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Associations between PACAP levels and psychophysiological indicators of fear and arousal in adults with posttraumatic stress symptoms☆

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ABSTRACT

Posttraumatic stress disorder (PTSD) is associated with heightened fear responding and decreased fear regulation, as demonstrated with psychophysiological measures (e.g., autonomic function) and circulating biomarkers of stress, such as pituitary adenylate cyclase-activating polypeptide (PACAP). The current study examined associations between PACAP levels and psychophysiological indicators of arousal in a sample of 168 trauma-exposed adults with a range of PTSD symptoms. We also examined sex differences in these relationships. Psychophysiological indicators included fear-potentiated startle (FPS), heart rate (HR), and heart rate variability (HRV) during a fear acquisition and extinction paradigm. PACAP levels were derived from fasting blood samples. PACAP levels were positively correlated with FPS during acquisition and HR during extinction, and negatively correlated with HRV during extinction. There were no significant PACAP-by-sex interaction effects in FPS, HR, or HRV models. Our results in the total sample are consistent with prior work demonstrating associations between PACAP and psychophysiological arousal. They add to previous evidence that circulating PACAP levels are associated with biological markers of centromedial amygdala-dependent functioning. Future research is needed among larger samples, including longitudinal designs to better determine causal relationships between PACAP levels and fear-related arousal.

1. Introduction

Posttraumatic stress disorder (PTSD) is characterized by disrupted fear learning and symptoms of hyperarousal, intrusions, avoidance, and negative beliefs [1]. Individuals with PTSD exhibit heightened fear responding, coupled with a lesser ability to regulate fear, and these disruptions have been demonstrated with both peripheral psychophysiological measures and circulating stress biomarkers [2–6]. Among these, pituitary adenylate cyclase-activating polypeptide (PACAP) has garnered significant attention due to its translational relevance to fear and threat across humans and rodents [7–9]. Given the higher prevalence of PTSD in females [10], recent efforts have intensified to characterize sex differences in stress and fear-relevant biomarkers, including PACAP.

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PTSD has long been associated with altered stress physiology, such as exaggerated sympathetic arousal (e.g., elevated heart rate [HR]) and diminished parasympathetic control (e.g., low heart rate variability [HRV]) at rest and during challenge or stress (e.g., threat of shock) [2,3,11]. Further, people with PTSD exhibit higher fear-potentiated eye blink startle (FPS) to conditioned stimuli during fear acquisition [12–16], and higher FPS during early and late phases of fear extinction [4,17–20]. Few studies have examined sex differences in fear-relevant psychophysiology [21,22], but a recent study found that trauma-exposed females who developed PTSD demonstrated higher HR and lower HRV during fear extinction compared to males with and without PTSD [23].

PACAP is a neuropeptide that is centrally involved in the stress response [7,24,25]. It regulates the autonomic nervous system, promoting increased arousal during exposure to stress or threat (e.g., increased HR and blood pressure, pupil dilation). PACAP also facilitates the release of stress-related neurochemicals (e.g., epinephrine, dopamine) [24,25]. As such, PACAP is highly expressed in neural structures that regulate stress responding. For example, the bed nucleus of the stria terminalis, a central relay of stress circuits, expresses high levels of PACAP peptide and transcripts and is heavily innervated by PACAP-immunoreactive afferents [26–31]. The amygdala, a brain region that is implicated in fear responding, expresses high levels of PACAP receptors and receives dense PACAP fiber projections from stress-related nuclei [29,30,32]. Ressler and colleagues [6] found higher circulating PACAP levels in people with PTSD relative to controls, with notable sex differences. Specifically, a polymorphism in an estrogen response element of the PACAP type 1 receptor predicted PTSD diagnosis and severity only in female participants [6]. Moreover, higher PACAP levels correlated with higher FPS during fear acquisition among female but not male participants. Another study found that circulating PACAP levels were associated with general anxiety, again in female but not male participants [33]. Our recent work demonstrated that higher PACAP levels were associated with greater resting-state functional connectivity of the amygdala, particularly in trauma-exposed females [34]. Collectively, these findings point to sex differences in PACAP associations with psychophysiological indicators of stress and fear.

