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Event-related potentials to acoustic startle probes during the anticipation of predictable and unpredictable threat

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Abstract

The startle reflex is a robust measure of defense system activation. Startle probes also elicit ERP P300 and N100 responses that capture attentional engagement. The startle probe-elicited P300 and N100 have been primarily examined during affective picture viewing paradigms, and no study has examined these measures in the context of a threat anticipation task or in relation to threat predictability. In the present study, 131 participants completed a no (N), predictable (P), and unpredictable (U) threat-of-shock task, and the startle eye blink reflex, P300, and N100 responses to the startle probe were measured. We also examined several psychometric properties of these psychophysiological measures. Results indicated probe P300 attenuation during the P and U relative to N condition. In contrast, probe N100 enhancement was present only for the U condition. The P300 and N100 decreased (i.e., habituated) at comparable rates across the different threat conditions. The startle reflex also decreased, but only startle during the U (and not P) condition continued to differ from the N condition by the end of the task. Internal consistency of the ERP measures was acceptable and comparable to the startle reflex. Finally, the startle reflex was correlated with the probe N100, but not P300, across threat conditions. This study is one of the first to use startle probe ERPs to demonstrate that a context of potential threat also elicits attentional engagement. Furthermore, this study provides novel evidence that the probe N100 may provide a measure that is uniquely sensitive to unpredictable threat.

Descriptors: Event-related potential, P300, Predictability, N100, Startle reflex

The startle reflex is a robust psychophysiological indicator of defense system activation (Lang, 1995). In humans, startle is typically assessed by presenting a loud acoustic noise and measuring the eye blink response using electromyography (EMG; Blumenthal et al., 2005). Affective picture viewing studies have demonstrated that the startle reflex is modulated by emotional valence, such that, relative to neutral pictures, startle is potentiated when viewing unpleasant pictures and attenuated when viewing pleasant pictures (Lang, Bradley, & Cuthbert, 1990). The startle reflex has also been examined in the context of fear conditioning, such that startle is potentiated in the presence of threat cues (i.e., CS+) relative to safety cues (i.e., CS-; Davis, 1986; Grillon, Ameli, Woods, Merikangas, & Davis, 1991).

Predictability is an important feature of threat that has been suggested to impact defense system activation and differentiate the emotional states of fear and anxiety (Barlow, 2000; Grillon, Baas, Lissek, Smith, & Milstein, 2004; Hamm & Weike, 2005). Fear is associated with predictable threat and prepares an organism for more immediate fight, flight, or immobilization responses. In contrast, anxiety is elicited when the perceived threat is less certain (or present) and requires a sustained state of vigilance and defensive preparedness. To test the distinction between fear and anxiety, Grillon and colleagues developed the no, predictable, and unpredictable threat (NPU-threat) startle paradigm (Schmitz & Grillon, 2012). The task consists of three different conditions: (1) no threat (no aversive stimulus delivered), (2) predictable threat (aversive stimulus signaled by short duration cue), and (3) unpredictable threat (aversive stimulus not signaled). In each condition, participants view a short duration cue that is separated by a variable interstimulus interval (ISI), and these phases are examined separately as they delineate between cued and contextual responding, respectively. Across several studies, the startle reflex has been shown to be potentiated in anticipation of predictable and unpredictable threat relative to no threat (Grillon et al., 2004; Shankman, Robison-Andrew, Nelson, Altman, & Campbell, 2011). Furthermore, several anxiety disorders (e.g., panic disorder, posttraumatic stress disorder) are characterized by increased startle in anticipation of unpredictable threat, but evidence for increased startle in anticipation of predictable threat has been mixed (Grillon et al., 2008, 2009; Shankman et al., 2013).

The startle reflex provides valuable information on defense system activation; however, signals of threat also capture attention—and it is unclear whether attention is also modulated by the

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predictability of threat. The current study focused on ERP components elicited by the startle probe that index attentional processes. Specifically, a small number of studies have examined variation in the startle probe-elicited P300 (i.e., probe P300), a positive deflection of the ERP signal that is maximal at centroparietal sites and occurs around 300 ms after the onset of the startle probe (Putnam & Roth, 1990; Roth, Dorato, & Kopell, 1984; Sugawara, Sadeghpour, Traversay, & Ornitz, 1994). The probe P300 is attenuated while viewing both pleasant and unpleasant relative to neutral pictures due to greater allocation of attentional resources to the arousing foreground stimuli (Bradley, Codispoti, & Lang, 2006; Cuthbert, Schupp, Bradley, McManis, & Lang, 1998; Schupp, Cuthbert, Bradley, Birbaumer, & Lang, 1997). In addition, the startle probe-elicited N100 (i.e., probe N100) is a negative deflection in the ERP signal that is maximal around frontocentral sites and occurs around 100 ms after the onset of the startle probe. The probe N100 is thought to reflect early sensory processing and is enhanced while viewing unpleasant relative to pleasant or neutral pictures (Cuthbert et al., 1998). Together, EMG and ERP responses elicited by the startle probe can be used to differentially assess defensive mobilization and the interplay of emotion and attention, respectively. However, the probe P300 and N100 have been primarily examined using affective picture viewing paradigms (Bradley et al., 2006; Cuthbert et al., 1998; Schupp et al., 1997), and no study has examined these ERP responses in the context of a threat anticipation task or whether they differ as a function of the predictability of threat.

