INIA Stress and Chronic Alcohol Interactions: Glucocorticoid Antagonists in Heavy Drinkers

NCT02989662

3/17/2021

Date: March 17, 2021
Principal Investigator: Mary E. McCaul
Application Number: IRB00138426

JHM IRB - eForm A – Protocol

- **Use the section headings to write the JHM IRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.**
- **When submitting JHM IRB eForm A (new or revised), enter the date submitted to the field at the top of JHM IRB eForm A.**

****

1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Alcohol use disorders affect millions of Americans, increasing morbidity and mortality with substantial negative health, social and economic impact. Despite decades of research, there are still only 3 FDA approved medications for alcohol use disorder (AUD), all of which have very modest effects with a high prevalence of relapse post-treatment. Clearly there is urgent need for identification of treatment medications that target novel mechanisms of alcohol action.

In preclinical and clinical models, stress plays a central role in initiation, maintenance and relapse to alcohol use. The HPA axis is one of the main stress response pathways. Like stress, alcohol activates the HPA axis to release glucocorticoids in humans and laboratory animals. Glucocorticoids impact drug use behaviors through at least three mechanisms: amplifying the mesolimbic dopamine reward signal, increasing the stress peptide CRF causing anxiety and dysphoria, and increasing habit formation circuits.

Mifepristone (MIFE), a competitive antagonist at the glucocorticoid receptor (GR), is FDA approved or under investigation for treatment of several endocrine and psychiatric disorders. Preclinical studies have shown that MIFE attenuates alcohol-induced mesolimbic DA neurotransmission in mice and reduces voluntary alcohol intake in rats. In alcohol dependent rodents, MIFE blocks the escalation of alcohol intake and compulsive drug-seeking responses during abstinence; it also reduces alcohol-withdrawal induced hyper-excitability and memory deficits. There is a single human study of MIFE for AUD treatment, showing MIFE reduces alcohol cue-induced craving in alcohol dependent subjects. Over time, MIFE should permit "healing" of dopaminergic neurotransmission and other drug-seeking circuitry dysregulated from excessive exposure to cortisol.

We are proposing a Phase I study of MIFE that examines safety, drug-drug interactions, and preliminary evidence of efficacy in reduction of alcohol effects. We will compare the effects of active and placebo MIFE on: 1) fMRI measures of functional connectivity (FC) and alcohol cue-induced brain activation (CA) focused on brain reward and stress pathways, 2) alcohol craving and withdrawal symptoms during alcohol abstinence on the CRU; 3) stress-induced alcohol motivated responding, and 4) alcohol sensitivity/reward. Also, using a within-subject design, we will assess changes in fMRI before and after MIFE administration. Subjects will receive up to 1200 mg MIFE or matching placebo daily over a 6 day period. Age-matched healthy control subjects will also undergo MIFE dosing over a 4 day period and fMRI procedures on an outpatient basis. We predict that MIFE treatment will decrease alcohol withdrawal

symptoms, alcohol motivated responding following stress and subjective experiences of high and positive alcohol effects compared to placebo.

2. Objectives (include all primary and secondary objectives)

Primary aim: The primary study objective is to determine MIFE effects in AUD and HC persons on a variety of alcohol-related measures. We will examine if there are significant interactions between MIFE treatment concurrent with alcohol administration by measuring subjective responses, adverse events and cardiovascular responses (heart rate (HR), blood pressure (BP)), compared to placebo. We also will examine MIFE effects on fMRI, alcohol withdrawal symptoms and craving, and alcohol motivated responding.

Specific aims are to compare GR antagonism versus placebo for:

1. Changes in resting state FC and CA across key brain regions, focused on nucleus accumbens, amygdala and prefrontal cortex. Hypothesis 1a. GR antagonism will normalize the AUD resting state FC and CA to more closely resemble HC scans. Hypothesis 1b. Magnitude of FC and CA normalization induced by GR antagonism will predict the magnitude of reductions in self-reported CRU alcohol craving and withdrawal in AUD subjects. Hypothesis 1c: The extent of FC and CA dysregulation on day 1 is related to alcohol exposure history.
2. Reduction of alcohol withdrawal symptoms and craving and improvement in low mood during early alcohol abstinence. Hypothesis 2a: GR antagonism will decrease alcohol withdrawal symptoms and craving compared to placebo. Hypothesis 2b: GR antagonism will improve negative mood relative to placebo.
3. Reduction of stress-induced alcohol motivated responding. Hypothesis 3a: Compared to placebo, GR antagonism will decrease alcohol craving and the number of earned drinks following social stress. Hypothesis 3b. The magnitude of FC and CA changes induced by GR antagonism will predict number of drinks earned following stress.
4. Reduction of alcohol sensitivity and reward. Hypothesis 4a: Compared to placebo, GR antagonism will reduce the positive subjective responses and attenuate alcohol-induced increases in heart rate and skin temperature following alcohol administration. Hypothesis 4b: Magnitude of FC and CA changes induced by GR antagonism will predict intensity of self-reported alcohol subjective and physiological effects.
5. Side-effects will be closely monitored in both AUD and HC participants.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Mechanisms through which cortisol may influence drug related behaviors.

DA Reward circuitry. Glucocorticoid receptors (GRs) are abundantly expressed in mesolimbic dopaminergic neurons of the reward system (Harfstrand et al., 1986; Morimoto et al., 1996). Plasma glucocorticoids levels are positively associated with the magnitude of dopamine release (DAR) (Oswald et al., 2005; Wand et al., 2007; Niwa et al., 2013) and modulate postsynaptic DA receptors as well as increase synaptic strength in midbrain DA neurons. Rodent studies have shown that cortisol, through the epigenetic mechanism of cytosine methylation, increases tyrosine hydroxylase expression in the mesolimbic system resulting in robust dopamine release (Niwa et al., 2013). Rodent studies have also demonstrated that administration of CORT increases the reinforcing effects of many abused drugs by acting on the mesolimbic DA system (Deroche et al., 1995; Piazza and Le Moal, 1996;

Cho and Little, 1999; Barrot et al., 2000). Thus, medications that would either reduce cortisol levels or block cortisol actions should attenuate DAR, reduce the positive subjective effects of acute alcohol ingestion, and alleviate negative mood and craving during early alcohol abstinence.

Extrahypothalamic stress systems. Second, CORT increases the expression of extra-hypothalamic sources of CRF and thereby promotes stress-induced relapse (Schulkin et al., 1998; Shepard et al., 2000). This circuitry is hypothesized to play an important role in mediating the relationship between stress and reward dysfunction, and to produce negative emotional states that motivate habitual drug-seeking (Gray and Bingaman, 1996; Makino et al., 2002; Koob, 2009; Haass-Koffler and Bartlett, 2012). Up regulation of CRF by CORT contributes to anxiety-like behaviors and enhanced or exaggerated emotional and fearful reactivity to perceived stress; in turn, anxiety and negative emotional states facilitate the initiation, maintenance and relapse back to alcohol use (Sinha, 2013). Increasing levels of anxiety and arousal are reported with increasing levels of drug craving as blood drug levels decrease in the addicted person (Foltin and Haney, 2000). MIFE administration has anxiolytic effects in various animal stress models (Korte et al., 1995; Sufka et al., 2009; Jakovcevski et al., 2011). Anxiolytic actions of MIFE may be especially helpful during cessation of alcohol use, when there is heightened nervousness and restlessness and greater sensitivity to stress as a relapse trigger.

Habitual learning. Secretion of CORT in response to acute stress affects learning and memory. CORT has been shown to induce a bias for promoting habit forms of learning and memory in lieu of goal-directed performance (van Stegeren et al., 2010; Smeets, 2011; Everitt and Robbins, 2013). These effects of cortisol on habit-based learning may be a primary basis for the relationship between cortisol, vulnerability to development of alcohol dependence as well as relapse after abstinence. We posit that cortisol blockade will reduce habitual learning related to chronic alcohol use and help extinguish drug seeking behaviors.

Potential Efficacy of MIFE for Alcohol Treatment.

Alcohol Preclinical Research. Support for the potential of MIFE for treating AUD comes from preclinical studies in rodents. In a rodent model, mifepristone has been shown to normalize DA levels as well as block excessive release of DA after methamphetamine challenge (Niwa et al., 2013), thereby having the potential to attenuate the reinforcing effects of alcohol and other drugs of abuse. Relatedly, MIFE has been shown to reduce voluntary alcohol ingestion (Koenig and Olive, 2004), and block the development of ethanol-induced conditioned place preference, a measure of alcohol reward, as well as associated adaptations in DA D2 receptors in the frontal cortex (Rotter et al., 2012). MIFE also attenuates stress-induced and alcohol-induced motor sensitization (Roberts et al., 1995). Rats exposed to chronic intermittent alcohol vapor develop physical dependence, escalate voluntary alcohol intake, and show persistent alcohol seeking responses during protracted abstinence; these effects are blocked by MIFE (Richardson et al., 2008; Vendruscolo et al., 2012; Silva and Madeira, 2012). Likewise, MIFE reduces the severity of alcohol withdrawal (Vendruscolo et al., 2012; Sharrett-Field et al., 2013) and prevents alcohol-withdrawal induced hyper-excitability and memory deficits (Jacquot et al., 2008). MIFE administration into the central amygdala suppresses yohimbine-induced reinstatement of alcohol-seeking (Simms et al., 2012). MIFE also has anxiolytic effects in various animals models of stress-induced anxiety (Korte et al., 1995; Sufka et al., 2009; Jakovcevski et al., 2011). Thus, MIFE treatment during early abstinence may reduce anxiety, craving and alcohol-seeking behaviors that are associated with altered HPA axis activity and relapse to heavy drinking.

Alcohol Clinical Research. In humans, MIFE is used medically as an emergency contraceptive, for the treatment of endometriosis and progesterone sensitive tumors. More

recently MIFE (300-1200 mg) was approved for treatment of Cushing's syndrome, a serious endocrine disorder caused by endogenous hypercortisolism (DeBattista and Belanoff, 2006). MIFE doses of 300-1200 mg/day have also been effective in clinical trials of other disorders associated with cortisol dysregulation including psychotic depression and bipolar depression (Blasey et al., 2011; Watson et al., 2012). We are aware of only one randomized placebo-controlled clinical trial of 7-day 600 mg/day MIFE treatment in non-treatment seeking alcohol dependent subjects (ClinicalTrials.gov identifier: NCT01548417). A meeting abstract of this ongoing study reported that MIFE reduced alcohol cue-induced craving (Mason, 2012).

In addition to examining the behavioral and psychopharmacologic effects of GR antagonism, we will measure functional connectivity (FC) and alcohol cue-induced brain activation (CA) in the presence and absence of GR blockade in both AUD and HC participants. Resting-state FC research has identified a number of networks including those associated with the sensory/motor system, executive functioning as well as visual and auditory processing (Khanna et al., 2015). Although agnostic regarding direction of connections, FC can identify neural systems that are coupled and share functional properties as measured by a positive or negative correlation in the resting state (rs) BOLD signal over time between the regions.

Measurements at rest and during alcohol cues have already advanced our understanding of the neural correlates of alcohol craving and relapse risk. Han and co-workers (2015) showed that AUD subjects have positive FC between the dorsolateral prefrontal cortex (DLPFC), cingulate, and cerebellum, and negative FC between the DLPFC and the orbitofrontal cortex. In this study, AUD participants also showed positive FC between the DLPFC, temporal lobe and striatal areas. Beck and co-workers (2012) showed that alcohol-related cues elicited increased activation in brain areas associated with attentional bias toward these cues (the ventral tegmental area extending into the subthalamic nucleus and ventral striatum) and that, in patients who remained abstinent, increased activation and connectivity were observed in brain areas associated with processing of salient or aversive stimuli. In a particularly rigorous study, Sinha and co-workers (2013) identified that disrupted vmPFC/ACC function plays a role in relapse. Most recently, the GR gene has been identified as a primary regulator of a network of brain areas involved in alcohol reward, including the mPFC, NAcc, central nucleus of the AM, and ventral tegmental area (Repunte-Canonigo et al., 2015). As we would hypothesize, stress in the absence of alcohol also modulates AM- mPFC conductivity (Quaedflieg et al., 2015). We will extend these finding by measuring resting and CA patterns before and during GR blockade, with a particular focus on NAcc, AM and PFC, (see Fig 3), allowing comparisons between human findings from the proposed study and animal findings from other grants funded as part of the NIAAA INIA consortium. We also will perform exploratory analyses of whole brain functional connectivity. This combination of analyses will provide unprecedented information of how glucocorticoids alter synchronously interacting neural circuitry in persons with AUD and provide exciting new therapeutic targets for AUD medication development.

