Repurposing alpha1 noradrenergic antagonists for alcoholism treatment

Principal Investigator: John Curtin, PhD
Co-Investigator: Aleksandra Zgierska, MD
Study Coordinators: Jesse Kaye, MS & Cindy Colombo, MS

The University of Wisconsin – Madison

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Study Protocol
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Project Summary

The first objective of the current proposal is to implicate norepinephrine alpha1 receptor involvement in reactivity to stressful events in humans by using a sophisticated laboratory stress task in conjunction with an alpha1-blocker, Prazosin. The second objective is to determine whether Prazosin, an FDA-approved blood pressure medication, is effective at reducing stress-reactivity among abstinent alcoholics who are trying to quit drinking. No currently available pharmacotherapy treatment options for alcoholism are specifically designed to prevent relapse caused by stress, which is a common hurdle in the way of attaining long-term recovery. As the pharmaceutical industry has drastically reduced its investment in developing novel medications to treat alcoholism in recent years, it is becoming increasingly essential to identify currently available drugs with known neurobiological mechanisms, such as Prazosin, that may be effective treatment alternatives for addiction.

The current study aims to evaluate the effects of Prazosin on stress-reactivity in alcoholics in early abstinence versus healthy volunteers. Participants will take either Prazosin or a placebo pill at two laboratory sessions, after which they will complete a stress task. The task will consist of three conditions exposing participants to unpredictable shock, predictable shock, or no shock. The eye blink startle response will be measured as a physiological index of the participants' reactivity to the stressful task (i.e., predictable and unpredictable shocks). Previous research has consistently demonstrated that drugs that reduce the stress response, such as alcohol and benzodiazepines, reduce the startle response specifically to unpredictable stressors. Furthermore, drug deprivation among drug dependent individuals (e.g., nicotine, marijuana or alcohol) selectively increases the startle response during unpredictable stressors. This is an attractive lab task as very similar methods (e.g. unpredictable shock) and measures (e.g., startle) have been used extensively in rodents and non-human primates, so the field has a rich understanding of the neurobiology involved in this stress system. In particular, the neurotransmitter norepinephrine has been critically implicated in the stress response, and Prazosin, a drug that blocks norepinephrine alpha1 receptors, has been shown to reduce stress-induced relapse in rodent models of alcoholism. This study will examine whether Prazosin reduces the startle response during unpredictable stressors in abstinent alcoholics in early recovery vs. healthy volunteers. These findings would suggest that norepinephrine alpha1 receptors are involved in stress-reactivity in humans and that Prazosin may be an effective treatment of stress-induced relapse for alcoholics pursuing abstinence.

Given the tremendous cost associated with conducting large scale clinical trials to vet treatments for addiction, the current proposal represents an efficient laboratory-based screening procedure to evaluate the potential efficacy of novel pharmacotherapies. This type of translational research aims to expand treatment options for the eighteen million people in the United States who suffer from an alcohol use disorder.

Background and Significance

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DRUG ADDICTION, NOREPINEPHRINE, AND UNPREDICTABLE STRESSORS

Stressors are potent instigators of relapse to drug use in clinical research with abstinent drug dependent humans and preclinical relapse models (i.e., stressor-induced reinstatement) in rodents. In rodents, brain norepinephrine (NE) levels are elevated in response to both discrete stressors[1–6] and drug deprivation[7,8]. Similarly in humans, plasma and CSF NE-metabolite levels are elevated during alcohol withdrawal[9–11] and in response to acute stressors[12–14]. Manipulations that increase central nervous system (CNS) NE levels (e.g., yohimbine, NET inhibitor, NE injections) increase drug-seeking behavior in rodents and non-human primates across a wide class of drugs including nicotine[15], alcohol[16], cocaine[17–19], heroin[20], and methamphetaime[21]. Although all central noradrenergic receptor classes (post-synaptic α1 & ß; autoreceptor α2) have been implicated in the etiology of addiction, NE-α1 receptors may be particularly important in stress-induced relapse. Prazosin, a selective noradrenergic NE-α1 receptor antagonist, blocked escalation of drug-seeking behavior in rodent models of dependence for alcohol[22], cocaine[23], opioids[24], and nicotine[25]. Prazosin also reduces baseline alcohol consumption[22], particularly in alcohol-preferring genetic strains of rats[26–28]. Prazosin, but not ß antagonists or α2 agonists, dose-dependently reduced dysfunctional brain reward thresholds observed during nicotine withdrawal[25]. Most critically, systemic administration of Prazosin blocks reinstatement of alcohol self-administration that is induced by unpredictable footshock stressors or directly increased NE via yohimbine[29]. Thus NE-α1 antagonists have broad treatment relevance for reducing stressor-induced relapse in drug addiction.