The present study examined the startle reflex and probe P300 and N100 during the NPU-threat task (Schmitz & Grillon, 2012). Similar to unpleasant pictures (Bradley et al., 2006; Cuthbert et al., 1998; Schupp et al., 1997), we hypothesized that the probe P300 would be attenuated and the probe N100 would be enhanced during the threat conditions relative to no threat condition. As previously mentioned, no studies have examined the probe P300 and N100 in relation to predictable versus unpredictable threat; therefore, we did not have specific hypotheses regarding differences between these conditions.

The present study also had two exploratory aims. First, we examined several psychometric properties of the startle EMG and ERP responses during the NPU-threat task. First, we tested whether the startle reflex and probe P300 and N100 responses during the task decreased (i.e., habituated) over time. This was tested using multilevel modeling to examine the time course of responding during the different threat conditions. Second, the present study compared the internal consistency of the startle reflex and probe P300 and N100 across the different threat conditions as a function of increasing trial numbers (Meyer, Bress, & Proudfit, 2014). The NPU-threat task was designed to examine the startle reflex in anticipation of predictable versus unpredictable threat and typically includes 6 to 12 trials per condition (Grillon et al., 2004; Schmitz & Grillon, 2012; Shankman et al., 2011)—consistent with guidelines on measurement of the startle reflex (Blumenthal et al., 2005). However, it is not clear if ERP responses are stable with so few trials; therefore, these analyses aimed to shed light on whether the startle probe EMG and ERP measures for the NPU-threat task have comparable psychometric properties and the ideal number of trials necessary to achieve stable indicators of defense system activation and ERP measures of attention. Third, while several studies have examined affective modulation of the startle reflex and probe P300 and N100 (Bradley et al., 2006; Cuthbert et al., 1998; Schupp et al., 1997), no study has reported the within-subjects relationship between these measures to determine whether they may reflect similar or different processes. Thus, we also examined the correlation between the startle reflex, probe P300, and N100 across the different threat conditions.

Method

Participants

The sample included 131 introduction to psychology students from the University of Illinois–Chicago who participated for course credit. Exclusion criteria were an inability to read or write English, history of head trauma with a loss of consciousness, or being left-handed (as confirmed by the Edinburgh Handedness Inventory; range of laterality quotient: +10 to +100; Oldfield, 1971). The sample was college-aged ($M = 19.36$, $SD = 2.02$), predominately female (64.9%), and ethnically diverse, including 38.2% Caucasian, 28.2% Hispanic, 22.1% Asian, and 11.5% African American.

Stimuli Presentation

Stimuli were administered using PSYLAB (Contact Precision Instruments, London, UK). Acoustic startle probes were 40-ms duration, 103-dB bursts of white noise with near-instantaneous rise time presented binaurally through headphones. Electric shocks were 400 ms in duration and administered to the wrist of the participant's left (nondominant) hand. Shock intensity was determined ideographically using a workup procedure for each participant (see below).

Procedure

After electrode placement, participants were seated in an electrically shielded, sound-attenuated booth approximately 3.5 ft from a 19-in computer monitor. Participants first completed a 2.5-min baseline habituation task during which nine acoustic startle probes were administered. Next, shock intensity was determined using a workup procedure where participants received increasing levels of shock, until they reached a level they described as "highly annoying but not painful" (maximum shock level was 5 mA). The mean shock level across the entire sample was 2.25 mA ($SD = 1.21$). At the end of the task, participants rated how intense, annoying, and anxiety provoking the shocks were on a scale ranging from 1 (*not at all*) to 7 (*extremely*), and the degree to which they would avoid the shocks on a scale ranging from 1 (*would definitely not avoid*) to 7 (*would definitely avoid*).

The NPU-threat task was a variant of that used by Grillon and colleagues (Schmitz & Grillon, 2012) and has been described elsewhere (see Nelson, Bishop, Sarapas, Kittles, & Shankman, 2014; Nelson & Shankman, 2011). Briefly, the task included three within-subjects conditions: no shock (N), predictable shock (P), and unpredictable shock (U). Text at the bottom of the screen informed participants of the current condition by displaying "no shock" (N), "shock at 1" (P), or "shock at any time" (U). Each condition lasted 90 s, during which a 6-s visual countdown (CD; i.e., cue) was presented five times. The ISI (i.e., time between CDs during the 90-s condition) ranged from 7 to 17 s, during which only the text describing the condition was on the screen. In the N condition, no shocks were delivered. In the P condition, participants received a shock every time the CD reached 1. In the U condition, shocks were administered at any time. Startle probes were presented both during the CD (1–5 s following CD onset) and ISI (5–14 s following ISI onset). The time intervals between shocks and subsequent startle probes were always greater than 10 s to ensure that subsequent probes were not affected by prior shocks.