The current study examined associations between PACAP levels and psychophysiological indicators of fear, as well as sex differences in these relationships, in a symptomatic trauma-exposed sample with a range of PTSD symptom severity. Psychophysiological indicators included FPS, HR, and HRV during fear conditioning (acquisition and extinction phases). We hypothesized that 1) higher PACAP levels would be associated with higher FPS and HR, and lower HRV; 2) PACAP-FPS associations would be stronger in females versus males; 3) PACAP-HR and HRV associations would be stronger in females versus males.

2. Methods

2.1. Participants and procedure

Participants were 168 adults (65 % female) aged 19–54 (*M*age = 30.14 years) with PTSD symptoms from a DSM-5 Criterion A trauma exposure. Data were collected from 2019 to 2023 as part of a parent study investigating the relationships of stress peptides with individual differences in fear and arousal-related phenotypes in trauma-exposed adults with a range of PTSD symptoms. Participants needed to be able to provide written informed consent, be 18–55 years old, and meet diagnostic criteria for PTSD or subthreshold PTSD (meeting diagnostic criteria for at least two PTSD symptom) [35]. Exclusion criteria were confounding hormonal medical history (e.g. menopause, hormone replacement therapy), inability to have blood drawn, current antipsychotic medication (unless prescribed only for sleep), history of schizophrenia or schizoaffective disorder, current moderate-to-severe alcohol or substance use disorder, psychotic disorder, anorexia, obsessive-compulsive disorder, or manic or mixed mood episode. Because the parent study included a neuroimaging component [34,36], additional exclusion criteria were left-handedness, confounding neurological disorder, and history of moderate or severe traumatic brain injury, in order to reduce neural variability associated with these factors. All study procedures were approved by the Institutional Review Board. After providing informed consent, participants completed a clinical interview of PTSD symptoms, a blood draw, and the FPS paradigm.

2.2. PACAP

Fasting blood samples were scheduled between 8 and 10 AM, following informed consent. Samples were collected in EDTA tubes, centrifuged for 15 minutes at 3500 rpm, and plasma was stored in a -80C freezer. PACAP assays were conducted using double antibody sandwich ELISA immunoassays (Cat. No. HUFI02692, AssayGenie). The intra-assay variability was ~9 %, the assay midpoint was 1.1 fmol, and a detection limit from the linear range of the standard curve was 0.2 fmol. Four samples were excluded due to undetectable PACAP levels. Outliers with extremely high (3 standard deviations above the mean; n = 2) or unreliably low (below reliable assay detection levels; n = 1) PACAP levels were Winsorized to the next highest or lowest reliable/non-outlier values, as previously described [33,34].

2.3. Psychophysiology measures

We used a well-validated fear conditioning paradigm with habituation, acquisition, and extinction phases [Fig. 1; 4,19,36]. Habituation consisted of 12 108 dB startle probes (noise alone [NA]). During acquisition, startle probes were delivered, as well as 12 conditioned stimuli (CS; colored shapes) that were paired with an air blast (CS+) and 12 that were never paired (CS-). During extinction, no air blasts were delivered. Acquisition consisted of three counterbalanced conditioning blocks with four trials of each type (NA, CS+, CS-) per block. Each CS was presented for six seconds and co-terminated with the startle probe. For the CS+ , a 250 ms 140 p.s.i. air blast was delivered to the larynx 0.5 seconds later. After 10 minutes, the extinction phase began, consisting of four counterbalanced blocks with four trials of each type (NA, CS+, CS-) and no air blast. All trials were on a fixed schedule; the intertrial interval was 9–22 seconds. Psychophysiology measures were collected throughout the FPS paradigm using Biopac MP150 for Windows. Ag/AgCl electrodes were used to measure HR, HRV, and FPS.