Unfortunately, "stress" remains ill-defined and inconsistently operationalized in both basic research and clinical research on drug addiction in humans. Research on stress responding implicates central nervous system, endocrine, and peripheral biological systems that produce changes in affect, arousal, and attention[30–33]. However, research is rapidly accruing to suggest that the CNS negative affect component of the stress response, and more specifically, acute negative affective response to a subset of stressors characterized by stressor unpredictability, may provide a critical mechanism to account for stressor-induced relapse among drug dependent rodents and humans[34]. These unpredictable stressors (i.e., ambiguous, low probability, temporally imprecise stressors) appear to produce phenomenologically distinct affective responding via partially separable neural mechanism relative to predictable stressors (i.e., well-defined, high probability, imminent stressors)[35].

STARTLE POTENTIATION DURING STRESSOR EXPOSURE

Programmatic affective neuroscience research has relied heavily on startle potentiation as a primary measure of defensive system activation to parse the neural mechanisms involved in response to unpredictable vs. predictable stressors. The use of startle potentiation to index affective response to stressors among rodents, non-human primates, and humans has provided an important animal-human translational bridge in this research[36–38]. As such, we have detailed knowledge of the neurobiology of the startle response and its potentiation[37–40]. Startle potentiation also can be measured with both minimal disruption of task-related processes and reduced influence by demand characteristics than measures under volitional control (e.g., self-report).

In preclinical models, startle reflex potentiation during unpredictable stressors has strongly implicated NE and corticotropin-releasing factor (CRF) sensitive pathways through the lateral divisions of the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST)[35,41,42]. In contrast, distinct pathways through the medial division of the CeA appear responsible for startle potentiation during predictable stressors[35,43,44]. A large corpus of research implicates CRF as a critical mediator of the stress response[35,45] and NE is a powerful modulator of extrahypothalamic CRF[46]. Prazosin reduces startle potentiation in rodents due to manipulations of CRF[46] and other stress-relevant neurotransmitter systems (e.g., DA[47]). In humans, the startle response is potentiated by pharmacological challenge that elevates NE levels via yohimbine in healthy controls and particularly in drug dependent populations[48,49]. However, the effect of Prazosin on startle potentiation has never been examined in humans to date. Thus, startle potentiation during unpredictable stressors represents 1) a psychophysiological index of heightened response to stressors, 2) is sensitive to CNS NE system activation in rodents, 3) has well known neurobiological substrates in rodents, and 4) and can be assessed in rodents, non-human primates, and humans, positioning it as an attractive translational measure.

THE NO SHOCK, PREDICTABLE SHOCK, UNPREDITABLE SHOCK (NPU) TASK

Research in affective neuroscience has relied extensively on cued stressor (e.g., threat of electric shock) tasks to explicate psychological and neurobiological mechanisms involved in the negative affective response to stressors in

animals and humans. Christian Grillon at NIH has developed a cued-stressor task called the "No Shock, Predictable Shock, Unpredictable Shock" (NPU) task to carefully contrast response to unpredictable vs. predictable stressors[50,51]. Predictable shock conditions involve administration of 100% cue-contingent, imminent electric shock. Unpredictable shock conditions involve temporally uncertain administration of shock. Startle potentiation during unpredictable shock (relative to no-shock blocks) provides the primary measure of negative affective response to stressors that in rodents engages both NE system activity and elicits etiologically relevant behaviors for addiction (i.e., stressor-induced reinstatement). This task represents a direct translation of methods and measures used by Davis, Walker and colleagues to parse neural mechanisms involved in response to unpredictable vs. predictable stressors[35,43]. We will use this task in the proposed research with humans.