Thus, there is clear evidence for the role of glucocorticoids in the development and maintenance of alcohol use disorders. To date, there has been very limited research targeting the HPA axis for treatment of this disorder. MIFE is a novel and promising pharmacotherapy.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Study Overview: This is a randomized, double-blind inpatient or outpatient human laboratory study. Procedures will be completed as a 7-day inpatient protocol in AUD subjects (N=75) and a 4-day outpatient protocol in an age and gender-matched comparison group of healthy control

(HC) subjects (N=75). On day 1, AUD subjects will be administered an alcohol beverage to control time from last drink before undergoing the first fMRI session. Two hours after alcohol administration, resting-state and alcohol-cued activity will be measured. Following the fMRI session on day 1, all subjects will be randomized to once-daily MIFE or placebo administration. They will also complete cognitive assessment tasks such as the Wisconsin Card Sorting Task (WCST) and two parts of the Wechsler Adult Intelligence Scale-III (WAIS-III), the digit span and the digit symbol substitution tests. They will receive their first medication dose on day 1. Over the next 3 days, AUD participants are monitored as they go through supervised alcohol withdrawal. On day 4, all subjects will complete a second fMRI session to quantify FC changes as a function of alcohol withdrawal and medication administration across key brain regions recently shown to be under GR regulatory control. They will also repeat cognitive assessment tasks. On day 5, AUD subjects complete the Trier Social Stress Test (TSST) followed by the Alcohol Motivated Response (AMR) procedure, during which subjects work for either their preferred alcohol beverage or money. On day 6, AUD subjects will participate in a cumulative alcohol administration procedure during which subjective and physiological responses to alcohol are repeatedly assessed. AUD subjects are discharged on day 7 after completion of the cognitive assessment tasks and a brief intervention. AUD participants are followed for two weeks post discharge. HC participants are followed for one week post second fMRI.

Table 1. Study Day	**AUD Procedures**	**HC Procedures**
Pre-admission	Assessment, Medical History, Vital Signs, blood work	Assessment, Medical History, Vital Signs, blood work
Day 1	Updated assessment, blood work, urine pregnancy test; alcohol administration and fMRI session 1 MIFE/placebo randomization and dosing; Cognitive Assessment	Updated assessment, blood work, urine pregnancy test; fMRI session 1 MIFE/placebo randomization and dosing; Cognitive Assessment
CRU stay	Days 2-6: Alcohol withdrawal monitoring	
MIFE/placebo dosing	Days 2-6	Days 2-4
Day 4	fMRI session 2; Cognitive Assessment	fMRI session 2; Cognitive Assessment
Day 5	TSST and AMR	
Day 6	Alcohol sensitivity session	
Day 7	Cognitive Assessment; Discharge	
Day 11		OP safety follow-up visit
Day 14	OP safety follow-up visit	
Day 21	OP safety follow-up visit	

All visits will be completed remotely where possible (ex. Assessment and follow-up visits), although many procedures require in person assessment, including an inpatient stay for AUD participants. For all in-person visits, staff will use appropriate PPE (face mask/shield, gowns, gloves) and will practice physical distancing. Participants will be required to wear facemasks and if they do not have one, a facemask will be provided by our staff. Office spaces will be cleaned by sanitizing surfaces between office visits.

Assessment.

All potential subjects will be screened by telephone using a standardized initial questionnaire, which includes demography, drug and alcohol use patterns and associated problems, and a brief personal medical and mental health status. This telephone screen addresses the major inclusion/exclusion criteria for the study and quickly rules out respondents

who disqualify. We typically telephone screen 5 persons for each participant scheduled for in-person assessment.

Subjects who appear eligible based on phone screen will be invited to provide written informed consent. Teleconsent will be used as opposed to in person consenting where possible to reduce unnecessary in person encounters specifically for a consent procedure.

We will be using DocuSign, the Institution's approved and 21 CFR Part 11-compliant software, to obtain a secure electronic signature. We will send the consent to the participant via DocuSign, providing a participant-specific code in advance of sending the document via DocuSign, that will be required when the participant accesses and signs the consent. The consent discussion may take place via phone or video conference (e.g. Zoom). Participants will be given adequate time to consider the research study and ask questions prior to signing the consent form. When ready to sign, the participant will enter their code, verifying that the person signing the consent is the person that we spoke with previously, and sign the consent within the DocuSign system. (Note: For studies requiring multiple signatures, e.g. two parental signatures or if a witness is required, all individuals will receive codes in order to sign). Once the participant has electronically signed, the study team member obtaining informed consent will be notified that the electronic form is ready for his or her signature. Once signing is completed by all parties, both the study team and the participant can download the signed consent as a PDF. The study team will also have access to the audit log and the Certificate of Completion. The study team will load the signed consent into Epic.

In instances where Docusign cannot be executed or the potential participant declines to use it, Teleconsent will still be used as opposed to in person consenting where possible to reduce unnecessary in person encounters specifically for a consent procedure. Participants will be provided with a copy of the Informed Consent prior to the teleconsent meeting either via email, fax, mail or previously provided during an in person visit.

The consent designee must verify the participant physically signed the consent document either by viewing via video conference, obtaining a photo of the signed consent document; or obtaining verbal confirmation from the participant that he/she signed the consent form or agreed to participate electronically. The participant will sign and date/time the informed consent document. The document is then mailed, emailed or faxed to the consent designee. The participant will be asked to return the original signed document on their first in person visit. If the Informed Consent form is mailed to the consent designee by the participant the IRB-approved consent designee will sign the copy, which they possess after the participant has acknowledged signature on their copy. Once the original is received by the consent designee the copies will be attached to make a single document. In all other instances, once received, the IRB-approved consent designee signs, dates/times the informed consent document.

After the Informed Consent process is completed, the IRB approved study team member files the consent document in EPIC, including a note confirming the consent process. The entire consent document is also then filed in the research record.

To the extent possible, assessment instruments will be completed remotely, either by interview with the study coordinator or on paper to be returned with the signed consent form.

Assessment instruments include the following. The MINI International Neuropsychiatric Interview 7.0 (Sheehan et al., 1998) will be used to assess the presence/absence of alcohol or other substance use disorders, as well as mood, anxiety and psychotic disorders to establish

study eligibility. The pattern, amount and route of administration of alcohol use are characterized using the 90-day Time Line Follow Back (TLFB) (Sobell and Sobell, 2003; Robinson et al., 2014). Subjects complete the Alcohol Use Disorders Identification Test (AUDIT) (Bohn et al., 1995) to characterize alcohol-related problems. The Fagerström Test for Nicotine Dependence (FNDT) (Heatherton et al., 1991) characterizes smoking quantity/frequency and severity of nicotine dependence. The Shipley-2 Vocabulary evaluation and or the Rapid Estimate of Adult Literacy in Medicine (REALM Health Literacy Test) will be used to determine reading grade level.

Initial assessment findings will be reviewed and those participants who still appear to be eligible for study participation will be invited to come to the research office for blood work, urine toxicology and a medical history and vital signs. A urine toxicology screen is obtained to check for recent drug use. Women will complete urine pregnancy testing. Subjects will undergo a medical history , and standard laboratory tests (complete blood count, comprehensive metabolic panel) and a 12-lead ECG. In rare cases, MIFE can cause low blood potassium and cardiac changes. We will exclude individuals with potassium levels below the normal range or who have any clinically significant abnormality on the ECG. The ECG and medical records will be reviewed by the study physician (Dr. Wand). Psychiatric and behavioral assessments will be reviewed by Dr. McCaul, Co-PI and a licensed psychologist. We are eliminating all sections of the brief physical examination (PE) from the protocol except vital signs to enhance safety for staff nurse and physician. We have determined that the PE findings have never contributed to ruling out participation by participant. The detailed medical history will be obtained. We expect to assess 3 people for each eligible participant; thus the total assessed sample will be 450 subjects. Because of the risk of confounding by smoking rates and severity among AUD subjects, we will recruit an equal number of HC smokers, matched on Fagerstrom scores.

General CRU procedures:

For AUD subjects, this study will be completed as a 7 day (6 night) inpatient protocol on the Bayview Clinical Research Units (CRU). We will conform with all COVID-related safety procedures implemented by the CRU.

On Day 1 prior to completion of any study procedures, subjects will undergo a urine toxicology for recent alcohol and drug use; a drug positive other than marijuana will result in rescheduling study procedures. Female subjects will complete a urine pregnancy test; positive subjects will be excluded from further study procedures. Vital signs (BP and HR) and laboratory tests (CBC and CMP) will be obtained. Updated assessment measures will include an interim timeline follow-back of alcohol use.

During the Bayview CRU stay, participants will be allowed to smoke; subjects will be smoking abstinent for 2 hours prior to study procedures. They will have the option to receive a transdermal patch (21 mg) while off the CRU to complete study procedures.

Daily at 8:00 pm, participants will complete self-reports of alcohol craving, including Visual Analog Scale for alcohol and tobacco craving (VAS); Penn Alcohol Craving Questionnaire (PACS) (Flannery et al., 1999), Beck Depression Inventory-II (BDI-II), Beck Anxiety Inventory (BAI), Obsessive Compulsive Drinking Scale (OCDS), and Alcohol Urge Questionnaire (AUQ). Nursing staff will complete clinician-administered instruments for alcohol withdrawal assessment (see below). Participants will have PRN access to over the counter medications to treat headache, gastrointestinal upset and body aches.

Each day of the CRU stay, the CRU nurse will administer the Systematic Assessment for Treatment of Emergent Events (SAFTEE) (Johnson et al., 2005). This standardized, structured

instrument is specifically adapted for use in substance use disorder clinical trials, to collect information on incidence and severity of medication side effects and adverse events.

To determine if the degree of elevation of CORT in response to GR blockade by MIFE correlates with any of our outcome measures, each morning upon wakening and at bedtime on the CRU, a salivary cortisol sample is obtained. Baseline samples will be collected on day 1, prior to beginning study medications. Measurement of CORT in saliva is not invasive and multiple samples per day are easily obtained. Unlike serum CORT, salivary CORT is unbound to protein and reflects the physiologically relevant fraction. For each sample, subjects chew a pad for 90 sec, which they then place into a capped salivette. Saliva is harvested by centrifugation and stored in a cryotube at -70 until assayed in duplicate (Diagnostic Systems Laboratories; Webster, Texas). The intra- and inter-assay coefficients of variation are below 5%. As we expect variability in CORT within and across MIFE doses, these data will be included in our analyses.

Because MIFE is occasionally associated with hypokalemia, serum potassium level is obtained before study procedures on day 1 and again on day 7 and at the 1 week follow-up visit. Potassium replacement will be addressed as needed.

Outpatient procedures for HC: Healthy control subjects will complete the study as a 4-day outpatient protocol.

On day 1, the study procedures will be similar to those for inpatient participants. However, the outpatient participants will not receive alcohol prior to the fMRI and will be given take-home mifepristone doses and instructed on at-home medication observation and saliva collection procedures at the end of the day.

On days 2 and 3, participants will be asked to complete a video teleconference with nursing staff or a research coordinator. Video teleconference will be on a HIPAA compliant telemedicine app or website (participant preference) and does not require the participant to enter any potentially identifying information. The participant is free to enter their study ID or a pseudonym to enter the virtual waiting room to connect with the provider. This service uses end-to-end encryption and no identifying information is recorded. On this video teleconference, nursing staff will administer the Systematic Assessment for Treatment of Emergent Events (SAFTEE) (Johnson et al., 2005). This standardized, structured instrument is specifically adapted for use in substance use disorder clinical trials, to collect information on incidence and severity of medication side effects and adverse events. Nursing staff will also administer the adrenal insufficiency questionnaire. Participants will then be observed taking their dose of MIFE.

To determine if the degree of elevation of CORT in response to GR blockade by MIFE correlates with any of our outcome measures, each morning upon wakening and at bedtime participants will be asked to collect a salivary cortisol sample. Baseline samples will be collected on day 1, prior to beginning study medications. Measurement of CORT in saliva is not invasive and multiple samples per day are easily obtained. Unlike serum CORT, salivary CORT is unbound to protein and reflects the physiologically relevant fraction. For each sample, subjects chew a pad for 90 sec, which is then placed into a capped salivette. Saliva is harvested by centrifugation and stored in a cryotube at -70 until assayed in duplicate (Diagnostic Systems Laboratories; Webster, Texas). The intra- and inter-assay coefficients of variation are below 5%. As we expect variability in CORT within and across MIFE doses, these data will be included in our analyses.

Because MIFE is occasionally associated with hypokalemia, serum potassium level is obtained before study procedure at 7:00 am on day 1 and at the 1 week follow-up. Potassium replacement will be addressed as needed. MIFE levels will also be tested at day 4.

On day 4, outpatient HC participants will return to the office in the morning and complete the study procedures.

Alcohol Withdrawal Management:

AUD subjects will complete medically supervised alcohol detoxification as in our earlier studies (McCaul, Wand, Eissenberg et al., 2000; McCaul et al., 2001; Bencherif et al., 2004; Weerts et al., 2008; Weerts et al., 2011; Wand et al., 2013; Weerts et al., 2013). Participants will be required to wear a mask before nurse, staff or housekeeping enter the room. All AUD subjects will receive intravenous D5W NS over the first 12 hours following admission. The Clinical Institute Withdrawal Assessment - Alcohol Revised (CIWA-Ar) (Sullivan et al., 1989), which includes 10-items (nausea/vomiting, tremor, paroxysmal sweats, anxiety, agitation, tactile disturbances, auditory disturbances and visual disturbances), will be completed by CRU nurses every 4 hours while the participant is awake. This well-validated clinical tool will assess alcohol withdrawal symptom severity, and is the gold standard to guide benzodiazepine (BZ) administration during symptom-driven withdrawal management (Saitz et al., 1994). We have successfully studied alcohol withdrawal in our prior research. Scores on the CIWA-Ar are sufficiently elevated that we are able to see reductions in withdrawal severity as a result of research pharmacological intervention. If the CIWA score is ≥12 the subject is given intravenous diazepam (5mg) or lorazepam (1mg). The CIWA score is repeated 1 hour after the first diazepam dose. If the score remains ≥10 then a second intravenous diazepam dose (5mg) or lorazepam dose (1mg) is given. This procedure is repeated one more time and if the CIWA-Ar score remains ≥10 then the participant is transferred to the Bayview ED and terminated from the protocol. Based on our previous study, we anticipate that few subjects will require BZ treatment since we exclude subjects who report a history of withdrawal complications. If systolic blood pressure increases above 180 and/or diastolic blood pressure increases above 105, we may administer atenolol 25 mg po and then titrate as needed. Subjects will be removed from the protocol for seizures, hallucinations, or disorientation or following three doses of diazepam or lorazepam. At that point in time, the physician on call will be contacted. The subject will be transported to the Johns Hopkins Bayview Emergency Department for care. The subject is now terminated from the study.

