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Testing neurophysiological markers related to fear-potentiated startle

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ABSTRACT

Fear-potentiated startle (FPS) paradigms provide insight into fear learning mechanisms that contribute to impairment among individuals with posttraumatic stress symptoms (PTSS). Electrophysiology also has provided insight into these mechanisms through the examination of event-related potentials (ERPs) such as the P100 and LPP. It remains unclear, however, whether the P100 and LPP may be related to fear learning processes within the FPS paradigm. To this end, we tested differences in ERP amplitudes for conditioned stimuli associated (CS+) and not associated (CS-) with an aversive unconditioned stimulus (US) during fear acquisition. Participants included 54 female undergraduate students (mean age = 20.26). The FPS response was measured via electromyography of the orbicularis oculi muscle. EEG data were collected during the FPS paradigm. While the difference between CS+ and CS- P100 amplitude was not significant, LPP amplitudes were significantly enhanced following the CS+ relative to CS-. Furthermore, the LPP difference wave (CS+ minus CS-) was associated with FPS scores for the CS- during the later portion of fear acquisition. These findings suggest that conditioned stimuli may have altered emotional encoding (LPP) during the FPS paradigm. Thus, the LPP may be a promising neurophysiological marker that is related to fear learning processes.

1. Introduction

Posttraumatic stress symptoms (PTSS) are associated with significant impairment across multiple domains of functioning (Magruder et al., 2004; Norman et al., 2007). Some intermediate phenotypes that have emerged as underlying mechanisms of this dysfunction are exaggerated startle and impaired fear inhibition, which relate to the fear learning process (a form of classical conditioning). Researchers have been able to examine fear learning processes primarily through the use of fear-potentiated startle (FPS) paradigms (e.g., Grillon and Morgan, 1999; Norrholm et al., 2011; Sijbrandij et al., 2013). FPS paradigms are based on classical conditioning principles where an aversive unconditioned stimulus (US) is repeatedly paired with a conditioned stimulus (CS+), resulting in an FPS response mediated by the amygdala and the sympathetic nervous system (SNS). FPS is an indicator of fear conditioning and is defined as the relative increase in auditory startle response (i.e., typically to a white noise burst) when it is paired with a CS + versus when it is presented by itself (i.e., the acoustic startle reflex is potentiated by the pairing of the startle probe with the feared CS+ (e.g., Norrholm et al., 2011). In FPS paradigms, exaggerated startle refers to increased eyeblink startle to a CS+, while impaired fear inhibition refers to heightened startle to stimuli that are not paired with a US (CS-). Research has demonstrated that civilians and Veterans with PTSS exhibit greater FPS to a CS+ (exaggerated startle; Grillon and Morgan, 1999; Jovanovic et al., 2009, 2010) and a lowered ability to distinguish between danger (CS+) and safety (CS-) cues compared to individuals without PTSS (poor fear inhibition; Jovanovic et al., 2009; Sijbrandij et al., 2013).

Electrophysiology, which provides excellent time resolution in the milliseconds (ms) range, also has been used to clarify mechanisms that underlie fear learning processes. In particular, research on event-related potentials (ERPs) such as the P100 suggests that early visual processing may be involved in fear/aversive conditioning. Pizzagalli et al. (2003) demonstrated that P100 and N100 amplitudes were larger for fearful faces paired with an aversive shock (CS+) compared to those that were not. Results from this study suggest that emotionally salient

