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Emotion Regulation and Potentiated Startle Across Affective Picture and Threat-of-Shock Paradigms

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Abstract

Past studies beginning with Jackson and colleagues (2000) document increases and decreases in emotionally-potentiated startle by way of instructing participants to enhance or suppress their emotional responses to *symbolic* sources of threat (unpleasant pictures). The present study extends this line of work to a threat-of-shock paradigm to assess whether startle potentiation elicited by threat of *actual* danger or pain is subject to emotion regulation. Results point to successful volitional modulation for both *Affective-Picture* and *Threat-of-Shock* experiments with startle magnitudes from largest to smallest occurring in the enhance, maintain, and suppress conditions. Successful regulation of startle potentiation to the threat of shock found by the current study supports the external validity of the Jackson paradigm for assessment of regulation processes akin to those occurring in the day-to-day context in response to *real* elicitors of emotion.

Keywords

emotion regulation; startle; psychophysiology; anxiety; fear

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Emotionally-enhanced startle, referring to the potentiation of the startle reflex when the aversive motivational system is activated by an unpleasant or anxiogenic foreground (Bradley, Cuthbert, & Lang, 1990; Grillon, Ameli, Woods, Merikangas, & Davis, 1991; Vrana, Spence, & Lang, 1988), has become a widely used objective index of negative emotion. More recently, volitional self-regulation of emotionally-enhanced startle has been demonstrated (Dillon & Labar, 2005; Jackson, Malmstadt, Larson, & Davidson, 2000; Piper & Curtin, 2006). In these studies, participants displayed a complete reduction in startle potentiation elicited by unpleasant pictures from the International Affective Picture System

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(Lang, Öhman, & Vaitl, 1988) following the simple instruction to 'suppress' emotional responses. Because all applications of Jackson's regulation paradigm to date employ emotional pictures representing *hypothetical* affective scenarios, a question remains regarding the efficacy of volitional suppression for neutralizing fear-potentiated startle elicited by the threat of *actual* physical danger or pain. Given that the value of experimental paradigms testing emotion regulation lies in their ability to generate inferences regarding the workings of emotion regulation to *real* sources of emotion arising in the day-to-day context, it is necessary to establish whether effects from Jackson's paradigm extend beyond regulation of symbolic sources of aversiveness to actual threat of danger.

One possibility is that negative emotion to actual physical threat will be less subject to willful suppression. Though emotion regulation is generally adaptive and considered an essential component of mental health (Gross & Munoz, 1995), down-regulation of negative emotion to potent and imminent danger may serve to prolong exposure and vulnerability to survival threats and may not have been naturally selected over the course of evolution. In turn, negative emotion elicited by actual physical threat or pain (i.e., electric shock) may be less subject to volitional suppression than that elicited by more hypothetical or symbolic sources of threat (i.e., unpleasant pictures).

The notion that negative emotion generated by threat of shock may be more difficult to terminate via willful suppression derives support from data revealing more robust startle potentiation elicited during threat of shock relative to unpleasant pictures (Lissek, Orme, Mcdowell, Johnson, & Grillon, 2004), as well as findings that expression of fear-potentiated startle elicited by instructed (Baas et al., 2002) or conditioned threat of shock (Scaife, Langley, Bradshaw, Szabadi, 2005) is difficult to reduce with anti-anxiety benzodiazepines (but see Bitsios, Philpott, Langley, Bradshaw, & Szabadi, 1999; Graham et al., 2005; Riba et al., 2001). Additionally, because levels of startle modulation elicited by emotional pictures and threat of shock are uncorrelated (Greenwald, Bradley, Cuthbert, & Lang, 1998) and because responses to negative pictures and threat of shock have been linked to dissociable neural substrata (Funayama, Grillon, Davis, & Phelps, 2001), it is plausible that regulation of emotional responses to unpleasant pictures versus threat of shock involves separable processes and the ability to suppress emotion from the former may not necessarily indicate the ability to suppress emotion from the latter.

The aim of the current study was to replicate the findings of Jackson et al. (2000) using IAPS pictures and to extend this line of work to a *threat-of-shock* paradigm (Grillon et al., 1991) with the particular focus on whether startle potentiation elicited by genuine physical threat can be abolished through willful suppression. In addition to startle EMG data, both subjective reports of experienced difficulty to suppress/enhance and qualitative reports of regulation strategies were collected to further characterize potential differences in regulation across paradigms.

Though suppression effects in the Threat paradigm were a primary focus, comparing levels of enhancement across paradigms was of additional interest. Given findings of greater state anxiety during the Threat versus Picture paradigm (Lissek et al., 2004) and the documented positive relationship between state anxiety and escalation or catastrophizing of negative emotion (e.g., Granot & Goldstein-Ferber, 2005), it logically follows that negative emotion to threat of shock might be more subject to willful enhancement. Additionally, the ability to enhance startle potentiation to threat of shock was of interest given concerns that threat of shock may elicit 'ceiling' magnitudes of startle (Bradley, Moulder, & Lang, 2005; Grillon et al., 2006; Grillon & Baas, 2003). For example, the lack of differentiation between anxiety patients and healthy controls found in several studies assessing startle potentiation to discrete, threat-of-shock cues (Grillon & Morgan, 1999; Grillon, Morgan, Davis, &

Southwick, 1998; Pole, Neylan, Best, Orr, & Marmar, 2003) could be attributable to ceiling effects producing maximal outputs in the startle system among all participants, leaving little room for elevations in startle magnitudes among patients versus controls. Testing whether startle magnitudes elicited during threat of shock can be increased through voluntary attempts to enhance negative emotion, will provide evidence for or against the possibility of such a ceiling effect.

A final aim of the current effort was to further assess differences in the magnitude of unregulated emotionally-potentiated startle across instructed threat-of-shock and unpleasant pictures. Though stronger potentiation to instructed threat-of-shock versus unpleasant pictures might be expected given the greater aversive salience of the former (Lissek et al. 2004), the only study to date assessing startle modulation within both an instructed threat-of-shock and picture paradigm found startle potentiation of equal magnitudes to threat-of-shock and unpleasant pictures (Bradley et al., 2005). In that study, shock electrodes remained attached during assessment of startle potentiation to unpleasant pictures. Given that startle is potentiated by the simple presence versus absence of shock electrodes (Grillon & Ameli, 1998), startle increases to negative pictures may have been influenced by the presence of shock electrodes. As such, in the present study shock electrodes were attached during the Threat but not Picture experiment so as to assess magnitudes of potentiation to unpleasant pictures independent of potentiation associated with the presence of shock electrodes.

In sum, the current study was undertaken to test predictions that 1) startle potentiation elicited by threat of shock versus unpleasant pictures would be less subject to volitional suppression but more subject to enhancement, 2) subjective reports of task difficulty would reveal greater ease of suppressing in the Picture paradigm and greater ease of enhancing in the Threat paradigm, 3) distinct regulation strategies would be used for modulating emotion in the Picture and Threat runs, and 4) potentiation of the startle reflex during threat of shock would be stronger than that elicited by unpleasant pictures.