MIFE Administration:

MIFE has a rapid onset of action and achieves peak plasma levels within 1 - 2 hrs of oral dosing. Peak plasma levels and bioavailability increase as a function of dose but are not directly proportional to dose. However, plasma concentrations of MIFE metabolites do increase in direct proportion to dose; metabolites have antiglucocorticoid activity and contribute to long-acting drug effects (Pomara et al., 2002). MIFE is effective with once-a-day dosing. The half-life is 40 hrs following a single dose and 84.6 hrs following multiple doses. Following discontinuation of MIFE, plasma levels are detectable for up to 2 weeks (Heikinheimo et al., 2003). For this study, we selected to use the high MIFE dose of 1200mg, based on recent clinical trials for other neuropsychiatric disorders associated with HPA-axis dysfunction, and that 1200 mg MIFE has been safely administered over extended periods (MIFE Investigator Brochure, Corcept Therapeutics). This study only exposes subjects to MIFE for up to 6 days. We are not planning to reduce MIFE dose during such a brief treatment period. If a subject experiences significant medication side-effects or any serious adverse event or if the participant declines to continue medication, then we will discontinue that participant from the study. It

should be noted that inpatient subjects are under continuous nursing observation throughout the CRU stay and thus adverse events can be identified and addressed rapidly. HC subjects will be instructed to call the study doctor if they have any adverse events. Subjects will be randomized to active MIFE and placebo on a 2:1 ratio.

In the proposed study, MIFE will be administered under an IND (Wand 124365). MIFE (300 mg) and matching placebo will be provided by Corcept Therapeutics, Menlo Park, CA. We will overencapsulate the medication and placebo as necessary. The Johns Hopkins Bayview Investigational Drug Services (IDS) will maintain the inventory and dispense the medication. MIFE absorption and elimination are not affected by age, body weight, BMI, race or sex (Mifepristone Investigator Brochure, Corcept Therapeutics).

Since MIFE has potent antiprogestational effects, women participants will undergo a urine pregnancy test at the time of the initial alcohol sensitivity and prior to the first MIFE dose. On day 1, participants will be dosed after the fMRI session under nursing observation. On all subsequent days, inpatient participants will be dosed once daily at 9 am under nursing observation and HC outpatient participants will be given take home medication and asked to take the medication under nursing observation via video teleconference. Blood draws for MIFE trough and peak plasma level determinations will be drawn at 15 minutes before dosing and 90 minutes after dosing, respectively. MIFE blood draws will be completed for outpatient HC participants on day 4 only. Subjects can discontinue taking study medication at any time, ending study participation. Discontinued subjects will be asked to complete post-discharge follow up.

fMRI scans for functional connectivity and alcohol cue activation on Days 1 and 4: Both AUD and HC participants undergo fMRI sessions. On day 1, all subjects report to the IPSAR office at 9 am, complete interim assessment procedures, urine drug and pregnancy screens and receive a calorie-controlled breakfast. AUD and HC smokers with tobacco use disorder (TUD) will receive a nicotine patch (21mg). At 10 am on day 1 only, AUD subjects will receive an alcohol drink in a standardized calorie-free mixer targeting a blood alcohol level of 80mg% based on gender and BMI. We have included this drink for several reasons. Most importantly, we want to standardize time from last drink to start of scanning across AUD subjects, since subjects will come for admission with varying abstinence durations. Secondly, we want to minimize the impact of both alcohol intoxication and withdrawal on this initial scan, and so have attempted to time the scan to begin when BAL is at or near 0mg%. Finally, we anticipate that this pre-scan drink will act as a primer for the alcohol cuing procedure employed in the study. At 1 pm, all subjects undergo rs-FC and CA fMRI procedures.

fMRI Scanning procedures: All studies will be conducted at 3T in the F.M.Kirby Research Center for Functional Brain Imaging. We will follow all COVID-related safety procedures implemented by the Kirby Research Center. We will counter-balance for handedness and always use the right hand for responses.

fMRI scanning. fMRI data will be acquired at 3.0 Tesla using gradient-echo EPI BOLD. Typical parameters are: 47 axial slices with TR = 3000 ms; TE = 30 ms; flip angle =85 degrees; field of view = 214 (LR) x 214 (AP) mm2; nominal acquisition voxel size 3 x 3 mm2; matrix size = 72x 72, slice thickness = 3 mm, no slice gap, yielding coverage in the inferior-superior direction of 141 mm. These parameters replicate those used in the MGH-USC Human Connectome Project (www.humanconnectomeproject.org).

In-plane structural scans. Anatomical scans are acquired to overlay functional activation onto anatomical structures. A 3D magnetization-prepared rapid acquisition with a gradient echo (MP-RAGE) sequence is used for this (TR = 8.0 ms, TE = 3.7 ms, Flip = 8 deg, Inter-shot time = 3000 ms, FOV =256 mm, 200 z-phase encoding steps, with a linear profile acquisition order, 1

mm slice thickness, TI = 854 ms, matrix = 256 x 182, rFOV = 78%, 1 average, for a total scan duration of 6 min 25 sec with SENSE reduction factor of 2.)

rs- fMRI Brain Connectivity methods, including pre-processing, artifact detection, physiological noise removal using CompCor methods, and generation of seed-based connectivity maps will be similar to those in our Preliminary Studies, using scan parameters described above.

rs- fMRI analysis. Motion statistics and artifact frequency will be compared to insure that there are no group differences. If necessary, outlier subjects will be removed to achieve equality, or, if this is not possible, motion covariates will be used in analyses. We will determine connectivity using a priori specified seed voxels derived from two sources. The first is from coordinates from previously published studies (Muller-Oehring et al., 2015), or anatomical regions derived from published probability atlases (Hammers et al., 2003; Amunts et al., 2005). Primary regions of interest (ROIs) include ventral striatum/NAcc, mPFC, and central nucleus of the AM (Repunte-Canonigo et al., 2015). Additional seed regions of interest may be identified from group differences observed in the alcohol cue task described below. Connectivity will be measured using the peak cross-correlation between the seed region and other brain regions. These cross correlation results obtained for each subject can then be used in a second-stage analysis for making population inferences and group comparisons. For example, the correlation between the resting-state time series from NAcc and the time series from all other brain regions can be used to create a NAcc correlation volume for each subject. The differences in these correlation volumes before and after drug treatment can be compared for the placebo and drug groups in order to characterize group-dependent differences in altered NAcc connectivity resulting from GR antagonist treatment. We hypothesize that medication groups will exhibit significant differences relative to the AUD placebo group and that fewer differences in connectivity will be observed between HCs and medication groups than between HCs and the AUD placebo group, ie. drug treatments will tend to normalize connectivity toward patterns seen in HCs. In addition to seed-based analyses, we will perform exploratory analyses of whole brain functional connectivity using independent component analyses. Group comparisons will be performed using methods developed and published by our collaborator, Dr. Pekar (Calhoun et al., 2001; Calhoun et al., 2004). Most recently, Dr. Pekar and collaborators (Nebel et al., 2015) have used these methods to identify functional connectivity ‘signatures' associated with individual differences in symptom severity in children with autism.

fMRI during Alcohol-related Cues. Several investigations, including studies conducted by our consultant Dr. Myrick, have found that AUD adults exhibit increased brain activation in response to alcohol-related cues in reward circuitry regions, including ventral striatum, anterior cingulate, and medial prefrontal region, as well as in parietal and temporal regions (Schacht et al., 2013). These changes in brain activation have been shown to be sensitive to medication effects, including aripiprazole and naltrexone (Myrick et al., 2008; Myrick et al., 2010). To promote robust and rigorous conduct of the study, Dr. Myrick has agreed to serve as a consultant for this project and to provide the visual stimuli that were used in his studies to elicit ventral striatal activations.

Alcohol-Cue Methods: As described in reports by Myrick et al. (2004, 2008) six 120-second epochs of stimuli will be presented to subjects during fMRI scanning, using scan parameters described above. Each epoch will contain four 24-second blocks of alcoholic beverage pictures (beer, wine, or liquor), nonalcoholic neutral beverages, visual control pictures, and rest. Each epoch will have a different order of these stimuli and epoch order will be counterbalanced across subjects. Each block will have 5 pictures presented for 4.8 s each. After each block there will be a 6 second period where the subject will indicate the magnitude of alcohol craving rating (ranging from not at all to greatest ever) by pressing one of 4 MRI compatible response buttons. Subjects will be given a sip of their preferred spirits in non-carbonated juice through a straw prior to scanning, as described by Myrick et al. (2010).

Beverage pictures will be obtained from the Normative Appetitive Picture System supplemented by additional pictures from advertisements to prevent picture repetition. Visual control pictures will be scrambled versions of the alcohol pictures. Rest blocks will consist of a crosshair fixation. Visual stimuli will be back-projected to subjects using a MRI-compatible projector. A PC in conjunction with Eprime software will be used to generate visual stimuli and control experimental parameters.

Alcohol-cue fMRI Data Analysis: Standard pre-processing, including timing correction, motion correction, anatomical/functional coregistration, spatial normalization into MNI space (via segmentation), and spatial smoothing, as well as whole brain statistical analyses of fMRI data will be performed using the SPM12 software package. Following pre-processing, parameter estimation involves deriving least square estimates of the fit between regressor(s) and the fMRI timeseries at each voxel. Regressors consist of events of interest (e.g., alcoholic and nonalcoholic picture presentation) convolved with the hemodynamic response function. Population inferences will be performed on contrasts of parameter estimates for each subject (e.g., alcoholic pictures minus nonalcoholic pictures) using a random effects model that is appropriate for population inference. Corrections for multiple voxel comparisons will be accomplished using False Discovery Rate (Genovese et al., 2002) methods. Artifact detection software (Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC)) from head motion will be used to exclude artifactual timepoints and output from this software can be used to test whether there are motion or task-correlated-motion differences between groups. If such differences exist, outlier subjects will be removed to eliminate differences, or motion parameters will be entered as covariates in statistical tests. In addition to whole-brain methods, ROI analyses will be performed to test specific hypotheses of this proposal. Specific ROIs will include the ROIs examined under rs-FC as well as ROIs defined from prior fMRI studies of cue-induced activation (Neto et al., 2008; Myrick et al., 2008; Schacht et al., 2013; Muller-Oehring et al., 2015).

Cognitive Assessment: To study cognitive effects of mifepristone, all subjects will complete the cognitive battery of assessments on days 1 and 4. AUD participants will additionally repeat the cognitive battery on day 7 prior to discharge. The battery will include the Wisconsin Card Sorting Task (WCST) and two parts of the Wechsler Adult Intelligence Scale-III (WAIS-III), the digit span and the digit symbol substitution test (DSST). The WCST asks people to sort cards based on different criteria with the only feedback being whether the classification is correct or not. The classification rule changes over time and this task measures how well people can adapt to the changing rules (Puente, 1985). The WAIS-III digit span task requires subjects to repeat verbatim a string of digits that sequentially increases in length until the consecutive failure of two trials of the same digit span length (Wechsler et al., 1997). The WAIS-III digit symbol substitution test requires the rapid copying of symbols paired with numbers in 90 seconds (Wechsler et al., 1997). All three tests are available for computer administration. Computer tablets will be sanitized immediately after use.

Trier Social Stress Test (TSST) and Alcohol Motivated Responding (AMR) session on Day 5: To examine GR antagonist effects on stress-induced motivation to drink, AUD subjects undergo the TSST followed by an AMR session on day 5. The TSST consists of a public speaking component and a mental arithmetic component (Kirschbaum et al., 1993) and is one of the most validated human stress procedures (Kirschbaum et al., 1993). Confederates who are unknown to the study participants are used to implement the stress components. We engage JHU SOM employees to carry out these procedures since they are available on-site and the task is brief, requiring 10 minutes of their time. We have typically identified confederates through flyers posted in the SOM. No data are collected on the confederates; all data are generated by the study participants. Confederates are not provided with any information on the

identity of the study participants and do not have access to study files or data. Confederates and participants will be required to wear a facemask before, during and after the procedure. The conference room in which the procedure takes place allows for physical distancing.