The task consisted of two presentations of each 90-s condition (N, P, U), during which the CD appeared five times. Participants received startle probes during four out of the five CD and ISI presentations. Conditions were presented in one of the following orders (counterbalanced): PNUPNU or UNPUNP. All participants received 20 electric shocks (10 during P, 10 during U), and 48 startle probes (16 during N, 16 during P, and 16 during U) during the CD and ISI (with an equal number of startle probes occurring during the CD and ISI).

EMG Recording and Processing

Startle eye blink EMG was recorded using Neuroscan 4.4 (Compumedics, Charlotte, NC) and measured from two 4-mm Ag/AgCl electrodes placed over the orbicularis oculi muscle below the right eye. EMG was recorded using a band-pass filter of DC-200 Hz and a sampling rate of 1000 Hz. Offline, EMG data were rectified and then smoothed using a finite impulse response filter with a band-pass of 28–40 Hz. Peak amplitude of the startle blink reflex was determined in the 20- to 150-ms time frame following the startle probe onset relative to baseline (average baseline EMG level for the 50 ms preceding the startle probe onset). Blinks were scored as nonresponses if EMG activity during the 20- to 150-ms poststimulus time frame did not produce a blink peak that was visually differentiated from baseline activity. Blinks were scored as missing if the baseline period was contaminated with noise, movement artifact, or if a spontaneous or voluntary blink began before minimal onset latency and thus interfered with the probe-elicited blink response. Startle analyses were conducted using blink magnitude (i.e., averages include values of 0 for nonresponse trials) as this is a more conservative estimate of blink response (Blumenthal et al., 2005).

EEG Recording and Data Processing

EEG was recorded using Neuroscan 4.4 (Compumedics, Charlotte, NC) and measured from Ag/AgCl electrodes in a 64-channel stretch-lycra electrode cap. The ground electrode was at the frontal pole (AFz) and the online reference was near the vertex (between Cz and CPz). Electrodes placed at the right supra- and infraorbital sites were used to monitor vertical eye movements, and electrodes placed at the right and left outer canthi were used to monitor horizontal eye movements. Electrode impedances were under 5000 Ω, and homologous sites (e.g., F3/F4) were within 1500 Ω of each other. EEG was recorded through a Neuroscan Synamps2 data acquisition system at a gain of 10K (5K for eye channels) with a band-pass of DC-200 Hz and digitized continuously at a sampling rate of 1000 Hz. Offline, EEG data were rereferenced to the average of the left and right mastoid and band-pass filtered from 0.1 to 30 Hz. Eye blink and ocular corrections were conducted using established standards (Gratton, Coles, & Donchin, 1983).

A semiautomatic procedure was employed to detect and reject artifacts. The criteria applied were a voltage step of more than 50 μV between sample points, a voltage difference of 300 μV within a trial, and a maximum voltage difference of less than 0.50 μV within 100-ms intervals. These intervals were rejected from individual channels in each trial. Visual inspection of the data was then conducted to detect and reject remaining artifacts.

The EEG was segmented for each trial beginning 200 ms before the startle probe and continuing for 1,200 ms. The baseline was the 200 ms prior to the onset of the startle probe. Separate grand averages were conducted for each level of condition (N, P, U) and cue (CD vs. ISI), producing six different ERP averages (N_{ISI}, N_{CD}, P_{ISI}, P_{CD}, U_{ISI}, U_{CD}). The P300 was scored as the average activity at CPz (where it was maximal) between 260–320 ms, and the N100 was scored as the average activity at FCz (where it was maximal) between 90–130 ms.

Data Analysis

Twelve participants were excluded from analyses due to equipment failure ($n = 5$), excessive EEG artifacts that resulted in less than 50% usable trials ($n = 2$), outlier startle values ($n = 2$) (Hoaglin, 1986; Hoaglin & Iglewicz, 1987; Tukey, 1977), or current psychotropic medication use (antidepressant, $n = 2$; stimulant, $n = 1$), leaving a final sample of 119 participants. Startle and ERP data were analyzed using separate Condition (N, P, U) × Cue (CD vs. ISI) repeated measures analysis of variance (ANOVA). Condition and cue main effects were followed up by examining simple effects. Condition × Cue interactions were followed up by conducting separate repeated measures ANOVAs for each level of cue (CD vs. ISI). For the ERP analyses, we conducted identical but separate Condition × Cue repeated measures ANOVAs for the probe P300 and N100. To examine response habituation, we conducted mixed growth models examining the slope of responding across time, within individuals. Multilevel modeling is well suited for this purpose as it allows time to be modeled continuously, accounts for the variability in duration between startle probes, and handles missing data by weighting slope estimates by the number of observations (Goldstein, 2011). In the present study, the mixed growth models used restricted maximum likelihood (REML) estimation and an unstructured covariance matrix. All analyses were conducted in IBM SPSS Statistics, Version 22.0 (Armonk, NY).

