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State anxiety and affective physiology: effects of sustained exposure to affective pictures

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Abstract

Effects of sustained exposure to emotional stimuli on affective reactions and their recovery were examined to determine whether increasing exposure to a specific emotional content (e.g., unpleasant) cumulatively affects physiological responses; and whether motivational activation persists following sustained exposure. Participants viewed pleasant, neutral, and unpleasant IAPS pictures, presented in blocks separated by an inter-block interval. With increasing exposure to unpleasant pictures, startle magnitude showed greater potentiation, and corrugator EMG activity increased. Both affective startle and corrugator modulation persisted following exposure to unpleasant pictures. The cumulative effects of sustained exposure to unpleasant pictures were enhanced for those reporting higher state anxiety, consistent with the hypothesis that sustained aversive exposure leads to increased defensive activation. These findings suggest sustained exposure to unpleasant pictures may induce a short-term mood state, and may be a useful paradigm to study individuals who vary in symptoms of anxiety.

Keywords: State anxiety; Response sensitization; Habituation; Startle; Corrugator EMG; Skin conductance

1. Introduction

Previous work has indicated that during exposure to individual affective pictures, phasic facial electromyographic (EMG) indices of emotion, including the acoustically elicited startle eye blink response and corrugator supercilii activity, as well as initial heart rate deceleration, are heightened when viewing unpleasant, compared to pleasant, pictures. Furthermore, electrodermal reactivity is reliably greater when viewing emotional (pleasant

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or unpleasant), compared to neutral, pictures (e.g., Lang et al., 1993; Bradley et al., 2001). These responses are hypothesized to reflect activation of underlying brain systems that mediate appetitive and defensive motivational behavior, respectively (Lang, 1995).

In this study, we explored the emotional effects of exposure to a sustained series of emotional pictures. Important questions concern whether such exposure to unpleasant (or pleasant) emotional content has a cumulative effect on physiological responses, and second, whether defensive or appetitive activation is maintained when the stimuli are withdrawn. Thus, the research considers the special effects of massed emotional stimulation, either pleasant or unpleasant, on the development of a tonic affective state, and furthermore, assesses the extent to which this pattern may persist following exposure.

Two previous studies have examined psychophysiological responses during and after sustained affective picture presentations. Bradley et al. (1996) presented blocks of 24 pleasant, neutral or unpleasant pictures in which each picture was shown for 6 s and was followed by a 6 s inter-picture interval. Startle response magnitude was greater 1 s following the offset of unpleasant, compared to pleasant or neutral, pictures. Thus, at least immediately after picture offset, blinks were modulated in the absence of an unpleasant picture stimulus. In addition, corrugator EMG activity increased as more unpleasant pictures were presented, suggesting a cumulative effect of prior pictures on this facial frown response. Sutton et al. (1997) also presented blocks of 24 similarly rated affective pictures. In their study, each picture was shown for 12 s and affective startle modulation was explored at different time points within each picture. They found that startle potentiation was greater for startle probes presented later in each picture presentation compared to probes presented soon after picture onset.

In the current study, rather than measuring startle responses at different times within a single picture presentation or immediately after picture offset, startle was measured both early and late in a continuous picture series. Corrugator and zygomatic EMG activity, and autonomic responses (heart rate, skin conductance) were measured continuously. Furthermore, the persistence of affective responding was examined over relatively long (i.e., 30–60 s) intervals post-picture viewing, again with early and late assessment. Finally, participants' self-evaluated anxiety (state and trait anxiety) prior to the experiment was determined and their relationship assessed to both picture response magnitude and the persistence of these emotional effects after stimulation.

Several alternative hypotheses were evaluated: first, does response sensitization occur, resulting in a cumulative increase in affective modulation as more pictures of the same hedonic valence were presented? This view is supported by previous research on sustained exposure to pictures (e.g., Bradley et al., 1996) as well as by studies showing that prolonged exposure to uncontrollable stress in rodents, and electric shock in humans, results in the response sensitization of defensive systems (Figueiredo et al., 2003; Hamm and Stark, 1993). Second, prolonged exposure could lead to diminution in reactivity, presumably due to habituation. For instance, some data suggest that repeated processing of erotic film clips results in the loss of pleasure attenuated startle magnitude (Koukounas and Over, 2000). Third, it is possible that emotional reflex reactions are driven primarily by the specific stimulus, and these responses do not change with increased exposure. In this case, affective differentiation would also terminate after exposure.

Because studies have reported that participants with higher state anxiety show greater fear-potentiated startle (Grillon et al., 1993), we expected that those reporting greater state anxiety prior to the experiment might show more pronounced affective responses during unpleasant picture viewing, and that their reactions would be more sustained.

2. Methods

2.1. Participants

Participants were 37 (23 females, 14 males) introductory psychology students at the University of Florida who received course credit for participation. The Health Science Center Institutional Review Board at the University of Florida approved this experiment, and each participant provided written informed consent prior to participation.