Immediately following the TSST, subjects begin the AMR session. We have successfully developed an alcohol motivated responding (AMR) model using progressive ratio operant conditioning procedures widely used in preclinical laboratories and adapted for human research. At the start of the session, participants receive a primer drink consisting of one-half of a standard drink of their preferred alcohol. Response requirements (i.e., number of mouse clicks) increase with each earned reinforcer over a total of 10 reinforcement opportunities. During each response set, subjects elect to work for their preferred type of alcohol (0.5 standard drink) or money ($1.00). At baseline and at 10-min intervals during the AMR session, subjects provide a saliva sample for CORT measurement and complete the two-item Tiffany Brief Craving Scale (Tiffany et al., 2000) (How badly would you like an alcoholic drink right now?; Rate your current desire to drink alcohol) on a visual analog scale (VAS) from 0 (not at all) to 10 (extremely). At the end of the 60 min AMR period, participants receive earned drinks and/or vouchers for earned money. AMR sessions last for 60 min or until no response is made for 10 min, whichever comes first. If the session is terminated, subjects do not gain access to alcohol until the end of the 60-min session. The maximum amount of alcohol that can be earned is 5 standard drinks (SD), earned in 10 response sets (0.5 SD each set). Subjects drink their preferred type of alcohol; alcohol volume is standardized to ensure equivalency across subjects for the amount of absolute alcohol that can be earned. Drinking is paced to ensure that subjects cannot exceed 0.5 SD every 5 minutes or the total consumption of all 5 SDs in a 50-minute period. Subjects also are provided their preferred mixer and snacks.

Alcohol Sensitivity Session:

On day 6, AUD subjects will participate in an alcohol sensitivity session to assess subjective and cardiovascular responses to oral alcohol administration after stabilization on MIFE/placebo. On the morning of the procedure, subjects receive a calorie-controlled breakfast and lunch to control for dietary effects on alcohol absorption. We will obtain a blood sample for MIFE plasma level determination prior to the alcohol session.

In this cumulative alcohol dosing procedure, placebo and three active alcohol drinks are administered at timed intervals to increase BAL progressively (0, 0.03, 0.067, 0.10%) during a single session. Alcohol doses for each subject are determined using a Computerized Blood Alcohol Calculator (CBAC©) which adjusts for age, height and gender differences in body water and duration of drink consumption. This allows us to target similar BALs in males and females. Investigational Drug Services prepares each 120 mL drink by mixing the appropriate mL of 95% ethanol and a non-caloric sweetened beverage using a w/v metric. The placebo drink is blinded by floating 1 mL of ethanol on top of the beverage and by placing an ethanol-soaked wristband around the glass to deliver a strong alcohol odor. Active alcohol drinks are prepared in the same manner. Subjects are monitored continuously by staff, who also ensure consumption of each drink is paced over 10 min. Staff will physically distance from the participant during alcohol consumption when participants will have to remove their masks.

Subjects complete a computerized battery of subjective assessments at baseline before alcohol consumption (-45 minutes) and every 45-min thereafter. The battery requires 10 minutes for completion and includes the (1) Drug Effect Visual Analog Scale (VAS) (Uhart et al., 2013) , (2) Brief Craving Scale (Cox et al., 2001), (3) Biphasic Alcohol Effects Scale (BAES) (Martin et al., 1993), (4) Subjective High Assessment Scale (SHAS) (Schuckit, 1980), (5) POMS (D. M. McNair et al., 1992; D. McNair and Heuchert, 2003) Tension Anxiety subscale. At time 0, the placebo drink is administered. Active alcohol drinks are administered at 45 min intervals

(+45, +90 and +135). Breath alcohol levels are determined at baseline (-15) and 15 min after consumption of each drink. Heart rate, skin conductance and skin temperature are recorded continuously using a noninvasive monitor; blood pressure is recorded every 5 min. Participants also complete a brief battery of psychomotor/reaction time tasks to measure level of intoxication.

Periodically throughout the session, we will perform measurements of blood alcohol levels using a handheld breathalyzer device. The technique has the potential to aerosolize water vapor during the participant's exhalation. The staff member running these sessions will be wearing full PPE attire. The staff member will leave the research room each time the participant exhales into the breathalyzer. Participant then removes disposable mouth piece and places it in a basket containing a red bag designed to hold toxic materials. A fresh mouth piece is attached by staff after re-entering procedure room.

After the session, subjects return to the Clinical Research Unit for monitoring and data collection. A staff member must accompany the participant on the car ride from the JHH campus to the Bayview CRU. Staff and participants will use face masks and physically distance to the extent possible during the ride. The subjective assessment battery and an Alcohol Hangover Scale (McCaul, Turkkan et al., 1991a) are completed hourly for 4 hours post session and once the next morning.

We have safely administered alcohol in a range of doses to social drinkers and heavy drinkers (Turkkan et al., 1988; McCaul et al., 1991a; McCaul, Turkkan et al., 1991b). We have demonstrated reliable alcohol dose-related increases in heart rate and subjective effects of alcohol, and these effects are sensitive to medications used for the treatment of alcohol use disorder (e.g., naltrexone, acamprosate) (McCaul et al., 2000; McCaul, Wand, Rohde et al., 2000; McCaul et al., 2001; Brasser et al., 2004). We have used single dose and cumulative alcohol dose procedures in our research. Since it requires multiple sessions to generate full alcohol dose-effect functions when each dose is administered within a single session, we selected the cumulative alcohol dosing procedure for this study as it minimizes subject research burden and reduces dropout rates. In addition, since an alcohol dose effect function is generated in a single session, downward, leftward and rightward dose shifts in response to MIFE treatment can be detected.

As shown in Figure 1, cumulative alcohol dosing procedures generate highly reproducible BALs and subjective effects, with little between-subject variability. Subjective effects track BAL across the ascending and descending limb of the curve. Using this procedure, we have shown that the GABRA2 gene influenced alcohol subjective and physiological effects (Uhart et al., 2013). To date, we have completed this procedure over 150 subjects.

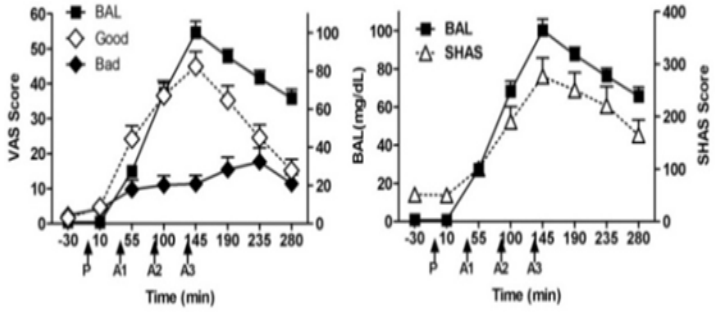

Figure 1. *Time course of BAL and self-rated effects on the VAS and SHAS. Arrows indicate times of placebo*

(P) and each alcohol drink (A1, A2, A3) consumed.

Discharge and Follow-up:

AUD subjects are discharged on day 7 following cognitive assessment and participation in a brief motivational intervention for alcohol use; information on treatment availability will be provided prior to discharge from the CRU. Under the supervision of Dr. McCaul, the intervention is delivered by Master's-level staff following procedures outlined in NIAAA Clinician Guide to Brief Intervention.

All female participants are advised to use nonhormonal birth control (e.g., condoms) for the duration of a one month period following discharge. All male participants will also be advised to use condoms for the duration of the follow-up visits and for at least 5 half-lives of the drug plus 90-days after the last dose of mifepristone. All subjects will be given a supply of condoms.

AUD subjects return weekly for 2 follow-up visits. HC subjects return one week post-second fMRI for a follow-up visit. As described above, MIFE is a long acting compound; plasma levels will likely remain elevated throughout the first two weeks post-discharge at the time of the follow-up visits. At each follow-up visit, participants provide self-reported alcohol use using TLFB procedures for the period since they were last seen, complete self-report measures of alcohol craving (as listed under the inpatient stay), medication side effects (SAFTEE) and adverse events. In addition, we will obtain a blood spot for PEth determination as a biomarker of recent drinking intensity. We also will get a blood sample for MIFE level and, at week 1 follow-up, a sample will be obtained to test for potassium level. Subjects will be reminded of the importance of use of nonhormonal birth control and/or condoms and will be provided with condoms.

b. Study duration and number of study visits required of research participants.

Each subject will participate in an initial assessment, and a possible second assessment visit for completion of medical history, vital signs and laboratory tests. AUD participants will be admitted to the CRU for 6 nights. HC participants, who participate in the study on an outpatient basis, will continue MIFE dosing and salivary sample collection at home. For AUD participants, there are two follow-up visits at 1 and 2 weeks following CRU discharge. For HC participants, there is one follow-up visit at 1 week following the second fMRI. It is anticipated that subjects will complete all study procedures within a 6 month period.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Staff and subjects will be blind to the alcohol and MIFE doses administered during this study to reduce expectancy effects.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

Subjects are not seeking treatment for their AUD; they will not currently be engaged in therapy.

e. Justification for inclusion of a placebo or non-treatment group.

A placebo MIFE dose is included for comparison with the active drug effects and to control for expectancy effects.

f. Definition of treatment failure or participant removal criteria.

A participant will be removed from the study if s/he experiences a moderate or severe adverse event, is noncompliant with study or CRU rules, or if it is determined by the study physician to be unsafe to continue.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

MIFE dosing is discontinued at the time of discharge from the study.

5. Inclusion/Exclusion Criteria

Healthy, non-treatment seeking, 21-60 years old, male and female subjects will be recruited through the media. Recruitment uses a mix of newspaper, radio, and on-line advertisements. Based on the 2011 census, the Baltimore metropolitan area is mostly Caucasian (61%) or African-American (30%); the remaining 9% is Asian (4%) and persons reporting two or more races (5%); 8.8% of the population report Hispanic ethnicity.

This study will include two groups of participants – persons with AUD and healthy controls. AUD subjects will have a current DSM 5 alcohol use disorder and will be actively drinking at least 50% above NIAAA recommended guidelines (women >10 drinks/week and men >20 drinks/week) and at least 5 binge drinking episodes in the past 30 days. Recent heavy drinking will be confirmed by PEth blood spot analysis => 50 ng/mL. HC subjects will not meet DSM 5 criteria for AUD and will not be drinking above NIAAA recommended guidelines as confirmed by PEth blood spot analysis < 50 ng/mL. We expect to consent 450 subjects in order to accrue 150 subjects into the study procedures (N=75 for AUD and N=75 healthy controls). Historically, our retention in the CRU has been outstanding, with over 90% of subjects completing the protocol.

Additional Inclusion Criteria for all subjects:

- Nontreatment seeking volunteers;
- 21 – 60 years old;
- English speaking;
- 5th grade literacy to ensure ability to read study assessment instruments;
- no current serious psychiatric or health problems that would contraindicate study participation;
- BMI > 19 and < 40; medication dosing is not weight-adjusted. We have restricted the weight range of participants to reduce weight-associated variability;
- For women, negative urine pregnancy test prior to initiating MIFE treatment and agreement to use nonhormonal birth control for 2 weeks following last MIFE dose. A medical record of surgical sterilization or elevated FSH level confirming post-menopausal status is required to document non-childbearing potential;
- No adult seizure history;
- No serious alcohol withdrawal complications in prior alcohol withdrawal episodes;

- Some non-drinking days on 90-day TimeLine Followback Drinking Calendar;
- Study Physician and PI recommendation.

Exclusion criteria for all subjects:

- Women on hormonal birth control, or who are pregnant, nursing or planning pregnancy; women with unexplained vaginal bleeding;
- Current DSM 5 mood, anxiety or psychosis diagnosis or currently treated with psychiatric medication;
- Current substance use disorder except alcohol, marijuana (mild) or tobacco; current illicit drug use other than marijuana;
- History of serious alcohol withdrawal symptoms (e.g., seizures, hallucinations) or history of inpatient, medicated alcohol withdrawal management, and CIWA-Ar score at time of assessment ≥ 12;
- Elevation of aspartate aminotransferase or alanine aminotransferase exceeding 5 X the upper limit of normal;
- History of clinically significant ECG abnormality or cardiovascular illness;
- Potassium levels below normal range at screening or CRU admission;
- Use of CYP3a inhibitors in the 3 months prior to study participation, including simvastatin, lovastatin, and CYP3A substrates with narrow therapeutic ranges, such as cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus; itraconazole, nefazodone, ritonavir, nelfinavir, indinavir, atazanavir, amprenavir, fosamprenavir, boceprevir, clarithromycin, conivaptan, lopinavir, posaconazole, saquinavir, telaprevir, telithromycin, or voriconazole; midazolam, triazolam, sildenafil;
- Any illness, condition, or medication use known to interact with MIFE metabolism (as outlined in the FDA package insert) that, in the opinion of the study investigators and the admitting physician, would preclude safe and/or successful study completion;
- History of metal implantation that would preclude MRI scanning, including implants, pumps, pacemakers.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Mifepristone. MIFE has been developed by Corcept Therapeutics and is currently FDA-approved or under investigation for hormone sensitive tumors and various neuropsychiatric disorders. MIFE acts as an antagonist at GR and progesterone receptors (PR) (DeBattista and Belanoff, 2006). We are examining effects of MIFE 1200 mg and matching placebo. This MIFE dose has been safely used over extended administration in humans. In our pilot data, we observed an effect of this dose on subjective responses to alcohol administration. MIFE has demonstrated a strong safety profile in severely medically compromised patients with Cushing Syndrome, suggesting that it will be well tolerated in clinical populations with less morbidity. Indeed, very few adverse experiences have occurred in clinical trials with diverse psychiatric populations, including AUD, bipolar, schizophrenia and dementia patients (Yararbas and Pogun, 2011; Watson et al., 2012; Howland, 2013; Morgan and Laufgraben, 2013; Mason, 2014). At present, the most clinically significant barrier to MIFE use is that it cannot easily be used in women on hormonal birth control.

