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BRIEF REPORT

Startle potentiation in aversive anticipation: Evidence for state but not trait effects

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Abstract

The present study was undertaken to determine whether aversiveness contributes to startle potentiation in anticipation of affective pictures above and beyond the effects of emotional arousal. Further, participants high in trait anxious apprehension, which is characterized by worry about the future, were expected to show especially pronounced anticipatory startle responses. Startle blink reflex was measured during warning stimuli that predicted the valence of ensuing aversive/unpleasant, pleasant, or neutral pictures. Startle magnitude was larger in anticipation of aversive than of pleasant pictures and smallest in anticipation of neutral pictures. Enhanced startle potentiation was not found in anxious apprehension subjects. These data suggest that the aversive nature of stimuli contribute to the potentiation of startle above and beyond the effects of emotional arousal, which may be a universal phenomenon not modulated by individual differences.

Descriptors: Anticipation, Startle reflex, Anticipatory anxiety, Anxious apprehension, Emotion

Anticipation of impending aversive events often serves an important adaptive function of alerting the organism so that appropriate emotional, cognitive, and physical preparations can be made. The psychological and physiological parameters that mechanistically govern this process remain largely unknown and are an area of active investigation. One physiological measure that has shown promise in elucidating some of the mechanisms involved is the startle reflex. Indeed, potentiation of eye-blink startle has been documented in a number of paradigms employing anticipatory periods. Extant evidence suggests a number of factors modulating this phenomenon (e.g., Lipp, Siddle, & Dall, 2000).

Several studies suggest that affect is an important factor in the augmentation of startle during anticipation. Grillon and colleagues have convincingly demonstrated potentiation of startle in humans during anticipation of electric shock (Grillon, Ameli, Foot, & Davis, 1993; Grillon, Ameli, Merikangas, Woods, & Davis, 1993; Grillon, Ameli, Woods, Merikangas, & Davis, 1991; Grillon & Davis, 1995). Subjects were informed that shock might be delivered to their wrists during threat periods but would not be during safe periods. Acoustic startle probes elicited larger blink magnitudes during threat than safe periods that were up to 2 min in duration. Startle potentiation has also been observed in anticipation of noxious noise blasts (Patrick & Berthot, 1995; Skolnick & Davidson, 2002). Of further relevance, increased startle in rats was also observed for conditioned stimuli that precede the onset of shock by intervals ranging from 4 to 51.2 s (Davis, Schlesinger, & Sorenson, 1989; Siegel, 1967).

To examine whether anticipation of positive stimuli is also characterized by augmented startle, Sabatinelli, Bradley, and Lang (2001) measured eye-blink startle to a 6-s warning light predicting erotic pictures. A sample of 32 male university students with elevated snake fear exhibited startle potentiation of equivalent magnitude in anticipation of erotic and of snake pictures when compared to neutral pictures of household items. Their findings suggest that startle potentiation accompanying anticipatory processes is modulated by the emotional arousal of anticipating pictures varying in emotional content. Other recent data provide further evidence that anticipation of both negative and positive events are accompanied by potentiation of startle (Patrick, 1999; Skolnick & Davidson, 2002). Although extant data indicate that emotional arousal contributes to the potentiation of startle during anticipation, research is needed that systematically examines whether hedonic valence modulates startle during anticipatory processes above and beyond the impact of emotional arousal.

The robust startle potentiation in anticipation of shock documented in previous research (e.g., Grillon et al., 1991) could be completely attributable to emotional arousal; however, an alternative hypothesis is that the aversive nature of such anticipation

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further contributes to startle potentiation. In a paradigm using simple warning symbols to predict the unpleasant/aversive, pleasant, or neutral content of ensuing pictures, the present study tested this hypothesis by employing aversive and pleasant pictures that were equated for emotional arousal based on published arousal rating norms for the pictures (Lang, Bradley, & Cuthbert, 1999).

