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ORIGINAL INVESTIGATION

Acute prazosin administration does not reduce stressor reactivity in healthy adults

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Abstract

Rationale Norepinephrine plays a critical role in the stress response. Clarifying the psychopharmacological effects of norepinephrine manipulation on stress reactivity in humans has important implications for basic neuroscience and treatment of stress-related psychiatric disorders, such as posttraumatic stress disorder and alcohol use disorders. Preclinical research implicates the norepinephrine alpha-1 receptor in responses to stressors. The No Shock, Predictable Shock, Unpredictable Shock (NPU) task is a human laboratory paradigm that is well positioned to test cross-species neurobiological stress mechanisms and advance experimental therapeutic approaches to clinical trials testing novel treatments for psychiatric disorders.

Objectives We hypothesized that acute administration of prazosin, a noradrenergic alpha-1 antagonist, would have a larger effect on reducing stress reactivity during unpredictable, compared to predictable, stressors in the NPU task.

Methods We conducted a double-blind, placebo-controlled, crossover randomized controlled trial in which 64 healthy adults (32 female) completed the NPU task at two visits (2 mg prazosin vs. placebo).

Results A single acute dose of 2 mg prazosin did not reduce stress reactivity in a healthy adult sample. Neither NPU startle potentiation nor self-reported anxiety was reduced by prazosin (vs. placebo) during unpredictable (vs. predictable) stressors.

Conclusions Further research is needed to determine whether this failure to translate preclinical neuroscience to human laboratory models is due to methodological factors (e.g., acute vs. chronic drug administration, brain penetration, study population) and/or suggests limited clinical utility of noradrenergic alpha-1 antagonists for treating stress-related psychiatric disorders.

Keywords Stress · Prazosin · Noradrenaline · Startle response · Startle potentiation · Posttraumatic stress disorder · Alcohol use disorder

Introduction

Initial excitement for prazosin as a promising treatment for posttraumatic stress disorder (PTSD) and alcohol use disorder (AUD) has recently been tempered by larger clinical trials, which failed to show improvement in clinical outcomes (Petrakis et al. 2016; Raskind et al. 2018; Simpson et al. 2018). Prazosin is a norepinephrine alpha-1 (NE-α1) antagonist originally developed as an antihypertensive medication that has widespread actions in both the peripheral and central nervous systems. Prazosin's ability to penetrate the blood-brain barrier and the well-documented role of NE in arousal, sleep, and stress spurred researchers to test prazosin as a novel treatment for PTSD (for review, see Hendrickson and Raskind 2016). Early studies demonstrated positive clinical outcomes related to nightmares, sleep disturbance, and patients' overall functioning in PTSD (Raskind et al. 2003, 2007, 2013). Follow-up studies suggested prazosin may reduce relapse in

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AUD (Simpson et al. 2009; Fox et al. 2012), which is highly comorbid and shares stress-related etiology with PTSD (McCarthy and Petrakis 2010; Gilpin and Weiner 2017). Understanding why the findings of most early small trials failed to replicate and examining if prazosin may improve other symptom targets remain pressing scientific and clinical questions (Krystal et al. 2017; Haass-Koffler et al. 2018).

Efforts to repurpose prazosin grew from robust animal neuroscience literature that clearly demonstrated NE's broad role in coordinating the body's response to stress (Berridge and Waterhouse 2003; Arnsten 2009). In rodents, brain NE levels are elevated in response to discrete stressors (Pacák et al. 1995; Galvez et al. 1996). Similarly, manipulations that increase NE release or NE-receptor binding elicit arousal and stress-related behaviors (Varty et al. 1999; Berridge 2008). The NE system and neural circuits can develop sensitized responses to acute stressors following exposure to prolonged or intense stressors or chronic alcohol/drug use (Smith and Aston-Jones 2008; Koob 2009; Rajbhandari et al. 2015). Although dysregulation in these NE neural systems may occur via multiple pathways, the resulting exaggerated stress reactivity may represent a transdiagnostic feature and viable treatment target for both PTSD and addiction. Indeed, preclinical rodent models of stress-induced reinstatement of alcohol-seeking behavior have found promising effects of prazosin (Lê et al. 2011; Funk et al. 2016). Despite this neuroscientific foundation, repurposing prazosin has proceeded largely without basic psychopharmacology research in humans and has failed to include mechanism-relevant clinical outcomes such as exaggerated stress reactivity (but see Fox et al. 2012; Verplaetse et al. 2017).

Research is rapidly accruing to suggest that stress reactivity, and more specifically, acute response to a subset of stressors that are unpredictable (vs. predictable), may provide a critical mechanism to account for many maladaptive outcomes among stress-related psychiatric disorders (e.g., relapse, Kaye et al. 2017; Koob 2009). These unpredictable (i.e., ambiguous, ill-defined) stressors appear to produce phenomenologically distinct responses via overlapping yet partially separable neural mechanisms relative to predictable (i.e., well-defined, imminent) stressors (Davis et al. 2010). Unpredictable stressors and NE manipulations are ubiquitous in behavioral neuroscience animal models that probe anxiety-like and drug-seeking behaviors. For instance, unpredictable footshock and yohimbine challenge are widely used to instigate reinstatement of previously extinguished drug-seeking behavior, a model for stress-induced relapse (for review see Mantsch et al. 2016). Moreover, unpredictability is a cardinal feature of the typical stressors that humans experience in their daily lives (e.g., financial security, interpersonal conflicts); these types of stressors often exacerbate PTSD symptoms and precede relapse in addiction. As such, examination of NE mechanisms in stress reactivity in humans may benefit from evaluation of tasks that can parse unpredictable vs. predictable stressors.