While this is clearly important clinically, there are several factors that mitigate the concern: 1) at present, there are very few effective AUD medications, so the potential benefit of alcohol cessation for women of child bearing age may outweigh this limitation; 2) the elevated HIV risk of persons with substance use disorders makes condom use a priority in this population, thus nonhormonal birth control methods are advocated; and 3) at present, Corcept is investigating medications without progestational blockade effects; however, these compounds are still under review at the FDA. Importantly, safety findings from the proposed research will be highly applicable to these new compounds and so a proof-of-concept study of MIFE is fully warranted at this time.

Alcohol. One of the aims of the project is to examine MIFE effects on alcohol sensitivity, including subjective intoxication and heart rate. We are using a cumulative dosing procedure for alcohol administration that is based on targeted breath alcohol levels (BrAL). The maximum target BrAL is 0.10 mg/dL; this is achieved by administration of approximately 4 standard drinks over a 90 minute period. This alcohol dose is below the typical amount self-administered by participants but sufficient to produce subjective euphoric and sedating effects.

Alcohol is available at the conclusion of the AMR session. A maximum dose of 5 standard drinks of the participant's preferred type/brand of alcohol is paced at 0.5 standard drinks every 5 minutes to slow intoxication.

Nicotine patch. Active nicotine patch (21mg) is the standard of care for management of nicotine withdrawal.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Mifepristone is FDA-approved to terminate intrauterine pregnancies of up to 49 days gestation. It also has been FDA-approved for treatment of refractory Cushing Syndrome patients. More recently, it has been under investigation for treatment of various neuropsychiatric disorders, including AUD, bipolar, schizophrenia and dementia patients (Yararbas and Pogun, 2011; Watson et al., 2012; Howland, 2013; Morgan and Laufgraben, 2013; Mason, 2014). A small-scale pilot study of MIFE in AUD has been conducted. Additional research on MIFE/alcohol interactions are warranted at this time.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

During the alcohol sensitivity session, subjects will drink 3 doses of alcohol intended to bring their terminal BrAL to 0.10 mg/dL. Also, at the conclusion of the AMR session, participants can consume up to 5 standard drinks of their preferred alcohol type/brand, based on the number of alcohol reinforcers earned during the session. Beverages containing more than 7% alcohol are not regulated by the FDA; they are regulated by the Bureau of Alcohol, Tobacco, Firearms and Explosives. These beverages are legally sold to adults.

7. Study Statistics

a. Primary outcome variables:

- adverse events and side-effects;

- fMRI changes in re-FC and CA across brain regions under GR regulatory control;
- Reduction in TSST-induced alcohol motivation and drinking;
- cardiovascular responses (HR, BP, ECG) during alcohol sensitivity sessions;
- alcohol euphoric and sedating effects during sensitivity sessions using VAS and Adjective Scales.

b. Secondary outcome variables will examine the effects of MIFE on a variety of psychological measures during alcohol abstinence, alcohol sensitivity sessions and outpatient visits. Secondary outcome measures include:
   - Craving for alcohol during the inpatient stay, assessed using VAS, PACS, OCDS, and AUQ;
   - Alcohol withdrawal symptom severity, assessed using the CIWA-Ar;
   - Mood and personality assessments during the inpatient stay (BPRS, BDI, and BAI);
   - Alcohol use during the outpatient follow-up, assessed using timeline followback self-report and PEth levels.

c. Statistical plan including sample size justification and interim data analysis.

Primary and secondary outcome variables will undergo data cleansing and Corrective/Preventive Measures based confirmation of accuracy and missing data. We will perform exploratory data analyses initially (stem-leaf displays, box plots, histograms, and Q-Q plots) and, if necessary, appropriate data transformations will be performed.

Power analyses: All power estimates are based on type I error =0.05, Power > 0.8. Specific aims are to compare GR antagonism versus placebo for:

Aim 1. Changes in rs-FC and CA across key brain regions under GR gene regulatory control. From the data obtained in preliminary fMRI studies (4.1c), we extracted each subject's connectivity measure (r value converted to Fisher Z score) between left NAcc and 6 mm ROI in the right inferior parietal cortex. Mean and SD connectivity measurement of 0.134, .082 were obtained from alcohol subjects and -.058, .062 were obtained for controls. We made the following assumptions: (a) t1 connectivity will be that of Alc mean (.134) for drug and placebo groups; (b) t2 connectivity will not change for placebo group; (c) t2 connectivity for the drug group will normalize by at least one-third toward HC values, ie: t2(expt group) = Alc mean – (Alc mean – HC mean)/3 = 0.07; (d) SD for each cell is the pooled SD (.076); (e) the correlation between repeated measurements (t1 and t2) is 0.5. Using these assumptions a partial eta squared of 0.1563 was calculated for the group x time interaction for a repeated measure ANOVA. Using this estimate with GPower3 (Faul et al., 2007) software we calculated that 25 subjects per group will result in 83% power for detecting a significant interaction at $p < .05$.

Cue-induced Activation: Myrick et al (2008) showed that AUD compared with HC subjects showed increased ventral striatal activation to alcoholic beverage pictures relative to neutral beverage pictures, and that treatment with a combination of naltrexone and ondansetron significantly decreased CA activation. Their paper provided mean and SD information for placebo and drug group ventral striatal activation that was used to estimate effect size for the present proposal for the interaction for a repeated measures anova with between group factor of drug treatment (placebo vs drug) and within group measure of time (baseline and after

treatment). Because this study was performed on a 1.5T scanner, the contrast to noise ratio was multiplied by a factor of 1.3, as described by Hoenig et al.,(2005) to accurately reflect scanning at 3T in the present study. These calculations revealed a partial eta squared value of 0.185, assuming that the drugs in the present study produce at least a comparable effect to the naltrexone/ondansetron treatment. Using this estimate with GPower3 (Faul et al., 2007) software we calculated that 25 subjects per group will result in 90% power for detecting a significant interaction at $p < .05$.

Aim 2. Reduction of alcohol withdrawal symptoms and craving, and improvement in low mood during early alcohol abstinence. Power for this aim was based on Vendruscolo et al (2015) (Fig 2A) in which MIFE effects were examined on VAS craving scores. Our hypotheses test if placebo (size = n) is different from MIFE. Comparing placebo effects (mean=11.5 (SD=2.1) to active drug effects (mean=9.3 (SD=2.2), total sample size required for the proposed aim is 39 (i.e.,20 in each group).

Aim 3. Reduction of stress-induced alcohol motivation and drinking. Power for this aim was based on Vendruscolo et al (2015) (Fig 2B) in which MIFE effects were examined on drinking during an outpatient treatment phase of the study. This calculation is based on between-subject calculation which overestimates the sample size requirement. Comparing placebo effects (mean (SEM) = 38.0 (10.2)) to active drug effects (mean (SD) = 27.5 (10.2)), total sample size required is 32. Using data from our AMR session in which mean number of drinks is 4.33 (sd = 3.17), we are powered to observe a 50% or greater reduction in drinks earned within the session.

Aim 4. Reduction of alcohol reward and sensitivity. Based on our preliminary data (4A1, Aim 3) and a sample of 50 GR antagonist vs 25 placebo subjects, we are powered to detect a difference of 3.0 degrees (3.3%) in peak skin temperature. Further, using data from our current alcohol sensitivity procedure (mean peak VAS total=168 (SD=118); mean peak liking=47.9 (SD=36.9)), we are powered to observe a 45 - 50% or greater reduction in alcohol subjective effects.

Aim 5. Side-effects. Power calculation is based on the assumption that the number of side-effect reports and adverse events follows a Poisson distribution. In preliminary data (Corcept Investigator Brochure), an average of 12 events was reported in MIFE treated subjects. We estimate that 22 subjects in each group will give power > 0.8 for this statistical test.

Statistical Analyses: For aims 1 – 4, AUD and HC subjects will be separately grouped.

Aim 1: CA will be measured from contrasts of parameter estimates derived from event-related analysis. FC will be measured using the peak cross-correlation between seed regions and other ROIs. In both cases, results obtained for each subject can then be used in a second-stage analysis for making population inferences and group comparisons (as was done in Fig 6). For example, the correlation between the resting-state time series from NAcc and the time series from all other brain regions can be used to create a NAcc correlation volume for each subject. Using repeated-measure ANCOVA with between-subject factors of drug group (Placebo, GR antagonist) and alcohol history (AUD, HC) and within-subject factors of time (scan 1, scan 2), differences in these correlation volumes before and after drug treatment can be compared for the medication and AUD vs. HC groups in order to characterize group-dependent differences in altered NAcc connectivity resulting from the medication intervention. Potential confounding factors will be examined and added to the model as covariates if necessary. Multi-linear regression will be used to test the correlation between post-treatment FC and CA with the self-reported alcohol craving and withdrawal measures (VAS, OCDS) in AUD subjects. Finally, we test the correlation between FC and CA dysregulation and alcohol exposure history collected from 90-day Time line follow-back (TLFB). Daily alcohol use data collected from TLFB will be summarized as number of drinking days, number of binge drinking days, and number of drinks

per drinking day for all subjects. Then linear regressions models will be constructed to test the correlation between these drinking measures and FC and CA measures from scan 1.

Aim 2: The main outcomes are CIWA-Ar scores for alcohol withdrawal, VAS and OCDS scores for alcohol craving, and Profile of Mood States 2-Short Version subscale scores. The normality of the measures will be examined first and two sample t-test, or equivalent non-parametric methods will be used to compare outcomes between GR antagonist-treated and placebo-treated groups. We will also use ANCOVA model to adjust for potential confounding factors as covariates.

Aim 3. AMR variables include: total number of alcohol drinks earned; total number of responses during the 60-minute session; distribution of responses for alcohol versus money; progressive ratio breakpoint for alcohol (maximum completed ratio value); response rate (responses/min) and response latency to compete the ratio for alcohol and money. We also measure alcohol craving using the Tiffany Craving Scale; these data will be summarized using peak score and area under the curve. The normality of the outcome measures will be examined first and two sample t-test, or equivalent non-parametric methods will be used to compare the outcomes between GR antagonist-treated group and placebo-treated group.

Aim 4: Subjective and physiological data will be collected repeatedly during the alcohol sensitivity session. We will test outcome measures between the two groups using mixed-effect linear regression with random intercept. Baseline measures will be examined as potential confounding factors and added to the model if needed. We will summarize the baseline adjusted area under the curve and peak values of the outcome measures and compare between the two groups using repeated measure ANCOVA model.

Aim 5: We use the SAFTEE to collect comprehensive side-effect information. We will construct negative binomial generalized linear regression model with the number of events as the dependent variable and grouping and other covariates as independent variables. The model will give the difference in the logs of expected numbers of side effect events between the two treatment groups. We will then also make comparisons taking into consideration the duration and severity of the side effects, which are both collected in SAFTEE for each reported side effect event. A summary side-effect score will be calculated as the sum of the duration multiplying the severity of each side effect event.

Secondary analyses: Another objective of this study is to generate power estimation for a full-size future clinical trial.

d. Early stopping rules.

Individual subjects will be discontinued from the protocol if they experience a moderate-to-severe adverse reaction to MIFE. They also will be discontinued for repeated rule violations on the CRU. The study will be discontinued if there is a pattern of MIFE-related AEs or SAEs.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Subjects will be carefully screened and fully informed of study procedures and risks prior to participation in any study procedures. This study involves several procedures, each of which entails some risk of discomfort or side-effects. It also employs several drugs, each of which entails some risk. These risks are discussed by procedure and medication. Participants will receive a thorough description of all potential risks in the consent form.

Assessment procedures: The major disadvantage is the time taken to complete study instruments and questionnaires. Our experience with these evaluations indicates they are acceptable to participants. This study involves questions about dangerous or illegal behavior, psychiatric history, a medical history, and laboratory tests. There is a small risk that participants will become upset during the assessment interview. There also is a risk of breach of confidentiality if the responses were to be disclosed.

Blood draws and urine collection: We will collect blood for health assessment and medication levels. Blood chemistries and urinalysis are performed at baseline, at the time of admission to the CRU (or day 1 for HC participants completing the study on an outpatient basis), and on protocol days 4 – 6. Urinalysis for drug toxicology assessment is performed to identify illicit drug use. A blood spot for PEth testing is obtained for detection of recent heavy drinking; PEth blood spot is collected at assessment and each follow-up visit. Urine pregnancy tests are completed in female subjects at screening and on day 1. A urine pregnancy test is obtained prior to the initial MIFE dose. Blood draw procedures involve minimal risks such a slight risk of discomfort at the intravenous site. A small amount of bleeding under the skin will produce a bruise in about 5% of cases. The risk of temporary clotting of the vein is about 1%. The risk of infection or significant blood loss is less than 1 in 1000. In rare cases, fainting could occur.

Alcohol Withdrawal: Applicants with a history of prior inpatient medicated withdrawal, alcohol withdrawal-related seizures, or other serious alcohol withdrawal symptoms will be excluded from participation. Eligible participants will undergo monitored alcohol abstinence and medically supervised alcohol withdrawal on the CRU.

Mifepristone (MIFE) Administration: Since MIFE blocks the progesterone receptor, treatment of a pregnant woman would result in the termination of pregnancy. All females must have a negative urine pregnancy test prior to MIFE initiation; women on hormonal birth control, who are pregnant, nursing or planning pregnancy, and women with unexplained vaginal bleeding are excluded from study participation. In addition, women must agree to use a non-hormonal form of birth control for at least 1 month after stopping MIFE treatment. We will provide female participants with condoms at each outpatient follow-up visit. Women may experience endometrial thickening or unexpected vaginal bleeding; there is a low likelihood of these events.