Based on findings of individual differences in anxiety influencing various psychophysiological indices during anticipation, including startle magnitude (Grillon, Ameli, Foot, et al., 1993), electroencephalographic activity (Davidson, Marshall, Tomarken, & Henriques, 2000), and heart rate (Thayer, Friedman, Borkovec, Johnson, & Molina, 2000), subjects high in trait anxious apprehension, a form of anxiety characterized by worry about the future and theoretically linked to abnormalities in anticipatory processing (Nitschke, Heller, & Miller, 2000), were expected to show larger anticipatory startle responses to aversive pictures than asymptomatic controls. To test the specificity of this predicted effect, comparisons were made to four additional groups characterized by distinct affective symptomatology (anxious arousal, anhedonic depression, mixed anxiety/depression, defensiveness) who were predicted not to show these differences.

Method

Participants

Participants were 81 men selected from more than 2,000 University of Wisconsin–Madison Introductory Psychology students based on their responses to the Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990) and the Mood and Anxiety Symptom Questionnaire (MASQ; Watson et al., 1995). Those scoring above the 80th percentile on a target scale (PSWQ, MASQ Anxious Arousal, MASQ Anhedonic Depression) and below the 50th on the other two scales were assigned to the anxious apprehension ($n = 12$), anxious arousal ($n = 14$), and anhedonic depression ($n = 18$) groups. Students scoring above the 80th on all three scales were assigned to mixed anxiety/depression. Those scoring below the 50th were classified as controls ($n = 12$) if they scored less than 17 on the Marlowe–Crowne Social Desirability scale (MCSD; Crowne & Marlowe, 1964) administered at the startle session and as defensive ($n = 12$) if not. An additional 17 men were dropped due to insufficient startle responses. Females were also tested (129 with scorable startle); however, because the aversive and pleasant pictures were not equated for emotional arousal using female norms (Lang et al., 1999), their data are not highlighted here.

Materials

Three categories of picture stimuli designed to elicit positive, negative, or neutral emotions were chosen from Shows 1 through 12 of the International Affective Picture System (Center for the Study of Emotion and Attention, 1999).[1] Pictures were selected such that aversive and pleasant pictures were of opposite valence, $t(82) = -68.24$, $p < .001$, but both highly arousing, $t(82) = 0.91$, $p > .36$ (see Table 1). Neutral pictures had arousal ratings that were much lower than either aversive or pleasant pictures and valence ratings that were average. Of the 126 pictures used, there were 42 of each picture category.

Procedure

Upon arrival at the laboratory, participants were seated in a comfortable chair and informed consent was obtained. Participants were positioned approximately $\frac{1}{2}$ m from a 17-in. NEC-6FG multisync monitor upon which pictures were displayed. Prior to the picture presentation, electrodes for recording startle responses were placed and impedances checked. In order to familiarize the participants with the procedure and habituate them to the acoustic startle probe, participants then viewed an introductory set of nine pictures, during eight of which startle probes were presented.

Pictures were presented in six blocks of 21 pictures, with 7 pictures of each valence included in each block. The presentation of the pictures and acoustic startle probes were controlled by in-house software on a 100-MHz Pentium PC. Pictures were presented in a quasi-random order, with the constraint that not more than two stimuli of a given valence were presented consecutively. Pictures were presented for 6 s each. Immediately prior to each picture, a large warning stimulus that indicated the valence of the coming picture was presented for 4 s. A plus sign predicted positive pictures, a minus sign predicted negative pictures, and a circle predicted neutral pictures. Following the pictures, a blank screen was presented for 7–13 s (mean interstimulus interval was 10 s).