To parse the neural mechanisms involved in response to unpredictable vs. predictable stressors, affective neuroscience has relied heavily on startle potentiation, an important animal-human translational bridge (Davis et al. 2010). As such, we have detailed knowledge of the neurobiology of the startle response and its potentiation. In preclinical rodent models, startle potentiation during unpredictable stressors has implicated NE- and corticotropin-releasing factor (CRF)-sensitive pathways through the lateral divisions of the central amygdala and bed nucleus of the stria terminalis (Walker et al. 2009; Davis et al. 2010). In contrast, distinct pathways through the medial division of the central amygdala appear responsible for startle potentiation during predictable stressors (Walker and Davis 1997; Davis et al. 2010). NE is a powerful modulator of extrahypothalamic CRF and many stress-related behaviors (Berridge and Dunn 1989; Gresack and Risbrough 2011). In rodents, acute prazosin pretreatment reduces startle potentiation elicited by direct administration of CRF, suggesting that CRF-enhanced startle is NE-α1 dependent (Gresack and Risbrough 2011). Prazosin administration prior to unpredictable stressors (e.g., restraint and inescapable tail-shock) reduces subsequent increases in startle response in rodents (Manion et al. 2007). In humans, the startle response is potentiated by pharmacological challenge that elevates NE levels via yohimbine in healthy adults and patients with PTSD or alcohol/drug addiction (Morgan et al. 1993, 1995; Stine et al. 2001). Thus, startle potentiation during unpredictable stressors (1) represents a psychophysiological index of heightened response to stressors, (2) has well known neurobiological substrates in rodents, and (3) can be assessed across species, positioning it as an attractive translational measure. However, the effect of an NE-α1 antagonist on startle potentiation has not been examined in humans to date.

Grillon and colleagues developed the No Shock, Predictable Shock, Unpredictable Shock (NPU) task to contrast responses to unpredictable vs. predictable stressors (Schmitz and Grillon 2012). Predictable shock conditions involve administration of 100% cue-contingent, imminent electric shock. Unpredictable shock conditions involve temporally and probabilistically uncertain administration of shock. Startle potentiation during unpredictable shock (relative to no-shock blocks) provides the primary measure of stressor reactivity. This task represents a direct translation of preclinical methods and measures to parse the neural mechanisms involved in response to unpredictable vs. predictable stressors (Davis et al. 2010).

The NPU and related tasks have been used to identify common phenotypic characteristics of stress-related disorders and to probe pharmacological effects of anxiolytic agents (Schmitz and Grillon 2012; Shankman et al. 2013; Kaye et al. 2017). Individuals with PTSD, AUD, and panic disorder

display elevated startle potentiation to unpredictable stressors but not predictable stressors (Grillon et al. 2008, 2009b; Gorka et al. 2013; Moberg et al. 2017). This hypersensitivity to unpredictable stressors is not indicative of psychopathology broadly, as it is not observed in major depressive disorder or generalized anxiety disorder (Grillon et al. 2009b; Shankman et al. 2013). Pharmacological manipulations with expected anxiolytic effects (i.e., acute benzodiazepines and alcohol, chronic SSRIs) selectively reduce startle potentiation during unpredictable (vs. predictable) stressors (Grillon et al. 2006, 2009a; Moberg and Curtin 2009; Bradford et al. 2013). These studies support the utility of startle potentiation during unpredictable stress in the NPU task as a sensitive testbed to detect transdiagnostic perturbations in stress-related disorders and screen potential novel medications to target these processes. Examining prazosin's effects on startle potentiation in the NPU task would be particularly informative considering conflicting reports on the efficacy of prazosin as a novel treatment for PTSD/AUD and the paucity of basic psychopharmacology research on how prazosin affects stress reactivity in humans.

The current double-blind, placebo-controlled, crossover randomized controlled trial ($N = 64$) examined the effects of acute prazosin administration on stress reactivity during unpredictable and predictable stressors in the NPU task in healthy adults. This is the first study, to our knowledge, to examine the impact of prazosin on the startle response in humans, a physiological measure of stress reactivity. We examined startle potentiation and self-reported anxiety during the NPU task to include translational and subjective markers of stress reactivity. We hypothesized that prazosin (vs. placebo) would have a larger effect on reducing stress reactivity during unpredictable (vs. predictable) stressors. Positive results would suggest greater NE-α1 receptor involvement in acute responses to unpredictable relative to predictable stressors. Further, if NE-α1 antagonism reduces stress reactivity in humans, this could provide guidance for prioritizing outcomes in future clinical research (e.g., exaggerated startle in PTSD, stress-related relapse in addiction).

Materials and methods

Open science and preregistration

We took several steps to follow emerging open science guidelines to promote transparency and reproducibility. We preregistered the study design and data analysis plan prior to the start of data collection (Open Science Framework: https://osf.io/m8jmp/, ClinicalTrials.gov NCT02966340). We have reported how we determined our sample size, all data exclusions, all manipulations, and all measures in the study (see Supplement, Simmons et al. 2012). Finally, we have made the data, analysis code, and other study materials publicly available (https://osf.io/un6h6/).

Participants

We recruited 64 participants (32 female) from November 2016 to March 2018 from the greater community (see Supplement for CONSORT diagram and a priori power calculations).[1] Participants were 18 to 46 years old (mean age = 23 years, $SD = 5.3$ years). The racial composition of the sample was 64% White, 19% Asian, 6% Black, and 11% Other Race (8% Hispanic/Latino). We excluded those who self-reported: uncorrected auditory or visual problems; colorblindness; pregnancy, breastfeeding, or unreliable contraception in women; current medication with direct noradrenergic action (e.g., NE beta blockers, NE alpha2 agonists, NE alpha1 agonists, psychostimulants, SNRIs); current medication with acute anxiolytic or sedative properties (e.g., benzodiazepines, zolpidem); current medications with interactions with prazosin that increase side effect potential (e.g., sildenafil, trazadone); medical or psychiatric conditions that would contraindicate electric shock exposure or prazosin administration; substance use disorder other than tobacco; or severe, persistent mental illness. We excluded those with a blood alcohol concentration > 0.00%, non-negative urine pregnancy test (female only), heart rate < 56 or > 100 bpm, systolic blood pressure < 100 or > 160 mmHg, orthostatic hypotension, or symptoms upon standing (e.g., dizziness, lightheadedness, etc.) at any study visit. We compensated participants $390 for completing the study ($15 screening visit, $150 per study visit, $75 completion bonus).

