Endogenous Salivary α-Amylase does not Interact with Skin Conductance Response during Fear Extinction in Posttraumatic Stress Disorder

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Abstract

Posttraumatic Stress Disorder (PTSD) is associated with elevated noradrenergic signaling, which has an impact on emotional learning and memory. Fear extinction is thought to underlie the processes of exposure therapy, however the relationship between noradrenaline and extinction in PTSD is unclear. Participants with PTSD (*n* = 21), trauma-exposure without PTSD (TC; *n* = 36), and non-trauma-exposed controls (NTC; *n* = 27) completed a fear conditioning and extinction paradigm, and conditioned fear was indexed by skin conductance response (SCR). Salivary α-amylase (sAA) collected at baseline and immediately post-fear acquisition was used as an index of noradrenaline, and we ~~were~~ examined whether sAA in response to fear acquisition was a moderator between fear extinction and PTSD symptoms. While there was a significant increase in sAA from baseline to post-fear acquisition, this was not modulated by group. Compared to ~~the comparison groups~~TC and NTC, the PTSD group displayed a slower decline in SCRs during early extinction, which generalized across stimulus type, and was not moderated by sAA. These findings suggest that the relationship between fear extinction and PTSD symptoms does not change as a function of sAA levels; however previous research suggests other processes of fear learning may be associated with noradrenergic activity in PTSD.

Keywords: PTSD; trauma; extinction; salivary α-amylase; sympathetic arousal.

1. Introduction

Hyperactive noradrenergic signaling is considered a hallmark correlate of Posttraumatic Stress Disorder (Zoladz and Diamond, 2013), a chronic psychiatric condition that can develop following a traumatic event (American Psychiatric Association, 2013). Noradrenaline (NA) is a catecholamine released from the adrenal glands during situations of stress and physical arousal, and PTSD is often shown to be associated with elevated NA, relative to controls (Geracioti et al., 2001; Pietrzak et al., 2013; Yehuda et al., 1998; Yehuda et al., 1992). Elevated noradrenergic signaling is associated with enhanced emotional memory formation (Mueller and Cahill, 2010), and excessive sympathetic responding to a traumatic event may result in stronger trauma memories (Zoladz and Diamond, 2013). Impaired extinction of psychophysiological fear conditioning is widely considered to be a prominent feature of the fear-related symptoms of PTSD (Briscione et al., 2014; Pitman et al., 2012; Zuj et al., 2016b), and research is emerging to suggest a relationship between sympathetic arousal and fear extinction in PTSD. Further, fear extinction is considered to be the underlying theoretical basis of exposure therapy, and understanding the relationship between these processes is an important step in translating information from the laboratory to treatment.

Prospective research shows that increased sympathetic arousal (indexed by higher resting heart rate) in the early stages post-trauma predicts increased PTSD symptom development (Bryant et al., 2000; Shalev et al., 1998). Alternatively, Videlock et al. (2008) measured plasma NA levels in emergency room admissions, finding that participants who developed greater PTSD symptoms at 5-months post-admission had lower plasma NA in the emergency room. A recent cross-sectional study, however, found that patients with current PTSD diagnosis had significantly higher urinary NA secretion than controls (Wingenfeld et al., 2015). While it is commonly agreed that PTSD is associated with increased sympathetic

arousal, the literature is somewhat inconsistent regarding the relationship between NA secretion, fear extinction, and PTSD symptoms (Zuj et al., 2016b).

Consistently elevated noradrenergic activity in the aftermath of trauma is thought to contribute to the persistence of fear-related symptoms of PTSD (Zoladz and Diamond, 2013), and the use of pharmacological drugs to block NA receptors may prove useful in the treatment of PTSD. Fitzgerald et al. (2015) recently showed propranolol, a β1/β2-adrenoceptor antagonist, enhanced fear extinction in rats when administered immediately following fear conditioning. In healthy humans, propranolol appears promising when administered immediately following a reactivated conditioned fear memory to target memory reconsolidation (Kindt et al., 2014; Soeter and Kindt, 2011, 2012). Further, a recent clinical study in humans showed that propranolol use by participants undergoing six treatment sessions for PTSD showed lower psychophysiological responses (indexed by skin conductance response (SCR) and heart rate response) to reactivated trauma memories, compared to control groups that did not receive propranolol (Brunet et al., 2014). These findings support an earlier pilot study showing that a 7-day course of propranolol in the acute aftermath of trauma resulted in lower PTSD symptoms two months post-trauma, compared to individuals who did not receive propranolol (Vaiva et al., 2003). Propranolol acts to block noradrenergic signaling, however Fitzgerald et al. (2015) highlight that the mechanism by which propranolol improves PTSD symptoms is largely unknown. It is possible that propranolol and the blockade of NA receptors reduce PTSD symptoms by enhancing the extinction of exaggerated emotional fear memories associated with the trauma. That is, we suggest that NA levels may moderate the relationship between fear extinction and PTSD symptoms.