Results

Shock Ratings

Participants rated the shocks as moderately intense ($M = 4.53$, $SD = 1.15$), annoying ($M = 5.79$, $SD = 1.27$), and anxiety provoking ($M = 4.98$, $SD = 1.48$). Participants also reported that they would avoid receiving the shocks again to a high degree ($M = 5.23$, $SD = 1.58$). Shock ratings were not associated with any psychophysiological measure (ps $> .10$).

EMG Startle Reflex

Figure 1 displays the startle reflex means (and standard errors) for the different threat conditions and cues. Results indicated a main effect of condition, $F(2,236) = 78.54$, $p < .001$, G-G$\epsilon = .70$, $\eta_p^2 = .40$, and cue, $F(1,118) = 43.99$, $p < .001$, $\eta_p^2 = .27$, and a Condition × Cue interaction, $F(2,236) = 12.88$, $p < .001$, G-G$\epsilon = .91$, $\eta_p^2 = .10$. The Condition × Cue interaction was followed up by conducting separate repeated measures ANOVAs for each level of cue (CD vs. ISI). During the CD, startle differed between conditions, $F(2,236) = 66.54$, $p < .001$, G-G$\epsilon = .81$, $\eta_p^2 = .36$, due to greater startle during P_{CD} and U_{CD} relative to N_{CD}, $F(1,118) = 26.90$ $p < .001$, $\eta_p^2 = .19$; $F(1,118) = 92.16$, $p < .001$, $\eta_p^2 = .44$, respectively, and greater startle during U_{CD} relative to P_{CD}, $F(1,118) = 53.42$, $p < .001$, $\eta_p^2 = .31$. Startle during the ISI also differed between conditions, $F(2,236) = 72.98$, $p < .001$, G-G$\epsilon = .67$, $\eta_p^2 = .38$, due to greater startle during U_{ISI} relative to N_{ISI} and

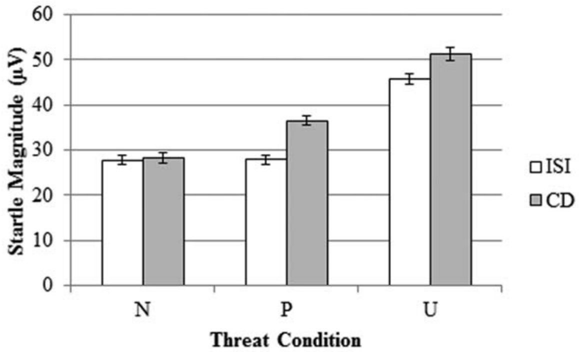

Figure 1. Startle magnitude across different levels of threat condition (N, P, U) and cue (CD vs. ISI). Error bars represent standard error. CD = countdown; ISI = interstimulus interval; N = no threat; P = predictable threat; U = unpredictable threat.

P_{ISI}, $F(1,118) = 82.63$, $p < .001$, $\eta_p^2 = .41$; $F(1,118) = 79.53$, $p < .001$, $\eta_p^2 = .40$, respectively, which did not differ, $F(1,118) = 0.71$, *ns*, $\eta_p^2 = .01$.

For the habituation analyses, we conducted a three-level mixed growth model examining the slope of the startle reflex across time, within individuals, and included condition and cue as within-subjects factors. Time was coded as the second the startle probe occurred relative to the start of the task (onset of task = 0 s). Figure 2 displays the slopes and intercepts of the mixed growth model for the startle reflex. There was a main effect of time, $t(5158.21) = -2.28$, $b =$ -0.04, $p < .05$, and cue that approached significance, $t(5121.35) = -1.95$, $b = -7.98$, $p < .06$, and Condition × Cue, $t(5141.12) = 5.26$, $b = 9.81$, $p < .001$, and Condition × Time interactions, $t(5100.28) = 2.72$, $b = 0.02$, $p < .01$, and a Cue × Time interaction that approached significance, $t(5072.90) = 1.85$, $b = 0.02$, $p < .07$, that were qualified by a Condition × Cue × Time interaction, $t(5090.15) = -4.17$, $b = -0.02$, $p < .001$. The Condition × Cue × Time interaction was followed up by conducting separate Condition × Time interactions for each level of cue (CD vs. ISI). For the ISI, there was a main effect of condition, $t(2397.47) = 7.06$, $b = 8.68$, $p < .001$, and time, $t(1369.28) = -2.06$, $b = -0.02$, $p < .05$, indicating that the startle reflex during the N_{ISI}, P_{ISI}, and U_{ISI} all decreased at a comparable rate. For the CD, there was a main effect of condition, $t(2577.31) = 13.73$, $b = 18.98$, $p < .001$, and a Condition × Time interaction, $t(2525.40) = -6.35$, $b = -0.02$, $p < .001$. The Condition × Time interaction for the CD was followed up by examining the time main effect separately for each level of condition (N, P, U). Results indicated that the startle reflex decreased over time during all three conditions, but the P_{CD}, $t(118.55) = -7.54$, $b = -0.06$, $p < .001$, and U_{CD}, $t(120.40) = -8.55$, $b = -0.06$, $p < .001$, decreased at a greater rate relative to the N_{CD}, $t(118.19) = -5.44$, $b = -0.03$, $p < .001$.

