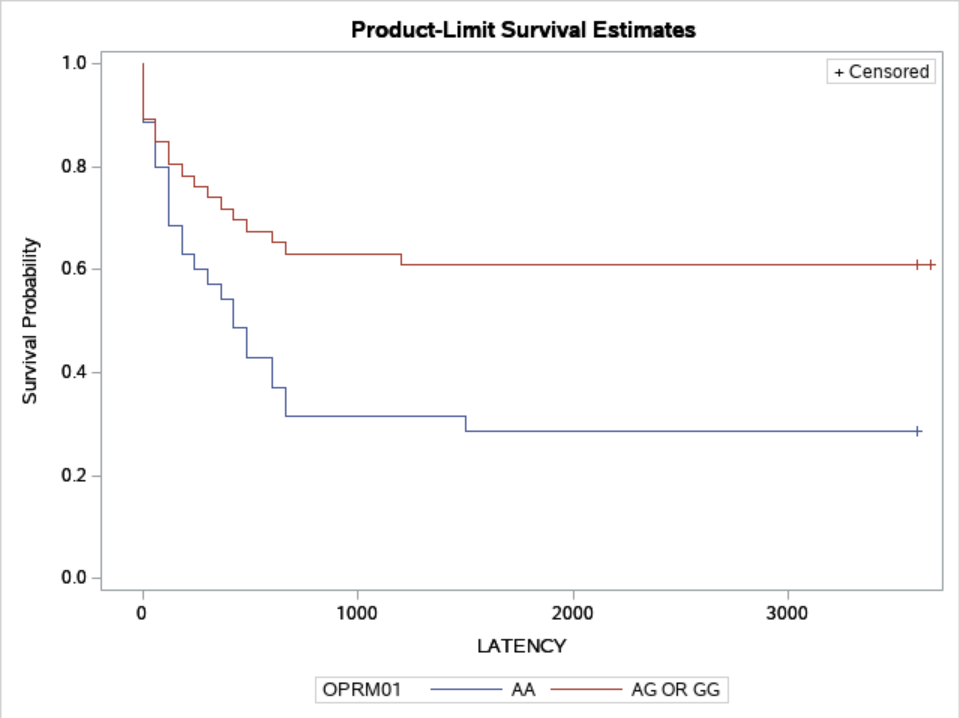
Preprocessing of imaging data was conducted using FMRIB's Software Library (FSL 5.0) (www.fmrib.ox.ac.uk/fsl). Motion correction was performed using FSL's MCFLIRT with the middle volume as the reference image and normalized correlation as the cost function. FSL's Brain Extract Tool (BET) was used to remove skull and non-brain tissue from both the structural and functional scans (Pruim et al., 2015). To reduce the effect of physiological noise and motion, including that associated with swallowing, data were denoised using ICA-AROMA, with a non-aggressive approach (Pruim et al., 2015). Images were preprocessed using high-pass temporal filtering (100 s cutoff) through FSL's FMRI Expert Analysis Tool (FEAT, Version 5.63), and smoothed with a 6 mm full width half maximum Gaussian kernel. Data for each subject were registered to the MBW, followed by the MPRAGE using affine linear transformations, and then normalized to the Montreal Neurologic Institute (MNI avg152) template. Registration was further refined using FSL's nonlinear registration tool (FNIRT) (Andersson, Jenkinson, & Smith, 2007). Of the 48 participants who completed both scans, six participants were excluded from analyses due to excessive head motion (>2 mm translation) and one participant was excluded due to poor registration. Thus, the final analyses include 41 participants.

All first-level analyses of imaging data were conducted within the context of the general linear model (FSL's FEAT). Regressors for each task condition were formed by convolving delta functions representing the 5 sec period of taste delivery with a double-gamma hemodynamic

response function (HRF). The temporal derivative of this function was also included as a covariate to account for small temporal shifts in the hemodynamic response. Six motion regressors representing translational and rotational head movement were also entered as regressors of no interest. "Spike" regressors were created for each image with a frame displacement value above threshold (75% percentile plus 1.5*inter-quartile range) using FSL's fsl_motion_outliers. FSL's root mean square intensity difference of volume N to volume N+1 (DVARS) calculation indicated that across participants, average DVARS across task trials ranged from .12 to .63, with mean of .27 and SD of .11.

Multilevel Cox Proportionality of Hazards

For dichotomous and categorical variables, Kaplan-Meier plots of log(-log(survival)) versus log(latency) were used to assess the assumption of proportionality of hazards for cox models. These plots indicated that these assumptions were met for OPRM1 and medication condition. For continuous variables, plots of Schoenfeld residuals versus latency were generated. Analyses examining latency as a function of the continuous variable*log(latency) were used to test the proportional hazards assumption. The interaction of this time-varying covariate and ventral striatum activation (Parameter estimate = -.04, SE = .02, p = .10, Hazard Ratio = .96), as well as for drinks per drinking day (Parameter estimate = .10, SE = .10, p = .30, Hazard Ratio = 1.11) indicated that the proportional hazards assumption was met. Plots are depicted in **Figures S1a-S1d**.



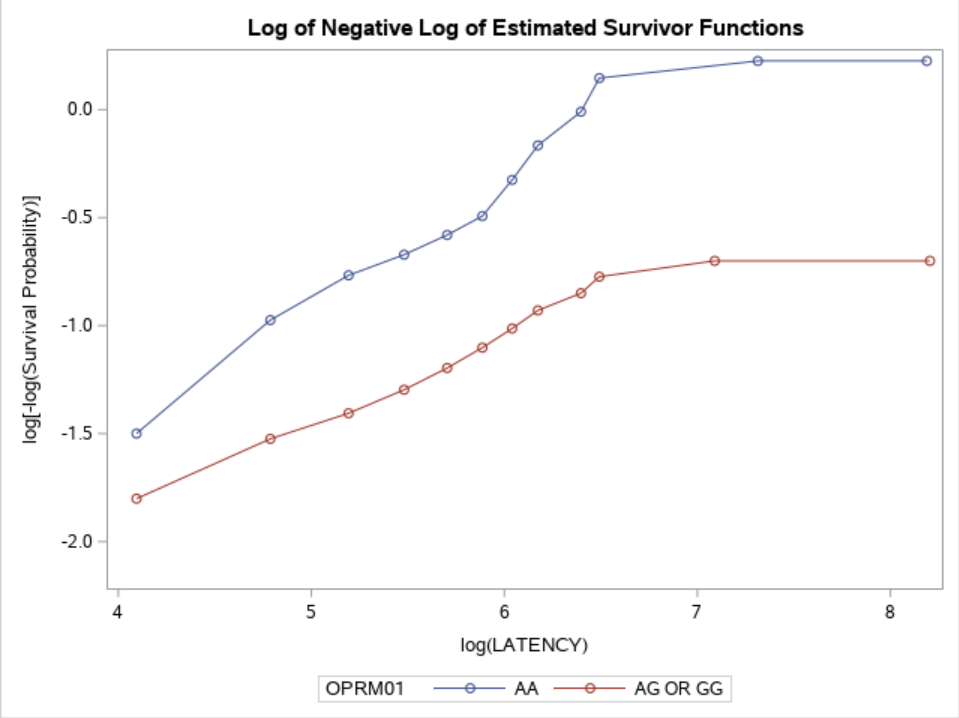
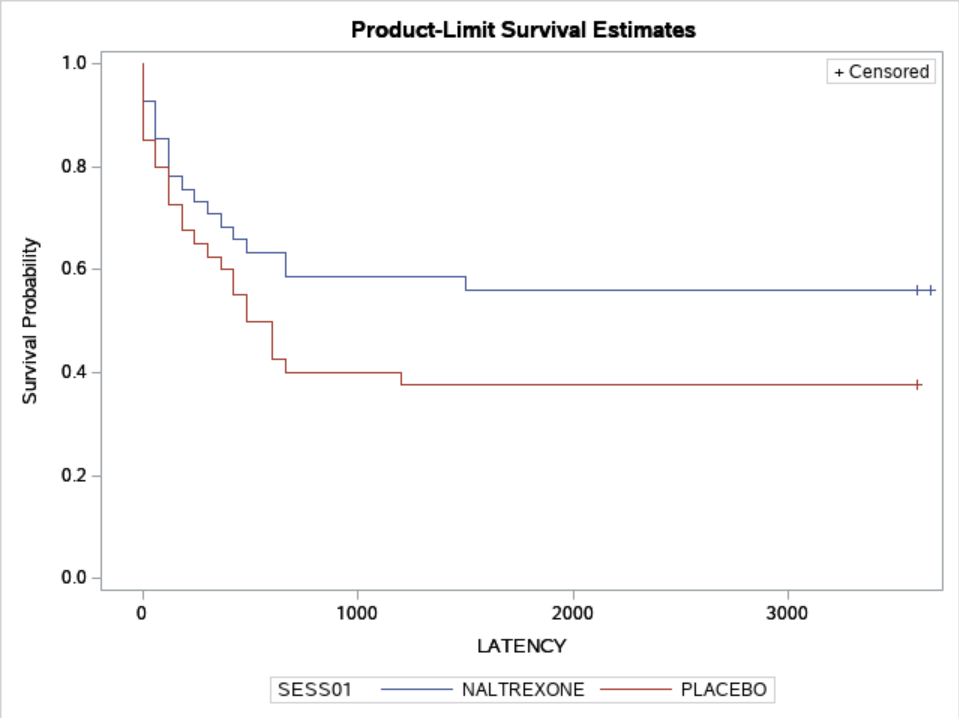


