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Review

# Physical activity does not disturb the measurement of startle and corrugator responses during affective picture viewing

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**Abstract**

Healthy college females ($n = 24$) completed counterbalanced conditions of 20 min of very low and low intensity cycling exercise and seated rest. Startle and corrugator supercilii responses, and baseline orbicularis oculi and corrugator supercilii electromyographic (EMG) activity, were measured during each exercise condition while participants viewed pleasant, neutral and unpleasant pictures. The exercise conditions did not alter the magnitude of the startle or corrugator responses compared with the resting control condition. Baseline orbicularis EMG increased slightly and baseline corrugator EMG increased significantly, during the low intensity exercise condition. In conclusion, low intensity physical activity is not sufficient to alter emotional responsiveness as assessed by the acoustic startle eyeblink response and corrugator supercilii EMG responses during the viewing of pleasant, neutral, and unpleasant pictures. Despite modestly increased baseline EMG levels, reliable startle and corrugator EMG responses can be obtained in healthy college females during picture viewing while performing low intensity cycling exercise.


*Keywords:* Physical activity; Startle response; International Affective Picture System; Perceived exertion; Muscle pain

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## 1. Introduction

The acoustic startle response has been shown repeatedly to be modulated by an organism's motivational state, both in rats and in humans (Davis et al., 1999; Lang et al., 1998). Fear-potentiated acoustic startle has been demonstrated in rats, and in humans acoustic startle eyeblink response attenuation and augmentation during the viewing of pleasant and unpleasant pictures, respectively, has been reported consistently in the literature (Bradley, 2000; Davis et al., 1999; Lang et al., 1998). To date, most studies have been conducted while human participants were in a stationary seated position or while rats were not moving in their cages. Thus, the aims of this experiment were: (1) to examine baseline electromyographic (EMG) activity in the orbicularis oculi and corrugator supercilii muscles during cycling exercise; (2) to examine if startle eyeblink responses could be reliably measured during cycling exercise; and (3) to examine if cycling exercise altered the established effects of affective picture viewing on startle eyeblink and corrugator supercilii responses.

There are two proposed neural pathway for the acoustic startle reflex, the primary pathway from the cochlea to the facial motor nucleus (Davis et al., 1999), and a modulatory pathway from the amygdala that may, depending on the motivational state of the organism, modify the output to the facial motor nucleus (Davis et al., 1999). The consistently observed affective startle modulation effect has been attributed to the manipulation of this secondary pathway. This brings up several possibilities regarding the potential effects of motor activity on the startle response: motor activity could interfere with the primary acoustic startle pathway, resulting in a complete lack of a startle reflex, or it could affect only the modulatory pathway, resulting in an altered pattern of affective modulation. In addition, the motivational state associated with any particular motor activity, such as moving away from a painful stimulus or approaching a plateful of appetizing food, may interact with the primary and secondary startle reflex pathways.

Rodent research has indicated that movement coincident with the startle eliciting stimulus drastically attenuates the startle response (Wecker and Ison, 1986). However, these movements have been related to consuming, grooming, face washing, and sniffing behaviors. Thus, it is not clear if the appetitive motivational state of the animal contributed to this effect or if the reduction in startle amplitude was related to motor activity alone. It is clear that there is a need to distinguish effects of motor activity from motivated activity.

Human acoustic startle responses have been observed in the biceps femoris, rectus femoris, tibialis anterior, and soleus muscles during walking (Nieuwenhuijzen et al., 2000) and during simultaneous isometric contractions of multiple skeletal muscles (Aniss et al., 1998). It is unclear, however, if reliable startle eyeblink responses can be measured in humans during dynamic activation of the quadriceps muscle group. Also uncertain is whether the activation of a muscle group mobilized in response to threat alters the established effects of unpleasant emotional foreground stimuli on startle eyeblink magnitude. In order to more completely address the effects of motor activity on emotional responsiveness, EMG activity of the corrugator supercilii, a muscle known to be active in response to various unpleasant stimuli (Fridlund and Cacioppo, 1986), was also recorded. A tertiary purpose was to examine baseline tension in the orbicularis oculi and corrugator supercilii muscles during cycling exercise.