PRELIMINARY EVIDENCE

Research from our lab provides compelling evidence that unpredictable stressors elicit startle potentiation that is attenuated by anxiolytic drugs and exacerbated by abstinence in drug dependent users. Alcohol administration robustly suppresses startle potentiation during tasks involving unpredictable stressors, including the NPU task[52,53]. Similarly, benzodiazepines reduce startle potentiation to unpredictable stressors[54]. Furthermore, these anxiolytic effects are specific to unpredictable stressors as these drugs produce minimal effects on startle potentiation to predictable stressors. These studies provide evidence that startle potentiation in the NPU and similar tasks is a sensitive index of the effects of pharmacologic agents on negative affective response to stressors.

In a parallel line of research we have demonstrated that these tasks have proved effective at differentiating drug deprived, dependent users from non-dependent healthy controls for a variety of drugs including tobacco[55], marijuana[56], and alcohol[57]. Cigarette smokers displayed elevated startle response selectively during unpredictable stressors (vs. predictable stressors) after 24-hours of nicotine deprivation compared to non-deprived smokers[55]. Dependent marijuana users show significantly increased startle potentiation during unpredictable stressors after three days of abstinence relative to non-deprived smokers and non-smoker controls[56]. Furthermore, alcoholics in 1-8 weeks early abstinence show larger startle potentiation to unpredictable stressors (vs. predictable stressors) compared to healthy controls in a variant of the NPU task[57]. This demonstrates that these tasks provide an objective non-invasive physiological biomarker of the effects of drug deprivation on affective responses to unpredictable stressors that represents an etiologically relevant cross-drug phenomenon.

We believe these data in humans, in combination with ample evidence from rodent models, supports our use of the NPU task to advance translational research aimed at identifying mechanisms for novel pharmacological treatments of stress induced relapse mechanisms in addiction. We also provide this preliminary evidence to confirm that our laboratory has the necessary expertise to work with clinical samples of drug dependent users, acutely administer drugs safely in the laboratory, and measure psychophysiological response (including startle potentiation) during stressor tasks[55–58].

REPURPOSING PRAZOSIN

Though Prazosin has been clinically available for forty years only recently has human research focused on addiction treatment. Fox and colleagues[59] demonstrated that among treatment-seeking alcohol dependent patients, Prazosin (16mg/day, 4wks) reduced self-reported alcohol craving and negative affect in response to guided imagery exposure to stress. This is the first evidence in humans implicating NE-α1 in stress-induced relapse mechanisms in addiction. However, this measure of stress imagery reactivity has less direct ties to preclinical models of stress reactivity than our model of startle potentiation in the NPU task. One additional study by Simpson et al[60] found that Prazosin (16mg/day, 6wks) reduced the number of drinks per day and number of days drinking in the final weeks of an RCT for alcohol dependence treatment. These first positive findings in humans instill excitement regarding Prazosin, particularly since they likely underestimate the true potential efficacy of NE-α1 antagonists because they do not focus specifically on stress-induced relapse outcomes. These studies demonstrated that in drug dependent samples Prazosin has a good safety profile and was generally well tolerated with minimal side effects, which is an important consideration given that aversive side effects are often a primary factor limiting the clinical effectiveness of psychiatric medications. Further considerations that increase the excitement for repurposing Prazosin for addiction treatment is that it is the most lipid soluble NE-α1 antagonist increasing its ability to cross the blood-brain barrier, is highly selective for the NE-α1 receptor (K_i=0.12-0.31), has a better side effect profile than other NE receptor modulators (e.g., Clonidine), and it is available in generic formulation[61–64]. Doxazosin another NE-α1 antagonists reduces alcohol drinking in alcohol-preferring rats[65], and is being investigated for cocaine dependence in humans[66,67], but Prazosin is the only drug shown to specifically reduce stress-induced relapse in rats and has preliminary human evidence in alcoholism. The use of Prazosin is not contraindicated with