Due to the anti-progestin effects of mifepristone and given the lack of adequate data on the potential levels of mifepristone in semen, male subjects will be required to employ barrier protection with their female partners. Males should use barrier protection during intercourse for at least 5 half-lives of the drug (half life = 18 hours) plus 90-days (the duration of one spermatogenic cycle in men and residence time for unejaculated sperm) after last dose of mifepristone to avoid risk of anti-progestin effects transmitted to female sexual partners. We will provide male participants with condoms for at least 94-days after the last dose of mifepristone.

Based on prescribing information for MIFE treatment of Cushing Disease, MIFE may produce prolongation of the QT interval of the electrocardiogram (ECG) and use of MIFE with QT interval-prolonging drugs is contraindicated. A recent evaluation of the cardiac safety of three doses of 1200 mg MIFE given every 12 hrs to subjects found no clinically meaningful QT prolongation in healthy subjects (Corcept Therapeutics Investigator Brochure).

Based on data in individuals with Cushing Disease, MIFE may cause adrenal insufficiency. Symptoms of adrenal insufficiency may include abdominal pain, vomiting, muscle weakness and fatigue, depression, extremely low blood pressure (hypotension), weight loss, changes in mood, and shock (adrenal crisis). There has been no evidence of the development of adrenal insufficiency during short-term administration of MIFE doses up to 1200 mg in clinical trials for other psychiatric disorders.

MIFE has been shown to infrequently cause low blood potassium (hypokalemia) in patients with Cushing Syndrome. Symptoms of low potassium may include muscle weakness, aches, or cramps, abnormal or irregular heartbeats (palpitations). Although hypokalemia has not been observed in other patient populations, we will exclude subjects with potassium levels below the normal range at screening or day 1.

MIFE administration also has been associated with the possibility of developing a rash and/or a dry mouth. Both side effects are mild-to-moderate in severity and both have been found to discontinue when drug administration is terminated.

Use of MIFE with strong CYP3A inhibitors is counter indicated, as concomitant use can increase mifepristone plasma levels. Subjects using CYP3A inhibitors in last 3 months are ruled out prior to study enrollment.

Alcohol administration: Alcohol may produce mood and behavioral effects that are dysphoric and that can result in impairment in performance and judgment and, in cases of overdose, ethanol can produce medically serious toxic effects. Prior to the fMRI on day 1, subjects receive an alcohol drink in a standardized calorie-free mixer targeting a blood alcohol level of 80mg% based on gender and BMI. Following the Alcohol Motivated Responding Session, during the alcohol self-administration period, the maximum dose of alcohol that subjects can self-administer is five standard drinks over 50 minutes; this dose will produce a blood alcohol level of approximately 120mg/dL. In the alcohol sensitivity session, the proposed alcohol dose is one that produces a blood ethanol level of approximately 100 mg/dL. Study subjects are those who regularly drink doses of alcohol greater than the amount used in this study, reducing the likelihood of serious adverse events. These are medically safe but behaviorally intoxicating levels, associated with impairment of psychomotor and cognitive performance and with emotional and behavioral effects that may range from sedation and drowsiness to agitation, irritability, depression, and emotional lability. These effects dissipate as blood ethanol levels decline.

Nicotine Patch: Participants have the option to receive a nicotine patch on session days. The nicotine patch dose selected for study in this protocol is FDA-approved and has been shown to have a low incidence rate of serious side-effects or adverse events in clinical trials with nicotine-dependent patients. Less than 5% of smokers have to stop using a nicotine replacement product because of side effects. Side effects of nicotine patches may include: skin rash at the location of the patch; sleep problems when using a 24-hour patch, such as having trouble sleeping or having especially vivid dreams. On rare occasions, there have been reports of severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); fast or irregular heartbeat; mouth, teeth, or jaw problems; pounding in the chest; severe diarrhea, dizziness, nausea, vomiting, or weakness.

Functional MRI: Subjects may experience discomfort while lying in the MRI machine for the scans. Subjects may experience claustrophobia before entering the MRI machine and during the performance of the MRI while lying in the MRI machine. Subjects may experience fear and discomfort during the MRI due to the noise of the machine.

Radiation Exposure: If a subject has had metal removed from the eyes or head in the past; or if the subject has worked as a machinist, welder, or metal worker and did not routinely wear eye protection, or had an incident in which metal fragments got in the eye, we will require an x-ray of the orbits/head to verify that there is no remaining metal in the eyes or head before conducting any MRI scanning. This will involve 3 exposures of an X-ray procedure that will result in a total exposure of 0.03 rem. We anticipate that this procedure will rarely need to be done, but if it is necessary, the total exposure is less than the 0.3 rem that the average person in the United States receives each year from natural sources. The risk from the radiation exposure in this study is therefore very small.

Trier Social Stress Test: Subjects will participate in the Trier Social Stress Test as part of the experimental protocol. This test combines the stressors of public speaking and mental arithmetic and has been shown to elevate a variety of physiological measures, including neuroendocrine and autonomic variables. Participants are monitored carefully throughout the procedure. It is possible but extremely unlikely that a subject will evidence clinically significant symptoms of anxiety or grossly elevated autonomic reactivity.

Rating Scales and Questionnaires: The major disadvantage is the time taken to complete study instruments and questionnaires and a possible breach of confidentiality. Our past experience with these evaluations indicates they are acceptable to patients.

b. Steps taken to minimize the risks.

Subjects with any eligibility contraindications are excluded from participation. Subjects are permitted to discontinue their participation at any time. Subjects are carefully and continually monitored throughout their participation. In case of a medical adverse event, the study physicians Dr. Wand or Dr. Andrews are available by cell phone for assistance. In the event of psychological distress, Dr. McCaul, a licensed psychologist, or Dr. Andrews, a licensed psychiatrist, is available to meet with the participant.

Insuring protocol comprehension: We exclude potential subjects below the 5th grade reading level because of concerns about their ability to adequately comprehend the informed consent document and participate in study procedures. Many of our behavioral/subjective measures are paper and pencil, self-report instruments and require basic literacy and language skills (e.g., English is not primary language). If subjects are not at a 5th grade reading level, they have difficulty responding accurately to the study questionnaires.

Psychosocial assessments: The risk of distress or personal discomfort elicited during assessment is minimized by the use of standardized assessment procedures widely and successfully used in many research settings. Also, all study staff are trained in nonjudgmental interview techniques. Employees are carefully trained to monitor subjects for any adverse effect and to contact an Investigator immediately if such an event

occurs. If serious psychological concerns (e.g., suicide risk) arise, staff has been trained to immediately refer these to Dr. McCaul, a licensed psychologist, or a study physician.

Medically supervised and alcohol withdrawal and monitored abstinence: Subjects will be closely monitored by nursing and physician staff for alcohol withdrawal signs and symptoms. All AUD subjects will receive iv administration of 1 liter of normal saline with vitamins over the first 12 hours following admission. On days 1 – 4, the Clinical Institute Withdrawal Assessment - Alcohol Revised (CIWA-Ar), which includes 10-items (nausea/vomiting, tremor, paroxysmal sweats, anxiety, agitation, tactile disturbances, auditory disturbances and visual disturbances), will be completed by CRU nurses every 4 hours while the participant is awake. This well-validated clinical tool will assess alcohol withdrawal symptom severity and is the gold standard to guide benzodiazepine (BZ) administration during symptom-driven withdrawal management (Saitz et al., 1994). If the CIWA score is ≥12 the subject is given diazepam (5mg). The CIWA score is repeated 1 hour after each dose. If the score remains ≥10 then another diazepam dose (5mg) is given up to a maximum of three doses. Based on our previous study, we anticipate that few subjects will require BZ treatment since we exclude subjects who report a history of withdrawal complications. If systolic blood pressure increases above 180 and or diastolic blood pressure increases above 105, we may administer atenolol. The most common symptoms of atenolol include dizziness, lightheadedness, tiredness, drowsiness, depression, nausea and diarrhea. Less common but more serious side-effects include: shortness of breath, and swelling of the hands, feet, ankles or lower legs. Subjects who experience seizure activity or delirium tremens will be discontinued from the study; also subjects will be discontinued on recommendation by the study physician. To date we have successfully withdrawn over 98% of our alcohol-dependent subjects without medications requiring protocol termination. Exclusion of subjects with a history of alcohol withdrawal complications, and/or high CIWA scores at assessment is a successful strategy to help ensure subject safety. In the event of a severe reaction or medical emergency, the participant will be transported to near-by emergency services.

Mifepristone administration: The use of MIFE in this study will be under FDA IND 124365; Dr. Wand, Co-Principal Investigator, holds the IND. We are taking multiple precautions to ensure that MIFE is not administered to women who are pregnant or planning pregnancy. Alcohol is highly toxic and pose significant risk to fetal development. We believe it is imperative to study therapeutic agents in women of childbearing age to further the development of potential treatments for this population.

- Only persons with no medical exclusions will be enrolled for study participation.
- Subjects are under continuous observation while on the Clinical Research Unit. Subjects not on the CRU are checked on daily while receiving MIFE.
- Medication side effects and adverse effects are formally monitored daily on the CRU for inpatient subjects, on the phone for outpatient subjects, and during outpatient visits via the SAFTEE. In case of an emerging or adverse event, the study physician is available by cell phone for assistance.
- Women who use hormonal birth control, are pregnant, planning to become pregnant or are nursing are excluded from study participation. All females must have a negative urine pregnancy test prior to MIFE initiation. Females must use a non-hormonal medically acceptable method of contraception (e.g., condoms, IUD, diaphragm) for a one month period following discharge, unless the subject has had a surgical sterilization. We will provide female participants with condoms for the month following study participation. Women who provide a report of surgical sterilization or

who provide a lab report with FSH level greater than 25 confirming post-menopausal status may be exempt from pregnancy testing.

- Due to the anti-progestin effects of mifepristone and given the lack of adequate data on the potential levels of mifepristone in semen, male subjects will be required to employ barrier protection with their female partners. Males will be instructed to use barrier protection during intercourse for at least 5 half-lives of the drug plus 90-days (the spermatogenic cycle in men and residence time for unejaculated sperm) after last dose of mifepristone to avoid risk of anti-progestin effects transmitted to female sexual partners.
- Women who develop unexplained vaginal bleeding during the study will be excluded for further study procedures. Female study subjects will be educated to report and be evaluated for unexplained vaginal bleeding.
- Subjects with potassium levels below the normal range at screening will be excluded. We will also monitor potassium on days 1 and 7 (AUD participants only), and at the 1 week follow-up. Potassium will be administered if clinically needed.
- Based on data from studies and clinical trials, which encompass over 1500 subjects and patients treated for one to two weeks with MIFE doses ranging from 300 mg/day to 1800 mg/day, the risk for development of adrenal insufficiency in persons without Cushing Syndrome is low. Specifically, in studies of psychotic depression and in volunteers with antipsychotic drug induced weight gain as well as healthy volunteers and subjects with hepatic and renal impairment who participated in PK, PD, and DDI studies, there were no AEs of adrenal insufficiency reported (Personal Communication, Corcept Therapeutics). Still, in the current study, subjects will be monitored for the development of the signs and symptoms of adrenal insufficiency (weakness, nausea, increased fatigue, hypotension) during the CRU stay and during outpatient visits. Once on the CRU and following MIFE administration, we monitor for hypoadrenalism by measuring blood pressure and heart rate along with all vital signs q6 hours. For inpatient participants, we also will be asking questions q6 hours regarding emergence of symptoms of hypoadrenalism, including the development of fatigue, nausea, vomiting, abdominal pain, loss of appetite, light headedness, arthralgias, and myalgias. Outpatient participants will be asked these questions once daily either in person or by phone. If an AUD subject on the CRU is thought to be developing hypoadrenalism, we will discontinue the MIFE, support blood pressure with intravenous fluids and infuse 8mg of dexamethasone intravenously. Outpatient healthy control participants will be provided with oral dexamethasone 8 mg to take home. They will be given an instruction sheet (see supplemental study documents) that advises them of the symptoms of adrenal insufficiency and advises them to call Dr. Wand if these symptoms start to develop. This dose is more than adequate to reverse the glucocorticoid receptor blockade. The proposed single dose of dexamethasone is routinely used in the determining the etiology of Cushing Syndrome. In patients with impaired glucose tolerance or frank diabetes mellitus, this dose of dexamethasone can transiently increase blood sugar; however, in our study, participants with impaired glucose tolerance are excluded. Dexamethasone will only be used in the unlikely event of adrenal crisis in which we do not anticipate any negative side-effects from the treatment.
- Dr. Wand, a board certified neuroendocrinologist, has extensive clinical experience in treating patients with adrenal insufficiency and will manage care if needed.
- Subjects will be educated about the signs/symptoms of adrenal insufficiency including unusual tiredness or weakness, nausea and/or vomiting, dizziness when standing, aches and pains, loss of appetite, and being depressed. Subjects will be

instructed to report these symptoms and to follow up with the physician if they experience any of them.

Nicotine Patch: Some subjects may develop a skin rash at the location of the nicotine patch. This may be a reaction either to the sticky backing on the patch or to the nicotine. Moving the patch to a different part of your body or using a nonprescription antihistamine cream, ointment, or gel (such as Benadryl) may relieve some of the discomfort. People who experience significant allergic reaction to the patch will be discontinued and will be provided with medical care to reverse symptoms.