## 2. Method

### 2.1. Participants

Twenty-eight female students were recruited from exercise science courses at the University of Georgia and received extra course credit for participation. A female sample was employed because females show more reliable affective modulation of facial EMG during picture viewing compared with males (Bradley et al., 2001). All participants signed an informed consent form approved by the Institutional Review Board. Four volunteers were excluded from participation. Two participants were excluded because they reported the use of anti-depressant medication and a third potential participant was excluded because she reported the current use of methylphenidate (Ritalin); a fourth was excluded because she reported a complete lack of sleep during one night immediately prior to a testing session. All other participants were medication free, except ten who reported taking oral contraceptives. Our prior findings showed no effect of oral contraceptives on startle and corrugator responses (Smith et al., 2002). The final sample ($n = 24$) had a mean ($\pm$ S.D.) age of 21 (1 year), height of 165 (7 cm), weight of 64.2 (14.5 kg), $\dot{V}O_{2peak}$ of 38.2 (8.3 ml kg$^{-1}$ min$^{-1}$) (normative value = 35.2, American College of Sports Medicine, 1995), and a self-reported 7-day physical activity history of 122 (17 kJ kg$^{-1}$ per day) (comparative value = 150.4 (14.4); Dishman and Steinhardt, 1988). In addition, these participants had a mean ($\pm$S.D.) trait anxiety score of 36.1 (8.1) (normative value = 40.4 (10.2); Spielberger et al., 1983) and a BDI score of 7.1 (6.4) (normative value = 10.9 (8.1); Beck et al., 1961).

Based on an expected moderate effect size (Cohen's d = 0.8) for the exercise × valence interaction, moderate ($r = 0.4$) average correlations between repeated measures across exercise conditions and large average correlations ($r = 0.8$) between repeated measures for valence, 24 subjects provided an a priori power of 0.94 to detect a significant 3 (exercise) × 3 (valence) interaction at $\alpha = 0.05$ (Potvin and Schutz, 2000).

### 2.2. Experimental design

A repeated measures design was employed. The participants each completed four days of testing. Two repeated measures factors were employed, exercise condition [rest, very low intensity cycling (30 watts at 60 rpm = 14 $\pm$ 2% peak power output, ~ 20% $\dot{V}O_{2peak}$), and low intensity cycling (86 $\pm$ 14 watts at 60 rpm = 40% peak power output, ~ 50% $\dot{V}O_{2peak}$)] and picture valence [pleasant, neutral, and unpleasant]. The primary dependent variables were (1) startle eyeblink response magnitude (microvolts) and (2) corrugator EMG responses during each exercise condition while viewing pleasant, neutral and unpleasant slides; and (3) SAM ratings of valence, arousal, and dominance in response to each exercise condition. Ratings of perceived exertion and heart rate (HR) were obtained during each exercise condition as a manipulation check on the exercise intensity.

### 2.3. Materials

Four different slide shows were constructed from 84 color pictures chosen from the International Affective Picture System (IAPS; Center for the Study of Emotion and Attention, 1999). The same slide shows that were used in a previous study were employed here to facilitate comparisons to prior work (Smith et al., 2002). One slide show was viewed twice on the first day of testing; once during a self-assessment Manikin (SAM; Lang, 1980) rating procedure and again during an acoustic startle eyeblink accommodation procedure. Participants then viewed different slide shows during each of the three exercise conditions on subsequent test days.

Each slide show contained 21 slides, seven from each of three valence categories (pleasant, neutral, and unpleasant). Pleasant and unpleasant slides with the highest arousal ratings were chosen from the IAPS (the Appendix A lists the IAPS identification numbers for the pictures employed). All four slide shows were constructed to reflect similar mean valence ratings based on SAM norms. The 21 slides in each slide show were arranged into three blocks of seven slides. The order of the slides in each block was determined pseudo-randomly by shuffling the slides by hand. Each block of seven slides contained six slides that were presented with the acoustic stimulus (two pleasant, two neutral, and two unpleasant slides) and one slide from one of the three valence categories that was presented with no acoustic stimulus.