Impaired fear extinction learning is widely considered a prominent feature of the fear-related symptoms of PTSD (Briscione et al., 2014; Pitman et al., 2012; Zuj et al., 2016b).

Research is largely consistent that NA modulates emotional learning and memory, and this effect also translates to fear extinction learning (Mueller and Cahill, 2010). Pharmacological studies in humans show that administration of yohimbine (an α^2-receptor antagonist that increases NA release) to healthy controls prior to fear acquisition results in a stronger conditioned fear trace that was resistant to extinction (Soeter and Kindt, 2012). Alternatively, rodent studies show that yohimbine is associated with context- and dose-dependent enhancements in fear extinction when administered immediately prior to extinction (Cain et al., 2004; Morris and Bouton, 2007). Behavioral research in humans shows that strategically timed stress induction tasks (and associated increases in sympathetic arousal) have the capacity to strengthen fear acquisition (Antov et al., 2013), and enhance fear extinction (Antov et al., 2015), when the stress task is performed immediately prior to the respective acquisition/extinction phase. Mueller and Cahill (2010) argue that NA modulation on extinction is strongest when the relationship between the conditioned stimulus (CS) and the unconditioned stimulus (US) is most predictable.

α-amylase is an enzyme found in saliva that has been shown to increase in association with sympathetic arousal (Nater and Rohleder, 2009). As such, salivary α-amylase (sAA) has been proposed as an indicator of NA levels (Chatterton et al., 1996; Rohleder and Nater, 2009), and has been used in a number of studies to examine the relationship between NA and intrusive memories in PTSD. For example, a recent study found the interaction between sAA and salivary cortisol to predict greater frequency of negative intrusive memories in a sample with PTSD, compared to trauma-exposed and non-exposed controls (Nicholson et al., 2014). Another recent study with healthy controls found that elevated sAA (following a cold pressor test) predicted greater frequency of intrusive memories to negative images in men but not women (Bryant et al., 2013). These findings suggest that sAA may present as a useful marker for noradrenergic signaling in fear-related features of PTSD.

The current study used a standardized differential fear conditioning and extinction paradigm to investigate the effect of endogenous sAA activity on fear extinction. Specifically, we were interested in the role of sAA in response to fear conditioning as a moderator between fear extinction learning ability and PTSD symptom severity in a sample with current PTSD, trauma exposure without PTSD, and non-trauma-exposed controls. Based on recent research using sAA as an indicator of NA in intrusive memories (Bryant et al., 2013; Nicholson et al., 2014), and the role of noradrenergic signaling in fear conditioning and extinction (Antov et al., 2015; Antov and Stockhorst, 2014; Soeter and Kindt, 2012), we predicted that sAA levels would moderate the relationship between fear extinction learning and PTSD symptoms. Specifically, we hypothesized that poorer fear extinction learning ability would be associated with greater PTSD symptom severity, and that this relationship would be stronger with lower sAA levels in response to fear conditioning.

2. Method

2.1. Participants

Eighty-four participants aged 18-63 years (M = 27.6 years, SD = 11.4) and comprising 37 males and 47 females were involved in the study. Participants were recruited from University of Tasmania undergraduate populations and from local psychology clinics. The results from a subset of the participants in the current study have been reported elsewhere,[1] examining hours-since-waking, endogenous cortisol reactivity, and negative appraisals as potential moderators of fear extinction (Zuj et al., 2017a; Zuj et al., 2016a; Zuj et al., 2017b).

[1] There are considerable differences in the sample~~s~~ presented in the current paper compared to previous studies from our lab. Specifically, the current study included 12 of 15 PTSD, 18 of 33 TC, and 12 of 22 NTC subjects from Zuj et al. (2016a); and 14 of 21 PTSD and 19 of 33 TC subjects from Zuj et al. (2017a). The current paper has the greatest overlap with Zuj et al. (2017b), with 17 of 18 PTSD, 31 of 33 TC, and 25 of 27 NTC participants. In total, 6 PTSD, 14 TC, and 12 NTC subjects have been included in all four studies. ~~compared to Zuj et al. (2016a), and Zuj et al. (2017a). The current paper has greater overlap with Zuj et al. (2017b), and this is reflected in the strong similarity of findings in the fear conditioning and extinction paradigm.~~

Participants were allocated to groups on the basis of exposure to a criterion A stressor (American Psychiatric Association, 2013) using the Traumatic Events Questionnaire (TEQ; Vrana and Lauterbach, 1994).

