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Effects of anticipated emotional category and temporal predictability on the startle reflex

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ABSTRACT

Anticipated emotional category and temporal predictability are key characteristics that have both been shown to impact psychophysiological indices of defensive motivation (e.g., the startle reflex). To date, research has primarily examined these features in isolation, and it is unclear whether they have additive or interactive effects on defensive motivation. In the present study, the startle reflex was measured in anticipation of low arousal neutral, moderate arousal pleasant, and high arousal unpleasant pictures that were presented with either predictable or unpredictable timing. Linear mixed-effects modeling was conducted to examine startle magnitude across time, and the intercept at the beginning and end of the task. Across the entire task, the anticipation of temporally unpredictable (relative to predictable) pictures and emotional (relative to neutral) pictures potentiated startle magnitude, but there was no interaction between the two features. However, examination of the intercept at the beginning of the task indicated a Predictability by Emotional Category interaction, such that temporal unpredictability enhanced startle potentiation in anticipation of unpleasant pictures only. Examination of the intercept at the end of the task indicated that the effects of predictability and emotional category on startle magnitude were largely diminished. The present study replicates previous reports demonstrating that emotional category and temporal predictability impact the startle reflex, and provides novel evidence suggesting an interactive effect on defensive motivation at the beginning of the task. This study also highlights the importance of examining the time course of the startle reflex.

1. Introduction

Psychophysiology has played a prominent role in the measurement of individual differences in emotion and motivation. For example, the startle eye blink reflex is a widely used tool for assessing defensive motivation (Blumenthal et al., 2005; Grillon and Baas, 2003). The startle reflex is modulated by current emotional state, and this has often been demonstrated during the presentation of emotional pictures. Specifically, the startle reflex is potentiated while viewing unpleasant pictures relative to neutral pictures, but is attenuated while viewing pleasant pictures relative to neutral pictures (Lang et al., 1990; Lang, 1995). The *anticipation* of viewing temporally predictable emotional pictures produces a different pattern of results, such that the startle reflex is increased in anticipation of both pleasant and unpleasant pictures relative to neutral pictures (Dichter et al., 2002; Sabatinelli et al., 2001; Sege et al., 2014). These findings suggest that while the startle reflex is sensitive to stimulus valence during picture perception, the anticipation of both pleasant and unpleasant pictures relative to neutral pictures primes defensive motivation.

Predictability is another feature of stimuli that has been suggested to impact defensive motivation (Davis et al., 2010; Grillon et al., 2004). In the laboratory this has often been examined using a no, predictable, and unpredictable (NPU) threat task (Schmitz and Grillon, 2012) that includes three within-subject conditions: no threat (no aversive stimulus is presented), predictable threat (aversive stimulus is signaled by a short duration cue), and unpredictable threat (aversive stimulus is unsignaled). Across all three conditions the startle reflex is measured as a psychophysiological indicator of defensive motivation. A growing number of studies have found that the startle reflex is potentiated in anticipation of both predictable and unpredictable threat relative to no threat (Grillon et al., 2004; Moberg and Curtin, 2009; Nelson and Shankman, 2011)—though startle potentiation appears to be larger in anticipation of unpredictable than predictable threat (Gorka et al., 2016; Nelson et al., 2015).

The NPU task has been exclusively used with aversive or threatening stimuli, including shocks (Bradford et al., 2013; Nelson et al., 2015; Shankman et al., 2013), noises (Nelson and Hajcak, 2017; Schmitz et al., 2011), airblasts (Grillon et al., 2004), and a breathing occlusion (Schroijen et al., 2016). However, predictability has also been shown to impact the processing of appetitive or pleasant stimuli (Berns et al., 2001). Although the NPU task includes a no threat comparison

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condition, it is unclear whether predictability also modulates defensive motivation in anticipation of pleasant and neutral stimuli. Emotional category and temporal predictability are orthogonal characteristics that have both been shown to impact the startle reflex in anticipation of viewing pictures, but no study has examined whether they produce additive or interactive effects on defensive motivation. If predictability impacts defensive motivation irrespective of emotional category, then it is possible that paradigms like the NPU task could be employed with less noxious (i.e., neutral or pleasant) stimuli. However, if there is an interaction between emotional category and predictability, such that unpredictability enhances defensive motivation to a greater degree for more aversive relative to less aversive stimuli, it would suggest that emotional category is an important characteristic to take into consideration in experimental design.