Alcohol Administration Sessions: Our laboratory has substantial experience administering alcohol to human subjects under a variety of experimental and dosing conditions. In conducting alcohol administration procedures in heavy or dependent drinkers, we are taking several important steps to ensure the safety and well-being of subjects participating in this protocol. First, we will follow the guidelines of the National Institute on Alcohol Abuse and Alcoholism for alcohol administration to persons with an alcohol use disorder. Second, we recruit only persons who are not seeking treatment and who are volunteering for remuneration. Third, we house subjects on the CRU under nursing observation to reduce potential harm during alcohol withdrawal and alcohol intoxication. Fourth, the dose of alcohol that will be available during the alcohol sensitivity session is well below doses routinely self-administered by the participants and drinking will be paced to prevent rapid ingestion of the available alcohol.

All alcohol doses are administered under doctor's orders and utilize pharmacy-grade ethanol or commercially available alcohol. Participants are monitored carefully throughout the laboratory session; should a subject evidence a medical or behavioral adverse effect, a medical staff member will be called immediately and the subject will receive appropriate evaluation and treatment. Thus, the careful choice of doses, the medical observation and monitoring, and the use of human volunteers who regularly drink doses of ethanol comparable to and higher than the ones selected for this study reduce the likelihood of serious adverse ethanol-induced effects.

Blood draws: Experienced medical personnel using sterile equipment will perform the blood draws, minimizing risks associated with venipuncture. The total volume of blood drawn in the protocol is very low and is not expected to lead to discomfort or health concerns.

Trier Social Stress Test: Following completion of the AMR and drinking session, subjects are debriefed about the TSST stress procedures.

fMRI risks: Participants will be escorted to KKI scan room by one of our staff members while maintaining physical distancing. Throughout the time at the KKI facility, we will follow all COVID-related safety policies of the center, including physical distancing, use of PPE and sanitizing. After the KKI technician positions the volunteer in the scanner, our staff person will position the hand-held response device and show the participant how to make responses during the alcohol-cued fMRI. Staff members will leave scan room remaining outside the room monitoring subject’s responses and safety. This space is large enough to accommodate one KKI staff member and one of our staff members. Subjects will be monitored by video camera throughout the scanning procedure and

questioned as to their state via intercom between each scan. The staff member will monitor the participant for adverse events or distress. Subjects will be provided with a squeezeball signaling device in the MRI in case adverse conditions develop during a scan. Experimenters will immediately stop the scan and attend to the subject if the squeezeball signal is activated by the subject. Should a participant feel discomfort we will reposition her/him and cushion the subject with padding. If subjects are markedly bothered by claustrophobia, then they will be reassured that the procedure is not harmful. If subjects experience claustrophobia unresponsive to reassurance, then study procedures will be discontinued. To deaden the noise of the MRI, subjects are asked to insert ear plugs before entering the MRI. The Kirby Center for Functional Brain Imaging at the Kennedy Krieger Institute has a crash cart nearby, and in case of medical emergencies the code team at Kennedy Krieger will be called and we will arrange emergency transportation to the JHH ED.

Confidentiality: Our staff is well trained in the matters of confidentiality. Subject numbers will be used to code all data forms for computer entry and storage. Study findings are reported using group data only. No information about subjects will be provided to anyone outside of the study including family members, third persons or organizations.

All members of our research group have completed the Health Insurance Portability and Accountability Act (HIPAA) compliance training courses relating to human subjects research. All data including information obtained during screening that may be linked to an individual's personal identification (names, addresses, outpatient medical record numbers) will be in a hard copy format and stored in a locked cabinet in a locked room located in a restricted access office. All laboratory data stored electronically will utilize the study code and will be in password-locked files on a LAN server operated by Johns Hopkins University School of Medicine with the latest security measures employed. Access to the subjects' study identification codes or other information will be restricted to the Principal Investigators, the research coordinator and, upon written request, to the Institutional Review Board or other regulatory agencies. Subjects will be advised that the results may be published in manuscript(s) but their identities will remain anonymous.

All remote contacts will use a HIPAA compliant telemedicine app or website (participant preference) and will not require the participant to enter any potentially identifying information. This service uses end-to-end encryption and no identifying information is recorded by the service itself.

COVID infection risk: Study activities will be conducted remotely as opposed to in person when possible to reduce unnecessary in-person encounters. For all in-person visits, we will follow HEIC clinical standards for maintaining staff and participant safety. Staffwill use appropriate PPE (face mask/shield, gowns, gloves) and practice physical distancing. Participants will wear face masks. Any equipment that participants are asked to handle to complete study procedures (e.g., MRI compatible response buttons) will be cleaned prior to use. Office spaces will be cleaned by sanitizing surfaces between office visits. We will limit the number of people within spaces (e.g. KKI MRI suite). For procedures that require contact (e.g. phlebotomy, obtaining vital signs), we will limit the amount of time the contact lasts and ensure both staff and patients use appropriate PPE.

A staff member must accompany the participant on the car ride from the JHH campus to the Bayview CRU. Staff and participants will use face masks and physically distance to the extent possible during the ride.

Blood alcohol levels measured by breathalyzer are obtained serially during the alcohol sensitivity session. The staff member running these sessions will be wearing full PPE attire. The staff member will leave the research room each time the participants exhales into the breathalyzer. Participant then removes the disposable mouth piece and places it in a basket containing a red bag designed to hold toxic materials. A fresh mouth piece is attached by staff after re-entering procedure room.

When utilizing both the CRU and the imaging suites, we will follow policies to limit COVID infection exposure risk established by those locations.

c. Plan for reporting unanticipated problems or study deviations.

Dr. McCaul has responsibility for communication with the JHU SOM IRB and will be responsible for reporting of AEs and SAEs. SAEs that are deemed to be severe and have a high probable or definite relationship to study procedures will be promptly reported to the Johns Hopkins Medical Institute IRB, Corcept Therapeutics, and the FDA.

Dr. McCaul also is responsible for notifying Corcept of (a) any serious and/or unexpected adverse drug experiences (as those terms are defined in 21 C.F.R. § 312.32(a), as amended), and (b) all adverse events of special interest (AESI) that occur during the course of the Study no later than twenty-four (24) hours after receipt of such information. AESI shall mean any of the following events, irrespective of the level of severity:

1. Major adverse cardiovascular events including death related to coronary or cerebrovascular disease, acute myocardial infarction, stroke, and revascularization (coronary or cerebral)
2. Retinopathy
3. Endometrial hyperplasia and/or abnormal vaginal bleeding of any cause

The PI shall notify Corcept of all serious adverse events each time that a report is made to the IRB.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Our staff is well trained in the matters of confidentiality including maintenance of genotyping information. At present there is no known health implication for the polymorphism under study. Subject numbers will be used to code all data forms for computer entry and storage. Study findings are reported using group data only. No information about subjects will be provided to family members, third persons or organizations. Our study personnel are trained in the protection of human subject confidentiality and are HIPAA trained and certified.

e. Financial risks to the participants.

All costs will be covered by NIAAA grant funds.

9. Benefits

a. Description of the probable benefits for the participant and for society.

This study is conducted for the advancement of science. It will provide critical safety information and examine pharmacokinetic interactions between MIFE and alcohol. It will also determine whether MIFE can be safely used for outpatient treatment of patients who may continue to use alcohol. Finally, it will provide preliminary findings of MIFE effects on alcohol craving, anxiety and intoxication.

This Phase 1 study will provide critical safety information and examine drug-drug interactions between MIFE and alcohol. Outpatient clinical trials that will meet requirements for FDA new drug approval cannot be pursued without this safety information.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will be paid $75 for the initial in-person assessment procedures. If all eligibility assessment procedures are not completed during the first assessment visit, we pay participants an additional $25 to come in a second time to complete procedures.

Subjects who are eligible to continue in the study will be paid for completed procedures and their time and effort in the study. AUD Subjects receive $15/day as basic CRU earnings and an additional $10 per day for compliance with CRU and study procedures.

Payments for AUD study completers
Assessment $75
CRU stay – 7 days X $25/day ($15/ day + $10/day for following protocol rules) = $175
Alcohol self-administration and sensitivity sessions = $75/session X 2 = $150
fMRI = $100/scan X 2 = $200
Follow-up visits X 2 = $100
Bonus - $200
Subject payment expense: $900

Payments for outpatient HC study completers
Assessment $75
Medication/questionnaire compliance = 4 days X $10/day = $40
Blood draw on day 4 = $10
fMRI = $100/scan X 2 = $200
Follow-up visit = $50
Bonus - $100
Subject payment expense: $475

Subjects are paid for the eligibility visit in cash after completing the assessment. If the subject is eligible for a bonus payment, it will be dispensed in cash on the day of discharge. Subjects will receive a check for the remaining amount of money that was earned during the rest of the study.

If the Investigator ends the subject's participation because s/he broke study or unit rules or the subject drops out of the study, s/he will be paid only the amount earned to date for completed procedures and the CRU stay. If the participant leaves during the CRU stay, s/he will not be paid any money until the scheduled discharge day, and will forfeit future possible earnings and bonus payments.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

Costs for all procedures and drugs will be covered by the study.

REFERENCES

Amunts, K., Kedo, O., Kindler, M., et al., 2005. Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: Intersubject variability and probability maps. Anat. Embryol. 210, 343-352.

Barrot, M., Marinelli, M., Abrous, D.N., et al., 2000. The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. Eur.J.Neurosci. 12, 973-979.

Beck, A., Wüstenberg, T., Genauck, A., et al., 2012. Effect of brain structure, brain function, and brain connectivity on relapse in alcohol-dependent patients. Arch.Gen.Psychiatry. 69, 842-852.

Bencherif, B., Wand, G.S., McCaul, M.E., et al., 2004. Mu-opioid receptor binding measured by [11 C] carfentanil positron emission tomography is related to craving and mood in alcohol dependence. Biol.Psychiatry. 55, 255-262.

Blasey, C.M., Block, T.S., Belanoff, J.K., et al., 2011. Efficacy and safety of mifepristone for the treatment of psychotic depression. J.Clin.Psychopharmacol. 31, 436-440.

Bohn, M.J., Krahn, D.D., Staehler, B.A., 1995. Development and initial validation of a measure of drinking urges in abstinent alcoholics. Alcoholism: Clinical and Experimental Research. 19, 600-606.

Brasser, S.M., McCaul, M.E., Houtsmuller, E.J., 2004. Alcohol effects during acamprosate treatment: A dose-response study in humans. Alcoholism: Clinical and Experimental Research. 28, 1074-1083.

Calhoun, V.D., Adali, T., Pearlson, G., et al., 2001. A method for making group inferences from functional MRI data using independent component analysis. Hum.Brain Mapp. 14, 140-151.

Calhoun, V.D., Adalı, T., Pekar, J.J., 2004. A method for comparing group fMRI data using independent component analysis: Application to visual, motor and visuomotor tasks. Magn.Reson.Imaging. 22, 1181-1191.

Cho, K., Little, H., 1999. Effects of corticosterone on excitatory amino acid responses in dopamine-sensitive neurons in the ventral tegmental area. Neuroscience. 88, 837-845.

Cox, L.S., Tiffany, S.T., Christen, A.G., 2001. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. Nicotine Tob.Res. 3, 7-16.

DeBattista, C., Belanoff, J., 2006. The use of mifepristone in the treatment of neuropsychiatric disorders. Trends in Endocrinology & Metabolism. 17, 117-121.

Deroche, V., Marinelli, M., Maccari, S., et al., 1995. Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. J.Neurosci. 15, 7181-7188.

Everitt, B.J., Robbins, T.W., 2013. From the ventral to the dorsal striatum: Devolving views of their roles in drug addiction. Neuroscience & Biobehavioral Reviews. 37, 1946-1954.

Faul, F., Erdfelder, E., Lang, A., et al., 2007. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods. 39, 175-191.

Flannery, B., Volpicelli, J., Pettinati, H., 1999. Psychometric properties of the Penn alcohol craving scale. Alcoholism: Clinical and Experimental Research. 23, 1289-1295.

Foltin, R.W., Haney, M., 2000. Conditioned effects of environmental stimuli paired with smoked cocaine in humans. Psychopharmacology (Berl.). 149, 24-33.

Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage. 15, 870-878.

Gray, T.S., Bingaman, E.W., 1996. The amygdala: Corticotropin-releasing factor, steroids, and stress. Critical Reviews™ in Neurobiology. 10.

Haass-Koffler, C.L., Bartlett, S.E., 2012. Stress and addiction: Contribution of the corticotropin releasing factor (CRF) system in neuroplasticity. Frontiers in Molecular Neuroscience. 5, 1-13.

Hammers, A., Allom, R., Koepp, M.J., et al., 2003. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. Hum.Brain Mapp. 19, 224-247.

Han, J.W., Han, D.H., Bolo, N., et al., 2015. Differences in functional connectivity between alcohol dependence and internet gaming disorder. Addict.Behav. 41, 12-19.

Harfstrand, A., Fuxe, K., Cintra, A., et al., 1986. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc.Natl.Acad.Sci.U.S.A. 83, 9779-9783.

Heatherton, T.F., Kozlowski, L.T., Frecker, R.C., et al., 1991. The Fagerström test for nicotine dependence: A revision of the Fagerstrom Tolerance Questionnaire. Br.J.Addict. 86, 1119-1127.

Heikinheimo, O., Kekkonen, R., Lähteenmäki, P., 2003. The pharmacokinetics of mifepristone in humans reveal insights into differential mechanisms of antiprogestin action. Contraception. 68, 421-426.