2.4. Psychophysiology data processing

Data were processed using MindWare software. For HR and HRV, data were visually inspected and corrected, and variables were derived by spectral analysis of 1-min epochs. A Hamming window function and log transformation were used, and standard recommendations were followed to determine settings for the high-frequency HRV band (0.12–0.40 Hz) [37]. Electromyogram data were visually inspected for artifacts (e.g., double blinks); segments without identifiable eye blinks were removed. Startle trials with a negative peak amplitude or a peak amplitude that was below the average baseline amplitude were treated as non-responses with an amplitude of 0. Data were sampled at a rate of 1 kHz, amplified by a gain of 2000, and filtered using 28 Hz low and 500 Hz high pass filters. For FPS, data were averaged over the last two acquisition blocks (“late acquisition”), consistent with our prior work, as it represents the phase of acquisition where fear learning peaks [4,20,23,38]. HR and HRV were averaged over time for the entirety of each phase, while FPS variables were calculated in reference to each CS. Following recommendations from the literature [39], we measured reflexive eyeblinks during a response window of 20–120 ms. For each block, FPS was calculated as: (peak startle magnitude for the CS) – (peak startle magnitude for NA [i.e., baseline startle]).

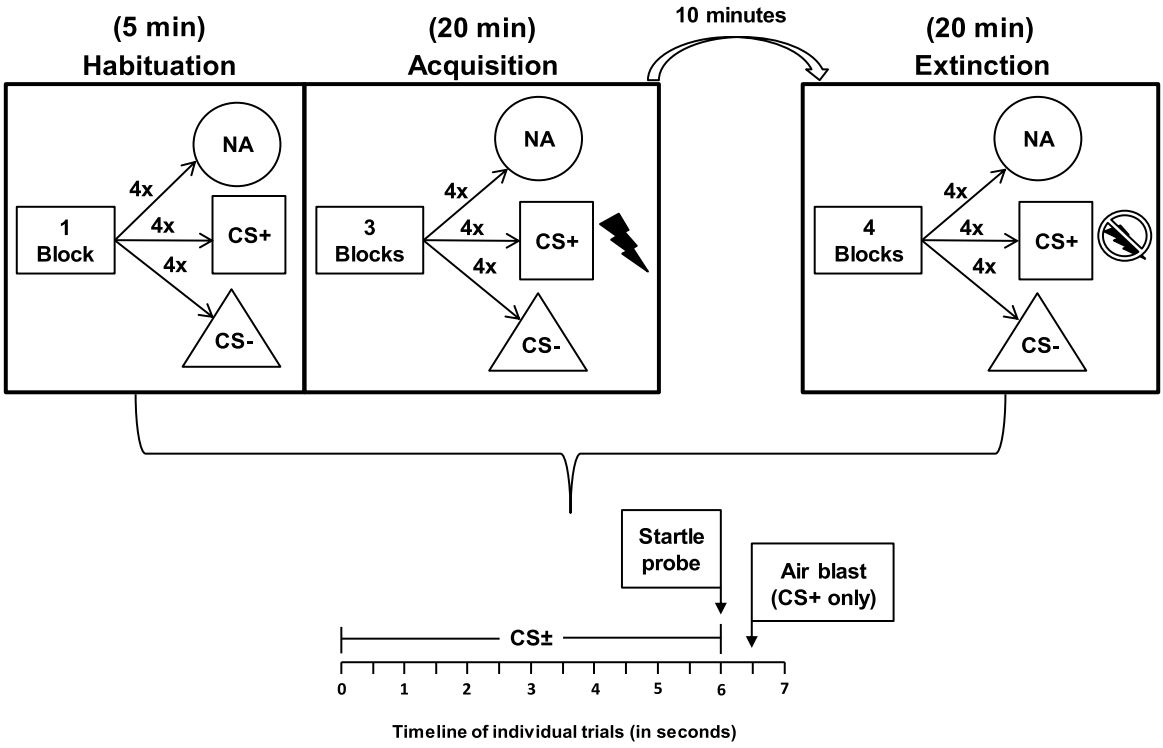

Fig. 1. Overview of fear-potentiated startle paradigm. *Note.* NA = noise alone; CS = conditioned stimulus.