The present study employed a within-subjects design and examined the effect of anticipated emotional category and temporal predictability on defensive motivation. To this end, the startle reflex was measured during the anticipation of low arousal neutral, moderate arousal pleasant, and high arousal unpleasant pictures that were presented with either predictable or unpredictable timing. The startle reflex is an advantageous tool for examining online defensive motivation, but there are two features that are important to consider. First, startle paradigms often involve missing values due to blink or motion artifacts (e.g., participant is blinking while a startle probe is being delivered), which can vary between participants and/or experimental conditions. Second, the startle reflex habituates (i.e., decreases) over time (Gorka et al., 2015; Nelson et al., 2015), and condition effects may be more or less pronounced at different times during the task. To address these issues, the present study employed linear mixed-effects modeling to examine startle magnitude across time, and the intercept at the beginning and end of the task. Multilevel modeling is an advantageous analytic approach 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).

We had three primary hypotheses. First, we hypothesized the startle reflex would be potentiated in anticipation of both moderate arousal pleasant and high arousal unpleasant pictures relative to low arousal neutral pictures. Second, we hypothesized that the startle reflex would be potentiated in anticipation of pictures that were presented with unpredictable relative to predictable timing. Finally, we hypothesized there would be an Emotional Category by Predictability interaction, such that unpredictable relative to predictable timing would enhance startle potentiation more in anticipation of high arousal unpleasant pictures compared to low arousal neutral and moderate arousal pleasant pictures. There were no specific hypotheses whether these effects would be present across time, at the beginning of the task, and/or at the end of the task.

2. Method

2.1. Participants

The sample included 95 undergraduates from Stony Brook University who participated for course credit. Exclusion criteria were a history of hearing loss or an inability to read or write English. The sample was on average 20.34 years old (SD = 1.93) and was comprised of 64 females. The racial/ethnic distribution was 32.6% Caucasian, 8.4% African-American, 12.6% Latino, 36.8% Asian, and 9.5% 'Other'. All participants provided informed consent and the study protocol was approved by the Stony Brook University Institutional Review Board.

2.2. Stimuli

Forty-eight color low arousal neutral, moderate arousal pleasant, and high arousal unpleasant pictures (16 per category) were selected from the International Affective Picture System (IAPS; Lang et al., 2008).[1] Each picture was presented for a duration of 2 s. Acoustic startle probes were presented using PSYLAB (Contact Precision Instruments, London, UK) and consisted of a 103 dB burst of white noise with near instantaneous rise time and a duration of 40 ms presented binaurally through headphones.

2.3. Procedure

After electrode placement, participants completed a 180-s baseline habituation task during which four acoustic startle probes were administered. Next, participants received instructions and completed two blocks of the picture-viewing task.