Methods

Participants

Fifty healthy participants (19 males, 31 females) with a mean age of 27.59 (*SD* = 8.94), and average state and trait anxiety scores of 32.10 (*SD*=8.91) and 34.16 (*SD*=8.92), respectively (State and Trait Anxiety Inventory: Spielberger et al., 1983) were recruited from the community via newspaper advertisement and reimbursed for their time. Prior to participation, participants gave written informed consent that had been approved by the NIMH Human Institutional Review Board. Inclusion criteria included: (1) no past or current Axis-I psychiatric disorder as per Structured Clinical Interview for DSM-IV, (SCID-I/NP: First, Gibbon, Spitzer, & Williams, 2001) administered by a staff psychologist, (2) no medical condition (i.e., cardiovascular, endocrine, or neurological diseases, current or past history of malignancies) or treatment for such conditions that interfered with the objectives of the study as determined by a staff physician during a physical exam, (3) no current use of psychoactive medications or other drugs altering central nervous system function, and (4) no current use of illicit drugs as per self-report and confirmed with a urine test.

Physiological apparatus

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments). Startle-blink EMG was recorded with two 6-mm tin cup electrodes placed under the right eye and amplifier band width was set to 30-500 Hz. Startle was elicited by a 40-ms duration, 102 dB(A) burst of white-noise with a near instantaneous rise time presented binaurally through headphones.

Stimuli

International Affective Picture System images (IAPS: Lang, Öhman, & Vaitl, 1988) used in the picture viewing paradigm were 28 unpleasant (valence = 2.61, arousal = 6.43, dominance = 3.46) and 14 neutral pictures (valence = 4.86, arousal = 2.89, dominance = 5.92) from the picture set employed by Jackson and colleagues (2000)[1]. Unpleasant pictures consisted of threat (e.g., pointed gun, lunging snake), mutilation (e.g., severed limbs, bloody bodies) and disgusting contents (roaches on a pizza) with proportions of each content reflecting those found in the picture set of Jackson et al. (67% threat, 25% mutilation, 8% disgust). As in Jackson's study, fewer neutral pictures were needed because only one of three regulation conditions (maintain) applied to neutral pictures, whereas three regulation conditions applied to negative pictures (suppress, maintain, enhance). The threat-of-shock component included one picture of the word “SAFE” (safety cue) and another displaying the word “SHOCK?” (threat cue). Three of 24 “SHOCK?” trials coterminated with the delivery of an electric shock (100-ms, 3-5μA) produced by a constant current stimulator and administered to the right wrist. For both picture viewing and threat-of-shock paradigms, three digitized male voices instructing participants to “suppress”, “maintain”, or “enhance” their emotional reactions followed presentation of unpleasant/threat pictures. The “maintain” instruction always followed neutral/safe stimuli. Such instructions were presented binaurally through the same headset delivering acoustic startle-probes. Pictures from both paradigm were 30 × 40 cm in size (length × width) and were presented at a viewing distance of approximately 46 cm.

Design

A within subjects design was employed whereby each participant underwent Picture and Threat (of shock) paradigms. The Picture and Threat runs were conducted in separate blocks with 24 negative/threat (negative) trials and 12 neutral/safe trials in each block. The design for the Picture and Threat blocks were identical, with the exception that shock electrodes were attached only during the Threat run. A schematic outline of the design used across paradigms is displayed in Figure 1. Trials included a negative or neutral/safe image presented for 12 sec, a one-word regulation instruction (suppress, maintain, or enhance) presented 4 sec post-image onset, and a startle probe delivered at either 3, 7, or 12 sec post-image onset. An additional 6 pictures were followed by instructions but not startle probes (4 negative [2 maintain, 1 suppress, and 1 enhance instruction], 2 neutral/safe [both maintain instructions]). All 12 probed neutral/safe pictures were followed by the maintain instruction with 3 startle probes delivered at either 7 or 12 sec post-image onset and an additional 6 given 3 sec after image onset. Of the 24 probed negative pictures, one-third were followed by each instruction type (8 suppress, 8 maintain, 8 enhance). Additionally, 2 of 8 probed negative pictures in each instruction type were probed at 3 sec post-image onset and 6 of 8 were probed, post-instruction, at either 7 (3 trials) or 12 sec (3 trials) post-image onset. Given that 2 of 8 negative pictures from each of three instruction sets were probed with 3 sec latencies, a total of 6 pre-instruction startle responses elicited during negative pictures and 6 pre-instruction startle responses elicited during neutral/safe pictures were available to assess the basic (unregulated) valence effect on startle. Probed trials were presented in a quasi-random order where no more than 5 pictures of the same valence and no more than 3 instructions or probes of the same class were presented consecutively. In addition to startle probes following image onset, 6 probes were delivered in the inter-trial-interval (ITI) between pictures in each block to assess baseline startle magnitudes across Picture and Threat runs. The experiment consisted of one Threat block and one Picture block. Order of blocks was counterbalanced such that the Threat block was first for half of the participants

[1]Please contact authors for a list of the specific IAPS pictures used in the current study.

(n = 23) and the Picture block was first for the other half (n = 24). Additionally order of blocks was entered as an independent variable in all relevant analyses to verify whether receiving one block before the other influenced levels of regulated or unregulated startle potentiation to threat of shock or unpleasant pictures.

Procedure

Participants underwent a screening session that consisted of the SCID-I/NP (First et al., 2001) a physical exam, and self-report questionnaires. Within two weeks of screening, participants returned for the testing session at which time EMG electrodes and headphones were placed, a habituation sequence consisting of nine startle probes (ITI = 18-25 sec) was run to reduce initial startle reactivity, and participants were given an explanation of the study including a modified version of the regulation instructions published by Jackson and colleagues (2000)[2]. Briefly, participants were told that pictures would appear on the computer monitor during which instructions to 'suppress', 'enhance', or 'maintain' their emotional response to the picture would be delivered via headphones. Participants were asked to stay focused on the picture during regulation attempts and that regulation should not be accomplished by generating an emotion other than the one elicited by the image. Finally, participants were informed that they would receive a bonus $50 in addition to their prearranged compensation if they both minimized and maximized their physiological arousal by 10% relative to their resting baseline. As was done by Jackson and colleagues (2000), this monetary incentive was included to motivate participants to apply themselves in their regulator efforts in ways similar to how they might apply themselves in day to day living where regulation efforts are motivated by real life consequences. Though participants believed the bonus was contingent on regulation performance before and during the experiment, at study completion all participants were given the $50 bonus regardless of regulation performance.