General procedures

University of Wisconsin (UW) Madison Health Sciences Institutional Review Board approved all procedures. We determined preliminary eligibility during a phone screening and screening visit. At this visit at UW-Madison, we explained the study purpose and procedures and obtained written informed consent. Eligible participants completed two subsequent overnight study visits at the UW Hospital separated by approximately two weeks (mean = 12.6 days, range = 4–35 days, median = 8 days). At each study visit, we reassessed the self-reported and objectively measured eligibility criteria, and the study physician completed a medical history and physical exam. The procedures were identical at both study visits except where noted (see Fig. 1a for Study Procedures Flowchart).

[1] We preregistered a sequential recruitment plan to enroll an equal number of participants with AUD in early recover if we confirmed our hypothesis that prazosin reduced stress reactivity to unpredictable (vs. predictable) stressors in health adults (https://clinicaltrials.gov/ct2/show/NCT02966340).

Participants were administered prazosin or placebo (see "Prazosin dosing" section) and 60 min post-dose were seated in a dimly lit room approximately 45 in in front of a 20-in CRT computer monitor. Participants completed the General Startle Reactivity Task (75 min post-dose) and NPU Task (90 min post-dose). At the first study visit only, participants completed a battery of questionnaires on an iPad (Apple Inc.) using Qualtrics software (Provo, UT, USA) to assess demographics, trait affect, and broadband personality traits. Participants were admitted overnight to the hospital for safety monitoring and discharged the following morning after medical assessment. Participants were debriefed at the final study visit.

Prazosin dosing

Participants were orally administered 2 mg prazosin at one study visit and placebo at the other visit (randomly assigned order was counterbalanced between subjects). Participants and study staff were blind to drug administration order.[2] Participant blinding was assessed after the NPU Task; participants reported which pill they believed they received that day on a 5-point Likert scale (1 = "No Medication"; 5 = "Study Medication").

Shock sensitivity assessment

We coded our experimental tasks in MATLAB using the Psychophysics Toolbox extensions (Kleiner et al. 2007). At the screening visit, we measured participants' subjective tolerance using standard procedures from our laboratory (Kaye et al. 2016). Participants rated a series of 200 ms electric shocks of increasing intensity (7 mA maximum) administered to the distal phalanges of the second and fourth fingers of one hand. We used participants' subjective maximum tolerated shock from this procedure during the NPU task to control for individual differences in subjective shock tolerance. We used the same shock level in the NPU Task at both study visits.

General startle reactivity

We measured participants' resting startle response prior to initiating the NPU task at both study visits to assess their general startle reactivity (75 min post-dose). Participants viewed a white fixation cross in the center of the black screen while nine acoustic startle probes were presented, separated by 13–20 s (task length: 2.5 min). No other images were displayed on the screen, and no shocks were delivered. General startle reactivity was calculated as the mean raw startle response during this procedure (excluding first three habituation probes). We assessed general startle reactivity to evaluate individual differences in startle response and to determine if prazosin (vs. placebo) affects the startle response prior to the threat context.[3]

No shock, predictable shock, unpredictable shock task

Participants completed a version of the No Shock, Predictable Shock, Unpredictable Shock (NPU) task with demonstrated adequate psychometric properties for repeated administrations (see Fig. 1b; Kaye et al. 2016). During the NPU task, participants viewed a series of colored square "cues" displayed in the center of a computer screen with a black background. We presented cues in a blocked design with three conditions: No Shock (N), Predictable Shock (P), and Unpredictable Shock (U). Each shock condition was presented twice and separated by no shock conditions. Condition order was counterbalanced both within- and between-subjects (i.e., two condition orders: PNUNUNP, UNPNPNU), and participants completed the same order at both study visits. All blocks included six cues presented for 5 s separated by a variable inter-trial-interval (ITI; mean 17 s, range 14–20 s). A white fixation cross remained in the center of the monitor during the cues and ITI. We administered a 200-ms electric shock 200 ms prior to cue offset during every cue in the predictable shock conditions, so that the cue "predicted" that the shock would occur in several seconds. We administered electric shock at pseudorandom times during both cues and ITIs in the unpredictable shock condition (2 or 4.8 s post-cue onset and 4, 8, or 12 s post-cue offset), so that the occurrence of the shock was unpredictable by the participant. Twelve electric shocks were administered in each predictable and unpredictable shock condition. No electric shock occurred during the No Shock condition. We took several steps to ensure participants clearly understood the differences between task conditions based on our previously published methods (see Supplement and Kaye et al. 2016). Each block lasted approximately 2.5 m and the entire task lasted approximately 20 m. After the NPU task, participants retrospectively reported their anxiety/fear during each condition on a 5-point Likert scale (1 = "Not at all anxious/fearful," 5 = "Very anxious/fearful").