To determine whether the N_{CD}, P_{CD}, and U_{CD} conditions differed at the beginning versus end of the task, we extracted each participant's estimated intercept from the mixed growth models. The intercept was coded two different ways—once as the second the startle probe occurred relative to the start of the task (to get the beginning intercept) and as the second the startle probe occurred relative to the end of the task (to get the ending intercept). We then tested for differences between these intercepts using a repeated measures ANOVA with condition (N_{CD}, P_{CD}, U_{CD}) as the within-subjects factor.

For the intercept at the beginning of the task, results indicated a main effect of condition, $F(2,236) = 71.20$, $p < .001$, $\eta_p^2 = .38$, such that the U_{CD} was greater than the P_{CD}, $F(1,118) = 36.69$, $p < .001$, $\eta_p^2 = .24$, and N_{CD}, $F(1,118) = 134.10$, $p < .001$, $\eta_p^2 = .53$, and the P_{CD} was greater than the N_{CD}, $F(1,118) = 36.91$, $p < .001$, $\eta_p^2 = .24$. These results mirrored those for the average startle reflex. For the intercept at the end of the task, results again indicated a main effect of condition, $F(2,236) = 30.73$, $p < .001$, G-G$\epsilon = .75$, $\eta_p^2 = .21$, such that the U_{CD} was greater than the P_{CD}, $F(1,118) = 41.52$, $p < .001$, $\eta_p^2 = .26$, and N_{CD}, $F(1,118) = 32.54$, $p < .001$, $\eta_p^2 = .22$, but the P_{CD} and N_{CD} no longer differed, $F(1,118) = 0.06$, *ns*, $\eta_p^2 < .01$. Thus, while the P_{CD} and U_{CD} were potentiated relative to the N_{CD} at the beginning of the NPU-threat task, by the end of the task only the U_{CD} (and not the P_{CD}) continued to differ from the control condition (N_{CD}).

ERPs

The P300 was evident at centroparietal sites and was maximal approximately 280 ms after the startle probe (see Figure 3). Results indicated a main effect of condition, $F(2,236) = 20.63$, $p < .001$, G-G$\epsilon = .95$, $\eta_p^2 = .15$, such that the P300 was attenuated during P_{CD+ISI}, $F(1,118) = 43.70$, $p < .001$, $\eta_p^2 = .27$, and U_{CD+ISI}, $F(1,118) = 25.00$, $p < .001$, $\eta_p^2 = .18$, relative to N_{CD+ISI}, while P_{CD+ISI} and U_{CD+ISI} did not differ, $F(1,118) = 0.17$, *ns*, $\eta_p^2 < .01$. There was no main effect of cue, $F(1,118) = 0.33$, *ns*, $\eta_p^2 < .01$, or Condition × Cue interaction, $F(2,236) = 0.87$, *ns*, $\eta_p^2 = .01$.

The N100 was evident at frontocentral sites and was maximal approximately 110 ms after probe presentation (see Figure 4). Results also indicated a main effect of condition, $F(2,236) = 35.81$, $p < .001$, G-G$\epsilon = .91$, $\eta_p^2 = .23$, such that the N100 was enhanced during U_{CD+ISI} relative to N_{CD+ISI}, $F(1,118) = 53.81$, $p < .001$, $\eta_p^2 = .31$, and P_{CD+ISI}, $F(1,118) = 38.32$, $p < .001$, $\eta_p^2 = .25$, which did not differ, $F(1,118) = 1.71$, *ns*, $\eta_p^2 = .01$. There was no main effect of cue, $F(1,118) = 2.11$, *ns*, $\eta_p^2 = .02$, or Condition × Cue interaction, $F(2,236) = 0.04$, *ns*, $\eta_p^2 < .01$.

For the habituation analyses, we conducted two-level mixed growth models examining the slope of the ERP measures across time, within individuals, and including condition as a within-subjects factor. Time was coded as the second the startle probe occurred relative to the start of the task (onset of task = 0 s). For the P300, there was a main effect of condition, $t(5182.04) = -4.07$, $b = -1.88$, $p < .001$, and time, $t(2278.66) = -2.80$, $b = -0.01$, $p < .01$, but no Condition × Time interaction, $t(5221.57) = 0.73$, $b < 0.01$, *ns*. Similarly, for the N100 there was a main effect of condition, $t(5156.70) = -4.02$, $b = -1.84$, $p < .001$, and

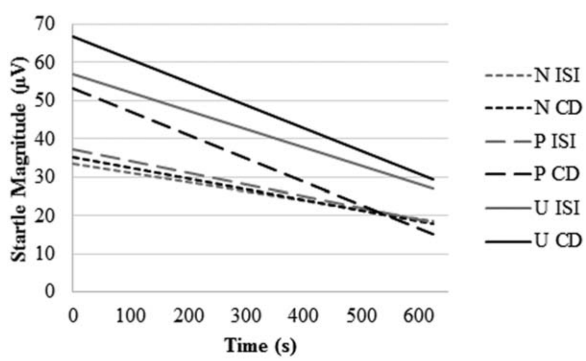

Figure 2. Startle magnitude habituation (i.e., rate of change over time) across different levels of threat condition (N, P, U) and cue (CD vs. ISI). CD = countdown; ISI = interstimulus interval; N = no threat; P = predictable threat; U = unpredictable threat.

