Prazosin during threat discrimination boosts memory of the safe stimulus

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Word count: 2532

Financial support

Funding was provided by NIMH grant MH105515 and a Klingenstein-Simons Fellowship Award in the Neurosciences to D.S.; NIMH grant MH105414 to R.L.C.; and Swiss National Science Foundation grant SNF 161077 to P.H. The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Keywords: Prazosin; noradrenaline, threat; fear conditioning; extinction; memory; PTSD

Abstract

The alpha-1 adrenoreceptor antagonist prazosin has shown promise in treatment of posttraumatic stress disorder (PTSD) symptoms, but its mechanisms are not well understood. Here we administered prazosin or placebo prior to threat conditioning (day 1) and tested subsequent extinction (day 2) and re-extinction (day 3) in healthy human participants. Prazosin did not affect threat conditioning but augmented stimulus discrimination during extinction and re-extinction, via lower responding to the safe stimulus. These results suggest that prazosin during threat acquisition may have influenced encoding or consolidation of safety processing in particular, subsequently leading to enhanced discrimination between the safe and threatening stimuli.

Pavlovian threat conditioning is a prominent model for understanding the significance of threat discrimination (i.e., the distinction of threatening and safe stimuli) learning and memory in posttraumatic stress disorder (PTSD). This model assumes that a conditioned stimulus (CS+, a formerly neutral stimulus such as a shape or a sound) is associated with an unconditioned stimulus (US, the aversive event) during threat learning, enabling the CS+ itself to become an unsafe cue and trigger a defensive response, while the control stimulus (CS-, a safe cue) may acquire inhibitory properties as it had never been paired with the US. Threat conditioning studies in PTSD generally found increased responses to the CS- but no consistent effect on the discrimination of threat (Duits et al., 2015). The effect of threat learning can be quantified by the amount of discrimination between the unsafe and the safe cue during threat conditioning, and subsequent presentations of the CS+ without the US induce extinction learning.

The alpha-1 adrenergic receptor antagonist prazosin has shown promise in the treatment of PTSD. Prazosin attenuates noradrenaline effects at central postsynaptic alpha-1 adrenergic receptor after peripheral administration (Menkes *et al.*, 1981) and has been shown to reduce PTSD symptoms including nightmares, poor sleep quality, hyperarousal, and impaired global function (Ahmadpanah *et al.*, 2014; George *et al.*, 2016; Germain *et al.*, 2012; Raskind *et al.*, 2000, 2002, 2003, 2007, 2013; Taylor *et al.*, 2008) which may involve abnormally heightened activity of the noradrenergic central nervous system (Raskind *et al.*, 2016; Southwick *et al.*, 1993).

Previous threat conditioning studies in humans have shown that learned threat memory resisted extinction training following noradrenergic stimulation by yohimbine before threat

acquisition, while the initial learning of threat was unaffected (Soeter and Kindt, 2011, 2012). Yohimbine is an alpha-2-adrenergic antagonist that stimulates central noradrenergic activity by blocking the alpha-2-adrenergic autoreceptor, and its physiological effects typically include an increase in systolic and diastolic blood pressure. Prazosin, on the other hand, has antihypertensive effects by blocking alpha-1-adrenergic receptors. Although the central actions of alpha-1-adrenergic receptors with respect to threat learning are not fully understood, the opposite physiological effects of yohimbine and prazosin suggest that prazosin may have opposite effects on extinction learning compared to yohimbine.