2.3.1. Picture-viewing task

The picture-viewing task was a variant of the NPU threat task (Schmitz and Grillon, 2012), modified so that participants anticipated viewing neutral, pleasant, and unpleasant pictures that were presented with either predictable or unpredictable timing. The task used a within-subjects design and contained six different conditions: predictable neutral picture, unpredictable neutral picture, predictable pleasant picture, unpredictable pleasant picture, predictable unpleasant picture, and unpredictable unpleasant picture. Text at the bottom of the computer monitor informed participants of the current condition by displaying the following information: "neutral picture at 1," "neutral picture at any time," "pleasant picture at 1," "pleasant picture at any time," "unpleasant picture at 1," or "unpleasant picture at any time." Each condition lasted 63 s, during which a 5-s visual countdown was presented four times. The interstimulus interval (i.e., time between countdowns) ranged from 6 to 12 s ($M = 9$ s) during which only the text describing the condition was on the screen. In the predictable condition, pictures were presented when the countdown reached 1. In the unpredictable condition, pictures were presented at any time (during the countdown or interstimulus interval). Across both types of trials participants always knew the emotional category (neutral, pleasant, or unpleasant) of the picture that was about to appear. Startle probes were presented during both the countdown (1 to 4 s following countdown onset) and interstimulus interval (4 to 10 s following interstimulus interval onset). During the task instructions, participants completed one practice trial of each condition (predictable neutral picture, unpredictable neutral picture, predictable pleasant picture, unpredictable pleasant picture, predictable unpleasant picture, and unpredictable unpleasant picture). No startle probes were administered during the practice trials, and the pictures shown during the practice trials were not included in the actual task.

The task consisted of two presentations of each 63-s condition, during which the countdown appeared four times. Participants received startle probes during three out of the four countdown and interstimulus interval presentations. Emotional category order and predictability order were counterbalanced across participants. Each condition (predictable neutral picture, unpredictable neutral picture, predictable pleasant picture, unpredictable pleasant picture, predictable unpleasant picture, and unpredictable unpleasant picture) was presented twice across two blocks, with a short break (30 s) between blocks. All participants received 72 startle probes, with an equal number of startle probes occurring during each condition (12 each) and across the countdown and interstimulus interval (36 each).

[1] The IAPS images included objects (7002, 7003, 7010, 7012, 7017, 7021, 7025, 7032, 7040, 7052, 7061, 7090, 7175, 7211, 7235, 7950) for neutral pictures (valence $M = 5.00$, $SD = 0.22$; arousal $M = 2.95$, $SD = 0.70$), food (7200, 7330, 7340, 7350, 7405, 7451, 7461, 7470) and affiliative scenes (2091, 2154, 2156, 2158, 2274, 2391, 2550, 4626) for pleasant pictures (valence $M = 7.26$, $SD = 0.54$, arousal $M = 4.89$, $SD = 0.66$), and mutilation (3030, 3051, 3071, 3000, 3100, 3110, 3170, 3266) and threat scenes (2811, 6242, 6244, 6250, 6350, 6510, 6560, 9425) for unpleasant pictures (valence $M = 2.10$, $SD = 0.49$, arousal $M = 6.58$, $SD = 0.60$).

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After the end of the task, participants made picture valence and arousal ratings to confirm they were perceived as belonging to the intended emotional category. Participants were shown each picture that was presented during the task in a random order, and rated its valence on a 7-point Likert scale ranging from 1 (*very unpleasant*) to 7 (*very pleasant*), with 4 representing neutral, and its arousal on a scale ranging from 1 (*not at all arousing*) to 7 (*extremely arousing*).

2.4. EMG recording and processing

Startle eye blink electromyography (EMG) was recorded using PSYLAB and measured from two 4-mm sintered Ag/AgCl electrodes placed over the orbicularis oculi muscle beneath the left eye. EMG activity was sampled at 1000 Hz and filtered between 30 and 500 Hz. Offline, EMG activity was rectified in a 200 ms window, beginning 50 ms before the onset of the startle probe, and a 6-point running average was applied to the rectified data to smooth out sharp peaks. Peak amplitude of the startle reflex was determined in the 20 to 150-ms time frame following the startle probe onset relative to baseline (i.e., average EMG activity in the 50 ms preceding the startle probe onset). Blinks were scored as nonresponses if EMG activity during the 20 to 150-ms post-probe time frame did not produce a blink peak that was at least 3 μV in amplitude and could be visually discerned from the surrounding EMG 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. The present study examined blink magnitude (i.e., averages include values of 0 for nonresponse trials) as it is a more conservative estimate of the startle response (Blumenthal et al., 2005).