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information (shock contingency) may modulate early processing as indexed by the P100/N100. Building on this work, Liu et al. (2012) demonstrated that in addition to reflecting early visual processing of stimuli, the P100 effect appears to be temporally dynamic. Using single-trial analysis, P100 amplitudes for a CS+ and CS- initially decreased during conditioning, and subsequently increased before exhibiting a final period of habituation. The initial decrease may reflect that learning was not yet established, and thus an increase in P100 was not observed at this early point in the conditioning phase. In contrast, the subsequent increased amplitude for the CS+ reflects that visual processing of the CS+ was enhanced as learning progressed. Relative to the CS-, P100 amplitude for the CS+ demonstrated a faster rate of increase, suggesting that individuals responded to the CS+ more quickly given its greater emotional salience (Liu et al., 2012). A recent study provided additional support for the relevance of the P100 in fear learning processes. Using a fear conditioning paradigm with faces as conditioned stimuli, Muench et al. (2016) reported that P100 amplitudes were enhanced for a self-threatening CS+ (i.e., fearful face directed towards participants) that had not been extinguished relative to an extinguished CS+. Overall, these studies suggest that early visual processing, indexed by the P100, may be implicated in fear learning processes.

In addition to early visual processing, other ERP research has demonstrated that components implicated in later emotional encoding may be important to consider in fear/aversive conditioning. The late positive potential (LPP)—an ERP that indexes elaborative encoding of emotional information—is greater for emotionally salient information (e.g., words: Auerbach et al., 2015a, 2016; images: Bondy et al., 2017; Cuthbert et al., 2000; Kujawa et al., 2015; Schupp et al., 2000; for a review, see Hajcak et al., 2010). Particularly relevant to the current study, larger LPP amplitudes to unpleasant versus neutral stimuli have been found in individuals with high versus low PTSS (Lobo et al., 2014), providing further evidence that PTSS is associated with hyperarousal. There is a body of literature related to fear/aversive conditioning and the LPP (along with other late ERPs) that provide a foundation for the current study. In an early study using an aversive conditioning paradigm with masked stimuli, Wong et al. (1994) found that unpleasant stimuli (CS+) were associated with a greater P300 amplitude than were pleasant stimuli (CS-; the P300 is thought to reflect attention allocation to emotionally salient stimuli; Hajcak et al., 2010). By using masked stimuli that were not perceptually accessible, they demonstrated that participants expected the US (an electric shock) without being overtly aware of the contingency. In a follow-up study, Wong et al. (2004) replicated this using a modified paradigm; the P300-LPP component was significantly increased for a CS+ compared to a CS- following aversive conditioning. Similarly, recent studies using fear/aversive conditioning paradigms have demonstrated that LPP amplitude is increased for a CS+ compared to CS- in the fear learning/conditioning (Panitz et al., 2015) and post-conditioning/extinction phases (Pastor et al., 2015).

Previous research has implicated ERPs such as the P100 and LPP in fear learning processes. While these processes appear relevant to CS+/CS- discrimination, they have not been tested in relation to FPS variables such as exaggerated startle and fear inhibition. There are several advantages to examining these relationships using FPS: 1) in comparison to skin conductance response (also used as an index of fear learning), the FPS response is quicker, more stable, and accounts for baseline startle (e.g., Glover et al., 2011); 2) FPS has a well-defined neural circuit and is strongly linked with amygdala activation (e.g., Davis, 1994; LaBar et al., 1998); and 3) unlike other physiological indices such as skin conductance response or certain ERPs, the FPS response is heightened only for stimuli associated with an aversive US (regardless of contingency awareness), making it a more specific marker of fear. Thus, studying FPS variables along with ERPs that are implicated in fear discrimination is essential to integrating physiological markers of fear learning processes.

Towards achieving this goal, the current study examined the P100 and LPP in the context of fear acquisition using an FPS paradigm administered to a non-clinical sample. First, we hypothesized that early visual processing (P100 amplitude) would be significantly greater for a conditioned stimulus associated with an aversive US (CS+) compared to one that was not (CS-). Second, we tested whether emotional encoding (LPP amplitude) would be significantly greater for the CS+ compared to the CS-. Given that poor fear discrimination is caused by heightened arousal to all stimuli (both dangerous and safe; i.e., hypervigilance), we hypothesized that worse discrimination between the CS+ and CS- (P100 and LPP difference scores) would be significantly associated with exaggerated startle (higher FPS to CS+) and poor fear inhibition (higher FPS to CS-) during the conditioning paradigm.