Startle probes occurred at 4.5 s post cue-onset on a pseudorandom subset of 8 cues and 13, 14, or 15 s post-cue offset during 4 ITIs in both shock conditions (no shock condition: 12 cues and 6 ITIs). Startle probes occurred a minimum of 12.5 s after another startle eliciting event (e.g., shock or startle probe). Serial position of startle probes across the three

[2] The University of Iowa Pharmaceuticals prepared and over-encapsulated study drug and matching placebos. The University of Wisconsin Pharmaceutical Research Center implemented and maintained the randomization and blind. At visit 1, participants were randomized 1:1 to Drug Order (A: visit 1 prazosin and visit 2 placebo; B: visit 1 placebo and visit 2 prazosin) and NPU Task Order (four condition and startle probe counterbalancing orders), stratified by Sex.

[3] In accordance with our pre-registration, we excluded and replaced two participants with general startle reactivity at their first study visit of < 5 μV (non-responders).

a

Screening Visit	Randomization	Study Visits 1 & 2		
Consent Eligibility Shock Sensitivity	Order A: Prazosin, Placebo Order B: Placebo, Prazosin	Drug Administration (0 min)	General Startle Reactivity (75 min)	NPU Task (90 min)

b

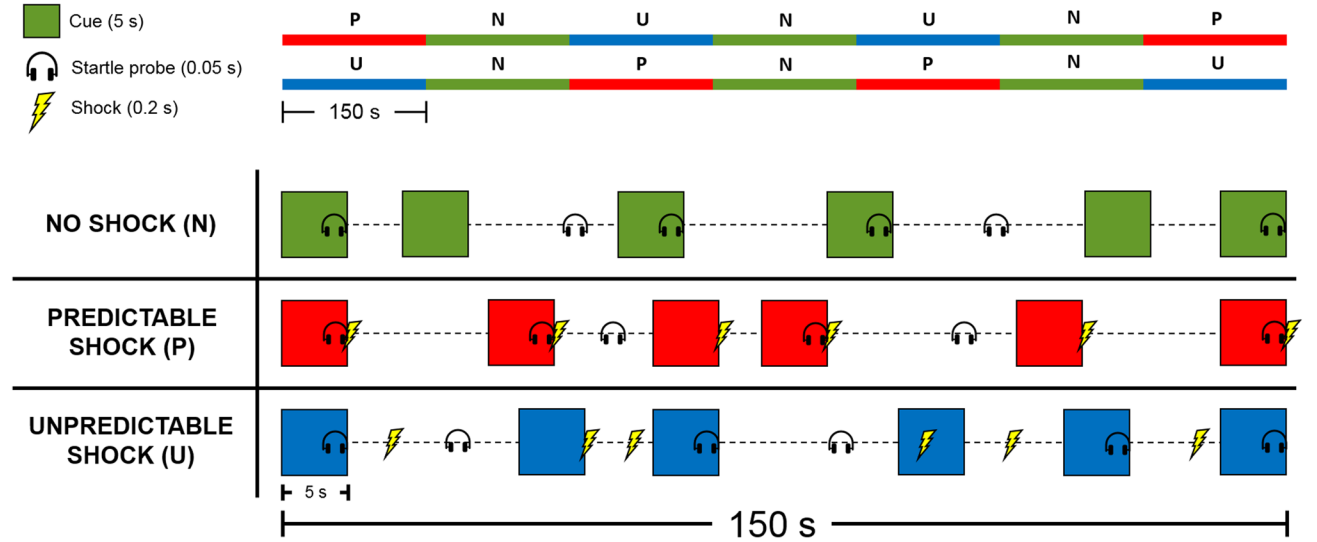

Fig. 1 Study procedures flowchart and No Shock, Predictable Shock, Unpredictable Shock (NPU) Task**. a** This figure displays the procedures completed at each visit in this within-subjects crossover design. Screening visit procedures included obtaining informed consent, preliminary eligibility determination, and shock sensitivity assessment. At study visit 1, we randomly assigned participants to drug administration order (between-subjects). All participants received both prazosin and placebo (within-subjects), one at each study visit. Participants randomized to order A ($n = 34$) received 2 mg prazosin at study visit 1 and placebo at study visit 2. Participants randomized to order B ($n = 30$) received placebo at study visit 1 and 2 mg prazosin at study visit 2. At study visits 1 and 2, participants were orally administered a pill and completed the General Startle Reactivity Task (75 min post-dose) and NPU Task (90 min post-dose)**. b** In the NPU task, participants viewed a series of colored square "cues" displayed briefly on a computer screen. We presented cues in a blocked design with three conditions: No Shock (N), Predictable Shock (P), and Unpredictable Shock (U). The upper panel displays counterbalanced conditions both within- and between-subjects. Participants completed the same condition order at both study visits. The lower panel displays examples of each condition. All blocks included six cues presented sequentially for 5 s separated by a variable inter-trial interval (ITI; 14–20 s). In No Shock, we instructed participants that no electric shocks would be administered at any time. In Predictable Shock, we instructed participants that they would receive a shock at the end of every cue, but never during the ITI, so that the cue "predicted" that the shock would occur in several seconds. In Unpredictable Shock, we instructed participants that they could receive a shock at any time, during both the cues and ITIs, so that the occurrence of the shock was unpredictable to the participant. We measured the eye-blink startle response elicited by "startle probes" (5 ms acoustic white noise) presented binaurally over headphones. We calculated startle potentiation during cues separately in Predictable and Unpredictable Shock conditions as the differences between response to startle probes during the shock conditions and no-shock conditions (i.e., predictable startle potentiation = predictable cue – no shock cue). After the NPU task, participants retrospectively reported their subjective anxiety/fear during each condition cue. A figure legend is displayed in the left panel. Panel **b** was modified with permission from Schmitz and Grillon (2012). Used with permission of Springer Nature

conditions for both cues and ITIs was counterbalanced within-subjects to account for habituation. We used two different orders of the serial position of startle probe, counterbalanced between-subjects.

