Effects of Cortical Dopamine Regulation on Drinking, Craving, and Cognitive Control

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Protocol

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A. SPECIFIC AIMS, BACKGROUND AND SIGNIFICANCE

1) Alcohol use disorder, prefrontal dopamine, and catechol-*O*-methyltransferase

Alcohol use disorder (AUD) is a devastating disease characterized by dysregulated motivation for alcohol and loss of control over consumption. In the brain, motivated behavior and cognitive control are mediated, in part, by dopamine (DA) signaling between the striatum and prefrontal cortex (PFC) (1). In humans, the PFC is a large and heterogeneous region with relatively high expression of both D_1- and D_2-like DA receptors. It comprises regions that underlie processing of learned reward values, including the orbitofrontal cortex (OFC) and ventromedial PFC (vmPFC) (2), as well as regions that underlie decision-making and inhibitory control, including the inferior frontal gyrus (IFG) (3) and dorsomedial and dorsolateral PFC (dmPFC and dlPFC) (4). These cortical areas interact in networks with striatal subregions to exert control over motivated behavior (5). The relationship between cortical DA and cognitive control has been hypothesized to be inverted-U-shaped (see **Figure 1**), with both high and low tone associated with poor control (6-8) (though other shapes for this relationship have also been proposed; 9). Thus, dysregulated inactivation of cortical DA, leading to excessive synaptic accumulation or removal, may contribute to loss of control in AUD and other addictive disorders.

Dysregulation of DA function in AUD has been studied for many years. Early human positron emission tomography (PET) studies found decreases in striatal D_2 receptor availability (10) and DA transmission (11) among alcoholics, relative to controls, but were limited by the lack of DA radioligands that bound in PFC. Decreased striatal D_2 availability was correlated with increased vmPFC response to alcohol cues (12), but direct ascertainment of cortical DA and its relationship to alcohol use was not possible. Recently, however, a PET study that used a novel cortical DA ligand, [11C]FLB 457, reported that prefrontal DA transmission was substantially down-regulated among recently abstinent alcoholics, particularly in the vmPFC, OFC, and dlPFC (13). These findings accord with recent animal data from the Charleston ARC, which suggest that chronic intermittent alcohol exposure profoundly disrupts the ability of D_2-like receptors in the vmPFC to regulate pyramidal neuronal firing—independent of changes in receptor expression (14).

In most brain areas, including the striatum, DA is inactivated primarily through active reuptake at the presynaptic dopamine transporter (DAT). In the PFC, however, the DAT is not highly expressed (15), and the principal method of DA inactivation is degradation by the enzyme catechol-*O*-methyltransferase (COMT) (16). A single nucleotide polymorphism (SNP) in *COMT*, the gene that encodes this enzyme, has been associated with differential COMT function. Specifically, the met (A) allele of the val158met SNP (rs4680), which engenders a valine to methionine amino acid substitution, is associated with a three- to four-fold reduction in COMT efficacy (17), and thus greater synaptic DA accumulation, relative to the val allele, which is associated with low PFC DA tone (see **Figure 1**).

The val158met SNP is among the most thoroughly studied genetic variants in psychiatry (18; 19). Although initial studies showed an association with cognitive function, subsequent meta-analyses found no effect on this phenotype (20). Associations with alcohol-related phenotypes have also been mixed (21-24). More recent studies suggest, however, that *COMT* val158met variation may moderate response to DAergic medications, including olanzapine (25), methylphenidate (26), haloperidol (27), the D_2 antagonist sulpiride (28), and the COMT inhibitors tolcapone (29-32) and entacapone (33). Given the evidence of dysregulation of DA function among individuals with AUD, it seems likely that a DAergic medication might reduce drinking in this population, and that the val158met SNP might moderate this response.

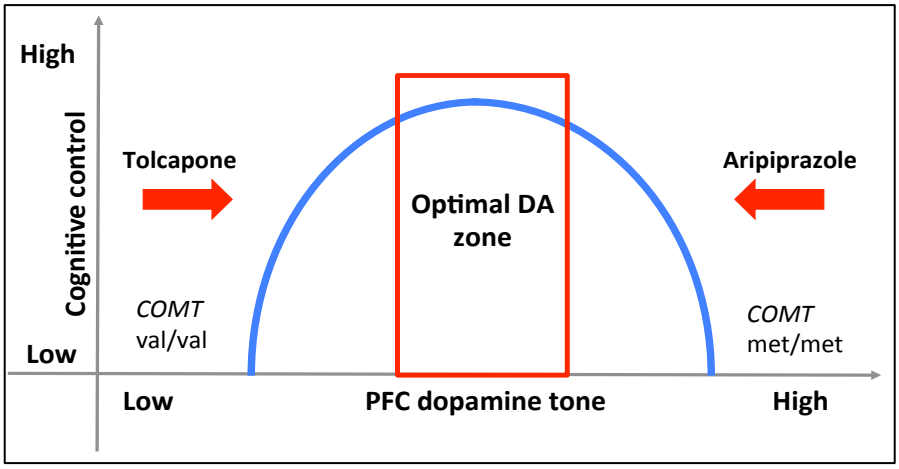


Figure 1. Conceptual diagram of the relationship between PFC DA tone and cognitive control, and the effects of *COMT* val158met variation on DA tone in this region. Our preliminary data suggest that **aripiprazole**, a DA partial agonist that we have tested in the current ARC funding period, may shift met-allele homozygotes into the optimal DA zone for control over drinking. Conversely, **tolcapone**, a COMT inhibitor and novel medication for AUD that we propose to test here, may shift val-allele homozygotes into this zone.

B. Aripiprazole preliminary data and progress report

During the current ARC funding period, we tested this pharmacogenetic hypothesis by evaluating the interactive effects of *COMT* val158met variation and aripiprazole (APZ), a D_2 partial agonist that is believed to stabilize cortical and striatal DA transmission, possibly by reducing both when they are upregulated (34) (although it also has serotonergic effects). We administered APZ to non-treatment-seeking subjects with early-stage alcohol dependence (i.e., individuals ages 21-40). To date, 60 subjects (mean age = 27, 75% male, average of 9 drinks/drinking day) have completed the study, in which they were randomized to APZ (titrated to 15 mg daily) or placebo for eight days, and completed alcohol cue-reactivity and stop-signal (N = 23) fMRI tasks on day 7 and an alcohol self-administration paradigm (bar lab) on day 8. We used the cue-reactivity and stop-signal tasks because they assess motivated behavior and cognitive control and because PET studies suggest that these tasks strongly elicit cortical and striatal DA release (35; 36). We assessed the effects of APZ and *COMT* variation on the number of drinks, out of eight possible, that subjects chose to consume in the bar lab and on brain activation elicited by alcohol cues, as well as activation related to response inhibition.

Our preliminary data indicate that APZ trended toward reducing both drinks per drinking day during the first six days on medication ($F(1, 57) = 3.15$, $p = .08$) and drinking in the bar lab ($F(1, 57) = 2.50$, $p = .12$). However, the effect of APZ on bar-lab drinking was driven almost entirely by an interaction between medication group and variation at the *COMT* val158met SNP ($F(2, 53) = 3.35$, $p < .05$; see **Figure 2**). Met-allele homozygotes who received APZ chose to consume, on average, two drinks, while those who received placebo chose to consume over seven drinks. While preliminary, this finding could account for some of the mixed findings from previous clinical trials of APZ for alcohol dependence (e.g., 37): APZ might be effective primarily for "early stage" individuals who are homozygous for the *COMT* met allele. Importantly, covarying for APZ-related side effects (e.g., sedation) did not reduce the statistical significance of this finding.