Hoenig, K., Kuhl, C.K., Scheef, L., 2005. Functional 3.0-T MR assessment of higher cognitive function: Are there advantages over 1.5-T imaging? Radiology. 234, 860-868.

Howland, R.H., 2013. Mifepristone as a therapeutic agent in psychiatry. J.Psychosoc.Nurs.Ment.Health Serv. 51, 11-14.

Jacquot, C., Croft, A.P., Prendergast, M.A., et al., 2008. Effects of the glucocorticoid antagonist, mifepristone, on the consequences of withdrawal from long term alcohol consumption. Alcoholism: Clinical and Experimental Research. 32, 2107-2116.

Jakovcevski, M., Schachner, M., Morellini, F., 2011. Susceptibility to the long-term anxiogenic effects of an acute stressor is mediated by the activation of the glucocorticoid receptors. Neuropharmacology. 61, 1297-1305.

Johnson, B.A., Ait-Daoud, N., Roache, J.D., 2005. The COMBINE SAFTEE: A structured instrument for collecting adverse events adapted for clinical studies in the alcoholism field. Journal of Studies on Alcohol, Supplement. S15, 157-167.

Khanna, N., Altmeyer, W., Zhuo, J., et al., 2015. Functional neuroimaging: Fundamental principles and clinical applications. The Neuroradiology Journal. 28, 87-96.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test': A tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology. 28, 76-81.

Koenig, H.N., Olive, M.F., 2004. The glucocorticoid receptor antagonist mifepristone reduces ethanol intake in rats under limited access conditions. Psychoneuroendocrinology. 29, 999-1003.

Koob, G.F., 2009. Neurobiological substrates for the dark side of compulsivity in addiction. Neuropharmacology. 56, 18-31.

Korte, S.M., De Boer, S., De Kloet, E., et al., 1995. Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus-maze. Psychoneuroendocrinology. 20, 385-394.

Makino, S., Hashimoto, K., Gold, P.W., 2002. Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress. Pharmacology Biochemistry and Behavior. 73, 147-158.

Martin, C.S., Earleywine, M., Musty, R.E., et al., 1993. Development and validation of the biphasic alcohol effects scale. Alcohol.Clin.Exp.Res. 17, 140-146.

Mason, B., 2014. A Glucocorticoid antagonist shows therapeutic potential for alcohol dependence in a POC human laboratory study. Alcohol and Alcoholism (Oxford, Oxfordshire). 48, 175.

Mason, B., 2012. A human laboratory study of mifepristone treatment for alcohol dependence. Neuropharmacology. 38S, 53.

McCaul, M.E., Turkkan, J.S., Svikis, D.S., et al., 1991a. Alcohol and secobarbital effects as a function of familial alcoholism: Extended intoxication and increased withdrawal effects. Alcoholism: Clinical and Experimental Research. 15, 94-101.

McCaul, M.E., Turkkan, J., Svikis, D., et al., 1991b. Familial density of alcoholism: Effects on psychophysiological responses to ethanol. Alcohol. 8, 219-222.

McCaul, M.E., Wand, G.S., Eissenberg, T., et al., 2000. Naltrexone alters subjective and psychomotor responses to alcohol in heavy drinking subjects. Neuropsychopharmacology. 22, 480-492.

McCaul, M.E., Wand, G.S., Rohde, C., et al., 2000. Serum 6-beta-naltrexol levels are related to alcohol responses in heavy drinkers. Alcoholism: Clinical and Experimental Research. 24, 1385-1391.

McCaul, M.E., Wand, G.S., Stauffer, R., et al., 2001. Naltrexone dampens ethanol-induced cardiovascular and hypothalamic-pituitary-adrenal axis activation. Neuropsychopharmacology. 25, 537-547.

McNair, D., Heuchert, J., 2012. POMS 2. Educational and Industrial Testing Service. MultiHealth Systems, Inc.

McNair, D., Heuchert, J., 2003. POMS: Profile of Mood States: Technical Update.

McNair, D.M., Droppleman, L.F., Lorr, M., 1992. Edits Manual for the Profile of Mood States: POMS.

Morgan, F.H., Laufgraben, M.J., 2013. Mifepristone for management of Cushing's syndrome. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 33, 319-329.

Morimoto, M., Morita, N., Ozawa, H., et al., 1996. Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: An immunohistochemical and in situ hybridization study. Neurosci.Res. 26, 235-269.

Muller-Oehring, E.M., Jung, Y.C., Pfefferbaum, A., et al., 2015. The resting brain of alcoholics. Cereb.Cortex. 25, 4155-4168.

Myrick, H., Anton, R.F., Li, X., et al., 2004. Differential brain activity in alcoholics and social drinkers to alcohol cues: Relationship to craving. Neuropsychopharmacology. 29, 393-402.

Myrick, H., Anton, R.F., Li, X., et al., 2008. Effect of naltrexone and ondansetron on alcohol cue-induced activation of the ventral striatum in alcohol-dependent people. Arch.Gen.Psychiatry. 65, 466-475.

Myrick, H., Li, X., Randall, P.K., et al., 2010. The effect of aripiprazole on cue-induced brain activation and drinking parameters in alcoholics. J.Clin.Psychopharmacol. 30, 365-372.

Nebel, M.B., Eloyan, A., Nettles, C.A., et al., 2015. Intrinsic visual-motor synchrony correlates with social deficits in autism. Biol.Psychiatry.

Neto, L.L., Oliveira, E., Correia, F., et al., 2008. The human nucleus accumbens: Where is it? A stereotactic, anatomical and magnetic resonance imaging study. Neuromodulation: Technology at the Neural Interface. 11, 13-22.

Neuroimaging Informatics Tools and Resources Clearinghouse (NITRC), Artifact Detection Tools (ART).

Niwa, M., Jaaro-Peled, H., Tankou, S., et al., 2013. Adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids. Science. 339, 335-339.

Oswald, L.M., Wong, D.F., McCaul, M., et al., 2005. Relationships among ventral striatal dopamine release, cortisol secretion, and subjective responses to amphetamine. Neuropsychopharmacology. 30, 821-832.

Piazza, P.V., Le Moal, M., 1996. Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu.Rev.Pharmacol.Toxicol. 36, 359-378.

Pomara, N., Doraiswamy, P.M., Tun, H., et al., 2002. Mifepristone (RU 486) for Alzheimer's disease. Neurology. 58, 1436.

Puente, A., 1985. Wisconsin card sorting test. Test critiques. 4, 677-682.

Quaedflieg, C., van de Ven, V., Meyer, T., et al., 2015. Temporal dynamics of stress-induced alternations of intrinsic amygdala connectivity and neuroendocrine levels. PloS one. 10, e0124141.

Repunte-Canonigo, V., Shin, W., Vendruscolo, L.F., et al., 2015. Identifying candidate drivers of alcohol dependence-induced excessive drinking by assembly and interrogation of brain-specific regulatory networks. Genome Biol. 16, 1-13.

Richardson, H.N., Lee, S.Y., O'Dell, L.E., et al., 2008. Alcohol self-administration acutely stimulates the hypothalamic-pituitary-adrenal axis, but alcohol dependence leads to a dampened neuroendocrine state. Eur.J.Neurosci. 28, 1641-1653.

Roberts, A.J., Lessov, C.N., Phillips, T.J., 1995. Critical role for glucocorticoid receptors in stress- and ethanol-induced locomotor sensitization. J.Pharmacol.Exp.Ther. 275, 790-797.

Robinson, S.M., Sobell, L.C., Sobell, M.B., et al., 2014. Reliability of the Timeline Followback for cocaine, cannabis, and cigarette use. Psychology of addictive behaviors. 28, 154.

Rotter, A., Biermann, T., Amato, D., et al., 2012. Glucocorticoid receptor antagonism blocks ethanol-induced place preference learning in mice and attenuates dopamine D2 receptor adaptation in the frontal cortex. Brain Res.Bull. 88, 519-524.

Saitz, R., Mayo-Smith, M.F., Roberts, M.S., et al., 1994. Individualized treatment for alcohol withdrawal: a randomized double-blind controlled trial. JAMA. 272, 519-523.

Schacht, J.P., Anton, R.F., Myrick, H., 2013. Functional neuroimaging studies of alcohol cue reactivity: A quantitative meta-analysis and systematic review. Addict.Biol. 18, 121-133.

Schuckit, M.A., 1980. Self-rating of alcohol intoxication by young men with and without family histories of alcoholism. J.Stud.Alcohol. 41, 242-249.

Schulkin, J., Gold, P.W., McEwen, B.S., 1998. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: Implication for understanding the states of fear and anxiety and allostatic load. Psychoneuroendocrinology. 23, 219-243.

Sharrett-Field, L., Butler, T.R., Berry, J.N., et al., 2013. Mifepristone pretreatment reduces ethanol withdrawal severity in vivo. Alcoholism: Clinical and Experimental Research. 37, 1417-1423.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., et al., 1998. The Mini-International Neuropsychiatric Interview (MINI): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J.Clin.Psychiatry. 59, 22-33.

Shepard, J.D., Barron, K.W., Myers, D.A., 2000. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res. 861, 288-295.

Silva, S.M., Madeira, M.D., 2012. Effects of chronic alcohol consumption and withdrawal on the response of the male and female hypothalamic–pituitary–adrenal axis to acute immune stress. Brain Res. 1444, 27-37.

Simms, J.A., Haass-Koffler, C.L., Bito-Onon, J., et al., 2012. Mifepristone in the central nucleus of the amygdala reduces yohimbine stress-induced reinstatement of ethanol-seeking. Neuropsychopharmacology. 37, 906-918.

Sinha, R., 2013. Modeling relapse situations in the human laboratory. Behavioral Neurobiology of Alcohol Addiction. 13, 379-402.

Smeets, T., 2011. Acute stress impairs memory retrieval independent of time of day. Psychoneuroendocrinology. 36, 495-501.

Sobell, L.C., Sobell, M.B., 2003. Alcohol consumption measures. In: J.P. Wilson (Ed.), Assessing Alcohol Problems (2 Ed.) National Institute on Alcohol Abuse and Alcoholism, Rockville, MD.

Sufka, K.J., Warnick, J.E., Pulaski, C.N., et al., 2009. Antidepressant efficacy screening of novel targets in the chick anxiety-depression model. Behav.Pharmacol. 20, 146-154.

Sullivan, J.T., Sykora, K., Schneiderman, J., et al., 1989. Assessment of alcohol withdrawal: The revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). Br.J.Addict. 84, 1353-1357.

Tiffany, S.T., Carter, B.L., Singleton, E.G., 2000. Challenges in the manipulation, assessment and interpretation of craving relevant variables. Addiction. 95, 177-187.

Turkkan, J.S., Stitzer, M.L., McCaul, M.E., 1988. Psychophysiological effects of oral ethanol in alcoholics and social drinkers. Alcoholism: Clinical and Experimental Research. 12, 30-38.

Uhart, M., Weerts, E.M., McCaul, M.E., et al., 2013. GABRA2 markers moderate the subjective effects of alcohol. Addict.Biol. 18, 357-369.

van Stegeren, A.H., Roozendaal, B., Kindt, M., et al., 2010. Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. Neurobiol.Learn.Mem. 93, 56-65.

Vendruscolo, L.F., Estey, D., Goodell, V., et al., 2015. Glucocorticoid receptor antagonism decreases alcohol seeking in alcohol-dependent individuals. J.Clin.Invest. 125, 3193-3197.

Vendruscolo, L.F., Barbier, E., Schlosburg, J.E., et al., 2012. Corticosteroid-dependent plasticity mediates compulsive alcohol drinking in rats. J.Neurosci. 32, 7563-7571.

Wand, G.S., Oswald, L.M., McCaul, M.E., et al., 2007. Association of amphetamine-induced striatal dopamine release and cortisol responses to psychological stress. Neuropsychopharmacology. 32, 2310-2320.

Wand, G.S., Weerts, E.M., Kuwabara, H., et al., 2013. The relationship between naloxone-induced cortisol and delta opioid receptor availability in mesolimbic structures is disrupted in alcohol-dependent subjects. Addict.Biol. 18, 181-192.

Watson, S., Gallagher, P., Porter, R.J., et al., 2012. A randomized trial to examine the effect of mifepristone on neuropsychological performance and mood in patients with bipolar depression. Biol.Psychiatry. 72, 943-949.

Wechsler, D., Coalson, D.L., Raiford, S.E., 1997. WAIS-III: Wechsler Adult Intelligence Scale. Psychological Corporation San Antonio, TX.

Weerts, E.M., Kim, Y.K., Wand, G.S., et al., 2008. Differences in δ-and μ-opioid receptor blockade measured by positron emission tomography in naltrexone-treated recently abstinent alcohol-dependent subjects. Neuropsychopharmacology. 33, 653-665.

Weerts, E.M., Wand, G.S., Kuwabara, H., et al., 2011. Positron emission tomography imaging of mu-and delta-opioid receptor binding in alcohol-dependent and healthy control subjects. Alcoholism: Clinical and Experimental Research. 35, 2162-2173.

Weerts, E.M., McCaul, M.E., Kuwabara, H., et al., 2013. Influence of OPRM1 Asn40Asp variant (A118G) on [11C]carfentanil binding potential: Preliminary findings in human subjects. Int.J.Neuropsychopharmacol. 16, 47-53.

Yararbas, G., Pogun, S., 2011. Tamoxifen and mifepriston modulate nicotine induced conditioned place preference in female rats. Brain Res.Bull. 84, 425-429.