Title: Dismantling MBRP: Identifying Critical Neuroimmune Mechanisms of Action

NCT#: NCT02994043

Version Date: 14 JULY 2020

University of Colorado Boulder

TITLE: Mindfulness Based Relapse Prevention

PROTOCOL VERSION DATE: 14 July 2020
VERSION: 17

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In addition to the individuals listed above, undergraduate and professional research assistants will play an active role in the data collection phase of this study. Research assistants will assist with the study appointments and procedures (i.e., conducting phone screens to determine participant eligibility, collecting informed consent, running the MRI scan, performing the required laboratory techniques, questionnaire administration and blood draw). Therapists will be selected from the graduate program in clinical psychology at the University of Colorado and will have a master's degree in clinical psychology and previous training in the fundamentals of psychological intervention provided by Dr.

Hutchison or Dr. Dimidjian. All personnel have completed CITI training, and have been trained in responsible conduct of research, biosafety training and study specific procedures/guidelines.

I. OBJECTIVES

Please note that the grant supporting this project is funded by the NIAAA and IRB approval of the protocol is needed as soon as possible.

The goal of this study is to examine the effectiveness of Mindfulness Based Relapse Prevention (MBRP) versus Relapse Prevention (RP) for the treatment of Alcohol Use Disorders (AUD) by examining neurobiological, immunological, epigenetic, and microbial characteristics of AUD. We will compare the effectiveness of MBRP compared to RP by examining changes in DNA methylation of key genes implicated in alcohol dependence, expression of inflammatory cytokines, changes in number and diversity of microbial populations found in the gut, liver function tests, and connectivity and cue-elicited BOLD activation within reward and control circuits of the brain. In addition, we will explore how expression of inflammatory cytokines might influence the relationship between alcohol consumption, brain activation and connectivity within reward and control regions. This research will dismantle the effects of MBRP from the effects of RP in the treatment of AUDs and elucidate the mechanisms that mediate the effects of MBRP. From a larger perspective, the findings of this investigation will increase the body of knowledge about how MBRP works and will advance a biobehavioral conceptualization of the development and maintenance of alcohol dependence. Ultimately, this work should lead to the development of interventions that target epigenetic and immune system changes.

Specific Aim 1.
Comparing the effectiveness of MBRP (n=113) to RP (n=113).

- Hypothesis 1. It is hypothesized that MBRP will demonstrate superior effectiveness, as compared to RP, by reductions in heavy drinking days at the end of treatment (8 weeks) and longer term (20 and 32 weeks) decreases in liver function tests (8, 20, and 32 weeks).

Specific Aim 2.
Examining the putative molecular mechanisms underlying the effects of MBRP versus RP.

- Hypothesis 2. Based on studies suggesting that MBIs alter epigenetic regulation of key genes implicated in alcohol dependence (i.e., DRD2, SLC6A3, DBH) and reduce inflammatory pathway activation (i.e., IL-8, IL-6, TNFα), it is hypothesized that MBRP treatment will demonstrate significant decreases in methylation of key genes as well as decreases in inflammation biomarkers.
- Hypothesis 3. It is also hypothesized that individuals who receive MBRP, as compared to RP, will demonstrate greater LECN/RECN connectivity as well as reduced cue-elicited BOLD response in the striatum.

Specific Aim 3.
The third specific aim will determine whether the effectiveness of MBRP vs. RP on clinical outcomes is mediated by the effect of MBRP on molecular mechanisms, and, in turn, their effects on network connectivity and BOLD response.

- Hypothesis 4. It is hypothesized that the effects of MBRP on clinical outcomes (Hypothesis 1) will accrue through two routes: the effects of MBRP on reductions in inflammatory markers

(Hypothesis 2) which in turn influence LECN/RECN connectivity; and the effect of MBRP on methylation of key genes which in turn influence cue-elicited BOLD activation in the striatum (Hypothesis 3). These effects will be tested in the context of a structural equation modeling framework.

Exploratory Aim 1.

Comparing gut microbiota populations pre and post intervention for MBRP vs. RP

- Hypothesis 5. We will test the hypothesis that there will be differences in gut microbiota between MBRP and RP groups both before and after 8 weeks of treatment, as measured by microbial DNA collected from fecal samples.

II. BACKGROUND AND SIGNIFICANCE

Alcohol use disorders (AUDs) are associated with socioeconomic costs of approximately $234 billion in the United States alone (Rehm et al., 2009). Despite decades of research, the best treatments have proven to be only modestly successful. Meta-analyses suggest that common psychosocial treatments, such as 12 step facilitation, motivational enhancement approaches, and cognitive behavioral therapies lead to 12 month abstinence rates of 17 to 35% (Miller, Walters, & Bennett, 2001). Results from Project COMBINE, one of the largest, most carefully controlled clinical trials to date, indicated that naltrexone and medication management and a combined behavioral intervention were superior to other conditions, but only produced a 12 month abstinence rate of approximately 20% (Anton et al., 2006).

Clearly, there is room for improvement. While a number of psychosocial treatments are modestly effective, it is largely unknown ***how*** these treatments exert their effects. New studies designed to probe the therapeutic mechanisms that drive the efficacy of existing treatments are needed to further refine efforts to more effectively target those mechanisms. Information about the active mechanisms may also be used to develop measures that will provide an early signal about whether a given treatment is working, which could be instrumental in deciding whether to switch treatments. Finally, knowledge about the mediating mechanisms can be used to identify critical associated domains of patient heterogeneity, which in turn can be used to identify the patients most likely to respond to a particular treatment.

Recent funding announcements from NIAAA have acknowledged the significance of research on treatment mechanisms. For example, PAR 14-051 "Mechanisms of Behavior Change" is an acknowledgement of the need for exactly this type of research, as it calls for "*research that bridges the gap between basic science processes and clinical treatment by increasing our understanding of how treatment impacts specific mechanisms operating at the cognitive, neurobiological or genetic level of analysis in relation to alcohol-related outcomes."* The current protocol will first review the critical neurobiological factors that underlie the etiology and maintenance of AUDs with an emphasis on cutting edge research published in the last few years as well as our own preliminary data. This framework will then be used to discuss how existing treatments may impact these neurobiological mechanisms. In the context of this critical review, we will argue that Mindfulness Based Relapse Prevention (MBRP) emerges as the candidate with the strongest support as a treatment that impacts these mechanisms.

Mechanistic Framework - Perturbations in Control and Reward Regions in the Brain.

In order to provide a conceptual foundation for the aims and hypotheses of the present application, it is important to contextualize the putative effects of existing treatments in a broader conceptual model that integrates molecular, neural, and clinical constructs associated with relapse. For a number of years now, scientists have emphasized neuroadaptations in the control and reward networks of the brain and the interplay between the two (Karoly, Harlaar, & Hutchison, 2013; Koob & Volkow, 2010). The model presented below (see Figure 1) is grounded in both basic animal neuroscience and human cognitive neuroscience and identifies two primary brain networks that influence an individual's alcohol and drug use behavior. The control network (top of Figure 1) consists of prefrontally distributed areas, including the orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), and inferior frontal gyrus (IFG). The cognitive neuroscience literature has consistently implicated these areas in reflection and control over impulsive decisions, evaluation of the magnitude of reward, and the urge to drink or use drugs (Bechara & Van Der Linden, 2005; Goldstein & Volkow, 2002; Wong et al., 2006). The reward network (bottom of Figure 1) is involved in the anticipation and processing of reward and has been studied extensively in the animal and human literature (Berridge & Robinson, 1998; Kalivas & Volkow, 2005). Research has repeatedly implicated specific structures (e.g., striatum, anterior cingulate, precuneus) in the motivation to use drugs and the attribution of incentive salience to drug cues, and this is consistent with our own human neuroimaging studies (Claus et al., 2011). While there has been substantial research on reward and control networks and how they may be related to treatment outcomes, there has been much less work on the molecular mechanisms that underlie the neuroadaptations in these networks, especially in clinical settings. In this application, we argue that the molecular precursors of these adaptations should be primary targets for intervention development.

Molecular Mechanisms Underlying Alcohol-Induced Adaptations in Reward and Control Circuitry.

As described in Figure 1, emerging evidence suggests that molecular mechanisms such as epigenetic regulation and changes in immune system function, particularly inflammatory processes, are involved in the effects of alcohol on control and reward circuits. We will discuss the evidence for the immune system first, followed by the role of epigenetic regulation.

At first glance, the link between AUDs and immune processes may not be obvious. However, recent studies have suggested that perturbation of the immune system is a critical mechanism in the etiology of AUDs (for reviews see Mayfield, Ferguson, & Harris, 2013; Leclercq et al., 2012; Leclercq et al, 2014). More specifically, alcohol produces deleterious effects on peripheral and central immune signaling pathways, particularly through activation of lipopolysaccharide (LPS)-induced TLR4 signaling cascades that span the gut, liver, and brain, and coincide with the release of cytokines and other pro-inflammatory pathways in the brain (Fernandez-Lizarbe, Pascual, & Guerri, 2009). Repeated activation of pro-inflammatory pathways and microglia is

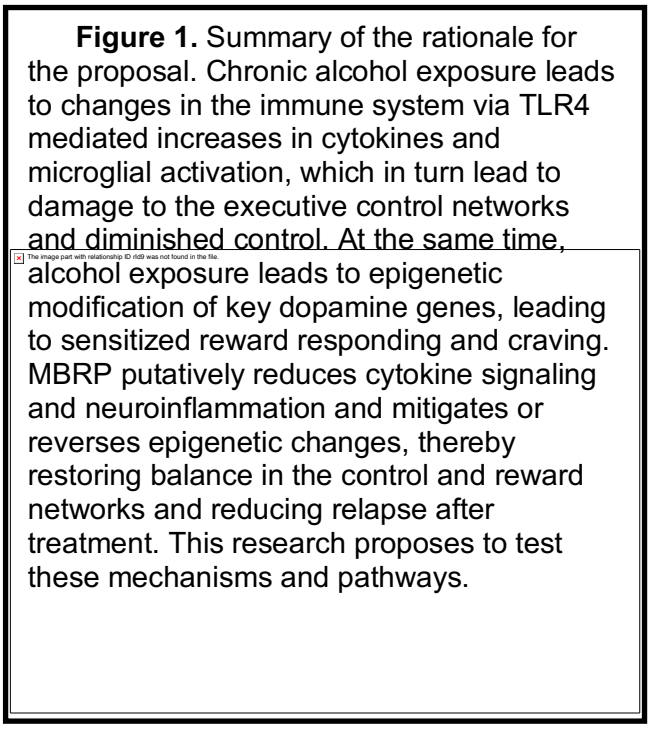


Figure 1. Summary of the rationale for the proposal. Chronic alcohol exposure leads to changes in the immune system via TLR4 mediated increases in cytokines and microglial activation, which in turn lead to damage to the executive control networks and diminished control. At the same time, alcohol exposure leads to epigenetic modification of key dopamine genes, leading to sensitized reward responding and craving. MBRP putatively reduces cytokine signaling and neuroinflammation and mitigates or reverses epigenetic changes, thereby restoring balance in the control and reward networks and reducing relapse after treatment. This research proposes to test these mechanisms and pathways.

associated with increased neuroinflammation and neural damage in frontal brain regions involved in executive function and control (Alfonso-Loeches et al., 2010; Guerri & Pascual, 2010). These neuroinflammatory processes have also been implicated in the etiology of a number of other neurodegenerative diseases as well as the deleterious effects of aging on cognitive function (Smith, Das, Ray, & Banik, 2012; Ownby, 2010; Lyman et al., 2014).

While animal models have indicated that acute and chronic alcohol use is associated with increased levels of LPS circulating in the blood, there have also been studies in humans demonstrating that acute binge drinking releases LPS which in turn ligates TLR4 and activates the release of cytokines and inflammation. Specifically, a recent study in humans tested the effects of an acute binge episode on circulating LPS levels (Bala et al., 2014). The study estimated that the physiological dose of LPS after a binge episode was 100 pg/ml. As part of the study, peripheral blood mononuclear cells (PBMCs) were isolated from subjects and challenged ex-vivo with a 100 pg/ml dose of LPS. The study observed significant induction of cytokines (TNFα, IL-6, MCP1). Thus, these data are also consistent with the notion that the alcohol/LPS/TLR4/neuroinflammation pathway is a central mechanism in the etiology of AUDs.

While the neuroinflammatory effects of alcohol likely underlie changes in control circuits, epigenetic regulation of genes and proteins involved in the reward system are also clearly important factors in the etiology of AUDs. Epigenetics is a term that refers to the potentially reversible biochemical processes that regulate gene transcription and expression without altering the DNA sequence. Recent studies in animals and humans have suggested that epigenetic mechanisms may mediate the effect of alcohol and drugs on long lasting adaptations in the brain (Nestler, 2014; Starkman, Sakharkar, & Pandey, 2012; Warnault & Ron, 2013). Modern epigenetic research has focused primarily on how changes to DNA influence chromatin structure (Goldberg, Allis, & Bernstein, 2007). DNA methylation and histone acetylation are two epigenetic processes that have been shown to modify chromatin and exert downstream effects on gene transcription. DNA methylation tends to occur at unmethylated cytosine guanine (CpG) dinucleotides, clusters of which are often located in promoter, or 5' regions, of many human genes, and have come to be known as CpG islands (Jones & Takai, 2001). Unusual patterns of methylation in humans have been associated with numerous diseases (Santos, Mazzola, & Carvalho, 2005), including cancer, schizophrenia, and a variety of other psychiatric conditions including AUDs (Shukla et al., 2008). While still a nascent area of research, there are a growing number of studies on epigenetics and alcohol abuse in humans that have focused on DNA methylation. With respect to AUDs, previous research has observed increased methylation levels in alcohol-dependent patients compared with controls in a number of genes (e.g., Hillemacher et al., 2009; Zhang et al., 2013). In addition, a recent genome-wide methylation study in our lab implicated methylation of the DRD2 promoter (Harlaar et al., 2014).

Our most recent data suggest that epigenetic changes in the DRD2 promoter are strongly associated with DA D2 receptor binding in vivo using [C^{11}] raclopride PET, associated with BOLD response to alcohol cues using fMRI, and associated with clinical measures of impaired control over alcohol use (see preliminary studies). This is critical because mesolimbic dopamine activation has long been implicated as a major mechanism in alcohol and drug-related reward (Koob & Volkow, 2010). Even more specifically, the DA D2 receptor and the DRD2 gene are arguably the most important targets in this system. DRD2 expression is reduced in the nucleus accumbens among high-alcohol-preferring mice (Bice et al., 2008) while over-expression is associated with low-ethanol self-administration (Thanos et al., 2001). In humans, positron emission tomography (PET) studies have found that decreased striatal DA D2 receptor availability is associated with substance abuse more generally and alcohol dependence specifically (Volkow et al., 2009; Heinz et al., 2004) Alterations in

dopamine function have also been linked to downstream endophenotypes associated with reward processing, inhibitory control, and learning (Koob & Volkow, 2010). Thus, findings across animal and human studies, using PET and fMRI, suggest that changes in D2 receptor availability and function are related to substance abuse and may underlie changes in reward response and impaired control, hallmarks of addiction. Treatments that have the potential to reverse or mitigate changes in the epigenetic regulation of the DRD2 and other genes could have substantial impact.

Treatment Targets: Immune System Inflammation and Epigenetic Regulation.

To summarize, epigenetic regulation and immune system activation are two critical molecular mechanisms that lead to changes in reward and control neural systems that have long been acknowledged as central to the development and maintenance of AUDs. In our reading of the extant literature, we examined existing medications and psychosocial interventions that have at least some efficacy in the treatment of AUDs and have also demonstrated safety. Naltrexone, varenicline, gabapentin, acamprosate, and topiramate were scrutinized. While many of these putatively target the neural reward system by virtue of their action at key receptor sites, there is little evidence to suggest that any of these medications target the underlying molecular mechanisms (i.e., epigenetic gene regulation) and/or inflammatory pathways. Topiramate is one possible exception given that one study has suggested that topiramate may have HDAC inhibitory activity (Eyal et al., 2004). However, the evidence is not particularly strong. In terms of medications that are known to alter epigenetic regulation and inflammation but have yet to be studied in the context of AUDs, most have extremely undesirable side effect profiles (e.g., voronistat). Finally, many of the existing anti-inflammatory medications also have serious side effects, which make them less attractive as candidate medications. For example, medications like diclofenac and vioxx (now withdrawn) are known to be associated with substantial increased risk of heart attacks and strokes (McGettigan & Henry, 2011). With respect to existing psychosocial treatments, many (e.g., motivational interventions, CBT) directly target cognitive control over alcohol use. Thus, existing pharmacotherapies and psychosocial treatments do not appear to target epigenetic regulation and immune system function. However, there is one relatively new psychosocial treatment with an increasingly robust evidence base suggesting effects on immune system function as well as epigenetic regulation.

A growing number of studies indicate that Mindfulness Based Interventions (MBIs) are associated with alterations in inflammatory genes and proteins (e.g., IL-6, IL-8, TNF-α) and epigenetic regulation (e.g., histone deacetylases) across a range of patient populations (Rosenkranz et al., 2013; Roberts et al., 2014; Antoni et al., 2012; Black et al., 2013; Bower et al., 2014; Pace et al., 2009; Carlson, 2003; Bhasin et al., 2013; Carlson et al., 2007; Kaliman et al., 2014; Witek-Janusek et al., 2008). For example, mindfulness based stress reduction (MBSR) has been shown to reduce inflammatory cytokine activation and increase anti-inflammatory cytokine activation in cancer patients (Carlson, 2003; Bhasin et al., 2013) with effects persisting up to 1 year (Carlson et al., 2007). Finally, a few studies have also suggested a dose effect, such that more frequent mindfulness practice appears to increase the longevity of intervention effects on inflammatory biomarkers such as TNF-α (Kaliman et al., 2014), which is consistent with other studies demonstrating associations between increased mindfulness practice and cytokine reductions (Bower et al., 2014).