Prior to the Threat run, shock electrodes were attached and a shock workup procedure was completed to establish a level of shock that was "highly annoying but not painful". Shock electrodes were unattached during the Picture run. Following Picture and Threat blocks, participants rated the overall level of anxiety induced by negative and neutral/safe picture-sets using one 10-point scale reflecting their overall level of anxiety to negative pictures and a second 10-point item reflecting their overall level of anxiety to neutral/safe pictures. Additionally, participants qualitatively described their regulation strategies and rated on a 10-point scale "how difficult it was to suppress/enhance" their emotional response to the negative conditions in the Picture and Threat runs.

Assessing Regulation Strategies

Participants' were asked to write down qualitative descriptions of the strategies they used to enhance and suppress negative emotion following both Picture and Threat runs ("What strategy did you use to suppress/enhance your emotion to the negative-picture/threat of shock"). Prior to analysis, such qualitative descriptions were coded at the individual level with a coding scheme developed from the data. Coding categories consisted of regulation strategies endorsed by more than 2 participants and the majority of participants endorsed multiple categories. Table 1 displays suppress and enhance categories derived from the data for Picture and Threat paradigms.

[2]Complete regulation instructions for the threat-of-shock paradigm are available upon request to the corresponding author.

Data Analysis

Startle EMG was rectified and then smoothed (20-ms moving window average). The onset latency window for the blink reflex was 20-100-ms and the peak magnitude was determined within a window of time extending from the time of response onset to 120 ms. Additionally, the average baseline EMG level for the 50 ms immediately preceding delivery of the startle stimulus was subtracted from the peak magnitude. EMG magnitudes were standardized using within subject *T*-score conversions to normalize data and to reduce the influence of between subjects variability unrelated to psychological processes. Because similar results were obtained with the raw and *T*-scored data only the results of inferential analyses of the *T*-scored data are presented. Data were averaged across participants to form grand averages for each cell in the design matrix (i.e., paradigm/instruction/negative vs. neutral [safe]/probe latency). In order to test effects of volitional regulation, startle responses to negative pictures probed with 7 and 12 sec latencies were averaged and analyzed using a 3 (Instruction) × 2 (Paradigm: Threat and Picture) × 2 (Order: Picture vs. Threat first) multivariate analysis of variance (MANOVA) with repeated measures. Additionally, in order to test the basic (unregulated) valence effect on EMG magnitudes across paradigms, startle responses elicited 3 sec post-image onset were analyzed within a 2 (Valence: Negative and Neutral/ Safe) × 2 (Paradigm: Picture and Threat) × 2 (Order: Picture vs. Threat first) MANOVA with repeated measures. MANOVAs were computed using Wilk's Lambda and were followed, when necessary, by paired samples *t*-tests. Although only one dependent variable was included in each analysis, MANOVA was chosen because it affords protection against sphericity without performing the univariate correction (Tabachnick & Fidell, 1996). Alpha was set at .05 for all statistical tests.

Because two participants displayed no detectable EMG activity (μV for all blinks = 0), and standardized startle scores for a third participant exceeded three standard deviations above the sample mean for 4 of 7 data-points of interest in the Threat run, data from 47 of 50 participants underwent analysis.

Results

Emotion Regulation

Startle EMG—The Instruction × Paradigm × Order MANOVA revealed a main effect of Paradigm, $F(1,45) = 172.16$, $p < .0001$, indicating overall larger startle magnitudes during the Threat run, compared to the Picture run. Additionally, a main effect of Instruction, $F(2, 44) = 15.76$, $p < .0001$, $d = .57$[3], as well as a Paradigm × Instruction interaction, $F(2,44) = 4.91$ $p < .02$, $d = .32$, were found. The Paradigm × Instruction interaction was further assessed by testing the effect of Instruction in each paradigm separately. Startle magnitudes to the negative stimuli were increased linearly from suppress to maintain to enhance instructions for both picture, $F(1, 46) = 16.39$, $p < .0002$, $d = .58$, and threat experiments, $F(1, 46) = 23.35$, $p < .0001$, $d = .69$ (see Figure 2). Planned comparisons revealed increased magnitudes during the enhance relative to maintain instruction for Picture, $t(46)= 2.82$, $p = .007$, $d = .30$, and Threat runs, $t(46)= 3.35$, $p = .002$, $d = .48$, and attenuated startle to the suppress relative to maintain condition in the Picture, $t(46)= 2.27$, $p < .03$, $d = .33$, and Threat runs, $t(46)= 2.79$, $p < .009$, $d = .40$ (see Figure 2). Finally, whether or not participants completed the Threat or Picture run first did not interact with effects of instruction as the Instruction × Order and Instruction × Paradigm × Order interactions were nonsignificant (*p*'s > .18).

[3]Effect sizes for reported statistical results were estimated using the unbiased estimator *d*(Hedges & Olkin, 1985). This index was selected because it corrects for bias in estimation of the population effect size.

Because patterns of regulation were similar across paradigms, and because maintain minus suppress difference scores in the Threat versus Picture experiment were approximately equal, $t(46) = 1.31$, $p > .19$, $d = .20$, the Instruction × Paradigm interaction was likely due to larger enhance minus maintain difference scores found in the Threat versus Picture experiment , $t(46) = 2.37$, $p = .02$, $d = .34$. Tests of proportion change scores yielded results similar to tests of difference scores with greater proportion increase from maintain to enhance in the Threat paradigm, $t(46) = 2.28$, $p < .04$, $d = .33$, but no difference across paradigms in proportion decreases from maintain to suppress, $t(46) = 1.24$, $p > .23$, $d = .18$.

Controlling for differences in baseline startle magnitudes across paradigms: Given that baseline (ITI) startle magnitudes were larger in the Threat versus Picture blocks, paradigm differences in regulation may have been influenced by increases in baseline startle from Picture to Threat blocks. In turn, comparisons of instruction effects across paradigms were reanalyzed with increases in baseline from Picture to Threat (ITI_{Threat} minus $ITI_{Picture}$) covaried-out. Such reanalyses revealed a significant Paradigm × Instruction interaction, $F(2,44) = 6.99$ $p = .002$, $d = .38$, as well as significantly greater enhancing in the Threat paradigm whether operationalizing enhancing by enhance-maintain difference scores, $F(1, 45) = 7.74$, $p = .008$, $d = .40$, or enhance/maintain ratios, $F(1, 45) = 12.05$, $p < .002$, $d = .50$. Finally, no differences in suppression were found across paradigms whether indexing suppression as maintain-suppress difference scores, $F(1, 45) = 2.00$, $p > .16$, $d = .20$, or maintain/suppress ratios, $F(1, 45) = 1.24$, $p > .25$, $d = .16$. The comparable results from analyses with and without this statistical control demonstrate that regulation differences across paradigms were not driven by increased baseline startle in the Threat versus Picture paradigm.