The PTSD Checklist-Civilian version (PCL-C; Weathers et al., 1994) was used to estimate PTSD symptom severity according to the diagnostic criteria of the DSM-IV (American Psychiatric Association, 2000). These diagnostic criteria include at least one intrusive memory symptom, three avoidance symptoms, and two hyperarousal symptoms. The PCL-C for the DSM-IV was used in the current study as data collection began prior to the availability of diagnostic instruments for the DSM-5. All participants in the PTSD group displayed the above symptomatology, with the exception of four participants who displayed less than three avoidance symptoms, but showed greater severity of intrusive memories and hyperarousal symptoms. This criteria resulted in the PTSD group showing a minimum PCL-C total score of 40, with 52% showing a total score greater than 50. Participants who had experienced a criterion A stressor, but did not meet the minimum guidelines for PTSD in a general population sample (i.e., PCL-C total score < 30; National Center for Posttraumatic Stress Disorder, n.d.), were classified as trauma-exposed controls (TC). PCL-C total scores ranged from 17-29 in the TC group. Participants who reported no experience of a traumatic event were classified as non-trauma exposed controls (NTC).

The above criteria resulted in three groups: PTSD (n = 21), TC (n = 36), and NTC (n = 27). Participants in the PTSD and TC groups were exposed to a variety of environmental and interpersonal traumas, including war exposure (n = 5), accident (n = 21), natural disaster (n = 27), witness to serious injury or death (n = 36), assaulted or molested (n = 22), threatened or held captive (n = 12), and tortured or the victim of terrorism (n = 2). Mean years since trauma for the PTSD group was 10.1 years (SD = 12.8 years), and 10.1 years (SD = 10.9 years) for the TC group. Participants also completed the Depression Anxiety Stress

Scale – 21 item version (DASS; Lovibond and Lovibond, 1995). The University of Tasmania Social Science Human Research Ethics Committee and the Tasmanian Health and Medical Research Ethics Committee approved this study. All participants gave full informed consent prior to involvement, and all testing was conducted in a single session.

2.2. Fear conditioning and extinction paradigm

The differential fear conditioning and extinction paradigm in the current study has been used and described previously (Orr et al., 2000; Zuj et al., 2017a; Zuj et al., 2016a; Zuj et al., 2017b). The unconditioned stimulus (US) was a 500ms mild electric shock delivered to the first interroseous muscle of the dominant hand, and set to a level considered "highly annoying, but not painful" by each participants. Figure 1 displays the experimental paradigm used and relevant instructions provided to participants.