Participants viewed each slide show while seated on a Monark (model 818E) cycle ergometer in a 2.1 m $\times$ 2.7 m sound attenuated, environmentally controlled (23 $\pm$ 1 °C, 40 $\pm$ 3% relative humidity, 50 $\pm$ 2 lux illumination when the slide projector was off) chamber (Lingren, Inc.). The slides were projected from a Kodak Carousel 4600 projector located outside the chamber onto a white wall approximately 2.2 m in front of the participant. Each participant was provided standardized verbal instructions to maintain a consistent very light grip on the handle bars (based on a rating of perceived exertion corresponding to the verbal anchor 'very light'), to attend to each slide the entire time it was shown, and to ignore the occasional sounds from the headphones. The nine-point SAM was employed for affective ratings of the slides and in response to each exercise condition (Lang et al., 1999; Bradley and Lang, 1994). The SAM is a pictorial scale that assesses three dimensions of subjective affect; valence (pleasant to unpleasant), arousal (excited to calm), and dominace (feelings of being controlled vs. in control).

### 2.4. Procedures

Testing occurred on 4 days during a 1 week period. In order to minimize the potential effects of menstrual phase, participants were tested in their early follicular phase (i.e. during the week following the end of menses). Menstrual phase was confirmed by verbal and written self-report.

On day 1 participants read and signed the informed consent form, completed the medical history and anxiety and depression questionnaires, and provided responses to Blair's (1984) 7-day physical activity recall interview. All participants reported some experience with bicycling exercise. Participants were next prepared for measures of orbicularis oculi and corrugator supercilii EMG activity. Then, each participant sat in the environmental chamber on the cycle ergometer and listened to recorded instructions regarding the use of the SAM. The participants provided SAM ratings of valence, arousal, and dominance to pleasant, neutral, and unpleasant slides during an experimenter controlled (via IBM-PC) presentation of an IAPS slide show. Immediately after each picture was viewed the participants were visually prompted to rate their emotional response to the slide using the SAM. The purpose of these SAM ratings was to check whether the subjective ratings of pleasantness were consistent with the intended valence of the slides.

Participants viewed the same slide show again on day 1 while acoustic startle eyeblink responses were evoked. These responses were not scored or analyzed, but served as an accommodation trial to the noise stimulus. An analysis of data we collected in a previous study on three separate days when subjects viewed different pictures each day indicated that startle response magnitude decreased from day 1 to day 2, but remained stable from day 2 to day 3. Thus, more reliable measures of startle magnitude were obtained on the second and third days of testing, resulting in one-way random effects intra-class Rs = 0.94, 0.92 and 0.92 for pleasant, neutral, and unpleasant slides, respectively (data from Smith et al., 2002; see also Larson et al., 2000). Lastly on day 1, participants completed an exercise test to determine peak rate of oxygen consumption.

#### 2.4.1. Determination of $\dot{V}O_{2peak}$

The electronically braked cycle ergometer (Mijnhardt) continuously increased resistance at a rate of 20 W min$^{-1}$. Prior to each exercise session, a SensorMedics metabolic cart (Model 2900) was calibrated with concentrations of known gas samples (Scholander, 1947). The metabolic cart obtained measures of ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER; i.e. $\dot{V}CO_2/\dot{V}O_2$) every 20 s throughout the exercise test. HR was continuously displayed only to the experimenter with a UNIQ HR monitor. Ratings of perceived exertion using a validated 6–20 scale were obtained every 2 min during exercise until volitional exhaustion (Borg, 1998). Prior to exercise, explicit instructions for scale use were provided verbally (Cook et al., 1997). All except two participants met at least two of the following criteria: (1) a respiratory exchange ratio above 1.10; (2) a rating of perceived exertion of at least 18; and (3) a HR at least 90% of age predicted maximal HR (220 minus age).

### 2.4.2. Exercise conditions

The order of the exercise conditions on days 2, 3 and 4 was counterbalanced among participants. After the exclusion of four participants, each of the six possible orders of the exercise conditions were completed by four participants, except two orders (very low, low, rest and low, very low, rest) that were competed by three and five participants, respectively. There were no statistically significant effects of either the order of the exercise conditions or the day of testing on the dependent measures.