2. Method

2.1. Participants

A total of 83 undergraduate students were recruited through psychology courses at a Midwestern university. However, 26 participants did not attend the session, and for 3 participants, data were unusable because of equipment malfunction. Thus, the final sample included 54 female students aged 17–28 years (mean age = 20.26, SD = 2.61). Inclusion criteria for the study were 18 years of age or older, English fluency, and written consent. Participants were not selected on the basis of trauma exposure, and there were no specific exclusion criteria. The racial distribution included: 32 (59.3%) Caucasian, 14 (25.9%) African American, 4 (7.4%) Asian, and 2 (3.7%) Other. The majority of participants identified as non-Latino/Hispanic (88.9%).

2.2. Procedure

Undergraduate students in psychology courses were invited via email to participate; interested students were then scheduled for the FPS session. Upon arrival to the session, participants provided written consent, completed self-report measures, and were administered the FPS paradigm while electroencephalogram (EEG) data were recorded. Following the experiment, participants were debriefed and provided with a list of local counseling resources. Participants received 4 credits for their psychology course for completing the study. All procedures were approved by the university's Institutional Review Board.

2.3. Measures

2.3.1. State-trait anxiety inventory

The State-Trait Anxiety Inventory (STAI; Spielberger, 1983) is a 40-item self-report measure of state and trait anxiety. Items are rated on a 4-point Likert-type scale from 1 (*not at all*) to 4 (*very much so*), with higher scores indicating greater anxiety. Prior research has demonstrated high internal consistency and good test-retest reliability (Spielberger, 1983). Given that state anxiety has been associated with FPS in previous research (Grillon et al., 1993), state items from the STAI were administered after the informed consent to control for anxiety related to being in the presence of the psychophysiology chamber. The Cronbach's alpha in the current study was 0.89, which indicates excellent internal consistency.

2.4. FPS recording, data reduction, and analysis

Biopac MP150 for Windows (Biopac Systems, Inc., Aero Camino, CA) was used to collect psychophysiological data. Experimental stimuli were presented using SuperLab 4.0 for Windows (SuperLab, Cedrus, Corp., San Pedro, CA) and synchronized with psychophysiological data acquisition using a DIO card (Measurements Computing, Inc). The FPS response was measured via electromyography (EMG) of the right orbicularis oculi muscle and was identified as the maximum amplitude of

the eyeblink muscle contraction 20 to 200 ms after the startle probe was presented. Two 5 mm Ag/AgCl pre-gelled disposable electrodes were positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus. All impedances were less than 6 kΩ. EMG activity was acquired at a sampling rate of 1 kHz, amplified and digitized using the EMG module of the Biopac system. The startle probe was a 108-dB 40-ms burst of broadband noise with a near instantaneous rise delivered through headphones. Startle response was recorded during the entire FPS paradigm. Participants underwent a brief auditory screening to ensure that their hearing was within normal limits.

The FPS paradigm has previously been validated in both clinical and nonclinical samples (Glover et al., 2011; Jovanovic et al., 2012; Norrholm et al., 2006). The paradigm included two phases: acquisition (20 minutes) and extinction (25 minutes). The aversive US was a 250 ms airblast with an intensity of 140 pounds per square inch directed at the larynx. The conditioned stimuli (CS) consisted of different colored shapes presented on a computer monitor that was straight ahead, eye level, and one meter away from the participant (programmed with SuperLab). The startle probe was presented 6 seconds after initiation of the CS and was followed by the US 0.5 s later. The CS + (a blue square) was paired with the airblast, while the CS- (a purple triangle) was not. Shapes were not counterbalanced given that this procedure has not affected FPS variables in prior research (e.g., Jovanovic et al., 2010). The acquisition phase consisted of a habituation block with four trials of each CS (not reinforced with airblasts) and eight startle probes alone (noise alone [NA]), followed by three conditioning blocks with four trials of each type (NA, CS+, CS-) in each block (see Fig. 1). Participants were instructed to use a keypad to indicate whether or not they thought the US would follow each stimulus (the "+" button was pressed if they expected a CS to be followed by the US, the "-" button was pressed if they did not expect a CS to be followed by the US, and the "0″ button was pressed if they were not sure). The extinction phase consisted of six blocks of four trials of each type (NA, CS+ [*unreinforced*], and CS-) in each block. Given that this was part of a larger study evaluating the impact of a mindfulness exercise on extinction learning (presented in-between acquisition and extinction), results from the extinction phase are not presented due to potential effects of the exercise. Trials for all phases were on a fixed schedule. The inter-trial interval was between 9 and 22 seconds.