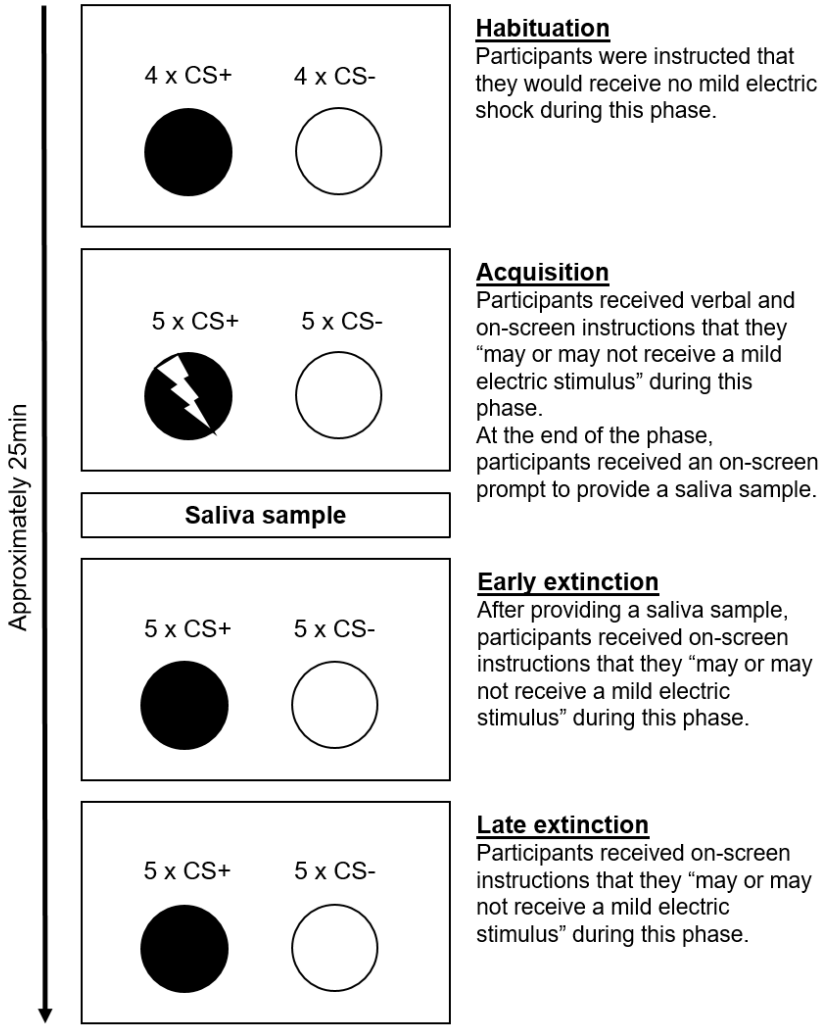

Figure 1. Each experimental phase consisted of five presentations of the CS+ and five presentations of the CS- (with four presentations of each stimulus in the habituation phase). Conditioned stimuli were self-made red and blue circles presented individually in the centre of a computer screen for 12s each, with an inter-trial interval ranging from 12-21s. Trial order was pseudo-random, with no more than two CS+/- presentations in a row. A 100% reinforcement schedule was used during the fear acquisition phase, whereby the US was

delivered immediately following the offset of every CS+ trial, as is commonly used in conditioning research (e.g., Jovanovic et al., 2014; Orr et al., 2000; Orr et al., 2014). Immediately following the acquisition phase, participants were instructed to provide a saliva sample, and when movement artefact normalized in the skin conductance recording, participants were then instructed to continue. Inter-phase intervals varied slightly between participants with some participants taking longer to provide saliva (intervals lasted no longer than 1 minute), and the entire paradigm took approximately 25 minutes to complete.

2.3. Skin conductance response

Skin conductance level was measured through a 22mV$_{rms}$, 75Hz constant-voltage coupler (FE116, ADInstruments) with bipolar electrodes on the intermediate phalange of the first and third fingers of the non-dominant hand, sampled at 512Hz and stored at 64Hz, and recorded in micro-Siemens (μS). Skin conductance response (SCR) to the CS+ and CS- was calculated by subtracting the mean SCL during the 2s prior to stimulus onset from the maximum SCL during the 12s stimulus duration. All SCR values were square-root transformed. For negative SCR values, the absolute value was transformed and given a negative sign (Milad et al., 2006) ~~and the absolute value of negative scores was transformed and the negative sign replaced~~.

2.4. Threat expectancy ratings

During the 12s stimulus presentation, participants were asked to rate their threat expectancy of the US on a 0-100 visual analogue scale (VAS; 0 “certain no electrical stimulus”; 100 “certain electrical stimulus”; as previously used by Lommen et al., 2013). Threat expectancy ratings are commonly included in studies of fear conditioning and extinction (e.g., Kindt and Soeter, 2013; Vervliet et al., 2007).

2.5. Salivary α-amylase

All participants provided saliva samples at the beginning of testing, and immediately post-fear acquisition. Endogenous noradrenergic activity was measured from sAA, which has received support from human and animal research as a valid biomarker of noradrenergic activity (Nater and Rohleder, 2009). Samples were collected immediately post-fear acquisition as sAA has an immediate peak latency response post-threat (Nater and Rohleder, 2009). Thawed samples were centrifuged at 1500 × *g* for 15 minutes to collect clear saliva, which was used for all assays without further processing. All saliva samples were brought to room temperature before adding to the assay wells. Samples were analyzed using a commercially available ELISA assay (Salimetrics, USA) according to the manufacturers instructions. All samples were analyzed in duplicate, and had an intra-assay variability of 5.6%, and an inter-assay variability of 6.3%. Baseline and post-acquisition sAA were square-root transformed to normalize distributions.

2.6. Statistical analyses

Three (group) × 2 (CS) × 5 (trial) mixed-model ANOVAs were conducted separately for each phase (with four trials each for habituation and acquisition). The first CS+/- trial of acquisition was omitted from statistical analyses as the US had not yet been encountered, and no fear learning would have occurred (Zuj et al., 2016a). Repeated measures ANCOVAs were conducted separately for early and late extinction, with a percentage of maximal conditioned responding serving as the covariate to correct for individual differences in conditioning. This value was computed according to Milad et al. (2006), whereby the largest differential conditioned response between the CS+ and CS- during acquisition was multiplied by 100. This value was mean centered prior to use as a covariate in line with previous

recommendations on conducting repeated measures ANCOVA (Aligna, 1982; Delaney & Maxwell, 1981). ~~The final CS+ trial of the acquisition phase was included as a covariate in the extinction learning ANOVAs to account for individual differences in fear acquisition.~~ All statistical analyses on SCR data used square-root transformed values. Greenhouse-Geisser corrections were made for within-subjects variables where necessary. Brown-Forsythe *F*-ratio adjustments were made where necessary, and pairwise comparisons were conducted with Bonferroni corrections or Games-Howell tests where appropriate. Moderation analyses were conducted using the PROCESS macro for SPSS (Model 1; Hayes, 2013). An alpha level of $\alpha = 0.05$ was used for all tests of statistical significance. Effect sizes are reported as Cohen's *d* following the criteria of 0.2, 0.5, and 0.8 as small, moderate and large effects, respectively (Cohen, 1988). Partial-eta squared (η_p^2) are reported for mixed-model ANOVAs.