2.5. Data analysis

Thirteen participants were excluded from analyses due to EMG artifacts in >50% of trials ($n = 5$) or equipment failure ($n = 8$), leaving a final sample of 82 participants. Picture ratings were analyzed using a one-way repeated measures analysis of variance (ANOVA); one participant was excluded from these analyses for not completing the picture ratings. Startle magnitude was analyzed using linear mixed-effects modeling of the slope of startle magnitude across time, within individuals, and included predictability (predictable vs. unpredictable timing), emotional category (low arousal neutral, moderate arousal pleasant, high arousal pleasant), and cue (countdown vs. interstimulus interval) as within-subjects factors. Time was coded as the time at which the startle probe occurred relative to the start of the task (onset of task = 0-s), and the models used restricted maximum likelihood (REML) estimation and an unstructured covariance matrix. To determine whether startle magnitude differed at the beginning and/or end of the task, we extracted each participant's estimated intercept from the models. The intercept was coded two different ways—once relative to the start of the task (to get the beginning intercept), and again 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 predictability, emotional category, and cue as the within-subjects factors. Greenhouse-Geisser epsilons (G-Gε) are reported for repeated measures analyses where assumptions of sphericity were violated. All analyses were conducted in IBM SPSS Statistics, Version 22.0 (Armonk, NY, USA).

3. Results

3.1. Picture ratings

As expected, the pictures differed in valence ratings, $F(2, 160) = 436.09$, $p < 0.001$, $\eta_p^2 = 0.85$, such that unpleasant pictures ($M = 2.36$, $SD = 1.36$) were rated as more unpleasant relative to pleasant pictures ($M = 7.31$, $SD = 0.97$), $F(1, 80) = 565.06$, $p < 0.001$, $\eta_p^2 = 0.88$, and neutral pictures ($M = 4.85$, $SD = 0.70$), $F(1, 80) = 224.66$, $p < 0.001$, $\eta_p^2 = 0.74$, and pleasant pictures were rated as more pleasant relative to neutral pictures, $F(1, 80) = 452.96$, $p < 0.001$, $\eta_p^2 = 0.85$. The pictures also differed in arousal ratings, $F(2, 160) = 83.53$, $p < 0.001$, $\eta_p^2 = 0.51$, such that unpleasant pictures ($M = 5.44$, $SD = 2.53$) were rated as more arousing relative to pleasant pictures ($M = 4.78$, $SD = 1.98$), $F(1, 80) = 5.67$, $p < 0.05$, $\eta_p^2 = 0.07$, and neutral pictures ($M = 2.05$, $SD = 1.49$), $F(1, 80) = 113.54$, $p < 0.001$, $\eta_p^2 = 0.59$, and pleasant pictures were rated as more arousing relative to neutral pictures, $F(1, 80) = 136.80$, $p < 0.001$, $\eta_p^2 = 0.63$.

3.2. Startle magnitude

The average number of missing trials for the predictable condition were: 7.32% ($SD = 14.37$) for the neutral trial interstimulus interval, 7.11% ($SD = 14.59$) for the neutral trial countdown, 7.32% ($SD = 13.88$) for the pleasant trial interstimulus interval, 6.50% ($SD = 13.55$) for the pleasant trial countdown, 6.71% ($SD = 14.31$) for the unpleasant trial interstimulus interval, 4.47% ($SD = 10.49$) for the unpleasant trial countdown; and for the unpredictable condition were: 5.29% ($SD = 11.38$) for the neutral trial interstimulus interval, 5.89% ($SD = 12.66$) for the neutral trial countdown, 5.08% ($SD = 11.32$) for the pleasant trial interstimulus interval, 4.47% ($SD = 11.43$) for the pleasant trial countdown, 5.49% ($SD = 12.58$) for the unpleasant trial interstimulus interval, and 6.71% ($SD = 14.07$) for the unpleasant trial countdown. To examine whether conditions differed on the average percentage of missing trials, we conducted a Predictability X Emotional Category X Cue repeated measures ANOVA with the average percentage of missing trials as the dependent variable. Results indicated no main effects or interactions for predictability, emotional category, or cue.