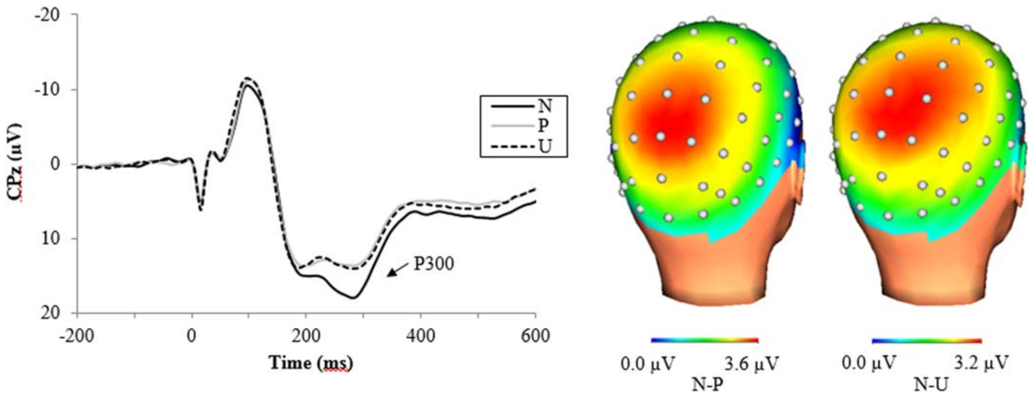

Figure 3. ERP grand-average waveforms at CPz for the N, P, and U conditions. The probe P300 was reduced during P and U compared to N; data were collapsed across CD and ISI phases of each condition. Head maps depict the scalp distribution of the increased probe P300 in N relative to the P (left head map) and U (right head map) conditions. ERP = event-related potential; ISI = interstimulus interval; ms = milliseconds; N = no threat; P = predictable threat; U = unpredictable threat.

time, $t(1703.00) = 5.17$, $b = 0.02$, $p < .001$, but no Condition × Time interaction, $t(5194.00) = -0.75$, $b > -0.01$, *ns*. Together, these results suggest that the P300 and N100 both decreased over time, but the rate of habituation did not differ between the N, P, and U threat conditions.

Internal Consistency

The internal consistency of the startle reflex and probe P300 and N100 was examined by calculating Cronbach's alpha as a function of the number of trials. Cronbach's alpha greater than .90 indicates excellent internal reliability, between .70–.90 indicates good internal reliability, between .50–.70 indicates moderate internal reliability, and less than .50 indicates low reliability. As shown in Figure 5, the startle reflex achieved excellent internal reliability, the probe N100 achieved good internal reliability, and the probe P300 achieved moderate internal reliability. Furthermore, all three measures reached relatively stable internal consistency by the fourth trial, although the P300 improved the most between trials 5–8. Overall, these analyses suggest that the probe P300 and N100 achieved acceptable internal consistency during the NPU-threat task.

Within-Subjects Correlation Between EMG and ERP Measures

Pearson's correlations were conducted between the EMG startle reflex and probe P300 and N100 averaged across the threat conditions. Startle was negatively associated with the N100, $r(119) = -.18$, $p < .05$, such that greater EMG startle reflex was associated with an enhanced N100, but was not associated with the P300, $r(119) = .05$, *ns*. Furthermore, there was no association between the P300 and N100, $r(119) = .09$, *ns*.

Discussion

The present study examined the startle reflex and probe P300 and N100 responses in anticipation of no, predictable, and unpredictable threat. Results indicated that the probe P300 was attenuated during both the predictable and unpredictable relative to no threat condition. In contrast, the probe N100 was enhanced during the unpredictable, but not predictable, threat condition. Thus, the probe P300 was decreased on trials in which there was a potential for any threat, whereas the probe N100 was specifically increased in the context of unpredictable threat. These results indicate that the startle probe ERPs could fully distinguish all three threat conditions.