3. Results

3.1. Descriptive and clinical data

Descriptive and inferential statistics are displayed in Table 1. One-way ANOVA revealed a trend-level between-group difference on age and sex. As expected, there were significant between group differences in PCL total scores, $F(2, 25.55) = 135.50$, $p < 0.001$, with the PTSD group having significantly higher mean PTSD symptom severity than the TC and NTC groups ($ps < 0.001$), who also significantly differed ($p < 0.001$). There was also a significant between-group difference on DASS subscale scores, with the PTSD group showing significantly greater depression, anxiety, and stress scores than the TC and NTC groups ($ps < 0.001$). The TC group had significantly higher levels of stress than the NTC group ($p = 0.003$), however there was no significant difference on depression ($p = 0.387$), or anxiety ($p = 0.159$).

Table 1

Mean scores and SDs of demographic, clinical and salivary measures

Measures	PTSD (n = 21)	TC (n = 36)	NTC (n = 27)	Test statistic	p
Demographic data					
- Age (years)	32.67 (14.62)	26.97 (9.76)	24.48 (9.67)	$F_{(2, 50.76)} = 2.93$	0.062
- Sex	13F, 8M	15F, 21M	19F, 8M	$\chi^2_{(2)} = 5.56$	0.062
PCL-C					
- Total	52.52 (11.38)	23.47 (3.87)	19.93 (2.56)	$F_{(2, 25.55)} = 135.50$	< 0.00
- Intrusive	3.00 (1.22)	0.28 (0.51)	0.00 (0.00)	$F_{(2, 81)} = 132.52$	< 0.00
- Avoidance	4.24 (1.81)	0.39 (0.69)	0.19 (0.48)	$F_{(2, 27.28)} = 84.48$	< 0.00
- Hyperarousal	3.52 (1.03)	0.44 (0.77)	0.15 (0.46)	$F_{(2, 45.66)} = 127.70$	< 0.00
DASS					
- Depression	9.43 (5.76)	2.28 (2.37)	1.52 (2.15)	$F_{(2, 30.88)} = 28.47$	< 0.00
- Anxiety	8.14 (4.26)	1.94 (1.87)	1.11 (1.67)	$F_{(2, 32.19)} = 39.33$	< 0.00
- Stress	13.62 (6.34)	4.89 (3.07)	2.56 (2.36)	$F_{(2, 32.98)} = 40.04$	< 0.00
AUDIT	6.86 (5.46)	6.22 (3.83)	6.11 (4.10)	$F_{(2, 55.50)} = 0.18$	0.837

Note: PTSD = Posttraumatic Stress Disorder; TC = Trauma-exposed control group; NTC = Non-trauma exposed control group; PCL-C Checklist-Civilian version; DASS = Depression Anxiety Stress Scale; AUDIT = Alcohol Use Disorders Identification Test.

3.2. Salivary α-amylase

A repeated measures ANOVA revealed there was a significant increase in sAA from baseline to post-acquisition, $F(1, 81) = 4.64$, $p = 0.034$, $\eta_p^2 = 0.054$, however there was no group × time interaction, $F(2, 81) = 2.18$, $p = 0.120$, $\eta_p^2 = 0.051$. Figure 2 displays mean sAA levels during baseline and post-acquisition.