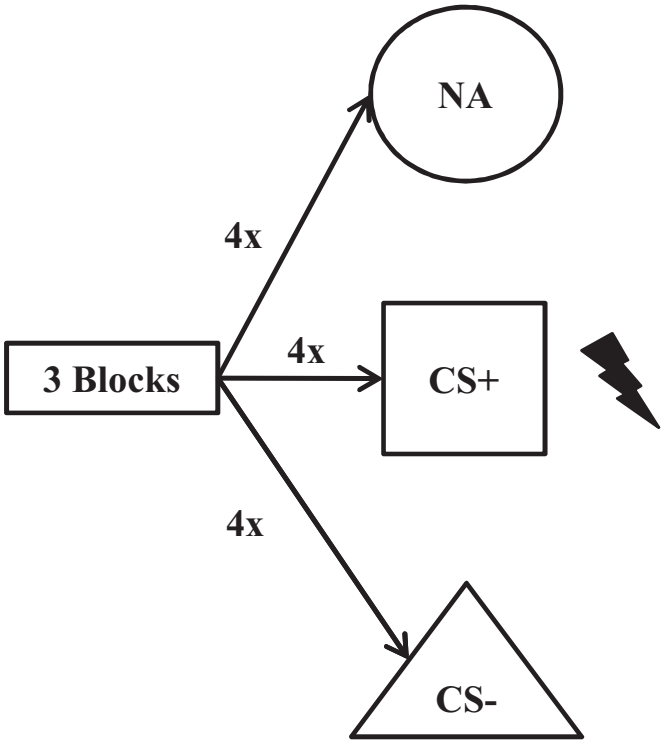

Fig. 1. Overview of stimuli presentations in the FPS acquisition phase
Note. NA = noise alone; CS+ = conditioned stimulus paired with airblast; CS- = conditioned stimulus not paired with airblast.

FPS was obtained from the raw Biopac recordings (EMG) using MindWare EMG software (MindWare Technologies, Inc). EMG signals were amplified by a gain of 1000. Screening of eyeblinks involved visually inspecting EMG data for double blinks and other artifacts. When necessary, segments of EMG data without an identifiable eyeblink were set to "0." Startle magnitude values were obtained from MindWare for each stimulus (e.g., amplitude of eyeblinks in response to each CS).

FPS was calculated using a difference score ([startle magnitude in the presence of a CS in each conditioning block] – [startle magnitude to NA]); Norrholm et al., 2011). Two FPS variables were used in the current study: FPS to the CS+ and FPS to the CS- during acquisition. Four participants had less than 75% usable FPS data and therefore, were excluded from analyses. Following the screening in MindWare, physiological data were exported to SPSS for further analysis.

2.5. EEG recording, data reduction, and analysis

EEG data were recorded during the FPS session from 9 International 10–20 system sites (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4) with a tin-electrode cap (Electro-Cap International Inc., Eaton, OH). An equipment failure precluded us from recording at the Pz site for most participants; we therefore excluded Pz from analyses. Electrode impedances were kept below 5 kΩ. Horizontal and vertical eye movements were recorded using electrooculogram (EOG) with electrodes placed at the outer canthi and lower orbital ridges. Each EEG and EOG channel was amplified by separate EEG100C and EOG100C modules (BioPac MP100C system, BioPac Systems, Goleta, CA) with an analog bandpass filter from 0.1 to 35 Hz. EEG and EOG data were sampled at 1 kHz (1,000 samples/sec). EEG and EOG data were gathered using AcqKnowledge 3.8.1 for the BioPac MP100C system.