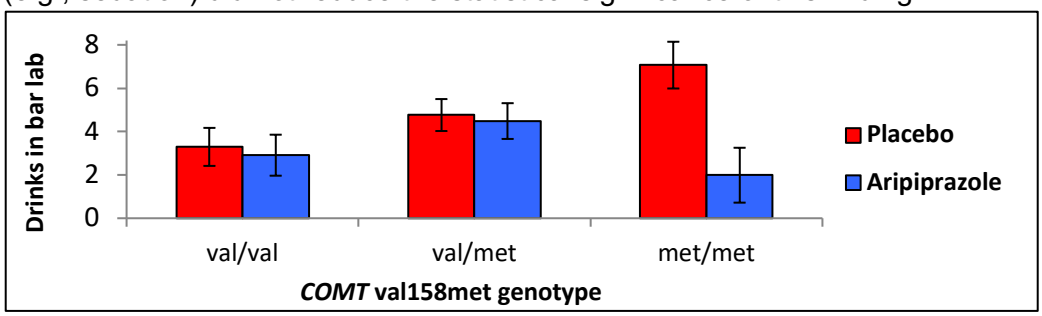


Figure 2. Interaction between APZ and *COMT* val158met genotype on bar-lab drinking. APZ reduced drinking among met-allele homozygotes ($F(1, 53) = 8.98$, $p = .004$), but was not significantly different from placebo for heterozygotes or val-allele homozygotes.

APZ and *COMT* val158met genotype also interacted in their effects on alcohol cue-elicited brain activation. A whole-brain analysis of this interaction identified a cluster in the right and medial OFC (Brodmann area [BA] 11) in which *COMT* genotype significantly moderated the effect of APZ on cue-elicited activation. APZ, relative to placebo, decreased cue-elicited OFC activation among met-allele homozygotes, but increased it among val homozygotes (see **Figure 3**). Further, OFC activation was positively associated with the number of drinks subjects consumed in the bar lab ($r(60) = 0.26$, $p < .05$), and when it was added to the model that tested the interactive effects of APZ and *COMT* genotype on bar-lab drinking, the interaction was no longer significant ($F(2, 52) = 2.00$, $p = .15$), suggesting that cue-elicited activation in this region mediated the effect.

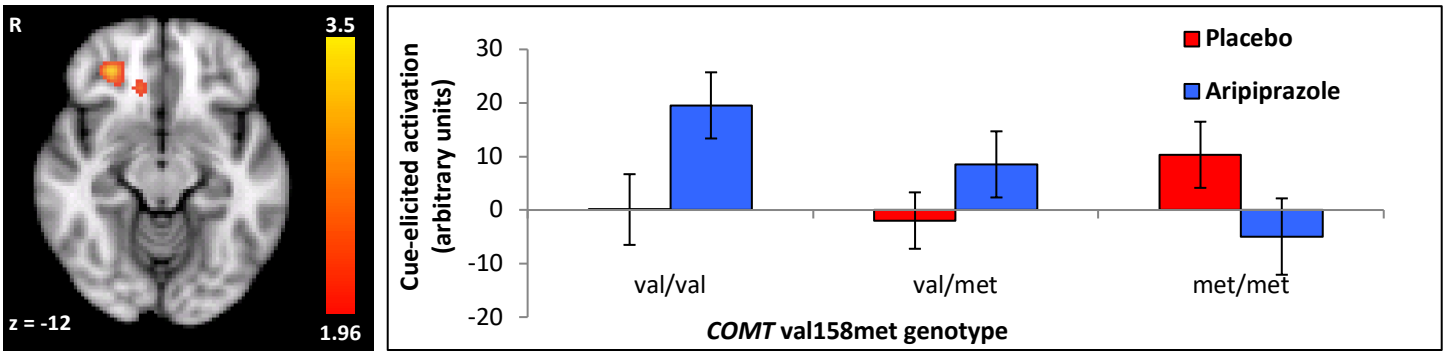
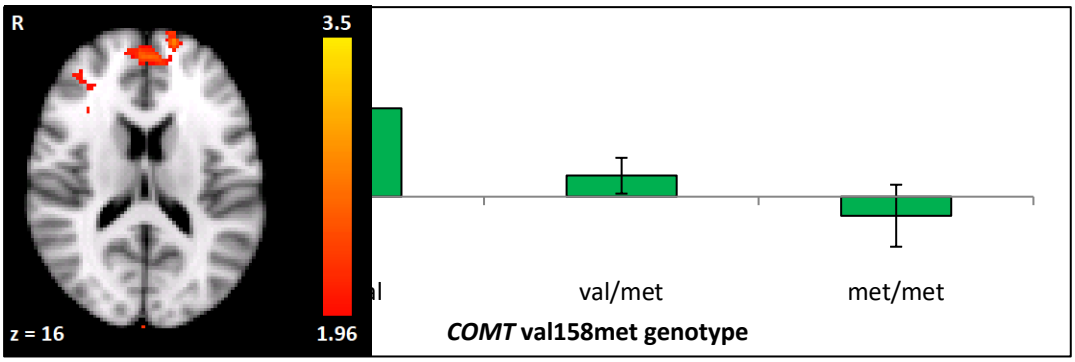


Figure 3. Interaction between APZ and *COMT* val158met genotype on alcohol cue-elicited OFC activation. The color scale indicates voxels in which these factors moderated cue-elicited activation (thresholded, voxelwise, at $z > 1.96$).

A subset ($N = 23$) of the subjects in the current ARC study were also administered a response inhibition (stop-signal) fMRI task (38). For this task, activation to task trials on which subjects failed to inhibit a response was contrasted with activation to trials on which they successfully inhibited a response (i.e., unsuccessful vs. successful inhibitions). A whole-brain analysis indicated that, although APZ did not significantly affect activation associated with this contrast, *COMT* genotype did, such that, irrespective of medication, val-allele homozygotes, relative to heterozygotes and met homozygotes, displayed greater dmPFC (BA 9) activation during unsuccessful inhibition (see **Figure 4**). This finding is consistent with other reports that *COMT* val-allele carriers demonstrate dysregulated cortical activation indicative of compromised cognitive function (39). Activation of dmPFC did not mediate the interaction of APZ and *COMT* genotype on bar-lab drinking, but this conclusion is limited by the current sample size.



of *COMT* val158met genotype on dmPFC activation associated with unsuccessful response scale indicates voxels in which *COMT* genotype significantly moderated the unsuccessful > ontrast (thresholded, voxelwise, at $z > 1.96$)

Collectively, the current data suggest that a novel pharmacogenetic interaction between a DAergic medication (APZ) and *COMT* variation is mediated by changes in cue-elicited cortical activation, such that APZ appears to be more effective in reducing drinking and cue-elicited activation among met-allele homozygotes. Interestingly, while APZ's effect on drinking was not mediated by changes in brain activation related to cognitive control (or the lack thereof), val-allele homozygotes, relative to heterozygotes and met homozygotes, demonstrated greater cortical activation during failed response inhibition. Thus, these findings raise the question of whether a different

medication might be more effective in reducing drinking among *COMT* val homozygotes, who derived little drinking reduction from APZ, and whether such a medication might exert its effects through changes in a reward-related phenotype, such as cue reactivity, or through a control-related phenotype, such as response inhibition. Because the *COMT* val allele is associated with relatively lower cortical DA, one possibility is that a medication that increases cortical DA could "rescue" cognitive control and reduce drinking among *COMT* val-allele homozygotes.