Figure S1a. Kaplan-Meier curves for OPRM1 genotype vs. time and log(-log(survival)) versus log(latency)



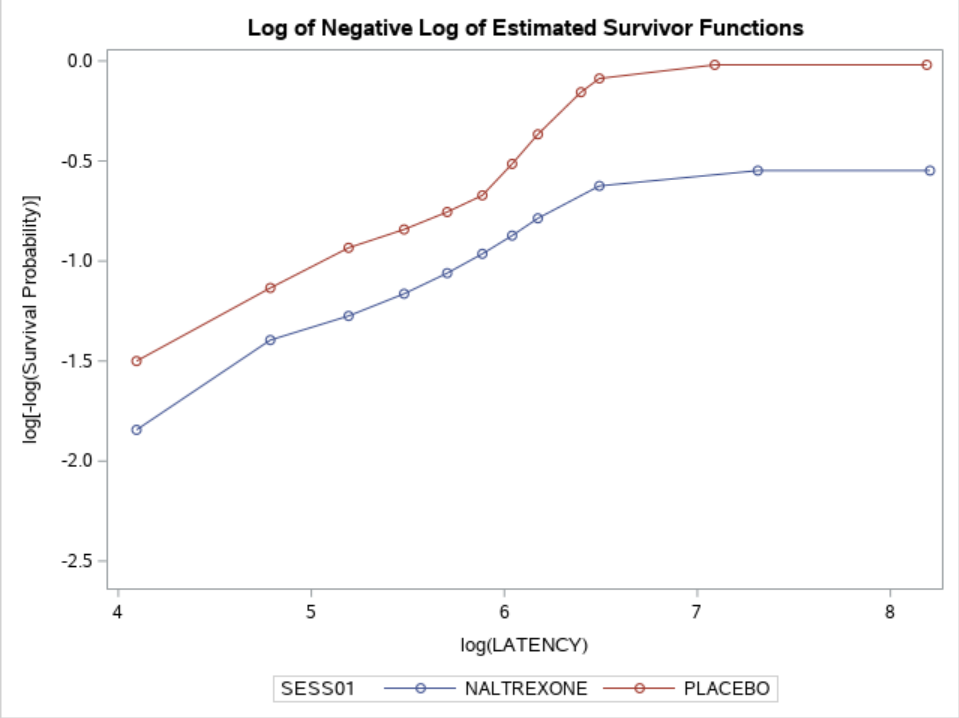


Figure S1b. Kaplan-Meier curves for medication condition (SESS01) vs. time and log(-log(survival)) versus log(latency)

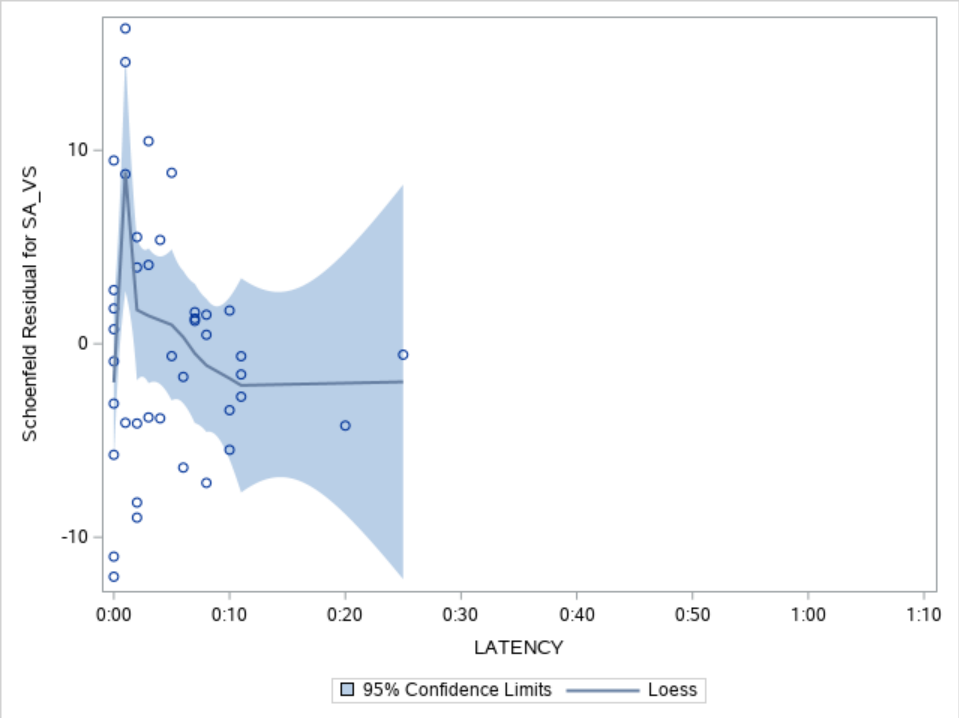


Figure S1c. Loess curve for Schoenfeld residuals of ventral striatum activation (SA_VS) vs. time.

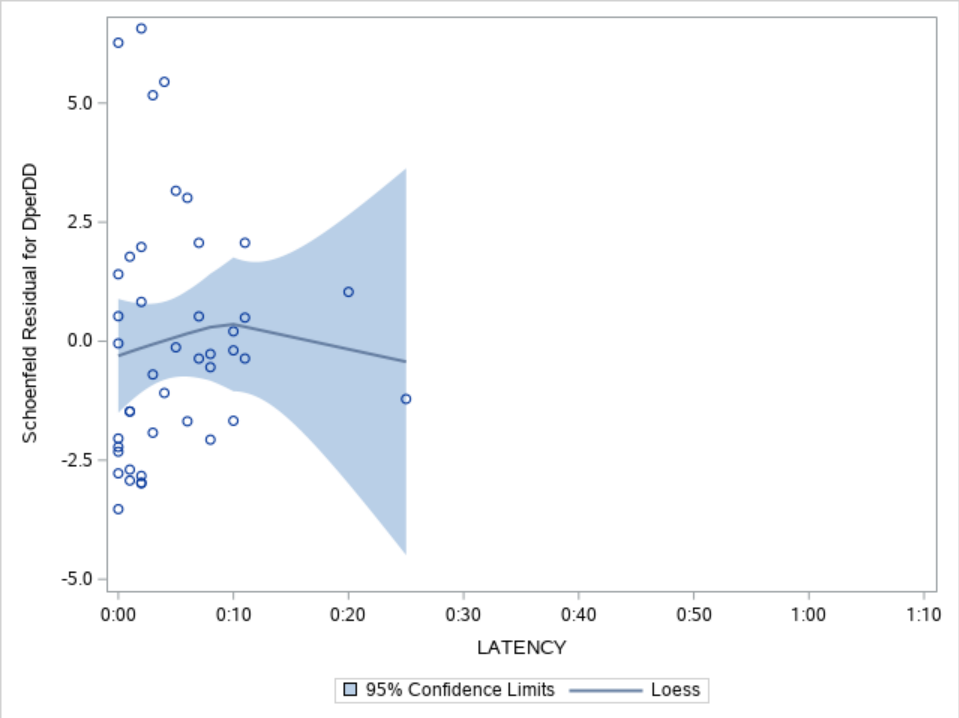


Figure S1d. Loess curve for Schoenfeld residuals of Timeline Follow-Back Drinks per Drinking Day (DPDD) vs. time