It is important to note that this research has been conducted across a range of patient populations, rather than specifically with AUD patients. In addition, these studies have used a range of assays for immune system function. Some assays are more sophisticated and others less so. Finally, the choice of immune targets has not always been consistent. The most recent studies on the effects of MBIs have begun to incorporate more sophisticated methods for measuring immune system changes by examining

changes in gene and protein expression in PBMCs. In fact, three recent studies used procedures very similar to those proposed in this application and deserve a more thorough review including the observed effect sizes (expressed as Cohen's d). One of these studies was focused on the effects of MBI on cytokine production in PBMCs without a lipopolysaccharide (LPS) challenge (Witek-Janusek et al., 2008). In this study, an 8-week MBSR intervention (n=38) was compared to a control intervention (n=28) in a sample of breast cancer survivors. The results indicated that MBSR resulted in significantly lower levels of inflammatory cytokines in PBMCs across the trial (IL-4, d=.89 ; IL-10, d=.55). The effects were most pronounced immediately after the intervention and at the 4-week follow-up. There was no effect on IL-6. Another study examined the acute effects of a mindfulness meditation condition (n=19) versus a control condition (n=21) on expression of genes involved in epigenetic regulation (i.e., HDAC inhibitor genes) and inflammatory pathways in PBMCs (Kaliman et al., 2014). In this study, mindful meditation produced robust changes in genes involved in epigenetic regulation (HDAC2, d=.71; HDAC3, d=1.0; HDAC9, d=.92) and pro-inflammatory genes (RIPK2, d=.95; COX2, d=.52). Finally, the last study examined the effect of an MBI for the treatment of insomnia (Irwin et al., 2015). Unlike the first two studies, PBMCs were isolated and TLR4 receptors were stimulated with LPS before assaying for inflammatory markers. The MBI condition (n=48), compared to the control condition (n=25), reduced inflammatory biomarkers at 4 months (TNFα, d=.59 ; IL-6, d=.57) and 7 months (TNFα, d=.64 ; IL-6, d=.64) after the intervention. These studies were selected because they are most similar to the proposed study in terms of testing the effect of MBIs on immune system biomarkers in PBMCs. In all three studies, the effect sizes for the impact of MBIs on inflammatory biomarkers and epigenetic regulation ranged from moderate to large, indicating that MBIs may have strong effects on inflammation and epigenetic regulation. Finally, it is important to note that the last study described above is particularly relevant to the proposed work because it utilized LPS stimulation of TLR4 receptors, which is exactly the pathway that has been implicated in the development and maintenance of AUDs (see Figure 1 above). Thus, the proposed research will feature a vertically integrated assessment of epigenetic regulation, neurogenic inflammation including basal and LPS stimulated changes in inflammatory gene expression in PBMCs, immunoassays of cytokines in PBMCs, and downstream effects on structure and function of control circuits in the brain.

In sum, our literature review yielded close to a dozen studies suggesting that MBIs may influence two key neurobiological mechanisms that are critical to the etiology and maintenance of AUDs. Given the growing body of evidence suggesting that MBIs impact mechanisms that appear to be critical in the maintenance of AUDs, the question becomes whether there is an MBI that has demonstrated efficacy in the treatment of AUDs. In fact, a recent review of 24 clinical studies on substance use disorders suggests that MBIs represent a promising intervention (Chiesa & Serretti, 2014). One specific MBI, known as Mindfulness Based Relapse Prevention (MBRP) combines mindfulness components with relapse prevention and was manualized several years ago (Bowen, Chawla, & Marlatt, 2011). Perhaps most importantly, recent randomized control trials (RCTs) testing the effects of MBRP have provided the best evidence to date that MBRP is an efficacious treatment (Bowen et al., 2014; Witkiewitz et al., 2014). For example, in a large (n=286) RCT published in JAMA Psychiatry, MBRP resulted in superior alcohol and substance use outcomes as compared to a relapse prevention (RP) intervention only, and treatment as usual (Witkiewitz et al., 2014). The effect sizes for the MBRP versus RP in these two RCTs ranged from d=.2 to d=.4. (small to moderate). Thus, there is clearly a strong evidence base emerging around the efficacy of MBRP over and above RP in isolation, although much work remains to be done.

Alcohol and the Gut Microbiome

Prior research suggests that bacteria in the gut may influence numerous aspects of human health. The effects of alcohol on gut microbiota are currently not well understood, however several recent human and animal studies have linked chronic alcoholism with altered microbiota composition in the gut (Mutlu et al., 2012; Yan et al., 2011). Further, progressive changes in the gut microbiome appear to coincide with the progression of liver cirrhosis caused by inflammation (Bajaj et al., 2014). Not surprisingly, the gut microbiome may represent a potential treatment target for alcohol-related liver disease (Hartmann, Chen, & Schnabl, 2012). This field of research is still in its infancy, thus further investigation is needed. In particular, studies controlling for factors such as age, gender and diet may shed further light on the possible influence of heavy alcohol consumption on the microbiota composition of the gut.

Summary of Significance, Impact, and Innovation

For the last two decades, the conventional wisdom has underscored the importance of targeting dopamine circuits, either directly or indirectly, by using medications that act as antagonists, agonists, or mixed agonists at dopamine receptors (e.g., aripiprazole, quetiapine) or other receptor systems that may modulate dopamine function indirectly (e.g., naltrexone, ondansetron, varenicline). Given the extant body of work, the results have been somewhat disappointing, as only naltrexone eventually received FDA approval as a treatment for AUDs. Rather than targeting receptors that influence neural circuits, it may be more effective to target the molecular mechanism, at the level of transcription, which underlies changes in dopamine receptors and the function of the reward circuit (e.g., epigenetic modifications). Likewise, perturbation in immune system function is just now emerging as an important target.

Importantly, what has been largely overlooked in most research on AUDs is that there may be psychosocial interventions that exert top down control over epigenetic and inflammatory mechanisms and could be equally or more effective than medications while having fewer side effects. Although this has been largely untested in the alcohol field, there are close to a dozen studies in other patient populations to suggest that MBIs may have exactly this type of effect. If psychosocial interventions, such as MBRP, are demonstrated to be efficacious and the active ingredients can be harnessed and focused, the field will potentially see much greater success.

Thus, the significance and impact of the first aim of the proposed research stems from the simple notion that understanding how a given treatment may be effective informs efforts to increase the effectiveness of that treatment. The proposed study will serve to dismantle the effects of the mindfulness components from the relapse prevention components and test innovative and unique hypotheses about how the mindfulness components work in an AUD patient population. Specifically, the first aim will isolate the effect of Mindfulness from Relapse Prevention by comparing MBRP to RP only. Two published studies to date suggest that MBRP is more effective than RP only (Bowen et al., 2014; Witkiewitz et al., 2014). However, these two studies were conducted in samples of individuals with a variety of substance use disorders, rather than AUDs specifically. Thus, the first aim is significant because it will be the first large, randomized trial to dismantle the effect of MBRP from RP in a sample of individuals with a primary diagnosis of AUD. The second aim will isolate the effects of mindfulness on key neurobiological mechanisms by determining whether MBRP influences epigenetic regulation of key genes and influences immune system function as compared to RP. The third aim will determine whether the effect of mindfulness on drinking outcomes is mediated by the effect of mindfulness on epigenetic regulation and immune system function. Because no other clinical scientists are targeting epigenetic and immune system changes in AUD treatment trials (to our knowledge), this study and this program of research are highly innovative. In addition, our vertical integration of

epigenetic regulation, immune system function including an ex-vivo LPS challenge of PBMCs, a neuroimaging assessment of changes at the neural circuit level, and clinical assessments is innovative and unique from a methodological perspective.

From a clinical perspective, identification of the molecular mechanisms that mediate the effects of mindfulness in AUD patients can then be leveraged in a variety of ways. This information may be used in future studies to test how MBIs might be refined to increase the effect of the intervention on these mechanisms. For example, does increasing the dose/practice of mindfulness increase the effects of the intervention on inflammatory pathways and/or epigenetic regulation of key genes? Would booster sessions at regular intervals after the initial 8-week training increase the effects of mindfulness practice on the immune system? Would combining MBRP with other interventions that putatively target the same mechanisms (e.g., exercise) increase the effects? All of these questions can be asked and answered once we have verified the underlying mechanisms and identified measurable biomarkers. Finally, knowledge about the mechanisms may also be used to develop an important intermediate and inexpensive test of treatment success. For example, if changes in immune system function or epigenetic regulation at 4 weeks are highly predictive of relapse after treatment, tests of immune system function or epigenetic regulation could be used to determine whether the treatment is having an effect early in the process, allowing treatment providers to alter the dose and/or treatment for non-responders. As an analogy, a primary care physician will prescribe a treatment to prevent heart disease (e.g., a statin) and then order a cholesterol test to determine if the statin is working. If not, the dose is increased or a different treatment is prescribed. In the same way, tests of immune function or epigenetic regulation may be used to check on the effect of the AUD treatment early in the process.

Due to the COVID-19 situation we are collecting remote data only and will return to our original protocol when it is safe to do so. The remote version of this study will use telehealth therapy and thus the following references have been included for BACKGROUND AND SIGNIFICANCE:

Although in-person psychotherapies have always been the norm, telehealth is increasingly becoming a more common, convenient and effective alternative. A study of evidence-based psychotherapy for PTSD and depression found that home-based telehealth is a treatment with clinical outcomes matching those of in-person clinic-based delivery systems (Acierno et al., 2016). A more recent systematic review of numerous studies looking at telemedicine's effectiveness in substance use disorders showed this method of therapy had high patient satisfaction and was an effective alternative to in-person psychotherapy (Lin, Casteel, Shigekawa, et al., 2019). Finally, a study looking at the increasing use of telemedicine versus in-person therapy for opioid use disorder revealed that patients treated via telemedicine were more likely to stay in therapy than patients treated in-person (n = 1590; aOR = 1.27; 95% CI 1.14–1.41; $p < 0.001$). At the one-year mark, telemedicine patient's retention rate was 50%, while in-person patients were at 39% retention (Eibl et al., 2017).

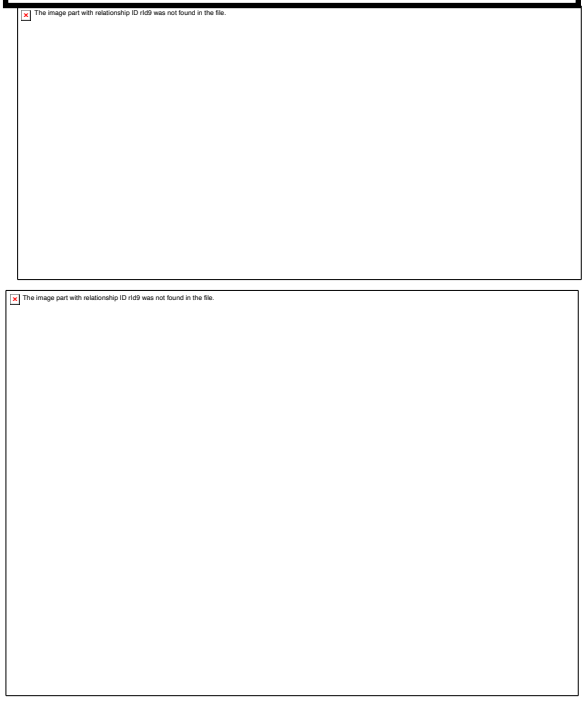
III. PRELIMINARY STUDIES

Epigenetic Mechanisms.

To date, we have performed a number of experiments to validate our epigenetic approach and determine whether epigenetic modifications play a role in the etiology of AUDs. The results suggest that our approach is feasible and that epigenetics is involved in the etiology of AUD.

In order to validate that DRD2 methylation changes are functionally significant and that DNA methylation in peripheral tissue like blood is correlated with protein levels in the brain, we conducted a series of studies in which we sequenced bisulfite treated DNA to estimate the amount of methylation in a 655 base amplicon in the promoter region of the DRD2 that previously demonstrated an effect. These data and the data described below have been bundled into one manuscript and have been submitted for peer review. The first experiment examined the association between D2 binding potential using PET and the degree of methylation in this amplicon. To reduce Type I error, the first analysis examined the average methylation across the amplicon in relation to [^{11}C]raclopride binding (BP_{ND}) reflecting DA D2/D3 receptor availability in the nucleus accumbens. The correlation between DRD2 methylation and average of right and left accumbens BP_{ND} was r = 0.650, p = 0.022 (correlations for left and right individually were r = 0.55 and r = 0.72).

Figure 2. The correlation between methylation across 17 CpGs in the DRD2 promoter region and DA D2/D3 binding.



Representation of the functional relationship between the average methylation of these CpGs and striatal BP_{ND} are shown in the brain map and scatter plots in **Figure 2.**

DRD2 Methylation and BOLD Response to Alcohol Cues.

BOLD response to alcohol cues in the nucleus accumbens and caudate were extracted from each individual in a previously collected data set (n=78). Correlations between the BOLD response to alcohol cues in these structures and the average methylation of the 17 CpGs were calculated. Negative correlations were found between the average methylation of the 17 CpGs and the bilateral caudate (left: r = -0.273, p = 0.027; right: r = -0.305, p = 0.013) and nucleus accumbens (left: r = -0.247, p = 0.045; right: r = -0.241, p = 0.052).

DRD2 Methylation and Clinical Measures of Alcohol Dependence.

Again using the clinical sample (n=78), three commonly used measures of severity were negatively correlated with average methylation of the 17 DRD2 CpGs, controlling for age (AUDIT: r = -0.276 p = 0.015; Alcohol Dependence Scale: r = -0.295, p = 0.008).

Finally, our most recent epigenome-wide analyses indicate that the dopamine transporter gene (SLC6A3) and dopamine beta hydroxylase gene (DBH) are also important epigenetic targets, and thus, we have included these additional genes.

To identify treatment implications, we also examined DRD2 methylation as a predictor of treatment outcome in a subsample of 50 individuals from a previous trial with olanzapine (Littlewood et al., 2014). Methylation in the DRD2 promoter was associated with less ability to control alcohol use

over 12 weeks of treatment, regardless of treatment condition (unpublished data). These results also add support to the notion that epigenetic changes may play a role in relapse. Directly targeting these epigenetic changes may be beneficial. Finally, it is important to note that we have also conducted a number of clinical trials with AUD patients and tobacco users over the last 10 years (Hutchison et al., 2006; Littlewood et al., 2014). Our demonstration of the importance of epigenetic regulation of reward related genes, and our success with clinical trials, provides evidence regarding feasibility of the proposed trial in terms of recruitment, data collection, and study completion.

Immune Function.

While our program of research has focused more on epigenetic regulation, there is a substantial amount of data in the literature to suggest the importance of the LPS/TLR4 signaling cascade in the etiology and maintenance of AUDs (Mayfield et al., 2013). Based on this literature, we recently examined the association between cytokines and key alcohol phenotypes, including connectivity in the left and right executive control networks (LECN/RECN). Our previous work suggests that these two networks are important in the etiology of AUDs (Karoly, Weiland, Sabbineni, & Hutchison, 2014). To generate effect sizes on the association between cytokines (TNF, IL-6, IL-8) and neural network connectivity, we assayed these markers in the plasma of 30 subjects in one of our existing datasets. The correlations with connectivity in the LECN and RECN ranged from r = -.24 to r = -.55 for TNF and IL-8. It is also important to note that the samples were not fresh, and the measures were not as sophisticated as the proposed approach that involves the collection of PBMCs. We now routinely collect PBMCs in one of our studies on aging and inflammation (PI: Bryan) and we routinely utilize an assay that involves stimulating the PBMCs with LPS to examine the effects of LPS on inflammatory gene expression and cytokines. Given the superiority of these methods, we expect to see larger effect sizes in the proposed study.

IV. RESEARCH STUDY DESIGN

Overview and Design of the Proposed Study.

A 2 (Treatment**:** 8 weeks of MBRP vs. 8 weeks of RP) x 5 (Time: Baseline, 4, 8, 20, 32 weeks) mixed factorial design, where treatment is a between-subjects factor and time is a within-subjects factor, will be used to test the treatment outcome hypothesis in Aim 1. Subjects will be randomly assigned to receive MBRP or RP for a period of 8 weeks such that there are equal numbers of patients in each group. An intermediate assessment of the effects of treatment on immune system function and epigenetic regulation of dopamine genes will be collected at 4 weeks. Follow-up assessments will be obtained at 8 weeks (the end of treatment), 20 weeks (12 weeks after the end of treatment), and 32 weeks (24 weeks after the end of treatment).

Treatment:

Rationale for Treatment Conditions.

MBRP was chosen as the experimental treatment because there is mounting evidence that MBIs have a significant effect on immune system function, including on targets that have been implicated in the maintenance of AUDs. To our knowledge, there are no other examples of psychosocial

interventions or pharmacotherapies that influence the immune system and epigenetics that are as well supported as MBRP. RP was chosen as the control condition in order to dismantle the effects of MBRP from RP, thereby isolating the effects of the mindfulness components. Our decisions regarding the overall length of treatment (8 weeks), length and content of individual sessions, subject selection, assessments, and follow-ups at 5 and 8 months after the end of treatment were based on recent trials of MBRP and RP (Bowen et al., 2014; Witkiewitz et al., 2014).

MBRP and RP Implementation.