Table 1 displays means and standard deviations for startle magnitude across different levels of predictability, emotional category, and cue. Linear mixed-effects modeling indicated a main effect of predictability, $t(5408.10) = 3.07$, $b = 1.22$, $p < 0.01$, such that startle magnitude was greater during unpredictable relative to predictable timing trials, a main effect of emotional category, $t(5385.84) = 5.67$, $b = 1.39$, $p < 0.001$, such that startle magnitude was greater during the high arousal unpleasant picture trials relative to the moderate arousal pleasant, $t(3563.40) = 3.52$, $b = 1.72$, $p < 0.001$, and low arousal neutral picture trials, $t(3538.68) = 6.01$, $b = 1.47$, $p < 0.001$; startle magnitude was also greater during the moderate arousal pleasant picture trials compared to the low arousal neutral picture trials, $t(3539.46) = 2.41$, $b = 1.19$, $p < 0.05$, a main effect of cue, $t(5375.78) = -4.89$, $b = -1.92$, $p < 0.001$, such that startle magnitude was greater during the interstimulus interval relative to the countdown, and a main effect of time, $t(82.41) = -11.77$, $b = -0.02$, $p < 0.001$, indicating that startle magnitude habituated (i.e., decreased) across the task. Results also indicated an Emotional Category X Time interaction, $t(5377.32) = -4.23$, $b = -0.004$, $p < 0.001$. As shown in Fig. 1, startle habituation was greater during the high arousal unpleasant and moderate arousal pleasant picture trials relative to the low arousal neutral picture trials, $t(3528.07) = -4.12$, $b = -0.004$, $p < 0.001$; $t(3531.80) = -4.51$, $b = -0.009$, $p < 0.001$, respectively. Startle habituation did not differ between the moderate arousal pleasant and high arousal unpleasant picture trials, $t(3532.75) = 0.16$, $b = 0.0003$, *ns*.

For the intercept at the beginning of the task, results indicated a main effect of cue, $F(1, 81) = 23.42$, $p < 0.001$, $\eta_p^2 = 0.22$, such that startle magnitude was greater during the interstimulus interval relative to the countdown. Results also indicated a main effect of predictability, $F(1, 81) = 10.46$, $p < 0.01$, $\eta_p^2 = 0.11$, and emotional category, $F(2, 162) = 38.00$, $p < 0.001$, $\eta_p^2 = 0.32$, which were qualified by a Predictability X Emotional Category interaction, $F(2, 162) = 4.21$, $p < 0.05$, G-Gε = 0.79, $\eta_p^2 = 0.05$. As shown in Fig. 2 (top), during the predictable timing trials, startle magnitude was greater during the high arousal unpleasant and moderate arousal pleasant picture trials relative to the low arousal neutral picture trials, $F(1, 81) = 22.57$, $p < 0.001$, $\eta_p^2 = 0.22$; $F(1,$

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Table 1
Startle magnitude across different levels of predictability, emotional category, and cue.

	Predictable timing						Unpredictable timing					
	Low arousal neutral		Moderate arousal pleasant		High arousal unpleasant		Low arousal neutral		Moderate arousal pleasant		High arousal unpleasant	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Interstimulus interval	29.70	22.29	34.35	23.13	35.08	22.98	30.26	22.85	34.40	23.02	36.92	23.52
Countdown	28.21	22.51	32.25	22.31	32.93	23.23	29.83	21.81	32.75	22.23	35.61	22.68

Note. *M* = mean, *SD* = standard deviation.

