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Fear reacquisition and symptoms of combat-related PTSD: Specificity and preliminary examination of the influence of the 5-HT3A receptor gene

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ABSTRACT

Several studies have examined the acquisition and extinction of fear in PTSD in the context of Pavlovian conditioning. However, research examining reconditioning of fear following extinction, a form of post-extinction re-emergence of conditioned behavior is limited. Although the 5-HT3A receptor gene polymorphism has been linked to trauma responses, its influence on the re-emergence of conditioned fear among those exposed to trauma remain unclear. In the present study, combat-exposed veterans (N = 114) completed a differential fear conditioning task in which one colored rectangle (CS+) predicted a loud scream (US), whereas a different colored rectangle (CS-) predicted no US. Acquisition, extinction, and post-extinction reconditioning effects indexed by conditioned anxiety, US expectancy, and skin conductance response were examined. Associations with allelic variation in the serotonin 5-HT_3 gene, *HTR3A* (rs1062613) were also examined. Participants rated the CS+ as significantly more anxiety inducing and associated with greater US expectancy than the CS- during acquisition. The CS+ also elicited a stronger skin conductance response than the CS- during acquisition. A significant decrease in anxiety and US expectancy in response to the CS+ was observed after extinction and a re-emergence of conditioned responses to the CS+ was observed during reacquisition. Although a diagnosis of PTSD was characterized by greater anxiety to the CS + but not the CS- during acquisition and extinction, those with and without a PTSD diagnosis did not differ in the reacquisition of fear following extinction. Subsequent preliminary analysis did show that increased posttraumatic symptoms and cognitions were associated with increased US expectancy at reacquisition for the CS+ and CS- among CC carriers but not among T carriers of *HTR3A* (rs1062613). These findings suggest that posttraumatic symptoms among trauma exposed veterans with the CC polymorphism of the *HTR3A* gene may be associated with stronger reconditioning of fear following extinction.

Posttraumatic stress disorder (PTSD) is a persistent, maladaptive response to a traumatic event that includes symptoms such as hypervigilance, avoidance, negative mood, and intrusive memories (American Psychiatric Association, 2013). Though trauma can take many forms, PTSD is commonly studied within the context of combat trauma. Combat trauma is quite common among service members; however, the majority of those who experience combat trauma do not subsequently develop PTSD (Thomas et al., 2010). This discrepancy suggests the presence of risk factors that may predict who is more likely to develop PTSD. Indeed, previous research has identified several PTSD risk factors, including gender (Tolin & Foa, 2006), history of physical disease (Tortella-Feliu et al., 2019), low social support (Brewin, Andrews, & Valentine, 2000), and genetic variants (Hawn et al., 2019; Roby, 2017). In addition to identifying risk factors, research has also examined underlying mechanisms of pathological fear in PTSD through a fear conditioning framework. In the acquisition phase of the traditional fear conditioning paradigm, one conditioned stimulus (CS+) is repeatedly paired with an aversive stimulus (unconditioned stimulus; US), while another conditioned stimulus (CS-) is never paired with the US. With repeated pairing, the CS+ then becomes a signal for the US and eventually elicits a similar response (conditioned response; CR) even in the absence of the US, whereas the CS- is a learned safety signal. In the extinction phase, the CR can be extinguished by repeatedly presenting the CS+ without the US, resulting in new learning of the lack of "danger" associated with the CS+ (Duits et al., 2015; Hofmann, 2008).

Fear conditioning has been a useful framework for understanding the etiology of PTSD. Conceptually, the trauma can be considered an US, and the continued fear response in PTSD patients can be considered a

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CR. Similar stimulus contingencies may then be modeled in the laboratory. As an example within the context of combat trauma, loud noises (CS+) may be paired with a traumatic combat experience (US) and may then elicit physiological arousal or intrusive memories of the event (CR). An important component of PTSD may then be a tendency to acquire such a conditioned response (i.e., "conditionability" [Orr, Metzger, & Pitman, 2002]), impaired extinction, and/or overgeneralization of fear learning. Results from one meta-analysis comparing fear conditioning responses in individuals with anxiety-related disorders (largely PTSD) to healthy controls found no differences in responses to the CS+ during acquisition (Duits et al., 2015), suggesting that those with PTSD do not exhibit increased "conditionability." Instead, those with anxiety-related disorders exhibit small increases in fear responses to the CS-, potentially suggesting overgeneralization of the fear response to neutral stimuli. Further, individuals with anxiety-related disorders demonstrate sustained fear responses to the CS+ during extinction, suggesting impaired safety learning in individuals with PTSD. The role of fear overgeneralization and impaired extinction in PTSD is further supported by studies finding these effects are associated with increased PTSD symptom severity (Jovanovic et al., 2009; Norrholm et al., 2011). Impaired extinction learning may be particularly important to understanding PTSD etiology, as studies examining fear conditioning prior to trauma exposure indicate impaired extinction learning pre-trauma predicts PTSD symptoms post-trauma (Guthrie & Bryant, 2006; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013).

Fear conditioning paradigms may also be useful for better understanding a phenomenon evident in both experimental and clinical settings known as return of fear, or the reemergence of an extinguished fear association (Rachman, 1989). Understanding predictors of return of fear may improve outcomes for exposure therapy for PTSD, which is largely predicated on extinction learning. Considerable research has shown a return of fear effect in fear conditioning paradigms in healthy samples (see Vervliet, Craske, & Hermans, 2013 for a review). Similar work in individuals with PTSD finds evidence for fear renewal following extinction, such that the CR returns when the CS is presented in a different context (Milad et al., 2008; Wicking et al., 2016). Another potential mechanism for return of fear is reacquisition, which describes the rapid return of the CR to the CS when the CS is re-paired with the US (Kehoe & Macrae, 1997). Such re-pairing may occur within the context of combat-related PTSD, such as a fireworks display in the veterans' environment eliciting a panic attack. Thus, identifying predictors of reacquisition may both inform models of PTSD etiology and improve exposure interventions. Although fear reacquisition has been examined in animal models (Williams & Lattal, 2019), no study to date has examined fear reacquisition and its determinants within a conditioning paradigm in veterans.

Although post-extinction re-emergence of conditioned fear has not been extensively studied in the experimental psychopathology literature, it may be a meaningful construct for investigation given that it describes the susceptibility of those with PTSD to relapse after exposure to a mild stressor. Much like the ability to acquire and extinguish conditioned fear reactions, however, there is likely considerable inter-individual variation in the magnitude of post-extinction re-emergence of conditioned fear among trauma exposed individuals. The study of genetic variants has revealed an important source of inter-individual variation that may inform how fear conditioning processes explain symptoms of PTSD (Londsdorf & Kalisch, 2011). Indeed, candidate gene studies have identified genetic factors that may be relevant in predicting fear conditioning responses among those with PTSD (Amstadter et al., 2009). For example, previous research has found increased fear response to the CS- and impaired fear extinction in Met/Met carriers of the catechol-O-methyltransferase (COMT) val158met polymorphism with PTSD (Deslauriers, Toth, Der-Avakian, & Risbrough, 2018; Seth Davin Norrholm et al., 2013). Similarly, slower extinction learning is seen in Met-allele carriers of the BDNF Val66Met polymorphism who have PTSD (Felmingham et al., 2018). Notably, this polymorphism has also been found to predict worse treatment response to exposure therapy in PTSD (Felmingham, Dobson-Stone, Schofield, Quirk, & Bryant, 2013), highlighting the clinical significance of research examining gene × environment interactions. There is increasing evidence suggesting that serotonin modulates affective responses following extreme stress, such as those that precede PTSD (Connor & Davidson, 1998; Zhao et al., 2017). Although the rs1062613 single-nucleotide polymorphism (SNP) in the upstream regulatory region of the 5-HT3A gene has been identified to be functionally important in understanding psychiatric disorders more broadly (Barnes, Hales, Lummis, & Peters, 2009) and trauma responses more specifically, (Jang, Lee, Huh, & Chae, 2015), its interactive effects with fear conditioning processes in the context of PTSD is unclear.