3) Rationale for the COMT inhibitor tolcapone as a medication to treat AUD

As noted above, COMT is the primary mechanism for DA inactivation in the PFC; thus, medications that inhibit this enzyme could achieve the aim of increasing cortical DA. Tolcapone, a brain-penetrant, reversible COMT inhibitor (40) that is FDA-approved for the treatment of Parkinson's disease (41), offers an opportunity to test this hypothesis. Tolcapone acutely increases DA concentrations in both the periphery and the PFC (42). Among Parkinson's patients, it is normally co-administered with levodopa (L-DOPA) to reduce peripheral metabolism of the latter before it enters the brain. As described in greater detail below, there are issues with tolcapone's hepatotoxicity that likely preclude extended use of this medication to reduce heavy drinking in the general clinical population. Thus, this proposal presents a sub-acute treatment study in which tolcapone is used as a pharmacogenetic proof-of-concept probe. Tolcapone's efficacy in reducing drinking would suggest that COMT inhibition is a possible pharmacological target for AUD, and other brain-penetrant, non-hepatotoxic COMT inhibitors could be developed to achieve similar or enhanced therapeutic results.

Data from animal models support the idea that tolcapone might be effective in increasing cognitive control and reducing drinking. Tolcapone increased DA release in the PFC during executive functioning tasks (42; 43), and in an animal model of cue-elicited drinking, it was recently reported to reduce alcohol consumption among both alcohol-preferring (P) and high-drinking Wistar rats (44). When administered independently of L-DOPA, tolcapone appears to have relatively limited effects on striatal DA or motor function (45; 46), likely because the greater expression of DAT in the striatum, relative to COMT, counteracts its acute effects. This differential regional effect further raises the question of whether, among individuals with AUD, tolcapone's effects on drinking might be mediated through changes in a reward-related mechanism, such as cue reactivity (as we have shown for APZ), or through a more cortically-dependent mechanism, such as cognitive control.

Human studies offer additional evidence that tolcapone enhances cognitive function, and suggest that this effect may be specific to individuals homozygous for the val (higher activity) allele of the *COMT* val158met SNP, who have lower cortical DA and thus fall on the left side of the inverted-U-shaped function discussed above (see **Table 1**). Among healthy controls, tolcapone, given either acutely or over an eight-day span, improved performance on executive functioning, working memory, and set-shifting tasks, as well as pre-pulse inhibition of the startle response, a phenomenon believed to reflect cognitive efficiency (29; 30; 32). In two of these studies, beneficial cognitive effects were largely specific to val-allele homozygotes (29; 30), and in the third, these effects were moderated by variation at another *COMT* SNP that also regulates COMT activity (32). Two more recent studies of healthy controls also reported the effects of tolcapone on cognitive tasks more relevant to addictive behavior. An acute dose of tolcapone (200 mg) decreased risky decision-making only among val-allele homozygotes, and also improved working memory performance among these individuals (31). Further, the same acute dose of tolcapone reduced impulsive choice on a delay-discounting task (47). Thus, among individuals with AUD, tolcapone might act to "rescue" impaired cognitive control over heavy drinking.

Table 1. Human studies of tolcapone and *COMT* val158met effects

First author, year	*N*	Behavior	Tolcapone effect	*val158met* moderation
Controls				
Apud, 2007 (29)	47	Executive functioning Working memory Set-shifting	**Improved** **Improved** **Improved**	None None Only among val/val
Giakoumaki, 2008 (30)	23	Prepulse inhibition Working memory Set-shifting	**Improved** **Improved** **Improved**	Only among val/val Only among val/val Only among val/val
Roussos, 2009 (32)	25	Prepulse inhibition Working memory Set-shifting	**Improved** **Improved** **Improved**	More among val/val, val/met* None* None*

Farrell, 2012 (31)	67	Working memory Decision making	**Improved** **Improved**	Only among val/val Only among val/val
Kayser, 2012 (47)	23	Delay discounting	**Reduced**	Not tested
Clinical populations (addictive disorders)				
Grant, 2013 (48)	24	Pathological gambling	**Reduced**	More among val/val, val/met
Ashare, 2013 (49)	20	Cigarette smoking Craving, withdrawal Working memory	None None **Improved**	None None More among val/met
*Moderation by *COMT* rs4818 SNP, with greater tolcapone effects in group with high-activity (low DA) allele				

Data from two recent studies of individuals with addictive disorders also support this putative function for tolcapone (see **Table 1**). Among pathological gamblers, eight weeks of tolcapone (100 mg, 3x/day) reduced gambling urges relative to baseline, particularly among val-allele homozygotes (48). Interestingly, many of these individuals were also active drinkers, and median self-reported drinking frequency on the AUDIT decreased from 2-3x/week at baseline to weekly or less at study completion (Wilcoxon signed-rank test for this difference: $Z = -3.49$, $p < .001$) (J. Grant, personal communication). Among cigarette smokers, eight days of tolcapone (200 mg, 3x/day), relative to placebo, did not reduce smoking, craving, or withdrawal, but did improve cognitive function (working memory) (49).

Several of the studies noted above also employed neuroimaging paradigms; collectively, they suggest that tolcapone's effects may be mediated through changes in cortical activation and the connectivity of corticostriatal networks. These studies have primarily reported tolcapone-mediated reductions in cortical activity during cognitive tasks, and have been interpreted as evidence of improved cortical efficiency, although not all effects have been consistent, and few studies have examined interactions with *COMT* genotype. Among healthy controls, tolcapone, relative to placebo, decreased dlPFC and dmPFC activation during working memory and attentional control (29; 50) and reduced corticostriatal connectivity during a delay-discounting task (47). Similarly, among smokers, tolcapone, relative to placebo, reduced dlPFC and dmPFC activation related to working memory; interestingly, this effect occurred only among *COMT* val-allele homozygotes (49). However, tolcapone also increased dlPFC and dmPFC activation during the delay-discounting task noted above, and among pathological gamblers treated with tolcapone, improvement in gambling urges was related to increased fronto-parietal activation, relative to a baseline scan, during an executive planning task (48). Overall, neuroimaging data suggest that tolcapone may improve cognition through alterations in cortical activation and corticostriatal network connectivity.

4) Tolcapone preliminary data

Given our preliminary data with APZ and the literature reviewed above, we sought to evaluate the effects of tolcapone among non-treatment-seeking individuals with AUD. The MUSC Institutional Review Board for Human Research approved an open-label pilot safety and feasibility study with similar parameters to the study proposed here (i.e., same dosing schedule and bar-lab paradigm). To date, five non-treatment-seeking subjects with normal liver function have enrolled in the study, with no significant adverse events and minimal liver enzyme elevation observed in only one subject, who was discontinued from the study before the bar lab. Urinary riboflavin measurements indicate that all subjects have been adherent to the protocol. Subjects' drinking decreased from 80% heavy drinking days (i.e., ≥ 5/4 drinks/day for men/women) during the 90 days prior to the study to 50% heavy drinking days while on medication (paired *t*-test: $t(3) = 2.85$, $p = .065$); while very preliminary, this trend lends further support to the hypothesis that tolcapone will reduce drinking.

B. RESEARCH DESIGN AND METHODS

1) Timeline and general design of the study

Enrollment and data collection will be conducted during years 1-4 and the first half of year 5; the study blind will be broken in the second half of year 5, and data analysis will begin at that point. Ninety non-treatment-seeking subjects (20 per year in years 1-4, and 10 in year 5) with *DSM-5* Alcohol Use Disorder (AUD) will be randomized, on the basis of their *COMT* genotype, to tolcapone (titrated to 200 mg, 3x/day) or placebo for seven days, and will complete the procedures detailed in **Table 2**. Immediately prior to randomization (day 1), subjects will undergo a pre-treatment fMRI scan, during which anatomical, alcohol cue-reactivity, and stop-signal scans will be acquired. If more than one week has passed between a subject's initial assessment by the ARC Clinical

Intake and Assessment Core, urine drug screening and pregnancy testing will be repeated, as will measures of recent alcohol consumption (e.g., Timeline Follow-back and Obsessive Compulsive Drinking Scale). On study day 7, subjects' drinking over the previous six days will be assessed, and the fMRI procedures will be repeated. Blood will be drawn on days 1 and 7 to assess tolcapone effects on COMT activity in red blood cells. On day 8, subjects will be counseled regarding alcohol problems and encouraged to participate in treatment if indicated. Subjects will be compensated $300.