Specifically, threat discrimination learned under prazosin, and thus under attenuated noradrenergic effects on alpha-1 adrenergic receptors, may reverse the extinction effect of yohimbine: compared to placebo, threat discrimination should be unaffected during acquisition but easier to subsequently extinguish, i.e., higher responses to CS+ compared to CS- during acquisition in both the prazosin and placebo group, but a faster decay of the CS+ response during extinction and re-extinction in the prazosin group compared to the placebo group, without affecting the CS-. Contrary to this prediction, however, studies in rodents found that alpha-1 adrenoreceptor antagonists enhanced threat acquisition and impaired threat extinction (Bernardi and Lattal, 2010; Cain *et al.*, 2006; Do-Monte *et al.*, 2010; Lazzaro *et al.*, 2010), raising the concerning possibility that prazosin might in fact work against extinction-based treatments (Do-Monte *et al.*, 2010; Maren, 2011). Notably, additional noradrenergic effects on beta- and alpha-2 adrenergic receptors may have also contributed to these memory processes (Do-Monte *et al.*, 2010).

To clarify the effects of prazosin on threat acquisition and extinction in healthy humans, we used a randomized double-blind between-within-subjects experimental design over three consecutive days, conducted in the same context (Fig. **1**): Threat learning on day 1, threat extinction on day 2, and re-extinction test on day 3; the re-extinction test examined whether participants retrieve the learned threat discrimination (learned on day 1) or the extinction memory (learned on day 2). On day 1 (acquisition), participants were randomly assigned to receive either placebo or 3 mg of prazosin (1 mg capsule followed by 2 mg capsule 30 min later) two hours before threat acquisition (at the expected peak plasma prazosin level). Immediately before and every 30 minutes until 90 minutes after the administration of the study drug, blood pressure and heart rate were measured. In addition, we measured the trait anxiety subscale of the Spielberger State-Trait Anxiety Inventory (STAIT; Spielberger *et al.* 1983) before the experiment. Forty healthy human participants provided written informed consent and were compensated. The experiment was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai. The final sample consisted of 38 participants, one of which did not show up for the third day (Table 1).

During threat conditioning, two colored squares were presented for 4 s on each trial, one of which was paired with a mild electric shock in 43% of the trials (CS+) while the other was never paired with a shock (CS-). On day 2 (extinction) and day 3 (re-extinction) no shocks were delivered but the stimulating bar electrodes were connected to the participant's non-dominant wrist. Skin conductance response (SCR) to the stimuli was measured throughout with Ag-AgCl electrodes, filled with standard NaCl electrolyte gel, and attached to the middle phalanges of the second and third fingers of the non-dominant hand. SCR signal

was amplified and recorded with a MP150 BIOPAC Systems skin conductance module connected to a PC. Data were continuously recorded at a rate of 200 samples per second. Shocks were delivered using a Grass Medical Instruments SD9 stimulator and stimulating bar electrodes attached to the participant's non-dominant wrist. Shock intensity was calibrated up to a maximum of 60V to reach a level described by participants as "uncomfortable, but not painful".

The outcome measure was the psychophysiological arousal response to the CSs, indexed by the estimated anticipatory sudomotor nerve activity (aSNA) amplitude (Bach *et al.*, 2010). Estimates of aSNA indicate the anticipation of an aversive event within the time window of stimulus presentation. These were calculated by inverting a forward model that describes how (hidden) SNA translates into an (observable) SCR using a variational Bayes approximation. A unit increase in aSNA corresponds to an increase in SCR of 1 μ S. This method uses summary statistics across all available trials (i.e., average aSNA per condition and stimulus) to demonstrate successful experimental manipulations such as threat conditioning, and has shown to be more sensitive compared to a conventional SCR base-to-peak analysis (Bach, 2014; Bach *et al.*, 2010, 2015; Staib *et al.*, 2015).

We used linear mixed models with restricted maximum likelihood estimation for all analyses, due to their well-established advantages over conventional analysis of variance (e.g., Gueorguieva and Krystal, 2004). Mixed models efficiently allow for a full analysis of the data even in the presence of missing data (allowing us to include the participant that did not complete day 3). We used the estimates of aSNA as outcome measure and the R software (R Core Team, 2016) and the packages lme4 (Baayen *et al.*, 2008; Bates *et al.*, 2015; Bates,