EEG data were processed with BrainVision Analyzer 2.04 software (Brain Products, Germany). EEG data were referenced to the average reference, and offline filters (0.1–30 Hz) were applied. An independent component analysis (ICA) transform was used to identify and remove eye movement and eyeblink artifacts. The following criteria were used for the ICA: whole data, classic PCA sphering, infomax ICA, energy ordering, and 512 convergence steps. For each trial, EEG data were segmented 200 ms before and 1200 ms after stimulus onset. Consistent with prior research (Auerbach et al., 2015a, b, a semi-automated procedure to reject intervals for individual channels used the following criteria: (a) a voltage step > 50 μV between sample rates, (b) a voltage difference > 300 μV within a trial, and (c) a maximum voltage difference of < 0.50 μV within a 100 ms interval. In addition to these semi-automated procedures, all trials were visually inspected for manual artifact identification and removal. Three participants were removed from analyses due to poor EEG data quality. The P100 was calculated as the average mean amplitude at Cz 75–125 ms poststimulus for each stimulus type during acquisition. The LPP was calculated as the average mean amplitude at Cz 600–1200 ms poststimulus for each stimulus type during acquisition. To determine the internal consistency of the P100 and LPP data, we conducted split-half reliability tests. The Spearman-Brown Prophecy Formula (Nunnally et al., 1967) was used to correct this correlation because the total number of items included in the averages are split in half (reliability = $2 * r_{odd/even} / 1 + r_{odd/even}$). The Spearman-Brown coefficient was evaluated where: > 0.80 = good/excellent, 0.70 - 0.79 = acceptable, and < 0.60 = poor. SPSS 21.0 (SPSS, Inc., Chicago, IL) was used to conduct analyses.

Table 1
Bivariate correlations among study variables.

	1	2	3	4	5	6	7	8	9	10	11	12
1. Age	–											
2. Race	0.071	–										
3. STAI	−0.017	−0.217	–									
4. P100 difference score	−0.016	−0.191	0.416*	–								
5. LPP difference score	0.015	−0.344*	0.222	0.635**	–							
6. N200 difference score	0.080	−0.223	0.133	0.560**	0.489**	–						
7. FPS for CS+ Block 1	−0.097	−0.079	0.091	0.132	−0.155	0.014	–					
8. FPS for CS+ Block 2	0.053	−0.030	−0.296*	0.067	0.099	0.097	0.364*	–				
9. FPS for CS+ Block 3	−0.082	−0.215	0.181	0.140	−0.155	−0.083	0.589**	0.116	–			
10. FPS for CS- Block 1	−0.101	−0.166	−0.093	−0.134	−0.152	−0.144	0.567**	0.123	0.371*	–		
11. FPS for CS- Block 2	0.045	0.069	−0.077	0.221	0.022	0.008	0.244	0.698**	0.062	0.050	–	
12. FPS for CS- Block 3	−0.141	0.188	−0.065	0.063	−0.295*	-0.084	0.449**	0.514**	0.495**	0.262	0.703**	–
Mean	20.264	–	35.315	2.756	2.674	3.896	23.831	22.503	36.608	20.157	11.983	9.988
SD	2.610	–	8.689	10.405	6.370	12.244	24.409	39.875	49.471	22.283	35.932	23.976
Minimum	17	–	20	−18.469	-11.379	−27.409	−10.330	−148.429	−1.012	−15.732	−107.777	−34.218
Maximum	28	–	54	30.103	14.248	36.073	109.416	145.920	227.740	87.980	176.411	138.352

Note. *$p < 0.05$; **$p < 0.01$; LPP = late positive potential; CS+ = conditioned stimulus reinforced; CS- = conditioned stimulus non-reinforced; FPS = fear-potentiated startle; STAI = State-Trait Anxiety Inventory.