The present study employed a differential fear conditioning task in which one colored rectangle (CS+) predicted a loud scream (US), whereas a different colored rectangle (CS-) predicted no US to examine acquisition, extinction, and post-extinction reconditioning among combat-exposed veterans. Responding on the task was indexed by conditioned anxiety, US expectancy, and skin conductance. It was predicted that those with a diagnosis of PTSD would be characterized by greater excitatory fear conditioning to the CS+ during acquisition compared to those without a PTSD diagnosis, and that this effect would endure through extinction, as revealed by a group by CS interaction at both stages of conditioning. Similarly, it was predicted that those with a diagnosis of PTSD would be characterized by greater reconditioning to the CS+ following extinction compared to those without a PTSD diagnosis. The present study also explored the extent to which the rs1062613 polymorphism of the 5-HT3A gene may represent a novel candidate gene for gene × environment interactions in predicting reconditioning effects following fear extinction among combat-exposed veterans. One study found that CC carriers of the rs1062613 polymorphism of the 5-HT3A who had also experienced childhood sexual trauma demonstrated increased loudness dependence of auditory evoked potential, which is thought to reflect central serotonergic neurotransmission (Jang et al., 2015). Although there are advantages to a genome wide association approach to research questions of this nature, the candidate gene approach employed in the present is focused on a specific gene with existing knowledge of the gene's biological function and impact on specific traits. As a preliminary exploratory test, the extent to which posttraumatic symptoms among combat-exposed veterans are associated with increased fear reacquisition particularly among CC carriers of the rs1062613 polymorphism of the 5-HT3A gene was examined.

1. Method

1.1. Participants

The sample ($N = 114$) consisted of combat-exposed veterans from Operation Enduring Freedom and Operation Iraqi Freedom. Two participants who screened negative for manic episodes and psychotic symptoms prior to enrollment met criteria on the MINI (see below) once enrolled in the study and were excluded from analyses, leaving a sample of 112 participants. The mean age of participants was 31.89 years ($SD = 7.70$), ranging from 21 to 63 years. Among the combat-exposed veterans, 32.1% met criteria for PTSD. The majority of participants were male ($n = 100$; 89.3%), and the racial composition was as follows: Caucasian ($n = 88$; 78.6%), African American ($n = 10$; 8.9%), Hispanic/Latino ($n = 9$; 8.0%), American Indian/Alaska Native ($n = 1$; 0.9%), multiethnic ($n = 2$; 1.8%), and other ($n = 2$; 1.8%). Approximately half (59.1%) of the sample was married, and the average level of educational attainment was some college (47.3%).

1.2. Measures

Posttraumatic Cognitions Inventory (PTCI; Foa, Ehlers, Clark, Tolin, & Orsillo, 1999). The PTCI is a 33-item self-report scale of trauma-related

beliefs (i.e., posttraumatic cognitions) about the self (e.g., "I am a weak person"), the world (e.g., "People can't be trusted"), and self-blame (e.g., "The event happened because of the way I acted"). Items are rated on a Likert scale from 1 (*totally disagree*) to 7 (*totally agree*). The PTCI demonstrated excellent internal consistency in the present study (α = 0.96).

Posttraumatic Stress Disorder Checklist (PCL; Weathers, Litz, Herman, Huska, & Keane, 1993 , October). The PCL is a 17-item measure of DSM-IV (APA, 1994) PTSD symptoms in the past month. The PCL consists of three subscales (i.e., re-experiencing, avoidance/numbing, arousal). Items are rated on a Likert scale from 1 (*not at all*) to 5 (*extremely*). The PCL demonstrated excellent internal consistency in the present study (α = 0.95).

The *Combat Exposure Scale* (CES; Keane et al., 1989) is a 7-item scale that assesses various dimensions of combat-related stress. The CES uses a 5-point Likert scale (1–5) and assesses the duration and intensity of exposure to a variety of warzone stressors and traumatic events. The CES had marginally acceptable internal consistency in the present study (α = 0.63).

The *State Trait Anxiety Inventory–Trait Version, Form Y* (STAI-T; Spielberger, 1983) is a 20-item scale that measures the enduring or chronic experience of anxiety (e.g., "I feel nervous and restless"). Items are rated on a Likert scale from 1 (*not at all*) to 4 (*very much so*). The STAI-T had good internal consistency in the present study (α = 0.92).

The *Empirical Valence Scale* (EVS; Lishner, Cooter, & Zald, 2008) is a labeled magnitude scale designed for rating subjective experiences. In contrast to the equidistant verbal labels of visual analogue or Likert-like scales, the verbal labels on the EVS are spaced according to prior research assessing how participants rate the verbal labels themselves on a 0–100 scale. Participants rated anxiety and US expectancy in response to the CSs and fear in response to the US using the unipolar version of the EVS scale. The unipolar version of the scale contains the following labels and corresponding values: not at all (0), barely (7), slightly (12), mildly (24), moderately (38), strongly (70), extremely (85), and most imaginable (100). These labels are placed on a line (without the corresponding numeric values). Ratings are made by clicking anywhere on the line with a mouse.

1.3. DNA collection and genotyping

Saliva samples were collected from study participants using Oragene vials. DNA was extracted from Oragene vials by the University DNA Resources Core. Genotyping of the selected single nucleotide polymorphisms (SNPs) was performed using the Sequenom genotyping platform. Standard quality control procedures (duplicates, positive controls, blanks) were performed. Genotype efficiency was evaluated and only SNPs with GE > 95% were included. Hardy-Weinburg equilibrium (HEW) was calculated for each of the SNPs. All SNPs were in HWE ($p > .05$). The genotype frequencies of CC, CT, and TT polymorphisms in the 5-HT3A SNPs were 58% ($n = 64$), 34% ($n = 38$), and 8% ($n = 9$), respectively. The allele frequencies are in line with previous studies that concluded that the T allele is less frequent than the C allele (Hammer et al., 2012). The group size of the TT genotype ($n = 9$) was too small to examine between-subject effects (Gatt, Nemeroff, Schofield, Paul, & Williams, 2010); therefore, consistent with previous research (Jang et al., 2015), T (CT and TT) and CC carrier groups were categorized for analysis.

1.4. Materials and apparatus

Pink and green rectangles that subtended 2.5 × 2 degrees of visual angle were presented as conditioned stimuli (Van Damme, Crombez, Hermans, Koster, & Eccleston, 2006). The sound of a woman screaming (750 ms duration; 50 ms rise/fall; 90 dB) was played over earbud headphones as the unconditioned stimulus (Neumann & Waters, 2006). Ratings of the CSs and US were made on the same modified empirical valence scale. The stimuli were presented on a 17″ monitor (75 Hz; 1280 x 1024 resolution) at a viewing distance of 82 cm. Stimuli were presented using MATLAB's Psychophysics toolbox (Brainard, 1997).

1.5. Procedure

The university's Institutional Review Board approved all aspects of this study. All participants provided written informed consent prior to participation. Participants were recruited through advertisements placed throughout the community for veterans who had been exposed to a combat-related trauma. Potential participants were screened for combat-related trauma exposure using the Combat Exposure Scale (CES; Keane et al., 1989) and the PTSD module of the Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1997). The MINI is a structured diagnostic tool that assesses 17 disorders consistent with the fourth edition of the Diagnostic and Statistical Manual of Mental Disorder (DSM-IV; American Psychiatric Association, 1994). After endorsing exposure to a trauma, veterans were asked to briefly describe the trauma to ensure combat-related trauma exposure. Eligible participants came into the laboratory to complete the remaining study requirements. Prior to study participation, all participants were required to present a valid military ID to ensure the trauma was combat-related. Following informed consent, participants provided a saliva sample and then were administered the full MINI (Sheehan et al., 1997) by a post-baccalaureate research assistant who was trained and supervised by a licensed clinical psychologist. Participants were excluded from the present study if they met diagnostic criteria for any of the following: bipolar disorder, intellectual disability, psychosis, attention-deficit hyperactivity disorder, developmental disorder, and/or current or past neurological disease. After completing the MINI, participants were seated at a standard laboratory computer to complete the study measures and the conditioning task.

The conditioning task consisted of the following stages: *Habituation.* Participants viewed 5 non-reinforced presentations (6 s) of each CS. During all stages, CSs were presented at the center of the screen in a pseudorandom order that prevented more than 2 consecutive presentations of the same CS (Lissek et al., 2009) and were preceded by a fixation cross (1.5 s) and followed by an ITI, varied randomly between 12 s and 18 s. *Acquisition.* During this stage, the CS+ was reinforced. On CS+ trials, the US was played during the last 750 ms of CS+ presentation, such that the CS+ and US co-terminated; on CS- trials, the US was not played. The acquisition phase was divided into two blocks, both with 5 presentations of CS+ trials, and 5 presentations of CS- trials. Between blocks, US expectancy was rated for each CS. *Extinction.* The acquisition procedure was repeated, but without US presentation (i.e., CS+ reinforcement). *Reacquisition.* The acquisition procedure was repeated immediately after extinction, but with only one block of trials.