2005) and lsmeans (Lenth, 2016) for all analyses. A log-transformation was applied on the aSNA estimates to correct for unequal variances (heteroscedasticity). Fixed effects included drug (placebo, prazosin), session (acquisition, extinction, re-extinction) and stimulus (CS+, CS-) as well as their interactions. In addition, a mean-centered linear term for trial was entered to account for the time-effect in each session. Significance of fixed effects was assessed using likelihood ratio tests against a χ^2 distribution and maximum likelihood as estimation method. A maximal random effects structure including random intercept and slopes for session and stimulus was included to avoid inflated type-1 errors (Barr *et al.*, 2013). Random slopes for session, stimulus, and the session x stimulus interaction for each subject were included to account for random variation between subjects' in the effects of interest. An unstructured variance-covariance matrix was used to allow for correlation between the random effects. The significance threshold was set at .05, two-tailed.

To assess whether there were group differences in threat discrimination in any of the sessions, we tested for an interaction of drug (placebo, prazosin) x session (acquisition, extinction, re-extinction) x stimulus (CS+, CS-) and found that the 3-way interaction was significant ($\chi 2$ (2) = 7.26, P = .03; Fig. **1**) and driven by enhanced stimulus discrimination (CS+ vs. CS-) in the prazosin group compared to placebo during extinction and re-extinction but not acquisition (Fig. 2). Specifically, both groups showed successful acquisition, indicated by stronger response to the CS+ compared to the CS- during acquisition and effect sizes between medium and large (Placebo: t(18) = 2.9, P = 0.0097; Cohen's d = 0.66; Prazosin: t(18) = 4.1, P = 0.0007; Cohen's d = 0.94). However, in the placebo group, the stimulus discrimination was not significant during extinction (t(18) = 1.55, P = 0.14) and re-extinction

($t(17) = 1.3$, $P = 0.21$) whereas for the prazosin group, aSNA for CS+ was significantly higher compared to aSNA for CS- in the prazosin group during extinction ($t(18) = 5.04$, $P = 0.0001$) and re-extinction ($t(18) = 4.76$, $P = 0.0002$; Fig. 2), confirming the 3-way interaction. These results indicate that threat acquisition was successful with no group differences, but stimulus discrimination differed between the groups during extinction and re-extinction, with augmented discrimination in the prazosin compared to the placebo group. Notably, these results were consistent with the results obtained by using the conventional manually scored base-to-peak SCR data (see Supplementary Results).

Next, we asked whether changes in response to one stimulus in particular drove the discrimination, i.e., we tested for differences between the placebo and the prazosin group in aSNA responses to CS+ as well as for aSNA responses to CS- (Fig. 1). We found that responses to the CS- were significantly lower in the prazosin group compared to the placebo group in extinction ($t(36.04) = -2.24$, $P = 0.031$) and re-extinction ($t(34.05) = -2.9$, $P = 0.007$) but not acquisition ($t(39.74) = 0.44$, $P = 0.66$), whereas CS+ did not significantly differ between groups in any session (all $P > 0.84$) or phase within sessions (all $P > 0.71$). These results indicate that prazosin boosts threat discrimination during extinction and re-extinction via effects on the safe stimulus. Detailed analysis of early and late phases in each stage confirmed that prazosin had no significant effects during acquisition. The protocol induced extinction and re-extinction in both groups, with responses to both stimuli gradually decreasing from early to late phases, but responses to the CS- were significantly lower in the prazosin compared to placebo group during late extinction and throughout re-extinction (see Supplementary Results).

Thus, prazosin effects during threat memory formation may change the fate of memory: threat discrimination learned under prazosin would be harder to extinguish over time. Consistent with the rodent data (i.e., potentiating threat memory), and in contrast to the expected effects of alpha-1 adrenergic receptor blockade in humans (i.e., reducing threat memory), we found that the prazosin group compared to placebo showed enhanced threat discrimination memory during extinction and re-extinction, driven by lower responding to the safe stimuli, with no effects during acquisition.