2.2. Materials and design

One hundred and forty-four pictures were selected based on their normative valence ratings from the International Affective Picture System (IAPS), a collection of standardized photographic materials (Center for the Study of Emotion and Attention [CSEA-NIMH], 1999; Lang et al., 1999). Thirty-six pictures were pleasant, 72 were neutral, and 36 were unpleasant in content.[1] Pictures of the same hedonic valence content were shown in a series of 36 pictures. Each participant viewed four series of pictures. An initial practice series of neutral pictures began the experiment for all participants. This was followed by three critical series consisting of pleasant, neutral, or unpleasant pictures. For all participants the neutral series occurred in the middle position; the order of the pleasant and unpleasant pictures (i.e., first or third in the critical series) was counterbalanced across participants. In each series, 36 pictures were presented in three blocks of 12 pictures each, in which each block of 12 pictures was separated by a 30 s inter-block interval. Each of the three series was separated by a 60 s inter-series interval.

Each of the 12 pictures in each block was shown for 6 s, followed by a 1.5 s inter-picture interval. Thus, each block of 12 contiguous pictures lasted 90 s; an entire series of 36 pictures lasted 390 s (6.5 min, including inter-block and -series intervals). Acoustic startle probes were presented over headphones 2 s after picture onset during the 2nd and 11th picture of each block of 12. Picture order was counterbalanced such that, across subjects, each picture was presented once at both the 2nd and 11th position in each block of 12. Two

[1] IAPS identification numbers for the pictures used. Neutral 1: 6150, 7100, 2190, 7035, 7550, 2280, 7000, 7130, 2200, 7040, 7700, 2383, 7009, 7500, 2220, 7090, 5532, 2440, 7002, 7140, 2210, 7050, 7710, 2385, 7006, 7495, 2215, 7080, 5531, 2381, 7004, 7491, 2214, 7060, 5530, 2372. Unpleasant: 1300, 3000, 2730, 3500, 3062, 9320, 1050, 3010, 3160, 3530, 3064, 9373, 1201, 3060, 9300, 6350, 3110, 9570, 1120, 3015, 3170, 6550, 3071, 9340, 1052, 3053, 9008, 6313, 3130, 9042, 1220, 3030, 3266, 6560, 3102, 9040. Neutral 2: 7010, 7510, 2221, 7110, 5500, 2480, 7020, 7545, 2230, 7150, 5510, 2570, 7034, 7640, 2271, 7235, 5731, 2516, 7025, 7560, 2240, 7170, 5520, 2485, 7031, 7496, 2270, 7211, 5395, 2410, 7030, 7620, 2250, 7175, 5455, 2630. Pleasant: 5830, 4611, 5270, 2070, 4660, 8190, 2050, 4650, 5660, 2071, 4664, 8170, 1811, 4659, 5628, 4610, 4689, 5594, 1460, 4652, 5890, 1463, 4666, 8350, 4641, 4658, 5626, 1722, 4680, 5611, 1440, 4653, 5623, 1710, 4672, 7580.

additional startle probes were presented during each inter-block interval, one at 2 s and another at the end of the inter-block interval (30 s), or the end of the inter-series interval (60 s). In addition, three startle probes were presented prior to picture viewing in order to accustom the participant to the startle stimulus.

2.3. Apparatus and physiological response measurement

Digitized versions of the IAPS pictures were displayed using VPM stimulus control software (Version 11.2, Cook, 2001) running on an IBM computer. Pictures were shown on a 17-in. color monitor situated approximately 1.0 m from the participant. Picture onset was virtually instantaneous and pictures were shown in 24-bit color. The acoustic startle stimulus was a 50 ms, 95 dB(A) burst of white noise with instantaneous rise time, generated by a Coulbourn (Coulbourn Instruments, L.L.C., Allentown, PA) S81-02 white noise generator and presented over matched Telephonics (Telephonics Corp., Huntington, NY) TDH-49 headphones.

A second IBM-compatible computer running VPM software controlled physiological data acquisition (Cook, 2001). Physiological signals were continuously sampled at 50 Hz beginning 3 s before each series of pictures. Activity over the orbicularis oculi muscle was sampled at 1000 Hz from 50 ms prior to the onset of the noise stimulus to 250 ms after stimulus offset. Blink responses were measured from the left orbicularis oculi muscle using sensormedics silver–silver chloride miniature electrodes and the placement recommended by Fridlund and Cacioppo (1986). The raw EMG signal was amplified by 30,000 and frequencies below 90 Hz and above 250 Hz were filtered, using a Coulbourn S75-01 bio-amplifier. The raw signal was rectified and integrated using a Coulbourn S76-01 contour following integrator, with a calibrated time constant of 123 ms.

Activity over the corrugator supercilii (above left eye) and zygomaticus major (left cheek) muscles were measured with sensormedics miniature silver–silver chloride electrodes using the placement recommended by Fridlund and Cacioppo (1986). The raw EMG signals were amplified by 30,000 and frequencies below 90 Hz and above 1000 Hz were filtered, using a Coulbourn S75-01 bio-amplifier. The raw signals were rectified and integrated using a Coulbourn S76-01 contour following integrator, with a time constant of 500 ms.