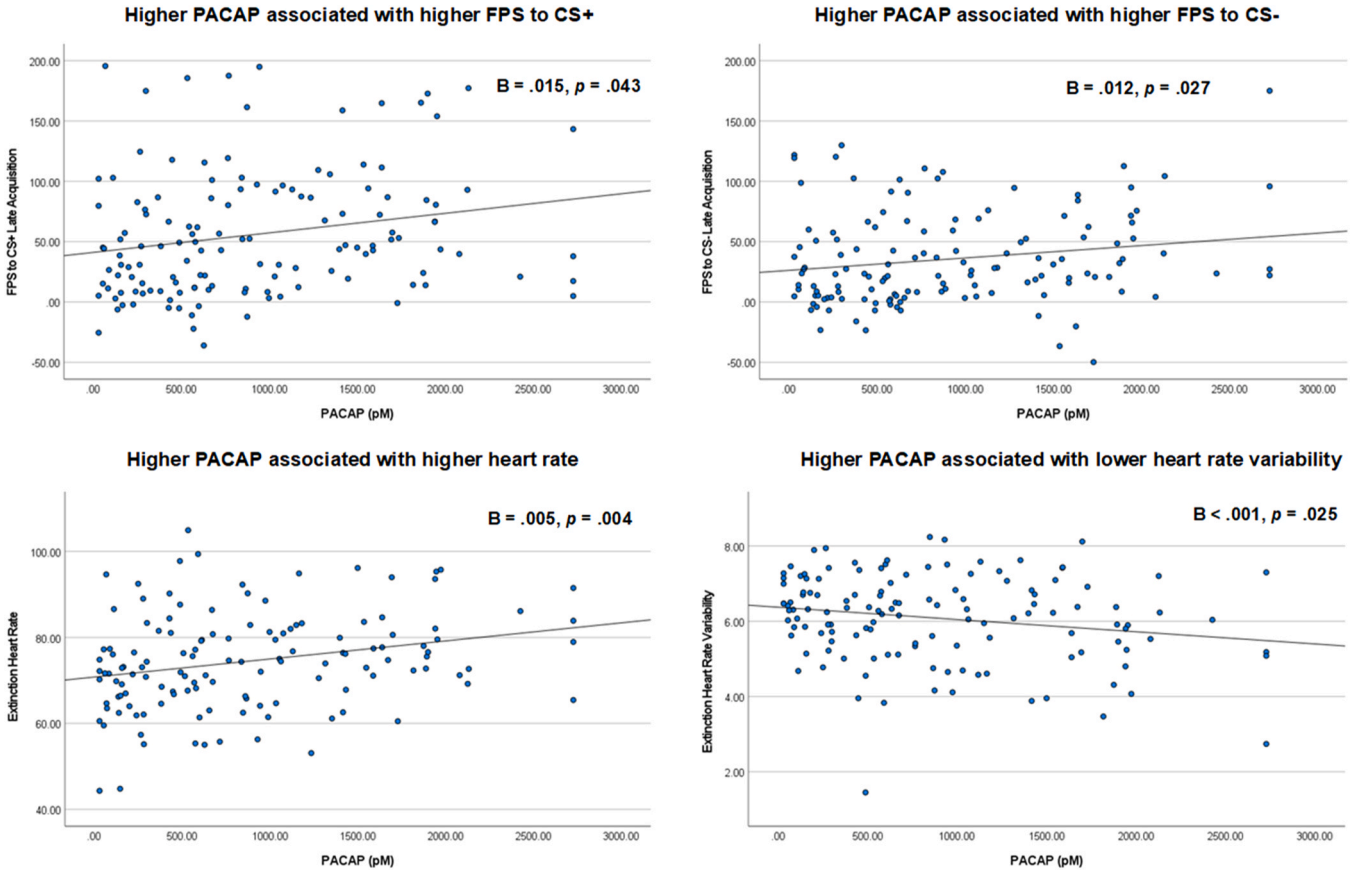

Fig. 2. Scatterplots of PACAP associations with fear and arousal physiology. Uncorrected p-values shown.

2.5. Clinical Measures

The Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) [40] was administered to determine PTSD diagnosis. The PTSD Checklist for DSM-5 (PCL-5) [41] was used to determine PTSD severity, consisting of 20 items that assess DSM-5 symptoms, each rated on a scale from 0 to 4, with higher scores indicating more severe symptoms.