How can prazosin interfere with extinction? Potentially, prazosin acts upon the encoding of the memory, affecting both inhibitory and excitatory plasticity, which is later recruited for extinction (Clem and Schiller, 2016). Some clues may arise from evaluating the effects of prazosin on threat learning and extinction, especially since alpha-1 adrenergic receptors are abundant in the lateral nucleus of the amygdala (LA), a key region of synaptic plasticity in threat learning and extinction (LeDoux, 2000). The LA receives inputs from the locus coeruleus that contain noradrenaline and exhibit tonic and phasic firing in response to aversive stimuli (Tully and Bolshakov, 2010). However, the role of noradrenaline in the modulation of threat learning is less clear. On the one hand, it has been shown that noradrenaline may suppress feed-forward inhibition of threat conditioning thalamic pathway, thereby enhancing learning-related plasticity (Ehrlich *et al.*, 2009; Tully *et al.*, 2007). On the other hand, alpha-1 adrenergic receptors in the LA may inhibit descending output from the central nucleus of the amygdala to brain regions controlling arousal and defensive responses (Braga *et al.*, 2004; Pape and Pare, 2010). As prazosin has a short half-life of only 3 hours, the results cannot be explained by direct action of prazosin in extinction or re-extinction,

suggesting that alpha-1 receptor blockade may alter the long-term consequences of newly acquired threat associations. In addition, state-dependent learning effects instead of a drug effect of prazosin appears to be an unlikely explanation, given that we found the drug effect in extinction and again in re-extinction.

Rodent studies indicate that prazosin may in fact reduce amygdala's inhibitory tone (Bernardi and Lattal, 2010; Cain *et al*., 2006; Do-Monte *et al*., 2010; Lazzaro *et al*., 2010). Thus, although alpha-1 adrenergic receptor blockade during threat learning is an unlikely model for PTSD since it should counteract symptoms induced by elevated sympathetic activity, prazosin may capture certain conditions and individual differences in alpha-1 adrenergic receptor activity during the experience of a traumatic event, which may affect the development of PTSD in interesting and important ways. The current results indeed suggest that learning threat discrimination under prazosin is less flexible and better remembered over time.

Lower responding to the CS- but not CS+ drove the effect of prazosin on threat discrimination. Although it may seem as if prazosin augmented memory for the safe stimulus (i.e., decreased aSNA in response to the CS-) without affecting threat memory, a few considerations should be noted. The discrimination between safe and unsafe cues is adaptive only during acquisition when threat is imminent. During extinction, both cues are safe and the learned discrimination is no longer adaptive. A recent meta-analysis (Duits *et al.*, 2015) showed that anxiety patients exhibit less discrimination than controls in acquisition (when it is adaptive) and more discrimination in extinction (when it is maladaptive). Our results show that the prazosin group, but not placebo, persistently maintained the discrimination between

the CS+ and the CS-, never fully extinguishing the difference between the stimuli despite two consecutive extinction sessions, which is maladaptive. It might be problematic, therefore, to argue that prazosin has positive effects by augmenting memory for safety learning when the net result is abnormally persistent threat discrimination. Nevertheless, to fully disentangle these competing interpretations, future studies should examine administration of prazosin prior to extinction, when the CS+ undergoes safety learning, and examine whether this would diminish or augment subsequent stimulus discrimination. Consistent with the latter possibility, rodent studies found impairments in extinction learning when prazosin was administered prior to extinction training (Do-Monte *et al.*, 2010) and between repeated extinction sessions (Bernardi and Lattal, 2010), indicating it does not enhance but rather counteracts safety learning.

The findings of this study may have clinical relevance as prazosin is often prescribed for the treatment of PTSD symptoms (Ahmadpanah *et al.*, 2014; George *et al.*, 2016; Germain *et al.*, 2012; Raskind *et al.*, 2000, 2002, 2003, 2007, 2013; Taylor *et al.*, 2008), and prolonged exposure therapy is currently the most effective behavioral therapy in PTSD (McLean and Foa, 2011). If prazosin is combined with or followed by extinction-based treatments, it might influence re-experienced or new memories in the course of therapy. In addition, as extinction was only indirectly affected in this study through memory encoding or consolidation, the clinical implications may be more relevant for individuals who are taking alpha-1 blockers when they are traumatized (or who receive them immediately after the trauma) or for individuals with innately reduced alpha-1 signaling in defensive brain circuits.