81) = 16.54, $p < 0.001$, $\eta_p^2 = 0.17$, but startle magnitude did not differ between the high arousal unpleasant and moderate arousal pleasant picture trials, $F(1, 81) = 0.04$, *ns*. In contrast, during the unpredictable timing trials startle magnitude was greater during the high arousal unpleasant and moderate arousal pleasant picture trials relative to the low arousal neutral picture trials, $F(1, 81) = 70.66$, $p < 0.001$, $\eta_p^2 = 0.47$; $F(1, 81) = 11.35$, $p = 0.001$, $\eta_p^2 = 0.12$, and startle magnitude was greater during the high arousal unpleasant picture trials relative to the moderate arousal pleasant picture trials, $F(1, 81) = 11.52$, $p = 0.001$, $\eta_p^2 = 0.12$. We also followed-up the Predictability X Emotional Category interaction by examining the impact of unpredictability on the startle magnitude separately for the different emotional categories. During the high arousal unpleasant picture trials, startle magnitude was greater during the unpredictable relative to predictable timing trials, $F(1, 81) = 22.98$, $p < 0.001$, $\eta_p^2 = 0.22$; however, during the low arousal neutral and moderate arousal pleasant picture trials startle magnitude did not differ during the unpredictable relative to predictable timing trials, $F(1, 81) < 0.01$, *ns*; $F(1, 81) = 0.58$, *ns*, respectively.

For the intercept at the end of the task, results indicated a main effect of cue, $F(1, 81) = 10.61$, $p < 0.01$, $\eta_p^2 = 0.12$, such that startle magnitude was greater during the interstimulus interval relative to the countdown. Results also indicated a main effect of emotional category, $F(2, 162) = 4.62$, $p < 0.05$, $\eta_p^2 = 0.05$, which was qualified by a Predictability X Emotional Category interaction, $F(2, 162) = 3.09$, $p < 0.05$, $\eta_p^2 = 0.04$. As shown in Fig. 2 (bottom), during predictable timing trials startle magnitude was decreased during the moderate arousal pleasant picture trials relative to the high arousal unpleasant and low arousal neutral picture trials, $F(1, 81) = 17.84$, $p < 0.001$, $\eta_p^2 = 0.18$; $F(1, 81) = 4.35$, $p < 0.05$, $\eta_p^2 = 0.05$, but startle magnitude did not differ between the high arousal unpleasant and low arousal neutral picture trials, $F(1, 81) = 3.41$, *ns*. During the unpredictable timing trials startle magnitude did not differ between the different emotional categories. We also followed-up the Predictability X Emotional Category interaction by examining the impact of unpredictability on the startle magnitude separately for the different emotional categories. During the moderate arousal pleasant picture trials, startle magnitude was greater during the unpredictable relative to predictable timing trials, $F(1, 81) = 3.93$, $p = 0.05$, $\eta_p^2 = 0.05$; however, during the low arousal neutral and high arousal unpleasant picture trials startle magnitude did not differ unpredictable relative to predictable timing trials, $F(1, 81) = 1.38$, *ns*; $F(1, 81) = 1.84$, *ns*, respectively.

3.3. Picture ratings and startle magnitude

Finally, we examined the association between startle magnitude and average self-report valence and arousal ratings across all pictures. To this end, self-report ratings were added to the linear mixed-effects models, and separate analyses were conducted for the arousal and valence ratings. Results indicated a main effect of arousal ratings, $t(78.95) = 2.11$, $b = 3.39$, $p < 0.05$, such that greater arousal ratings were associated with greater startle magnitude. There were no other main effects of interactions involving arousal or valence ratings.