Self-report CR assessment. At the end of the habituation, acquisition, extinction, and reacquisition stages, participants retrospectively rated how anxious the CSs made them feel and how much they expected the scream. At the end of the experiment, participants were presented the scream one additional time, and then rated how afraid the scream made them feel. The term *anxious* was used with the CSs and *afraid* with the US, because anxiety involves anticipating a threat (e.g., waiting for a possible scream), whereas fear involves encountering a threat (e.g., hearing a scream; Lang, Davis, & Öhman, 2000).

Psychophysiological CR assessment. A psychophysiological monitoring system (Biopac Systems; Goleta, CA) was used to collect electrodermal activity (EDA) data. EDA was measured (10 kHz sampling rate) using a pair of radio-translucent electrodes (1 cm diameter, Biopac Systems) attached to the thenar and hypothenar eminences of participant's left palm during the Pavlovian fear conditioning task. All EDA data were preprocessed using Biopac Acknowledge 4.1 software. A 1 Hz low pass filter was applied and data were resampled at 250 Hz. EDA was used to measure autonomic arousal in response to the CSs at habituation, conditioning, extinction, and reacquisition. To exclude responding to

the US and to match the period of measurement for the CS+ and CS–, data after 5.2 s were omitted. Thus, EDA in response to the CSs was measured from 2 s to 5.2 s (3.2 s window).

1.6. Data analytic approach

Consistent with similar Pavlovian conditioning studies (e.g., Armstrong & Olatunji, 2017; Lissek et al., 2009), separate analyses were conducted for each stage of the conditioning paradigm (habituation, acquisition, extinction, reacquisition). 2 (CS: CS+, CS–) X 2 (diagnostic status: PTSD+, PTSD-) mixed-effects analyses of variance (ANOVAs) were conducted to examine US expectancy, subjective anxiety, and physiological arousal in response to the CS between those with and without PTSD. Significant CS by diagnostic status interactions were followed up with independent samples t-tests to examine group differences in response to the CS+ and CS-. To further probe change in CS responding across conditioning phases, exploratory analyses were conducted for the extinction and reacquisition phases in which time (i.e., acquisition to extinction; extinction to reacquisition) was added to the model.

Consistent with the view that genetic influences do not fit categorical diagnoses (PTSD vs no PTSD) and that more dimensional approaches can maximize precision and statistical power in the search for genetic variants linked to psychopathology (Waszczuk et al., 2020), influences on conditioned responses during reacquisition were further examined with moderation analyses using continuous measures of PTSD symptoms. More specifically, twelve moderation models were tested using the PROCESS macro (Hayes, 2013) to examine whether *HTR3A* (rs1062613; CC vs T carrier) influenced the relationship between posttraumatic cognitions and PTSD symptoms and US expectancy, subjective anxiety, and physiological arousal during reacquisition. Continuous predictor variables were mean-centered prior to analysis. Significant interactions were probed with a simple slopes analysis (Aiken & West, 1991) examining the impact of the predictor variable on conditioned responses during reacquisition for CC and T carriers separately.

2. Results

2.1. Descriptive statistics

Veterans with and without PTSD did not significantly differ on age, gender, ethnicity, education, income, time since last tour of duty, combat exposure, *HTR3A* (rs1062613) carrier status, or ratings of fear in response to the US (p's $> .05$). However, there was a significant association between diagnostic status and marital status, such that those with PTSD were more likely to have been divorced. As expected, veterans with PTSD reported significantly higher PTSD symptoms and trait anxiety than veterans without PTSD (p's $< .001$). See Table 1 for descriptive statistics.

2.2. Habituation

Expectancy. The main effects of CS, $F(1, 110) = 2.12$, $p = .15$, $\eta^2_p = .02$, and diagnostic status, $F(1, 110) = 0.001$, $p = .97$, $\eta^2_p < .001$, and the interaction between CS and diagnostic status, $F(1, 110) = 0.688$ $p = .35$, $\eta^2_p = .01$, were not significant.

Anxiety. The main effect of CS, $F(1, 110) = 1.44$, $p = .23$, $\eta^2_p = .01$, and diagnostic status, $F(1, 110) = 2.70$, $p = .10$, $\eta^2_p = .02$, and the interaction between CS and diagnostic status, $F(1, 110) = 0.02$, $p = .90$, $\eta^2_p < .001$, were not significant.

EDA. The main effects of CS, $F(1, 102) = 0.003$, $p = .96$, $\eta^2_p < .001$, and diagnostic status, $F(1, 102) = 0.42$, $p = .52$, $\eta^2_p = .004$, and the interaction between CS and diagnostic status, $F(1, 102) = 0.02$, $p = .89$, $\eta^2_p < .001$, were not significant.

Table 1
Means/standard deviations for demographics and symptoms for those with (+) and without (−) posttraumatic stress disorder (PTSD).

	PTSD+ (n = 36)	PTSD- (n = 76)	
Age	32.54 (8.00)	31.59 (7.60)	$t(109) = .60$, $p = .55$, $d = .12$
Gender			$\chi^2(1) = 1.97$, $p = .16$, $V = .13$
Male	83.3%	92.1%	
Female	16.7%	7.9%	
Ethnicity			$\chi^2(5) = 3.04$, $p = .69$, $V = .17$
White	80.6%	77.6%	
Black	5.6%	10.5%	
Latino	11.1%	6.6%	
American Indian	0%	1.3%	
Multiracial	0%	2.6%	
Other	2.8%	1.3%	
Asian American	0%	0%	
HTR3A (rs1062613)			$\chi^2(1) = .01$, $p = .92$, $V = .01$
CC carrier	58.3%	57.3%	
T carrier	41.7%	42.7%	
Marital status			$\chi^2(2) = 14.65$, $p < .01$, $V = .37$
Married	45.7%	65.3%	
Single	14.3%	25.3%	
Divorced	40.0%	9.3%	
Income			$\chi^2(9) = 11.45$, $p = .25$, $V = .32$
Under $20K	14.3%	8.0%	
$20–29K	14.3%	8.0%	
$30–39K	28.6%	24.0%	
$40–49K	20.0%	14.7%	
$50–59K	5.7%	12.0%	
$60–69K	2.9%	4.0%	
$70–79K	5.7%	5.3%	
$80–89K	8.6%	4.0%	
$90–99K	0%	4.0%	
Over $100K	0%	16%	
Education			$\chi^2(5) = 4.70$, $p = .56$, $V = .21$
Some high school	0%	1.3%	
High school degree	5.7%	10.7%	
Some college	60.0%	41.3%	
College degree	28.6%	32.0%	
Masters degree	5.7%	13.3%	
Doctoral degree	0%	1.3%	
Months since last tour	68.47 (116.082)	38.12 (35.73)	$t(38.14) = 1.53$, $p = .14$, $d = .42$
CES	22.89 (8.51)	20.67 (8.14)	$t(109) = 1.31$, $p = .19$, $d = .27$
Comorbidity			
MDD	44.4%	1.3%	$\chi^2(1) = 35.29$, $p < .001$, $V = .56$
Any anxiety-related disorder	63.9%	21.1%	$\chi^2(1) = 19.75$, $p < .001$, $V = .42$
Panic disorder	38.9%	5.3%	$\chi^2(1) = 20.48$, $p < .001$, $V = .43$
Agoraphobia	41.7%	10.5%	$\chi^2(1) = 14.52$, $p < .001$, $V = .36$

(*continued on next page*)

Table 1 (*continued*)

	PTSD+ (n = 36)	PTSD- (n = 76)	
Social anxiety disorder	19.4%	1.3%	χ^2 (1) = 12.10, p < .001, V = .33
OCD	11.1%	0%	χ^2 (1) = 8.76, p < .001, V = .28
GAD	8.3%	6.6%	χ^2 (1) = .11, p = .74, V = .03
PCL	55.56 (12.52)	32.13 (9.92)	t (110) = 10.70, p < .001, d = 2.17
PTCI	122.16 (35.74)	76.67 (23.80)	t (50,21) = 6.94, p < .001, d = 1.16
STAI	50.61 (11.76)	35.66 (8.88)	t (54.60) = 6.77, p < .001, d = 1.51
US afraid rating	582.69 (217.06)	523.00 (141.84)	t (49.83) = 1.590, p = .124, d = .35

Note. CES = Combat Exposure Scale; MDD = Major depressive disorder; OCD = obsessive compulsive disorder; GAD = generalized anxiety disorder; PCL = PTSD Checklist; PTCI = ;STAI = State Trait Anxiety Inventory; US = Unconditioned stimulus.