first line treatments for alcohol dependence and has been found to increase the effectiveness of Naltraxone to reduce alcohol consumption in rodent models[68]. Finally, there has been increased excitement in the field over the possibility that Prazosin may be an effective treatment for PTSD[69–73]. This may lead to fruitful lines of future research within the NIMH RDoC initiative that aims to focus on transdiagnostic dimensions, such as heightened stress-reactivity, which may represent a common feature in PTSD and addiction. In summary, in addition to the strong preclinical evidence implicating NE-α1 activation as a key mechanism involved in stress-induced relapse; there is mounting support from human studies to justify further examination of the potential for Prazosin to alleviate the exaggerated stress reactivity observed in alcohol and drug dependent individuals pursuing abstinence.

DIVERSITY AND HEALTH DISPARITIES

There are clear health disparities in alcoholism and substance use disorders that manifest as greater negative health consequences and cost for low SES and racial minority populations[74]. The stress-induced relapse mechanisms that are the focus of the current grant may be particularly problematic for low SES populations where significant life stressors are more prevalent. Developing and repurposing medications that can treat this particular mechanism of relapse will have a relatively greater benefit to individuals who are at risk to experience more frequent and intense stressors.

Innovation

This research is innovative in its attempt to directly translate research findings from animal models to the examination of human addiction etiology and treatment ("bench to bedside"). There is substantial evidence implicating stress-related neurocircuitry in the etiology of addiction and relapse in rodents but the evidence in humans is much more limited[7,34,45,75]. This application uses a well-validated translational task with strong connections to the preclinical literature. The use of reliable biomarker of neuroadaptations resultant from chronic drug use that are also sensitive to the acute effects of both prescription and non-prescription anxiolytic drugs (e.g., benzodiazepines and alcohol) allows for generalizations across a wide class of drugs that alter the stress response[52,54,76].

This research is also innovative in its attempts to 'repurpose' Prazosin from its original FDA approved use for hypertension to a new purpose of relapse prevention in alcoholism. Thomas Insel, director of NIMH, clearly argues that given substantial cuts in R&D budgets for new psychiatric drugs at most major pharmaceutical companies the near term outlook of novel molecular targets is grim[77,78]. Therefore, it is critical to take advantage of existing compounds acting on neurotransmitter systems that have been implicated in the etiology of psychiatric disorders. The current proposal is one clear example of this theme to screen Prazosin for repurposing for the treatment of stress-induced relapse in alcoholism by using this cost effective biomarker as a surrogate endpoint.

This laboratory approach of identifying biomarkers of stress-reactivity that can be measured along a continuum from normal to abnormal addresses the current paradigm shift being advocated by the NIMH's Research Domain Criteria (RDoC). RDoC calls for a dimensional approach to studying the roots of human behavior at multiple levels of analysis that cuts across DSM diagnostic categories [77,79–82]. The current proposal aims to examine a number of the constructs (e.g., responses to acute threat (fear) or potential harm (anxiety)) defined within the Negative Valence System, using a paradigm recommended by the NIMH workgroup[83]. Indeed, although this paradigm is used to study abnormal processes in abstinent alcoholics, it represents a framework to evaluate abnormal stress-reactivity that spans not only multiple drugs of addiction (e.g., tobacco, marijuana); but likely other disorders (e.g., PTSD) that may share a common neuroadaptation that manifests as a heightened stress response.

Study Objectives and Aims

Objectives

First Objective: To confirm NE-α1 involvement in startle potentiation during unpredictable stressors in humans with alcoholism and matched controls. This will support the use of this laboratory assay that implicates NE mechanisms as a surrogate endpoint in this 'proof-of-concept' trial aimed at identifying novel treatment targets.

Second Objective: Provide preliminary evidence that Prazosin is effective at reducing stressor-reactivity, particularly among alcoholics in early abstinence, thereby advancing efforts to repurpose Prazosin for treatment of stressor-induced relapse in addiction.