Controlling for differences in unregulated startle potentiation across paradigms: As will be reported more fully below, the basic, unregulated valence effect on startle potentiation was larger in the Threat versus Picture paradigm ($p < .0001$). In order to control for the influence of this difference in unregulated potentiation on effects of instructions across paradigms we reanalyzed the Paradigm × Instruction interaction, as well as relevant simple effects, while covarying-out increases in unregulated potentiation from Picture to Threat paradigms (Threat minus Picture [unregulated] startle potentiation). Results reveal a trend for a Paradigm × Instruction interaction, $F(2, 44) = 2.66$, $p = .08$, $d = .23$, a trend for greater enhancement in the Threat versus Picture paradigm, $F(1, 45) = 2.86$, $p < .10$, $d = .24$, and no difference in suppression across paradigms, $F(1, 45) = .93$, $p > .34$, $d = .14$. That the Paradigm × Instruction interaction and the enhancement effect across paradigms fell below significance after controlling for paradigm differences in unregulated startle potentiation, suggests that different patterns of volitional regulation across paradigms were highly influenced by paradigm differences in the basic, unregulated effect of startle potentiation. Specifically, the greater enhancing found in the Threat paradigm does not seem to be a function of greater up-regulation of negative emotion to threat of shock as much as it reflects increases in unregulated fear-potentiated startle from Picture to Threat paradigms.

Assessing the completeness of suppression of startle potentiation in the Picture versus Threat paradigm: In the Picture paradigm, startle magnitudes elicited during the negative/suppress condition were not significantly potentiated relative to magnitudes elicited during the neutral/maintain condition, $t(46) = .56$, $p > .57$, $d = .08$, whereas the threat/suppress condition in the Threat experiment yielded startle magnitudes significantly larger than those evoked in the safe/maintain condition, $t(46) = 4.64$, $p < .0001$, $d = .67$ (see Figure 2). Such results suggest a persistence of startle potentiation during suppression attempts in the Threat but not Picture paradigm. Though this paradigm difference in persistence of startle potentiation during suppression is supported by a Paradigm × Suppress interaction when defining suppress by the negative(threat)/suppress minus neutral(safe)/maintain contrast,

$F(1,46) = 21.38$, $p < .0001$, this interaction fell below significance after covarying paradigm differences in the basic, unregulated effect of startle potentiation, $F(1,45) = 21.38$, $p = .13$, indicating that this paradigm difference in persistence of potentiation during suppression is the result of greater unregulated potentiation to threat of shock which likely heightened the difficulty of full suppression in the Threat paradigm.

Relation between regulation in Picture and Threat paradigms: Degrees of regulation across Picture and Threat paradigm were uncorrelated for both effects of suppress, and enhance, whether operationalizing regulation effects with difference scores (maintain-suppress: $r(47) = .05$, $p > .74$, $d = .10$; enhance-maintain: $r(47) = .09$, $p > .54$, $d = .18$) or proportion change scores (maintain/suppress: $r(47) = .06$, $p > .65$, $d = .12$; enhance/maintain: $r(47) = .13$, $p > .89$, $d = .26$), indicating that the ability to regulate in one paradigm was independent of the ability to regulate in the other.

Self-Report Data—Participants' ratings of regulation difficulty from 1-10, with 10 being the most difficult, revealed greater difficulty suppressing emotions in the Threat versus Picture experiment (Threat average = 5.65, $SD = 1.96$; Picture average = 4.87, $SD = 1.95$), $t(46) = 2.08$, $p < .044$, $d = .30$, and greater difficulty enhancing in the Picture versus Threat experiment, (Picture average = 5.13, $SD = 2.08$; Threat average = 3.83, $SD = 2.19$), $t(46) = 3.24$, $p < .003$, $d = .46$.

Regulation Strategies—Table 1 displays suppress and enhance categories for Picture and Threat paradigms, the number of participants endorsing a given category, and paired sample *t*- tests assessing differences in regulation strategies across paradigms. In order to control for type II error resulting from the multiple comparisons included in this table, a Bonferroni correction was applied to the criterion *p* value ($p = .05/13 = .004$). As can be seen, depersonalizing the negative stimulus was a more frequently used strategy to suppress emotional responses in the Picture versus Threat experiment, $t(47) = 4.42$, $p < .0001$, $d = .63$), and personalizing the negative stimulus was more frequently used for enhancing responses in the Picture versus Threat experiment, $t(47) = 7.55$, $p < .0001$, $d = 1.08$). Additionally lowering and raising the perceived probability of a negative outcome was used more frequently to suppress and enhance, respectively, in the Threat versus Picture paradigm (both *p*'s < .003), and focusing on the shock or most negative aspect of the image was a more frequently used enhance strategy in the Threat versus Picture paradigm ($p < .0001$). Finally, dichotomous codes ('1' if present and '0' if absent) for each of seven suppress and each of six enhance strategies were then correlated with continuous levels of suppression (maintain – suppress difference and maintain/suppress proportion) and enhancement (enhance – maintain difference and enhance/maintain proportion) of startle potentiation, respectively. Results revealed no relation between use of any of the strategies and levels of regulation (all *p*'s > .11).

The Basic (Unregulated) Valence Effect

Emotionally Potentiated Startle—Startle was potentiated by negative versus neutral/safe stimuli when the reflex was probed before the instruction to regulate (i.e. 3-sec latency) in both picture viewing, $t(46) = 2.82$, $p = .007$, $d = .40$, and threat-of-shock paradigms, $t(46) = 11.75$, $p < .0001$, $d = 1.69$. Additionally, a significant Valence × Paradigm interaction was found, $F(1, 45) = 94.52$, $p < .0001$, $d = 1.39$, indicating that threat of shock elicited stronger potentiation of the reflex at the 3 sec probe-latency time (see Figure 3). Furthermore, Valence × Order, Paradigm × Order, and Valence × Paradigm × Order interactions were all nonsignificant (*p*'s > .13) indicating that receiving electric shocks before the picture run did not significantly influence levels of startle potentiation to negative cues across paradigms. Finally, levels of startle potentiation elicited by threat of shock and unpleasant pictures were

uncorrelated, $r(47) = .02$, $p > .86$, $d = .04$, suggesting that startle potentiation to threat of shock was independent of potentiation elicited by unpleasant pictures.