Consistent with the treatment manuals and previously published papers, a goal of abstinence is encouraged but not required (see Bowen et al., 2014). For scheduling logistics, we will not have a group format but instead each treatment will be provided individually. As noted in the budget justification, the consultants (Dr. Sarah Bowen and Dr. Katie Witkiewitz) along with Dr. Sona Dimidjian (Co-I), will be on-site at the inception of the study to provide feedback on the refinement and implementation of MBRP and RP. Dr. Bowen and Dr. Witkiewitz will continue to provide expert supervision and consultation throughout the study. Dr. Sona Dimidjian, who is widely known for her expertise with MBIs, will be onsite for supervision and training throughout the trial. Therapists will be selected from the graduate program in clinical psychology at the University of Colorado and will have a master's degree in clinical psychology and previous training in the fundamentals of psychological intervention provided by Dr. Hutchison or Dr. Dimidjian. At the inception of the study, Dr. Bowen will travel to Boulder to provide a two-day workshop on MBRP. At a separate time, Dr. Witkiewitz will travel to Boulder to provide a two-day workshop on RP. In advance of the training workshops, prospective therapists will study the relevant manual (MBRP or RP). The workshops will contain both didactic and role-play exercises. *Therapists will be cross-trained in the interventions and will lead an equal number of both conditions to prevent therapist effects*. After training, prospective therapists will be required to demonstrate competency in MBRP or RP by submitting audio recordings of pilot therapy sessions with patients recruited through the University of Colorado training clinic, who will not be part of the proposed study. The recordings will be reviewed by Dr. Bowen and either Dr. Dimidjian or Dr. Hutchison and rated using both the Yale Adherence and Competence Scale as well as the MBRP competence scale, using a 7-point Likert scale as described in previous reports (Bowen et al., 2014). Therapists who demonstrate an average rating of 5 across the duration of the 8-week treatment will be certified to be a therapist for the trial. During the course of the trial, weekly supervision meetings will be convened and an on-site supervisor (Dr. Hutchison or Dr. Dimidjian) will review at least one audio recording per week using the same rating scales to ensure consistency in the treatment throughout the duration of the trial. Dr. Bowen will visit Boulder once per year to assess overall fidelity and progress of the trial. MBRP will consist of 8 weekly 1-hour sessions provided in an individual format, whereas RP will consist of the same format, time, location, and amount of assigned homework but will not contain the mindfulness content provided during MBRP.

Participants will be instructed to arrive at the CINC 10 minutes prior to their scheduled appointment and meet in Room 129. Participants will fill out a quick check-in survey on an iPad before their appointment begins in order to inform therapists of the extent to which they completed their home practice the week before. The check-in survey will also allow participants to record any questions they have for their therapist before the appointment begins. The room will have two chairs with a coffee table in between the chairs, along with meditation cushions. Water will be available in the room. At each session, the therapist will engage and guide the participant in a discussion and exercise. The session will last one hour.

MRI and Connectivity:

MRI Data Collection (Baseline and Week 32).

Before treatment begins and at the 6 month follow-up appointment (week 32), subjects will participate in an fMRI cue exposure session (Claus et al., 2011). Participants who are determined eligible to have an MRI scan will undergo a structural scan, using a Siemens 3T Trio, followed by a functional MRI scan that involves alcohol cue exposure. Our cue procedure is consistent with NIAAA guidelines and less risky than previous studies that have examined this mechanism in a sample receiving treatment (e.g., Myrick et al., 2008; Spagnolo et al., 2014; see protection of human subjects section). **Structural MRI (sMRI):** We will acquire a high-resolution protocol sufficient to permit accurate tissue classification and anatomical parcellation. For optimal contrast between gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF) at 3T, we will use a multi-echo MPRAGE (MEMPR) sequence with the following parameters: TR/TE/TI = 2300/2.74/900 ms, flip angle = 8°, FOV = 256x256 mm, Slab thickness = 176 mm, Matrix = 256x256x176, Voxel size =1x1x1 mm, Number of echos = 4, Pixel bandwidth =650 Hz, Total scan time = 6 min. **Functional MRI (fMRI) – Cue-Elicited BOLD:** fMRI scans will be collected with single-shot full k-space echo-planar imaging (EPI) with ramp sampling correction using the intercomissural line (AC-PC) as a reference (TR: 2.0 s, TE: 27ms, α: 70^{o}, matrix size: 64×64, 32 slices, voxel size: $3 \times 3 \times 4$ mm^3). Image-based higher order automatic shimming will be employed. FMRI data will be acquired with a 32-channel coil. Scanner instability and signal-to-noise ratio during fMRI scanning on a phantom will be monitored weekly throughout the project period. Automated analysis of scanner instability with the Weiskoff method is routinely performed. We have used our alcohol cue task successfully in over 600 individuals (Claus et al., 2011). It is reliable and well validated. The task compares the taste of an individual's favorite alcoholic beverage with a sweet, appetitive control taste (litchi juice). The task was designed to minimize movement by only administering 1 ml of each beverage through Teflon tubing that is attached to a computer controller gustometer, over each 24 second trial. It is important to note that the total amount of the alcoholic beverage is only 12 ml (less then a teaspoon). The task itself involves a block design with twelve pseudorandomly ordered alcohol and control trials that are presented in two consecutive runs. In order to examine whether the effects of MBRP differ from those of RP, we will calculate the signal change for alcohol minus the control condition in the precuneus, putamen, and anterior cingulate and use the signal in these regions of interest (ROIs) in the structural equation models (see analysis section).

Resting State Connectivity (Baseline and Week 32).

Before treatment begins and at the 6 month follow-up appointment (week 32), a 10 minute resting state scan will be collected to derive measures of functional connectivity in the left and right executive control networks (LECN/RECN) following our previously published procedures (Weiland et al., 2014). These networks will be identified using functionally defined ROIs/nodes. The nodes for the LECN include: dorsolateral prefrontal cortex (DLPFC), left middle/superior frontal gyrus (MFG), left superior parietal gyrus/angular gyrus (PAR), left inferior/middle temporal gyri (TL), right crus I/crus II/Lobule VI (CE), and left thalamus (TH). The RECN nodes include: right DLPFC, right MFG, right PAR, right medial superior frontal gyrus (mSFG), left CE, and right caudate (CU). Functional connections between nodes, each pairwise correlation defined as an edge, will be calculated and used to create a correlation matrix between the time-series of all nodes within each network for each subject. A Fisher r-to-z transformation will be applied to r-values to yield z-scores. Connectivity strength as a primary global measure of connectivity for each network will be calculated as the mean of all pairwise

correlations between nodes within each network as well as other graph theory metrics (e.g., degree, efficiency, centrality). The framewise displacement (FD) for each subject will be calculated across the entire resting state run from the image motion parameter and used a covariate in the analyses.

Blood Collection and DNA Methylation:

DNA Collection, Extraction, Storage, and Sequencing (Baseline, Week 4, 8, 20, 32).

We will retain an aliquot of whole blood (approximately 10 mL) for DNA extraction. After extraction, DNA will be quantified and stored at -80° for future analysis. DNA will be collected at baseline, 4, 8, 20, 32 weeks so that we can monitor epigenetic changes over time.

Bisulfite Sequencing / Estimation of Methylation.

Bisulfite specific PCR primers will be designed using MethPrimer primer design software freely available at http://www.urogene.org/methprimer/. PCR amplification will use Accuprime™ *Taq* DNA Polymerase High Fidelity (Life Technologies™) in 50 ul reactions according to the manufacturer's instructions using 20 ng of converted genomic DNA with a final forward and reverse primer concentration of 200 nm each. PCR products will be purified using DNA Clean and Concentrator™-5 (Zymo Research) and quantified. Dual indexed libraries will be prepared using Illumina Nextera XT DNA Sample Preparation kit**.** Denatured and diluted libraries will be sequenced on the Illumina MiSeq benchtop sequencer with the sequencing by-synthesis technology per manufacturer's protocol. Data will be demultiplexed on the MiSeq instrument automatically, and zipped FASTQ files will be generated per sample, per read. Data will be accessed either in the run analysis folder locally on the instrument, or through BaseSpace beta. Custom scripts then determine the ration of methylation at each CpG in the amplicon. Average methylation across the CpGs identified in of our preliminary data will be used as the primary methylation measure in the analyses below.

Blood Draw Collection and LPS Stimulation:

Blood Levels of Cytokines (Baseline, Week 4, 8, 20, 32).

As noted in the background and in our description of our preliminary studies, we expect that MBRP will reduce biomarkers related to inflammation during the course of treatment and it is hypothesized that changes in inflammation will mediate the effects of MBRP on changes in connectivity in the brain, neurocognition, and drinking outcomes. Blood samples drawn at each assessment (baseline, 4, 8, 20, 32 weeks) will be assayed for IFNg, IL-1a, IL-1b, IL-6, IL-2, IL-4, IL-8, IL-10, IL-12, and TNF. Previous studies in animals (Mayfield et al., 2013) and humans (Bala et al., 2014), as well as our own preliminary data, suggest that cytokine activation is associated with the pathophysiology of AUDs. This is the same assay used in our preliminary studies.

Liver Function Panel Tests (Baseline, Week 8, 20, 32).

As stated in the study background, LPS-induced pro-inflammatory cascades span the brain, gut, and liver (Fernandez-Lizarbe, Pascual, & Guerri, 2009). Additionally, enzyme levels detected in a liver function panel (LFT) can indicate possible liver damage and cirrhosis cause by alcohol abuse (Bellentani et al., 1997). We expect to see changes in liver function tests as a result of decreased alcohol consumption and pro-inflammatory markers associated with RP and MBRP therapy. The LFT will also provide an additional objective measure of alcohol consumption to supplement the self-report measures we will collect. To detect differences pre and post-treatment as well as differences between

RP and MBRP treatment groups, we will have a liver function panel test performed on a 2mL sample of each participant's blood collected at baseline, 4, 8, 20, and 32 weeks. Liver function panels will be performed by Boulder Community Hospital Reference Lab.

Lipopolysaccharide (LPS)-PBMC challenge.

We also expect MBRP to impact the responses of blood monocyte cells to an LPS challenge. This may represent a more accurate test of the effects of the treatment, since LPS stimulation of PBMCs simulates the TLR4 inflammation cascade that putatively mediates the neuroinflammatory effects of alcohol. To that end, 40 ml of whole blood will be drawn at each assessment point. Peripheral blood mononuclear cells (PBMC) will be separated using density gradient centrifugation. Cells will be counted and viability assessed using trypan blue exclusion. PBMCs will be exposed to LPS (0, 0.1, 1, 10 and 100 ng/ml) for 20h in a 96 well v-bottom plate. Protein levels of cytokines and chemokines (IL1,TNF, IL6, IL4, IL10, IFNg, MIP-1a) will be measured in supernatant using a multiplex ELISA assay (Aushon Biosystems, Billerica MA). mRNA levels of the same cytokines and chemokines will be measured in cell lysates using real-time PCR. We have successfully performed the PMBC isolation and LPS challenge in other projects.

Gut Microbiome Sample Collection and Microbial DNA Extraction:

Fecal Sample Collection, Storage, Extraction, and Sequencing (Baseline, Week 8)

We expect to see changes in both the number and diversity of microbiota populations found in the gut as a result of treatment and reduced alcohol consumption. Because inflammation, liver function, and the gut microbiome are heavily intertwined, we will examine the relationship between changes in the gut microbiome with changes in inflammatory biomarkers in the blood (cytokines and blood monocyte cell response to LPS challenge) and liver function as a result of both RP and MBRP therapy. Fecal samples will be collected from participants using at-home kits and then stored at -80°. Microbial DNA will be extracted from fecal samples and then analyzed to determine how many and which bacterial species are present in the gut. Gut microbiome data will be collected at baseline and 8 weeks so that we can monitor changes in number and diversity of microbial DNA pre and post-intervention.

<u>Due to the COVID-19 situation we are collecting remote data only and will return to our original protocol when it is safe to do so. The remote RESEARCH STUDY DESIGN will be as follows:</u>

Because of the remote nature of the study, we will not be collecting the data mentioned above: MRI data, microbiome samples, or blood data.

We will be administering the parts of this study that can be done remotely through contact with a research assistant: All therapy sessions (mentioned above), and the Validating Self-report Measures listed directly below.

Additionally, due to the necessity of the remote nature attributable to COVID: All participants will need access to the internet whether by computer, smartphone, or other device such that they can complete this study. A research assistant will send unique links to participants that they will simply click on in order to participate, applications such as: Zoom (an internet application that allows us to see

and talk with the participant), Qualtrics and REDCap (online survey platforms), and DocuSign (an internet application that allows us to send forms for signatures).

Validating Self-report Measures:

Biochemical Outcome Measures (Baseline, Week 8, 20, 32).

Following the procedures and recommendations outlined in a report on biochemical verification of abstinence, tests of plasma **%CDT and GGT** will be used as an unbiased, biological outcome variable that will be used to validate self-report measures (Anton, Lieber, & Tabakoff, 2002) (Martínez-Raga et al., 2002).

Measures and Assessments:

Individual Difference Measures (Baseline).

- A Demographics Questionnaire will be used to collect information on age, sex, marital status, SES, occupation, income, education, and race.
- The Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES) will be used to measure individual differences in motivation at baseline.
- Drinking History is measured with 12 items assessing lifetime drinking history such as current drinking, age of onset, and number of previous of attempts to quit/reduce drinking.
- A Smoking History Questionnaire will be used to collect information on frequency and quantity of nicotine use and will be used to determine whether the treatment groups differ on nicotine consumption.
- A Cannabis Self-Treatment questionnaire will be used to better characterize our population in case participants are self-treating with cannabis.

Self-Report Outcome Measures (Baseline, Week 4, 8, 20, 32).

- Heavy Drinking Days (HDD), Drinks per Drinking Day (DDD), and Percent Days Abstinent (PDA) as well as any comorbid drug use (e.g., tobacco use) will be obtained at each assessment using the Timeline Follow Back (TLFB), which is a calendar-based assessment of daily alcohol and drug use (Sobell et al., 1979). The TLFB has been shown to have good psychometric characteristics among a variety of drinker groups and can generate variables that provide a wide range of information about an individual's drinking (e.g., pattern, variability, and magnitude of drinking). HDD is the primary behavioral outcome that will be analyzed. However, secondary outcome measures of interest will also be collected.
- The Impaired Control Scale – Failed Control (ICS-FC) is a 25-item scale that is used to measure the severity of alcohol dependence with respect to dimensions such as impaired control over drinking, awareness of compulsion to drink, etc. (Heather, Booth, & Luce, 1998).
- The Alcohol Dependence Scale (ADS) has been used widely and found to have excellent predictive value with respect to DSM diagnosis (Kivlahan, Sher, & Donovan, 1989).
- The Pennsylvania Alcohol Craving Scale (PACS) is a 5-item measure of craving that will be completed at baseline and at each follow-up session to provide a measure of craving (Flannery, Volpicelli, & Pettinati, 1999). In a recent study, the PACS was found to be a

slightly better predictor of drinking as compared to the OCDS and AUQ (Flannery, Poole, Gallop, & Volpicelli, 2003).

- The Drug Use Questionnaire assesses individual behavior with regard to frequency and quantity of drug use. This measure will be used to assess whether smoking and drug use behavior changes during the course of clinical trial.
- The Beck Depression Inventory-II (BDI-II) consists of 21 scaled statements designed to assess symptoms of depression, with a coefficient alpha of .91 (Beck, Steer, & Carbin, 1988) The BDI-II will be administered at baseline and at last follow-up to examine changes in comorbid depression and covary baseline differences if necessary.
- The Beck Anxiety Inventory (BAI; Beck et al., 1988) consists of 21 items, each describing a common symptom of anxiety. The items are summed to obtain a total score that can range from 0 to 63. The BAI will be administered to examine comorbid anxiety and covary baseline differences if necessary.
- The Five Facet Mindfulness Questionnaire (Baer et al., 2006) will determine the extent to which MBRP increases mindfulness practice as a proximal mediator of the effects of the intervention. These data will be used as a manipulation check.
- The extent to which patients engage in key aspects of mindfulness will be collected using the 10 item Mindfulness Practice Questionnaire (e.g., Witkiewitz, Lustyk, & Bowen, 2013) on a weekly basis.
- The CHIME (Bergomi, Tschacher, & Kupper, 2015) consists of three items and will determine the number of minutes of mindfulness practice per week each patient is currently engaging in. Minues of current meditation practice will be calculated using the following compound variable: (weekly frequency x average session duration). The data will be used to co-vary for patients who are already engaging in frequent mindfulness meditation practice at baseline if necessary.
- The Multidimensional Personality Questionnaire (Tellegen, 1982, Tellegan & Waller, 2008) consists of 18 True/False items of statements describing negative emotionality and stress reaction. The questionnaire will be administered to examine comorbid negative emotionality and used to co-vary baseline differences if necessary.
- The Ruminative Response Scale (Treynor, Gonzalez, & Nolen-Hoeksema, 2003) consists of 22 items scoring how often the patient engages in ruminative thinking associated with depression. The data will be used to examine comorbid negative emotionality and used to co-vary baseline differences if necessary.
- The Effortful Control Scale Derryberry & Rothbart, 1988, Evans & Rothbart, 2007) consists of 35 items that measure the degree to which the patient exhibits behavioral control. The data will be used to co-vary baseline differences in behavioral control if necessary.
- The Drinking Motives Questionnaire (Cooper, 1994, Kuntsche et al., 2006, Young-Wolff et al., 2009) consists of 20 items used to assess patients' varying motives for drinking alcohol. The Drinking Motives Questionnaire will be used to assess whether motives for drinking alcohol change from baseline following treatment.
- Alcohol use will be evaluated with a variation of the measure used by White and Labouvie (1989). First, participants will be asked if they have ever had an alcoholic drink (with instructions that define one alcoholic drink as "one beer, one glass of wine, or one serving of hard liquor either by itself or in a mixed drink"). Those (likely well over 90%) who

answer that they have previously had alcohol before will be asked to rate: (1) their frequency of use in the last three months on a 9-point scale ranging from "never" to "every day", (2) their typical quantity of drinks in one sitting on a 10-point scale ranging from "no drinks" to "more than 20 drinks", and (3) their frequency of getting drunk when drinking in the past three months on a 5-point scale ranging from "never" to "always."