To accomplish these objectives we propose three specific aims:

Specific Aims

AIM 1: Examine the effects of a NE-α1 antagonist (Prazosin) on responses to unpredictable stressors.

1.1 Prazosin (2mg vs. placebo) will reduce negative affective response to unpredictable stressors measured via startle potentiation, translating preclinical to clinical neuroscience.

1.2 Prazosin (2mg vs. placebo) will reduce negative affective response to unpredictable stressors measured via self-report, identifying uniquely human phenomenology.

AIM 2: Confirm evidence of exacerbated stress reactivity in abstinent alcoholics (vs. matched controls).

2.1 Abstinent alcoholics (vs. matched controls) will display elevated negative affective response to unpredictable (vs. predictable) stressors measured via startle potentiation, conceptually replicating previous findings that provide a laboratory assay of stress-induced relapse mechanisms in addiction.

2.2 Abstinent alcoholics (vs. matched controls) will display elevated negative affective response to unpredictable stressors measured via self-report.

AIM 3: Examine the differential effects of Prazosin in abstinent alcoholics vs. matched controls.

3.1 The predicted effects of Prazosin on reducing negative affective responses to unpredictable stressors as measured via startle potentiation (Aim 1.1) and self-report (Aim 1.2) will be moderated by alcoholism, such that the effects of Prazosin will be larger in abstinent alcoholics than matched volunteers.

Study Population

Participant Population and Recruitment

The site of the proposed research is the University of Wisconsin-Madison. Prospective participants will be recruited through the greater Madison community via paper fliers and online advertisements. Paper flyers will be posted in a variety of locations throughout the UW campus and community (e.g., laundromats, restaurants, gas stations, etc.). Paper flyers will also be posted at targeted locations to recruit recovering alcoholics including community support groups (e.g., AA meeting locations) and local in- and out-patient treatment facilities for alcoholism (e.g., Gateway Recovery, Tellurian, ARC, Connections Counseling, etc.). Participants over the age of 50 will be excluded to limit potential extraneous variance related to normal aging processes. To decrease the risk of adverse psychological reactions in response to startle probes and electric shock, participants will be excluded if they have a lifetime diagnosis of serious and persistent mental illness (SPMI; e.g. bipolar disorder, schizophrenia, any psychosis).

Thirty-six alcohol dependent participants in early protracted abstinence (at least 1 week but no more than 8 weeks since last alcohol use) will be recruited for the study. Thirty-six non-alcoholic participants will also be recruited to will serve as the comparison group. Participants in the alcoholic and control groups will be matched on selected characteristics including age, sex, education level, daily nicotine use, and Axis I psychopathology. These characteristics will be matched at the group level such that the average and range of both groups will be comparable.

Inclusion Criteria

All participants:
Can read and write in English.
Ages of 18-50 years.

Alcoholic Participants:
The alcoholic participants will have at least one but no more than eight weeks completely free from alcohol consumption.

Control Participants:
The control participants must not meet criteria for any current or lifetime history or substance dependence other than nicotine dependence.

Exclusion Criteria

Exclusion criteria are divided into three broad categories of Medical, Psychiatric/Behavioral, and Medications/Therapies. These criteria are all implemented to protect human subject safety, except where noted with an asterisk (*) denoting scientific/theoretical reasons.

Medical exclusion
•Self-reported acute or unstable illness precluding a safe and reliable study participation; medical conditions precluding participation will include, for example, unstable angina, history of myocardial infarction, history of congestive heart failure, chronic renal or hepatic failure, pancreatitis, Meniere's disease, benign positional vertigo, narcolepsy.
•A pre-existing hypotension (systolic less than 100) or orthostatic hypotension (systolic drop greater than 20mmHg after two minutes standing or any drop accompanied by dizziness).
•Systolic blood pressure greater than 160
•Tachycardia >=100
•Allergy or previous adverse reaction to prazosin or other alpha1-NE antagonist.
•Women of childbearing potential (see definition below) must agree to use one of the following forms of birth control until after study completion. Acceptable birth control is defined as the following methods of contraception: abstinence; hormonal contraceptives (e.g. combined oral contraceptives, patch, vaginal ring, injectables, and implants); intrauterine device (IUD) or intrauterine system (IUS); vasectomy of partner and tubal ligation; "single" barrier methods of contraception (e.g. male condom, female condom, cervical cap, diaphragm, contraceptive sponge) with use of spermicide; or "double barrier" method of contraception (e.g. male condom with diaphragm, male condom with cervical cap).
•Women who are breastfeeding will be excluded.
•*Color blindness. [Excluded because colored images identify critical differences between predictable, unpredictable, and no-shock conditions in the NPU stress-reactivity task.]