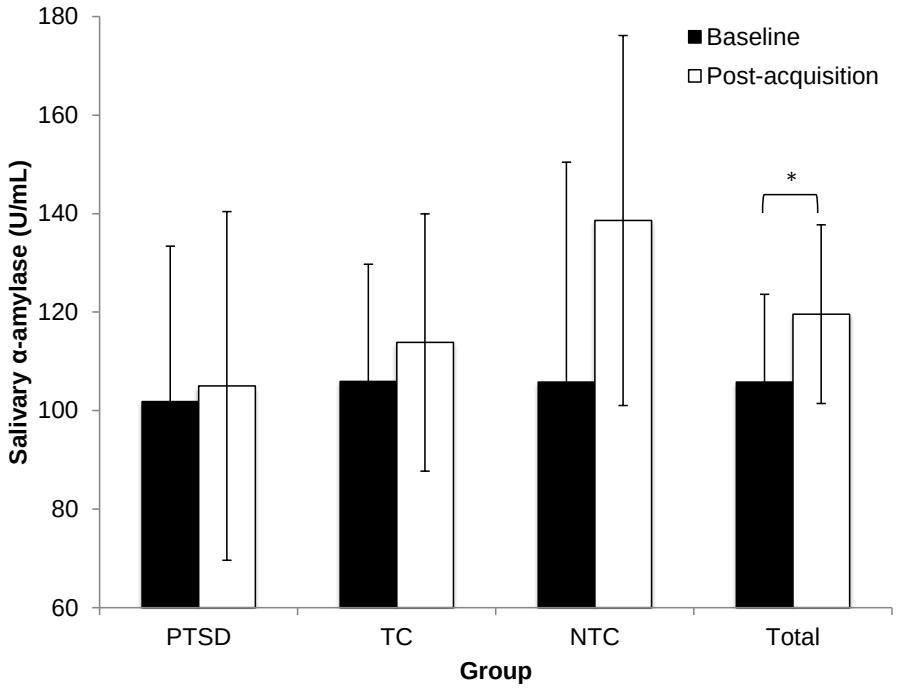

Figure 2. Baseline and post-acquisition sAA levels for each group and total levels. On average, all groups showed an increase in sAA levels from baseline to post-acquisition, however these levels did not differ significantly between groups. Figure shows raw sAA levels. * $p < 0.05$. Error bars represent 95% confidence intervals.

3.3. Threat expectancy

Self-reported US-expectancy ratings collected during the fear conditioning and extinction paradigm indicated a significant CS × trial interaction during the acquisition phase $F(3.40, 254.88) = 103.38$, $p < 0.001$, $\eta_p^2 = 0.580$, $\varepsilon = 0.850$, with differential responding increasing across trials, pooled across groups. During early extinction, there was also a significant CS × trial interaction, $F(3.46, 262.58) = 10.29$, $p < 0.001$, $\eta_p^2 = 0.119$, $\varepsilon = 0.864$, with differential responding decreasing over early extinction. During late extinction there was still a significant CS × trial interaction as differential responding continued to decrease, $F(3.63, 275.49) = 3.84$, $p = 0.006$, $\eta_p^2 = 0.048$, $\varepsilon = 0.906$. Furthermore, ANOVA revealed a significant group main effect during late extinction, with the PTSD group reporting higher average US-expectancy ratings than the TC and NTC groups, pooled across CS and trials, $F(2, 76) = 4.76$, $p = 0.011$, $\eta_p^2 = 0.111$.

3.4. SCR amplitude

Habituation. Mixed-model ANOVA revealed a significant main effect of trial, $F(2.89, 233.80) = 3.08$, $p = 0.030$, $\eta_p^2 = 0.037$, $\varepsilon = 0.962$, with SCRs decreasing over the four trials, pooled across groups (see Figure 3). No further main effects or interactions were significant.

Acquisition. There was a significant CS main effect, $F(1, 81) = 79.90$, $p < 0.001$, $d = 0.84$, with the CS+ eliciting, on average, a significantly larger SCR ($M = 0.90$, 95% CI [0.79, 1.02], $SD = 0.52$) than the CS- ($M = 0.48$ [0.37, 0.59], $SD = 0.49$). Further, there was a significant main effect of trial, $F(3.64, 294.84) = 15.12$, $p < 0.001$, $\eta_p^2 = 0.157$, $\varepsilon = 0.910$. Importantly, there was a significant group × CS interaction, $F(2, 81) = 4.42$, $p = 0.015$. Tests of simple effects revealed no between-group simple main effect for the CS+, $F(2, 81) = 0.27$, $p = 0.763$, $\eta_p^2 = 0.007$, however there was a significant between-group effect in responding to

the CS-, $F(2, 81) = 3.35$, $p = 0.040$, $\eta_p^2 = 0.076$. This effect shows that there were no significant differences between the PTSD and TC groups in responses to the CS- ($p = 0.993$), however both the PTSD and TC group displayed significantly greater responding to the CS- than the NTC group ($p = 0.041$, and $p = 0.020$, respectively).