2.3. Acquisition

Expectancy. There was a significant main effect of CS, F (1, 110) = 373.99, p < .001, η^2_p = .77, such that the CS+ elicited higher US expectancy than the CS-. The main effect of diagnostic status, F (1, 110) = 2.03, p = .16, η^2_p = .02, and the interaction between CS and diagnostic status, F (1, 110) = 1.97, p = .16, η^2_p = .02, were not significant.

Anxiety. There was a significant main effect of CS, F (1, 110) = 169.94, p < .001, η^2_p = .61. Although a main effect of diagnostic status was not significant, F (1, 110) = 2.17, p = .14, η^2_p = .02, Fig. 1 shows that there was a significant interaction between CS and diagnostic status, F (1, 110) = 8.26, p < .05, η^2_p = .07. Results of follow-up independent samples t-tests indicated that veterans with PTSD reported increased anxiety in response to the CS+ than veterans without PTSD, t (110) = 2.45, p < .05, 95% CIs [137.58, 14.40], d = 0.50. In contrast, veterans with and without PTSD did not significantly differ in anxiety in response to the CS-, t (85.47) = −0.90, p = .37, 95% CIs [−19.39, 51.46], d = −0.17.

EDA. There was a significant main effect of CS, F (1, 102) = 67.98, p < .001, η^2_p = .40, such that the CS+ elicited higher physiological arousal than the CS-. Although the main effect of diagnostic status was not significant, F (1, 102) = 2.03, p = .16, η^2_p = .02, there was a trend-level interaction between CS and diagnostic status, F (1, 102) = 3.91, p = .05, η^2_p = .04. However, results of follow-up independent samples t-test indicated that veterans with and without PTSD did not differ significantly in physiological arousal to the CS+, t (54.04) = 1.58, p = .12, 95% CIs [-.35, .04], d = 0.36, or to the CS-, t (102) = −0.20, p = .84, 95% CIs [-.04, .05], d = −0.04.

2.4. Extinction

Expectancy. There was a significant main effect of CS, F (1, 109) = 93.46, p < .001, η^2_p = .46, such that the CS+ elicited higher US expectancy than the CS-. Although there was also a trend level effect of diagnostic status, F (1, 109) = 3.41, p = .07, η^2_p = .03, there was not a significant interaction between CS and diagnostic status, F (1, 109) = 0.92, p = .34, η^2_p = .01. Results of a 2 (CS) by 2 (diagnostic status) by 2 (time) mixed ANOVA to examine partial extinction (i.e., decreased US expectancy from acquisition to extinction) revealed a significant CS by time interaction, F (1, 109) = 163.57, p < .001, η^2_p = .60. Results of follow-up paired samples t-tests indicated a significant decrease in US expectancy in response to the CS+ from acquisition to extinction, t (110) = 15.99, p < .001, 95% CIs [158.52, 203.37], d = 1.5, suggesting extinction of the conditioned response. There was also a significant increase in US expectancy in response to the CS- from acquisition to extinction, t (10) = −2.30, p < .05, 95% CIs [−50.82, −3.76], d = −0.22. However, there was not a significant CS by time by diagnostic status interaction, F (1, 109) = 0.30, p = .59, η^2_p = .003.

Anxiety. There was a significant main effect of CS, F (1, 109) = 45.36, p < .001, η^2_p = .29, such that the CS+ elicited higher anxiety than the CS-, and there was a trend level effect of diagnostic status, F (1, 109) = 2.92, p = .09, η^2_p = .03, such that those with PTSD reported higher anxiety than those without PTSD during extinction. There was a trend-level interaction between CS and diagnostic status, F (1, 109) = 3.15, p = .08, η^2_p = 03. Results of follow-up independent samples t-tests indicated that veterans with PTSD reported increased anxiety in response to the CS+ than veterans without PTSD, t (109) = 2.16, p < .05, 95% CIs [4.53, 106.65], d = 0.44. In contrast, veterans with and without PTSD did not significantly differ in anxiety in response to the CS-, t (109) = 0.79, p = .43, 95% CIs [−26.16, 60.44], d = 0.16.

Results of a 2 (CS) x 2 (diagnostic status) x 2 (time) mixed ANOVA to examine partial extinction (i.e., decreased anxiety from acquisition to extinction) revealed a significant CS by time interaction, F (1, 109) = 84.93, p < .001, η^2_p = .44. Follow-up analyses indicated a significant decrease in anxiety in response to the CS+ from acquisition to extinction, t (109) = 10.10, p < .001, 95% CIs [87.27, 129.86], d = 0.96. In contrast, there was not a significant change in anxiety in response to the CS- from acquisition to extinction, t (109) = −1.56, p = .12, 95% CIs [−34.03, 4.11], d = −0.15. There was also a trend-level CS by time by diagnostic status interaction, F (1, 109) = 3.95, p = .05, η^2_p = .04. However, follow-up analyses revealed no significant difference in anxiety response to the CS+ between those with and without PTSD, t (109) = 1.12, p = .26, 95% CIs [−19.68, 71.20], d = 0.23, and no significant difference in anxiety in response to the CS-, t (109) = −1.56, p = .12, 95% CIs [−72.33, 8.62], d = −0.31, (see Table 2 and Fig. 1).

EDA. The main effects of CS, F (1, 102) = 0.003, p = .96, η^2_p < .001, and diagnostic status, F (1, 102) = 0.01, p = .94, η^2_p < .001, and the interaction between CS and diagnostic status, F (1, 102) < .001, p = .97, η^2_p < .001, were not significant, indicating a full extinction of the conditioned physiological response (see Table 2 and Fig. 1).

2.5. Reacquisition

Expectancy. There was a significant main effect of CS, F (1, 109) = 217.90, p < .001, η^2_p = .67, such that the CS+ elicited greater expectancy of the US than the CS-, suggesting a reacquisition effect. The main effect of diagnostic status, F (1, 109) = 1.92, p = .17, η^2_p = .02, and the interaction between CS and diagnostic status, F (1, 1109) = 0.33, p = .57, η^2_p = .003, were not significant. Results of a 2 (CS) by 2 (diagnostic status) by 2 (time) mixed ANOVA to further examine reacquisition (i.e., increased US expectancy from extinction to reacquisition) revealed a significant CS by time interaction, F (1, 109) = 74.52, p < .001, η^2_p = .41. Follow-up paired samples t-tests indicated a significant increase in US expectancy in response to the CS+, t (109) = −8.01, p < .001, 95% CIs [−136.49, −82.37], d = −0.76, and a significant decrease in US expectancy in response to the CS-, t (109) = 4.57, p < .001, 95% CIs [29.37, 74.43], d = 0.43, suggesting successful and specific reacquisition of the conditioned response. There was not a significant CS by time by diagnostic status interaction, F (1, 109) = 0.01, p = .94, η^2_p < .001.

Anxiety. There was a significant effect of CS, F (1, 109) = 81.36, p < .001, η^2_p = .43, such that the CS + elicited higher anxiety than the CS-, suggesting a reacquisition effect. There was also a significant main effect of diagnostic status, F (1, 109) = 5.31, p < .05, η^2_p = .05, such that those with PTSD reported higher anxiety than those without PTSD, regardless of CS. However, there was not a significant interaction between CS and diagnostic status, F (1, 109) = 0.35, p = .56, η^2_p = .003. Results of a 2 (CS) by 2 (diagnostic status) by 2 (time) mixed ANOVA to further examine reacquisition (i.e., increased anxiety from extinction to reacquisition) revealed a significant CS by time interaction, F (1, 109) =