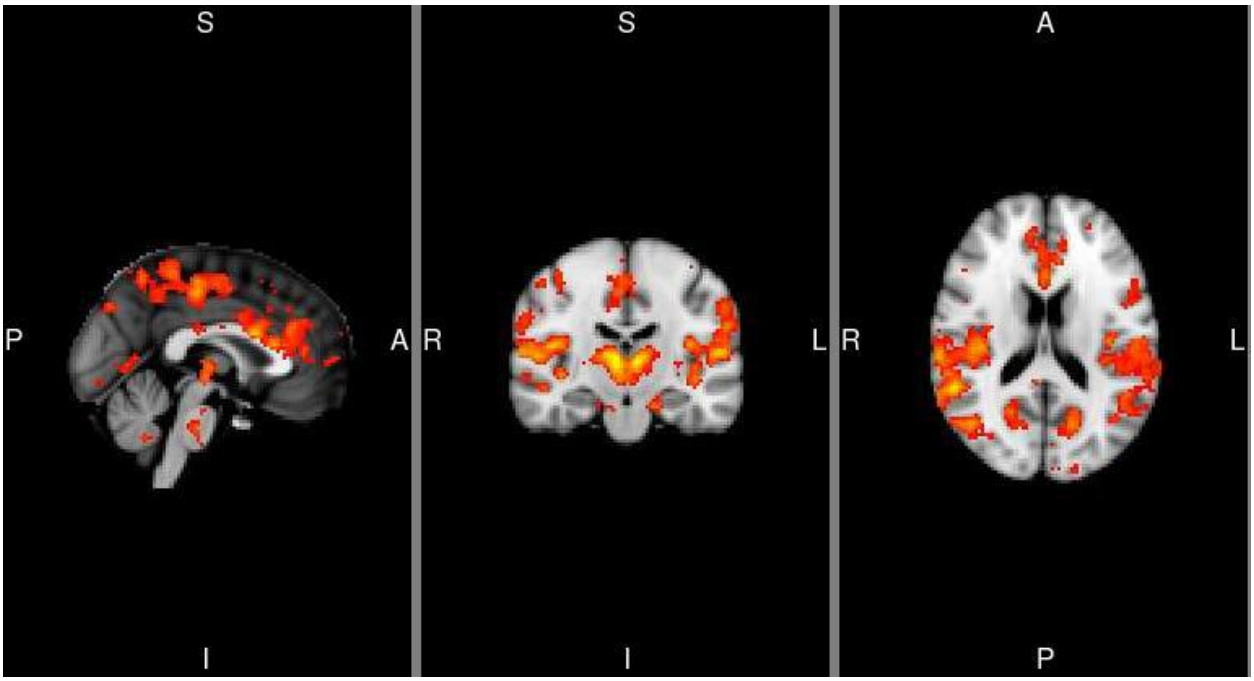


Figure S2. Uncorrected Whole-brain Alcohol > Water Taste task-related activation. MNI coordinates for depicted slices are X = 0, Y = -18, Z = 18. Color bar represents z-values. L=left, R=right, S=superior, I=inferior, A=anterior, P=posterior

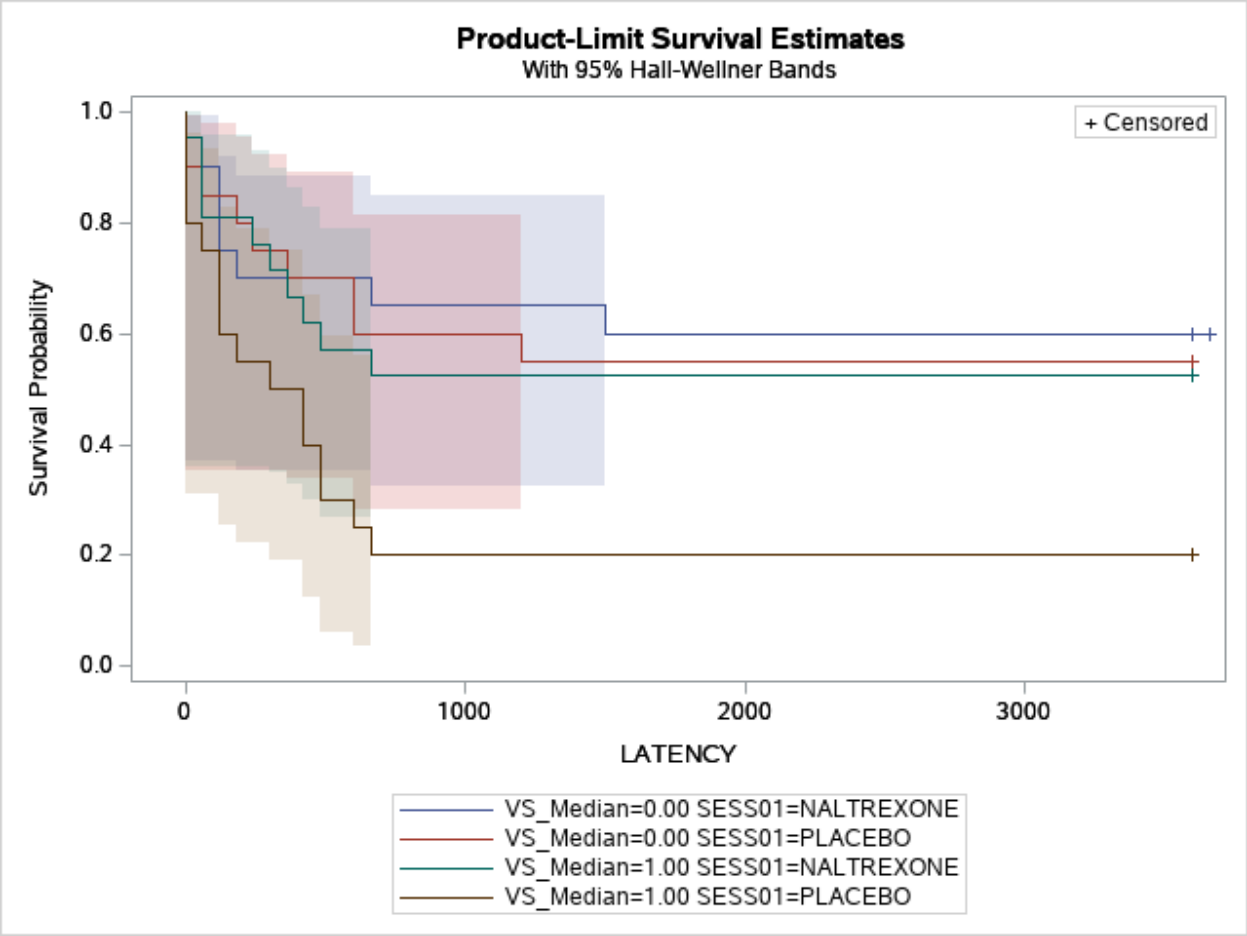


Figure S3. Cox proportional regressions stratifying latency to first drink by medication condition (SESS01) and ventral striatum median-split activation (VS_Median). Ventral striatum median-split activation (VS_Median; 0 = below median, 1 = above median) is for visualization purposes only.