Skin conductance electrodes were placed adjacently on the hypothenar eminence of the left palmer surface using sensormedics standard silver–silver chloride electrodes filled with a 0.5 M NaCl and Unibase cream (Warner Chilcott Laboratories, Morris Plains, NJ). The signal was recorded with a Coulbourn S71-22 skin conductance coupler calibrated prior to each session to detect activity in the range of 0–40 micro-Siemens (μS). The calibration value was used off-line to convert the digitized raw signal to skin conductance values in μS. Skin conductance responses were scored as the number of skin conductance responses greater than 0.05 μS.

The electrocardiogram was recorded from the left and right forearms, using 8 mm sensormedics silver–silver chloride electrodes filled with electrolyte paste. The signal was filtered using a Coulbourn S75-01 bio-amplifier, and a Schmitt trigger interrupted the computer each time it detected a cardiac R-wave. Inter-beat intervals were recorded to the nearest millisecond, and reduced off-line using VPM software (Cook, 2001) into heart rate

in beats per minute in half-second bins, weighting each interval by the fraction of time occupied (Graham, 1980).

2.4. Procedure

The participant sat in a recliner in a dimly lit room. After signing the informed consent form, the participant completed the state-trait anxiety inventory (STAI; Spielberger et al., 1983). Then, the sensors were placed on the participant and the participant was instructed that several series of pictures would be displayed, that each picture should be viewed the entire time it was on the screen, and that any brief noises heard over the headphones could be ignored. During the first 5 min, the participant did not see pictures and three noise probes were presented (not scored). The remainder of the session lasted 26 min (1560 s) and consisted of the viewing of the practice series and the three critical series. Immediately after the last series of pictures was viewed, the participant was again asked to complete the STAI-Y1 (state anxiety) and was instructed to ''indicate how you feel right now''. The participant was subsequently debriefed, paid credit, and thanked for his/her participation.

2.5. Data reduction and analysis

The eye blink data were reduced off-line using a VPM program (Cook, 2001) that implements a peak-scoring algorithm (Balaban et al., 1986) that scores the peak response for onset latency and amplitude. Trials with clear artifacts were rejected, while trials with no responses were scored as zero magnitude blinks. Reactions in corrugator, zygomatic, and heart rate were determined by subtracting the mean activity in the 3 s prior to each series of 36 pictures from that occurring at each half-second during the entire series of pictures and inter-block intervals. For skin conductance, the number of responses greater than 0.05 μS was determined (Dawson et al., 2000). For facial EMG activity and heart rate, mean values over the first six pictures (first half of exposure) and the last six pictures (second half of exposure) in a block of 12 pictures, and during each 30 s inter-block interval (four total, including the two 30 s periods during the last 60 s inter-series interval), were used to estimate reactivity.

Data were analyzed separately during picture blocks and inter-block intervals using SPSS Version 11 (Chicago, IL) general linear model repeated measures ANOVAs, with gender (Male, Female) and picture order (pleasant or unpleasant first) as between subject factors. For each block and inter-block interval, startle magnitude was analyzed during and following exposure using 2 (gender) × 2 (order) × 3 (valence: pleasant, neutral, unpleasant) × 2 (exposures: first half, second half) repeated measures ANOVAs. To assess startle magnitude across the entire series of pictures, 2 (gender) × 2 (order) × 3 (valence) × 3 (block) repeated measures ANOVAs were employed.

For corrugator, zygomatic, and heart rate changes, and number of skin conductance responses, a 2 (gender) × 2 (order) × 3 (valence) × 3 (block) × 2 (exposures) repeated measures ANOVA was employed during picture viewing. During the inter-block intervals (except for heart rate), a 2 (gender) × 2 (order) × 3 (valence) × 4 (block) repeated measures ANOVA was employed. Because order did not affect the results for corrugator, zygomatic, or heart rate changes, or the number of skin conductance responses, order was

replaced by anxiety group as a between subject factor in the statistical tests for the effects of anxiety. The Huynh–Feldt epsilon was employed to adjust the degrees of freedom in each repeated measures analysis with more than two levels. Due to missing data for some subjects, the startle responses during pictures were analyzed with $n = 33$ (12 males, 21 females), and during the inter-block interval with $n = 34$ (14 males, 20 females). Due to computer error, heart rate data were missing for two participants, and zygomatic EMG data were excluded from one participant whose responses were greater than 3.0 S.D. below the mean during the pleasant series.

3. Results

3.1. Startle magnitude

Startle magnitude reliably increased with exposure to an increasing number of unpleasant pictures in a block (valence × exposures $F(2,64) = 3.55$, $P = 0.035$, $\eta^2 = 0.100$; see Fig. 1). That is, when viewing unpleasant pictures, startle magnitude was larger for probes presented late in the block compared to probes presented early in the block ($F(1,32) = 9.16$, $P = 0.005$, $\eta^2 = 0.223$). There were no differences in reflex magnitude for probes presented early or late in a block when viewing either pleasant or neutral pictures (Fs < 1).