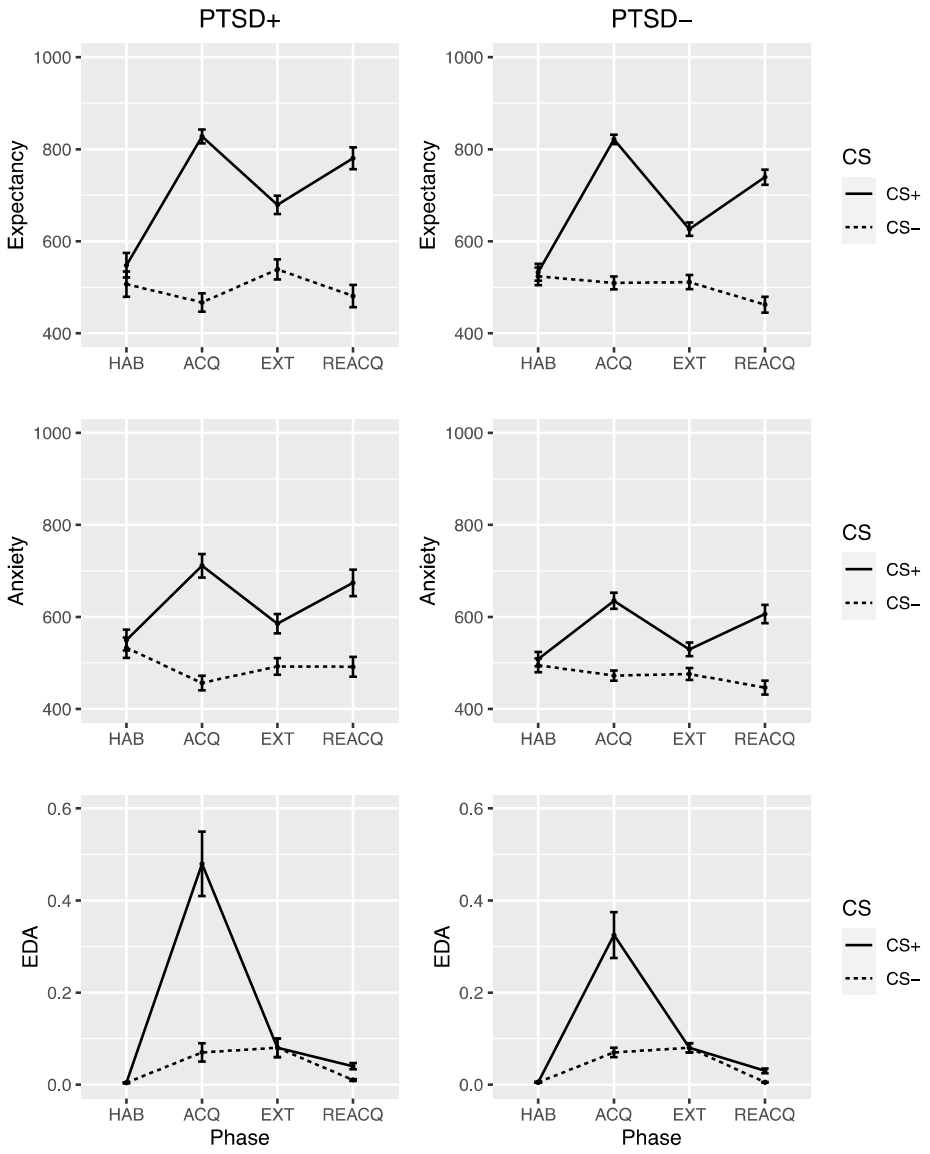

Fig. 1. US expectancy, anxiety, and EDA in response to the CS ± at each phase of conditioning (habituation, acquisition, extinction, reacquisition) for veterans with PTSD (PTSD+) and veterans without PTSD (PTSD-). Error bars represent ± 1 SE.

28.75, $p < .001$, $\eta^2_p = .21$. Follow-up paired samples t-tests indicated a significant increase in anxiety in response to the CS+, $t\,(109) = -7.22$, $p < .001$, 95% CIs [−102.45, −58.29], $d = -0.69$, and a trend-level decrease in anxiety in response to the CS-, $t\,(109) = 1.75$, $p = .08$, 95% CIs [−2.73, 43.49], $d = 0.17$. However, there was not a significant CS by time by diagnostic status interaction, $F\,(1, 109) = 0.19$, $p = .66$, $\eta^2_p < .002$.

EDA. There was a significant effect of CS, $F\,(1, 102) = 44.66$, $p < .001$, $\eta^2_p = .31$, such that the CS+ elicited higher physiological arousal than the CS-. The main effect of diagnostic status, $F\,(1, 102) = 2.22$, $p = .14$, $\eta^2_p = .02$, and the interaction between CS and diagnostic status, $F\,(1, 102) = 1.24$, $p = .27$, $\eta^2_p = .01$, were not significant. Results of a 2 (CS) by 2 (diagnostic status) by 2 (time) mixed ANOVA to further examine reacquisition (i.e., increased physiological response from extinction to reacquisition) revealed a significant CS by time interaction, $F\,(1, 102) = 11.13$, $p < .01$, $\eta^2_p = .10$. However, follow-up paired samples t-tests revealed a significant decrease in physiological response to the CS+, $t\,(103) = 5.99$, $p < .001$, 95% CIs [.03, .06], $d = 0.58$, and a significant decrease in physiological response to the CS-, $t\,(103) = 6.98$, $p < .001$, 95% CIs [.05, .09], $d = 0.68$. Thus, a reacquisition effect was not evident for physiological arousal. Further, there was not a significant CS by time by diagnostic status interaction, $F\,(1, 102) = 0.31$, $p = .58$, $\eta^2_p = .003$.

2.6. *Effects of HTR3A (rs1062613) and PTSD symptoms on fear reacquisition*

As shown in Table 3, there was a significant interaction effect between *HTR3A* (rs1062613) and PTSD symptoms to predict CS+ expectancy at reacquisition, $\Delta R^2 = 0.04$, $F = 4.14$, $p < .05$. Simple effects analysis revealed a significant positive relation between PTSD symptoms and US expectancy to the CS+ at reacquisition for CC carriers, such that increased PTSD symptoms were associated with increased US expectancy, $\beta = 2.35$, $t = 2.10$, $p < .05$. In contrast, there was no significant relation found between PTSD symptoms and US expectancy at reacquisition for T carriers (see Fig. 2). There was also a significant interaction effect between *HTR3A* (rs1062613) and PTSD symptoms to predict US expectancy to the CS- at reacquisition, $\Delta R^2 = 0.05$, $F = 5.61$, $p < .05$. Simple effects analysis revealed a significant positive relation between PTSD symptoms and US expectancy to the CS- at reacquisition for CC carriers, such that increased PTSD symptoms were associated with increased US expectancy, $\beta = 3.68$, $t = 3.24$, $p < .01$. In contrast,

Table 2
Means and standard deviations for conditioned responses at each conditioning phase.

PTSD+					
Phase of conditioning					
CR	CS	Habituation	Acquisition	Extinction	Reacquisition
US expectancy	**CS+**	547.75 (184.38)	827.63 (133.06)	679.02 (133.72)	780.39 (159.93)
	CS-	506.78 (187.57)	467.05 (112.67)	538.85 (144.56)	480.89 (165.61)
Anxiety	**CS+**	549.64 (154.18)	711.15 (157.20)	585.18 (129.99)	673.78 (193.00)
	CS-	533.06 (153.18)	456.50 (80.56)	492.41 (116.09)	491.64 (176.79)
EDA	**CS+**	.004 (.005)	.48 (.50)	.08 (.10)	.04 (.06)
	CS-	.004 (.005)	.07 (.11)	.08 (.11)	.01 (.02)
PTSD–					
Phase of conditioning					
CR	**CS**	**Habituation**	**Acquisition**	**Extinction**	**Reacquisition**
US expectancy	**CS+**	532.63 (146.16)	821.20 (60.77)	626.00 (114.81)	739.29 (132.39)
	CS-	523.79 (153.37)	509.37 (123.06)	511.18 (123.13)	462.19 (134.30)
Anxiety	**CS+**	508.14 (127.41)	635.16 (151.91)	529.59 (125.66)	606.01 (161.29)
	CS-	494.87 (122.44)	472.53 (102.14)	475.85 (103.67)	446.33 (102.16)
EDA	**CS+**	.005 (.005)	.32 (.40)	.08 (.11)	.03 (.03)
	CS-	.005 (.007)	.07 (.10)	.08 (.11)	.005 (.01)

Note. PTSD = posttraumatic stress disorder; CR = conditioned response; CS = conditioned stimulus; US = unconditioned stimulus; EDA = electrodermal activity.

Table 3
Model coefficients for the hypothesized moderations between rs1062613 and PTSD symptoms to predict expectancy in fear conditioning ($N = 110$).