Early extinction. With the percentage of maximal conditioned responding during acquisition included as a covariate, there was a significant main effect of CS, $F(1, 80) = 8.26$, $p = 0.005$, $d = 0.27$, with significantly larger SCR amplitude to the CS+ ($M = 0.60$ [0.51, 0.69], $SD = 0.41$) compared to the CS- ($M = 0.49$ [0.40, 0.58], $SD = 0.42$). There was also a significant main effect of trial, $F(3.77, 301.83) = 33.91$, $p < 0.001$, $\eta_p^2 = 0.298$, $\varepsilon = 0.943$, which was superseded by a significant group × trial interaction, $F(7.55, 301.83) = 2.96$, $p = 0.004$, $\eta_p^2 = 0.069$, $\varepsilon = 0.943$. ~~With the final CS+ trial of acquisition included in the analysis as a covariate, there was no significant main effect of CS, $F(1, 80) = 1.15$, $p = 0.287$, $d = 0.33$. There was, however a significant main effect of trial, $F(3.70, 296.02) = 9.23$, $p < 0.001$, $\eta_p^2 = 0.103$, $\varepsilon = 0.925$, and a significant group × trial interaction, $F(7.40, 296.02) = 2.31$, $p = 0.024$, $\eta_p^2 = 0.055$, $\varepsilon = 0.925$.~~ Tests of simple effects show the TC and NTC groups demonstrate significant reduction in SCRs from trial 1 to trial 2 ($p < 0.001$, and $p = 0.003$, respectively) and all further between-trial changes were non-significant, indicating rapid extinction in the TC and NTC groups. The PTSD group, however, showed a significant reduction in SCRs from trial 1 to trial 2 ($p = 0.021$) and from trial 2 to trial 3 ($p = 0.002$), with no significant between-trial changes after trial 3. These findings suggest that extinction learning is slower for the PTSD group, compared to TC and NTC groups (see Figure 3).

Late extinction. After controlling for the ~~final CS+ trial of acquisition~~percentage of maximal conditioned responding, the ANCOVA revealed ~~no significant main effects or interaction during the late extinction phase ($Fs < 1.53$, $ps > 0.05$).~~ a significant but small magnitude main effect of CS, $F(1, 80) = 4.74$, $p = 0.032$, $d = 0.19$, with the CS+ still eliciting

a larger SCR amplitude (M = 0.50 [0.40, 0.83], SD = 0.47) than the CS- (M = 0.42 [0.34, 0.51], SD = 0.40). Further, there was a significant main effect of trial, $F(3.30, 264.12) = 17.60$, $p < 0.001$, $\eta_p^2 = 0.180$, $\varepsilon = 0.825$

[INSERT FIGURE 3 ABOUT HERE]

Figure 3. Square-root transformed SCRs for the PTSD **(A)**, TC **(B)**, and NTC **(C)** groups. There was a significant group × trial interaction during the early extinction phase, with the PTSD group displaying a slower rate of extinction across the first three to four trials, compared to the TC and NTC groups, who showed a rapid attenuation of SCRs from trial 1 to 2. Error bars display 95% confidence intervals. * $p < 0.05$, ** $p < 0.01$.

3.5. Fear extinction and salivary α-amylase moderation

As used in a previous study from our lab (Zuj et al., 2017b), a difference score was calculated between trial 1 and trial 2 of early extinction separately for the CS+ and the CS-, which served as predictor variables in separate moderation analyses (Models 1 and 2, respectively; Hayes, 2013). This difference score was computed to account for rapid extinction during the early trials of extinction learning. PCL total score was the outcome variable, and the difference between baseline sAA and post-acquisition sAA levels was entered as the moderator variable. Model 1 did not predict a significant amount of variance in PCL total, $R = 0.23$, $R^2 = 0.055$, $F(3, 80) = 1.54$, $p = 0.210$. Further, Model 2 did not predict a significant amount of variance in PCL total, $R = 0.16$, $R^2 = 0.024$, $F(3, 80) = 0.67$, $p = 0.576$. There were no significant main effects or interactions for either model. This was still the case after age, sex, and salivary cortisol were included in the models as covariates.

4. Discussion

The aim of the current study was to investigate the potential for endogenous sAA (as an index of NA and sympathetic arousal) to serve as a key influence on the relationship between fear extinction learning and PTSD, with important clinical implications. First, while we found a significant increase in sAA from baseline to post-acquisition, this effect did not change as a factor of group, with all groups showing comparable increases in sAA. Second, the PTSD group showed slower fear extinction learning during the early extinction phase, compared to the TC and NTC groups. Third, the moderation analyses revealed that sAA levels ~~(both baseline and post-acquisition)~~ did not moderate the relationship between fear extinction learning and PTSD symptoms. Further, SCR amplitude to the CS+ and CS- during the early extinction phase did not predict PTSD symptom severity in this sample, irrespective of sAA.

From a theoretical perspective, the fear extinction model has demonstrated good predictive validity in the translation of behavioral/pharmacological interventions from the laboratory model (extinction) to the treatment model (exposure therapy), and vice versa (Scheveneels et al., 2016). The findings of the current study suggest that noradrenergic activity may not be a moderating factor of immediate extinction learning, however increased noradrenergic signaling is known to enhance emotional learning and memory processes (Mueller and Cahill, 2010). Previous research has used behavioral (e.g., Antov et al., 2015; Antov et al., 2013) and pharmacological tasks (e.g., Soeter and Kindt, 2012) designed to activate the stress response and subsequent catecholamine release. The current study involved the collection of endogenous sAA levels prior to and immediately following fear acquisition. While the US (in this case, a mild electric shock) appeared to increase sAA release, these levels showed no interaction with group or fear extinction learning ability. Previous research showing clear links between NA (or sympathetic arousal) and fear extinction directly

manipulated noradrenergic signaling either through pharmacological intervention or via stress-induction tasks (Antov et al., 2015). These research design differences may have led to greater changes in NA, compared to changes seen in the current study. There also appears to be large individual variability in sAA levels, possibly contributing to the lack of between-group differences.