Startle response measurement and quantification

We recorded eyeblink electromyogram (EMG) activity to the acoustic startle probes (50 ms, 102 dB) according to published guidelines (Blumenthal et al. 2005). We conducted data acquisition, offline processing, and artifact rejection using our previously published (Kaye et al. 2016) and preregistered criteria (see Supplement for details). We quantified the startle response as the peak amplitude 20–100 ms post-startle probe onset relative to a 50 ms pre-probe baseline. We calculated startle magnitude as the mean startle response during cues for each condition in the NPU task. We calculated startle potentiation during cues separately for unpredictable and predictable blocks as the difference between

response to probes during the shock and no-shock blocks (i.e., predictable startle potentiation = predictable cue − no shock cue).[4]

Preregistered analysis plan

We preregistered our a priori analysis plan prior to initiating data collection. We analyzed startle potentiation and self-reported anxiety in the NPU Task in separate general linear models (GLMs) with repeated measures for drug (prazosin vs. placebo) and NPU task condition (unpredictable vs. predictable). We report partial eta squared (η_p^2) and raw GLM parameter estimates (b) to document effect sizes. We evaluated additive covariates to increase power and report covariates included in the final models.[5] We used the standard $p < .05$ criteria for determining that results from all tests are significantly different from those expected if the null hypothesis were correct. We removed any model outliers identified as Bonferroni-corrected studentized residuals of $p < .05$.

Our preregistered hypothesis was that prazosin (vs. placebo) would have a larger effect on startle potentiation (primary outcome) and self-reported fear/anxiety (secondary outcome) during unpredictable vs. predictable stressors. We tested these hypotheses with separate models for each outcome with a two-way interaction of drug X NPU task condition. We report data analysis of our a priori preregistered hypothesis tests separately from all subsequent analyses for manipulation checks, robustness, and exploratory analyses. We accomplished data analysis and figure preparation with R within R-Studio.

[4] We analyze raw startle potentiation consistent with our preregistered analysis plan and numerous previous studies with this and related tasks (Moberg and Curtin 2009; Bradford et al. 2013, 2014; Kaye et al. 2016; Moberg et al. 2017). We report analyses of startle response during the no-shock blocks to confirm that observed effects result from shock threat rather than control condition (no-shock block) differences (see footnote 6 and 8). We do not standardize startle potentiation as it yields lower internal consistency and temporal stability than raw startle potentiation in the NPU task (Bradford et al. 2015; Kaye et al. 2016). Consistent with our previous studies, we also limit analyses to the cue period in predictable and unpredictable blocks to control for (i.e., match) the attentional demands associated with the visual foreground across these blocks (Lang et al. 1990).

[5] We collected a battery of other measures that were available to be used as either covariates or moderators in the analysis of the primary and secondary dependent variables (see Supplement). We utilize covariates to increase power to detect the focal effect in our analytic models. We preregistered to select covariates if we confirmed that the specific covariate (e.g., general startle reactivity, drug order, intolerance of uncertainty) significantly predicted the test of the primary hypothesis (i.e., two-way interaction between drug and NPU task condition). Any categorical between-subject factors were coded as unit-weighted, centered, orthogonal regressors (e.g., sex: male = − 0.5, female = 0.5). Any continuous/quantitative individual difference covariates were mean-centered. We conducted analyses separately for each dependent variable (e.g., startle potentiation, self-reported fear/anxiety potentiation) with only one covariate in the model at a time to determine covariate selection. We only used the covariate if it was a significant predictor of the drug X NPU condition interaction for each dependent variable separately (e.g., startle potentiation or self-reported fear/anxiety potentiation).

Results

NPU task preregistered analyses

NPU startle potentiation We analyzed startle potentiation in a GLM with repeated measures for drug (prazosin vs placebo) and NPU condition (unpredictable vs predictable shock), see Fig. 2a and Table S1. Test of our primary preregistered hypothesis showed that there was not a significant drug X NPU condition interaction, $\eta_p^2 = .006$, $b = 2.4$ μV, $t(63) = 0.64$, $p = .526$, indicating that prazosin (vs. placebo) did not have a larger effect on reducing startle potentiation during unpredictable (vs. predictable) threat.[6]

NPU self-report anxiety We analyzed self-reported fear/anxiety potentiation in a GLM with repeated measures for drug and NPU condition, see Fig. 2b and Table S1. Test of our secondary preregistered hypothesis showed that there was not a significant drug X NPU condition interaction, $\eta_p^2 < .001$, $b = 0.03$, $t(63) = 0.18$, $p = .857$, indicating that prazosin (vs. placebo) did not have a larger effect on reducing retrospective self-reported anxiety/fear during unpredictable (vs. predictable) threat.

NPU task exploratory analyses

We report the following non-preregistered exploratory analysis to characterize the data more fully and provide insights for future research.

NPU startle drug main effect We examined whether prazosin had an overall effect of reducing stress reactivity irrespective of stressor predictability. There was a significant main effect of drug on startle potentiation, $\eta_p^2 = .107$, $b = -8.0$ μV, $t(61) = -2.71$, $p = .009$, indicating that startle potentiation was larger following prazosin than placebo administration (see Fig. 2a).[7] Following our preregistered analysis plan for our primary analysis, we included general startle reactivity as a covariate and removed one model outlier.[8]

NPU self-report anxiety drug main effect There was not a significant main effect of drug on overall self-reported

[6] We did not include any covariates in models predicting the two-way interaction on startle potentiation (primary outcome) or self-reported anxiety (secondary outcome) as none met our preregistered decision threshold. We did not identify or remove any model outliers (i.e., Bonferroni-corrected studentized residuals, $p < .05$).