Acknowledgements

Author Contributions: Dr. Homan had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Homan, Lin, Soleimani, Murrough, Clem, Schiller. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Homan, Schiller. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Homan, Bach, Schiller. Obtaining funding: Homan, Clem, Schiller. Administrative, technical, or material support: Schiller. Study supervision: Schiller. The authors declare no competing financial interests. This work was supported in part through the computational resources and staff expertise provided by Scientific Computing at the Icahn School of Medicine at Mount Sinai.

Conflict of interest

Dr. Murrough declares no conflicts related to the current study. In the past 3 years, Dr. Murrough has provided consultation services to Novartis, Janssen Research and Development, and Genentech; he is named on patents pending for neuropeptide Y as a treatment for mood and anxiety disorders, a patent pending for the combination of ketamine and lithium for suicidal ideation, and a patent pending for ketamine plus lithium to extend the antidepressant response of ketamine. Dr. Homan declares no conflicts related to the current study. Dr. Homan has received speaker's fees from Neurolite and Takeda Pharma in the past 3 years. All other authors report no conflicts of interest.

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Table 1: Demographics, behavioral and physiological measurements by group.

Characteristic	Placebo (N=19)	Prazosin (N=19)
Age, mean (SD), y	27.2 (5.6)	26.3 (4.9)
Gender, (m/f)	7/12	9/10
Adverse events	1	2
Drop-outs	1	1
Heart rate baseline, mean (SD), beats/min	73.4 (10.3)[a]	77.8 (11.4)[a]
Heart rate 90 min, mean (SD), beats/min	66.4 (9.9)	72.4 (11.2)[a]
Systolic BP baseline, mean (SD), mm Hg	110.6 (8.4)[a]	112.2 (7.6)[a]
Diastolic BP baseline, mean (SD), mm Hg	66.4 (7.7)[a]	67.7 (8.6)[a]
Systolic BP 90 min, mean (SD), mm Hg	107.2 (10.5)	103.9 (11.6)[a]
Diastolic BP 90 min, mean (SD), mm Hg	67.8 (11.0)	67.2 (8.4)[a]
Shock intensity, mean (SD), V	4.2 (1.4)	4.2 (1.0)[a]
Response to the Shock, mean (SD), SNA	2.04 (0.81)	2.01 (1.0)
Response to the Shock, mean (SD), mS	0.98 (0.11)	0.90 (0.23)
STAI-T total score, mean (SD)	32.7 (9.2)[a]	32.7 (9.1)

Two male participants were excluded before the experiment due to presumable side effects to the study drug: one male participant reported dizziness and nausea 30 minutes after ingestion of the placebo pill, another male participant developed a syncope for 5 seconds 1 minute after taking the first capsule (1 mg) of prazosin. One female participant experienced syncope approximately 5 hours after drug ingestion that lasted 3 minutes, but was willing to complete the study. Additional monitored side effects included blood pressure drops in two males and nosebleed in one female. The groups did not differ in the physiological response to the drug, there were no significant differences in trait anxiety or shock intensity levels, and the groups did not significantly differ in their physiological responses to the shocks during threat learning. Absence of physiological changes after the prazosin doses we used in this study is in line with previous studies (reviewed in George *et al.*, 2016). There was no significant influence of heart rate, blood pressure, trait anxiety and shock intensity (all *P*s > 0.16) when included as covariates in our model and our primary finding, the 3-way interaction, remained significant whenever an additional covariate was included. It is therefore unlikely that the physiological or psychological baseline measures influenced threat processing.