- The AUDIT (Saunders, Aasland, Babor, De La Fuente & Grant, 1993) is used to detect less severe problem drinkers and addresses both current problems (problems within the last 3 months) and problems across an individual's lifetime. The AUDIT consists of ten questions that cover such domains as alcohol consumption, drinking behavior, adverse psychological reactions, and alcohol-related problems.
- Participants will be asked about their engagement in any 12-step programs (Tonigan, Miller & Conners, 1997), psychological services for alcohol and other problems, and use of other self-help materials. At baseline, participants will be asked about lifetime engagement in treatment. Because this study will be recruiting a population of heavy drinkers, it will be important for us to know whether or not any of our participants have previously received treatment for alcohol abuse. In future analyses, this information (e.g., prior treatment history) will be used as a covariate.
- The AUQ **(**Bohn et al., 1995) will be used to assess craving. The AUQ consists of eight items related to urge drink that are rated on a 7-point Likert scale with the extremes anchored by "Strongly Disagree" and "Strongly Agree." The AUQ has demonstrated internal consistency and reliability.
- The ImpSS (Zuckerman, 1996) consists of 19 items measuring impulsivity and sensation seeking that may predict responses to alcohol and drug problems. The sum of the symptoms endorsed is a proxy measure of dependence.
- The Fruits and Vegetable Screener is a 10-item measure that was developed by the National Cancer Institute to assess how many times in the previous month subjects consumed different types of fruits and vegetables, including potion size questions for every food item. This measure was shown to be a useful estimate for obtaining median intakes of fruit and vegetable servings in U.S. populations (Thompson et al., 2002). Fruit and vegetable consumption has been shown to influence inflammation (e.g., Holt et al., 2009), and dietary factors have also been linked with epigenetic changes (see Hardy & Tollefsbol, 2011). Thus, the inclusion of this measure allows fruit and vegetable consumption to be controlled for in inflammation and epigenetic analyses.
- The single-item, self-rated diet quality measure (Loftfield et al., 2015) consists of a single question asking "In general, how healthy is your overall diet?" Participants can respond with (1) excellent, (2) very good, (3) good, (4) fair, or (5) poor. The item is significantly associated with variation in objective measures of dietary intake ($P<.001$).
- The VAEQ assesses levels of voluntary exercise (Bryan and Rocheleau, 2002). Participants indicated how frequently they engaged in exercise activities in the past 3 months and past week. The exercise composite score used in these analyses was composed of 4 items: (i) "In the past 3 months, what is the average number of days per week that you engaged in aerobic exercise?" (ii) "In the past 3 months, what is the average number of total minutes per week that you engaged in aerobic exercise?" (iii) "In the past week, how many days did you engage in aerobic exercise?" and (iv) "In the past week, what is the total number of minutes that you engaged in aerobic exercise?" This measure has been used to quantify exercise participation in our previous alcohol studies (Karoly et al., 2013).

Given that exercise may impact inflammation (see Woods, Vieira, & Keylock, 2009) and epigenetics (seeNtanasis-Stathopoulos, Tzanninis, Philippou, & Koutsilieris, 2013), this measure (either alone or in combination with the Godin, see below) will allow us to control for exercise behavior in inflammation and epigenetic analyses.

- The Godin Leisure-Time Exercise Questionnaire (QLTEQ; Godin & Shephard, 1985) is used to generate 'activity scores' based on how many separate times (occasions) in a typical week participants engage in mild, moderate, or vigorous exercise for more than 15 minutes of their leisure time. The three levels of intensity are defined as follows: (1) mild exercise, defining features include, slightly elevated heartbeat, slightly elevated breathing rate (able to carry on a conversation effortlessly); (2) moderate exercise, defining features include accelerated heartbeat, moderately elevated breathing rate (able to carry on a conversation), and mild physical exhaustion; and (3) vigorous exercise, defining features include rapid heartbeat, heavy breathing (difficult or unable to carry on conversation), and physical exhaustion. Participants will be asked to answer how many separate times they engaged in mild, moderate, and vigorous intensity exercise over the past week (for each data collection time point). This measure could be used alone or in combination with the VAEQ to control for exercise participation in inflammation analyses.
- The PSS (Cohen, Kamarck, & Mermelstein,1983) is a 14-item scale that measures (1) the degree to which situations in someone's life are perceived as stressful. Stress is a known correlate of substance use and abuse and it will be crucial for us to obtain a measure of our participants' perceived stress levels, which may be associated with inflammatory processes (e.g., Hart & Kamm, 2002) and epigenetic changes (see Eric J Nestler, 2012). Thus, this measure will be used to control for stress in epigenetic and inflammation analyses.
- The PSQI (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) is a 19-item self-report scale that measures sleep quality over the past month. The 19 self-related items are combined to form seven "component" scores, each of which has a range of 0-3 points. In all cases, a score of "0" indicates no difficulty, while a score of "3" indicates severe difficulty. The seven component scores are then added to yield one "global" score, with a range of 0-21 points. In the present study, it will be important to control for sleep duration and/or quality, given that sleep is known to be associated with inflammation (e.g., Patel et al., 2009) and epigenetic factors (see Qureshi & Mehler, 2014). Thus, component scores, global scores and single item scores could all potentially be used as covariates in later analyses of these data. Scores from this measure will be included as covariates in inflammation and epigenetic analyses.
- The Leisure-Time Categorical Item (L-Cat) is a single item comprised of six descriptive categories ranging from inactive to very active (Kiernan, Schoffman, Lee, Brown, Fair, Perri, & Haskell, 2013). Each category consists of 1–2 sentences describing common activity patterns differing in frequency, intensity, duration, and types of activities, thus encompassing content validity (van Poppel, Chinapaw, Mokkink, van Mechelen, & Terwee, 2010). Respondents pick the category best describing their activity during the past month. It assesses national activity recommendations as well as multiple clinically relevant categories below and above recommendations, and incorporates critical methodological principles that enhance psychometrics (reliability, validity, sensitivity to change).
- The Pain Interference item bank is from the National Institutes of Health (NIH) and is part of the Patient Reported Outcomes Measurement Information System (PROMIS). The Pain Interference SF-8a, is a 8-item short-form questionnaire that measures the self-reported

consequences of pain on relevant aspects of one's life. This includes the extent to which pain hinders engagement with social, cognitive, emotional, physical, and recreational activities (Amtmann et al., 2010).

- Emotion Regulation Questionnaire (ERQ) is a 10-item scale that is designed to assess individual differences in the habitual use of two emotion regulation strategies: cognitive reappraisal and expressive suppression (Gross, John, 2003).

Because of the COVID-19 situation and the remote nature of the study, we will not be collecting the Neurocognitive data:

Neurocognitive NIH Toolbox Measures (Baseline & Week 8).

- **Flanker Inhibitory Control & Attention Test:** Tests inhibitory control and attention. The participant focuses on a particular stimulus while ignoring other stimuli. Five arrows are displayed in the middle of the screen, each pointing left or right. The participant selects the arrow that matches the middle arrow in the sequence.
- **Dimensional Change Card Sort Test:** Measures cognitive flexibility and attention. Two target pictures are presented that vary along two dimensions (e.g., shape and color). Participants are asked to match one of the two bottom cards to the card displayed at top, by either color or shape.
- **Pattern Comparison:** Measures processing speed. The test takes less than 90 seconds and requires participants to discern whether two side-by-side pictures are the same or not.
- **Picture Sequence Memory Test:** Tests episodic memory. Participants are asked to reproduce the sequence of pictures that is shown on the screen.
- **Picture Vocabulary Test:** A measure of receptive vocabulary administered in a computer-adaptive test (CAT) format. Participant matches picture with spoken word.
- **List Sorting Working Memory Test:** Participants have to say back the list of images they are given.

Power Analysis:

Sample Size Requirements.

Sample size was selected to permit analysis of the hypotheses at a two-tailed alpha of .05 and power level of at least .80 following Cohen (Cohen, 1988). G*Power 3.0.3 (Erdfelder, Faul, & Buchner, 1996) was utilized for some power calculations, while Monte Carlo simulation strategies formed the basis of our power analysis for the mediational model (Bryan, Schmiege, & Broaddus, 2007; Mackinnon & Dwyer, 1993).

The impact of attrition.

It is anticipated that some attrition will occur in this population, though we do not anticipate differential attrition by condition. A conservative estimate of 20% attrition over the course of data collection is assumed. Recently published evidence suggests an attrition rate of approximately 20% for other trials. In our own previous trials, we have seen attrition of around 20%. Thus, sample size will need to be increased by 20% over minimum requirements. After final attrition has been calculated, pretest differences on alcohol use and problems will be compared among individuals who finished the study versus those who did not and by condition, to determine whether these two groups differed in

systematic ways and whether these differences were related to treatment condition.

With respect to attrition during the follow-up period, all participants will update their address and phone number as well as the name and number of an individual who always knows how to reach them at every visit. The project coordinator will also contact each patient in between follow-up visits.

Power for Specific Aim 1 - Detecting Treatment Effects on Alcohol Use Outcomes.

The analyses of treatment effects will proceed via random coefficient regression utilizing a 2 (**Treatment:** 8 weeks of MBRP vs. 8 weeks of RP) x 5 (**Time**: Baseline, 4, 8, 20, 32 weeks) mixed general linear model. The primary outcome variable tested will be heavy drinking days (HDD), consistent with previous MBRP trials (Bowen et al., 2014). Secondary analyses will explore related outcomes such as drinks per drinking day, scores on the ICS, and liver function tests, with appropriate control of Type I error. In each model, the key effect of interest will be the treatment X time interaction. Given the effect sizes in previous trials (small d=.23 or f <.10 to moderate d=.45 or f=.20), we conservatively estimate a small effect size (e.g., f = .10). A mixed model testing the between (treatment) X within (time) subjects interaction with a total of 180 participants (90 per treatment group) will give us well over .90 power, assuming alpha of .05 and a correlation of approximately .50 between repeated assessments. Given that we expect 20% attrition over the course of the trial, we will initially recruit ~226 participants.

Power for Specific Aim 2 - Detecting Treatment Effects on Putative Molecular Mechanisms.

As reviewed above, the effect sizes for the impact of MBIs (and thus likely MBRP) on inflammatory biomarkers and epigenetic regulation ranged from moderate to large. Thus, for tests of the hypotheses under Specific Aim 2, that there will be treatment effects on both inflammatory markers and epigenetics, the effect sizes are in all cases larger than our estimated effects of treatment on outcomes. Thus, 226 participants are more than adequate for Hypothesis 2 of Specific Aim 2. In terms of Hypothesis 3 of Specific Aim 2, there are no data available to estimate the size of the direct effects of MBRP on cue-elicited BOLD activation or on network connectivity. With 226 participants, we will be able to detect a direct effect as small as *d*=.38 (a small to moderate effect) on change in BOLD activation and network connectivity as a result of MBRP versus RP.

Power for Specific Aim 3 – Mediational Analysis.

Aim 3 will involve a test of the hypothesis that the effectiveness of MBRP vs. RP on clinical outcomes is mediated by the effect of MBRP on the molecular mechanisms, and, in turn, their effects on network connectivity and BOLD response. This analysis will involve the use of structural equation modeling (Bryan et al., 2007) techniques of the mediational model posited in Figure 4. Based on our review of the literature (see above for details and effect sizes), we expect moderate to large paths (d=.60) for the effect of MBRP on epigenetics and inflammation. We expect small to moderate relationships between epigenetics and cue-elicited BOLD activation and between inflammation and network connectivity strength (d=.30). Finally, for paths relating BOLD activation and network connectivity strength to drinking outcomes we expect paths in the small to moderate range (d=.30). We expect moderate to large (d=.75) loadings of all indicators for their respective latent constructs (for example, the loadings of heavy drinking days, drinks per drinking day, and impaired control on the Drinking Outcomes latent variable). Power analyses for these hypotheses were conducted in Mplus and then in SAS following procedures for estimating the power of the likelihood ratio test of the significance of parameters in structural equation models (Muthén & Muthén, 2002). We utilized Monte Carlo simulation to generate a population covariance matrix based on the hypothesized

parameters in the model. For the smallest path in the model, assuming two-tailed alpha of .05, our power to detect a significant parameter estimate is .71 with 150 participants and .83 with 200 participants; with 226 participants we have .87 power. For the moderate to large paths, we have well over .99 power with 226 participants.

Data Analysis Plan:

Analyses will be conducted using SAS Version 9.3 and EQS for Windows Version 5.7b. We will conduct extensive analyses of any attrition encountered in the project to determine bias. However, note that in each of the analyses described herein, we will have the capability of using modern approaches to the handling of missing data (Schafer & Graham, 2002) including full information maximum likelihood (FIML) estimation of missing data within Proc Mixed in SAS and within EQS.

Descriptive analyses. The distributional properties of all continuously scaled variables will be examined for skewness and kurtosis to determine the need for normalizing transformations or for alternations to our analysis plan (i.e., generalized estimating equations in SAS or robust estimation in EQS) prior to the primary analyses.

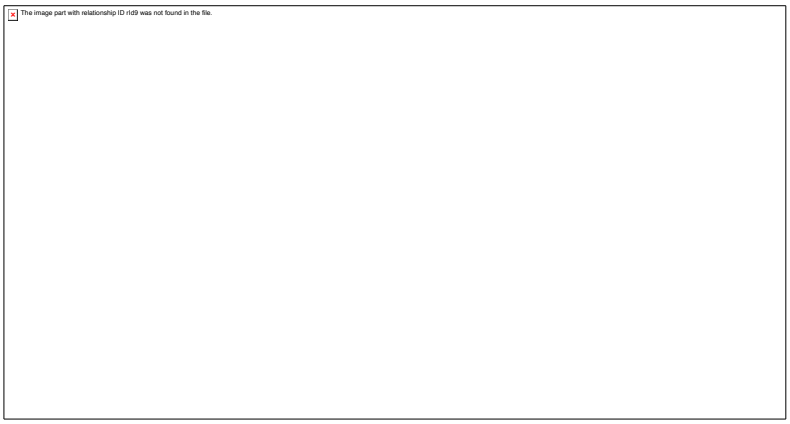
Pretest equivalence. To confirm the validity of random assignment, pretest equivalence of the two treatment conditions across demographics, drinking history, smoking, and all other baseline measures will be assessed via t-tests on continuous items and χ^2 tests of categorical items. We will use the Bonferroni approach (Keppel, 1991) to correct for alpha inflation with a familywise alpha of .05. Any variables on which the two groups are unequal at pretest will be covariates in all further analyses.

Specific Aim 1 - Detecting Treatment Effects on Drinking Outcomes.

The effect of the treatment on alcohol use outcomes (HYPOTHESIS 1) will be analyzed with a 2 (**Treatment:** 8 weeks of MBRP vs. 8 weeks of RP) x 5 (**Time**: Baseline, 4, 8, 20, 32 weeks) mixed general linear model with Heavy Drinking Days (HDD) as the primary outcome variable. We will also examine drinks per drinking day, ICS score, and liver function tests as dependent measures in each of 2 additional models, respectively. In the main analysis with HDD, a significant overall test of the treatment X time interaction will be followed by planned contrasts within the mixed model that allow us to test the superiority of MBRP over RP at the 8, 20, and 32 week follow-up points specifically. The FIML estimation procedure implemented in these analyses will allow us to utilize an "intent to treat" approach such that all subjects who are randomized will be included in the analyses (Schafer & Graham, 2002). It may be that the treatments are equally effective on drinking outcomes, and thus we will not see a direct effect of treatment on outcomes. Even in this case, it is possible that the mechanisms through which the treatments had their impact may differ. Thus, tests of Specific Aims 2 and 3 are still important and warranted.

Specific Aim 2 - Detecting Treatment Effects on Molecular Mechanisms.

The effect of the treatment on methylation and inflammation (HYPOTHESIS 2) will be analyzed with one 2 (**Treatment:** 8 weeks of MBRP vs. 8 weeks of RP) x 5 (**Time**: Baseline, 4, 8, 20, 32 weeks) mixed multivariate general linear model with methylation outcomes of DRD2, SLC6A3, and DBH and a second model with peripheral inflammation outcomes of IL-6, IL-8, and TNFα. The effect of the treatment on cue-elicited BOLD activation and network connectivity (HYPOTHESIS 3) will be analyzed with a 2-way multivariate analysis of variance (MANOVA; **Treatment:** 8 weeks of MBRP vs. 8 weeks of RP) with BOLD activation in the accumbens, caudate, putamen, and anterior cingulate regions as separate outcomes in the same analysis, and a second MANOVA with network connectivity



in the RECN and LECN as the outcomes. Note, again, that FIML estimation will be used to allow full utilization of available data in an intent to treat framework. It is also possible that the treatments are equally effective on drinking outcomes, and thus we will not see a differential effect of treatment on the mediators. Yet it is still important to know whether levels of methylation or inflammation post-treatment have any impact on changes in neurocognitive outcomes. Thus, the test of Specific Aim 3 is still critical, even in the case that both treatments are equally effective on both the mediators and drinking outcomes.

Specific Aim 3 - Mediational analyses.

Mediational analyses based on structural equation modeling with latent variables will be used to examine the mechanisms by which MBRP has its effects on behavioral outcomes related to alcohol use and dependence (See Figure 4; HYPOTHESIS 4) (Baron & Kenny, 1986; Bryan et al., 2007). Mediational analyses will utilize the methylation and inflammation data collected at 4 weeks, and changes in cue-elicited BOLD activation and network connectivity strength at 8 weeks as the mediators, and drinking outcome data collected at 20 weeks. The exogenous variable in the model is the contrast that compares MBRP to RP. Methylation, inflammation, and drinking outcomes are all conceptualized as latent variables, which means that they are not directly measured, but are rather inferred via the measurement of variables thought to be a consequence of the latent variable. Latent variables have advantages over measured variables in that they are more reliable and thus allow for higher power for the test of relationships between the underlying construct and other variables (Kline, 2010).

The model is then estimated in EQS, and both the fit of the model and the significance of the path coefficients are examined. If HYPOTHESIS 4 is supported, we will see significant paths from the treatment contrast to methylation and inflammation. Further, there will be significant paths from methylation to cue-elicited BOLD activation and from inflammation to network connectivity. Finally, we expect significant relationships between BOLD activation and network connectivity to drinking outcomes. Importantly, tests for the completeness of mediation of the treatment differences in drinking outcomes are employed through a one degree of freedom χ^2 test where a path directly from the treatment contrast to drinking outcomes is added to the model. A nonsignificant direct path and a nonsignificant change in χ^2 suggest that treatment effects on drinking outcomes were mediated through the series of molecular and brain-based mechanisms. A secondary test of mediation will utilize bootstrap methods to test the significance of, and confidence limits around, the mediated effect (Mackinnon & Dwyer, 1993). Similar mediational analyses have been widely used by Co-I Dr. Bryan (Bryan et al., 2007).