[7] There was not a significant effect of drug on startle response only during the No Shock condition, $\eta_p^2 = .039$, $b = 4.2$ μV, $t(61) = 1.57$, $p = .121$, suggesting that the main effect of prazosin on startle potentiation (i.e., shock cues minus no-shock cues) was not driven by a reduction in startle during No Shock.

[8] In the unadjusted model, there was not a significant main effect of drug on overall startle potentiation, $\eta_p^2 = .107$, $b = -6.8$ μV, $t(63) = -1.97$, $p = .053$, with no covariates included or outliers removed.

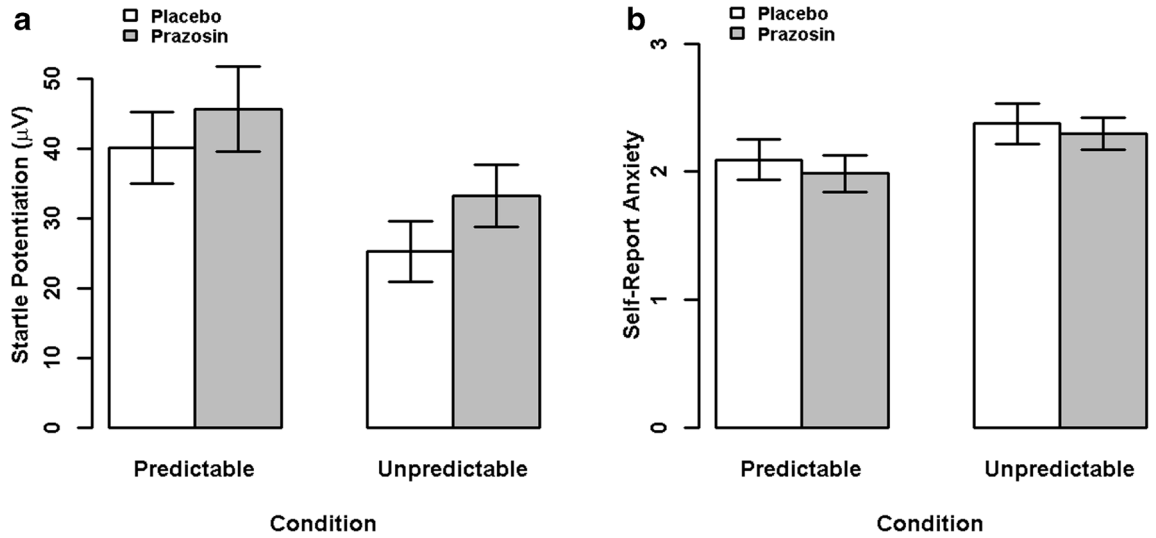

Fig. 2 Startle potentiation and self-reported anxiety potentiation by drug and NPU condition. Bars display **a** startle potentiation and **b** self-reported anxiety/fear potentiation to predictable and unpredictable shock (vs. no shock) following prazosin (gray) and placebo (white) administration. Confidence bars represent ± one standard error for point estimates of startle potentiation from the general linear models

fear/anxiety, $\eta_p^2 = .001$, $b = 0.03$, $t(62) = 0.27$, $p = .792$, indicating that prazosin did not affect overall self-reported fear/anxiety.[9]

General startle reactivity

We analyzed general startle reactivity in a GLM with repeated measures for drug. There was not a significant effect of drug on general startle reactivity (prazosin mean = 79.2 μV, placebo mean = 75.4 μV), $\eta_p^2 = .010$, $b = -3.8$ μV, $t(63) = -0.81$, $p = .421$, indicating that prazosin did not affect overall startle response prior to stressor exposure (i.e., NPU task).

Manipulation checks, robustness, post-hoc power, and exploratory analyses

We conducted follow-up analyses to evaluate the robustness, reliability, and internal validity of the NPU task, placebo blind, and peripheral effects of prazosin (see Supplement). These analyses support the effectiveness of the stressor and drug manipulations and evaluate alternative explanations for the primary results. We confirmed that 2 mg prazosin reduced blood pressure overall, with significant effect on diastolic by 1-h post-administration (Fig. 3), suggesting prazosin was physiologically active during the time window of the NPU Task (~ 1.5–2 h). We also report results from post-hoc Monte Carlo power simulation that indicate we had high power (> 99.9%) to detect a medium effect size for prazosin (see Supplement).

Discussion

Results of the current study indicate that a single acute dose of prazosin does not reduce stress reactivity in healthy adults. We did not find evidence to support our a priori hypothesis that acute administration of 2 mg prazosin (vs placebo) would decrease startle potentiation or self-reported anxiety to a greater degree during unpredictable than predictable stressors. Prazosin did not have differential effects on either measure of stress reactivity as a function of predictability. Robustness analyses (see Supplement) suggest these null results are not attributable to individual difference moderators (e.g., age, sex, baseline blood pressure, trait affect measures) or methodological factors (e.g., prazosin-placebo order, expectancy effects, shock intensity). Following a rigorous preregistered analysis plan in a well-powered efficient within-subjects cross-over study design increases confidence in our null results.

Exploratory follow-up analysis suggests prazosin may have acutely increased, rather than decreased, overall startle potentiation during threat of shock. This effect was robust to numerous analytic checks (see Supplement, e.g., no drug order moderation, between-subject drug effect observed at study visit 1 only). We did not, however, see this effect for self-reported anxiety potentiation. Given the exploratory nature of these tests, the conclusion that acute prazosin increases overall startle potentiation should be interpreted cautiously and awaits replication to bolster confidence in its reliability. Regardless, contrary to our hypothesis, prazosin did not decrease startle potentiation or self-report measures in the NPU task. Furthermore, prazosin did not

[9] We removed one model outlier, but there was still not a significant main effect of drug on overall self-reported anxiety/fear, $\eta_p^2 = .008$, $b = 0.09$, $t(63) = 0.70$, $p = .484$, with no outliers removed. We did not include any covariates in either model predicting the drug main effect on self-reported anxiety as none met our preregistered decision threshold. We also confirmed that was not a significant effect of drug on startle response during the No Shock condition, $\eta_p^2 = .013$, $b = 0.06$, $t(62) = 0.89$, $p = 0.375$.