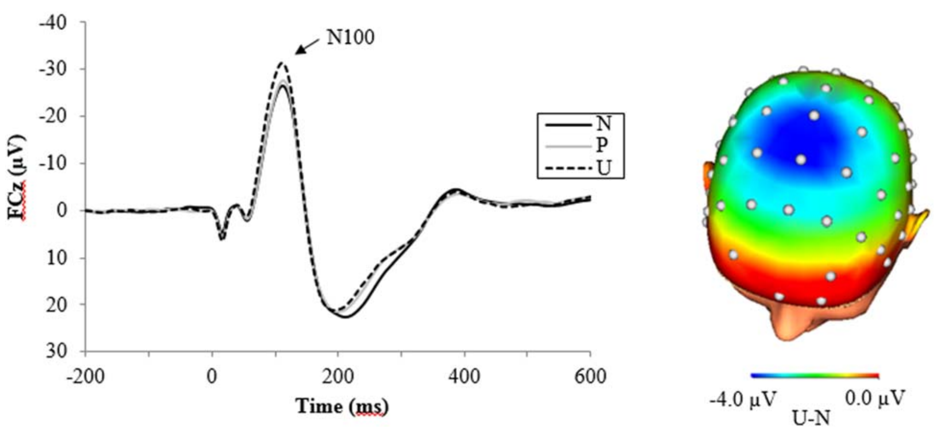

Figure 4. ERP grand-average waveforms at FCz for the N, P, and U conditions. The probe N100 was increased during U compared to P and N; data were collapsed across CD and ISI phases of each condition. The head map depicts the scalp distribution of the increased probe N100 in the U relative to N condition. ERP = event-related potential; ISI = interstimulus interval; ms = milliseconds; N = no threat; P = predictable threat; U = unpredictable threat.

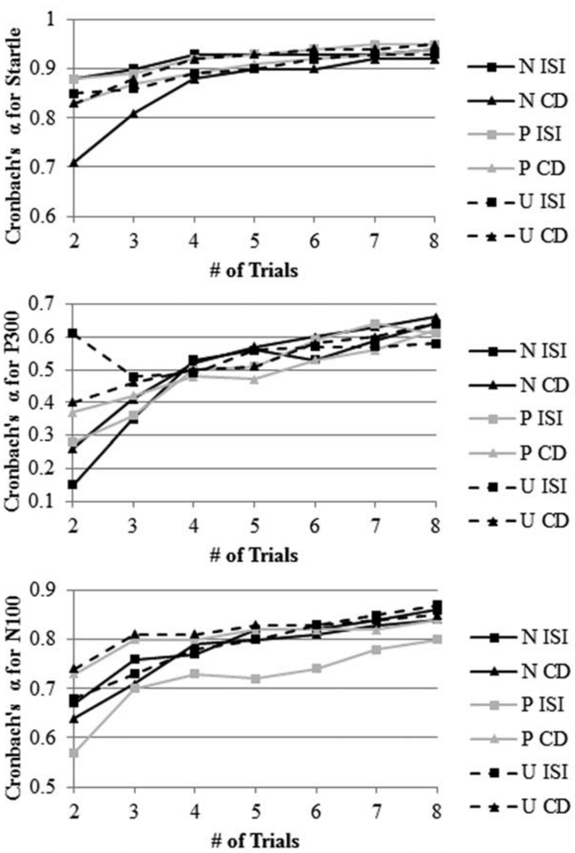

Figure 5. Cronbach's α for startle magnitude (top), P300 (middle), and N100 (bottom) as a function of the number of trials across different levels of threat condition (N, P, U) and cue (CD vs. ISI). CD = countdown; ISI = interstimulus interval; N = no threat; P = predictable threat; U = unpredictable threat.

There were several key differences between the startle reflex and probe ERP responses during the NPU-threat task. For instance, the startle reflex was potentiated in anticipation of predictable and unpredictable relative to no threat, and was also greater in anticipation of unpredictable relative to predictable threat. In contrast, the probe P300 was suppressed in anticipation of predictable and unpredictable threat relative to no threat to a comparable degree. Thus, while both measures were sensitive to the anticipation of threat, the EMG startle reflex was more sensitive to unpredictable threat while the probe P300 did not differ as a function of threat predictability. In addition, in contrast to the startle reflex and the probe P300, the probe N100 was uniquely enhanced to the anticipation of unpredictable threat. Together, these results indicate that the probe P300 and N100 are potentially useful markers of fear and anxiety and exhibit several characteristics that differ from the startle reflex.

The present study suggests that in the context of potential threat there is increased engagement with the task, which is reflected in an attenuated probe P300. These data extend previous work on probe P300 reduction during affective picture processing (Cuthbert et al., 1998; Schupp et al., 1997) to a threat anticipation paradigm for the first time. The probe P300 has been suggested to reflect the orienting response that indexes the allocation of attentional resources (Bradley, Keil, & Lang, 2012). Thus, probe P300 reduction in anticipation of potential threat indicates that attention is being directed away from the startle probe and toward the threat cues and/or context. Furthermore, these results indicate that the potential for any kind of threat, and not particular features of threat (e.g., predictability), is what draws attention to the environment. However, this study did not formally test other features of threat (e.g., controllability, intensity), and future research is needed to determine whether these parameters might influence the probe P300.

On the other hand, the present study provides novel evidence that the probe N100 is increased specifically in the context of unpredictable threat. The probe N100 has been proposed to reflect early cortical processing of sensory input to enhance such stimuli (Näätänen & Picton, 1987). The increased N100 may reflect an attentional component that primes early cortical processing of sensory input when the system is on the lookout for unpredictable danger. This pattern is different from that of the probe P300, which was attenuated to both predictable and unpredictable threat. Thus, evidence suggests that the anticipation of unpredictable threat primes early sensory processing of the environment, but the anticipation of threat in general engages later attention processing.