Predictor	Fear conditioning outcome					
	Y_1 (CS+ expectancy)			Y_2 (CS- expectancy)		
	Coeff	SE	*p*	Coeff	SE	*p*
X (PCL)	2.35	1.12	<.05	3.68	1.14	<.01
M (*HTR3A*)	−47.35	26.73	.08	7.26	27.24	.79
XM (PCL X *HTR3A*)	−3.55	1.74	<.05	−4.20	1.77	<.05
ΔR^2	.04		<.05	.04		<.05
Constant	778.11	17.43	<.01	468.96	17.77	<.01
	$R^2 = .07$			$R^2 = .09$		
	$F(3,106) = 2.75$, $p = .05$			$F(3,106) = 3.60$, $p < .05$		
Predictor	**Y_1 (CS+ anxiety)**			**Y_2 (CS- anxiety)**		
	Coeff	**SE**	***p***	**Coeff**	**SE**	***p***
X (PCL)	3.91	1.39	<.05	1.91	1.07	.07
M (*HTR3A*)	−2.84	33.21	.93	23.48	25.52	.36
XM (PCL X *HTR3A*)	−3.91	2.16	.07	−1.21	1.66	.47
ΔR^2	.03		.07	.005		.47
Constant	632.90	21.66	<.01	452.25	16.64	<.01
	$R^2 = .07$			$R^2 = .04$		
	$F(3,106) = 2.66$, $p = .05$			$F(3,106) = 1.57$, $p = .20$		
	Y_1 (CS+ EDA)			**Y_2 (CS- EDA)**		
X (PCL)	.0002	.0004	.59	<.0001	.0001	.99
M (*HTR3A*)	.001	.01	.50	.004	.002	.07
XM (PCL X *HTR3A*)	.0004	.001	.51	.0002	.0001	.17
ΔR^2	.004		.51	.02		.17
Constant	.03	.01	<.01	.004	.002	<.01
	$R^2 = .03$			$R^2 = .07$		
	$F(3,99) = .94$, $p = .43$			$F(3,99) = 2.50$, $p = .06$		

Note. PCL = PTSD Checklist; CS = conditioned stimulus.

there was no significant relation found between PTSD symptoms and US expectancy at reacquisition for T carriers (see Fig. 2).

There was likewise a trend-level interaction between *HTR3A* (rs1062613) and PTSD symptoms to predict anxiety in response to the CS + at reacquisition, $\Delta R^2 = 0.03$, $F = 3.26$, $p = .07$. Simple effects analysis revealed a significant positive relation between PTSD symptoms and anxiety in response to the CS+ at reacquisition for CC carriers, such that increased PTSD symptoms were associated with increased anxiety, $\beta = 3.91$, $t = 2.82$, $p < .05$. In contrast, there was no significant relation found between PTSD symptoms and anxiety in response to the CS+ at reacquisition for T carriers (see Fig. 3). Similar interaction effects between *HTR3A* (rs1062613) and PTSD symptoms were not observed in predicting anxiety in response to the CS- or EDA response to either CS during reacquisition.

2.7. Effects HTR3A (rs1062613) and posttraumatic cognitions on fear reacquisition

As shown in Table 4, there was a significant interaction effect between *HTR3A* (rs1062613) and posttraumatic cognitions to predict US expectancy to the CS+ at reacquisition, $\Delta R^2 = 0.07$, $F = 7.79$, $p < .05$. Simple effects analysis revealed a significant positive relation between posttraumatic cognitions and US expectancy to the CS+ at reacquisition for CC carriers, such that increased posttraumatic cognitions were associated with increased US expectancy, $\beta = 1.13$, $t = 2.09$, $p < .05$. In contrast, there was a trend-level negative relation found between posttraumatic cognitions and US expectancy at reacquisition for T carriers, such that increased posttraumatic cognitions were associated with decreased US expectancy, $\beta = -0.95$, $t = -1.84$, $p = .07$. (see Fig. 4). There was also a significant interaction effect between *HTR3A* (rs1062613) and posttraumatic cognitions to predict US expectancy to the CS- at reacquisition, $\Delta R^2 = 0.09$, $F = 10.63$, $p < .01$. Simple effects analysis revealed a significant positive relation between posttraumatic cognitions and US expectancy to the CS- at reacquisition for CC carriers, such that increased posttraumatic cognitions were associated with increased US expectancy, $\beta = 1.76$, $t = 3.19$, $p < .01$. In contrast, there was no significant relation found between posttraumatic cognitions and US expectancy at reacquisition for T carriers (see Fig. 4).

There was likewise a trend-level interaction between *HTR3A* (rs1062613) and posttraumatic cognitions to predict anxiety in response to the CS+ at reacquisition, $\Delta R^2 = 0.03$, $F = 3.30$, $p = .07$. Simple effects analysis revealed a significant positive relation between posttraumatic

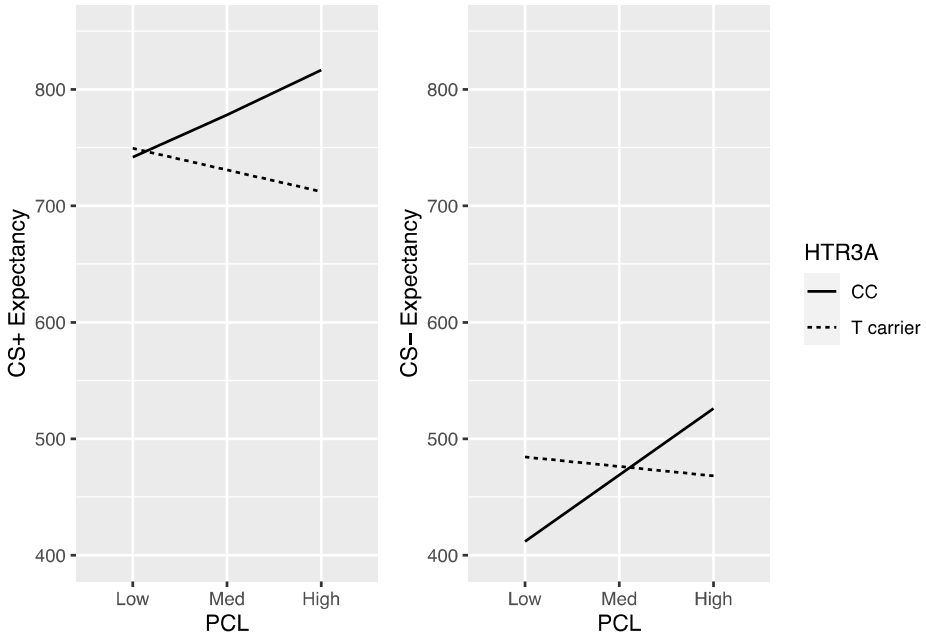

Fig. 2. Simple regression slopes of PTSD Checklist (PCL) scores predicting US expectancy in response to the CS ± at reacquisition at values of *HTR3A* (CC vs T). PCL scores were mean-centered prior to analysis, such that low, medium, and high represent the mean ± one standard deviation.

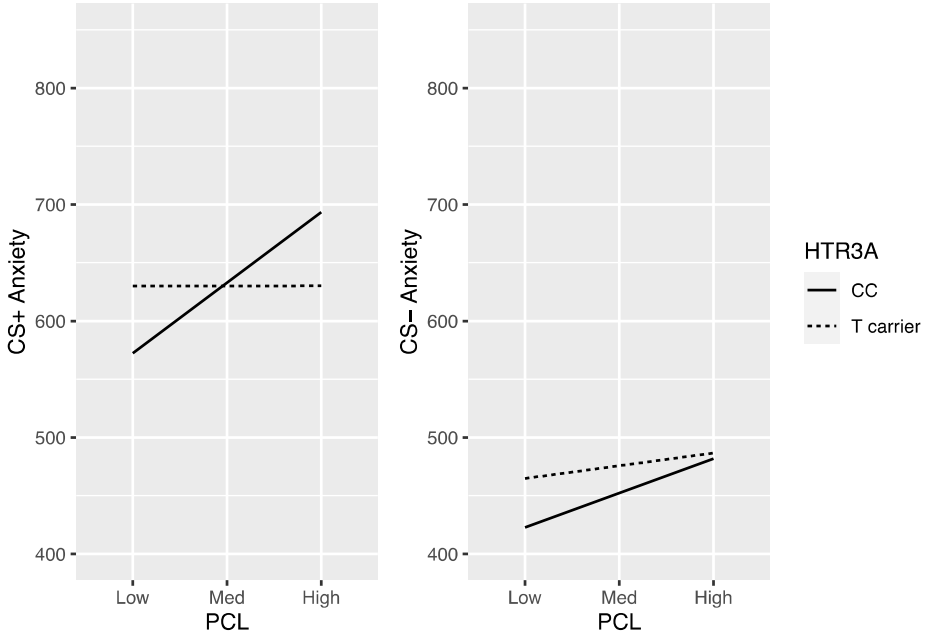

Fig. 3. Simple regression slopes of PTSD Checklist (PCL) scores predicting anxiety in response to the CS ± at reacquisition at values of *HTR3A* (CC vs T). PCL scores were mean-centered prior to analysis, such that low, medium, and high represent the mean ± one standard deviation.

cognitions and anxiety in response to the CS+ at reacquisition for CC carriers, such that increased posttraumatic cognitions were associated with increased anxiety, $\beta = 1.58$, $t = 2.30$, $p < .05$. In contrast, there was no significant relation found between posttraumatic cognitions and anxiety in response to the CS+ at reacquisition for T carriers (see Fig. 5). Similar interaction effects between *HTR3A* (rs1062613) and posttraumatic cognitions were not observed in predicting anxiety in response to the CS- or EDA response to either CS during reacquisition.