Table 2. Study visits and procedures

Visit	Procedure
Day 1	*Pre-treatment scan, blood draw, and medication randomization.* After the scan, subjects will receive a blister-pack containing study medication and will take the first dose at the study site in front of study personnel.
Day 7	*Post-treatment scan and blood draw.* Before the scan, drinking over the previous six days will be assessed. LFTs will be repeated on this day or the following day, after medication administration is complete.
Day 8	*Feedback session.* Subjects will receive motivational enhancement counseling related to their drinking.

2) Subject recruitment and selection

Subjects for this study will be similar to those recruited during our previous studies. Individuals who meet *DSM-5* criteria for AUD, but who are not seeking treatment, will be recruited via the ARC Clinical Intake and Assessment Core, which will also collect blood for DNA extraction and prospective *COMT* genotyping. For inclusion in this study, individuals will be required to be between ages 21 and 40 (to match the current ARC sample in which we have demonstrated an APZ by *COMT* val158met interaction), to be physically healthy with no history of liver disease and normal liver function (to reduce risk of tolcapone hepatotoxicity), to have no current Axis I psychiatric disorder, to be taking no psychoactive medications or medications contraindicated with tolcapone, to have no current non-alcohol substance use except nicotine, and to have no MRI contraindications (e.g., neurological disease, history of head injury, implanted metal).

Inclusion Criteria:

1. Age 21-40 (to focus on an age group still on a trajectory of increasing alcohol consumption).
2. Meets *Diagnostic and Statistical Manual of Mental Disorders, 5th edition* (*DSM-5*) criteria for current Alcohol Use Disorder.
3. Reports drinking, on average, at least 20 standard alcoholic drinks per week for at least the past 3 months.
4. Currently not engaged in, and does not want treatment for, alcohol-related problems.
5. Able to read and understand questionnaires and informed consent.
6. Lives within 50 miles of the study site.
7. Able to maintain abstinence from alcohol for two days (without the aid of detoxification medications), as determined by self-report and saliva alcohol measurements.

Exclusion Criteria:

1. Current *DSM-5* diagnosis of any other substance use disorder except Nicotine Use Disorder.
2. Any psychoactive substance use (except marijuana and nicotine) within the last 30 days, as indicated by self-report and urine drug screen. For marijuana, no use within the last seven days by verbal report and negative (or decreasing) urine THC levels.
3. Current *DSM-5* Axis I diagnosis, including major depression, panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, bipolar affective disorder, schizophrenia, dissociative disorders, eating disorders, or any other psychotic or organic mental disorder.
4. Current suicidal ideation or homicidal ideation.
5. Need for maintenance or acute treatment with any psychoactive medication, including antiepileptic medications.
6. Currently taking medication known to affect alcohol intake (e.g., disulfiram, naltrexone, acamprosate, topiramate).
7. History of severe alcohol withdrawal (e.g., seizure, delirium tremens), as evidenced by self-report and assessment with Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar).

8. Clinically significant medical problems such as cardiovascular, renal, gastrointestinal, or endocrine problems that would impair participation or limit medication ingestion.
9. Past alcohol-related medical illness, such as gastrointestinal bleeding, pancreatitis, or peptic ulcer.
10. Current or past hepatocellular disease, as indicated by verbal report or elevations of SGPT (ALT) or SGOT (AST) greater than the upper limit of the normal range at screening.
11. Females of childbearing potential who are pregnant (by urine HCG), nursing, or who are not using a reliable form of birth control.
12. Current charges pending for a violent crime (not including DUI-related offenses).
13. Lack of a stable living situation.
14. Presence of ferrous metal in the body, as evidenced by metal screening and self-report.
15. Severe claustrophobia or morbid obesity that preclude placement in the MRI scanner.
16. History of head injury with > 2 minutes of unconsciousness.

3) Medication safety, adherence, and dosing

a) Safety. Associations between variation at the *COMT* val158met SNP and a variety of psychiatric phenotypes (18) have suggested that tolcapone and other COMT inhibitors might be efficacious for individuals with psychiatric disorders, including depression (60) and schizophrenia (61; 62). However, despite this potential, several reports of severe liver toxicity have reduced enthusiasm for further study. There was no indication of toxicity in preclinical work with tolcapone, and clinical trial data suggested only a 1-3% greater incidence of abnormal elevation of liver enzymes (i.e., alanine transaminase [ALT] and aspartate transaminase [AST]) over placebo (63). Nevertheless, in the first years of clinical use, four of approximately 60,000 patients, dosed with tolcapone for two to four months, developed severe liver toxicity, with three deaths reported (63). Based on this, the FDA recommended routine liver function tests (LFTs) for patients receiving tolcapone (biweekly for the first year of treatment).

Most of the other side effects associated with tolcapone (sleep problems, hallucinations, dystonia) are related to its combined use with L-DOPA for the treatment of Parkinson's disease (64). Significant diarrhea, however, seems to be specific to tolcapone and has been reported even when used alone. Overall, other than idiosyncratic liver toxicity, tolcapone is well tolerated and a reasonably safe drug when used independently of L-DOPA, as demonstrated in the studies of smokers (49) and pathological gamblers (48) discussed above. Importantly, neither liver enzyme elevation nor other adverse events were noted in either of these studies.

Nonetheless, given past reports of liver toxicity, caution is in order and reduction of risk is required. Subjects in this study will take tolcapone for only seven days, a period shorter than the FDA's first recommended interval for LFTs (after two weeks of dosing). However, heavy-drinking subjects may be at increased risk for liver compromise. Therefore, only individuals who 1) report no history of past liver disease and 2) have normal liver enzymes prior to randomization will be enrolled in the study. In our current ARC study, 23% of subjects had elevated liver enzymes; thus, we do not anticipate that this exclusion criterion will unduly limit recruitment. Subjects will be contacted on day 4, after they have taken medication for three days, to inquire about their wellbeing, including any complaints of abdominal pain, gastrointestinal disturbance, extreme fatigue, or skin color change. If any liver-related complaints are present on day 4, subjects will be discontinued from further study. These symptoms will also be assessed in person on day 7, and LFTs will be repeated on day 7 or 8, after medication administration is complete; if liver-related complaints are present or liver enzymes are elevated at that time, we will continue to monitor until they return to normal, and/or consider a referral to a hepatologist. To date, we have used this procedure in ten individuals who completed a prior pilot study and over 70 who have been enrolled in the current protocol; it has worked well.

b) Adherence. Given that subjects will not be seeking treatment, adherence to medication ingestion could be of concern. To monitor adherence, study medication will be over-encapsulated with 25 mg of riboflavin (which, in our experience, yields urine levels 2-3x normal basal levels up to 24 hours after medication ingestion), and urinary riboflavin will be measured in the CNL. Urine will be collected twice at baseline (to obtain a more valid average daily output) and on day 7. A spectrofluorometric assay with known aqueous standards will be used to develop a standard fluorescence curve from which to read the fluorescence of urine samples. Riboflavin levels will be compared between medication and genotype groups; levels > 1500 ug/ml will be considered adherent. Although urinary riboflavin is not a perfect measurement of adherence,

c) Dosing. Subjects will be instructed to take medication capsules (each containing either placebo or 100 or 200 mg of tolcapone) in the morning, at noon, and in the evening on the schedule shown in **Table 3**. On day 7, subjects will take their final dose between 11 am and 2 pm, at least one hour before the final brain scan.