State anxiety was measured upon entry into the laboratory each day (STAI-Y1, Spielberger et al., 1983). Next, the participant was prepared for EMG recordings of orbicularis oculi and corrugator supercilii muscle activity and HR. This procedure lasted approximately 10 min. Each exercise condition, which was conducted within the chamber, lasted 20 min. The participants sat on the cycle ergometer at a seat height that was comfortable and allowed for nearly full leg extension with their hands on the handle bars. The participant either (1) did not pedal (rest/very low arousal), (2) pedaled at 60 rpm at 30 watts, which was equal to $14 \pm 2\%$ peak power output and $\sim 20\%$ $\dot{V}O_{2peak}$ (very low intensity exercise/low arousal), or (3) pedaled at 60 rpm with a resistance designed to equal 40% peak power output (low intensity exercise/moderate arousal). This last exercise condition was achieved by having the participants pedal at a power output of $86 \pm 14$ watts, which was $\sim 50\%$ of $\dot{V}O_{2peak}$.

At min 6, after a steady metabolic state had been achieved during all exercise conditions, each participant viewed one of 4 slide shows lasting 14 min while eyeblinks were evoked and corrugator EMG activity was recorded. SAM ratings of valence and arousal were obtained while seated on the cycle ergometer prior to each exercise condition, at min 5, and at min 20 during each exercise condition by referring to the recorded instructions for the SAM and asking each participant to "rate how the exercise (or sitting here) makes you feel right now" (instructions modified from the IAPS technical manual). Ratings of perceived exertion (Borg, 1998 6–20 RPE scale) and pain intensity (0–10 category scale; 0.5 = pain threshold and 1 = weak pain) (instructions for both from Cook et al., 1997), and HR (UNIQ HR monitor) were obtained after SAM ratings were made. Immediately after each test session the participants were asked to rate subjectively how well they paid attention to all the pictures that were presented. A 100 mm visual analog scale with the verbal anchors "no attention" (0 mm) and "best possible attention" (100 mm) placed at each end of the scale was employed for ratings of attention to the pictures. The attention ratings were used to confirm that participants subjectively were able to pay attention to the pictures equally well in all three exercise conditions. The leg muscle pain ratings were employed to describe leg muscle pain intensity during the exercise and rest conditions.

## 2.5. Data collection and reduction

Participants were prepared for EMG recordings of the orbicularis oculi and corrugator supercilii according to the recommendations by Fridlund and Cacioppo (1986). The skin surface was abraded lightly with fine-grained sand paper (220 grains $\times$ in.$^{-2}$) and alcohol swabs. Four miniature Beckman Ag–AgCl biopotential surface electrodes were filled with EC2 electrode cream and attached near the left eye. A fifth pre-gelled Ag–AgCl electrode (Eaton Electrode Co., Ann Arbor, MI) was placed medially on the forehead just below the hairline and serve as the common reference. Electrode impedance was verified as less than 5 kohms with a Grass Electrode Impedance Meter (Model EZM 4).

The acoustic stimulus was a 50 ms, 110-dB burst of white noise produced by a Grason–Stradler 455C noise generator (West Concord, MA) and passed through a Massey Dickson amplifier (Massey Dickson, Saxonville, MA) with a $<1$ ms rise/fall time. The acoustic stimulus was delivered binaurally through Sony Dynamic Stereo Headphones (Model MDR-V200). Prior to each experimental session the intensity of the acoustic stimulus was calibrated at the surface of the headphone using a General Radio 1551-C decibel meter (General Radio Co., Concord, MA).

Raw EMG signals were amplified 5000 times using Grass P511 amplifiers (Astro-Med, Inc., West Warwick, RI) with half-amplitude settings of 1 and 1000 Hz. The Grass P511 amplifiers were calibrated prior to each experimental session. A steep-rise impulse of 1 mV for a duration of a 500 ms was applied from an internal calibration source and the output of the calibration signal from the P511 amplifier was measured on an oscilloscope. Raw EMG signals were routed to an A/D board interfaced with an IBM-PC, digitized at 800 Hz, then displayed digitally using POLYVIEW version 2.0 software (Astro-Med, Inc.). Corrugator supercilii and orbicularis oculi EMG responses were integrated off-line using a time constants of 600 and 100 ms, respectively.