3. Discussion

As predicted, the fear conditioning paradigm employed in the present study led to the acquisition of discriminant conditioned responding, as participants reported greater US expectancy, anxiety, and displayed greater physiological arousal to the CS+ versus the CS–. In addition, the fear conditioning paradigm revealed fear learning tendencies that are associated with a diagnosis of PTSD. More specifically, a diagnosis of PTSD was characterized by greater anxiety to the CS+ but not the CS- during acquisition and extinction. This finding is consistent with models which posit that PTSD is characterized by increased "conditionability"—that is, a tendency to acquire more fear to neutral stimuli associated with traumatic events. This is operationalized in terms of greater excitatory fear learning to the CS+, such that the CS + elicits more anxiety than the CS– at acquisition, a pattern that has been

Table 4
Model coefficients for the hypothesized moderations between rs1062613 and posttraumatic cognitions to predict fear conditioning outcomes ($N = 110$).

Predictor	Fear conditioning outcome					
	Y_1 (CS+ expectancy)			Y_2 (CS- expectancy)		
	Coeff	SE	*p*	Coeff	SE	*p*
X (PTCI)	1.13	.54	<.05	1.76	.55	<.01
M (*HTR3A*)	−45.64	26.54	.09	8.06	27.19	.77
XM (PTCI X *HTR3A*)	−2.08	.75	<.05	−2.49	.76	<.01
ΔR^2	.07		<.05	.09		<.01
Constant	779.77	17.33	<.01	471.50	17.75	<.01
	$R^2 = .09$			$R^2 = .10$		
	$F(3,106) = 3.64$, $p < .05$			$F(3,106) = 4.08$, $p < .05$		
Predictor	**Y_1 (CS+ anxiety)**			**Y_2 (CS- anxiety)**		
	Coeff	**SE**	***p***	**Coeff**	**SE**	***p***
X (PTCI)	1.58	.69	<.05	.69	.53	.19
M (*HTR3A*)	−3.38	33.74	.92	26.28	25.81	.31
XM (PTCI X *HTR3A*)	−1.72	.95	.07	−0.97	.73	.18
ΔR^2	.03		.07	.02		.18
Constant	634.29	22.03	<.01	452.55	16.85	<.01
	$R^2 = .05$			$R^2 = .03$		
	$F(3,106) = 1.79$, $p = .15$			$F(3,106) = 1.07$, $p = .36$		
	Y_1 (CS+ EDA)			**Y_2 (CS- EDA)**		
X (PTCI)	.0001	.0002	.76	<.0001	<.0001	.89
M (*HTR3A*)	.05	.009	.54	.004	.002	.08
XM (PTCI X *HTR3A*)	.0002	.0002	.34	.0001	.0001	.18
ΔR^2	.009		.34	.02		.18
Constant	.03	.006	<.01	.004	.002	<.01
	$R^2 = .04$			$R^2 = .08$		
	$F(3,99) = 1.33$, $p = .27$			$F(3,99) = 2.95$, $p < .05$		

Note. PTCI = Posttraumatic Cognitions Inventory; CS = conditioned stimulus.

observed in individuals with PTSD compared to controls (e.g., Armstrong, Federman, Hampson, Crabtree, & Olatunji, 2021; Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Wessa & Flor, 2007). These findings also suggest that a diagnosis of PTSD may be characterize by a failure of extinction of fear responses. That is, PTSD may represent a failure to fully inhibit fear responses even after learning that the CS+ no longer predicts the US. Contrary to predictions, a diagnosis of PTSD was not characterized by greater reconditioning to the CS+ following extinction in individuals with combat exposure. Davis et al., (2000) suggest that PTSD may be better characterized by a failure to discriminate between the CS + and CS–, as seen in a tendency to acquire fear responding to *both* the CS+ and the CS–. In other words, rather than involving *enhanced* excitatory fear learning to the CS+ alone, PTSD may also reflect impaired inhibitory fear learning to the CS–. However, the present study failed to find robust support for this impaired inhibitory fear learning perspective of PTSD.

The findings did show that a diagnosis of PTSD was characterized by increased "conditionability" and failed extinction only for anxiety responses to the CS+. This may suggest that the differentiation of those with a diagnosis of PTSD from those without PTSD by excitatory fear learning to the CS+ may not be especially robust in the present study. Consideration of the group characteristics may also partially explain the lack of robust group differences in conditioned responding across the various levels of analysis. More specifically, the present study compared veterans with PTSD to an unselected sample of veterans without PTSD rather than a comparison with healthy controls. Many of the veterans without PTSD in the present study did meet diagnostic criteria for other disorders, including anxiety disorders which are also characterized by increases in the acquisition of fear learning (Lissek et al., 2005). In fact, previous research suggests that PTSD may not differ in conditionability from anxiety disorders (Duits et al., 2015). Accordingly, comparing veterans with PTSD to veterans without PTSD that may have other anxiety-related disorders likely limited group differences that may be observed in the present study. It is worth noting that many have questioned the research tradition of selecting patients from a single diagnostic group (mostly PTSD) and then comparing— in this case fear conditioning—between one clinical group and a control sample (Duits et al., 2015). Although current psychiatric nosology considers PTSD to be a discrete diagnostic category, empirical research suggests that it does not represent a discrete clinical syndrome (Ruscio, Ruscio, & Keane, 2002). Accordingly, a dimensional, rather than categorical, conceptualization of PTSD where continuous measures focused on evaluating the full range of PTSD symptom patterns may prove to be more informative (Broman-Fulks et al., 2006). In fact, adoption of a dimensional approach (see Supplementary Table 1) in the present study shows that PTSD symptoms corresponded more strongly with subjective (but not physiological) indicators of fear acquisition (but not fear

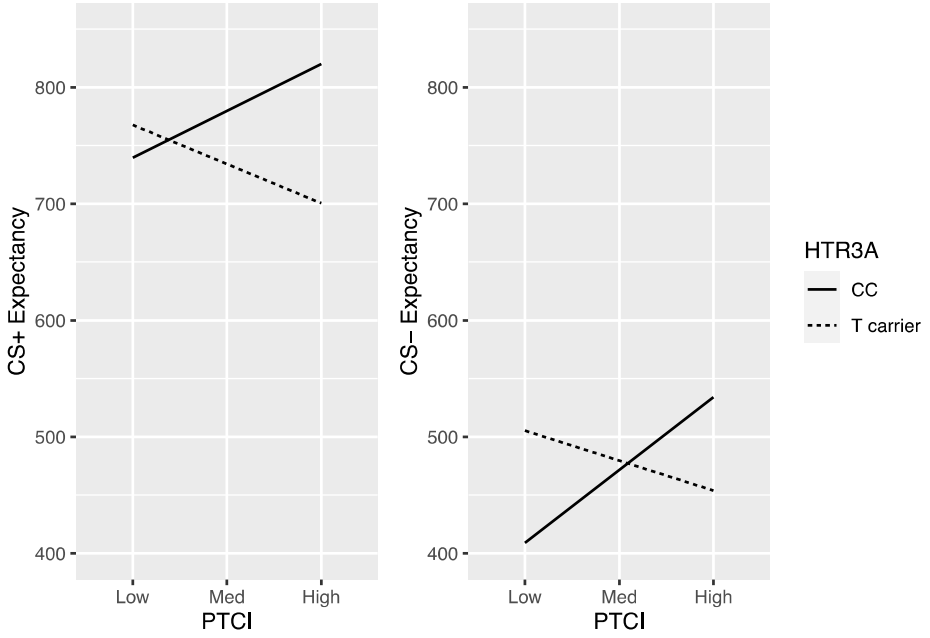

Fig. 4. Simple regression slopes of Posttraumatic Cognitions Inventory (PTCI) scores predicting US expectancy in response to the CS ± at reacquisition at values of *HTR3A* (CC vs T). PTCI scores were mean-centered prior to analysis, such that low, medium, and high represent the mean ± one standard deviation.