3. Statistical analyses

The parent study was designed to have greater than 95 % power to detect large associations (e.g., $r = 0.5$ for univariable associations) for analyses combining male and female participants, assuming a total of 160 participants contributing data. A power greater than 95 % was maintained for the current analysis of the combined sample, even accounting for the smaller-than-expected sample sizes of 129 for HR and HRV and 135 for FPS, and multiple testing adjustments. The parent study was not powered to detect sex differences in associations.

Pearson correlation analyses were conducted to test associations among PTSD symptom severity with PACAP levels, FPS, HR, and HRV (Hypothesis 1; parent study aims). Analyses of variance (ANOVAs) were used to examine potential sex differences in PTSD status, as well as differences in PACAP, FPS, HR, and HRV by PTSD status (Hypotheses 2 and 3; secondary analyses). Self-reported biological sex at birth was used in all analyses; no participants reported being transgender.

For our primary analyses, four regressions were conducted to associate the dependent variables of interest: FPS to the CS+ and CS- during late acquisition (two models), and HR and HRV during extinction (two models), with PACAP. Sex and age were included as control covariates in all models [42]. To assess for sex differences in PACAP associations, an interaction between PACAP level and sex was added to each model. A Hommel multiple testing correction was applied separately to significance tests for PACAP main effects and PACAP by sex interactions to account for our choice of four physiological outcomes. Statistical tests were two-sided with $p < 0.05$ as the statistical significance threshold. All 95 % confidence intervals reported are individual confidence intervals; corrected p-values are indicated in the text and table footnotes. Analysis was conducted using SPSS (version 28) and R (version 4.2.3) software.

4. Results

See Table 1 for descriptives of the sample. There were 157 participants with usable PACAP data, 143 with usable FPS data, and 139 with usable HR/HRV data. Pearson correlations showed that PTSD symptom severity (total PCL-5 score) was not significantly associated with PACAP levels, late acquisition FPS to the CS+ , late acquisition FPS to the CS-, or extinction HR or HRV. Of the total sample, 113 participants (67 %) met DSM-5 criteria for PTSD, and there was no sex difference in PTSD status (threshold versus subthreshold PTSD). There were no significant differences in PACAP, late acquisition FPS to the CS+ , late acquisition FPS to the CS-, or extinction HRV by PTSD status. Extinction HR was higher in participants with threshold compared with subthreshold PTSD at an uncorrected statistical level, $F(1138) = 3.98$, $p = .048$.

4.1. Fear Potentiated Startle

In the regression associating PACAP with FPS to the CS+ during late acquisition, there was a significant main effect of PACAP, controlling for age, B = 14.8, $t(3135) = 2.05$, p corrected= 0.04. Specifically, higher PACAP levels were associated with higher startle to the CS+ . The PACAP-by-sex interaction was not significant. In the regression associating PACAP with FPS to the CS- during late acquisition, there was a significant main effect of PACAP, controlling for age, B = 12.2, $t(3135) = 2.24$, p corrected = 0.04. Specifically, higher PACAP levels were associated with higher startle to the CS-. The PACAP-by-sex interaction was not significant. Table 2 shows FPS regression results in the total sample and separated by sex. Note that PACAP values have been divided by 1000 so that B values correspond to a 1000 pM increase.

Table 1
Demographic and clinical characteristics of the full sample and the sample stratified by sex assigned at birth.

