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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