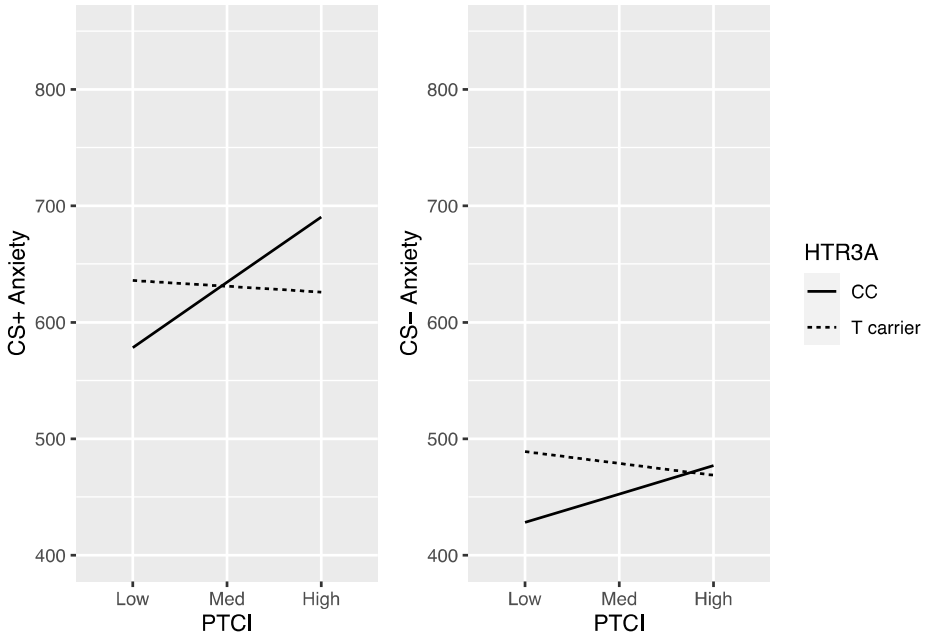

Fig. 5. Simple regression slopes of Posttraumatic Cognitions Inventory (PTCI) scores predicting anxiety in response to the CS ± at reacquisition at values of *HTR3A* (CC vs T). PTCI scores were mean-centered prior to analysis, such that low, medium, and high represent the mean ± one standard deviation.

extinction).

A major aim of the present study was to model the reconditioning of fear following extinction among trauma exposed veterans. Indeed, this process may be conceptualized as a form of post-extinction re-emergence of conditioned behavior that may be relevant to the maintenance of PTSD symptoms. Following a significant decrease in anxiety and US expectancy in response to the CS+ after extinction, the present study did find a re-emergence of these conditioned responses to the CS+ during reacquisition. Contrary to predictions, however, veterans with and without a diagnosis of PTSD did not differ in the reacquisition of conditioned responses. Consideration of the conditioning task may partially explain the lack of robust group differences at reacquisition. More specifically, group differences may not have been robustly observed due to a ceiling effect in conditioned responding. This may be best understood in terms of a "strong situation," a concept describing a situation that elicits such a reliable response that it conceals individual differences (see Lissek, Pine, & Grillon, 2006). As a strong situation, the conditioning task employed in the present study likely represents the presentation of unambiguous threat associations that evokes an adaptive fear response among those with and without PTSD alike. In other words, reinforcing 100% of the trials during reacquisition may have produced such a reliable contingency awareness leaving little room for robust group differences to be observed. This interpretation suggests that what may be required to detect more robust group differences is a "weak situation," a more ambiguous conditioning context where all reacquisition trials are not reinforced. Indeed, Lissek et al. (2006) suggest that "weak situations" (situations characterized by ambiguity or uncertainty) are more sensitive to detecting meaningful group differences in the psychobiology of fear and anxiety.

The present study also employed a more dimensional approach that examined continuous measures of the full range of PTSD symptoms and their association with fear learning at reacquisition. The findings showed that higher levels of PTSD symptoms (i.e., re-experiencing, avoidance/numbing, and arousal) and cognitions in the full sample were significantly associated with increased US expectancy and anxiety responses to the CS+ at reacquisition. Furthermore, *HTR3A* (rs1062613) moderated this effect such that the significant positive relation between PTSD symptoms and US expectancy and anxiety responses to the CS+ at reacquisition observed for CC carriers was not observed among CT/TT carriers. This pattern of preliminary findings is consistent with previous research showing that the CC genotype is associated with increased anxiety and amygdala hyper-responsiveness (Kilpatrick et al., 2011). Indeed, converging evidence has shown that the amygdala plays a central role in the expression of conditioned fear (Davis, Falls, & Gewirtz, 2000). However, the present study also found a significant positive relation between PTSD symptoms/cognitions and US expectancy and responses to the CS- at reacquisition for CC carriers but not for CT/TT carriers. This suggests that overgeneralization of expectancies to neutral stimuli at reacquisition may be associated with PTSD symptom severity among veterans who are CC carriers. The increased association between PTSD symptoms and US expectancy to the CS− (a safety signal) among CC carries at reacquisition is also consistent with research suggesting that impaired safety signal processing may contribute to PTSD (Jovanovic et al., 2009). The impaired safety signal perspective posits that those with PTSD may have deficits in inhibiting threat responding in the presence of a safety signal.

The inability to suppress conditioned fear in the context of safety may be due to a complex gene × environment interaction between one's individual predisposition(s) and environmental factors, such as early life stress and the frequency, degree, and intensity of traumatic event(s) (Jovanovic, Kazama, Bachevalier, & Davis, 2012). The present study suggests that one potential genetic determinant of the inability to suppress conditioned fear in the context of safety at reacquisition may be the CC genotype of the *HTR3A* gene. Of note is that this view is consistent with previous research showing that the *HTR3A* CC genotype is associated with a greater risk of psychopathology subsequent to experiencing trauma, possibly due to low central serotonin activity (Jang et al., 2015). Although there is strong evidence for the view that PTSD is characterized by excessive excitatory fear learning (Orr et al., 2000), deficits in inhibitory learning may be observed in PTSD when employing tasks that probe complex forms of inhibitory learning (e.g., safety transfer in conditional discrimination; see Jovanovic & Ressler, 2010). The present study suggests that consideration of the CC genotype of the *HTR3A* gene may also reveal inhibitory learning deficits to the CS– at reacquisition that may be associated with PTSD symptoms. Given that the present study is largely exploratory and preliminary, however, future research with larger samples will be needed to determine if the CC genotype of the 5-HT3A gene is a robust predictor of only reacquisition

or other experimental analogues that describe the return of fear and its implications for PTSD.

The present study offers important insights in the acquisition, extinction, and reacquisition of fear in relation to symptoms of PTSD. For example, the preliminary findings suggest that among trauma exposed veterans with the *HTR3A* CC genotype, reacquisition of fear following extinction is linked to PTSD symptom severity. However, the preliminary findings should be interpreted with study limitations in mind. Most notably is that as a candidate gene association study, the present study has a very small sample and statistical power is likely limited. Furthermore, multiple moderation analysis was conducted and based on a Bonferroni correction, a *p*-value of .004 would be the appropriate statistical threshold which would render only one of the gene × symptom interactions significant. This is further evidence that these findings should be considered preliminary until replicated in larger samples that can more adequately support multiple tests. There is also evidence highlighting existing genetic differences based on race/ethnicity (e.g., Huang, Shu, & Cai, 2015), and while the statistically significant gene × symptom interactions did remain unchanged in the present study when adding race/ethnicity as a covariate, future research employing larger more representative samples may shed more light on potential race/ethnicity differences on the moderated effects of the CC polymorphism of the 5-HT3A observed in the present study.

The moderated effects of the CC polymorphism of the 5-HT3A gene were also found to be specific to the self-report rantings of US-expectancy and anxiety. Although there is strong evidence for the validity of the US-expectancy measure for example (Boddez et al., 2013), the present findings require replication with more objective measures. While divergence between self-report and psychophysiological measures of fear conditioning is not uncommon in the PTSD literature (Blechert et al., 2007; Wessa & Flor, 2007), replication using additional physiological measures (i.e., fear potentiated startle) of conditioned fear responding would allow for greater confidence in the present findings. Another limitation is that US expectancy ratings were collected retrospectively, at the end of each conditioning phase. Future research assessing US-expectancy online during each trial may allow for more meaningful insight into the cognitive processes that characterize fear reconditioning in PTSD. Although the present study suggests that the CC genotype of the 5-HT3A gene could be relevant to understanding the relationship between US-expectancy at reacquisition and PTSD symptoms, future studies with larger sample sizes that consider a wider range of genetic characteristics are needed before definitive inferences can be made. The present study is also limited by the cross-sectional design. Indeed, it is unclear if the PTSD-related fear learning tendencies observed in the present study are a cause or consequence of PTSD and related symptoms. In fact, it may be that the present findings are more relevant for the maintenance of PTSD symptoms rather than the development of such symptoms. Prospective research designs that sample symptoms more dimensionally will be needed to better delineate the complex effects of gene × environment interactions on fear learning, unlearning, and relearning in PTSD.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brat.2022.104085.

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