SMOKING CUE-INDUCED NEURAL ACTIVATION AS A PREDICTOR OF SMOKING CESSATION OUTCOMES

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Abstract

Introduction: Heavy-drinking smokers experience significant barriers in smoking cessation. Combination varenicline plus naltrexone (VAR+NTX) may be an effective treatment targeting co-reinforcing smoking and drinking behavior. Increasingly, neuroimaging paradigms are explored as predictive translational tools in medication development. No studies to date, however have examined the predictive utility of neuroimaging responses for smoking cessation outcomes among heavy-drinking smokers. The current study therefore examines whether smoking cue-induced activation in 4 regions of interest are predictive of bioverified smoking abstinence and cigarettes per day reported at 6-months post-quit.

Methods: Participants in this secondary analysis (N = 19) were heavy-drinking smokers in a larger randomized, double-blind comparison trial of VAR+NTX versus VAR alone. Participants completed a neuroimaging smoking cue task after reaching a stable dose of their medication and prior to their quit date.

Results: Both medication conditions appeared to suppress activation attributable to the smoking > neutral conditions. Smoking cue-induced activation in Anterior Cingulate Cortex, Ventral Striatum, Orbitofrontal Cortex, and Anterior Insula were not significantly predictive of either bioverified smoking cessation rates or cigarettes per day reported at 6-month follow-up.

Conclusion: This secondary analysis was likely limited in power to detect the predictive validity of smoking cue-induced activation, or to compare medication conditions or subgroups of the sample. Data from this study may benefit future meta-analyses and data-driven studies that combine such available neuroimaging data to more definitely establish predictive validity of these paradigms.

INTRODUCTION

Recent surveys in the United States indicate that alcohol and tobacco consumption are highly comorbid; compared to those without an alcohol use disorder (AUD), individuals have 3.2 times greater odds of meeting criteria for a nicotine use disorder (Chou et al., 2016). Such co-consumption may also contribute to increased incidence of negative health outcomes such as multiple types of cancer (Dal Maso et al., 2016). Coupled with these health risks, greater alcohol use is associated with lower odds of smoking cessation (Toll et al., 2012), as well as faster lapses after initial smoking cessation attempts (Cook et al., 2012). Such heavy drinking smokers therefore represent a vulnerable subpopulation for which tailored interventions have been developed but remain needed to address significant barriers in smoking cessation (Kahler et al., 2017).

There is evidence that combination treatments can address smoking cessation difficulties among heavy-drinking smokers. Specifically, the use of smoking pharmacotherapies such as varenicline (VAR; agonist at the alpha4beta2 nicotinic acetylcholine receptor) in combination with alcohol pharmacotherapies like naltrexone (NTX; mu opioid receptor antagonist) may be useful in reducing alcohol-related smoking lapses. Relatively to monotherapy, VAR+NTX has been shown to reduce cravings for cigarettes during medication titration (Ray et al., 2014), reduce smoking after a priming alcohol dose (Roberts et al., 2018), and attenuate smoking topography behaviors among heavy-drinking smokers (Roche et al., 2015). To date, however, no large clinical trials have tested the efficacy of VAR+NTX against monotherapies for smoking cessation.

In addition to identifying promising treatments, another important avenue is improving the efficiency of the medication development pipeline. Recent efforts have included the

exploration of clinically translatable neuroimaging paradigms in treatment development. Within the smoking literature, several functional magnetic resonance imaging (fMRI) tasks have been used to elicit neural correlates of cigarette craving through the use of visual smoking cues (Brody et al., 2002). Studies have found that FDA-approved treatments for smoking cessation such as varenicline blunt smoking cue-induced activation in regions processing reward salience (i.e. ventral striatum and medial orbitofrontal cortex), as well as reduce self-reported cigarette cravings (Franklin et al., 2011). Varenicline has also been shown to downregulate functional connectivity in an amygdala-insula circuit in abstinent smokers at rest (Sutherland et al., 2013). Similarly, smokers treated with buproprion (an FDA approved treatment for smoking cessation) relative to placebo exhibit reduced smoking cue-induced activation in ventral striatum, orbitofrontal cortex, and anterior cingulate (Culbertson et al., 2011).

Beyond examining pharmacotherapy effects on smoking cue-induced activation, there is an increasing body of research examining whether neuroimaging outcomes can directly predict smoking cessation outcomes. Among treatment-seeking adult smokers enrolled in 8 weeks of behavioral intervention and nicotine patch, smoking cue-induced anterior cingulate cortex activation was positively associated an unsuccessful quit attempt (i.e. lapse) during the clinical trial (Janes et al., 2010). Within a 12-week clinical trial comparing the effectiveness of varenicline, bupropion, and placebo, smokers who demonstrated lower pre-quit striatum and medial prefrontal cortex activation in response to pleasant stimuli, relative to smoking cues, were less likely to be abstinent 6 months after the quit attempt across medications (Versace et al., 2014). Among smokers treated with 12 weeks of varenicline, those who relapsed during the treatment period exhibited increased resting-state connectivity among dorsolateral prefrontal cortex, temporal gyrus, and cerebellum compared to individuals who successfully quit (Shen et

al., 2017). Finally, in an experimental study in which smokers quit for 7-days after brief counseling session, smoking cue-induced anterior cingulate activation was positively associated with relapse likelihood (Allenby et al., 2020). These studies utilize different neuroimaging methods and tasks, but suggest that there may be common cue-induced responses that may be predictive of smoking cessation for multiple types of treatment.

A current gap in this literature is the examination of cue-induced activation predicting smoking cessation success among heavy-drinking smokers. The majority of smoking pharmacotherapy trials exclude participants with alcohol use disorder, and it is therefore not known whether these neuroimaging findings would translate for this subpopulation of smokers who experience significant barriers to quitting. A previous study in our lab has found that a combination of VAR+NTX, relative to placebo and naltrexone-alone, reduced smoking cue-induced anterior cingulate activation among heavy-drinking smokers (Ray et al., 2015). The current study extends these findings to examine whether pre-quit smoking cue-induced activation predicts smoking cessation success at 6-month post-quit, as well as time to lapse and time to relapse. Specifically, based on previous studies and emerging data on cue-induced cigarette craving (Janes et al., 2020; 2019; 2010; Sweitzer et al., 2016), we examine cue-induced activation in four regions of interest (ROIs) – anterior cingulate cortex (ACC), anterior insula (aINS), orbitofrontal cortex (OFC) and ventral striatum (VS), among a sample of heavy-drinking smokers in a double-blind, randomized comparison trial of VAR+NTX versus VAR alone. We anticipated that activation in these regions would be negatively associated with 6-month follow-up rates of smoking abstinence, and positively associated with cigarettes per day.

METHODS

Parent Study Design

The parent study for this secondary analysis was a 6-month (26-week) randomized, double-blind comparison trial of VAR (2mg) versus VAR (2mg) + NTX (50 mg) for smoking cessation and drinking reduction in a community sample of heavy-drinking smokers. Participants were screened, randomized, and received medication for a total of 12-weeks. During the initial weeks of medication titration, participants set smoking quit dates with a master's level clinician during a 30-45 minute counseling visit. Follow-up visits occurred at weeks 4, 8, 12, 16, and 26 weeks post-quit attempt. Participants were queried about medication side effects throughout the titration period by the study physician; no participants dropped out of the study due to side effects.