Abbreviations: BP, blood pressure; STAI-T, trait anxiety subscale of the Spielberger State-Trait Anxiety Inventory; CS, conditioned stimulus; SNA, sudomotor nerve activity; mS, microSiemens. [a] Due to one missing value, the measure was calculated only for N=18 participants.

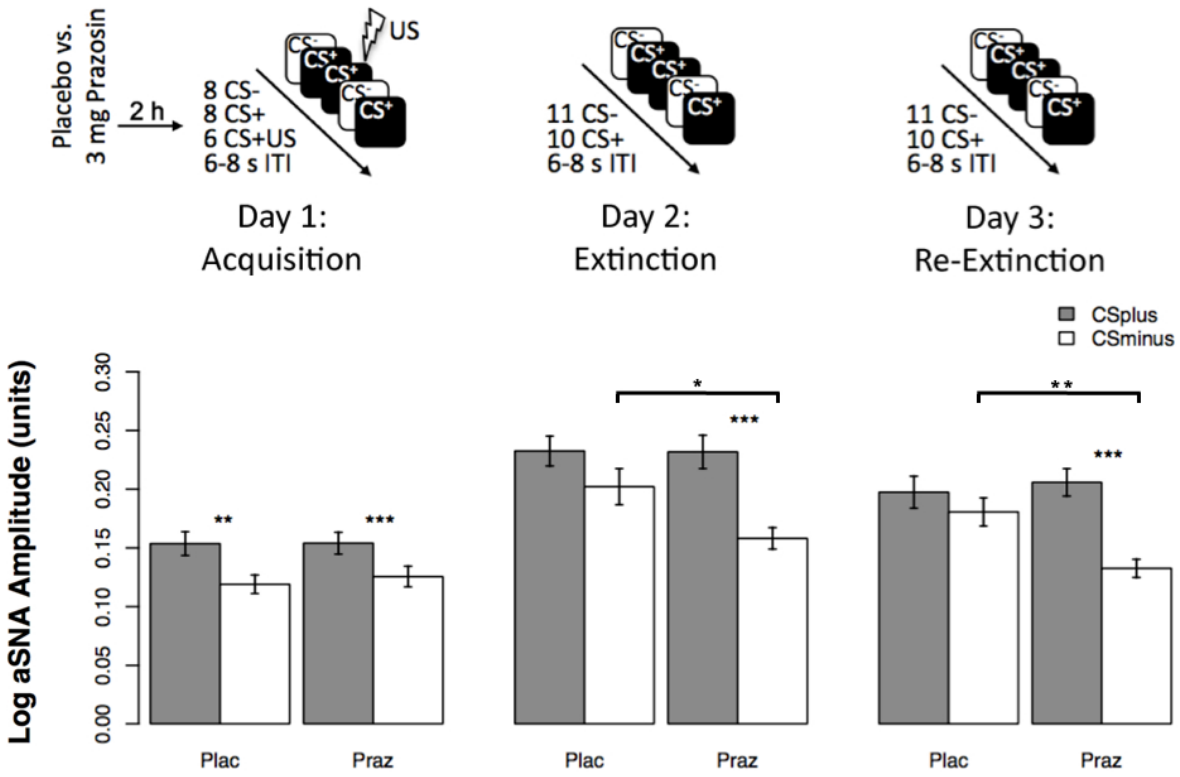

Figure 1: Experimental design and behavioral results. *Upper panel*. This was a randomized double-blind experimental design in healthy volunteers involving three consecutive days: Threat learning on day 1, threat extinction on day 2, and re-extinction test on day 3; the re-extinction test examined whether participants retrieve the threat discrimination (learned on day 1) or the extinction memory learned on day 2). On day 1 (acquisition), participants were randomly assigned to receive either placebo or 3 mg of prazosin (1 mg capsule followed by 2 mg capsule 30 min later) two hours before threat acquisition (at the expected peak plasma prazosin level). During threat conditioning, two colored squares were presented for 4 s on each trial, one of which was paired with a mild electric shock in 43% of the trials (CS+) while the other was never paired with a shock (CS-). On day 2 (extinction) and day 3 (re-extinction) no shocks were delivered but the stimulating bar electrodes were connected to the participant's non-dominant wrist. Extinction and re-