The startle probe EMG and ERP responses had relatively comparable psychometric properties. We found that in a typical variant of the NPU-threat task, which is calibrated for the startle reflex, there are a sufficient number of trials to examine startle probe ERPs. Specifically, the probe P300 and N100 both achieved acceptable internal consistency by the fourth trial. It is important to highlight, though, that the startle reflex and probe N100 had better overall internal consistencies relative to the probe P300. Future research is needed to examine other psychometric properties of the probe P300 and N100 (e.g., test-retest reliability, predictive validity) that have already been evaluated in the NPU-threat task using the EMG startle reflex (e.g., Shankman et al., 2013).

There were, however, habituation differences between the startle reflex and probe ERP responses. The probe P300 and N100 decreased over time but continued to demonstrate similar differences between the threat and no threat conditions. In contrast, the startle reflex also decreased over time, but did so differentially between the predictable and unpredictable threat conditions. Specifically, the multilevel modeling results indicated that at the beginning of the task the EMG startle reflex was potentiated during the predictable and unpredictable cue relative to the no threat cue. However, by the end of the task only the unpredictable (and not predictable) threat condition continued to differ from the no threat condition.

Animal research has indicated that the startle reflex to predictable and unpredictable threat are mediated by overlapping but distinct neural systems, specifically the central nucleus of the amygdala for predictable threat and the bed nucleus of the stria terminalis for unpredictable threat (Davis, 2006; Davis, Walker, Miles, & Grillon, 2010; Gray & McNaughton, 2000). In addition, the amygdala has been shown to habituate more rapidly to predictable relative to unpredictable stimuli (Herry et al., 2007). The present study's startle EMG and ERP results indicate that the anticipation of predictable threat becomes less aversive over time (as indicated by decreased startle), but it continues to engage attentional resources. It is important to note that one study found that, after multiple contiguous repetitions, the probe P300 no longer differed between emotional compared to neutral pictures (Ferrari, Bradley, Codispoti, & Lang, 2011). Therefore, it is possible that the probe P300 and N100, relative to the startle reflex, may be less likely to habituate in the short term, but both measures eventually fail to discriminate predictable (or present) threat relative to no threat.

The present study found evidence for a within-subject relationship between the startle reflex and the probe N100. Specifically, greater startle was associated with an increased N100 across all threat conditions, but was unrelated to the probe P300, and both the startle reflex and probe N100 demonstrated their strongest effects during the unpredictable threat condition. As previously mentioned, the startle reflex is modulated by both attention (Anthony & Graham, 1985; Hackley & Graham, 1987) and valence (Lang et al., 1990), and the probe P300 and N100 components appear to tap distinct aspects of attention. The correlation between the startle reflex and the probe N100 may suggest that startle is more associated with early (e.g., alerting) rather than later (e.g., executive control) components of attention. Both measures appear to index more automatic, reflexive aspects of aversive system activation and are particularly sensitive to the temporal unpredictability of threat. An alternative explanation for this association is that, since they occur very close in time, the blink reflex may have caused EEG artifacts that contributed to the probe N100. However, previous research has demonstrated that blink artifacts do not account for the probe N100 (Cuthbert et al., 1998), and the scalp distribution of the probe N100 (frontocentral, maximal at FCz) make it unlikely that this was due to eye blink artifacts, which tend to be more frontal (Gratton et al., 1983).

This study had several limitations that warrant consideration. The sample consisted of undergraduates and results may not generalize to all populations. In addition, the study design exclusively focused on the anticipation of predictable versus unpredictable electric shocks, and it is unclear whether similar results will be obtained for other types of aversive stimuli (e.g., unpleasant pictures). Finally, the probe P300 has been conceptualized as reflecting attention to aversive and appetitive stimuli that may be important for survival (Bradley et al., 2012), and several studies have found comparable P300 reduction during the viewing of pleasant and unpleasant pictures (Bradley et al., 2006; Cuthbert et al., 1998; Schupp et al., 1997) and environmental sounds (Keil et al., 2007). Future studies should determine whether there is comparable P300 suppression and/or N100 enhancement in anticipation of predictable versus unpredictable pleasant stimuli (e.g., pictures of food).

In summary, the present study found startle probe P300 and N100 modulation in the context of a threat anticipation task. Specifically, the probe P300 was attenuated in anticipation of predictable and unpredictable relative to no threat, while the probe N100 was enhanced in anticipation of unpredictable threat condition only. In addition, the startle probe ERPs demonstrated acceptable psychometric properties (e.g., internal consistency, habituation) that support their use in the NPU-threat task. Overall, the present study provides novel evidence that, unlike the startle reflex, startle probe ERPs can distinguish general threat anticipation (i.e., the probe P300) from the anticipation of unpredictable threat in particular (i.e., the probe N100), and suggests that anticipatory threat cues also recruit "motivated attention" and aversive system activation (Lang, Bradley, & Cuthbert, 1997).

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