Participant Recruitment

Participants for this study included treatment-seeking heavy-drinking smokers recruited in the Los Angeles metropolitan area through print, digital, and public transportation advertisements. Inclusion criteria were: 1) ages 21-65; 2) smoke at least 5 cigarettes per day (as assessed with the Timeline Follow-Back (TLFB) (Brown et al., 1998); and carbon monoxide reading greater than 4 ppm to verify smoker status at baseline; 3) be classified as a heavy drinker (Willenbring et al., 2009): for men, >14 drinks per week or at least 5 drinks per occasion at least once per month over the past 12 months. For women, >7 drinks per week of at least 4 drinks per occasion at least once per month over the past 12 months. Exclusion criteria were: 1) Clinically significant alcohol withdrawal, indicated by a score of at least 10 on the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-AR) (Sullivan et al., 1989); 2) lifetime history of psychotic or bipolar disorders; 3) meeting diagnostic criteria for substance use disorder other

than alcohol; 4) major depressive disorder with suicidal ideation. Female participants of childbearing age were also required to be practicing effective contraception and could not be pregnant or nursing. Additional exclusion criteria for the neuroimaging scan included: 1) history of epilepsy, seizures, or severe head trauma; 2) non-removable ferromagnetic objects in body; and 3) claustrophobia. All procedures were approved by the University of California, Los Angeles Institutional Review Board.

Medication Dosing Schedule

Medication titration for varenicline followed FDA guidelines for smoking cessation: 0.5 mg once daily for 3 days, 0.5 mg twice daily for 4 days, and 1 mg twice daily for the remainder of the 12-week treatment. For naltrexone, participants took 25 mg once daily for the first 5 days, and 50 mg for the remainder of the 12-week treatment. Study medications were tapered off after week 12.

Neuroimaging Session

Participants completed one neuroimaging session that was scheduled between day 9 and 12 of medication titration, to both reach a steady state on the target dose of the assigned medication and to scan prior to the counseling session and their scheduled quit attempt. At the visit, participants were breathalyzed to ensure a breath alcohol concentration of 0.00 g/dl, and were also permitted to smoke a cigarette one hour prior to the scan to control for smoking recency effects. A negative pregnancy screen for female participants was also required. To assess for cigarette and alcohol craving before the fMRI scan, participants completed a smoking craving

questionnaire (QSU) (Cox et al., 2001) and alcohol craving questionnaire (AUQ) (Bohn et al., 1995).

fMRI Task

The cigarette cues task employed in this study involved viewing blocks of videotaped cues from a first-person perspective. These videos are divided into those associated with smoking content (e.g. a person smoking a cigarette as they eat a meal) or neutral content (e.g. person writing in a journal), with each video lasting 45 seconds. The task was comprised of 12 total trials (6 cigarette and 6 neutral) pseudorandomly presented across participants, with the first video always being a neutral video. After each 45-second video, there was a 10-second cigarette-urge rating period, 1 second of response feedback, and a 10-second interstimulus period. Urge ratings were on a scale of 1 (no urge at all) to 4 (very high urge), and were indicated through a 4-button response box placed in the participants' right hand. Stimuli presentation and response collection was programed using MATLAB (Mathworks, Natick, MA) and Psychtoolbox (www.psychtoolbox.org). This task was developed by Brody and colleagues (Brody et al., 2002) and has been previously utilized in our research group to test the effects of pharmacotherapy on correlates of cue-elicited cravings (Ray et al., 2015).

Image Acquisition

Scanning took place at the UCLA Staglin Center for Cognitive Neuroscience on a 3.0T Siemens Prisma Fit scanner. A T2-weighted, high resolution matched-bandwidth (MBW) anatomical scan (Time to Repetition (TR) = 5,000 ms, time to echo (TE) = 34 ms, flip angle = 90 degrees, voxel size: 1.5 mm x 1.5 x 4 mm, field of view (FOV) = 192 mm^2, 34 slices, ~1.5

minutes) and a T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2,530 ms, TE = 1.74 ms, Time to Inversion (TI) = 1,260 ms, flip angle = 7 degrees, voxel size: $1mm^3$, FOV = 256 mm^2, ~6.2 minutes) were acquired for co-registration to the functional data. One run of a T2*-weighted echo planar imaging scan (TR = 2060 ms, TE = 34 ms, flip angle = 90 degrees, voxel size: 3mm x 3mm x 4mm, FOV = 192 mm^2, 390 TRs, ~13.39 minutes in duration) were acquired to examine the BOLD signal during the Smoking Cues Task. The first six TRs were discarded to allow for steady-state longitudinal magnetization to be reached.

Image Preprocessing

Preprocessing of imaging data was conducted using FMRIB's Software Library (FSL 5.0) (www.fmrib.ox.ac.uk/fsl). Motion correction was performed using FSL's MCFLIRT with the middle volume as the reference image and normalized correlation as the cost function. FSL's Brain Extract Tool (BET) was used to remove skull and non-brain tissue from both the structural and functional scans. Images were preprocessed using high-pass temporal filtering (100 s cutoff) through FSL's FMRI Expert Analysis Tool (FEAT, Version 5.63), and smoothed with a 5 mm full width half maximum Gaussian kernel. Data for each subject were registered to the MBW, followed by the MPRAGE using affine linear transformations, and then normalized to the Montreal Neurologic Institute (MNI avg152) template. Registration was further refined using FSL's nonlinear registration tool (FNIRT) (Andersson, Jenkinson, & Smith, 2007). Four participants were excluded from analyses due to excessive head motion (>3 mm translation), and one participant was excluded due to poor registration. The final analyses included 19 participants.

All first-level analyses of imaging data were conducted within the context of the general linear model (FSL's FEAT). Regressors for each task condition (smoke, neutral) were formed by convolving delta functions representing the 45 sec period for each block with a double-gamma hemodynamic response function (HRF). The temporal derivative of this function was also included as a covariate to account for small temporal shifts in the hemodynamic response. Six motion regressors representing translational and rotational head movement were also entered as regressors of no interest. "Spike" regressors were created for each image with a frame displacement value above threshold (75% percentile plus 1.5*inter-quartile range) using FSL's fsl_motion_outliers.

Analytic Plan

For the cigarette cues task, the main contrast of interest was the difference in activation corresponding to the cigarette cue videos relative to the neutral videos (Cigarette > Neutral), consistent with previous studies that have utilized this task (e.g. (Ray et al., 2015). Group-level mixed models utilized FSL's FLAME 2 (Woolrich et al., 2004) with outlier deweighting (Woolrich, 2008); Z-statistic images were thresholded with cluster-based corrections for multiple comparisons based on the theory of Gaussian Random Fields with a cluster-forming threshold of $Z > 2.3$ and a cluster-probability threshold of $p < 0.05$ (Worsley, 2001).

Linear and logistic regression models tested hypotheses regarding the translational value of cue-induced craving. Models separately examined VS, aINS, ACC, and OFC in predicting the following outcomes at 6-month follow-up: 1) 30-day TLFB cigarettes per day; and 2) bioverified point-prevalence abstinence (threshold of 5 parts per million CO in expired air) (Cheung et al., 2017). All models included medication condition (VAR or VAR+NTX). Due to limits in

statistical power, inclusion of variables of interest (assessment pre-scan cigarette craving, cannabis use status, sex, alcohol and cigarette dependence severity) was not feasible. Point-prevalence abstinence models were intent-to-treat, such that participants who dropped out during follow-up were considered to be non-abstinent.

RESULTS

Baseline comparisons

As indicated in Table 1, participants (N = 19) were majority male adults who smoked 16 cigarettes per day and 6 drinks per drinking day. As the data from this manuscript derive from a clinical trial comparing varenicline versus varenicline plus naltrexone, pre-test comparisons on demographic and substance use variables indicated that there were no significant differences between these two medication conditions on any of these variables except baseline TLFB cannabis use ($t(18)=2.97$, p = .01), such that individuals in VAR reported significantly greater cannabis use days than those in VAR+NTX (16.64 vs .38. mean days, respectively).