Table 3. Dosing schedule and safety precautions

	Dose	Frequency	Safety precautions
Day 1	100 mg	Twice daily	Subjects will take first dose of medication at study site, in front of study personnel.
Day 2	100 mg	3x daily	
Day 3	100 mg	3x daily	
Day 4	200 mg	3x daily	Subjects will be called and assessed for medication side effects/tolerability.
Day 5	200 mg	3x daily	
Day 6	200 mg	3x daily	
Day 7	200 mg	2x daily	LFTs repeated on Day 7 or 8 (after last dose of medication).

4) Test procedures

a) Pre-treatment scan session (day 1). Upon their arrival at the MRI suite, subjects give a breath or saliva sample for alcohol testing, to ensure they have not recently consumed alcohol, and administered the Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar; 65), to ensure they are not currently experiencing significant alcohol withdrawal symptoms. Subjects with a breath or saliva alcohol content > 0.02 will be rescheduled, and those with a CIWA-Ar score > 3 will not be scanned. However, in our experience, very few subjects arrive intoxicated or in alcohol withdrawal. Subjects will then be administered the Vocabulary and Matrix Reasoning subtests of the Wechsler Abbreviated Scale of Intelligence-II (WASI-II). For subjects who were referred from study 46264 (PI: Schacht), the WASI-II score from that study will be used, since this assessment cannot be repeated over short intervals. Scans will be performed with a Siemens 3.0T TIM Trio MR scanner with actively shielded magnet and high-performance gradients (45 mT/m, 200 T/m-sec). Each scan will last approximately 60 minutes and will consist of a high-resolution anatomical image, for subsequent stereotactic registration, and two gradient-echo echoplanar imaging (EPI) sequences: 1) an alcohol cue-reactivity task; and 2) three runs of a response inhibition task. Dr. Schacht will supervise all aspects of the proposed neuroimaging procedures, which are similar to those used in our current ARC project. Following the scan, blood will be collected for the red blood cell COMT activity assay. Subjects will then be randomized to tolcapone or placebo medication, and will take their first dose of medication at the study site.

1. Alcohol cue-reactivity task. This task, for which detailed methods are described in our past papers (e.g., 66; 67), reliably elicits OFC and vmPFC activation (68). Subjects are given a sip (10 mL) of 80-proof liquor mixed with juice (1:3 ratio), and are then shown pseudorandomly interspersed images of alcoholic (ALC) and non-alcoholic (BEV) beverages, visual control images, and fixation (see **Figure 5**). These stimuli were selected from a normative set, supplemented with images from advertisements, and matched by color, hue, and complexity. Stimuli are presented in six 120-s epochs, each consisting of four 24-s blocks of an image type (one block each of ALC, BEV, control, and fixation). Each block is followed by a 6-s washout period, allowing the hemodynamic response from the previous block to decline before the next is presented. A 12-m gradient-echo EPI sequence will be acquired (parameters: repetition/echo time (TR/TE)= 2200/35 ms; flip angle (FA)= 90°; field of view (FOV)= 192 mm^2; voxel size= 3.75 x 3.75 mm; 36 contiguous 3-mm-thick slices).

Figure 5. Sample alcohol (left) and beverage (right) images from the cue-reactivity task.

2. Response inhibition task. This task is adapted from a well-validated event-related stop-signal task (38) that reliably elicits dmPFC, dlPFC, and inferior frontal gyrus activation. We have used this task during the current ARC funding period. Subjects are presented with a series of trials on which they are instructed to respond as quickly as possible to a target stimulus (open circle). On 25% of trials, the target stimulus is followed by a "stop" stimulus (X: the "stop signal"), and subjects are instructed to attempt to withhold their responses on these trials (see **Figure 6**). The onset latency between the appearance of the target and the stop signal begins at 189 ms and increases by 67 ms (i.e., becomes more difficult) if the subject is able to withhold his or her response to that trial; conversely, onset latency decreases by the same amount if the subject fails to inhibit his or her response. This "psychophysiological staircase" procedure (69) ensures that all subjects perform the task equivalently, thereby removing behavioral variance and allowing isolation of the neural substrates of response inhibition. Responses must be made within 1 s of stimulus presentation, and the inter-trial interval varies randomly between 1 and 4 s, allowing subjects to develop a prepotent "go" response to the more frequent targets that are not followed by stop signals, but preventing rhythmicity in responding. To ensure a sufficient number of the less frequent stop trials, three 10-m gradient-echo EPI runs of the task will be acquired (parameters: TR/TE= 2000/25 ms; FA= 85°; FOV= 220 x 220 mm; voxel size = 3.44 x 3.44 mm; 32 contiguous 4-mm-thick slices).

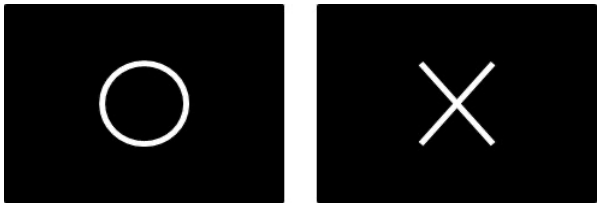


Figure 6. Target stimulus (left) and stop signal (right) from the response inhibition task.

b) Natural drinking period (days 1-6). Subjects will be given no specific instructions regarding their drinking during the first six days of study medication ingestion. On day 7, before the scan, subjects will report their drinking over the previous six days using the Timeline Follow-back calendar method (70).

c) Post-treatment scan session (day 7). Subjects will again give a breath or saliva sample for alcohol testing and assessed for alcohol withdrawal symptoms, and the functional sequences acquired during the pre-treatment scan will be repeated. At this visit or the following day (day 8), blood for LFTs and the COMT activity assay will also be collected.

d) Feedback session (day 8) and follow-up. The day after the second brain scan, subjects will be seen by a clinician for a 1-hour motivational enhancement session, using information from the GSC brief treatment, to motivate them to seek treatment for any problems related to their drinking. To ensure participation, subjects will not be compensated for study participation until after this session. If subjects express interest in treatment, one additional session will be offered. Subjects will also be shown the NIAAA "Rethinking Drinking" website. The Physical Symptom Checklist will be repeated at this time. If any major physical complaints are noted, the subject will be invited to return to the laboratory for an in-person evaluation by a study physician.

5) Data collection and analysis

a) Data collection and management

Self-report measures will be collected with the Research Electonic Data Capture (REDCap) system (http://project-redcap.org), implemented on a password-protected Apple iPad. REDCap is a mature, secure web application for building and managing online surveys and databases. Because no keystroke data entry is necessary, REDCap reduces the risk of data entry errors and saves staff time. Each subject will use the iPad to record answers to each questionnaire. In addition, the system reminds the subject to complete missing items before the next questionnaire is administered. Data collected using REDCap are automatically uploaded to a secure, password-protected online database, and can subsequently be exported into SPSS data files for analysis. Studies using REDCap for data collection in clinical research have been approved by MUSC's IRB, and the study PI has successfully implemented it in other research projects at MUSC.