The acoustic startle probe was a 40-ms burst of white noise at 95 dB with a nearly instantaneous rise time. Startle probes were generated with a Coulbourn S81-02 noise generator and a Coulbourn S82-24 audio-mixer power amplifier, and were delivered binaurally through Audio-Technica ATH-M3X headphones. There were nine trials for each valence with probes presented during the warning stimulus (1 s prior to picture onset) and nine with probes during the intertrial interval (6 s after picture offset). Data for three additional probes presented during or after picture presentation are

Table 1. *Valence and Arousal Ratings for Affective Pictures*

	Picture Category		
	Aversive/Unpleasant	Neutral	Pleasant
	Valence		
Men			
M	2.22^{a}	4.98^{b}	7.41^{c}
SD	0.38	0.37	0.32
Women			
M	1.64^{a}	5.05^{b}	7.56^{c}
SD	0.34	0.43	0.43
	Arousal		
Men			
M	6.23^{a}	2.89^{b}	6.11^{a}
SD	0.55	0.54	0.59
Women			
M	6.89^{a}	3.01^{b}	6.04^{c}
SD	0.56	0.43	0.56

Note: These ratings are from Lang, Bradley, and Cuthbert (1999). Within a row, means with different superscripts are significantly different from one another ($p < .05$).

[1]IAPS numbers used in this study were: Unpleasant: 3000, 3010, 3015, 3030, 3051, 3053, 3060, 3071, 3080, 3100, 3102, 3120, 3130, 3140, 3150, 3168, 3170, 3266, 3350, 3400, 3500, 3530, 6212, 6230, 6260, 3212, 6313, 6350, 6360, 6510, 6560, 6570, 9040, 9252, 9410, 9500, 9560, 9570, 9800, 9810, 9910, 9921; Neutral: 1670, 2620, 5510, 5520, 5531, 5532, 5533, 5534, 5731, 6150, 7000, 7002, 7006, 7009, 7010, 7025, 7030, 7034, 7035, 7040, 7050, 7060, 7080, 7090, 7100, 7130, 7140, 7150, 7170, 7190, 7207, 7217, 7224, 7233, 7234, 7235, 7490, 7500, 7700, 7710, 7920, 9210; Pleasant: 1710, 2216, 2391, 4599, 4660, 4670, 4680, 5260, 5270, 5450, 5460, 5470, 5480, 5621, 5623, 5629, 5700, 5910, 7230, 7270, 7502, 8030, 8034, 8080, 8170, 8180, 8185, 8190, 8200, 8210, 8300, 8340, 8370, 8380, 8400, 8420, 8470, 8500, 8501, 8502, 8510, 8531.

not presented here. Thirteen trials did not contain any startle probes, and 22 trials contained two probes, one early and one late. Probe times were quasi-randomly assigned for each trial with the constraint that no more than two of each probe time occurred consecutively. Following the startle paradigm, subjects completed the PSWQ and MASQ again and the MCSD.

Startle Recording and Quantification

Raw and integrated electromyography (EMG) was collected using two Sensormedics mini-electrodes placed on the inferior left orbicularis muscle. Electrodes were placed below the left eye approximately 36 mm apart (van Boxtel, Boelhouwer, & Bos, 1998). The impedance for the electrode pair was less than 20 KΩ. EMG signals were amplified 10,000 times and filtered with a bandpass of 1–800 Hz using SAI Bioelectric amplifiers (SA Instrumentation Co., Caroga Lake, NY). Raw EMG signals were then high-pass filtered at 30 Hz before being integrated and rectified using a Coulbourn S76-01 contour-following integrator with the time constant set at 20 ms. A 100-MHz Pentium PC running SnapStream software (HEM Data Corporation, Springfield, MI) and a 12-bit analog-to-digital board (Analogic Corporation, Wakefield, MA) were used to digitize and store all signals at 250 Hz throughout picture presentation. Recording equipment was calibrated before and after each session. The units for raw and integrated EMG were microvolts.

Peak magnitude was scored in a window between 20 and 120 ms following probe onset by subtracting EMG activity at reflex onset from peak amplitude. Approximately 14.8% of eyeblink reflexes were excluded due to an unstable baseline (50 ms preceding probe onset), or because reflex onset was prior to 20 ms following probe onset. Trials with no perceptible eyeblink reflex were assigned a magnitude of zero and included in analysis. Peak magnitude was *z*-transformed within subject, and outliers greater than three standard deviations were excluded.