4. Discussion

The present study examined the startle reflex in anticipation of low arousal neutral, moderate arousal pleasant, and high arousal unpleasant pictures that were presented with either predictable or unpredictable timing. Across the entire task startle magnitude was potentiated in anticipation of temporally unpredictable compared to predictable pictures. Startle magnitude was also potentiated in anticipation of moderate arousal pleasant and high arousal unpleasant pictures compared to low arousal neutral pictures, and startle potentiation was greater for high arousal unpleasant compared to low arousal pleasant pictures. Notably, the impact of temporal predictability on startle magnitude did not vary as a function of the emotional category of the pictures. When examining the intercept at the beginning of the task, however, there was evidence that unpredictability enhanced startle potentiation in anticipation of unpleasant but not neutral or pleasant pictures. Moreover, examination of the intercept at the end of the task indicated that the effects of emotional category and temporal predictability on startle magnitude were largely diminished. Together, these findings provide novel evidence suggesting an interactive effect of emotional category and temporal predictability on defensive motivation at the beginning of the task and highlight the importance of examining the time course of the startle reflex.

The present study replicated previous investigations which have reported startle potentiation in anticipation of both pleasant and

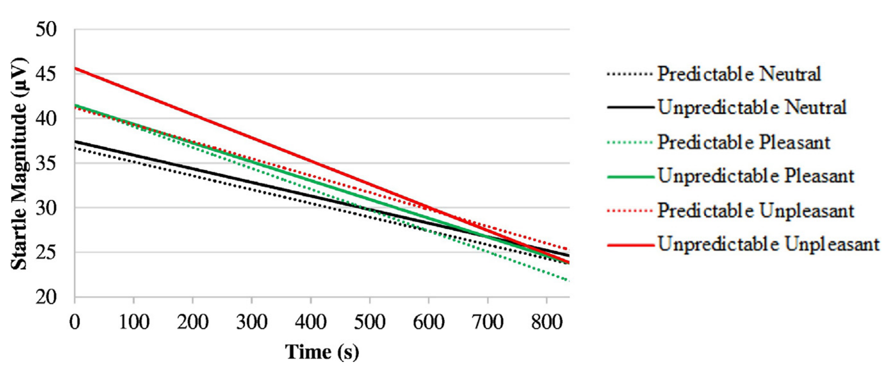

Fig. 1. Startle magnitude (y-axis) across different levels of emotional category (low arousal neutral, moderate arousal pleasant, high arousal unpleasant), predictability (predictable vs. unpredictable timing), and time (x-axis). Startle magnitude was collapsed across different levels of cue (countdown vs. interstimulus interval).

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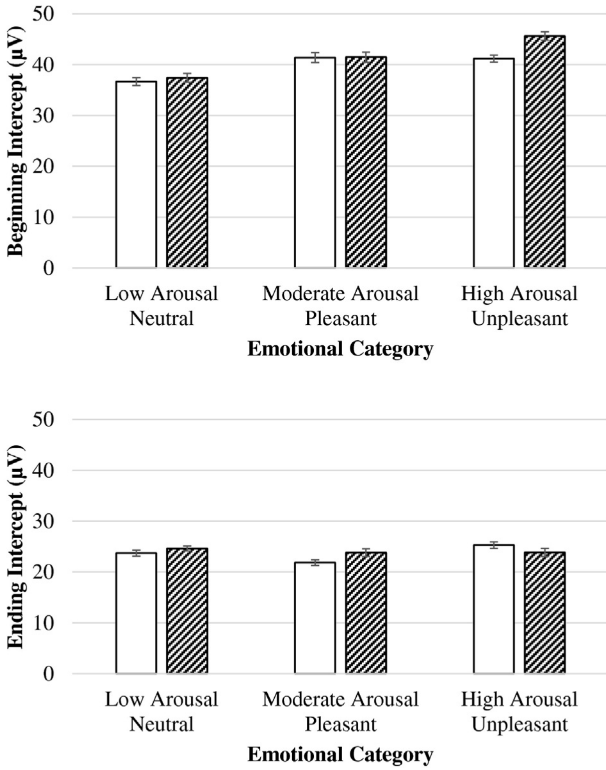