Main Effect of Task (Cigarette > Neutral Contrast)

Across participants, cigarette relative to neutral cues elicited one cluster of activation at the whole-brain level in the midbrain (see **Figure 1** and **Table 2a**; Neutral > Cigarette clusters also visible in **Figure 2** and **Table 2b**.). Neutral > Cigarette activation indicated significant clusters in the somatosensory cortex, motor cortex, temporal gyrus, and basal ganglia.

On average, participants reported that the cigarette cues condition elicited significantly greater craving than the neutral condition (Cigarette cue urge rating = 2.31, neutral urge rating =

1.52; paired samples *t*-test $t(17) = 4.78$, $p < .001$). Cigarette > Neutral ratings were not associated with activity for any of the ROIs (*p*s = .09-.89). There were no significant differences in clusters of activation when comparing VAR vs VAR+NTX for both Smoke > Neutral and Neutral vs Smoke contrasts.

6-Month Follow-Up

A total of 15 participants were retained through 6-month follow-up; the 4 participants who dropped out of the study were unable to be contacted and were coded as having returned to smoking. Of the remaining 15 participants, five met criteria for bioverified abstinence from cigarette smoking (breath CO 5ppm or lower).

In this subsample of individuals who completed the fMRI experiment, medication was not a significant predictor of point-prevalence abstinence (B(SE)) = -.12(1.06), $p = .91$). Separate models also demonstrated that all four ROIs (ventral striatum, anterior insula, anterior cingulate cortex, and orbitofrontal cortex) were not significantly predictors of 6-month point-prevalence abstinence (*p*s = .12-.60). As there were no significant models, planned covariates were not tested.

Similarly, for 30-day TLFB cigarettes per day, medication was not a significant predictor (B(SE)) = 4.77(7.21), $p = .52$). Separate models demonstrated that all four ROIs (ventral striatum, anterior insula, anterior cingulate cortex, and orbitofrontal cortex) were not significantly predictors of cigarettes per day (*p*s = .17-.64).

DISCUSSION

This secondary analysis explored the predictive utility of smoking cue-induced neural activation in predicting 6-month post-quit smoking outcomes, in a sample of treatment-seeking heavy-drinking smokers enrolled in a medication comparison trial. Planned analyses indicated that none of the four a-priori ROIs that have demonstrated smoking cue-induced activation (i.e. ventral striatum, anterior insula, anterior cingulate cortex, and orbitofrontal cortex) were predictive of bioverified smoking abstinence or reported cigarettes per day at 6-month follow-up.

This is the first study to examine the predictive value of cue-induced craving that utilizes scan data prior to quit but on a stable medication dosage. Previous studies have examined either pre-treatment cue reactivity (Owens et al., 2018) or utilized multiple scans to examine changes in cue reactivity from pre- to post-treatment (Janes et al., 2019). Both study designs have indicated that baseline limbic smoking cue reactivity, as well as reductions in cingulate cortex activation, can be used to predict smoking cessation success both during and after treatment. Additionally, unique experimental designs have been used to demonstrate that anterior cingulate cortex smoking cue reactivity during brief abstinence can predict relapse rates during a subsequent 7-day quit attempt (Allenby et al., 2020), as well as how slow nicotine metabolizers may have a weaker association between abstinence-induced caudate smoking cue reactivity and abstinence-induced subjective cigarette cravings (Falcone et al., 2016). Within the context of these studies, one possible interpretation of these studies is that longer-term abstinence (e.g. 6-months) may be less predictable than the short-term outcomes historically examined in this literature, particularly for a subgroup of heavy-drinking smokers that may experience greater barriers to quitting. Future examination of these relationships with larger samples is warranted. Notably, however, this study has some important limitations discussed further below.

This study also corroborates previous work on the impact of varenicline on smoking cue-induced correlates of craving. While a placebo comparison was not available in this superiority trial, it is notable that neutral > smoke comparison yielded multiple clusters of limbic and prefrontal activation. Such results are consistent with evidence that varenicline alone is sufficient to suppress activation in ventral striatum and medial orbitofrontal cortex (Franklin et al., 2011). Similarly, pilot work within our group has demonstrated that varenicline and naltrexone separately suppress nucleus accumbens smoking cue-induced activation among non-treatment-seeking heavy-drinking smokers (Ray et al., 2015). While sample sizes for each medication condition were too small for a sufficiently powered comparison, the current study adds to the growing literature on the impact of these pharmacotherapies on smoking cue-induced activation.

With these small contributions to the literature on the predictive utility of neuroimaging response and pharmacotherapy impacts on such response, this study has several critical limitations. For this reason, all analyses are likely underpowered to detect significant associations.. Larger samples are needed both to establish greater statistical power to detect zero-inflated or logit-based effects (Olvera Astivia, Gadermann, & Guhn, 2019), as well as to directly compare medication conditions to determine whether medication-induced changes translate into differences in abstinence and/or smoking rates. With these limitations, it is important to expand the literature on smoking cessation, given that there is no consistent consensus on important networks or regions that could represent potential treatment targets or mediators of abstinence. Smoking remains the leading cause of preventable deaths in the US, and expanding databanks of such neuroimaging-based data may useful in larger meta-analyses of predictors of smoking cessation, as well as in the use of data-driven and other big data methods of analysis (Cook et al., 2020; Frank et al., 2019).

Table 1. Sample characteristics (*N* = 19)

Variable	Statistic M(SD)
Sex (n(% female))	5 (26.3%)
Race/Ethnicity (n(%))	
Caucasian	9 (47.0%)
African-American	7 (36.8%)
Asian/Pacific Islander	2 (10.5%)
Latinx	1 (5.3%)
Age (M(SD))	42.95 (11.65)
Medication Condition (VAR, VAR+NTX)	A 11/B 8
Baseline TFLB Cigarettes per Day	16.00 (12.06)
FTND	4.95 (1.43)
Baseline TLFB Drinks per Drinking Day	6.02 (2.73)
AUDIT	18.16 (7.51)
Cannabis Use at Baseline (n(%))	8 (42.1%)
Baseline TLFB Marijuana Days	9.79 (14.13)
Pre-scan time since last cigarette (median hours)	150
Pre-scan QSU	26.00 (12.57)
Pre-scan AUQ	22.11 (7.39)
Smoking abstinence at 6 month FU (n(%))	5 (26.32%)
6-month FU TLFB Cigarettes per Day	10.55 (15.28)
Smoke > Neutral Ventral Striatum	.18 (.99)
Smoke > Neutral Anterior Insula	.01 (.54)
Smoke > Neutral Anterior Cingulate Cortex	.08 (.63)
Smoke > Neutral Orbitofrontal Cortex	.06 (.30)

Note. AUDIT = Alcohol Use Disorders Identification Test; AUQ = Alcohol Urge Questionnaire; CUDIT = Cannabis Use Disorder Identification Test; FTND = Fagerstrom Test for Nicotine Dependence; QSU = Questionnaire on Smoking Urges; TLFB = Timeline Follow-Back; VAR = varenicline; VAR+NTX = varenicline plus naltrexone

Table 2a. Significant clusters for the Smoke > Neutral condition

	Peak MNI coordinates					
Cluster region	**X**	**Y**	**Z**	**# Voxels**	**Max-Z**	***p*-value**
Hypothalamus	-8	-4	-12	280	3.29	.015

Table 2b. Significant clusters for the Neutral > Smoke condition

	Peak MNI coordinates					
Cluster region	**X**	**Y**	**Z**	**# Voxels**	**Max-Z**	***p*-value**
Somatosensory cortex	-56	-24	36	8398	5.69	1.36E-38
Sec. somatosensory cortex	62	-18	18	6860	5.08	1.62E-33
Occipito-temporal cortex	-10	-98	10	3399	5.03	2.22E-20
Primary motor cortex	-22	-18	72	2537	4.08	1.75E-16
Lateral occipital cortex	-54	-68	-4	861	3.79	3.58E-07
Precentral gyrus	40	-6	68	472	3.35	0.003
Occipito-temporal cortex	54	-68	-4	354	3.43	0.0030
Basal ganglia	-22	-56	-22	353	3.47	0.0031