3. Results

3.1. Descriptive statistics

General descriptive statistics and bivariate correlations are summarized in Table 1. Cohen's *d* effect sizes were interpreted based on ranges recommended by Cohen (1988): 0.2 = small, 0.5 = medium, 0.8 = large. With respect to the internal consistency of the ERP data, odd-even P100 trials were significantly correlated, $r = 0.394$, $p = 0.004$, and the corrected split-half reliability was 0.565, demonstrating poor internal consistency. Odd-even LPP trials were also significantly correlated, $r = 0.584$, $p < 0.001$, and the corrected split-half reliability was 0.737, demonstrating acceptable internal consistency.

3.2. Physiological Data: FPS, P100, and LPP

As indicated with a two-by-three ANOVA (CS type by block), participants were successfully fear conditioned and demonstrated significantly greater FPS to the CS+ compared to the CS-, and this difference increased with each block, $F(1,48) = 18.614$, $p < 0.001$, $\eta_p^2 = 0.279$ (main effect of CS type; see Fig. 2). For the P100, the difference between the CS+ and CS- was not significant but trended in the appropriate direction, $t(50) = 1.956$, $p = 0.056$, $d = 0.301$, and the latency for the CS+ and CS- did not differ significantly for the P100, $t(50) = 0.745$, $p = 0.460$, $d = 0.114$. In line with our hypothesis, the LPP amplitude following the CS+ was greater than the CS-, $t(50) = 2.998$, $p = 0.004$, $d = 0.336$ (see Fig. 3), and the LPP latency did not differ for the CS + and CS-, $t(50) = -1.627$, $p = 0.110$, $d = -0.279$.

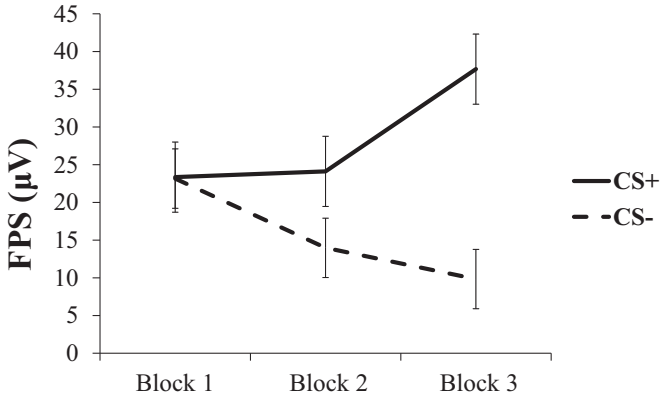

Fig. 2. FPS to the CS+ and CS- during acquisition
Note. CS+ = conditioned stimulus paired with airblast; CS- = conditioned stimulus not paired with airblast.

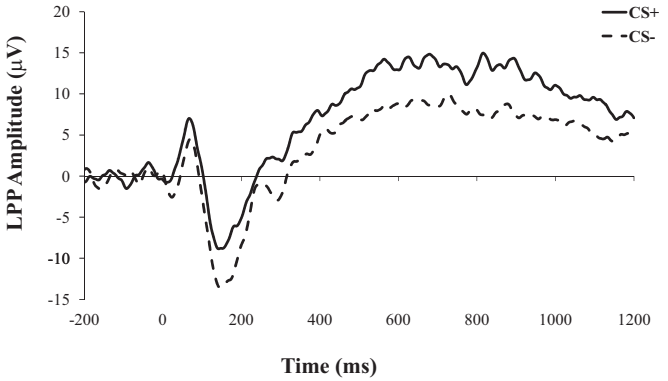

Fig. 3. LPP amplitude following CS+ and CS-
Note. CS+ = conditioned stimulus paired with airblast; CS- = conditioned stimulus not paired with airblast.