Psychological/Behavioral exclusion
•Self-reported lifetime diagnosis of serious and persistent mental illness, including schizophrenia, schizoaffective disorder, psychotic disorder NOS, delirium, bipolar Disorder, borderline personality disorder, or any neurocognitive disorder.
•Any current active substance use disorder other than tobacco.
•Control Participants Only: Lifetime history of substance dependence.

Medications/Therapies exclusion
•Current use of prazosin or other alpha1-NE antagonist (e.g., doxazosin, terazosin).
•Previous adequate trial of prazosin for alcohol use disorder or PTSD.
•Sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra) will not be permitted during the study because of increased risk of hypotension in combination with alpha1-NE antagonists.
•*Stimulants (e.g., d-amphetamine, methylphenidate) or alternative medications with stimulant properties (e.g., ephedra). [Excluded because these medications directly alter CNS noradrenergic neurotransmission.]
•*Beta-blockers (e.g., propanolol), alpha2 agonists (e.g., clonidine, guanfacine), and SNRI anti-depressants (e.g., venlafaxine, duloxetine). [Excluded because these medications directly alter CNS noradrenergic neurotransmission.]

Note: Women of childbearing potential are females who have experienced menarche and do not meet the criteria for women **not** of childbearing potential. Women n**ot** of childbearing potential are females who are permanently sterile (e.g., hysterectomy, bilateral oophorectomy) or postmenopausal. Postmenopausal is defined as 12 consecutive months with no menses without an alternative medical cause.

Research Design and Methods Overview

Overview of Study Procedures

Seventy-two participants (36 alcoholic & 36 controls) will be recruited to participate in a double-blind, placebo-controlled, mixed-design, cross-over study examining the effects of a NE-α1 antagonist (prazosin) on the affective response to stressors. ~~Both study visits will take place in the Clinical Research Unit (CRU) inpatient unit at the University of Wisconsin Hospital.~~ A screening visit will take place in the Addiction Research Center at the University of Wisconsin – Madison. At the ~~first study~~ screening visit general laboratory procedures and risks and benefits will be explained to the participants and informed consent will be obtained from all participants. Inclusion/exclusion criteria will be confirmed using interviews conducted by a nurse, graduate level clinician, self-report questionnaires and urine test for pregnancy. Study ~~Nursing~~ staff will collect ~~a medical history,~~ current medication, orthostatic vital signs, and urine pregnancy test to confirm eligibility. For both participant groups, research staff will first confirm that the potential participant is free from severe and persistent mental illness. Following this, the more in-depth Substance Use Disorders Module of the SCID-Research Version (SCID-RV[84]) will be administered to confirm the alcoholic participants' status with respect to an Alcohol Dependence diagnosis and no lifetime history of substance dependence for control participants. Participants will complete a shock sensitivity rating as per standard procedures from our laboratory.

Eligible participants will be scheduled for two study visits that will take place in the Clinical Research Unit (CRU) inpatient unit at the University of Wisconsin Hospital. Modifiable inclusion/exclusion criteria will be re-confirmed at both study visits by the above procedures as well as a medical history and physical exam. Participants will be randomly assigned to drug order (prazosin 1st vs. 2nd visit) in a stratified blocked schedule by sex and group (alcoholic vs. control) using urn randomization procedures[85]. The randomization and double-blind will be implemented and maintained by the UW Pharmaceutical Research Center, which is located in the same building in the UW hospital as all study visits in the CRU. All participants are told that they will receive both 2mg of prazosin and placebo, one agent at each study visit. Drug/placebo order is counterbalanced between participants. However, neither participants nor researchers will be aware of whether prazosin or placebo is administered at each visit.