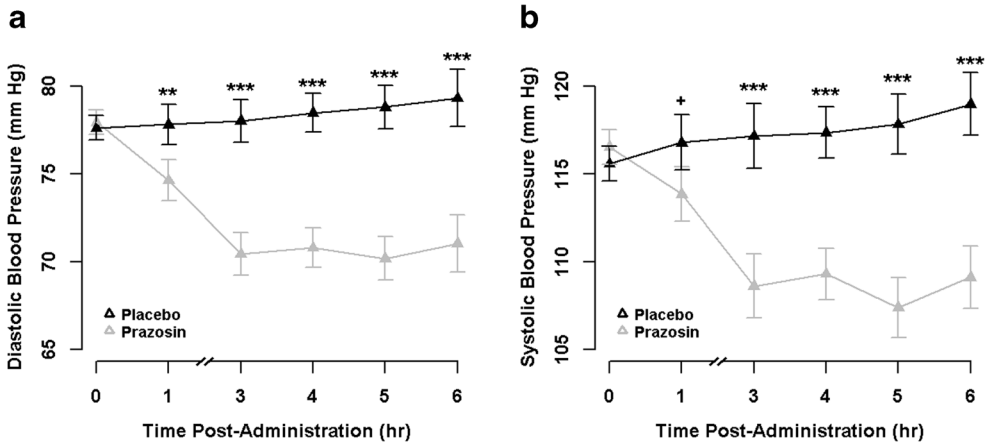

Fig. 3 Blood pressure by drug and time. Points display standing **a** diastolic and **b** systolic blood pressure (BP) by drug and time. Error bars represent ± 1 SE of the drug effect from separate covariate adjusted general linear models at each time point. We analyzed standing diastolic and systolic BP in separate general linear models with repeated measures for drug and time and baseline BP (mean-centered averaged across visits) as a between-subjects regressor. Prazosin produced a significant reduction in both diastolic and systolic BP overall (p's < .001). Furthermore, prazosin produced a significant reduction in diastolic BP at both 1-h (p = .008) and 3-h (p < .001). Prazosin produced a significant reduction in systolic BP by 3-h (p < .001), but non-significant change at 1-h (p = .067), consistent with the known greater effects on diastolic than systolic BP. See Supplement for additional analyses. $^{+}p < .10$; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$

affect startle response at baseline prior to stressor exposure in the NPU task (i.e., general startle reactivity) nor during the No Shock condition in the NPU task.

Current study strengths and limitations

Prior to initiating the current study, we comprehensively evaluated the psychometric properties of the NPU task in a large sample (n = 128) to confirm it was well-suited for repeated administration (Kaye et al. 2016). The current study confirmed that the NPU task was effective at eliciting robust stress reactivity across measures. Startle potentiation displayed good internal consistency in the NPU task (split-half reliability correlations > .8, see Supplement), bolstering our confidence in the reliability of this task-measure pairing. To maximize statistical power, we utilized a fully within-subjects design in a large sample size. We followed emerging open science recommendations by preregistering our a priori hypotheses to strengthen the validity of our results and we performed exploratory analyses to guide future research.

We conducted the NPU task when prazosin was most likely to be maximally active based on its pharmacokinetics and pharmacodynamics (Vincent et al. 1985). It remains possible that prazosin was not sufficiently active in the brain during this time due to insufficient dose or individual differences in first-pass metabolism, bioavailability, or blood-brain barrier penetrance.[10] This concern is reduced by our observation that prazosin lowered participants' blood pressure, indicating that the dose was physiologically relevant at least in the periphery when the NPU task occurred (see Fig. 2 and Supplement). We administered 2 mg, double the typical initial dose, to maximize our ability to detect acute effects while ensuring safety. However, this single dose may have been insufficient to impact stress reactivity robustly. Clinical doses to treat PTSD are typically higher (i.e., ≥ 10 mg), though the optimal therapeutic dose range (if any) remains unclear. However, these higher chronic doses may only be required when studying noisy clinical outcomes (e.g., AUD heavy drinking, PTSD hyperarousal symptoms), which arise from many mechanistic pathways. In contrast, we used a physiological measure (e.g., startle potentiation) that is tightly linked to putative NE stress mechanism affected by prazosin. As such, our use of this stress-mechanism focused measure in a controlled laboratory setting likely provided greater sensitivity to detect much smaller reductions in stressor reactivity. Further, it is possible that prazosin differentially affects stress reactivity following acute dosing versus chronic dosing used in clinical practice; similar to how 2 weeks of SSRI administration (but not acute administration) selectively reduces startle potentiation to unpredictable stress in the NPU task, mirroring their anxiolytic clinical profile in humans (Grillon et al. 2007, 2009a). Again, higher doses may be necessary to achieve long-lasting suppression of the stress system clinically. However, our study was designed to detect even the expected short-term suppression of stress reactivity at peak prazosin activity following an acute dose, consistent with the time course of peripheral prazosin administration on brain activity and behavioral responses in rodents (Darracq et al. 1998).

[10] Previous literature suggests that prazosin's peak effects on peripheral physiology and plasma concentration occur 1–4 h post-administration (Jaillon 1980). Unfortunately, there is limited research in humans to confirm the time course of effects in the brain (but see Rutland et al. 1980). Although prazosin is still widely used in rodent behavioral neuroscience research today to study the central nervous system, human studies have primarily examined peripheral physiology as prazosin was originally developed as an antihypertensive agent. Indeed, very few studies have examined basic acute effects of prazosin in humans since the 1970s.