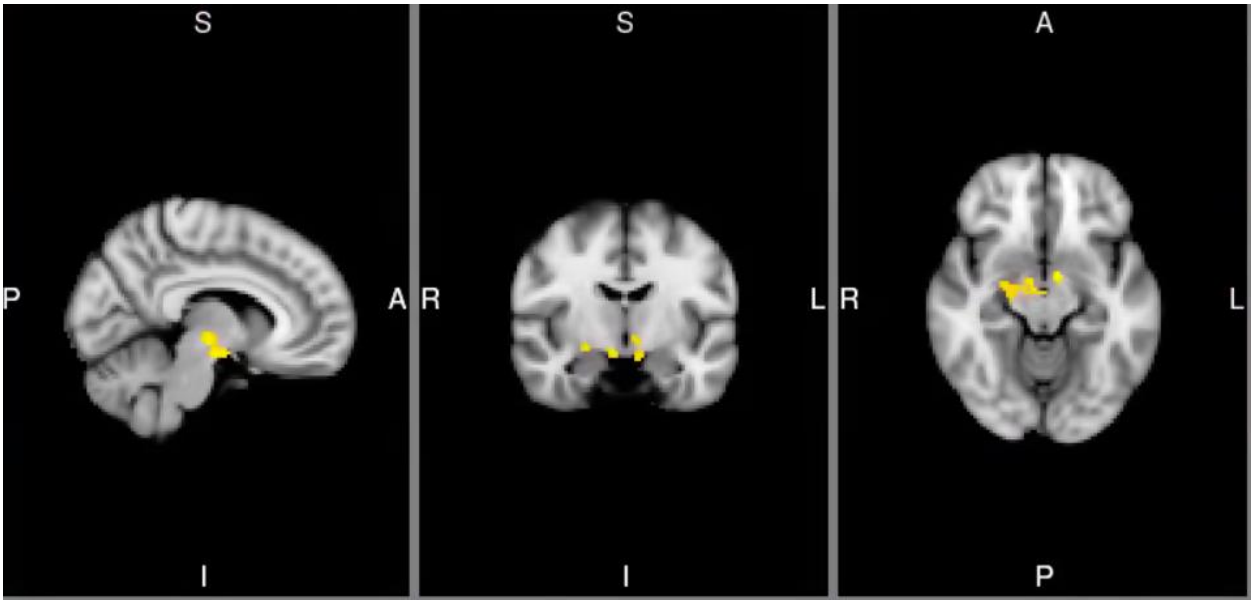

Figure 1. Whole-brain significant cluster for Smoke > Neutral cue. MNI coordinates for depicted slices are X=2 (left), Y=-10 (middle), Z=-10 (right). L=Left, R=right, S=superior, I=inferior, A=anterior, P=posterior.

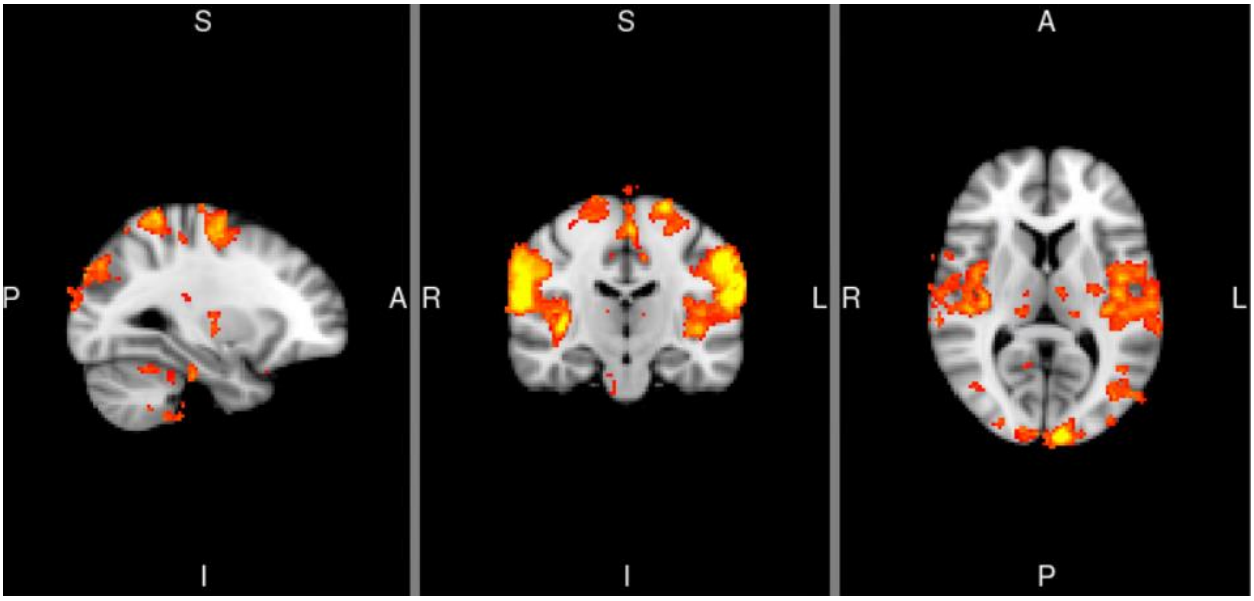

Figure 2. Whole-brain significant cluster for Neutral > Smoke cue. MNI coordinates for depicted slices are X=-28 (left), Y=-18 (middle), Z=8 (right). L=Left, R=right, S=superior, I=inferior, A=anterior, P=posterior.

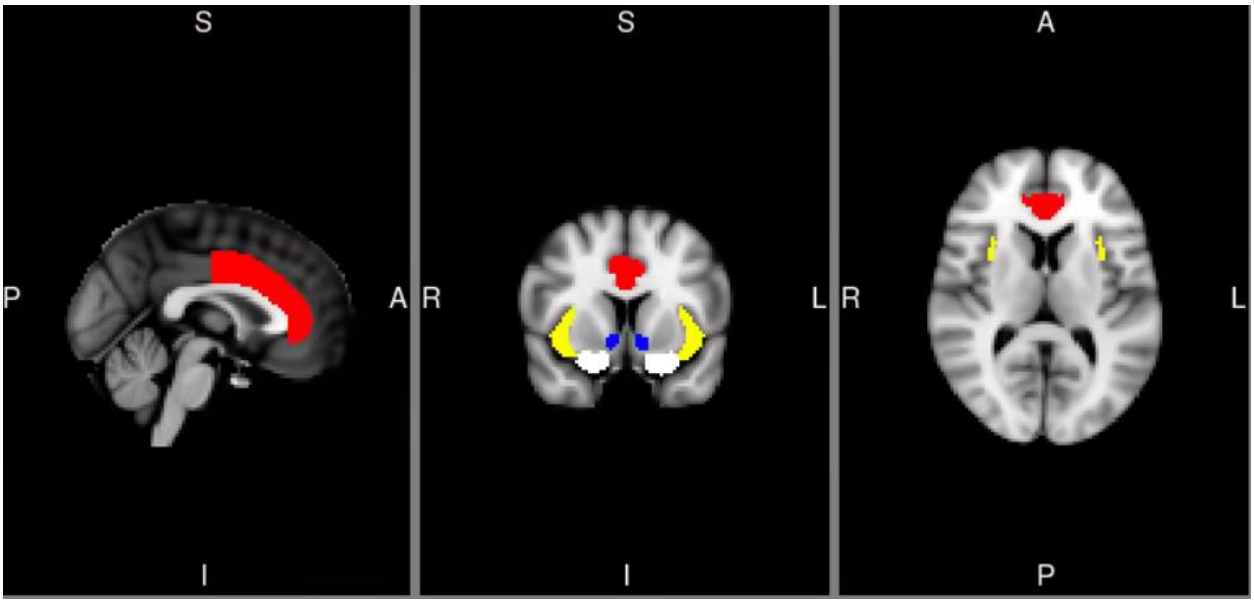

Figure 3. Anatomical region of interest mask for ventral striatum (blue), anterior insula (yellow), orbitofrontal cortex (white), and anterior cingulate cortex (red). ROI extracted from the Harvard Oxford atlas thresholded at 25% based on the maximum probability labels. MNI coordinates for depicted slices are X=0 (left), Y=10 (middle), Z=10 (right). L=Left, R=right, S=superior, I=inferior, A=anterior, P=posterior.