Characteristic	Total (N = 168)	Female (N = 109)	Male (N = 59)	Sex Difference
Age (years)	30.14 ± 8.75	29.02 ± 8.22	32.20 ± 9.37	$p = .024$
Race (%)				$p = .533$
Asian	7 (6 %)	6 (7 %)	1 (3 %)	
Black	8 (7 %)	6 (7 %)	2 (7 %)	
Native American	1 (1 %)	1 (1 %)	0	
White	81 (70 %)	59 (68 %)	22 (76 %)	
Bi-/multiracial	11 (9 %)	10 (11 %)	1 (3 %)	
Unknown or not reported	8 (7 %)	5 (6 %)	3 (10 %)	
Ethnicity (%)				$p = .877$
Hispanic	17 (15 %)	14 (16 %)	3 (10 %)	
Non-Hispanic	99 (85 %)	73 (84 %)	26 (90 %)	
Gender (%)				$p < .001$
Woman	103 (61 %)	103 (94 %)	0 (0 %)	
Man	57 (34 %)	1 (< 1 %)	56 (95 %)	
Non-binary or gender non-conforming	8 (5 %)	5 (5 %)	3 (5 %)	
Medication (%)				p's > .289
SSRI	30 (26 %)	25 (29 %)	5 (17 %)	
Mood Stabilizer	11 (9 %)	10 (11 %)	1 (3 %)	
Antipsychotic	1 (1 %)	0	1 (3 %)	
Benzodiazepine	7 (6 %)	6 (7 %)	1 (3 %)	
SNRI	8 (7 %)	7 (8 %)	1 (3 %)	
Tricyclic	1 (1 %)	0	1 (3 %)	
Stimulant	9 (8 %)	5 (6 %)	4 (5 %)	
Anti-hypertensive	3 (3 %)	2 (2 %)	1 (3 %)	
Atypical	11 (9 %)	9 (10 %)	2 (7 %)	
Antidepressant Anxiolytic	8 (7 %)	5 (6 %)	3 (10 %)	
PACAP (pM)	856.00 ± 691.10	1070.20 ± 688.80	397.62 ± 423.83	$p < .001$
PTSD Diagnosis (%)	113 (67 %)	76 (70 %)	36 (61 %)	$p = .253$
PTSD symptom severity	39.93 ± 14□29	40.75 ± 14□28	37.45 ± 14□27	$p = .283$

Note. * significant at $p < .05$; means ± standard deviations or N (%); PACAP: pituitary adenylate cyclase-activating polypeptide; SNRI: selective norepinephrine reuptake inhibitor; SSRI: selective serotonin reuptake inhibitor. PTSD diagnosis and total symptom severity were determined using the Clinician-Administered PTSD Scale for DSM-5

4.2. Heart Rate and Heart Rate Variability

In the regression associating PACAP with HR during extinction, there was a significant main effect of PACAP, controlling for age, B = 4.7, $t(3129) = 2.90$, p corrected = 0.02. Specifically, higher PACAP levels were associated with higher HR during extinction. The PACAP-by-sex interaction was not significant. In the regression associating PACAP with HRV during extinction, there was a significant main effect of PACAP, controlling for age, B = -0.4, $t(3129) = -2.27$, p corrected = 0.04. Specifically, higher PACAP levels were associated with lower HRV during extinction. The PACAP-by-sex interaction was not significant. Table 2 shows HR/HRV regression results in the total sample and separated by sex. Note that PACAP values have been divided by 1000 so that B values correspond to a 1000 pM increase.

5. Discussion

Our results are largely consistent with prior research indicating that PACAP is associated with higher psychophysiological arousal in trauma-exposed adults [6–8]. Specifically, higher PACAP levels were associated with heightened fear responding, diminished fear inhibition, and altered

Table 2
Estimated PACAP Coefficients for Main Effects and PACAP x Sex Interaction Models.

	Main Effects Model		Interaction Model			
	Total N = 135		Female Participants N = 80	Male Participants N = 38	Difference (Men – Women)	
Dependent variables	B (95 %CI)	*p*	B (95 %CI)	B (95 %CI)	B (95 %CI)	*p*
FPS to the CS+ in late acquisition	14.8 (0.5, 29.1)	0.04	14.9 (.000,.031)	14.3 (−22.7, 51.2)	−0.6 (−40.2, 39.0)	0.98
FPS to the CS- in late acquisition	12.2 (1.4, 22.9)	0.04	13.4 (1.8, 25.0)	4.8 (−22.7, 32.2)	−8.6 (−38.1, 20.9)	0.98
HR during extinction	4.7 (1.5, 7.8)	0.02	3.6 (0.2, 7.0)	10.6 (2.9, 18.4)	7.1 (−1.3, 15.4)	0.39
HRV during extinction	−0.4 (−0.7, −0.05)	0.04	−.0.3 (−0.6, 0.05)	−.0.7 (−1.5, 0.03)	−0.4 (−1.3, 0.4)	0.85

Note. *p* values reflect a Hommel multiple testing correction applied separately to results from main effects and interaction models; PACAP values have been divided by 1000 so that B values correspond to a 1000 pM increase; $N = 129$ for HR/HRV analyses; FPS = fear-potentiated startle; CS = conditioned stimulus; HR = heart rate; HRV = heart rate variability.

autonomic function. Our sex-based hypotheses were not supported by the regression analyses, suggesting that PACAP was associated with physiological arousal in both women and men, which is inconsistent with prior findings [6,33,34,42]. These findings offer additional insights into the link between stress peptide systems and physiological arousal in fear-based disorders.