The placebo blind was not completely effective, which can constrain interpreting the study results. However, follow-up tests confirmed the NPU task results did not differ by drug administration order (see Supplement). The results were also comparable when examining between-subject drug manipulation at the first study visit only. Furthermore, participants' self-reported expectancy of which drug they received did not moderate the effects of drug on NPU task results. These analyses support the robustness of the conclusions from the NPU task but do not rule out the potential impact of inadequate drug blind or expectancy effects.

Future directions and conclusions

To address some of the concerns and limitations from the current study, our research team is conducting a larger randomized controlled trial of another NE-α1 antagonist, doxazosin, on NPU task stress reactivity (ClinicalTrials.gov NCT02989493). This experimental medicine approach incorporates the NPU task into a traditional double-blind, placebo-controlled trial of AUD to examine clinical outcomes (e.g., heavy drinking days) as well as potential stress mechanisms. Doxazosin has a similar chemical structure to prazosin but has a more favorable clinical profile for use in psychiatric practice (e.g., longer half-life, once daily dosing). In this trial, participants complete the NPU task after titrating up to a therapeutic dose of doxazosin (8 mg) over several weeks. This could help clarify if the null effects in the current study may be due to an acute, single-dose administration or insufficient dose (vs. prolonged, higher-dose administration). Furthermore, this trial may be more likely to detect the effects of NE-α1 blockade in AUD patients who as a group show sensitized responses to unpredictable (vs predictable) stressors relative to healthy controls (as in the current study) (Gorka et al. 2013; Moberg et al. 2017). However, failure to detect effects of doxazosin on either NPU stress reactivity or clinical outcomes would cast serious doubt on the utility of NE-α1 antagonists as treatments for AUD and the translational nature of the NPU task.

We and others have proposed that the NPU task may be a viable surrogate endpoint to efficiently screen novel or repurposed pharmacotherapies targeting stress mechanisms in addiction and PTSD (Davis et al. 2010; Kaye et al. 2017). Acute administration of CRF_1 and NE-α1 antagonists in humans has not reduced startle potentiation during unpredictable stressors in the NPU task (current study; Grillon et al. 2015). This runs counter to predictions based on influential theories in behavioral neuroscience and raises questions regarding the utility of startle potentiation during unpredictable stress to identify cross-species neural mechanisms and/or candidate drug targets that successfully translate from rodent to human models (Davis et al. 2010; for critique see Shackman and Fox 2016).[11] While CRF_1 and NE-α1 antagonist effects on the NPU task have not confirmed predictions from rodent models, they do in fact appear to more closely align with emerging results from failed clinical trials for PTSD and AUD (Dunlop et al. 2017; Raskind et al. 2018; Simpson et al. 2018). The NPU task has been sensitive to effects of other medications (e.g., benzodiazepines) that do have anxiolytic clinical benefit in humans (Grillon et al. 2006, 2015). Thus, it remains possible that the NPU task is working as a surrogate endpoint should, correctly identifying effective versus ineffective treatments. Utilizing human laboratory measures to screen novel or repurposed pharmacotherapies earlier in the drug development process has potential to increase "fast-fails" at the phase 2a stage and save critical downstream resources (Grillon et al. 2015; Schwandt et al. 2016). However, there remain many important unanswered questions the field must rigorously address (e.g., meaningful prediction of clinical outcomes) for these laboratory measurement approaches (be it the NPU or other paradigms) to prove valuable as surrogate endpoints for clinical trials.

There remains an urgent need to develop treatments that target stress-related processes such as hyperarousal symptom cluster in PTSD and stress-induced relapse in addiction. Repurposing available NE medications held initial promise based on hypothesized stress mechanisms identified in preclinical behavioral neuroscience (Hendrickson and Raskind 2016; Krystal et al. 2017; Haass-Koffler et al. 2018). In spite of the burgeoning clinical trials literature of prazosin as a treatment for PTSD and AUD and increasing off-label prescribing, the current study is among the first experimental psychopharmacology study to investigate the effects of prazosin on stress reactivity in humans (Fox et al. 2012; Homan et al. 2017; also see Verplaetse et al. 2017). We failed to detect any indication that prazosin acutely reduces stress reactivity, measured via startle response and self-reported anxiety. These findings join with recent failures to replicate the treatment effects of prazosin for PTSD and AUD, suggesting the possibility that prazosin is a far less promising intervention

[11] Considerable preclinical research supports our study hypothesis that acute prazosin would selectively reduce startle potentiation during unpredictable (relative to predictable) shock. However, the most direct translational design of our current study in humans (i.e., acute prazosin effects on startle potentiation to unpredictable vs predictable shock) has not been performed in rodent models to date. We believe reverse-translation of our current study design in rodents, using parallel pharmacological manipulation (acute prazosin), stressor manipulation (unpredictable vs. predictable shock), and measurement (startle potentiation) is essential to clarify convergent or divergent results across species. Furthermore, additional psychopharmacology studies in both human and rodent models should address differences in acute vs chronic prazosin administration. Only recently have preclinical labs begun to examine the effects of chronic prazosin on relevant anxiety-like behaviors and alcohol use/seeking behaviors (Froehlich et al. 2013; Skelly and Weiner 2014; Rasmussen et al. 2017). However, no studies have examined chronic prazosin administration effects on startle response as the primary outcome measure.

for stress-related psychiatric disorders than originally believed (Petrakis et al. 2016; Raskind et al. 2018; Simpson et al. 2018; Kleinman and Ostacher 2019).

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Compliance with ethical standards

Conflict of interest The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, VA, or UW. The authors have no biomedical financial interests or potential conflicts of interest.

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