Corrugator baseline activity was defined as the mean integrated EMG (IEMG) activity in microvolts during the 1 s prior to slide onset. Corrugator responses were defined as the difference in microvolts between baseline IEMG activity and the mean IEMG activity during the 6 s slide interval. Two participants exhibited excessive corrugator activity throughout the very low intensity cycling condition. These data were discarded and the corrugator data were analyzed using $n = 22$. For eyeblink responses, the magnitude of the highest peak within 150 ms after the onset of the noise stimulus was determined manually according to the scoring criteria described by Balaban et al. (1986). There were no statistically significant differences between exercise conditions or slide valence categories for the number of missing blinks.

### *2.6. Data analysis*

Measurements of startle magnitude, corrugator supercilii responses, and baseline orbicularis and corrugator EMG were analyzed with 3 (exercise) × 3 (valence) repeated measures ANOVAs. HR and ratings of perceived exertion during each exercise condition, as well as SAM ratings of valence, arousal, and dominance during each exercise condition and in response to the pictures during the slide viewing session on day 1, were examined using 3 (exercise) × 3 (time) repeated measures ANOVAs. For all repeated measures ANOVAs the Greenhouse–Geisser epsilon (ε) was employed to adjust the degrees of freedom when the sphericity assumption was not met (i.e., if Mauchly's test of sphericity was statistically significant at $P < 0.05$) (Keselman, 1998). Effect sizes associated with $F$-statistics were expressed as eta-squared ($\eta^2$). Effect sizes based on mean differences were expressed as Cohen's d. The family-wise error rate was controlled using the Sidak adjustment when tests for simple effects and contrasts were conducted (Sidak, 1967).

## 3. Results

The analyses of the primary dependent variables showed that: (1) mean baseline orbicularis oculi EMG activity was slightly increased during exercise but was not significantly different across exercise conditions; (2) mean corrugator supercilii baseline EMG activity was significantly greater during low intensity cycling compared with during seated rest; (3) startle response magnitude was significantly larger during unpleasant and neutral slides compared with during pleasant pictures; however, startle response magnitude was not significantly different across the exercise conditions; and (4) corrugator supercilii responses were significantly greater during unpleasant compared with during pleasant pictures; however, corrugator supercilii responses were not significantly different across the exercise conditions.

### *3.1. Baseline EMG*

Mean orbicularis oculi EMG data prior to pleasant, neutral, and unpleasant slides during conditions of seated rest, very low intensity cycling, and low intensity cycling are shown in Table 1 (top panel). There was not a significant main effect for exercise for orbicularis oculi baseline EMG activity [$F(2, 46) = 2.387$, $P = 0.103$, $\eta^2 = 0.094$]. Non-significant effects for valence and the exercise × valence interaction [$F < 1$] also were found. Simple contrasts between the mean baseline orbicularis oculi EMG across valence categories revealed non-significant differences between the rest and very low intensity cycling condition ($P = 0.163$) and between the rest and low intensity cycling condition ($P = 0.146$).

Table 1
Mean (±S.D.) orbicularis oculi and corrugator supercilii baseline EMG (μV) during conditions of seated rest, very low intensity (14±2% peak power output), and low intensity (40% peak power output) cycling exercise 1 s prior to the presentation of each pleasant, neutral and unpleasant picture

| | Seated rest | Very low intensity | Low intensity |
|---|---|---|---|
| *Orbicularis oculi baseline EMG* | | | |
| Pleasant | 19.6 (12.5) | 30.5 (21.3) | 27.1 (17.5) |
| Neutral | 21.5 (14.3) | 27.8 (20.7) | 29.2 (24.8) |
| Unpleasant | 21.7 (12.8) | 30.3 (21.6) | 31.1 (21.0) |
| *Corrugator supercilii baseline EMG* | | | |
| Pleasant | 80.7 (46.0) | 102.4 (61.5) | 107.7 (62.6) |
| Neutral | 79.9 (45.0) | 109.0 (60.7) | 110.1 (61.7) |
| Unpleasant | 80.8 (43.3) | 99.3 (56.9) | 107.6 (63.0) |