Of particular relevance to the construct validity of emotionally-potentiated startle for the measurement of fear is the increase in startle magnitudes corresponding to increases in the anxiogenic quality of the experimental situation. Figure 3 displays such an increase (dotted white line) with startle magnitudes rising from A (no shock or shock electrodes, no negative pictures) to B (no shock or shock electrodes, negative pictures) to C (shock and shock electrodes, no threat cue) to D (shock and shock electrodes, threat cue signaling imminent shock delivery). This increase in startle magnitude from points A through D produced significant linear, $F(1,46) = 180.38$, $p < .0001$, $d = 1.93$, quadratic, $F(1,46) = 56.35$, $p < .0001$, $d = 1.08$, and cubic trends, $F(1,46) = 6.94$, $p = .01$, $d = .38$, with significant increases from A to B, $t(46) = 4.35$, $p < .0001$, B to C, $t(46) = 3.53$, $p=.001$, and C to D, $t(46) = 9.42$, $p < .0001$. Additionally, the increase from A to C, $t(46) = 5.50$, $p < .0001$, $d = .79$, in these trends reflects a significant increase from picture to threat ITI suggesting elevations in fear sensitization or contextual anxiety during threat-of-shock procedure due to the presence of the shock electrodes and administration of shocks (Greenwald et al., 1998;Grillon & Baas, 2003).

Subjective Measures of Anxiety Across Paradigms—Self reported anxiety in the Picture paradigm increased from neutral (mean =1.76; *SD* = 1.05) to negative (mean = 5.20; *SD* = 2.06) pictures, $t(46) = 13.09$, $p < .0001$, $d = 1.88$, and reported anxiety in the Threat paradigm increased from safe (mean =1.63; *SD* = 1.51) to threat (mean =6.33; *SD* = 2.03) cues, $t(46) = 15.43$, $p < .0001$, $d = 2.21$. Consistent with startle results, increases in reported anxiety from neutral/safe to negative/threat conditions were significantly larger in the Threat versus Picture paradigm, $F(1,45) = 10.76$, $p < .003$, $d = .47$.

Discussion

In the current study, one word regulation instructions (i.e., suppress, maintain, or enhance) produced linear changes in startle magnitude with enhance > maintain > suppress whether that emotion was elicited by unpleasant pictures or threat of shock. Such findings indicate that the ability to regulate emotionally-potentiated startle is not restricted to symbolic sources of emotion (unpleasant pictures) but remains robust during regulation of emotion generated by actual threat of danger (threat-of-shock). Importantly, successful regulation of startle potentiation to the threat of shock found by the current study supports the external validity of the Jackson paradigm for assessment of regulation processes akin to those occurring in the day-to-day context in response to *real* elicitors of emotion.

Emotion Regulation

That significant reduction of fear-potentiated startle to threat of shock via the suppress instruction was found, runs counter to the central hypothesis of the current study predicting that startle potentiation to threat of shock would be unresponsive to willful suppression. More consistent with this central hypothesis, emotionally potentiated startle to negative stimuli persisted during suppress attempts (relative to neutral/maintain) in the Threat but not Picture paradigm (see Figure 2) and participants reported greater difficulty suppressing the negative emotion elicited by the Threat versus Picture paradigm. Importantly, the persistence of startle potentiation during willful suppression of negative emotion to threat of shock versus unpleasant pictures was no longer significant after covarying out paradigm differences in magnitudes of the unregulated startle potentiation effect. Thus the differential absence of full suppression in the Threat versus Picture paradigm seems to have been driven

by the greater unregulated emotional-potentiation of startle to the threat of shock which heightened the difficulty of full suppression in the Threat paradigm.

Though similar linear effects of regulation on startle magnitudes were found for picture and threat runs (i.e., enhance > maintain > suppress), effects of enhancing on startle were stronger in the Threat experiment. At first look, this stronger enhance effect to threat of shock seems to support the prediction that stronger fear to actual relative to hypothetical threat would be more effectively increased (i.e., catastrophized: Granot & Goldstein-Ferber, 2005) through willful up-regulation of negative emotion. Nevertheless, secondary analyses of covariance indicated that this paradigm difference in enhancing was attributable to differences in magnitudes of unregulated startle potentiation across paradigms rather than to regulatory processes per se. However, it should be mentioned that less subjective difficulty enhancing negative emotion was reported for the Threat versus Picture paradigm, providing some level of support for the idea that negative emotion to real threat is more subject to up-regulation.

Though it is questionable whether current findings support the conclusion of greater willful enhancement during threat of shock, the robust enhancement of startle potentiation to threat of shock via instruction suggests that threat of shock (in the absence of willful enhancing) does not elicit ceiling levels of startle. Such results may lessen concerns that ceiling effects limit the use of fear-potentiated startle for assessing the additive effects of shock threat and other aversive stimuli (e.g., negative pictures) on startle potentiation (Bradley et al., 2005) as well as concerns that such ceiling effects might limit the use of fear-potentiated startle for testing the anxiolytic efficacy of pharmacological compounds (Grillon et al., 2006). Additionally, such results reduce the likelihood that ceiling effects on startle impede efforts to identify individual differences in startle potentiation to threat of shock. Nevertheless, the current findings only demonstrate that startle potentiation to threat of shock can be enhanced with startle probe latencies of 7- and 12-sec but do not shed light on the strength of this effect at earlier startle probe latencies (i.e., 3-sec post-threat-onset).

An additional regulation effect of interest was the finding that levels of regulation in the Picture and Threat paradigms were uncorrelated. Thus, whether a participant was able to suppress/enhance in the Picture paradigm had no relation to whether they were able to suppress/enhance in the Threat paradigm. This null relation suggests that participants could not rely on the same regulation processes to successfully modulate startle potentiation evoked by unpleasant pictures and threat of shock. Support for this idea comes from the finding that personalizing and depersonalizing the contents of negative pictures were commonly used enhancing and suppressing strategies in the Picture but not Threat experiment, whereas increasing and decreasing the perceived probability of negative outcomes were frequently used enhancing and suppressing strategies in the Threat but not Picture experiment. Typical depersonalizing strategies used to suppress emotion to unpleasant pictures involved reassuring oneself that the events portrayed in the pictures were not real and could not happen to them or their loved ones. Depersonalizing may not have been used in the Threat experiment because attempts to depersonalize such a personally salient event (i.e., imminent electric shock) likely seemed futile to participants. Similarly personalizing threat of shock may not have been used to enhance the response because the stressor was already maximally personalized.