Data storage and management for MRI data will be handled by the Center for Biomedical Imaging (CBI). A significant component of CBI is the informatics management system, which consists of an integrated system of Linux workstations surrounding a central core of Linux servers and a growing cluster facility, allowing network access for data retrieval and image analysis both locally and to CBI faculty working remotely. A full-time informatics person manages the system, providing system design, trouble-shooting, data export, software maintenance, and systematic backup. The system is stored at MUSC and is password-protected and encrypted for data integrity and confidentiality.

b) Sample size determination and power analysis. The effect size for APZ among *COMT* met-allele homozygotes in our preliminary data (d = 1.7) and the genotype frequencies for the *COMT* val158met SNP were used to determine sample size and statistical power for Aim 1. Power was calculated iteratively with repeated estimation from the SPSS (IBM, Armonk, NY) MANOVA package. This analysis indicated that power to detect a *COMT* by tolcapone interaction in which tolcapone's effect size among val-allele homozygotes is 1.3 or greater is 0.8 with 30 subjects per genotype group. We anticipate that, out of 140 subjects with normal liver enzymes referred by the **ARC Clinical Intake and Assessment Core**, approximately 31 will carry the met/met genotype, 65 the val/met genotype, and 44 the val/val genotype. Accordingly, val/met and val/val subjects will be chosen for inclusion in this project or the other clinical project on the basis of an *a priori* randomization table. Those not chosen for inclusion will be referred to Project 4.

For Aim 2, the path coefficients *a, b,* and *c'* will be used to estimate the strength of the causal relationship between each pair of variables (see **Figure 7**), and mediation will be determined by the significance of the joint (*a* * *b*) product: does the indirect path from the independent variables through the mediator to the dependent variable (drinking) explain significant variance? Fritz and Mackinnon (80) have conducted large-scale simulation to form empirical estimates of power for mediated effects similar to Cohen's (81) original analysis of effect size for simpler regression problems. For 90 subjects and medium effect sizes (d = 0.5) for the two arms of the indirect effect, power to detect a mediated effect is 0.8. Thus, power analyses for both Aims 1 and 2 suggest that a total N of 90 will yield ≥ 0.8 power to detect the hypothesized effects.

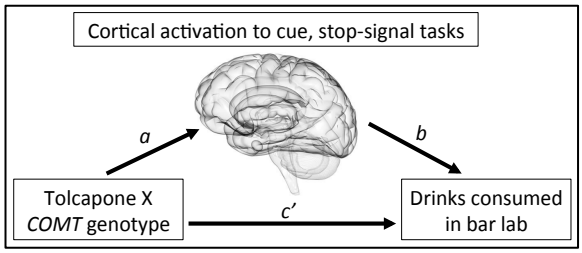


Figure 7. Proposed mediation pathway. The product *(a * b)* estimates how much of the effects of *COMT* genotype and tolcapone on bar-lab drinking are due to cortical activation to the cue or stop-signal tasks.

c) Randomization plan. *COMT* genotype will be used to prospectively randomize subjects to tolcapone or placebo medication. The *COMT* val/val genotype is more frequent among African-Americans (AA) than among European-Americans (EA), the two primary racial groups in the Charleston, SC area. In our preliminary data, 16% of subjects were AA and 83% were EA, consistent with the usual racial distribution of our studies. Val/val genotype frequency was 0.70 among AA and 0.25 among EA, consistent with dbSNP allele frequency estimates (Asian-Americans, who have comprised ~5% of our past study populations, have val/val frequencies similar to AA). To account for racial differences in val-allele frequency, we will stratify subjects by race (AA/Asian vs. EA) and *COMT* genotype (val/val, val/met, or met/met), and will then urn randomize subjects to tolcapone or placebo. Since some recent data suggest that the effects of both tolcapone (82) and *COMT* variation (83; 84) differ by sex, sex will be used as the urn variable within each of these six strata. The **ARC Biostatistics Core** statistician will oversee this process.

d) Aim 1: Evaluate tolcapone and *COMT* val158met effects on drinking in the natural environment during six days of treatment.

1. Analysis and expected results. Analyses for all aims will be conducted with consultation from the **ARC Biostatistics Core** statistician. The main dependent variable for Aim 1 will be drinking under natural conditions during days 1-6 (drinks per day). The general linear model (GLM) will be used to analyze the main effects of tolcapone and *COMT* genotype, as well as their interaction, on this variable. Pre-treatment drinking will be included as a covariate in these models, as will any salient demographic variables (e.g., age, cigarette smoking, family history of alcoholism). *We predict that tolcapone, relative to placebo, will reduce natural drinking, and will do so to a greater extent among COMT val-allele homozygotes.*

2. COMT activity assay. As part of this aim, we will also measure COMT inhibition peripherally, in red blood cells (since it is not possible to measure it in the brain), both to confirm that *COMT* val158met genotype indeed affects this construct among individuals with AUD and to evaluate medication adherence and/or individual variation in response to tolcapone. A previously described COMT activity assay (29) will be conducted in the CNL, and the GLM will be used to analyze effects of *COMT* genotype, medication, and their interaction on change in COMT activity between days 1 and 7. We will also evaluate whether, in the tolcapone group, level of COMT

inhibition is associated with amount of drinking in the natural environment, and will consider using this variable as a covariate in other analyses if it varies significantly between subjects.

e) Aim 2: Test tolcapone and *COMT* val158met effects on cortical activation related to alcohol cue reactivity and cognitive control, and evaluate whether these actions mediate its effects on drinking.

1. Preprocessing: Functional images from the cue and stop-signal tasks will be pre-processed with the FSL software package (FMRIB Analysis Group, Oxford, UK) (85); steps will include realignment to the first volume, smoothing with an anisotropic 8-mm FWHM kernel, high-pass filtering, resampling to 2-mm isotropic voxels, and stereotactic registration to the Montreal Neurological Institute 152-subject average template. Subjects with > 2 mm translational/2° rotational movement during either task will be excluded from analysis.

2. Cue task analysis: The averaged timecourse of the blood oxygen level dependent signal (BOLD) will be extracted from each subject's preprocessed images from *a priori* anatomically defined regions of interest (ROIs), defined as 6-mm-radius spheres around literature-based points in the vmPFC (BA 10) and OFC (BA 11). Hierarchical linear modeling (HLM v. 7.0, Scientific Software International, Skokie, IL) will then be used to calculate the difference in activation between the ALC and BEV blocks of these timecourses, and to analyze maximum likelihood estimates for ALC vs. BEV activation as a function of time, treatment, genotype, and the cross-level interactions of these factors. Specifically, the cue task stimuli will be nested within time (post- vs. pre-treatment scan); time will be nested within subject; and subjects will be nested within treatment (tolcapone vs. placebo) and *COMT* genotype (val/val vs. val/met vs. met/met).

3. Stop-signal task analysis: For each of the three task runs, trials will initially be classified as fixation, successful/unsuccessful hits (go trials), or successful/unsuccessful inhibitions (stop trials), and an event-related GLM, in which the hemodynamic response function (HRF) is convolved with the onset times of each trial, will be used to model each subject's preprocessed images with FSL FEAT v. 5.90. To eliminate effects of task-correlated motion, for each subject, the six motion parameters from the realignment will be also included in this GLM. For each subject, two contrast images will be generated for each run: 1) successful vs. unsuccessful inhibitions; and 2) go trials following unsuccessful inhibition trials on which the subject's reaction time increased vs. decreased, relative to the mean go trial reaction time (i.e., following an error, the subject's behavior became more vs. less restrained). The latter contrast, termed "post-error slowing", is an index of cognitive control (86). The three runs will be combined with a fixed-effects model, and a linear mixed effects model (FSL FLAME) will be used to test the interacting effects of time, treatment, and *COMT* genotype on both contrasts across the whole brain, with a voxelwise threshold of $z > 2.3$ and a cluster threshold of $p < .05$, corrected for the false discovery rate. For each subject, individual percent-signal-change (PSC) values for each contrast will also be extracted from *a priori* anatomically defined ROIs, defined as 6-mm-radius spheres around literature-based points in dmPFC (BA 9) and bilateral dlPFC (BA 46) and IFG (BA 47).