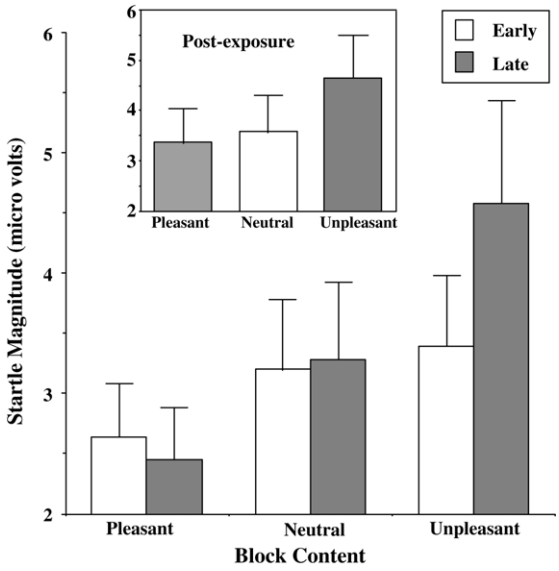

Fig. 1. Startle eye blink response magnitude (microvolts) as a function of affective content during picture blocks and following exposure in the inter-block intervals (inset). During picture viewing, the data are the mean for each startle probe position (early and late) averaged across the three picture blocks. There were no significant differences across time during the inter-block intervals, so the data during the post-exposure inter-block intervals are the averages across each probe time (2 and 30 s) in the inter-block intervals, and in the last inter-series interval (at 2 and 60 s).

Table 1
Startle magnitude (mean of the two trials per block; microvolts) and heart rate responses (average of 12 pictures per block; beats min^{-1}) across each pleasant, neutral, and unpleasant series

	Block 1	Block 2	Block 3
Startle magnitude—mean (±S.E.) per picture block across each series (microvolts)			
Pleasant	3.52 (0.82)	2.55 (0.46)	2.01 (0.34)
Neutral	3.70 (0.82)	3.03 (0.66)	3.28 (0.64)
Unpleasant	4.04 (0.74)	4.61 (1.12)	4.05 (0.74)
Heart rate—mean change (± S.D.) per picture from pre-picture baseline (beats min^{-1})			
Pleasant	−0.33 (1.70)	−0.12 (1.75)	−0.20 (1.63)
Neutral	0.18 (1.52)	−0.38 (1.56)	0.06 (1.59)
Unpleasant	−1.09 (1.87)	−0.60 (1.48)	−0.50 (1.56)

The change in startle reflex magnitude across the three blocks of pictures in a series (the average of the early and late startle responses in each block) differed by the valence content of the picture block (linear trend valence × block $F(1,29) = 4.26$, $P = 0.048$, $\eta^2 = 0.130$; see Table 1). For pleasant pictures, startle magnitude significantly decreased from blocks 1 to 3 ($F(1,31) = 5.28$, $P = 0.029$, $\eta^2 = 0.145$). For unpleasant (and neutral) pictures, however, reflex magnitude did not significantly change from the first to the third blocks (Fs < 1).

Affective modulation of startle magnitude persisted into the inter-block interval, including startle responses obtained both early and late in the interval at 30 s (as well as at 60 s during the inter-series interval) (valence $F(2,64) = 5.83$, $P = 0.005$, $\eta^2 = 0.154$) (see Fig. 1, inset). Specifically, following exposure to unpleasant pictures, startle reflexes were significantly larger than those elicited after exposure to either pleasant or neutral pictures ($P = 0.008$ and 0.011, respectively). This effect was primarily carried by those who viewed unpleasant pictures first (valence × order $F(2,64) = 6.65$, $P = 0.002$, $\eta^2 = 0.173$).

3.2. Corrugator EMG response

In general, corrugator EMG activity was heightened when viewing unpleasant, compared to pleasant ($F(1,32) = 11.03$, $P = 0.002$, $\eta^2 = 0.256$) or neutral pictures ($F(1,32) = 8.20$, $P = 0.007$, $\eta^2 = 0.204$; valence $F(2,64) = 8.93$, $P = 0.002$, $\eta^2 = 0.218$). More importantly, corrugator activity increased with increasing exposure to unpleasant pictures in a block (valence × exposures $F(2,64) = 9.71$, $P = 0.001$, $\eta^2 = 0.233$), with larger corrugator EMG changes elicited late, compared to early, in a block (see Table 2). For pleasant and neutral pictures, there were no differences in corrugator EMG activity across a block. The increase in corrugator activity from early to late in each unpleasant picture block was similar for each of the three picture blocks in a series (see Table 2).