Potential neural processes underlying unregulated and regulated emotion to the threat of shock and unpleasant pictures—Evidence for a dissociation between the neural underpinnings of emotional responses to unpleasant pictures and threat of shock comes from a study employing Picture and Threat paradigms to assess emotionally-potentiated startle in patients with unilateral medial temporal lobe damage (Funayama et al.,

2001). Patients unilaterally lost 70-80% of an amygdala and 100% of a hippocampus and parahippocampus during a procedure to treat medically refractory complex partial seizures of medial temporal lobe origin. Right unilateral temporal lobe patients displayed startle potentiation similar to healthy controls during anticipation of aversive electric shocks but failed to show startle potentiation to negative pictures. Left unilateral temporal lobe patients displayed the opposite pattern of results with normative potentiation to unpleasant pictures and no startle potentiation during the threat of electric shocks. Such results implicate right medial temporal lobe structures in emotional responding to hypothetically negative stimuli (e.g., unpleasant pictures) and left medial temporal lobe structures in emotional responding to actual threat of personally relevant harm or pain (e.g., shock). Given findings implicating lateral prefrontal cortex (PFC) activation during down-regulation of negative emotion (Ochsner, Bunge, Gross, & Gabrieli, 2002; Ochsner et al., 2004), successful suppression and enhancement in the picture paradigm may require willful access to PFC areas exerting inhibitory and excitatory inputs to right medial temporal lobe structures, whereas successful regulation in the Threat experiment may require access to PFC areas exerting such influence on left medial temporal lobe areas. These neurobiological inferences regarding regulation of emotion to actual versus hypothetical threat are speculative given the paucity of available data. Additionally, Funayama et al.'s findings may not only be driven by differences in neural processes elicited by threat of shock versus unpleasant pictures but may also reflect the difference between conditioned versus unconditioned processes. More specifically, Funayama's Threat paradigm, but not Picture paradigm, elicits acquisition of associative learning (colored squares associated with shock during Threat). The current Threat paradigm employing threat cues of the word ‘SHOCK?’ elicits fear of shock without the formation of a new association and thus conditioning processes are relatively absent in both paradigms employed by the current study. An additional difference between the current threat-of-shock paradigm and that of Funayama and colleagues (2001) is the absence of actual shock administration in Funayama until the very end of the study versus the presence of shock administration in the early, middle, and late portions of the current paradigm (3 shocks total). As a result, more habituation to the electric shock likely took place over the of course of the current study compared to the Funayama study, potentially leading to less strong threat related brain activation by the present threat paradigm. Future brain imaging studies assessing neural processes across the current Threat paradigm and Picture paradigm may elucidate paradigm-specific activations independent of conditioning processes and the absence of reinforcement of threat cues.

Unregulated Startle Potentiation Across Picture and Threat Paradigms

Baseline potentiation—The general increase in baseline startle in the Threat versus Picture experiment may be conceptualized as an effect of sensitization. In the context of fear and anxiety, sensitization is a time-limited enhancement in responsiveness to aversive or fear relevant stimuli when the fear state is already active (Groves & Thompson, 1970; Öhman & Mineka, 2001). In the Threat study, placing shock electrodes and administering shock activated the fear state leading to the ongoing enhancement in reactivity to intense, sudden, and perhaps aversive acoustic startle probes throughout the threat-of-shock run (i.e., sensitization).

Potentiation to threat cues and unpleasant pictures—Though fear sensitization processes may well account for the increase in baseline startle in the Threat paradigm, it is less likely to account for the potentiation of startle from the safety cue to threat-of-shock cue, as the sensitization from the shock electrodes was present during presentation of both cues yet probes during the threat cue potentiated startle above and beyond startle magnitudes elicited during the safety cue. Thus greater responding to the threat cue versus unpleasant pictures likely reflects greater phasic fear to the shock cue versus unpleasant pictures rather

than a general effect of fear sensitization. It should nevertheless be noted that pleasure attenuated startle to the safety cue may have inflated levels of emotionally potentiated startle to shock cues as potentiation was computed as a threat – safe difference score. Nevertheless, because effects of pleasure attenuated startle are weaker than those of emotionally potentiated startle, magnitudes of startle potentiation to threat are likely driven by fear-potentiated versus pleasure-attenuated startle effects. The possibility also exists that greater potentiation in the Threat paradigm was influenced by greater arousal evoked by threat-of-shock cues, as increasing arousal corresponds with increasing levels of startle potentiation (Bradley, Codispoti, Cuthbert, & Lang, 2001). Future studies are needed to assess the degree to which regulated and unregulated potentiation effects across paradigms were influenced by differences in evoked arousal.

The current finding that startle potentiation to negative cues across paradigms were uncorrelated replicates previous reports (Greenwald et al., 1998) and suggests that startle potentiation to threat of shock is independent of potentiation to unpleasant pictures. The marked increase in startle potentiation to negative cues in the Threat versus Picture experiment found in the current study contrasts results by Bradley and colleagues (2005) who found equal magnitudes of startle potentiation to instructed threat of shock and unpleasant pictures using a between groups manipulation. In the first group of participants, unpleasant pictures signaled risk for shock and pleasant pictures signaled safety, while in the second group unpleasant and pleasant pictures signaled safety and risk, respectively. Findings demonstrate equal startle magnitudes elicited in the presence of unpleasant pictures whether the unpleasant picture cued imminent shock delivery (Group 1) or signaled safety (Group 2). The critical difference between Bradley et al. (2005) and the current study may well be the presence and absence of shock electrodes, respectively, during assessment of startle potentiation to negative pictures. More specifically, the ambient threat produced by the presence of shock electrodes in Bradley et al. may well have elevated startle potentiation to the unpleasant pictures in Group 2 (even though such pictures signaled safety) by way of *expectation bias*, a cognitive distortion through which negative stimuli elicit biased expectancies of aversive outcomes (e.g., electric shock) in anxiogenic experimental environments (de Jong, Merckelbach, Bogels, & Kindt, 1998; Kennedy, Rapee, & Mazurski, 1997; Tomarken, Mineka, & Cook, 1989). That shock electrodes were removed during picture runs of the current study reduced the risk that startle potentiation to unpleasant pictures would be increased by illusory expectations of shock and may have contributed to the substantially larger difference in startle potentiation across Threat versus Picture paradigms in the current study.

Though Bradley et al. (2005) is the only existing study, to the knowledge of these authors, contrasting potentiated startle to instructed threat-of-shock and unpleasant pictures, a previous study found conditioned startle-potentiation to electric shocks to be equal in magnitude with startle potentiation to unpleasant pictures (Greenwald et al., 1998). The similar potentiation to shock conditioning and negative pictures found by Greenwald and colleagues may have resulted from two methodological characteristics of the study. For one, shock conditioning paradigms produce less robust fear-potentiated startle compared to instructed threat-of-shock (Grillon & Baas, 2003). Secondly, startle potentiation elicited during pictures in this study was operationalized as the difference in startle magnitudes from positive to negative pictures rather than neutral to negative. As such, startle potentiation to negative pictures was the product of both fear-potentiated and pleasure-attenuated startle and may have overestimated the unique effect of unpleasant pictures on the startle reflex. Though inconsistencies between present findings and those of past studies (Bradley et al., 2005; Greenwald et al., 1998) may be due to methodological differences, past findings demonstrate that under certain experimental conditions, unpleasant pictures and threat of shock elicit comparable levels of startle potentiation.