Bivariate correlations among the P100 and FPS variables were not significant. Therefore, we did not conduct further analyses with the P100. However, regression results indicated that the LPP difference score (CS+ minus CS-) was associated with FPS scores for the CS- (last conditioning block; $\beta = -0.295$, $p = 0.047$, $R^2 = 0.113$; see Figure 4). This suggests that greater discrimination between the danger signal (CS+) and safety signal (CS-) was associated with lower startle to the safety signal (CS-). When controlling for state anxiety (indexed by the STAI) and removing an outlier on the FPS to CS- variable in block 3, this relationship remained significant, $\beta = -0.351$, $p = 0.023$, $R^2 = 0.117$.

3.3. Exploratory analyses

Visual inspection of the LPP waveform (Fig. 3) suggested that amplitudes for the CS+ and CS- were significantly different at the N200 as well. Thus, exploratory analyses probed the N200. Odd-even N200 trials were significantly correlated, $r = 0.680$, $p < 0.001$, and the corrected split-half reliability was 0.810, demonstrating good-excellent internal consistency. Exploratory analyses comparing N200 amplitudes (160–210 ms post-stimulus) for the CS+ and CS- at Cz indicated the difference was statistically significant, $t(50) = 2.762$, $p = 0.008$, $d = 0.282$. N200 latency did not significantly differ for the CS+ versus CS-, $t(50) = -1.077$, $p = 0.287$, $d = -0.211$, and bivariate correlations among the N200 and FPS variables were not significant.

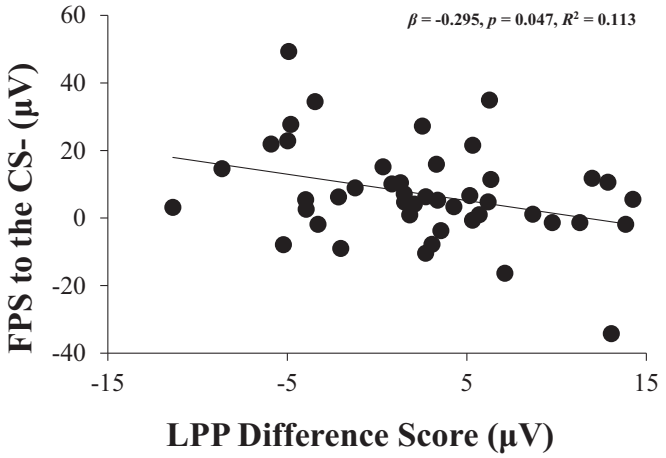

Fig. 4. Scatter plot of the relationship between the LPP difference score and FPS to the CS-
Note. CS- = conditioned stimulus not paired with airblast.

4. Discussion

The current study tested the relationship between FPS and the P100 and LPP in a sample of female undergraduate students. Results suggest that differences in LPP amplitude may be associated with fear discrimination during the fear acquisition phase of the FPS paradigm. Further, enhanced fear discrimination indexed by the LPP was related to better fear inhibition as indicated by FPS to the safety signal. These findings build upon prior ERP research in fear/aversive conditioning paradigms, and they represent an initial step in determining whether the LPP is a useful neurophysiological marker within the FPS paradigm, specifically.

In terms of our first hypothesis regarding early visual processing, the difference in P100 amplitude for the CS+ versus CS- was not significant ($p = 0.056$), but trended in the appropriate direction with a small effect size ($d = 0.301$). This may suggest that conditioning in the FPS paradigm led to differential visual processing of conditioned stimuli, which would support prior research with the P100 in other fear/aversive conditioning paradigms (e.g., Muench et al., 2016). Specifically, this finding could suggest that the negative valence of the CS+ (through its pairing with the US) led to increased visual attention towards it. As suggested by Muench et al. (2016), increased P100 amplitude for the CS+ versus the CS- may reflect that individuals successfully suppressed their fear response to the CS- by allocating less attention to it (because it was deemed to be "safe"). Alternatively, it is possible that the airblast was not emotionally salient enough to elicit early visual learning differences compared to other aversive USs, such as electric shock.