Heightened corrugator activity following exposure to unpleasant pictures persisted throughout each inter-block interval (valence $F(2,64) = 4.49$, $P = 0.021$, $\eta^2 = 0.123$). Corrugator EMG was significantly greater following sustained exposure to unpleasant pictures, compared to either pleasant ($F(1,32) = 5.52$, $P = 0.025$, $\eta^2 = 0.147$) or neutral ($F(1,32) = 4.90$, $P = 0.034$, $\eta^2 = 0.133$) picture blocks (see Table 2). The valence × block interaction during the inter-block intervals was not statistically significant ($P = 0.134$), nor

Table 2
Facial electromyographic, skin conductance, and heart rate responses during blocks of pleasant, neutral, and unpleasant pictures, and during inter-block intervals (IBI) and inter-series intervals (ISI) after picture exposure

	Block 1		IBI1	Block 2		IBI2	Block 3		ISI	
	First half	Second half		First half	Second half		First half	Second half	First half	Second half
Corrugator supercilii (μV)										
Pleasant	0.50 (1.17)	0.27 (1.49)	−0.13 (1.47)	0.54 (1.55)	0.43 (1.79)	−0.05 (1.71)	0.24 (2.30)	0.20 (2.20)	−0.34 (2.20)	−0.22 (2.43)
Neutral	0.67 (1.47)	0.93 (1.63)	0.20 (1.76)	0.95 (3.00)	0.81 (2.26)	−0.17 (1.69)	0.58 (1.84)	0.70 (2.20)	−0.37 (1.79)	−0.15 (2.12)
Unpleasant	3.15 (4.05)	3.89 (4.88)	2.07 (3.43)	3.25 (5.01)	4.14 (5.62)	1.69 (3.44)	3.09 (5.25)	3.95 (6.05)	2.03 (4.83)	0.63 (3.38)
Zygomaticus major (μV)										
Pleasant	0.74 (1.01)	0.84 (1.35)	0.39 (0.64)	0.79 (0.95)	0.66 (1.12)	0.46 (0.92)	0.51 (0.83)	1.09 (1.92)	0.55 (0.87)	0.33 (0.82)
Neutral	0.29 (0.47)	0.54 (1.26)	0.87 (1.60)	0.41 (0.60)	0.39 (0.70)	0.58 (0.79)	0.43 (0.76)	0.46 (0.85)	0.71 (0.87)	0.30 (0.93)
Unpleasant	0.40 (1.13)	0.40 (0.97)	0.33 (0.70)	0.55 (1.38)	0.39 (0.92)	0.46 (1.16)	0.24 (0.81)	0.36 (0.96)	0.46 (0.87)	0.34 (0.93)
SCRs (number > 0.05 μS)										
Pleasant	1.92 (1.89)	1.19 (1.97)	1.08 (1.71)	1.57 (1.76)	0.97 (1.52)	0.76 (0.98)	1.30 (2.04)	1.11 (1.47)	1.03 (1.36)	0.78 (1.40)
Neutral	1.49 (1.69)	1.19 (1.56)	1.38 (1.69)	1.22 (1.51)	1.03 (1.36)	1.16 (1.64)	1.32 (1.81)	1.08 (1.55)	1.54 (1.44)	0.84 (1.55)
Unpleasant	2.70 (2.50)	1.78 (2.33)	0.78 (1.20)	1.89 (2.13)	1.30 (1.73)	0.89 (1.35)	1.70 (2.22)	1.40 (1.89)	0.95 (1.37)	0.84 (1.24)
Heart rate—change from pre-series baseline (beats min^{-1})										
Pleasant	−1.23 (4.51)	−2.52 (5.03)	−1.18 (6.05)	−1.22 (3.83)	−2.39 (4.53)	−0.36 (4.64)	−1.53 (3.84)	−2.01 (4.68)	0.48 (3.86)	−0.09 (6.32)
Neutral	−2.09 (6.65)	−1.92 (7.20)	1.84 (8.35)	−0.91 (7.02)	−2.34 (6.83)	−0.19 (7.54)	−2.18 (7.74)	−1.53 (7.47)	0.20 (8.70)	−2.02 (8.40)
Unpleasant	−2.39 (6.46)	−3.32 (6.05)	−0.71 (5.90)	−1.76 (5.98)	−2.65 (6.91)	0.34 (6.90)	−1.44 (6.69)	−1.25 (7.12)	2.08 (7.48)	1.84 (4.44)

Across each row, the data are shown from the beginning to the end of each series. Values are expressed as mean change (±S.D.) in electromyographic activity (μV), skin conductance (number of responses greater than 0.05 μS), and heart rate (beats min^{-1}) across 45-s periods during picture blocks (six pictures), and 30-s periods during inter-block and -series intervals relative to a baseline 3 s prior to each series.

was the effect for block during the unpleasant series (unpleasant block $F(3,96) = 2.59$, $P = 0.076$, $\eta^2 = 0.075$). Nevertheless, a further examination of the unpleasant series showed corrugator EMG activity at 60 s following offset was not significantly different from zero ($t(35) = 1.12$, $P = 0.271$).