Understanding the Current Results within the Defense Cascade Framework

The defensive cascade in humans (Bradley et al., 2001) denotes a sequence of anxiety related responses (e.g., phasic changes in skin conductance and heart-rate) that progress as anxious arousal to a threat encounter increases from *pre-encounter* to *early post-encounter* (initial orienting to threat and the beginnings of defensive arousal) to *late post-encounter* (further increases in defensive arousal and preparation for action) to *overt action* (fight or flight) stages. The increasing levels of threat in this model have been conceptualized as a sequential increase in imminence of danger (Bradley et al., 2001; Fanselow, 1994). In this context, unpleasant pictures and threat of shock may represent distal and more proximal threat, eliciting early and late post-encounter responses, respectively. Greater startle potentiation to negative cues in the Threat versus Picture experiment is consistent with this framework as the early post-encounter phase is associated with moderate startle potentiation relative to baseline (after a very brief orienting-related decrease) and the late relative to early post-encounter phase is characterized by further increases in startle potentiation (Bradley et al., 2001). Though little data contrasting emotion regulation processes across defensive stages is available, present startle results suggest comparable magnitudes of willfull up- and down-regulation of emotion across late and early post-encounter stages. Self-report data however support a different conclusion and suggest that the stronger level of emotional reactivity in the late versus early post-encounter stage is accompanied by a greater difficulty to volitionally up- or down-regulate negative emotion.

Conclusion

The current study sought to assess the degree to which willful regulation of emotionally-potentiated startle to hypothetical threat, found by Jackson and colleagues (2000), extends to regulation of startle potentiation to actual threat. Results demonstrate that volitional attempts to suppress and enhance negative emotion led to decreased and increased startle potentiation to both unpleasant pictures (hypothetical threat) and threat of shock (actual threat), verifying the external validity of the Jackson method for examining regulation processes analogous to those occurring in the day-to-day context in response to *real* sources of danger or pain. Though initial analyses indicated greater enhancing and less full suppression of negative emotion elicited by threat of shock versus unpleasant pictures, such results became nonsignificant after covarying out paradigm differences in magnitudes of unregulated startle potentiation. Thus enhancing and suppressing differences across paradigms were a function of the stronger unregulated potentiation to threat of shock rather than to regulatory processes per se.

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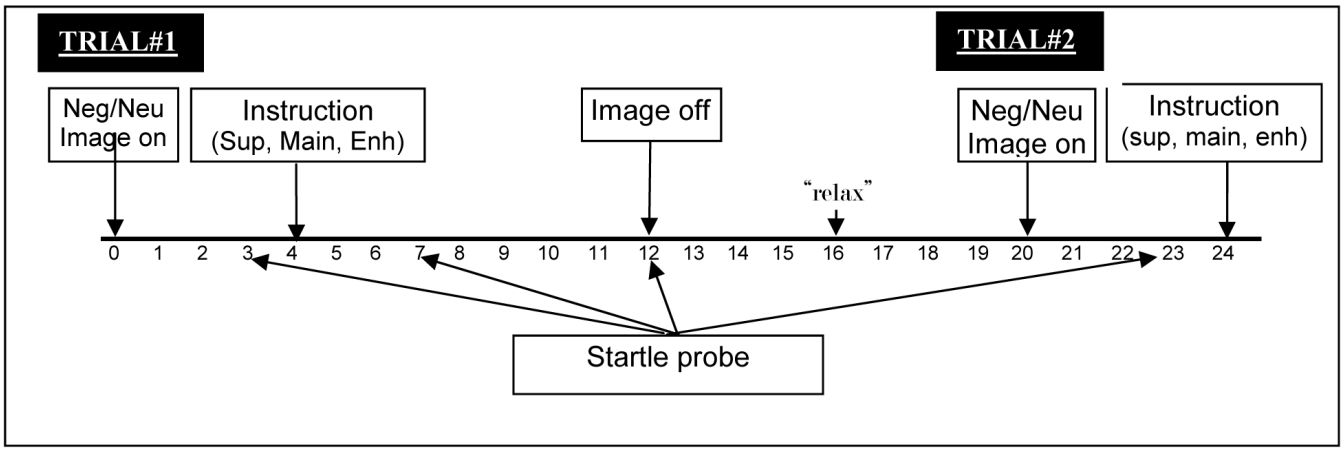
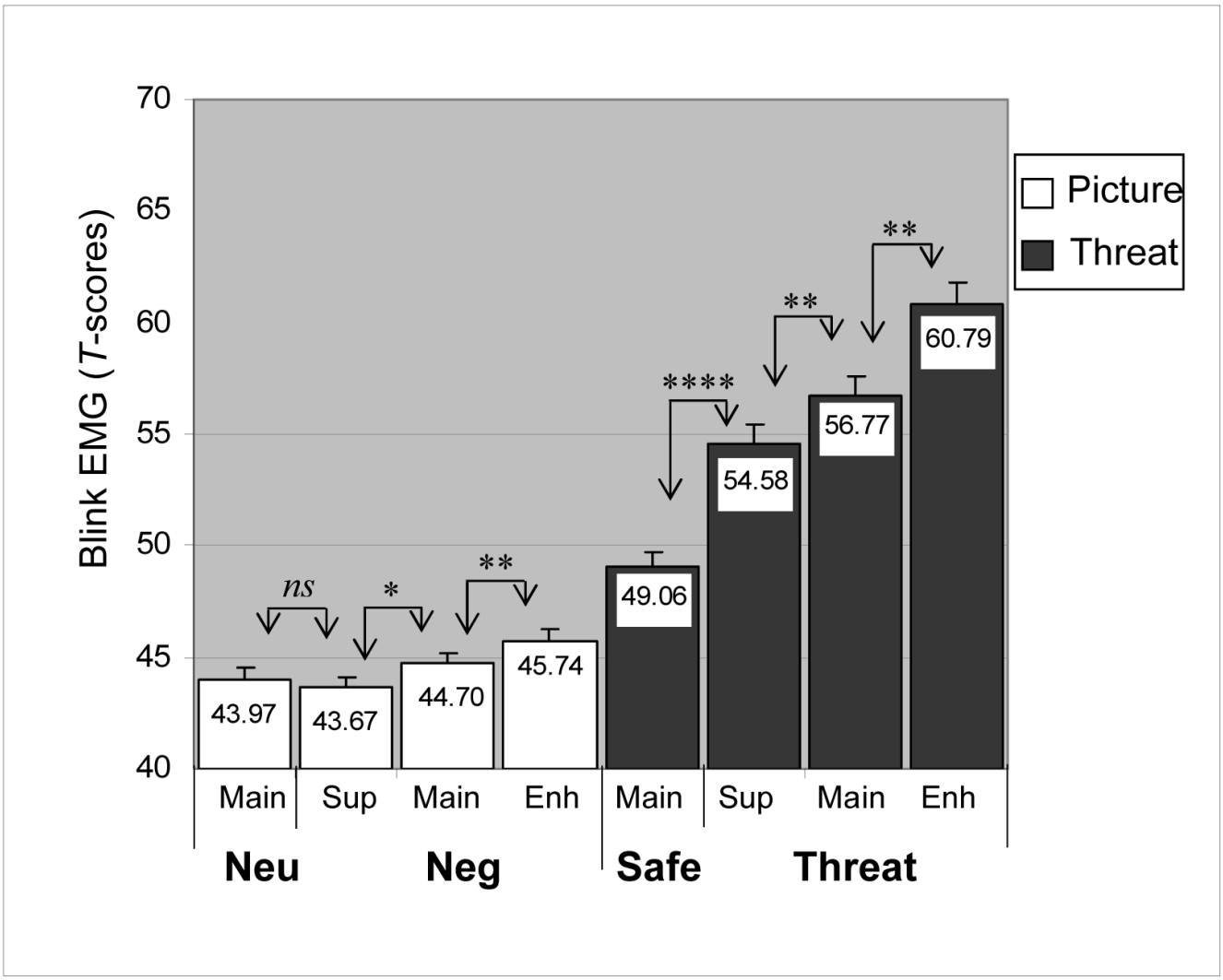


Figure 1.