4. Expected mediation results: We will test each of the vmPFC and OFC ALC vs. BEV coefficients and the dmPFC, dlPFC and IFG PSC values for the stop-signal contrasts (post- vs. pre-treatment) as individual mediators of the medication by genotype interaction on drinking (see **Figure 7**). To compare the effects of individual mediation by activation of any of the ROIs with the combined effects of cue-elicited and response-inhibition-related cortical activation, we will also test a multiple mediation model, in which the brain activation values described above are all tested in the same model. Using the SPSS AMOS package, we will model covariances between these ROI values (to partial out their shared variance), and will test whether the unique variance of the five ROIs collectively mediates the relationship between medication, genotype, and drinking to a greater extent than any individual ROI. To satisfy criteria for complete mediation, the medication and genotype variables must significantly predict the brain activation values, which must themselves predict significant variance in the drinking variables, and the medication by genotype interaction must be non-significant when the brain activation values are added to the model. If this interaction is still significant in the combined model, criteria for partial mediation can be satisfied if the indirect path between the interaction, the brain activation values, and drinking is also significant. *We predict that tolcapone, relative to placebo and to baseline, will reduce ALC vs. BEV activation of OFC and vmPFC and reduce dmPFC, dlPFC, and IFG activation for the successful vs. unsuccessful inhibitions and post-error slowing contrasts. Further, we predict that these effects will be greatest among val-allele homozygotes, that they will be related to drinking, and that they will mediate the medication by genotype interaction on drinking.*

f) Exploratory Aim: Explore tolcapone effects on cortical functional connectivity.

Analysis and expected results: Cortical functional connectivity during the cue and stop-signal tasks will be explored with the psychophysiological interaction (PPI) method (87), which analyzes connectivity between brain regions as a function of psychological context. Although our hypothesis is specific to corticostriatal connectivity, it is important to note PPI allows specification only of the initial "seed" regions; connectivity differences may exist between these seeds and multiple other regions, and we will examine all such differences. Briefly, BOLD signal timecourses will be extracted from each of the ROIs described above (seeds) and deconvolved from the canonical HRF, yielding an estimate of the neuronal signal in each seed that generated the observed HRF. In a separate model for each seed, the BOLD timecourse from that seed will be multiplied by the onset times and durations for the psychological conditions of interest (for the cue task, the ALC and BEV blocks; for the stop-signal task, successful and unsuccessful inhibitions) and reconvolved with the canonical HRF, yielding an interaction term that represents the BOLD signal in each seed only during the conditions of interest. This interaction term, along with the main effects terms (i.e., the onset times and durations for the conditions of interest, and the original seed timecourse) will be entered as regressors in a whole-brain GLM. Significant effects of the interaction terms in these GLMs will indicate brain regions whose signal fluctuates synchronously with the seed's signal specifically during the conditions of interest (i.e., ALC vs. BEV blocks and successful vs. unsuccessful inhibitions). *We predict that tolcapone, relative to placebo and to baseline, will decrease connectivity between striatum and OFC and vmPFC during alcohol cue exposure, and will increase connectivity between striatum and dlPFC, dmPFC, and IFG during response inhibition.*

C. PROTECTION OF HUMAN SUBJECTS

1) Risks to Human Subjects

a) Human Subjects Involvement, Characteristics, and Design

We anticipate that the ARC Clinical Intake and Assessment Core (CIAC) will refer 90 subjects to us for participation. Half of these subjects will be randomized to tolcapone, and the other half to placebo medication. We anticipate that 70% of subjects will be male and 30% female, and that approximately 20% of subjects will be racial and ethnic minorities. No special vulnerable populations will be involved in this research.

During assessment at the CIAC, subjects will receive liver function tests, and those with normal liver enzymes (transaminases) will be genotyped for the *COMT* val158met single nucleotide polymorphism (SNP). Due to the allele frequencies for this SNP, all subjects with the met/met genotype will be referred for participation, as will approximately half of the subjects with the val/met genotype and two-thirds of the subjects with the val/val genotype. Subjects with normal liver enzymes who have the val/met or val/val genotype will be randomly chosen for referral to this study or to the other clinical component of the ARC or an ARC pilot study.

b) Sources of Materials

1. The materials used for analysis in this protocol are verbal report, DNA, biologic specimens, and brain images. All reports and samples will be collected directly from the patients during the course of their participation in this study. Verbal report data will be collected from direct subject interview, questionnaires, and computer tasks. Biologic samples are both urine and blood samples. Urine samples are provided by natural means. Blood samples are obtained through standard venipuncture techniques. Brain images will be obtained via magnetic resonance imaging (MRI).

2. The CIAC will initially obtain blood samples, extract DNA, and genotype the *COMT* val158met SNP. If the CIAC subsequently refers a subject to this protocol, we will use this information to randomly assign the subject to tolcapone or placebo medication.

3. As detailed in the informed consent subjects sign upon intake at the CIAC, if the CIAC refers a subject to this protocol, information obtained during the CIAC assessment (i.e., interviews and questionnaires) will also be shared with the study team.

4. Upon their enrollment in this study, subjects will be assigned a study identification number that will subsequently be used to identify their data in lieu of other personal identifiers. Only the study PI(s) will have access to the database linking subjects' names to their identification numbers.

5. Data generated during the course of this study will be used specifically for the proposed project. Alcohol use data and identification of alcohol related problems will be used to provide educational information to subjects so that they may consider treatment options in an informed manner. If subjects request in writing that results of any evaluation or testing be shared with a third party, this request will be honored. All data will be collected and transferred according to HIPAA guidelines.

c) Potential Risks

There are several potential risks to subjects who participate in this study:

1. Individuals will be asked questions about their alcohol and other substance use as well as psychiatric symptoms. In addition, blood will be obtained for genetic analysis. All of this information is highly confidential and all attempts will be made to safeguard privacy and confidentiality.

2. Subjects may receive a study medication called tolcapone, which is approved by the FDA, but not for the treatment of alcohol problems or drinking. Unwanted side effects may occur with use of this medication. Tolcapone is FDA-approved for the treatment of Parkinson's disease, and is usually administered in combination with levodopa (L-DOPA). Tolcapone has also been tested in studies of patients with addictive behavior/disorders, including pathological gamblers and cigarette smokers. The most common side effects reported with tolcapone (sleep problems, hallucinations, dystonia) are related to its combined use with L-DOPA. Significant diarrhea, however, seems to be specific to tolcapone and has been reported even when used alone.

3. One very rare side effect associated with tolcapone is liver toxicity. While there was no indication of toxicity in preclinical work with tolcapone, clinical trial data suggested only a 1-3% greater incidence of increased liver enzymes (ALT and AST) over placebo. Nevertheless, in the first years of use, four out of 60,000 patients, dosed with tolcapone for two to four months, developed severe liver toxicity, with three deaths reported. Based on this, the FDA recommended routine liver enzyme monitoring in patients receiving tolcapone (every two weeks for the first year of treatment).

4. Subjects will be asked to abstain from alcohol the night before each MRI scan. Subjects with alcohol dependence may be at risk for alcohol withdrawal symptoms, depending on the extent of their prior alcohol use and their individual susceptibility. In our experience, most individuals who are at risk for alcohol withdrawal symptoms can be identified during the screening process based on their past history of such symptoms (e.g., seizure, delirium tremens). Given that this risk is a facet of subjects' pre-existing alcohol dependence, it is reasonably commensurate with the risk inherent to them in their daily lives should they choose to voluntarily reduce their drinking.