3.3. Zygomatic EMG responses

Zygomatic EMG activity was elevated when viewing pleasant, compared to unpleasant pictures ($F(1,32) = 4.45$, $P = 0.043$, $\eta^2 = 0.122$) but was not affected by duration of exposure (see Table 2). In addition, heightened zygomatic activity when viewing pleasant pictures was only found for women (gender × valence $F(2,64) = 3.85$, $P = 0.026$, $\eta^2 = 0.107$), compared to when viewing either neutral ($F(1,20) = 7.63$, $P = 0.012$, $\eta^2 = 0.276$) or unpleasant pictures ($F(1,20) = 13.95$, $P = 0.001$, $\eta^2 = 0.411$). There were no differences in zygomatic activity across the series or that persisted into the inter-block intervals.

3.4. Number of skin conductance responses

A greater number of skin conductance responses were elicited when viewing unpleasant, compared to pleasant ($F(1,33) = 4.48$, $P = 0.042$, $\eta^2 = 0.120$) or neutral pictures ($F(1,33) = 4.95$, $P = 0.033$, $\eta^2 = 0.130$; valence $F(2,66) = 3.64$, $P = 0.032$, $\eta^2 = 0.099$). Across a block of 12 pictures, the number of skin conductance responses decreased (exposures $F(1,33) = 21.56$, $P = 0.000$, $\eta^2 = 0.395$) as well as across the entire series of pictures (block $F(2,66) = 5.13$, $P = 0.009$, $\eta^2 = 0.135$; see Table 2).

A main effect of picture valence indicated that more skin conductance responses occurred following exposure to neutral, compared to pleasant ($F(1,33) = 14.79$, $P = 0.001$, $\eta^2 = 0.310$) or unpleasant picture blocks ($F(1,33) = 5.89$, $P = 0.021$, $\eta^2 = 0.151$) (valence quadratic trend $F(1,33) = 10.87$, $P = 0.002$, $\eta^2 = 0.248$).

3.5. Heart rate

Heart rate deceleration relative to the onset of each picture was more pronounced when viewing unpleasant and pleasant pictures compared to during neutral pictures (valence quadratic trend $F(1,31) = 15.12$, $P = 0.000$, $\eta^2 = 0.328$; see Table 1). There were no differences across the series during picture blocks.

Heart rate change following sustained exposure was affected by picture valence and by block (valence × block $F(6,174) = 2.66$, $P = 0.017$, $\eta^2 = 0.084$). For unpleasant pictures, heart rate tended to increase across blocks during the inter-block intervals (block $F(3,87) = 2.44$, $P = 0.070$, $\eta^2 = 0.078$), whereas heart rate decreased after neutral picture blocks (block $F(3,90) = 4.16$, $P = 0.008$, $\eta^2 = 0.122$). There was no difference after blocks of pleasant pictures (see Table 1).

3.6. Anxiety effects

Based on state anxiety (STAI-Y1) scores prior to the experiment, a median split was used to create two groups; those with low state anxiety (mean (±S.D.) score of 25.8 (3.6); 9

females, 9 males) and those with higher state anxiety (mean (±S.D.) score of 38.9 (5.8); 13 females, 5 males). A median split was also performed based on trait anxiety scores (STAI-Y2) (low trait anxiety mean (±S.D.) of 29.8 (2.6); higher trait anxiety mean (±S.D.) of 40.3 (5.9). The normative state and trait anxiety scores (±S.D.) for female college students are 38.8 (11.9) and 40.4 (10.2), respectively; and for male college students are 36.5 (10.0) and 38.3 (9.2), respectively (Spielberger et al., 1983). There were no differences between men and women for state or trait anxiety scores, nor did gender interact with anxiety group in any of the statistical analyses. In addition, there were no differences between the anxiety groups in baseline levels of orbicularis oculi or corrugator tension, and there were no baseline differences in heart rate or skin conductance level (micro-Siemens).

The low and higher state anxiety groups did not differ in startle magnitude during picture viewing or in the inter-block intervals (Fs < 1). A median split based on trait anxiety scores also did not reveal significant differences in startle magnitude between low and higher trait anxious participants (anxiety × valence $F(2,64) = 1.80$, $P = 0.177$, $\eta^2 = 0.053$; anxiety × valence × exposure $F(2,64) = 2.27$, $P = 0.116$, $\eta^2 = 0.065$). This null effect replicates the finding reported by Nitschke et al. (2002), in which participants high in trait anxious apprehension did not show exaggerated startle potentiation during the anticipation of an aversive stimulus.

Nevertheless, individuals who scored higher for state anxiety showed significantly more corrugator activity when viewing unpleasant pictures compared to the low state anxiety group (anxiety × valence $F(2,64) = 7.04$, $P = 0.007$, $\eta^2 = 0.180$; see Fig. 2, top left). The higher state anxiety group also showed a significantly greater increase in corrugator activity from early to late in unpleasant picture blocks (i.e., with increasing exposure; anxiety × unpleasant exposures $F(1,32) = 7.54$, $P = 0.010$, $\eta^2 = 0.191$; see Fig. 2, top left-inset). These two effects describe the significant anxiety × valence × exposure interaction found during picture viewing in corrugator EMG activity ($F(2,64) = 6.96$, $P = 0.004$, $\eta^2 = 0.179$). Following exposure to unpleasant pictures, the higher state anxiety group also showed more corrugator EMG activity compared to the low state anxiety group (anxiety × valence $F(2,64) = 4.89$, $P = 0.014$, $\eta^2 = 0.132$; unpleasant inter-block interval anxiety $F(1,32) = 7.37$, $P = 0.011$, $\eta^2 = 0.187$; see Fig. 2, top right). There were no differences in corrugator responses between groups based on trait anxiety scores.