Fig. 2. Startle magnitude intercept at the beginning (top) and end (bottom) of the task across different levels of emotional category (low arousal neutral, moderate arousal pleasant, high arousal unpleasant) and predictability (predictable vs. unpredictable timing). Startle magnitude was collapsed across different levels of cue (countdown vs. interstimulus interval).

unpleasant pictures relative to neutral pictures (Dichter et al., 2002; Sabatinelli et al., 2001; Sege et al., 2014). Startle potentiation in anticipation of unpleasant pictures was greater than in anticipation of pleasant pictures. This finding diverges from two previous studies that found no differences between startle potentiation in anticipation of pleasant and unpleasant pictures (Sabatinelli et al., 2001; Sege et al., 2014). It is important to note that unpleasant pictures were more arousing relative to pleasant pictures, and individual differences in arousal ratings were positively correlated with the startle reflex across all picture categories. These results suggest that the startle reflex, measured in anticipation of picture presentation, is sensitive to the expected arousal level of the impending stimulus. This interpretation is consisent with current theories on emotion and motivation suggesting that anticipation of emotionally salient stimuli impacts motivational states regardless of valence (Dichter et al., 2002; Sabatinelli et al., 2001).

However, there are two important caveats to consider. First, the startle reflex was measured during the anticipation phase, and participants were unable to predict the *specific* content or arousal level of the upcoming picture. Startle potentiation in anticipation of high-arousal stimuli may have been due to the pictures being perceived as more uncertain or unpredictable in content. Second, valence and arousal were confounding features of the pictures, such that unpleasant pictures were both more unpleasant and arousing relative to the neutral and pleasant pictures. Therefore, it is difficult to definitively conclude whether valence, arousal, or their combination contributed to increased startle potentiation during the high arousal unpleasant pictures. Future studies should examine the impact of temporal predictability on defensive motivation in anticipation of pleasant and unpleasant pictures that are matched on arousal level.

A small number of studies have examined the impact of uncertainty and unpredictability on the anticipation of viewing pictures (Shankman et al., 2014; Somerville et al., 2013), but these were functional magnetic resonance imaging investigations that focused on the anticipation of neutral or unpleasant pictures. The present study is the first to employ the NPU task using neutral, pleasant, and unpleasant pictures. Across all trials, temporal unpredictability enhanced defensive motivation. However, examination of the startle magnitude intercepts revealed a different pattern of results. Specifically, at the beginning of the task unpredictability only potentiated startle magnitude in anticipation of high arousal unpleasant pictures. Moreover, at the end of the task unpredictability only potentiated startle magnitude in anticipation of moderate arousal pleasant pictures, but this was primarily due to greater startle habituation during the predictable pleasant picture trials. These results suggest that temporal unpredictability potentiated startle magnitude to the greatest degree in anticipation of high arousal unpleasant pictures, and this effect was most pronounced at the beginning of the task.

The present study had several limitations that warrant consideration. First, the investigation was conducted in an undergraduate sample and this might limit the generalizability of the findings to other populations (e.g., individuals with clinical anxiety). Second, the timing of picture presentation is just one characteristic that can be unpredictable, and future studies should examine whether anticipated emotional category and other uncertain/unpredictable features (e.g., ambiguity of content) have additive or interactive effects on defensive motivation. Finally, self-reported valence and arousal picture ratings were collected retrospectively and may have been susceptible to demand characteristics.

In conclusion, the present study replicates previous reports demonstrating that temporal predictability and anticipated emotional category are important characteristics that impact the startle reflex, and provides novel evidence suggesting an interactive effect on initial defensive motivation. These findings suggest that emotional category is an important characteristic to take into consideration when utilizing pictures in the NPU task. Future studies are needed to further examine the relationship between predictability, different features of environmental stimuli (e.g., intensity), their impact on psychophysiological measures of emotion and motivation, and resulting individual differences such as anxiety.

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