All subsequent procedures will be identical at the first and second study visits except for the assessment of study eligibility and the drug administered. Alcoholic participants will complete a brief alcohol craving questionnaire, a 6 item subset of the 14-item version of the Desires for Alcohol Questionnaire (DAQ[86]) to determine baseline craving levels. Women of childbearing potential participants will provide a urine sample to verify they are not pregnant. All participants will then provide a breath sample to verify an initial blood alcohol concentration (BAC) of 0.00 (Alcosensor IV; Intoximeters, Inc.). All participants will be asked to refrain from consuming alcoholic beverages or any other psychoactive drugs for 24 hours prior to the experimental session, to avoid any acute effects of alcohol. Next all participants will ingest one pill with a full glass of water.

Research assistants will then prepare the participant for the experimental task with physiological sensors. Next participants will complete a baseline assessment of their blink EMG response and a shock sensitivity rating as per standard procedures from our laboratory. Based on the known pharmacodynamics of prazosin[87], participants will begin the NPU task (see below) exactly 90 minutes after drug administration. During the stress-reactivity task participants will view geometric shapes and words presented on a computer monitor. Participants will be asked to answer short questions during this procedure, which will be audio recorded for later scoring for accuracy. At various times during this procedure, brief electric shocks will be administered to the fingers of the participants' hand. The intensity of all shocks will be within the range that they indicated they could tolerate during the shock sensitivity procedure. In addition, acoustic white noise probes will be presented through earphones at random points during the procedure. These probes are used to elicit a startle blink reflex, which is used to index emotional response. After completion of the task, participants will complete questionnaires related to their emotional state during the task and a battery of self-report questionnaires to assess alcohol use history, trait affect and broadband personality traits.

After the NPU task participants will remain in the CRU inpatient unit overnight to monitor and assess for side effects under medical supervision by CRU staff. Participants second appointment will be scheduled 7 days (+/- 2 days) after the first study visit. Participants with unusable data at the first study visit (e.g., excessive physiological artifact, insufficient understanding of NPU task, startle non-responder) will not return for the second study visit. This study visit will be identical to the first study visit except for the drug administered (Prazosin vs. Placebo) and eligibility screening procedures. At the end of the first visit participants will be paid $150. At the second visit participants will be paid $150 for the study visit and a $75 bonus for study completion, debriefed, and released. Participants who do not complete the study (e.g., not eligible, refuse consent, withdraw, etc.) will be paid $25/hour for their time up to $150/visit. Alcoholic participants will be required to demonstrate that they are not experiencing significantly elevated levels of alcohol craving (Desires for Alcohol Questionnaire) before they are released, per standard lab procedures.

Table 1: Overview of Study Procedures

CATEGORY	PROCEDURE	STUDY VISIT 1 (Day 1)	STUDY VISIT 2 (Day 4-10)
SCREENING			
Eligibility	Consent, Inclusion/exclusion criteria	X	
Medical history/assessment	Medical history, concomitant medications, vital signs	X	
Alcohol Use	Timeline follow back; SCID - Substance Use Disorder	X	
	Blood alcohol concentration (BAC; via breath test)	X	X
Lab Chemistry	Urine pregnancy test	X	X
SAFETY			
Visit safety	Vital signs; Medication side effects	X	X
Acute craving (for study release)	6 item subset from Desire for Alcohol Scale	X	X
DRUG ADMINISTRATION			
Drug	Prazosin or placebo	2 mg prazosin or placebo	2 mg prazosin or placebo
OUTCOMES			
Stress reactivity	Startle potentiation and self-report negative affect from NPU task	X	X
Individual difference moderators	Self report: alcohol dependence severity, trait affect, personality trait	X	

Participant Reimbursement

All participants are paid $15 for time spent in the lab during the screening visit. All participants are paid $150/visit for time spent in the CRU for all study visits. Participants are provided a $75 bonus for completing all study visits. Participants who do not complete the study (e.g., not eligible, refuse consent, withdraw, etc.) will be paid $25/hour for their time at the CRU up to $150/visit. These modest inducements represent reasonable compensation for time spent in the study and cannot be considered coercive.