Exploratory Aim 1.

We will also conduct an additional exploratory analysis comparing populations of microbial DNA pre and post-intervention. We will control for age, gender, and diet, because these factors can differentially influence the gut microbiome as well. To do this, we will run a series of OLS multiple

regression models regression microbiome values on age, gender, fruit and vegetable consumption, and the contrast coded intervention (MBRP or RP) group variable both pre and post intervention. A follow-up analysis may include looking within an intervention group, and regressing age, gender, fruit and vegetable consumption and a continuous measure of alcohol use (i.e., total AUDIT score or TLFB drinks per drinking day) on the microbiome values to determine whether there is a dose-response relationship between alcohol intake and changes to the microbiome. We will also explore interactions between changes in the gut microbiome and changes in inflammation and liver function.

Differential attrition analyses.

Attrition analyses will be conducted after each follow-up data collection effort to provide assurance that differential attrition by treatment condition has not occurred. Following previously published procedures (Jurs & Glass, 1971), a series of ANOVAs of treatment (MBRP versus RP) X retention (retained, not retained) will be conducted on continuous baseline measures. Significant treatment X retention interactions identify measured variables on which differential attrition may have occurred. The logit model analog procedure will be applied to categorical baseline measures to test for differential attrition on categorical variables such as gender and ethnicity (Agresti, 1990). We will conduct these analyses to assure that differential attrition by treatment condition does not account for any of the effects of the treatments.

Secondary analyses.

In the context of the test of the model in Figure 4, we will conduct secondary analyses to test whether there are effects of methylation on network connectivity and of inflammation on BOLD activation. We will also conduct exploratory analyses to determine whether the treatments are differentially effective by gender, age, or race/ethnicity, and we will explore interactions between mechanisms with appropriate Type I error correction. Finally, we will examine the relationship of early changes in drinking behavior (within the first 2 weeks) and neural adaptations in the analysis plan and how the degree of mindfulness uptake relates to neural and clinical outcomes.

Project Timeline.

The proposed timetable for all data collection, analysis, and manuscript preparation is five years. Because the protocol is similar to clinical protocols we have used in previous studies and because we have existing experienced staff, we will only need 3 months to refine and implement the protocols. Thus, data collection will begin in earnest in April 2016. Consistent with previous trials, we expect to enroll about 6 patients per month during the first year and about 4 per month thereafter. Thus, we expect to enroll 50 patients during each of the first 4 years and 26 patients in the last year, for a total of 226 patients. The remainder of year 5 will involve data analysis and manuscript preparation.

V. FUNDING

This study is funded by a grant from NIAAA.

VI. ABOUT THE SUBJECTS

Participants for the proposed research are 226 individuals with an alcohol use disorder who will be recruited from the greater Denver/Boulder metropolitan area in the state of Colorado using the exclusionary criteria described below.

Criteria for inclusion in the study are:

1. Must be between the ages of *21 and 60* and provide informed consent;
2. Have a primary DSM-V diagnosis of alcohol use disorder;
3. Must be within 10 days of last drink;
4. Must have been drinking heavily (criteria dependent upon individual's age, gender, and BMI) for a consistent period of time;
5. Must have a breath alcohol level of 0 at screening (to sign consent form);
6. Not currently taking any medications for the treatment of psychiatric disorders, including substance use disorders, mood disorders, and psychosis;
7. Female subjects must not be pregnant, as indicated by a pregnancy test which will be administered at baseline (tests will be re-administered at the 32 week session prior to the MR scan);
8. Must not be positive for sedatives, opiates, cocaine, or amphetamine on drug screen at baseline;
9. Must not meet criteria for psychotic disorder, bipolar disorder, or a major depressive episode;
10. Must not have a Beck Depression Inventory (BDI) score of more than 28 (29-63 indicates severe depression);
11. Must not rate 2 or higher on BDI question 9 (assessment of suicidality);
12. Must have a Clinical Institute Withdrawal Assessment (CIWA) score less than 8 (indicating no need for medical detox). If CIWA is greater than 8, patient will be referred to detox and only accepted into study after detox is successfully completed.
13. Must have expressed a desire during their initial screen to reduce the number of drinks they regularly consume

VI. VULNERABLE POPULATIONS

This study does not include any vulnerable populations.

VII. RECRUITMENT METHODS

Advertisements in mass media outlets (e.g., newspaper, radio); through social media (e.g., Facebook); using online recruitment tools (e.g., ResearchMatch); via flyers given to local mental health providers, who offer limited or no substance use treatment (e.g., referrals for alternative treatment options [when alcohol is the primary presenting concern] or as an adjunct alcohol treatment [when the provider is addressing other mental health issues]); and direct mailings targeting the greater Front Range metropolitan area, which includes Boulder County, Denver, and suburbs of Denver, will be the primary sources of participants. Additionally, participants will be able to fill out an online screening showing their interest that will be directly advertised via a link to Redcap

on the mailings sent out. The online screening will be administered through Redcap and is password protected. Only the research team knows the password to this Redcap screening. This recruitment strategy is based on the successful recruitment in our previous trials. All of these advertisements will describe the opportunity to earn a maximum of $340 by participating in a study exploring mindfulness and biological mechanisms related to alcohol use. Men and women of all ethnic backgrounds will be recruited into the study. We expect that the proposed study will reflect the ethnic diversity of the greater Denver metropolitan area, such that approximately 25-35% of the final sample will represent Latino and non-Caucasian individuals. We will recruit 226 individuals. Our previous experience with clinical trials (e.g., Littlewood et al., 2014), as well as the experience of our research team members (Bowen, Witkiewitz, Dimidjian) with this specific intervention supports the feasibility of recruiting approximately 50 patients per year (see Bowen et al., 2014)

During the phone or online interview, participants will be screened for the inclusion criteria outlined above. Before asking any eligibility questions, the interviewer will obtain verbal consent from the potential participant. For the online interview, participants will be marking their electronic written consent to participate in the screening at the beginning of the survey. The potential participant will also be informed that some of the questions asked will concern their use of substances including alcohol, cannabis and illegal drugs. They will be told that they do not have to answer any question that makes them feel uncomfortable; however, not answering some or all of the questions on the phone or on the online screening, may result in their being ineligible to participate in the study. In accordance with the IRB's guidelines for pre-screening potential participants () only first names or initials will be collected to identify the potential participant during this phone screen (pre-screen). In regards to the online phone screen, the potential participant will be contacted on the phone after they complete the screen in order to receive an explanation about the study and to schedule them for their first appointment. For participants that do not meet criteria, they will be sent an email stating their ineligibility and will receive resources for alcohol and mindfulness therapies in their area. In order to protect participant confidentiality, the first eight pages of the phone screen form will be shredded immediately after the phone screen (pre-screen) is complete. Only the information relevant to contacting the participant on the phone screen will remain. This contact information will be important for us to retain so that we can reach participants concerning their appointments. Participant contact information will be stored in a separate research folder, apart from all other study documents. This folder will be stored in a locked filing cabinet CU CHANGE research lab at the Center for Innovation and Creativity (CINC). Each contact information sheet will be destroyed at the end of the study, which will minimize any risk of loss of confidentiality. For the online phone screen, the participant information will be kept on a password protected Excel document on a computer in Dr. Hutchison's laboratory which will be destroyed at the end of the study.

Due to the COVID-19 situation we are collecting remote data only and will return to our original protocol when it is safe to do so. The remote RECRUITMENT METHODS will be as follows:

Participants will be recruited using social media advertising and will go through a phone screen with a research assistant to confirm their eligibility and answer any questions they may have. If the participant is eligible, they will be scheduled for their next Zoom appointment.

VIII. COMPENSATION

We estimate that the completion of assessments and procedures across the duration of the trial, including therapy sessions, will total approximately 16 hours of time (an additional 2 hours for MRI). To compensate subjects for their time and travel costs, subjects who will not be scanning will be paid a maximum of $280 if they complete all therapy sessions and appointments and those subjects who will be scanned will be paid a maximum of $340. As a breakdown for compensation participants will be given:

- $30 for completing the Baseline Session (an additional $30 will be given to participants who under to the first MRI scan)
- $30 at the Intermediate Session (week 4)
- $30 for completing the End of Treatment Session (week 8)
- $15 for each therapy session they attended (week 1-3 and 5-7) ($90 total; will be given in total to participants at week 8)
- $50 for completing the full week 20 follow-up ($30 will be given for completing surveys, whether completed in our laboratory or remotely; $20 will be given for blood sample collection, which can only occur in our laboratory)
- $50 for completing self-report measures for the week 32 follow-up ($30 will be given for completing surveys, whether completed in our laboratory or remotely; $20 will be given for blood sample collection, which can only occur in our laboratory; an additional $30 will be given to participants who undergo the second MRI scan)

Due to the COVID-19 situation we are collecting remote data only and will return to our original protocol when it is safe to do so, the remote COMPENSATION will be as follows:

Compensation (via Amazon gift cards) for each study appointment goes as follows:

- Baseline session: $30
- Therapy sessions (weeks 1-3, 5-7): $15 per session ($90 total given at week 8)
- Intermediate session (week 4): $30
- End of treatment session (week 8): $30
- Follow-up session I (week 20): $50 for surveys
- Follow-up session II (week 32): $50 for surveys

IX. CONSENT PROCESS

Given that the phone screen portion of the study presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research study, the study team is requesting a waiver of written consent for the phone screen portion of the study. The member of the research team who is conducting the phone screen will say the following prior to asking any screening questions, in order to obtain verbal consent to conduct the screen: “Thank you for your interest in our research study investigating alcohol and mindfulness. Do I have your verbal consent for me to ask you a few questions to verify that you are qualified and tell you about the requirements before you come to the lab? It will take about 10-15 minutes of your time. If you have any questions you can contact Kent Hutchison at 303-492-8163 or the Institutional Review Board at 303-735-3702. Your participation in this pre-screening is voluntary.” The member of the study team will be available to answer any questions regarding the phone screen process, and the phone screen

will be immediately discontinued if the potential participant does not give verbal consent to answer the eligibility questions. For the online screening, the participant has the choice to exit out of the screening at any point without completing it. The study team will track individuals who give verbal consent to the phone screen in a non-identifiable manner (i.e., subjects who consent to be phone screened will be referred to as Subject-01, Subject-02, etc. on the tracking log). This tracking log will be stored in a password-protected file on a computer in Dr. Hutchison's laboratory.

When a participant arrives for his/her first session at the Center for Innovation and Creativity (CINC), a member of the research team (i.e., a trained research assistant or graduate student) will greet him/her in the first-floor lobby. The research assistant will take the participant to a private room and provide the participant with a copy of the informed consent document. A description of the MRI procedures is included in the consent form, and all participants will be given a verbal description of the MRI screening process and potential risks of MRI studies. Prior to asking the participant to sign the consent form, the trained research assistant and the participant will have a discussion regarding the research study. Additionally, the research assistant or graduate student will be available to answer any questions he or she may have about the study. Participation will be clearly stated as voluntary, with the option to withdraw at any time. There will be no deception involved with this study. After discussing the study and going over the consent form with the researcher, the participant will initial and sign the informed consent document.

Separate signatures will be obtained for participation in the study and broadly sharing gut metagenomic and phenotypic data. Individuals who opt not to have their gut metagenomic and phenotypic data broadly shared will still be allowed to proceed with participation in the study.

Due to the COVID-19 situation we are collecting remote data only and will return to our original protocol when it is safe to do so. The remote CONSENT PROCESS will be as follows:

When a participant arrives via Zoom for his/her first remote Baseline & consent session a member of the research team (i.e., a trained research assistant or graduate student) will greet him/her and welcome them to the study. The research assistant will provide the participant with a copy of the informed consent form via a link to the DocuSign document. Prior to asking the participant to sign the consent form, the trained research assistant and the participant will have a discussion regarding the research study. Additionally, the research assistant or graduate student will take the participant through the informed consent document, step by step, and give them an opportunity to ask any questions about their participation in the study. Participation will be clearly stated as voluntary, with the option to withdraw at any time. There will be no deception involved with this study. After discussing the study and going over the consent form with the researcher, and after all questions have been resolved, the participant will then show their ID to the researcher, over Zoom, to the confirm they are between 21-60 years old. Then the research assistant will use DocuSign to electronically sign the consent form and send it to the participant to sign. The participant will then send the signed DocuSign informed consent form back to the researcher. Signed consent forms will be stored securely on our server in a password protected folder.

X. PROCESS TO DOCUMENT CONSENT IN WRITING

In accordance with 45 CFR 46.117, an unsigned copy of the form used to document consent will also be given to the study participant.

XI. PROCEDURES

Screening for Eligibility/Scheduling

Participants will contact the researchers with their interest by phone, e-mail or by filling out the online screening. The researcher will respond with an overview of the study and answer any questions the potential participant has before screening the caller for preliminary eligibility. In the case that a potential participant requests more information via e-mail, the research assistant responding to the e-mail will ask the participant to call the lab phone number (same phone number provided on all study advertisements) for more information, or e-mail the study team a phone number at which the potential participant could be reached at and a desirable time for the research staff to call. Prior to being asked the phone screen questions, potential participants will be required to give their verbal consent, or in the case of the electronic online screening, written consent. They will be asked to state that they are willing to answer questions over the phone or through the online survey which will help researchers to determine their eligibility to take part in the study. The phone screening process will help to ensure participants have ample opportunity to be informed of the main study components, ask questions, and decide if they are willing to participate before they sign up for the study. For participants that complete the online screening, a research assistant will follow up with the participant on the phone to answer any questions and ask if the participant is willing to take part in the study. If the participants meet the study criteria during the initial phone interview or online screening, they will be invited to come to the CINC for the initial consenting session. They will be given the option of combining this session with the experimental baseline session. If participants choose to do a separate session for consenting, they will schedule their experimental baseline session upon completion of the consent/orientation session. Upon scheduling their first appointment, participants will be provided, via an e-mail attachment (see pre-appointment e-mail template), a letter that will include the time and date of the appointment. If participants are electing to combine both appointments, they will be instructed ahead of time not to drink alcohol within 48 hours, smoke marijuana within 48 hours, or consume caffeine or smoke cigarettes within 3 hours of the session. They will receive an email containing this information upon scheduling the session (see "Instructions for Appointment" document). If participants are doing the consent/orientation and study appointments on two separate visits, they will be given these instructions during the consent/orientation session.

COVID-19: Before interacting with research assistants, eligible participants will be asked additional questions related to COVID-19. These questions will be administered prior to any in-person appointment and by a research assistant over the phone (or at a distance of at least 6-feet if the participant is unable to complete the questions over the phone). These questions will be administered to protect staff and participants from the spread of COVID-19. The questions are a precaution based on current university and Centers for Disease Control and Prevention guidelines and may need to be updated as the understanding of COVID-19 evolves. These procedural questions are temporary in nature and will be lifted once university guidelines deem it unnecessary.

Informed Consent Appointment

Upon arrival for the informed consent appointment, participants will be met by a research assistant or graduate student who will escort them to a private consultation room where participants will be asked to complete and sign the informed consent form. If the participant is doing the consent and experimental baseline session in two separate visits, he or she will be informed at the consent session to refrain from drinking alcohol within 48 hours, smoking marijuana within 48 hours, or consuming caffeine or smoking cigarettes within 3 hours of the experimental baseline session, and the baseline session will be scheduled at the end of the consent appointment. The participant will also be given a copy of the Instructions for Appointment Document at the end of the consent visit. If the participant is doing the consent session and experimental session in the same visit, he or she will proceed with the study procedures after completing the informed consent process with the trained research assistant or graduate student.

Baseline Session

After arriving at the lab, qualified participants will (1) complete an informed consent form, and then (at either the same session or a separate session), participants will (2) complete a breath alcohol test, (3) complete a urine toxicology screen, (4) provide a blood sample for assays of peripheral inflammatory markers and DNA methylation, (5) fill out the baseline questionnaires, (6) and take several neurocognitive tasks. Participants will also be given a gut microbiome sample collection kit at the end of the session and will return it to their first scheduled session (7).

Subjects will be breathalyzed before every session starts, including all therapy sessions. Any subject that has a positive BAL or who reports drinking will be rescheduled for that appointment. A participant who blows a BAC over 0.00 will be given one chance to reschedule his/her appointment for the next day. If s/he comes in the next day with a breath alcohol greater than 0.00 then this session will be counted as "missed." If a subject accumulates more than one "missed" session, they will be automatically dropped from the study on the grounds that they failed to follow explicit study directions properly. Participants will not receive compensation if a session is terminated due to failure to follow the abstinence instructions. They will be informed of this contingency during the phone screen, consent visit and is stated the consent form.

A urine toxicology screen will be administered to insure that subjects have not recently used illicit drugs including, sedatives, opiates, cocaine, or amphetamines.

A member of our research team who has completed a certified training in phlebotomy will collect the blood sample. The blood draw procedures will involve collecting venous blood (40 ml) venipuncture of a peripheral arm vein using standard, sterile phlebotomy techniques. The samples will be tested for cytokine levels and epigenetics using equipment at the CINC.

Subjects will then complete baseline questionnaires related to their demographics, history of drinking, smoking and drug use, and alcohol craving (measures listed below). Also, subjects will take six neurocognitive tests using the NIH toolbox application, which will assess their cognitive function in terms of memory and attention. If not eligible for the MRI, subjects will be randomly assigned to receive MBRP or RP for the next 8 weeks at the end of their baseline session and will also be given their microbiome sample collection kits at the end of the session. They will return the sample kits at their next scheduled session. Subjects will be compensated $30 for completion of the baseline appointment.