Results

A repeated-measures MANOVA was conducted, with Valence (aversive/unpleasant, neutral, pleasant) as a within-subjects factor and Group (anxious apprehension, anxious arousal, anhedonic depression, mixed anxiety/depression, defensive, control) as a between-subjects variable. A main effect for Valence, $F(2,74) = 10.27$, $p < .001$, $\eta^2 = .217$, was characterized by both a linear trend, $F(1,75) = 7.72$, $p < .008$, $\eta^2 = .093$, consistent with a valence effect, and a quadratic trend, $F(1,75) = 17.02$, $p < .001$, $\eta^2 = .185$, consistent with an arousal effect (see Figure 1). Pairwise comparisons using least significant difference revealed larger anticipatory startle for aversive than pleasant, $p < .008$, and aversive than neutral, $p < .001$, with a trend for a greater response to pleasant than neutral, $p < .07$. Effects for Group or for Valence × Group were not significant, *ps* $> .20$. Identical effects emerged for the analogous MANOVA conducted on a subsample of 45 men who maintained group membership at the time of the laboratory session when PSWQ and MASQ scales used for subject selection were administered again.[2] Finally, analogous Valence × Group MANOVAs for the ITI probes revealed no differences for either sample, all *ps* $> .40$.

[2]Although not of utility in testing the hypothesis of a unique contribution of aversiveness to startle potentiation in anticipation of emotional pictures for women due to the difficulty of equating the arousal ratings for aversive and pleasant pictures, MANOVAs for the full sample of 129 women revealed the same effects. As for men, a main effect for Valence, $F(2,122) = 24.34$, $p < .001$, $\eta^2 = .285$, was characterized by both a linear, $F(1,123) = 24.25$, $p < .001$, $\eta^2 = .165$, and a quadratic, $F(1,123) = 29.98$, $p < .001$, $\eta^2 = .196$, trend, and all pairwise comparisons were significant (aversive vs. pleasant, $p < .001$; aversive vs. neutral, $p < .001$; pleasant vs. neutral, $p < .02$). Again, there were no effects for Group or for Valence × Group (*ps* $> .45$), and identical effects were found for the subsample of 59 women maintaining group membership at the laboratory session.

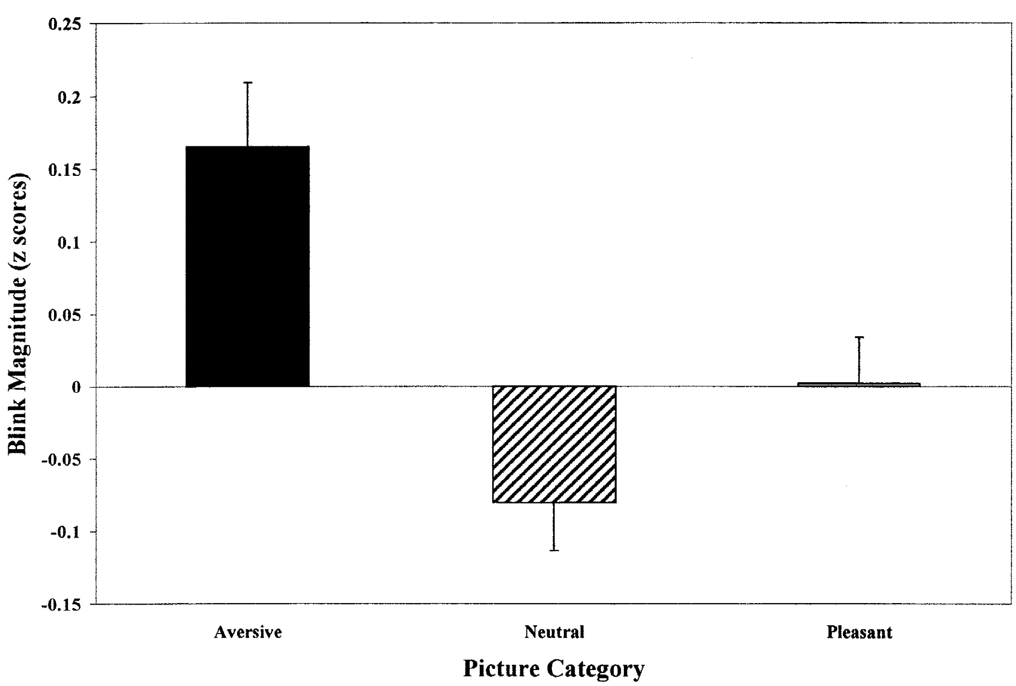

Figure 1. Mean blink magnitude (±*SE*) during warning symbols predicting aversive, neutral, and pleasant pictures.