Consistent with our second hypothesis, emotional arousal indexed by the LPP was significantly greater following a feared versus safe stimulus. This finding supports prior research demonstrating that individuals exhibit greater emotional arousal to unpleasant versus neutral stimuli (e.g., Auerbach et al., 2016; Bondy et al., 2017; Kujawa et al., 2015; Lobo et al., 2014), including stimuli presented in fear/aversive conditioning paradigms (e.g., Panitz et al., 2015; Pastor et al., 2015). In the current study, the CS+ represented an unpleasant, and specifically, fear-inducing stimulus. Our findings extend prior research by demonstrating that individuals exhibited a specific discrimination pattern similar to what has been observed with FPS variables in prior studies (e.g., Glover et al., 2013; Norrholm et al., 2006, 2011). Overall, these findings are consistent with prior ERP and conditioning studies and may suggest that stimuli in the FPS paradigm produce differential amplitudes for ERPs responsible for early visual processing as well as later emotional encoding (e.g., Muench et al., 2016; Panitz et al., 2015; Pizzagalli et al., 2003). Future research with clinical samples will be needed to test whether the current findings may be replicated, and whether they have relevance for fear learning processes among those with PTSS.

The finding that LPP discrimination was related to fear inhibition in block three of acquisition suggests that individuals who are better able to discriminate between emotional stimuli have a greater ability to inhibit fear in the presence of safety (i.e., if individuals better understand the difference between stimuli, they will be more able to inhibit fear in safe situations). It is well-established that poor fear discrimination and fear inhibition (as measured with FPS) are related processes that are both implicated in the development and maintenance of PTSS (Jovanovic et al., 2009, 2012). An important next step will be to determine if our findings regarding discrimination may be replicated and whether LPP discrimination is related to PTSS in clinical samples.

Our exploratory analyses with the N200 echo that of the LPP and suggest that differences for CS+/CS- amplitude are present among earlier ERP components. The N200 is a negative-going waveform thought to reflect conflict monitoring, such that amplitude is greater (more negative) for incongruent versus congruent stimuli (Kopp et al., 1996). In our study, N200 amplitudes were greater for the CS- compared to the CS+ (see Fig. 3, 160–210 ms poststimulus). This suggests that participants may have been expecting or attending more to the CS+, as it was associated with something threatening, while the CS- may have been considered task-irrelevant. Given that differences in N200 amplitude reflect cognitive control/inhibition (a top-down process), it may be relevant to future studies of fear learning processes (e.g., fear inhibition).

Findings should be interpreted in the context of the following limitations. First, the sample consisted of female undergraduate students, which limits the generalizability of our findings. Future studies would benefit from the examination of sex differences, especially as this may relate to better characterizing PTSS. Second, the habituation phase of the FPS paradigm has only four trials of each stimulus type. Given that at least eight trials are recommended for examining the LPP (Moran et al., 2013), examining differences in LPP to the CS+ and CS- during habituation is not warranted. Therefore, we cannot reliably determine whether significant differences were present prior to the acquisition phase. This also precludes us from determining whether counterbalancing would have impacted the LPP results. Third, the current study did not assess factors such as phase of the menstrual cycle, time of day, and food intake, which have been shown to influence LPP and other ERP amplitudes (O'Reilly et al., 2004; Polich and Kok, 1995; Zhang et al., 2015). We also did not assess the unpleasantness of the startle probe or US, which may influence startle responding and/or ERP amplitudes. Acquiring this information would improve the rigor and reproducibility in future studies. Last, an equipment failure with the Pz electrode precluded our ability to examine the LPP at this site. In future research, we aim to determine if our findings replicate at Pz.

In sum, our findings suggest that the LPP discriminated between experimental stimuli and was related to fear inhibition. The present study is an initial step towards determining whether the LPP (and potentially the P100 and N200) holds promise as a neurophysiological marker related to fear learning and discrimination processes. Future research with clinical populations will help to further understand how these ERPs may be used to examine processes underlying PTSS.

Conflicts of interest

None

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