MRI Scan I

All subjects who are eligible will undergo a structural scan, using a Siemens 3T Trio, followed by an fMRI scan that involves alcohol cue exposure. Female participants will be asked to take a

pregnancy test free of charge and must have a negative result in order to continue with participation in the study. Female participants may opt out of the urine pregnancy test and sign a waiver (one of the standard INC MRI scanning forms) indicating they understand that the risks associated with MRI and unborn fetuses is unknown and that they are confident that they are not currently pregnant.

The research assistant will go over the contraindications for fMRI screening form again with the participant to ensure that eligibility has not changed since the phone screen; if there is any question that a participant might not be safe to go into the scanner the appointment will be cancelled and rescheduled if possible.

Prior to going into the MRI scanner, the MRI technologist on duty will ask participants to remove all jewelry and metal objects from their pockets. Participants will be required to change into scrubs to prevent any possible risk from metallic objects or decorations in their clothing. Eligible participants will be escorted to a changing room where they will be asked to change into the hospital scrubs provided, and to lock up their belongings in the locker.

A trained MRI technician will position participants in the scanner and ensure that all safety procedures are followed. In an MRI scan, the subject lies down on a table and is placed into a long donut-shaped magnet. A specially designed coil will be placed around the head to provide better images (as is done with standard clinical examinations). As the MRI scan is performed, the subject will hear loud rapping and knocking noises that are normal for a MRI scan.

During the scanning session, we will acquire anatomical images for subsequent analysis as well as functional images during an alcohol cue task. The alcohol cue task compares the taste of an individual's favorite alcoholic beverage with a sweet, appetitive control taste (litchi juice). Subjects will be administered 1 ml of each beverage through Teflon tubing that is attached to a computer controller gustometer, over each 24-second trial. The total amount of the alcoholic beverage is only 1 ml (less than a teaspoon).

After the scan, a research assistant will escort the participants back to the changing room where their belongings are locked. Subjects in this group will be given their MBRP or RP condition assignment after the scan is complete in order to minimize any expectancy effects that may occur as a result of knowing their condition assignment before all pre-treatment measures have been taken. Subjects will be compensated $30 for completion of the MRI scan I appointment.

Goal-setting Session

Subjects will have a 1-hour individual session with a trained therapist before starting the 8-week long program, resulting in a total of 9 individual therapy sessions. The purpose of this being a chance for the participant to discuss with their therapist their goals for the next 8 weeks in terms of drinking reduction goals or abstinence goals. Subjects will take a BAC test before the session starts.

MBRP (or RP) Weekly Sessions

Subjects will meet with a trained therapist for 1-hour individual therapy sessions once a week for 8 weeks at the CINC in a private consultation room.

Subjects will be breathalyzed and complete their check-in survey before every session starts. If subjects are dropped due to accumulated "missed" sessions due to failure to follow the abstinence instructions, they will not be compensation for previous attended therapy sessions. They will be informed of this contingency during the phone screen, consent visit and is stated the consent form.

Subjects will be compensated $15 for each therapy session they complete (payment for each completed session is given to participants at week 8).

Intermediate Session (4 weeks)

At the intermediate session, subjects will continue with their usual session with a therapist (including a BAC test), and either before or after their appointment, complete a set of self-report outcome measures, and provide a blood sample. Subjects will be compensated $30 for completion of this session.

End of Treatment (8 weeks)

At the end of treatment session, subjects will continue with their usual session with a therapist (including a BAC test), and either before or after their appointment, complete a set of follow-up outcome measures including questionnaires and neurocognitive tests, and provide a blood sample. Subjects will be compensated $30 for completion of this session, plus an additional $15 for completing each therapy session (weeks 1-3 and 5-7), for a total of $90 if they have attended all 6 sessions. Participants will receive their second gut microbiome sample collection kits at the end of the session and will return them using a pre-paid, pre-addressed envelope that we will provide for them.

Follow-up Assessment I (20 weeks)

At this follow-up session, all subjects will take a BAC test, complete a set of self-report outcome measures and provide a blood sample. Subjects will be compensated $50 for completing this assessment ($30 for completing self-report measures and $20 for providing blood samples). For subjects who are unable to come into the laboratory, the option of completing self-report measures online (via Qualtrics) will be provided for a $30 compensation.

Participants who are unable to come into the laboratory will be sent an email, with an anonymous link to the same Qualtrics survey that would is administered at the follow-up assessments. All surveys are administered via Qualtrics and, thus, participants will already be familiar with navigating this online survey. The email will include the following instructions:

> As our study staff noted, your progress since finishing treatment is extremely important for us to understand how alcohol treatments work, and why they may work better for some individuals than others. Therefore, regardless of whether you feel you have improved, declined, or had no changes since completing treatment, your responses are equally valuable. To reimburse you for your time, we will provide a $30 gift card.
>
> If you choose to proceed, here is the link to your survey: [INSERT LINK]
>
> After clicking the link, please enter your study ID and treatment condition (see below). Please fill out this survey alone to ensure focus and anonymity. In addition, you may discontinue participation or refuse to answer any questions without penalty. Once you have completed the survey, study staff will be notified and you will receive your compensation within one week.
>
> Your study ID is: [INSERT ID]
> Your treatment condition is: [INSERT MBRP or RP]

Follow-up Assessment II (32 weeks) and MRI Scan II

At this follow-up session, all subjects will take a BAC test, complete a set of self-report outcome measures and provide a blood sample. Subjects will be compensated $50 for completing this

assessment ($30 for completing self-report measures and $20 for providing blood samples). For subjects who are unable to come into the laboratory, the option of completing self-report measures online (via Qualtrics) will be provided for a $30 compensation. The same email will be sent for the second follow-up assessment as provided above.

All subjects who are eligible and selected to participate in the MRI scan portion of the study, will complete the second MRI scan during this appointment and receive an additional $30 for completion of the second scan. Female participants will be asked to take a second pregnancy test free of charge and must have a negative result in order to go in the MRI. Following the MRI scan, a therapist will conduct an assessment with each participant after the cue exposure session to determine whether there is any residual craving or anxiety before leaving the session. Participants will also be provided with contact information for local AA and support groups for aftercare.

Check-in Session (33 weeks)

Those subjects who participated in the MRI scan will return for a visit with a therapist one week after their second follow-up session. Before this session starts, participants will take a BAC test. During this session, the therapist will assess whether there were any adverse events, including lapses that may have resulted from the alcohol cues administered during the MR scan. Any adverse events will be addressed in the session and reported to the PI. At the end of their last session (33 weeks), participation in the study is complete.

Name of instrument/tool/procedure	***Purpose (i.e. what data is being collected?***	***Time to Complete***
Questionnaire Measures Collected at Baseline Only		*10 minutes*
Demographics	• A demographics questionnaire will be used to collect information on age, sex, marital status, SES, occupation, income, education, and race.	
The Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES)	• SOCRATES (Miller & Tonigan, 1996) is an experimental instrument designed to assess readiness for change in alcohol abusers. The instrument yields three factorially-derived scale scores: Recognition (Re), Ambivalence (Am), and Taking Steps (Ts). It will be used to measure individual differences in motivation at baseline.	

Drinking History Questionnaire	• Drinking history is measured with 12 items assessing lifetime drinking history such as current drinking, age of onset, and number of previous of attempts to quit/reduce drinking.	
Smoking History Questionnaire	• Smoking History Questionnaire will be used to collect information on frequency and quantity of nicotine use and will be used to determine whether the treatment groups differ on nicotine consumption.	
		30 minutes

Questionnaire Measures Collected at Baseline, 4, 8, 20, 32 weeks

Timeline Follow Back (TLFB)	• The TLFB (Sobell, Maisto, Sobell, & Cooper, 1979) is an assessment method that obtains estimates of daily alcohol, cigarette, and drug use. The TLFB has been shown to have good psychometric characteristics with a variety of drinker groups and can generate variables that provide a wide range of information about an individual's drinking (e.g., pattern, variability, and magnitude of drinking). This instrument requires subjects to recall from memory the number of drinks consumed for each day over the prior 30 days as well as their use of tobacco products and recreational drugs. Studies with alcoholics have shown this instrument to be reliable in assessing drinking frequencies and other behaviors such as arrests and hospitalizations (Sobell et al., 1979).	

Heavy Drinking Days (HDD); Drinks per Drinking Day (DDD); Percent Days Abstinent (PDA)	• All three measures will be obtained at each assessment using the Timeline Follow Back (TLFB), a calendar-based assessment of daily alcohol and drug use (Sobell et al., 1979). The TLFB has been shown to have good psychometric characteristics among a variety of drinker groups and can generate variables that provide a wide range of information about an individual's drinking (e.g., pattern, variability, and magnitude of drinking). HDD is the primary behavioral outcome that will be analyzed.
Impaired Control Scale-Failed Control (ICS-FC)	• The Impaired Control Scale is a 25-item scale that is used to measure the severity of alcohol dependence with respect to dimensions such as impaired control over drinking, awareness of compulsion to drink, etc. (Heather, Booth, & Luce, 1998).
Alcohol Dependence Scale (ADS)	• The **ADS** has been used widely and found to have excellent predictive value with respect to DSM diagnosis (Kivlahan, Sher, & Donovan, 1989) and will be used to assess severity of alcohol use symptoms.
Pennsylvania Alcohol Craving Scale (PACS)	• The PACS (Flannery, Volpicelli, & Pettinati, 1999) is a 5-item measure that will provide a measure of craving at each session.
Drug Use Questionnaire	• The Drug Use Questionnaire assesses individual behavior with regard to frequency and quantity of drug use. This measure will be used to assess whether smoking

	and drug use behavior changes during the course of clinical trial.
Beck Depression Inventory-II (BDI-II)	• The BDI-II (Beck et al., 1996) is a 21-item measure of depression symptom severity in the past two weeks. BDI-II scores range between 0 and 63, with categorical depression ratings of "minimal" (0–13), "mild" (14–19), "moderate" (20–28), and "severe" (29–63). The BDI-II will be administered at baseline and at last follow-up to examine changes in comorbid depression and co-vary baseline differences if necessary.
Beck Anxiety Inventory (BAI)	• The BAI (Beck & Steer, 1990) consists of 21 items, each describing a common symptom of anxiety. The items are summed to obtain a total score that can range from 0 to 63. The BAI will be administered to examine comorbid anxiety and co-vary baseline differences if necessary.
Pain Interference - SF 8a	• The Pain Interference item bank is from the National Institutes of Health (NIH) and is part of the Patient Reported Outcomes Measurement Information System (PROMIS). The Pain Interference SF-8a, is a 8-item short-form questionnaire that measures the self-reported consequences of pain on relevant aspects of one's life. This includes the extent to which pain hinders engagement with social, cognitive, emotional, physical, and recreational activities (Amtmann et al., 2010).

The Five Facet Mindfulness Questionnaire	• The Five Facet Mindfulness Questionnaire (Baer et al., 2006) will determine the extent to which MBRP increases mindfulness practice as a proximal mediator of the effects of the intervention. These data will be used as a manipulation check.
Mindfulness Practice Questionnaire	• The extent to which patients engage in key aspects of mindfulness will be collected using the 10 item Mindfulness Practice Questionnaire (e.g., Witkiewitz, Lustyk, & Bowen, 2013) on a weekly basis.
Comprehensive Inventory of Mindfulness Experiences (CHIME)	• The CHIME (Bergomi, Tschacher, & Kupper, 2015) consists of three items and will determine the number of minutes of mindfulness practice per week each patient is currently engaging in. Minues of current meditation practice will be calculated using the following compound variable: (weekly frequency x average session duration). The data will be used to co-vary for patients who are already engaging in frequent mindfulness meditation practice at baseline if necessary.
Multidimensional Personality Questionnaire – Stress Reaction	• The Multidimensional Personality Questionnaire (Tellegen, 1982, Tellegan & Waller, 2008) consists of 18 True/False items of statements describing negative emotionality and stress reaction. The questionnaire will be administered to examine comorbid negative emotionality and used to co-vary baseline differences if necessary.

Response Styles Questionnaire - Ruminative Response Scale	• The Ruminative Response Scale (Treynor, Gonzalez, & Nolen-Hoeksema, 2003) consists of 22 items scoring how often the patient engages in ruminative thinking associated with depression. The data will be used to examine comorbid negative emotionality and used to co-vary baseline differences if necessary.
Emotion Regulation Questionnaire (ERQ)	• Emotion Regulation Questionnaire (ERQ) is a 10-item scale that is designed to assess individual differences in the habitual use of two emotion regulation strategies: cognitive reappraisal and expressive suppression (Gross, John, 2003).
Effortful Control Scale – Full Scale	• The Effortful Control Scale Derryberry & Rothbart, 1988, Evans & Rothbart, 2007) consists of 35 items that measure the degree to which the patient exhibits behavioral control. The data will be used to co-vary baseline differences in behavioral control if necessary.
Drinking Motives Questionnaire	• The Drinking Motives Questionnaire (Cooper, 1994, Kuntsche et al., 2006, Young-Wolff et al., 2009) consists of 20 items used to assess patients' varying motives for drinking alcohol. The Drinking Motives Questionnaire will be used to assess whether motives for drinking alcohol change from baseline following treatment.

Quantity/Frequency of Alcohol use	• Alcohol use will be evaluated with a variation of the measure used by White and Labouvie (1989). First, participants will be asked if they have ever had an alcoholic drink (with instructions that define one alcoholic drink as "one beer, one glass of wine, or one serving of hard liquor either by itself or in a mixed drink"). Those (likely well over 90%) who answer that they have previously had alcohol before will be asked to rate: (1) their frequency of use in the last three months on a 9-point scale ranging from "never" to "every day", (2) their typical quantity of drinks in one sitting on a 10-point scale ranging from "no drinks" to "more than 20 drinks", and (3) their frequency of getting drunk when drinking in the past three months on a 5-point scale ranging from "never" to "always."
Alcohol Use Disorders Identification Test (AUDIT)	• The AUDIT (Saunders, Aasland, Babor, De La Fuente & Grant, 1993) is used to detect less severe problem drinkers and addresses both current problems (problems within the last 3 months) and problems across an individual's lifetime. The AUDIT consists of ten questions that cover such domains as alcohol consumption, drinking behavior, adverse psychological reactions, and alcohol-related problems.
Prior Treatment Experiences Questionnaire (PTEQ)	• Participants will be asked about their engagement in any 12-step programs (Tonigan, Miller & Conners, 1997), psychological services for alcohol and other

		problems, and use of other self-help materials. At baseline, participants will be asked about lifetime engagement in treatment. Because this study will be recruiting a population of heavy drinkers, it will be important for us to know whether or not any of our participants have previously received treatment for alcohol abuse. In future analyses, this information (e.g., prior treatment history) will be used as a covariate.
	Alcohol Urge Questionnaire (AUQ)	• The AUQ **(**Bohn et al., 1995) will be used to assess craving. The AUQ consists of eight items related to urge drink that are rated on a 7-point Likert scale with the extremes anchored by "Strongly Disagree" and "Strongly Agree." The AUQ has demonstrated internal consistency and reliability.
	Impulsivity and Sensation Seeking Scale (ImpSS)	• The ImpSS (Zuckerman, 1996) consists of 19 items measuring impulsivity and sensation seeking that may predict responses to alcohol and drug problems. The sum of the symptoms endorsed is a proxy measure of dependence.
	Fruit and Vegetable Intake Screener (the "All-Day" version)	• This 10-item measure was developed by the National Cancer Institute to assess how many times in the previous month subjects consumed different types of fruits and vegetables, including potion size questions for every food item. This measure was shown to be a useful estimate for obtaining median intakes of fruit and vegetable servings in U.S. populations (Thompson et al.,

		2002). Fruit and vegetable consumption has been shown to influence inflammation (e.g., Holt et al., 2009), and dietary factors have also been linked with epigenetic changes (see Hardy & Tollefsbol, 2011). Thus, the inclusion of this measure allows fruit and vegetable consumption to be controlled for in inflammation and epigenetic analyses.
Self-Rated Diet Quality	• This single-item, self-rated diet quality measure (Loftfield et al., 2015) consists of a single question asking "In general, how healthy is your overall diet?" Participants can respond with (1) excellent, (2) very good, (3) good, (4) fair, or (5) poor. The item is significantly associated with variation in objective measures of dietary intake (P<.001).	

Voluntary Aerobic Exercise Questionnaire (VAEQ)	• The VAEQ assesses levels of voluntary exercise (Bryan and Rocheleau, 2002). Participants indicated how frequently they engaged in exercise activities in the past 3 months and past week. The exercise composite score used in these analyses was composed of 4 items: (i) "In the past 3 months,what is the average number of days per week that you engaged in aerobic exercise?" (ii) "In the past 3 months, what is the average number of total minutes per week that you engaged in aerobic exercise?" (iii) "In the past week, how many days did you engage in aerobic exercise?" and (iv) "In the past week, what is the total number of minutes that you engaged in aerobic exercise?" This measure has been used to quantify exercise participation in our previous alcohol studies (Karoly et al., 2013). Given that exercise may impact inflammation (see Woods, Vieira, & Keylock, 2009) and epigenetics (see Ntanasis-Stathopoulos, Tzanninis, Philippou, & Koutsilieris, 2013), this measure (either alone or in combination with the Godin, see below) will allow us to control for exercise behavior in inflammation and epigenetic analyses.