Those with higher state anxiety scores also showed a significantly larger number of skin conductance responses (anxiety $F(1,33) = 6.25$, $P = 0.0180$, $\eta^2 = 0.159$), and this effect was only significant when viewing unpleasant pictures (valence × anxiety $F(2,66) = 2.58$, $P = 0.083$, $\eta^2 = 0.073$; unpleasant series anxiety $F(1,33) = 10.04$, $P = 0.003$, $\eta^2 = 0.223$; see Fig. 2, bottom left), and was similar for both men and women ($F < 1$). The high state anxiety group also showed significantly more skin conductance responses in the inter-block intervals (anxiety $F(1,33) = 6.97$, $P = 0.013$, $\eta^2 = 0.174$), and this effect was largest following exposure to unpleasant pictures (unpleasant inter-block interval anxiety $F(1,33) = 13.61$, $P = 0.001$, $\eta^2 = 0.292$), but was also significant following exposure to pleasant pictures (pleasant inter-block interval anxiety $F(1,33) = 4.34$, $P = 0.045$, $\eta^2 = 0.116$; see Fig. 2, bottom right). An analysis based on a median split of trait anxiety scores confirmed that those with higher trait anxiety had a greater number of skin conductance responses after unpleasant pictures compared to those with low trait anxiety (trait anxiety × valence $F(2,66) = 4.35$, $P = 0.019$, $\eta^2 = 0.117$; unpleasant inter-block

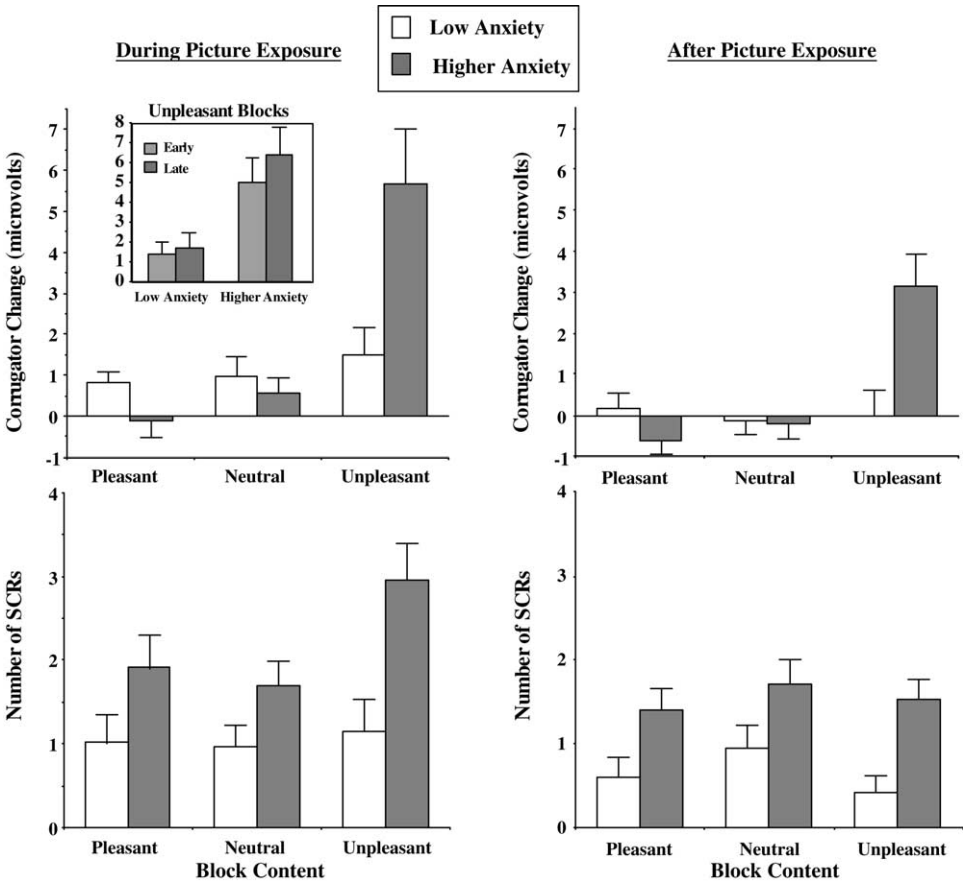

Fig. 2. Corrugator change (top left) and number of skin conductance responses (SCRs) (bottom left) during exposure to blocks of pleasant, neutral, and unpleasant pictures, and corrugator change (top right) and number of skin conductance responses (bottom right) following exposure to picture blocks, for those who scored low (n = 18; white bars) and higher (n = 18; gray bars) on a state anxiety questionnaire (STAI-Y1) prior to the experiment. The data are the mean change in microvolts from the pre-series baseline (±S.E.) for corrugator, and the mean number of SCRs (±S.E.) greater than 0.05 μS for skin conductance.

interval trait anxiety $F(1,33) = 7.89$, $P = 0.008$, $\eta^2 = 0.193$); however, the effect for trait anxiety was not significant during picture viewing.