Visit #	Time	Reimbursement

Screening Visit	1 hour	$15
Study Visit 1	3 to 5 hours + overnight	$150
Study Visit 2	2 to 4 hours + overnight	$150
Study Completion Bonus		$75
Total		$39075

Statistical Analysis and Timeline

Statistical Analysis

Analyses for quantitative dependent measures will be accomplished within General Linear Models using R[88]. In the sections below, we briefly describe the primary factors in statistical models to evaluate each Specific Aim. All models will include number of days abstinent at Visit 1 as an interactive between-subjects regressor to evaluate if drug effects and outcomes are comparable across alcoholics who vary in their previous duration of abstinence (1-8 weeks) on study initiation, although this has not moderated the effects on NPU task in our preliminary studies. Numerous individual difference measures (e.g., sex, mood/anxiety disorder comorbidity, tobacco use, trait affect, alcohol dependence severity) are available to add to these models as interactive between-subject regressors in secondary analyses to evaluate possible individual difference moderators of drug effects.

Unpredictable startle potentiation and self-reported negative affect from the NPU task across Visits 1-2 will be analyzed in separate general linear models with repeated measures on Drug (2 mg prazosin vs placebo) and Condition (unpredictable vs. predictable shock). Participant Group (alcoholic vs. control) and Drug Order (prazosin: visit 1 vs visit 2) will also be included as an interactive between subjects regressors to increase power. **Specific Aim 1** predictions will be supported by significant Drug X Condition interactions with prazosin producing selectively larger reduction in startle potentiation and self-reported negative affect during unpredictable relative to predictable shock. **Specific Aim 2** predictions will be supported by significant Group X Condition interactions with abstinent alcoholics displaying selectively larger startle potentiation and self-reported negative affect during unpredictable relative to predictable shock. **Specific Aim 3** predictions will be supported by significant Drug X Group X Condition interactions with prazosin producing selectively larger reduction in startle potentiation and self-reported negative affect during unpredictable relative to predictable shock to a greater degree among abstinent alcoholic than control participants.

Power Analyses for Sample Size Justification

Power analyses were conducted using the pwr package in R which calculates power based on the approach outlined by Cohen[89]. Power analyses were conducted to provide adequate power to detect comparable effect sizes observed in previous research from our laboratory that has used variants of the NPU task to examine acute drug effects (i.e., moderate dose of alcohol), drug deprivation effects (24 hours of nicotine deprivation among daily smokers), or differences between alcoholics in early abstinence (1-8 weeks) vs. matched controls. In the current study, N = 72 will provide 85% power to detect the effect of prazosin on startle potentiation during unpredictable (vs. predictable) stress in alcohol dependent participants (vs. controls) assuming a conservative small effect size ($\Delta R2 = .10$) in a model containing the predictors noted above ($R2 = .30$).

Timeline Justification

This project aims to be completed within three years. This goal is reasonable given our team's expertise in conducting psychophysiological laboratory research working with drug administration (i.e., alcohol and nicotine), community participants, and stress reactivity. Our lab has the experience and infrastructure to recruit and retain a sample of this size. We recently completed a pilot study for the current application that recruited 115 participants (58 alcoholic and 57 matched controls) over the course of 14 months. This study involved diagnostic interviews and stress-reactivity task over the course of two study visits. The current study will plan to initially consent eighty-five participants with a final sample size goal of seventy-two participants. Based on prior experience we anticipate that 85% of eligible participants will return to complete all study visits. A total of 157 complete visits (85 first, 72 second) will be conducted. If we conservatively expect to average 2 study visits per week to account for no-shows, failed eligibility criteria, and equipment failure we anticipate completing data collection within 1.5-2 years.

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