Godin Leisure Time Exercise Questionnaire	• The Godin Leisure-Time Exercise Questionnaire (QLTEQ; Godin & Shephard, 1985) is used to generate 'activity scores' based on how many separate times (occasions) in a typical week participants engage in mild, moderate, or vigorous exercise for more than 15 minutes of their leisure time. The three levels of intensity are defined as follows: (1) mild exercise, defining features include, slightly elevated heartbeat, slightly elevated breathing rate (able to carry on a conversation effortlessly); (2) moderate exercise, defining features include accelerated heartbeat, moderately elevated breathing rate (able to carry on a conversation), and mild physical exhaustion; and (3) vigorous exercise, defining features include rapid heartbeat, heavy breathing (difficult or unable to carry on conversation), and physical exhaustion. Participants will be asked to answer how many separate times they engaged in mild, moderate, and vigorous intensity exercise over the past week (for each data collection time point). This measure could be used alone or in combination with the VAEQ to control for exercise participation in inflammation analyses.
Perceived Stress Scale	• The PSS (Cohen, Kamarck, & Mermelstein,1983) is a 14-item scale that measures (1) the degree to which situations in someone's life are perceived as stressful. Stress is a known correlate of substance use and abuse and it will be crucial for us to obtain a

		measure of our participants' perceived stress levels, which may be associated with inflammatory processes (e.g., Hart & Kamm, 2002) and epigenetic changes (see Eric J Nestler, 2012). Thus, this measure will be used to control for stress in epigenetic and inflammation analyses.
	Pittsburgh Sleep Quality Index (PSQI)	• The PSQI (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) is a 19-item self-report scale that measures sleep quality over the past month. The 19 self-related items are combined to form seven "component" scores, each of which has a range of 0-3 points. In all cases, a score of "0" indicates no difficulty, while a score of "3" indicates severe difficulty. The seven component scores are then added to yield one "global" score, with a range of 0-21 points. In the present study, it will be important to control for sleep duration and/or quality, given that sleep is known to be associated with inflammation (e.g., Patel et al., 2009) and epigenetic factors (see Qureshi & Mehler, 2014). Thus, component scores, global scores and single item scores could all potentially be used as covariates in later analyses of these data. Scores from this measure will be included as covariates in inflammation and epigenetic analyses.
	The Leisure-Time Categorical Item (L-Cat)	• The Leisure-Time Categorical Item (L-Cat) is a single item comprised of six descriptive categories ranging from inactive

	to very active (Kiernan, Schoffman, Lee, Brown, Fair, Perri, & Haskell, 2013). Each category consists of 1–2 sentences describing common activity patterns differing in frequency, intensity, duration, and types of activities, thus encompassing content validity (van Poppel, Chinapaw, Mokkink, van Mechelen, & Terwee, 2010). Respondents pick the category best describing their activity during the past month. It assesses national activity recommendations as well as multiple clinically relevant categories below and above recommendations, and incorporates critical methodological principles that enhance psychometrics (reliability, validity, sensitivity to change).	
Neurocognitive NIH Toolbox Measures Collected at Baseline & End of Treatment (week 8)		*20 minutes*
Flanker Inhibitory Control & Attention Test	• Inhibitory control and attention. The participant focuses on a particular stimulus while ignoring other stimuli. Five arrows are displayed in the middle of the screen, each pointing left or right. The participant selects the arrow that matches the middle arrow in the sequence.	
Dimensional Change Card Sort Test	• Measures cognitive flexibility and attention. Two target pictures are presented that vary along two dimensions (e.g., shape and color). Participants are asked to match one of the two bottom	

	cards to the card displayed at top, by either color or shape.
Pattern Comparison	• Measures processing speed. The test takes less than 90 seconds and requires participants to discern whether two side-by-side pictures are the same or not.
Picture Sequence Memory Test	• Episodic memory. Participants are asked to reproduce the sequence of pictures that is shown on the screen.
Picture Vocabulary Test	• A measure of receptive vocabulary administered in a computer-adaptive test (CAT) format. Participant matches picture with spoken word.
List Sorting Working Memory Test	• Participants have to say back the list of images they are given.
MRI/fMRI Scan Collected at Baseline and 8 weeks	
MRI/fMRI In-task Measures	• The MRI data collection will take place at the CU Boulder's INC MRI Suite in the CINC. We will utilize a 2011 3T research-only Siemens Trio system (Erlangen, Germany) with a 32-channel head coil, multinuclear support, clinical features (extremity coil, shoulder array coil, spectrus injector), and additional software (e.g., BLADE, inline diffusion/BOLD, spectroscopy, cardiac, advanced functional software). Scanner instability and quality control on a phantom will be monitored weekly. All participants will undergo a structural scan, followed by a functional MRI scan, which

	includes the alcohol cue task.	
Structural MRI (sMRI)	• For this sequence, participants will be asked to just lie still and stay awake. No other actions on their behalf are needed. We will collect high-resolution anatomical images to permit accurate tissue classification and anatomical parcellation. For optimal contrast between gray matter (GM), white matter (WM) and cerebral spinal fluid (CSF) at 3T, we will use a multi-echo MPRAGE (MEMPR) sequence with the following parameters: TR/TE/TI = 2300/2.74/900 ms, flip angle = 8°, FOV = 256x256 mm, Slab thickness = 176 mm, Matrix = 256x256x176, Voxel size =1x1x1 mm, Number of echos = 4, Pixel bandwidth =650 Hz, Total scan time = 6 min.	*6 minutes*
Functional MRI (fMRI)	• fMRI scans will be collected with single-shot full k-space echo-planar imaging (EPI) with ramp sampling correction using the intercomissural line (AC-PC) as a reference (TR: 2.0 s, TE: 27ms, α: 70^{o}, matrix size: 64 × 64, 32 slices, voxel size: 3 × 3 × 4 mm^{3}). Image-based higher order automatic shimming will be employed. fMRI data will be acquired with a 32-channel coil. Scanner instability and signal-to-noise ratio during fMRI scanning on a phantom will be monitored weekly throughout the project period. Automated analysis of scanner instability with the Weiskoff method is routinely performed. We have used our alcohol cue task successfully in over 600 individuals (Claus et al.,	*6 minutes*

	2011). It is reliable and well validated. The task compares the taste of an individual's favorite alcoholic beverage with a sweet, appetitive control taste (litchi juice). The task was designed to minimize movement by only administering 1 ml of each beverage through Teflon tubing that is attached to a computer controller gustometer, over each 24 second trial. It is important to note that the total amount of the alcoholic beverage is only 12 ml (less then a teaspoon). The task itself involves a block design with twelve pseudorandomly ordered alcohol and control trials that are presented in two consecutive runs. In order to examine whether the effects of MBRP differ from those of RP, we will calculate the signal change for alcohol minus the control condition in the precuneus, putamen, and anterior cingulate and use the signal in these regions of interest (ROIs) in the structural equation models (see analysis section).	
Resting State Connectivity	• For this sequence, participants will be asked to just lie still and stay awake. No other actions on their behalf are needed. This resting state scan will be collected to derive measures of functional connectivity in the left and right executive control networks (LECN/RECN) following our previously published procedures (Weiland et al., 2014). These networks will be identified using functionally defined ROIs/nodes. The nodes for the LECN include:	*10 minutes*

	dorsolateral prefrontal cortex (DLPFC), left middle/superior frontal gyrus (MFG), left superior parietal gyrus/angular gyrus (PAR), left inferior/middle temporal gyri (TL), right crus I/crus II/Lobule VI (CE), and left thalamus (TH). The RECN nodes include: right DLPFC, right MFG, right PAR, right medial superior frontal gyrus (mSFG), left CE, and right caudate (CU). Functional connections between nodes, each pairwise correlation defined as an edge, will be calculated and used to create a correlation matrix between the time-series of all nodes within each network for each subject. A Fisher r-to-z transformation will be applied to r-values to yield z-scores. Connectivity strength as a primary global measure of connectivity for each network will be calculated as the mean of all pairwise correlations between nodes within each network as well as other graph theory metrics (e.g., degree, efficiency, centrality). The framewise displacement (FD) for each subject will be calculated across the entire resting state run from the image motion parameter and used a covariate in the analyses.		

Visit/Session	*Procedures/Tools*	*Location*	*Time to Complete*
Baseline Session	• Informed consent; Study orientation; BAC test; Urine drug test; Questionnaires; Neurocognitive	CINC	2 hours and 30 minutes

	tests; Blood draw; receive microbiome kit		
MRI Scan I	• BAC test; Pregnancy test; MRI	CINC	1 hour
Goal-setting Session	• BAC test; a goal setting session with a therapist to discuss drinking reduction goals for the next 8 weeks; return microbiome kit	CINC	1 hour
MBRP (or RP) Therapy Sessions (weeks 1-3 and 5-7)	• BAC test; Mindfulness Relapse Prevention or Relapse Prevention therapy session with a trained therapist	CINC	1 hour each session (6 hours total)
Intermediate Session (week 4)	• BAC test; MBRP (or RP) therapy session; Questionnaires; Blood draw	CINC	2 hours
End of Treatment Session (week 8)	• BAC test; MBRP (or RP) therapy session; Questionnaires; Neurocognitive tests; Blood draw; receive microbiome kit	CINC	2 hours
Mail-in Sample from Home	• Subject will mail-in their collection kit back to our lab in a pre-paid, pre-addressed envelope.	At Home	5 minutes

Follow-up Session (week 20)	• BAC test; Questionnaires; Blood draw	CINC	1 hour
Follow-up Session (week 32) & MRI Scan II	• BAC test; Questionnaires; Blood draw; Pregnancy test; MRI scan	CINC	1 hour (2 hours with scan)
Check-In Session (week 33)	• BAC test; a check-in session with a therapist to discuss potential relapse.	CINC	30 minutes
TOTAL TIME			*Approximately 16 hours (18* hours with MRI)

Due to the COVID-19 situation we are collecting remote data only and will return to our original protocol when it is safe to do so. The remote PROCEDURES will be as follows:

BASELINE SESSION: The first study appointment is an orientation and consent session, also referred to as the Baseline session. For this appointment, the participant will meet with a research assistant who will take them through the informed consent document and give them an opportunity to ask any questions about their participation in the study. The participant will show their ID over Zoom to the confirm they are between 21-60 years old. Then a research assistant will use Docusign to electronically sign the consent form and send it to the participant to sign. Signed consent forms will be stored securely on our server in a password protected folder.

The research assistant will then email the participant a link to the questionnaires. The participant will answer these questions while on Zoom with the research assistant. Participants will complete computerized questionnaires asking about substance use, psychological functioning and health behaviors. Then, each participant will be assigned to receive either MBRP or RP for an 8-week long program, and will be compensated with at $30 Amazon gift card for completing this appointment.

GOAL-SETTING SESSION: Participants will then be given some instructions about how to prepare for their next study session, which is called the Goal-setting Session. Before starting the 8-week long program, each participant will meet with a therapist for a 1 hour Goal-setting Session, resulting in a total of 9 individual therapy sessions. This will be an informational session where they will be introduced to their therapist, talk about goals for treatment, and learn about what they will be doing over the next 8 weeks.

THERAPY SESSIONS: For the therapy sessions, participants will meet with a therapist via Zoom once a week for 1 hour individual sessions. The therapist will guide each session by engaging the participant in an exercise and a discussion. Participants will be compensated $15 in Amazon gift

cards for every therapy session they attend. This compensation will be totaled for therapy sessions 1-3 and 5-7, and given to them at the end of their End of Treatment Session, at week 8 (see study table and compensation chart below).

INTERMEDIATE SESSION - 4 WEEKS: At the Intermediate Session, 4-week therapy session, participants will have their regular meeting with a therapist, and complete similar computerized questionnaires. The research assistant will again email the participant a link to the questionnaires. The participant will answer these questions while on Zoom with the research assistant. They will be compensated with a $30 Amazon gift card for this appointment.

END OF TREATMENT SESSION - 8 WEEKS: At the End of Treatment Session, week 8, participants will have their final session with their therapist and fill out questionnaires via a link sent by a research assistant over Zoom, and the research assistant will remain with the participant until they are finished. They will be compensated for all therapy sessions and receive an additional $30 Amazon gift card at the completion of this appointment.

FOLLOW-UP SESSIONS I & II: At the end of this week 8 session, they will schedule their final two follow-up appointments with the research assistant. These will be scheduled at 20 weeks (3 months post-treatment) and 32 weeks (6 months post-treatment). At these two appointments, participants will fill out questionnaires via a link, and will be compensated with a $50 Amazon gift card for completing the questionnaires at each of these two follow-up appointments. After this 32-week appointment, participation in this study will be complete.

Visit/Session	Procedures/Tools	Location	Time to Complete
Baseline Session	Informed consent; Study orientation; Questionnaires.	Remote/Zoom	1 hours and 30 minutes
Goal-setting Session	An introduction and goal-setting session with your therapist.	Remote/Zoom	1 hour
MBRP or RP, Individual Therapy Sessions (weeks 1-3 and 5-7)	Mindfulness Based Relapse Prevention or Relapse Prevention therapy session with a trained therapist.	Remote/Zoom	1 hour sessions (6 hours total)

Visit/Session	Procedures/Tools	Location	Time to Complete
Intermediate Session (at 4 weeks)	MBRP or RP therapy session; Questionnaires.	Remote/Zoom	2 hours
End of Treatment Session (at 8 weeks)	MBRP or RP therapy session; Questionnaires.	Remote/Zoom	2 hours
Follow-up Session I (at 20 weeks)	Questionnaires	Remote/Zoom	1 hour
Follow-up Session II (at 32 weeks)	Questionnaires	Remote/Zoom	1 hour
TOTAL TIME			Approximately 15 hours

XII. SPECIMEN MANAGEMENT

Specimen data will be stored indefinitely in locked freezers on site within the PI's laboratory designed specifically for storing biological specimens. All stored data will be coded with randomly generated numbers, and the master list linking the numbers to participants' names will be stored on a password protected server. For blood specimens being used for liver function tests, only research assistants who are authorized to work on the study will transport blood specimens from the CINC to Boulder Community Hospital Reference Laboratory in a sealed cooler. Identifying information associated with blood specimens will be removed before being transferred to BCH. Each specimen will be labeled with a general name (e.g. Research, P###), a randomly generated subject ID number, and a randomly generated date of birth to ensure confidentiality during specimen transport. At study closure, all links between participant name and number will be destroyed, at which point the specimen will be considered de-identified.

XIII. DATA MANAGEMENT

Strict standards of confidentiality will be maintained. MRI data will be electronically stored and analyzed using ID codes. If the data are published subjects will remain anonymous in all publications. Data will be stored indefinitely and will not be shared with other investigators without explicit permission from the CU Boulder IRB.

Signed consent forms will be stored in a locked filing cabinet in the PI's lab at the CINC. Liver function test results will be stored in the same locked filing cabinet. Electronically entered data from liver function test results will only be associated with the participant's subject ID number. All data from self-report and interview measures will be stored on password protected computers and on the PI's password protected server in the Department of Psychology & Neuroscience in Muenzinger Hall,

both of which are only accessible to research staff. Any identifying information will be destroyed after the follow-up session. After this, there will be no way to connect participant's names with participant data, at which point they will be considered de-identified.

Individuals who will have access to subject data include Dr. Kent Hutchison, research assistants working on the study, and personnel involved with scanning procedures at CINC and Georgia State University (see below).

Basic identifying information (name, address, phone number/email address) is collected from every research participant for the purpose of research logistics (schedule visits, etc.) and mailing of the radiological review letter, as appropriate. Participant names and other identifying information will be maintained in this restricted database, available only to authorized members of the research team for the duration of the study protocol.

Brain researchers within the Intermountain Neuroimaging Consortium (INC) are located at the University of Colorado Boulder in Boulder, CO and Georgia State University (GSU) in Atlanta, GA. COINS, a common tool utilized by researchers at INC, is a Neuroinformatics website used to register and store MR data. The COINS web service employs the highest security for registering participants, and requires each researcher to gain permission before being allowed access to COINS. Each researcher is given a portal where they may register participants for their own studies. Each researcher and/or their staff will be responsible for registering participants in COINS. Unique research subject identifiers (URSIs) are created for each participant in order to de-identify their imaging and research data according to good clinical practice. The URSIs are generated via the COINS database. Every participant is given an URSI, and all MR data are stored at INC and GSU using the URSI. Participants are asked to register for the MRI with personal information including their names, birthdate, phone number and address. This information is necessary in cases of incidental findings where GSU radiological review requires that the participant be contacted. GSU will maintain information associated with the URSI but not the scanning data (e.g., participant names and contact information) in a highly secure cloud computing service such as Amazon Web Services.

CU Boulder and GSU are currently involved in the storage and security of imaging data. Research staff at GSU will not be engaged in the research (they will not be interacting or intervening with subjects, nor will they obtain subject's private, identifiable data). Staff at GSU may be involved in helping researchers interpret the data collected during the protocol, but will not receive personal or identifiable data about the subjects. De-identified data will be managed through the COINS system. Access to personal information is restricted to PIs and project staff for the specific study in which the participant is enrolled and senior GSU and INC staff (for cases of incidental findings where a radiological review and letter to the participant would be necessary).

***Microbiome and Alcohol Data Sharing*:**

We plan to generate approximately 150 human gut microbiota metagenomes (i.e., microbiome). Per NIH policy of Genomic Data Sharing for this number of samples of non-human genetic data generated, data will be broadly shared for participants who consent. Specifically, de-identified gut metagenome data and phenotype data for consenting participants will be shared through GenBank, an NIH genetic sequence database at the National Center for Biotechnology Information that provides an annotated collection of publicly available DNA sequences. Data submission to GenBank will include individual-level microbiome data and phenotypic data related to alcohol use and depression. Separate signatures will be obtained for permission to broadly share these data.

XIV. WITHDRAWAL OF PARTICIPANTS

Situations in which the entire study may be terminated early include the following: If the Principal Investigator or other governing official discovers serious concerns about subjects' safety, inadequate performance or rate of enrollment (this includes a missed study session); because study objectives have been obtained according to pre-established statistical guidelines; or in the unlikely event that the Principal Investigator retires and no other additional investigators are able to succeed his role within the research project.

Though highly unlikely, the circumstances under which a participant would be withdrawn without his or her consent include: obviously not following instructions or behavior that is verbally or physically abusive towards research staff. Those who experience early withdrawal will receive prorated payment based on the number of sessions they completed.

XV. RISKS TO PARTICIPANTS

Risks Associated with Venipuncture.

There is a small risk of local hematoma, infection, and syncope associated with phlebotomy.

Risks Pertaining to the Collection of Genetic Material.

The collection of genetic material (i.e. from the blood) entails risks to confidentiality. Ethical guidelines for the collection and use of genetic material continue to develop; however, set guidelines for these purposes have yet to be officially set forth. The collection of genetic material has become common-place within several research disciplines, and entails only minimal risk to study participants.