Schematic drawing of the time-course of stimulus presentation including onset times for negative/neutral pictures (i.e., threat/safe cues or unpleasant/neutral pictures), regulation instructions, and startle probes. Sup = suppress, Main = maintain, Enh = enhance, Neg = negative, Neu = neutral. Startle probes occurred either 3-, 7-, or 12-sec post-image onset for any given trial.



*$p \leq .05$, ** $p \leq .01$, ***$p \leq .001$, ****$p \leq .0001$

Figure 2.

Average standardized startle magnitudes and SEM following regulation instructions across threat and picture paradigms. Sup = suppress, Main = maintain, Enh = enhance. Data from 7 and 12 s probe latencies were averaged together for each of 8 trial types below.

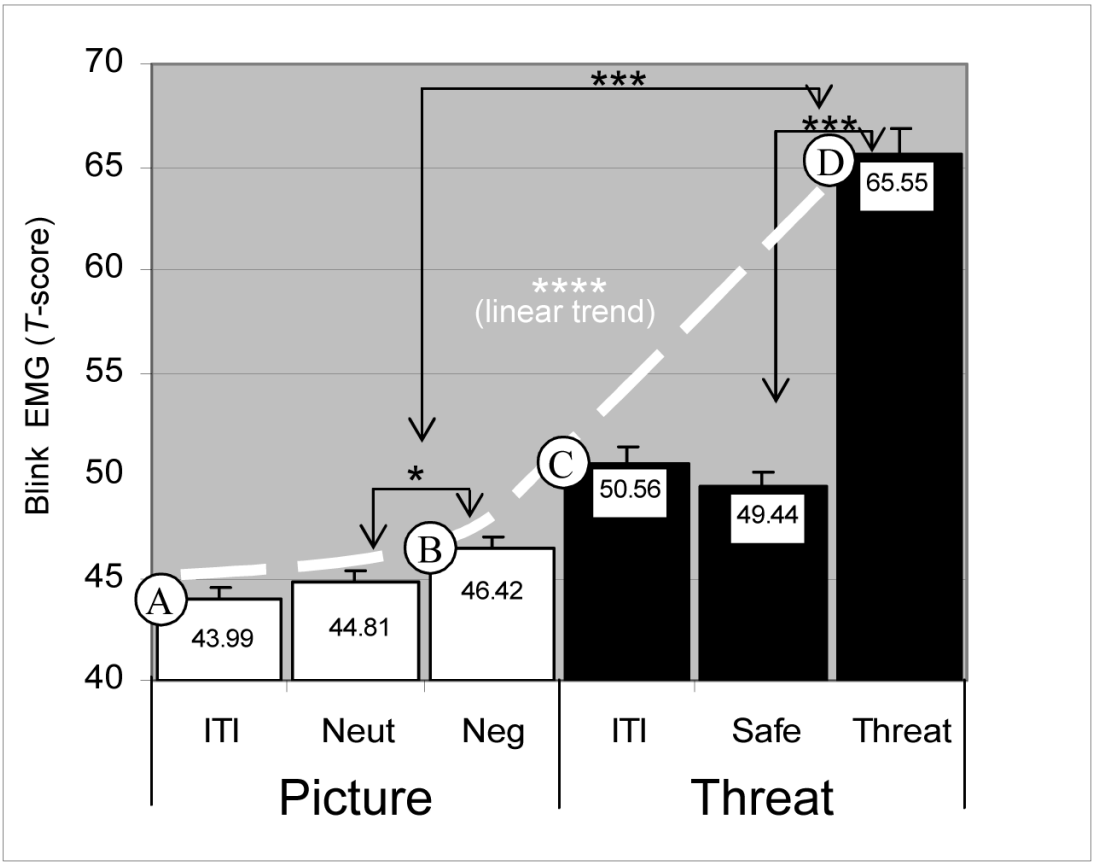


Figure 3.

Average standardized EMG magnitudes and SEM evoked during intertrial interval (ITI), neutral/safe, and negative stimuli across Picture and Threat paradigms. EMG magnitudes elicited during neutral/safe and negative stimuli consist of data collected at 3 sec post-image onset used to assess unregulated, emotionally potentiated startle. The white doted curve represents the curve-linear increase in startle magnitude as the anxiogenic quality of the experimental situation increased from A (no shock electrodes, no unpleasant pictures) to B (no shock electrodes, unpleasant pictures) to C (shock electrodes, no threat cue) to D (shock electrodes, threat cue signaling imminent shock delivery).

Table 1

Suppress and enhance strategies across Picture and Threat paradigms and the number of participants reporting the use of each.

Strategy	*n*(%)		*t(46)*
	Picture	Threat	
Suppress			
Slowed breathing	24 (52%)	27 (58%)	*ns*
Muscle relaxation	5 (11%)	2 (4%)	*ns*
Visual or cognitive avoidance of the image/shock	6 (13%)	6 (13%)	*ns*
Focusing on a positive/neutral aspect of the stimulus	8 (17%)	4 (9%)	*ns*
Lowering the perceived probability of a negative outcome	4 (9%)	22 (48%)	$p < .0001$
Increasing the perceived probability of a positive outcome	7 (15%)	4 (9%)	*ns*
Depersonalizing the negative stimulus	14 (30%)	0 (0%)	$p < .0001$
Enhance			
Increased breathing	16 (35%)	10 (22%)	*ns*
Muscle tensing	4 (9%)	4 (9%)	*ns*
Focus on the shock or the most negative aspect of the image	18 (39%)	35 (76%)	$p < .0001$
Increase the perceived probability of a negative outcome	10 (22%)	24 (52%)	$p < .003$
Decrease the perceived probability of a positive outcome	3 (7%)	4 (9%)	*ns*
Personalize the negative stimulus	27 (58%)	1 (2%)	$p < .0001$

Bonferroni correction applied significance level is $p = .05/13 = .004$. Thus results considered significant if $p \leq .05/13$ or .004.