4. Discussion

4.1. Cumulative defensive activation

Sustained exposure to an unpleasant affective context prompted cumulative effects on the magnitude of the startle eye blink and corrugator responses that support a response

sensitization hypothesis. With repeated exposure to different unpleasant exemplars, startle reflexes showed greater potentiation, and corrugator EMG activity increased. Furthermore, the cumulative effects of aversive exposure on corrugator EMG activity was greater for individuals with higher state anxiety, supporting an interpretation of increased defensive activation.

Both affective startle and corrugator modulation persisted at least 30 s following picture exposure. Blinks were larger following exposure to unpleasant pictures, compared to pleasant pictures, and corrugator activity remained significantly elevated following sustained exposure to unpleasant pictures. These findings extend those reported by Bradley et al. (1996), in which brief (i.e., 6 s) inter-trial intervals were explored. Moreover, these data are consistent with Garrett and Maddock (2001) who reported self-evaluative judgements of negative emotion persisted for several seconds following blocks of unpleasant pictures. In the current study, startle potentiation following unpleasant picture viewing persisted over at least 30 s, primarily for those subjects who saw the unpleasant pictures first. Perhaps the prior positive experience in the picture-viewing paradigm reduced the impact on those viewing unpleasant contents later. On the other hand, corrugator activity persisted following unpleasant blocks regardless of presentation order. Furthermore, the persistence of corrugator EMG activity following aversive exposure was significantly larger in those with higher state anxiety, consistent with the hypothesis that sustained exposure to an unpleasant context resulted in increasing defensive activation that persists after the foreground stimuli are no longer present.

4.2. Cumulative appetitive effects

Evidence for augmented appetitive processing during sustained exposure was somewhat weaker, as reflex magnitude did not decrease from early to late in a block of pleasant pictures. On the other hand, across the entire series of pleasant pictures (i.e., from the first to the third picture block) startle magnitude did significantly decrease, suggesting increasing appetitive activation with prolonged exposure. An alternative hypothesis, of course, is that this decrease is due to habituation, since, in general, the blink reflex decreases in magnitude with repeated startle elicitation (Blumenthal, 1997). However, the experiment was designed to reduce the effects of habituation; i.e., there were three noise-alone trials prior to picture viewing and an initial practice series of neutral pictures (a total of 15 noise stimuli). Under these conditions, the modulatory effects of emotion are less likely to be compromised (Bradley et al., 1993; Wedeking and Carlton, 1979). Furthermore, startle attenuation was specific to the pleasant series, and was not present in the middle neutral series. Thus, the decrease in reflex magnitude following prolonged exposure to pleasant pictures is most likely attributable to increasing appetitive activation.

4.3. Autonomic responses

There were significantly more skin conductance responses elicited during sustained exposure to unpleasant, compared to other, pictures. Moreover, this effect was greater for those reporting higher state anxiety both during and following picture exposure, again consistent with the idea that these reactions are mediated by defensive activation. Overall,

skin conductance level decreased across a series, presumably reflecting overall habituation in this electrodermal measure as the task context became more familiar (Montague, 1963).

Increased heart rate deceleration for unpleasant, compared to other, pictures was maintained across the exposure series, as expected if cardiac activity reflects initial orienting and stimulus intake that is heightened for threatening materials (Campbell et al., 1997). Following exposure to unpleasant pictures, heart rate increased. This apparent rebound could also reflect continued mental processing, as heart rate reliably increases during aversive imagery (e.g., Vrana and Lang, 1990).

4.4. Pictures and state anxiety

The current findings indicate that sustained exposure to unpleasant pictures prompts increasing startle potentiation and facial frowning that persist for up to 30 s following exposure, consistent with the idea that prolonged exposure to aversive stimuli leads to increasing defensive activation. An anxious mood state appeared to modulate affective reactions both during and subsequent to sustained aversive exposure. Although the participants in this study were not selected for mood disturbances, and in general were within the normal range for indices of state and trait anxiety, those who reported higher state anxiety showed larger increases in corrugator EMG activity, and a greater number of skin conductance responses both during and following exposure to unpleasant pictures. The possibility exists that the anxiety group difference is attributable to an abnormally reduced response by low state anxiety participants to the sustained unpleasant blocks. However, similar previous work with anxiety patients (Roth et al., 1990) suggests that it is indeed the higher anxiety group that is responsible for the effect. In summary, these data show that sustained exposure, particularly to unpleasant pictures, may serve as a useful short-term mood induction paradigm (see Bradley et al., 1996), and furthermore, that it may be an appropriate technique for understanding affective physiology among individuals who vary in symptoms of anxiety.

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