Risks Pertaining to Liver Function Panel Tests.

There is a risk that the liver function panel test will reveal a result concerning an individual research participant beyond the scope of the study protocol due to a critical value level obtained from the blood work. In the event of Boulder Community Hospital Reference Laboratory obtaining a critical value level result, personnel from BCH will contact Dr. Kent Hutchison with the subject number and test results. Dr. Kent Hutchison will then share this information with the participant, which will likely be distressing to them.

Risks Pertaining to fMRI Scans.

The MRI scanning procedure in a 3 Tesla system has been approved by the FDA and is safe for use in healthy human populations and there are no known risks associated with the visual or auditory stimuli presented during recording of functional MRI. There are however, several risks both physical and psychological, involved in the use of fMRI. These shall be explained to the participants during screening and consent processes:

Physical Risks and Discomforts.

- The magnetic field of the MR environment has the potential to cause burns or bodily injury if ferrous metal objects are implanted in the body, or if personal articles containing ferrous material are brought into the environment.
- Participants who could suffer potential risks include those with metal implants (pacemaker, metal rods, bone screws, orthodontics, metal flakes from metalworking).

- Participants who might be pregnant may be at risk during the MRI procedure. The risk of MRI to pregnant women and fetuses is currently unknown.
- Participants have the potential to experience discomfort due to scanner noise.
- Participants will be told that they may experience discomfort from lying in one position for a long time. The discomfort should subside once the scan is complete, but some subjects may be sore for a more extended duration.
- Participants will be informed about peripheral nerve stimulation (PNS/tingling) that they may experience during the scan. At sufficient exposure levels, peripheral nerve stimulation is perceptible as "tingling" or "tapping" sensations.

Psychological Risks and Discomforts.

- There is some risk of feeling nervous or claustrophobic while participants are in the scanner.
- There is a risk that the image will reveal an observation concerning an individual research participant that has potential clinical importance but is beyond the aims of this protocol. In the event of the confirmation of a significant anomaly in a participant's brain image, this information will likely be distressing to the participant.

Exposure to Alcohol.

During the fMRI scan at baseline and the second follow-up visit, participants are exposed to less than a teaspoon of an alcoholic beverage, there is a risk that participants may experience an urge to drink or it might cause cravings or anxiety. This risk is particularly relevant during the second MRI scan because many patients may have established abstinence or reduced their alcohol consumption substantially.

Risks Pertaining to Loss of Confidentiality and Privacy.

Confidentiality of participants is a priority for research staff and must be maintained unless the investigator obtains the express permission of the subject to do otherwise. Risks from breach of confidentiality include invasion of privacy, as well as social and economic risks. Economic risks include alterations in relationships with others that are to the disadvantage of the subject, and may involve embarrassment, loss of respect of others, labeling with negative consequences, or diminishing the subject's opportunities and status in relation to others. These risks include payment by subjects for procedures, loss of wages or income, and/or damage to employability or insurability.

Participants will be asked about illegal activities that they may have been involved in (i.e. illicit drug use). Participants will also be warned that there are some things that they might tell us that we CANNOT promise to keep confidential. Participants will be informed that we are required to report information like child abuse or neglect, crimes that they tell us they or others plan to commit, or harm planned against yourself or others.

Self-report data will be collected via individual laptop computers. Theft of the study computers is also a possibility, but an extremely unlikely risk.

Participants must be 21 years of age in order to participate in the study. For this reason, participants' driver's licenses will be checked at the beginning of the first study session to ensure that they are 21 years of age. In order to protect confidentiality, a copy of the license will not be retained.

Unanticipated risks.

Any experiment may involve risks that cannot be anticipated. If unanticipated risks occur, the investigators will follow the IRB guidelines for adverse event reporting the risks, discomforts, hazards

or inconveniences to the participants.

XVI. MANAGEMENT OF RISKS

Participants are informed of all procedures beforehand, and they must read the informed consent form and sign the consent form stating that they understand and agree to the procedures before beginning the study.

Protection against risks associated with Venipuncture.

The risks of hematoma and infection are minimized by having trained phlebotomist perform all blood draws along with using standard and sterile phlebotomy techniques.

Risks Pertaining to the Collection of Genetic Material.

The collection of genetic material has become commonplace within several research disciplines, and entails only minimal risk to study participants. All genetic material will be coded and stored with a randomly generated number. The master list linking the numbers to participants' names will be stored on a password-protected server. At study closure, all links between participant name and URSI will be destroyed, at which point the specimen/data will be considered de-identified. Participants will be informed, both verbally and in writing upon initial consent, of the risks of genetic research.

Risks Pertaining to Liver Function Panel Tests.

In the unlikely event of a critical value level test result, BCH will inform Dr. Kent Hutchison of the participant's randomly generated participant number and abnormal test result. Dr. Hutchison will immediately inform the participant of the test results and share a copy of the results for the participant to give to their primary care physician or a referred specialist. Participants will be informed of this risk associated with the liver function test during the consenting process and made aware that we will be unable to clinically interpret the results past informing them that they indicate critical value levels.

Risks Pertaining to fMRI Scans.

This protocol will be performed using an MR scanner employing pulse sequences and hardware that have been approved by the FDA for human clinical use. The field strength is 3 Tesla and all relevant operating characteristics (RF power deposition, rate of change of the field gradients, coil design) fall within the limits of FDA guidelines for NMR exposure.

In the case of an anomalous finding in a brain image, the following procedure is followed:

1. The technologist and/or research personnel flag potential abnormalities.
2. The MRI technologist notifies the INC Director of Operations, appropriate Georgia State University (GSU) personnel, and the P.I.
3. The scan gets queued to the radiologist worklist in COINS. All cases of suspected incidental findings are sent for formal neuroradiologic review at GSU.
4. The radiology review contains a written summary of the findings and classifies the referral status into one of these categories:
    - There is not enough information from the MRI scan to complete a full review. No obvious abnormalities found.
    - MRI shows nothing obvious that needs medical attention.

- MRI shows something that may or may not be of medical concern. Participants should consider discussing the enclosed report with their doctor.
- MRI shows something that needs to be brought to the attention of your doctor. Participants may also be contacted by the study team and/or appropriate GSU personnel about this report.

5. The PI will get an electronic copy of the radiology review (coded via URSI) as soon as the review is completed. If an urgent referral is recommended, the PI should discuss the review with the Medical Director prior to contacting the participant.
6. If a referral is recommended, the PI will contact the participant and explain that an unusual feature was observed in their scan. The PI provides the contact information for the Medical Director who reviewed the image (this information is in the letter mailed to the participant as well). Routine referrals are handled on a case by case basis and up to the PI/Medical Director to determine if the participant should receive a call in advance.
7. All cases reviewed will generate a formal radiology report, which is printed on letterhead and a copy of which is mailed to the participant. In the case of an urgent referral, someone from the study team or the Medical Director will contact the participant prior to the letter being mailed

Physical Risks and Discomforts.

- Participants who could suffer potential risks include those with metal implants (pacemaker, metal rods, bone screws, orthodontics, metal flakes from metalworking). Participants are carefully screened for these types of metal implants or any other bodily exposure to metal during informed consent, and again prior to the MR scan, using an MRI safety screening checklist. It will be clearly explained to participants that the magnetic field of the MR environment has the potential to cause burns or bodily injury if ferrous metal objects are implanted in the body, or if personal articles containing ferrous material are brought into the environment. MR Facility rules strictly forbid staff from entering the magnet room carrying metal objects.

- Female participants will be warned that the risk of MRI to pregnant women and fetuses is currently unknown. Study staff will explicitly inform all female participants of this fact and they will be given the option to complete a free urine pregnancy test. Female subjects who test positive will not be allowed to participate in the study. The pregnancy test will be strongly recommended by the study staff. If the participant wishes to dismiss the request to complete a urine pregnancy test, they must sign an agreement to acknowledge the risk and indicate that they do not believe they are currently pregnant. This will be required immediately prior to the MRI scan.

- Participants will be informed about potential discomfort they may experience due to scanner noise. This risk will be minimized by providing participants with earplugs and headphones. Ultimately, the volume of the noise is not great enough to pose a health risk, with or without earplugs/headphones.

- Participants will be told that they may experience discomfort from lying in one position for a long time. The discomfort should subside once the scan is complete, but some subjects may be sore for a more extended duration.

- Participants will be informed about peripheral nerve stimulation (PNS/tingling) that they may experience during the scan. At sufficient exposure levels, peripheral nerve stimulation is perceptible as "tingling" or "tapping" sensations. It will be made clear to participants that PNS symptoms usually subside shortly after the scan is completed. In addition, participants are given a squeeze ball to use in case of an emergency. They are informed that if they experience PNS related sensations or are otherwise uncomfortable, they can alert the MRI technologist via the squeeze ball and the technologist will stop the scan immediately.

Psychological Risks and Discomforts.

- There is some risk of feeling nervous or claustrophobic while participants are in the scanner. This risk will be minimized by (1) including exclusion criterion for claustrophobia in the phone pre-screen, (2) informing participants before they enter the scanning room that they may stop the scan at any time, (3) providing participants ear protection with headphones, a mirror to see out, a button to signal distress, and an intercom and (4) making sure the subject is lying comfortably with head and neck supported. Scan time will be kept to a minimum.

- There are psychological risks associated with potential incidental findings that may show up in the scan images. For example, there is a risk that an image may reveal an observation concerning an individual research participant that has potential clinical importance but is beyond the aims of the study. In the event of the confirmation of a significant anomaly in a participant's brain image, this information will likely be distressing to the subject and constitutes a psychological risk.

Exposure to Alcohol.

During the fMRI session, participants may experience a reaction to the alcohol presented to them during the scan. Such a reaction might manifest as craving or anxiety. These effects are almost always only temporary. We have used the same cue exposure task in over 600 participants; many of who were treatment-seeking patients who had established some measure of abstinence (required to be in our studies) prior to scanning. We have not observed any adverse effects from our cue exposure protocol in these individuals. It is important to understand that the entire task uses less than 1 tsp of an alcohol beverage. The consent form clearly notes that the cue exposure session takes place at baseline and at the second follow-up session (week-32) and clearly notes that patients can opt out of the cue exposure part of the study. The patients will be reminded again about the option to opt out of the MR scan. As we have done previously, we will conduct an assessment with each participant after the cue exposure session to determine whether there is any residual craving or anxiety. We also allow each patient to process his or her reaction to the alcohol cues with a therapist before leaving the experiment. Thus, the therapist and study staff will know immediately if there is an adverse reaction to the cues. The therapist will address any adverse reactions to the cues at that time. In addition, therapists will now see all patients for an additional week of therapy. Thus, a plan is also in place for follow-up with these patients. During this session at week-33, the therapist will assess whether there were any adverse events, including a lapse that may have resulted from the cues. Any adverse events will be addressed in the session and reported to the PI. These procedures ensure that the patient does not leave the lab in a vulnerable state. If more than five individuals during the entire trial report having a lapse within 48 hours of the cue exposure session, we will discontinue the cue exposure assessment.

Risks Pertaining to Loss of Confidentiality and Privacy.

Identifying information will be collected only for the purpose of contacting participants for their longitudinal follow-up assessments. All study computers are password protected and housed in the PI's lab space at either Muenzinger Hall or the CINC, which are both kept locked unless researchers or students are currently using the space. Further, there is no identifying information contained on the laptops. All identifying information (e.g., consent forms, contact information) is kept separate and secure from the data files and never on the same laptop and this information will be destroyed immediately after the last follow-up.

Participants will be informed that we are required to report information like child abuse or neglect, crimes that they tell us they or others plan to commit, or harm planned against yourself or others.

XVII. POTENTIAL BENEFITS

These studies are expected to add to the knowledge base of information on the development and treatment of alcohol use disorder. Given only a slight risk to participants and the greater possibility of long-term benefits to the knowledge base and to treatment interventions for alcoholics, the risks/benefits ratio seems reasonable. As a part of this study, all participants will have the opportunity to examine their own alcohol use behavior in the context of completing measurement instruments, and will have the opportunity to work with a trained therapist over the course of the trial. Participants will also receive the added benefit of a treatment (either MBRP or RP) that has demonstrated efficacy in the treatment of AUD. The risks associated with participating have been minimized via the procedures described above. Finally, a demonstration that MBRP is superior to RP and the identification of the mechanisms of action has great potential to benefit others.

XVIII. PROVISIONS TO MONITOR THE DATA FOR THE SAFETY OF PARTICIPANTS

We have taken a number of measures to ensure the confidentiality of the data and the safety of the participants. All data from the proposed study will be identified by a numerical ID code only. The information linking the numerical ID code to identifying information will be maintained separate and secure from the data themselves. At the conclusion of the final follow-up data collection, the list linking the ID code to identifying information will be destroyed.

It will be the responsibility of the PI to obtain and maintain approval of all procedures from the Internal Review Board (IRB). Study staff will report to the PI on a weekly basis regarding the procedures being employed in the protocol to assure that all IRB-approved procedures are being followed. Any adverse events will be reported to the PI immediately. The PI will immediately call the IRB to inform the secretary and the committee of the adverse event. The PI will submit a detailed description using the HRP-214 New Information Form in eRA to report all adverse events consistent instructions of CU Boulder IRB's List of Standard Operating Procedures document: (http://www.colorado.edu/vcr/sites/default/files/IRB-Policies-and-Procedures_05012013.pdf). Consistent with IRB policy, the reporting will occur immediately (within 24 hours) upon learning of a study-related death, study personnel will notify the IRB via e-mail by providing a brief summary of the event. Then, within 1 week (five business days), the PI or designee will submit a Reportable Event in eRA. (2) For any other problem or event requiring reporting to the IRB, the PI or designee will submit to the IRB a Reportable Event or Deviation in eRA as soon as possible, but no later than 10 working days from notification of event.

In addition, the proposed study will form and utilize a Data Safety and Monitoring Board (DSMB) at the University of Colorado. This DSMB is will consist of individuals with broad range of clinical and research experience. The DSMB members will include a psychiatrist (e.g., Dr. Chris Schenck) a clinical psychologist working in a non-profit addiction center (e.g., Dr. Annie Peters), and individual with extensive experience in research ethics who is also a member of the IRB (e.g., Dr. Thomas Kuntsman). The DSMB is intended to ensure the safety of our research participants, the validity of the data, and the appropriate termination of the proposed study based on any emergent significant benefits or risks. For these reviews the DSMB will review any adverse events and any safety differences that are emerging between the randomized arms at intervals of every three months. To facilitate this review, the PI will provide the DSMB with the data related to safety and efficacy. If the DSMB determines that there are significant emergent risks, the DSMB will make a recommendation to the PI and IRB that the study be closed for accrual of participants until a more detailed review can be conducted in conjunction with the PI and IRB. Ultimately, the study may be terminated depending on the outcome of this review.

The MRI technologists are not trained to identify potentially significant clinical anomalies in the brain images. Should the MRI technologist notice something he or she believes to be a potential anomaly in a brain image, he or she will follow the procedure noted in section XVII to ensure appropriate radiological review. The participant will be contacted if the radiologist recommends a scan to determine the clinical significance of any anomaly.

Lastly, a potential risk that the study team will be prepared to manage is participant endorsement of thoughts of suicide on the Beck Depression Invention (BDI-II). The BDI-II is used to measure depression symptomatology. Item number 9 from the BDI-II inquires about "suicidal thoughts or wishes" that participants may or may not be experiencing. In order to ensure the safety of our participants, a staff member will check the participant's response to item number 9 of the BDI-II before the participants leaves the building. If a participant has either responded "I would like to kill myself" or "I would kill myself if I had the chance," the research team member will immediately notify Dr. Hutchison (a licensed clinical psychologist). Dr. Hutchison or a master's-level clinician will immediately assess the participant for imminent suicide risk and provide him/her with a list of psychological services referral contacts. After the participant has been assessed for risk, the study coordinator will use the CU CHANGE lab's previously IRB approved 'Suicide Ideation Protocol' to connect the participant with an appropriate referral resource (i.e., 911 if in imminent danger, Boulder County Mental Health Center (BCMHC) if the participant is a community member, or Wardenburg Health Center and/or BCMHC if the participant is a CU Boulder student, staff, or faculty member). Dr. Hutchison himself or a research team member will follow up with Dr. Hutchison to ensure that all of the necessary measures had been taken to protect the safety of the participant and determine whether or not it is safe for the participant to continue their involvement in the study. If it is determined that the participant is at imminent risk and should not continue participating in the study, Dr. Hutchison will contact the participant and explain that for their safety, the research team does not feel that it is safe for him/her to continue participating in the study and will also answer any questions the participant may have about this decision.

XIX. PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

Fully informed consent will be sought to ensure that participants are aware of any possible risks. Subjects will be aware of all study procedures, measures, and data being collected throughout the

study. Data and observations will not be collected or obtained without their knowledge or consent. Participation in the research is completely voluntary, as is answering each particular question in all of the measures and providing each physiological measure.

XX. MEDICAL CARE AND COMPENSATION FOR INJURY

Participants will be informed to contact Dr. Hutchison immediately by phone (303-735-1304) should they feel that they have been harmed while participating in this study. They will be told that the cost for any treatment will be billed to them or their medical or hospital insurance. Information regarding compensation for injury is included in the informed consent document.

XXI. COST TO PARTICIPANTS

There are no costs to the participant for participation in this study aside from those accrued from transportation to and from study appointments. Participants will be compensated throughout the study, with a total of $280 if they complete all sessions. For those subjects who are eligible and selected to participate in the MRI scan portion of the study, they will be compensated an addition $60 ($30 per scan) with a total of $340 if they complete all sessions. At each session they will be compensated a given amount (described in IX.) that should cover all travel costs and parking is free at the CINC.

XXII. DRUG ADMINISTRATION

Not Applicable.

XXIII. INVESTIGATIONAL DEVICES

Not applicable.

XXIV. MULTI-SITE STUDIES

Not applicable.

XXV. SHARING OF RESULTS WITH PARTICIPANTS

There are no plans to share results of the study with individual participants.

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