The findings of the present study show specific group differences during early extinction learning, with a slower reduction in SCR amplitude to both the CS+ and CS- in the PTSD group, compared to the TC and NTC groups. These findings mirror the effects of a recent study from our lab, showing that endogenous cortisol reactivity was a significant moderator between fear inhibition to the safety signal during early extinction and PTSD symptom severity (Zuj et al., 2017b). The pattern of conditioning and extinction in the present study and in Zuj et al. (2017b) does, however, differ from previous conditioning and extinction studies in our lab (Zuj et al., 2016a; 2017a), which may be due to variations in skin conductance between changes in the sample. The findings of the current study require replication using alternative psychophysiological measures (i.e., fear-potentiated startle) to make robust conclusions.

The findings of the current study, suggest that fear extinction is not moderated by endogenous sAA. Here we also showed a significant group × CS interaction during fear acquisition, showing that the PTSD and TC groups demonstrated elevated fear responding to the CS- compared to the NTC group. Prior understanding suggests that impaired safety signal learning is related to PTSD (Jovanovic et al., 2012), however the current study suggests that reduced safety signal learning during the fear acquisition stage may be a consequence of trauma-exposure, rather than a PTSD-specific trait.

A key methodological factor that should be taken into account is that the current study examined immediate extinction training, whereby the extinction phase was conducted

immediately following fear acquisition training. Using a paradigm involving delayed extinction (for a recent review of different experimental paradigms, see Lonsdorf et al., 2017), may allow sufficient time for a sAA-moderated effect on extinction ability. Furthermore, assessing extinction recall the following day (or multiple days following extinction training) may also provide some insight into the relationship between endogenous sAA activity on extinction consolidation. Previous research used a cold pressor test prior to fear extinction learning to activate the stress response and enhance sympathetic arousal, resulting in a stronger extinction memory trace that was recalled the next day (Antov et al., 2015). In the current study, all groups displayed comparable increases in sAA levels post-acquisition, as well as similar patterns of extinction of SCR amplitude, save for the first few trials of the early extinction phase where the PTSD group displayed a slower attenuation of SCR amplitude. We speculate that these findings may be attributed to one of three possibilities: (1) the increase in sAA from baseline to post-acquisition was not large enough to detect an effect on fear conditioning and extinction; (2) the absence of a stress induction task decreased the sensitivity to reveal an interaction between sAA and fear extinction learning, or (3) noradrenergic enhancement may target a different fear learning process. We argue that the latter is more likely than the former options, as previous research has found propranolol administration during the reconsolidation window of reactivated fear memories reduces the return of fear (e.g., Kindt et al., 2014). Therefore we argue that increased noradrenergic signaling may be most effective when targeting the original fear memory, rather than extinction, which takes the form of a new inhibitory memory trace (Kindt et al., 2009). It is important to note that there were large levels of individual variability in sAA levels, and this explanation for the current findings is speculative.

A further finding of the current study that warrants explanation is the pattern of rapid extinction learning over the first three trials of the early extinction phase. A likely

explanation for this finding is that the 100% US-reinforcement schedule during the acquisition phase resulted in a highly predictable CS+-US relationship with little ambiguity. The extinction learning phases also revealed an increased SCR to the CS- as well as the CS+ during extinction, which may be due in part to the instructions presented to participants on-screen before each phase (i.e., "you may or may not receive a mild electric stimulus"). That is, potentially altering participants' expectations of the CS+-US contingency. A final explanation for rapid extinction could also be that participants initially learn no association between the US and either CS+/- during habituation, followed by learning the CS+-US relationship during acquisition, and finally reverting back to the initial learning of the habituation phase during extinction (i.e., that neither CS predicts the US).

In conclusion, the current study found that PTSD is associated with a slower reduction of generalized SCRs in the early trials of the early extinction phase. Further, while there was an overall increase in sAA levels post-fear acquisition, sAA did not differ as a function of group, and did not interact with early extinction changes in responding to the CS+ or CS-. Previous research suggests that noradrenergic stimulation via propranolol immediately following the reactivation of a fear memory has the capacity to prevent the return of fear (Kindt et al., 2014), suggesting that increased noradrenergic activity may cause a reduction in fear expression following fear reactivation, rather than fear extinction.

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