Discussion

Eye-blink startle was measured in anticipation of aversive, pleasant, and neutral pictures in an attempt to determine the impact of emotional valence and arousal on anticipatory startle responses. The larger startle found for both aversive and pleasant pictures than for neutral ones replicates the recent findings reported by Sabatinelli et al. (2001) suggesting that arousal potentiates startle during anticipation of emotional pictures. The larger startle for aversive than pleasant pictures demonstrates an incremental contribution of aversiveness (negative valence) in addition to that explained by arousal. Potentiation to the negative warnings was not differentially modulated by anxious apprehension, contrary to predictions, or by the presence of other individual differences tested—anxious arousal, anhedonic depression, defensiveness—but instead was robust across subjects regardless of group membership.

Replicating the arousal effect reported in the one previously published report examining startle in anticipation of emotional pictures by Sabatinelli et al. (2001) is impressive given the number of methodological differences with the current study (e.g., warning symbol used, length of anticipatory period, timing of anticipatory startle probes). Although both reports studied male students only, the sample recruited by Sabatinelli et al. was restricted to individuals scoring above the 85th percentile on a measure of snake fear, whereas our sample was comprised of students scoring either high or low on several affective self-report instruments. The IAPS pictures used also diverged substantially, with the content of the 45 pictures used by Sabatinelli et al. restricted to snakes, erotica, and household objects, whereas the 126 pictures used in the current study sampled from a large range of content within the aversive, pleasant, and neutral picture categories. The present study employed published norms to compare valence and arousal ratings of the aversive, pleasant, and neutral pictures presented, whereas Sabatinelli et al. did not report on valence and arousal ratings. If the erotic pictures were more arousing than the snake pictures for those subjects, that might cancel out the effects of aversiveness resulting in larger startle magnitude for the snake than erotic pictures under conditions where arousal is matched.

Consistent with findings of potentiated startle during anticipation of a noxious noise (e.g., Patrick & Berthot, 1995) and of shock in humans (e.g., Grillon et al., 1991) and in rodents (Davis et al., 1989), the present results may have implications for anticipatory anxiety, a construct invoked in related research (Grillon, Ameli, Woods, et al., 1993; Grillon & Davis, 1995; Grillon et al., 1991). Anticipatory anxiety may be elicited by the warning symbols predicting aversive pictures. Such a scenario would be consistent with the use of shock anticipation to infer anticipatory anxiety.

Trait measures of anxiety did not modulate potentiation to the negative warnings. Thus, whereas the paradigm devised here effectively elicited state increases in aversive anticipation, it did not prove useful for distinguishing individual differences in trait anxious apprehension, a type of anxiety characterized by worry. As predicted, none of the other individual difference variables investigated—anxious arousal, anhedonic depression, or defensiveness—were associated with systematic variations in startle magnitude in responses to the warning stimuli.

Future research sampling at multiple times during the anticipatory phase would allow a more precise estimate of the time course of anticipatory effects, and it is possible that anxious apprehensive individuals may exhibit earlier anticipatory effects. The paradigm featured here can be easily adapted to different experimental contexts and holds promise for investigating the neural circuitry and other physiological processes involved in the anticipation of and response to emotionally aversive and arousing stimuli (e.g., Nitschke et al., 2001).

In sum, robust state shifts in startle potentiation were found for aversive anticipation, with evidence of emotional valence and arousal contributing to blink magnitude. Moreover, trait differences in anxious apprehension, anxious arousal, depression, or defensiveness did not modulate this anticipatory effect. More generally, these startle findings suggest a critical role for aversiveness and arousal in anticipation of emotional events.

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