Our findings replicate prior research showing that PACAP levels are associated with exaggerated fear responding and diminished fear inhibition. Specifically, higher PACAP levels were correlated with higher FPS to the danger cue during acquisition and with the safety cue during extinction. We also extend the prior literature by demonstrating that PACAP associated with altered autonomic function, as indicated by higher HR and lower HRV during extinction. These results are compatible with previous imaging findings from this sample, which linked PACAP levels to greater resting-state connectivity of the amygdala, which facilitates startle responding [34]. Transcript expression levels of PAC_1 and $VPAC_2$ receptors in amygdala subnuclei are high [32,43], and direct PACAP activation or infusion into the amygdala induces stress-related behaviors [30,44,45]. Thus, PACAP may be crucial in regulating fear reflexes by modulating amygdala responses and signals to peripheral systems [46]. Our findings related to PACAP and HR/HRV are consistent with prior research indicating that PACAP promotes sympathetic activity [47]; however, other findings suggest that PACAP may have a cardioprotective role [48–50]. Overall, our results add to growing evidence that PACAP levels track with biological markers of amygdala-dependent and cardiac-autonomic functions, and suggest that PACAP levels are relevant to fear phenotypes across levels of analysis.

Contrary to our hypotheses, we did not observe significant PACAP-by-sex interactions in the FPS, HR, and HRV analyses. Because our study was not powered to detect interactions with sex, our results cannot rule out sex differences of physiological significance, but can rule out sex differences in PACAP associations with FPS of the magnitude observed in a prior study. Low power to detect PACAP-by-sex interactions is reflected in the widths of confidence intervals for sex differences, which include values of magnitude two or more times greater than the magnitude of the main effect of PACAP across measures. Prior research findings have suggested sex-specific PACAP effects [6,33,34], possibly due to estrogen-mediated mechanisms, though only one study [6] reported sex differences for psychophysiological indicators included in the current study, and without a test for interaction. Of note, based on a median split of PACAP values in our sample, our data are consistent with no more than a 39 μV greater increase in FPS to the CS+ and no more than 51 μV greater increase in FPS to the CS- for females than males associated with high versus low PACAP, compared to differences of > 60 μV and > 100 μV respectively in the prior study. Although our results are consistent with an influence of PACAP on physiological arousal that is similar in men and women, it remains important to consider that PACAP is modulated by estrogen, and that sex-specific effects may be sensitive to fluctuations in gonadal hormone levels. Thus, it may also be that overall stress peptide levels are associated with physiological arousal similarly in men and women, but that sex hormones impact PACAP variability within women. Studies with larger sample sizes, and matching biological sex groups and accounting for gonadal hormone levels, would be required to clarify the sex-specific effects of PACAP in fear physiology.

The interpretations of our study findings are limited by the composition of our sample, which was predominantly White, and thereby underpowered to robustly examine race effects. Additionally, the temporal association between PACAP and fear-relevant psychophysiology remains unclear; that is, we cannot determine whether elevated PACAP levels help drive the fear-related physiological responses observed, whether PACAP dysfunction is a physiological consequence of trauma, or both. To clarify the mechanistic role of PACAP in these physiological concomitants of post-trauma pathology, future research should consider longitudinal designs examining PACAP levels pre- and post-trauma exposure or measuring fear physiology following pharmacological manipulation of the PACAP system.

Overall, study findings were consistent with prior studies demonstrating associations between PACAP levels and psychophysiological arousal. Higher PACAP levels were associated with exaggerated fear responding and autonomic dysfunction, with no significant sex differences. These results suggest that circulating PACAP levels are indicative of psychophysiological indices of fear responding, and are congruent with our prior work showing their association with functional connectivity of the centromedial amygdala [34]. Additional studies are needed to extend these findings to larger and more representative samples.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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