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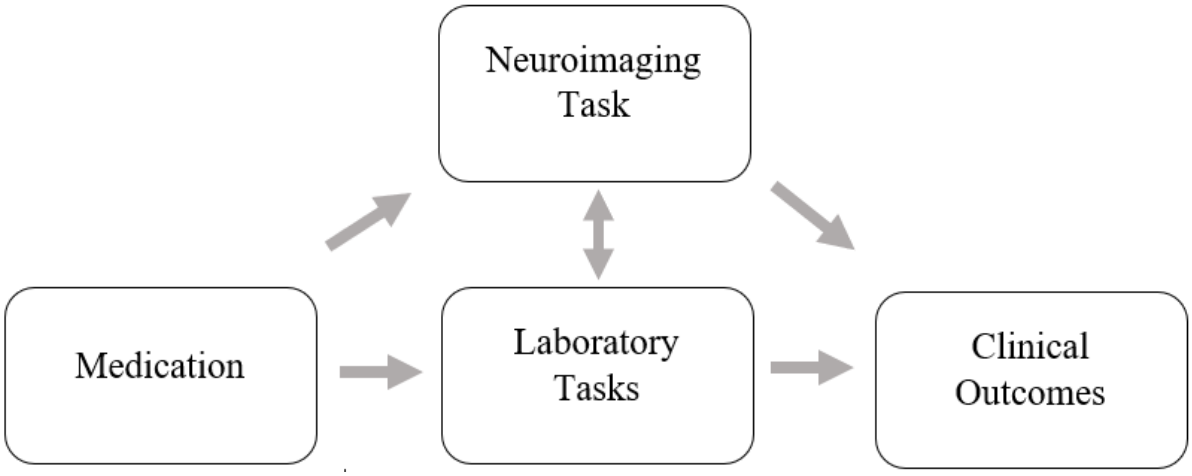
GENERAL SUMMARY

Increasingly, neuroimaging techniques are used to explore biological changes induced by pharmacotherapy. To date, the majority of research has been used to examine cross-sectional differences among those succeed in substance cessation versus those who relapse (Bell et al., 2014), as well as identify potential neural targets of both pharmacotherapy and psychotherapy that reduce substance craving and increase cognitive control (Cabrera et al., 2016; Konova et al., 2013). As discussed in the general introduction and illustrated in the figure below,



such work is critical in the medications development context, in order to 1) pinpoint accurate indicators of pharmacotherapy-induced neural change; 2) understand whether responses in current gold-standard experimental paradigms map onto responses to substance neuroimaging paradigms (i.e. demonstration of a link between neural response and behavior within a controlled environment); 3) outside of a laboratory context, whether neuroimaging responses hold any predictive value for substance use in complex, real-world cessation attempts, particularly over a longer timeframe. This dissertation adds to the nascent literature for each of these points.

Study 1 tested the effects of naltrexone relative to placebo on neural correlates of alcohol-induced cravings among a sample of non-treatment seeking heavy drinkers, and is the first study

to examine these effects among individuals of East Asian descent. This randomized, double-blind, crossover study utilized an alcohol taste-cues task did not elicit significant clusters of activation that may have been expected in striato-limbic pathways. Naltrexone relative to placebo did not significantly reduce activation in anterior cingulate cortex, ventral striatum, or orbitofrontal cortex. Naltrexone treatment enhanced functional connectivity in a key reinforcement-related pathway during alcohol versus water taste cues (i.e. ventral striatum with prefrontal cortex). These functional connectivity results corroborate naltrexone imaging results used with other substances of abuse, particularly with increased connectivity between either striatum or ventromedial prefrontal cortex and frontoparietal network (Elton et al., 2019), and suggest that naltrexone may increase top-down regulation of alcohol-induced processing of reward. This work is also consistent with research indicating that naltrexone normalizes local network inefficiencies among individuals with alcohol use disorder so that they more closely resemble healthy controls (Morris et al., 2018). Overall, this contribution to a compendium of studies demonstrates that naltrexone is an exemplar pharmacotherapy in improving neural connectivity for individuals with alcohol use disorder, and supports the broader study of pharmacotherapeutic effects on alcohol-induced neural activation.

Study 2 explored the translational potential of fMRI alcohol cue-induced neural activation as it relates to one of the most commonly utilized experimental paradigms, self-administration of alcohol. This study utilized the identical sample as study 1; for each medication condition, individuals completed a neuroimaging session on day 4 of titration. On titration day 5, they returned to the lab to complete a 60-minute alcohol self-administration paradigm, in which they were allowed to drink up to a BrAC of 0.06 with their preferred alcoholic beverage. Results demonstrated that after accounting for alcohol dependence severity, OPRM1 genotype, and

medication condition, ventral striatum activation was significantly associated with both latency to first drink, such that those with higher ventral striatum exhibited shorter latencies to consume their first drink. Additionally, ventral striatum activation was positively associated with the total number of drinks consumed in the session. This is one of the first studies to demonstrate the direct relationship between neural processing of the rewarding effects of alcohol and self-administration behavior in a laboratory setting. Notably, there were significant limitations including weak alcohol-elicited activation, sample size, and inability to administer carbonated beverages in the scanner, that require replication of these effects. Limitations notwithstanding, this study provides initial evidence for the second goal of this dissertation, and corroborate the convergence of neuroimaging and existing gold-standard administration outcomes.

Study 3 explored the potential predictive validity of smoking cue-induced activation on smoking cessation outcomes 6 months after a smoking cessation attempt. Specifically, this secondary analysis included neuroimaging data from a clinical trial comparing the effects of VAR (1 mg twice daily) + NTX (50 mg once daily) relative to VAR alone in an ongoing double-blind, randomized controlled study with a sample of treatment-seeking heavy-drinking smokers. Participants completed the neuroimaging session on days 9-13 of titration, during including a visual task assessing cue-induced smoking craving. Given the scant research on regions of interest important to such a translational inquiry, we examined the predictive validity of ventral striatum, anterior cingulate cortex, anterior insula, and orbitofrontal cortex. The total sample for this analysis was *N*=19; primary analyses indicated that none of the 4 ROIs were significantly predictive of either 30-day cigarettes per day or point-prevalence cigarette abstinence at 6-month follow-up. Notably, while the study design precluded inclusion of a placebo condition, results indicated that both medication conditions suppressed activation in cigarette relative to neutral

cues, corroborating previous work demonstrating that both varenicline and naltrexone may critically target cigarette cue-induced mesocorticolimbic activation related to subjective rewarding effects of smoking (Franklin et al., 2011; Ray et al., 2015). Additionally, while the external validity of this study's results are hampered by low sample size, larger future reviews examining the translational importance of cue-induced neural activation may benefit from the collection and analysis of this data.

Together, these three studies in this dissertation contribute to rapidly expanding areas of research focused on the integration of pharmacology and neuroimaging to refine addiction treatments. In particular, increasing numbers of neuroimaging reviews and meta-critiques of the literature are emphasizing elucidation of the specific clinical value of such research, to the point of testing combinations of pharmacotherapies and cognitive interventions with targeted brain stimulation (Hammond et al., 2019; Moningka et al., 2019). Similar efforts are being made in treatment development for other disorders such as depression (Cook et al., 2020; Spagnolo et al., 2020). In this vein, expansion of the types of studies that dissertation studies 2 and 3 represent is critical to streamlining the process of medication development and approval, as well as maximizing efficacy of pharmacotherapy in diverse and treatment-resistant populations of smokers and drinkers.

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