5. Subjects will undergo two MRI scans, which involve strong magnetic fields that may interact with metal in the body. Some subjects may also feel claustrophobic in the MRI scanner. In our experience, both metal in the body and claustrophobia can be identified during the screening process.

6. During each MRI scan, subjects will be exposed to alcohol-related cues. Some subjects may experience increased alcohol craving or an urge to drink, but generally no greater than under natural conditions. Based on our experience with alcohol cue exposure among alcoholics, we have developed a process to minimize this risk that is described below.

Although medication will be administered during the course of this study, this is purely an experimental paradigm, and subjects who participate will explicitly not be expecting "treatment." Consequently, there are no alternative treatments indicated.

2) Adequacy of Protection Against Risks

a) Recruitment and Informed Consent

1. Subjects for this study will be referred from the ARC Clinical Intake and Assessment Core.

2. Upon referral, potential subjects will be given an informed consent form that details the study

requirements, risks, and benefits. The study PI(s) and/or research assistant(s) will review the contents of the informed consent with the subject, and both the subject and one of these individuals will sign the document. A copy will be given to the subject and the original will be placed in the research record.

b) Protections Against Risk

1. To minimize risks to privacy of individuals and confidentiality of data, besides the study identification number assigned at the beginning of each subject's participation in the study, no other identifiers will be used. All research records will be stored in locked cabinets and only the faculty and staff listed on this grant application or their replacements will have access to those records. All laboratory tests will be coded only with a subject number to ensure confidentiality. To assure confidentiality of genetic samples, only the study identification number will be used.

2. To minimize the risk of serious alcohol withdrawal symptoms, potential subjects will be evaluated before inclusion in the study. Subjects with a Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) score > 3 will be excluded from scanning, and those with significant alcohol withdrawal (i.e., CIWA-Ar score > 10) will be referred for treatment at the MUSC Center for Drug and Alcohol Program's walk-in outpatient clinic. An inpatient substance detoxification unit is also available if necessary. Both units are less than a block away from the scanner and routinely accept admissions from addiction research studies. If, in the judgment of the investigator or subject, more acute treatment is necessary, the subject will be escorted to the MUSC Emergency Department, which is also less than a block away.

3. To minimize the risks of liver cell damage from study medications, we will only enroll individuals in the study who 1) report no history of past liver disease, including gastritis, pancreatitis, colitis, or peptic ulcer disease; and 2) have normal liver enzymes (ALT and AST) during screening, as determined by the CIAC assessment.

4. To further minimize the risks of liver damage, subjects who are randomized to tolcapone will take this medication for only seven days, which is shorter than the first FDA-recommended liver enzyme test date (two weeks of dosing). However, we recognize that those heavily consuming alcohol may be at increased risk for liver compromise. Therefore, we will contact subjects after they have taken medication for three days (i.e., on study day 4) to inquire about their wellbeing, including checking for any complaints of abdominal pain, gastrointestinal disturbance, extreme fatigue or skin color change. These symptoms will also be assessed in person on study day 7. Liver enzyme tests will be repeated on study day 7 or 8, after medication administration is complete. If any possible liver-related side effects are present on study day 4, subjects will be discontinued from further study. If liver enzymes are elevated above the upper limit of the normal range on study day 7 or 8, or possible liver-related side effects are present on this day, we will continue to monitor live enzymes until they return to normal and/or consider a referral to a hepatologist.

5. To minimize other risks associated with study medication ingestion, all subjects will be given a card that states that they are participating in a research study and may be taking tolcapone, and the name of the PI or his designate will be provided for emergency contact. During the course of the study, medical coverage will be provided for emergency situations. To minimize medication side effects, the dose of tolcapone will be titrated up over several days.

6. To minimize risks associated with MRI, all subjects will be screened for metal in their bodies, as well as for claustrophobia. Metal found, particularly around the head and neck area, will be exclusionary for participation. Metal in other areas of the body will be evaluated by trained technical staff prior to magnetic exposure. An anatomical brain image will be collected and reviewed for any significant brain pathology before proceeding with the scan. During the scan, subjects may stop the procedure at any time if they feel overly anxious or claustrophobic.

7. A motivational enhancement session will be scheduled for the day after the second scan session. During this session, the alcoholic subject will undergo a debriefing session regarding study participation and receive brief educational and motivational counseling about the risks of heavy alcohol consumption and

its effects on his/her life. The range of treatment services available will be explained. If a subject wishes to pursue treatment, an appropriate referral will be made that day or at any future time that the subject desires. The subject will be paid for study participation only after attending this debriefing, motivational, and educational session.

8. All serious adverse events will be reported to the IRB in a timely manner. In addition, at least once a year, a review of all adverse events reported will be undertaken and reviewed by the ARC Director and Scientific Director, and referred to an ARC Data and Safety Monitoring Board if necessary.

3) Potential Benefits of the Proposed Research to Human Subjects and Others

1. Subjects will be compensated for their participation, and may benefit from learning more about their alcohol consumption and the health consequences associated with it. Subjects may also benefit from receiving a referral to alcohol treatment services, if they express interest in such services.

2. The risks to subjects are reasonable in relation to the anticipated benefits they will gain. Risks to subjects can be satisfactorily minimized to keep the risk to benefit ratio acceptably low.

4) Importance of the Knowledge to Be Gained

1. Alcohol use disorder (AUD) is a devastating disease for individuals and society. Treatment studies to ascertain the relative benefits of medications to treat alcoholism pose risks to large numbers of subjects, and are quite expensive and time-consuming to conduct. This is particularly true when only a minority of exposed patients actually benefit from the treatment. Paradigms such as the one proposed in this protocol have the potential of providing information regarding the utility of drugs for the treatment of AUD that is both time efficient and cost effective. The use of genetic information to "tailor" specific medication to individual biological response (pharmacogenomics) is quickly becoming a very feasible and useful way of improving medication delivery to those who can most benefit from it.

2. The risks to subjects are reasonable in relation to the possibility that the knowledge gained from the proposed research will improve treatment options for AUD. Risks to subjects can be satisfactorily minimized to keep the risk to benefit ratio acceptably low.

5) Data and Safety Monitoring Plan

We recognize that this investigation does not constitute a clinical trial, but an experiment in non-treatment-seeking individuals. Nonetheless, a Data Safety and Monitoring Plan needs to be in place. The PI is responsible for the assessment, recording, and reporting of all adverse events associated with the study, whether routine or severe. Once per study year, at the time of the annual report, the project PI will work with the ARC's statistician to prepare a report of all study-related adverse events. The project PI will summarize these events and present them to the ARC Executive Committee (Scientific Director and Director) in a blinded fashion. If this committee deems it necessary, it will convene an independent review by a three-member Data Safety and Monitoring Board (DSMB) to review this report to determine if adverse events are of a different nature, or more than should be expected, from the study procedures or drugs being used. If a DSMB is assembled, it will have the authority to ask for more information, including the un-blinding of study data and/or more detailed reports. It will also provide a written report that could be submitted to the IRB. In addition, the report of the numbers and nature of events will be supplied to the NIAAA Project Officer at the time of the annual progress report. If a serious adverse event (SAE) occurs during the course of study participation, this SAE will be reported to the IRB within 48 hours of first knowledge of the event and will also be reported simultaneously to the NIAAA Project Officer. Also, during this annual review, recruitment, randomization, completion, and quality of the data will be reviewed. This plan is consistent with IRB policy at MUSC.

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