extinction started with a CS- presentation and thus involved one additional CS-; subsequent presentations of CS+ and CS- were counterbalanced. *Abbreviations*: CS, conditioned stimulus; US, unconditioned stimulus; ITI, intertrial interval. *Bottom panel.* **Stimulus discrimination summarized by group and stage show successful acquisition for both groups but persisting stimulus discrimination (mediated by effects on the safe stimulus) in the prazosin group in extinction and re-extinction.** Mean logarithmized anticipatory sudomotor nerve activity estimates (aSNA) for acquisition, extinction, and re-extinction after administration of placebo and prazosin. Error bars denote standard errors. *, $P < 0.05$; **, $P < 0.01$; ***; $P < 0.001$.

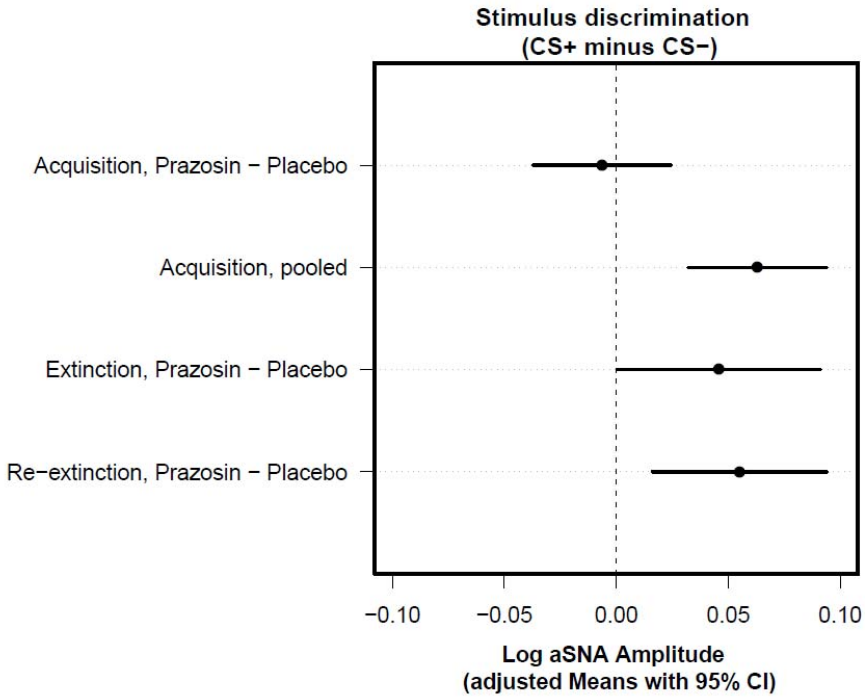

Figure 2: Planned contrasts for stimulus-discrimination differences between the prazosin and placebo groups for each session show effects specific for extinction and re-extinction but not acquisition. Confidence intervals that do not include zero (cross the vertical dashed line) indicate that the corresponding contrast is statistically significant. Adjusted means (indicating the mean response for each factor, adjusted for any other variables in the model) with 95% confidence intervals for each drug, session, and stimulus were calculated from the multilevel model output, and used to test the stimulus-discrimination (CS+ minus CS-) differences between prazosin and placebo for each session. Specifically, during acquisition, there was no significant group x stimulus interaction (t (38.42) = -0.40, P = .69), and there was evidence across groups for significant stimulus discrimination (t (38.42) = 4.07, P < .001). In contrast, when examining the subsequent stages, we found a significant group x stimulus interaction during extinction (t (39.3) = 2.06, P = .046) and re-extinction (t (38.42) = 2.86, P = .006). *Abbreviations*: CS, conditioned stimulus; CI, confidence interval.