Mean baseline corrugator supercilii EMG data prior to pleasant, neutral, and unpleasant slides during conditions of seated rest, very low intensity cycling, and low intensity cycling are shown in Table 1 (bottom panel). A significant main effect for exercise was observed for baseline corrugator supercilii EMG activity [$F(2, 42) = 3.694$, $P = 0.033$, $\eta^2 = 0.150$]. Contrasts between exercise conditions showed that corrugator baseline EMG activity was significantly greater during low intensity cycling compared with during seated rest ($P = 0.025$), and nearly significantly greater during very low intensity cycling compared with during seated rest ($P = 0.072$). As expected, there was no effect of slide valence [$F(2, 42) = 1.720$, $P = 0.191$, $\eta^2 = 0.076$] on baseline corrugator EMG activity (as the upcoming picture had not been presented), and no exercise × valence interaction [$F(4, 84) = 1.093$, $P = 0.350$, $\eta^2 = 0.049$, $\varepsilon = 0.581$].

### 3.2. Startle magnitude

Mean startle magnitude during pleasant, neutral, and unpleasant pictures during conditions of seated rest, very low intensity cycling, and low intensity cycling are shown in Fig. 1(top panel). A significant main effect for valence was observed for startle response magnitude [$F(2, 46) = 7.922$, $P = 0.007$, $\eta^2 = 0.256$, $\varepsilon = 0.578$]. Simple contrasts between valence categories showed that the mean startle response magnitude during pleasant slides was less than during both neutral ($P = 0.032$) and unpleasant slides ($P = 0.013$). There was neither a significant main effect for exercise [$F(2, 46) = 2.726$, $P = 0.076$, $\eta^2 = 0.106$] nor a significant exercise × valence interaction [$F(4, 92) = 0.989$, $P = 0.417$, $\eta^2 = 0.041$, $\varepsilon = 0.630$].

### 3.3. Corrugator response

Corrugator supercilii responses during pleasant, neutral, and unpleasant pictures during conditions of seated rest, very low intensity cycling, and low intensity cycling are shown in Fig. 1(bottom panel). A significant main effect of valence was observed for corrugator supercilii responses [$F(2, 42) = 7.802$, $P = 0.001$, $\eta^2 = 0.271$]. Simple contrasts between each valence category showed that the mean corrugator responses during unpleasant slides were significantly greater than during pleasant slides ($P = 0.007$). There was not a significant main effect for exercise [$F(2, 42) = 2.168$, $P = 0.140$, $\eta^2 = 0.094$, $\varepsilon = 0.782$] nor a significant exercise × valence interaction [$F(4, 84) = 1.666$, $P = 0.192$, $\eta^2 = 0.074$, $\varepsilon = 0.635$]. A separate one-way repeated measures ANOVA comparing exercise conditions during the viewing of unpleasant slides was conducted, followed by contrasts between exercise conditions. This analysis showed non-significant differences between corrugator responses to unpleasant slides during seated rest compared with during very low intensity cycling ($P = 0.073$) and low intensity cycling ($P = 0.328$).

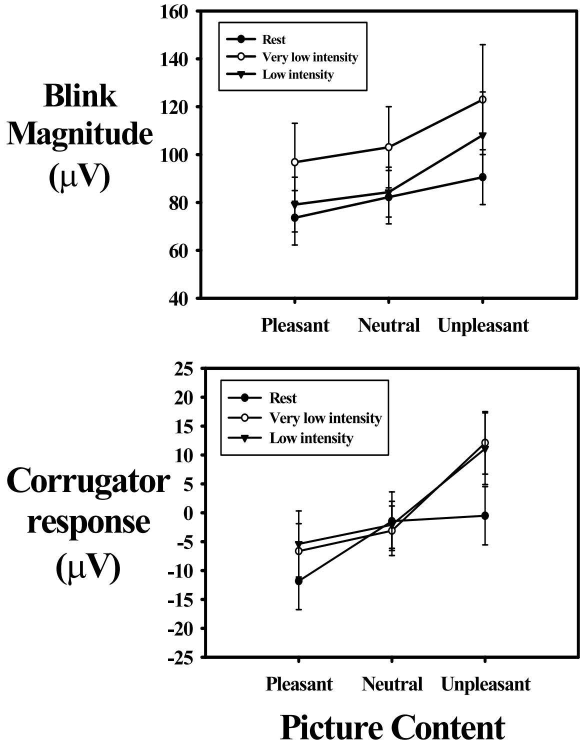


Fig. 1. Acoustic startle eyeblink magnitude (μV; top panel) and corrugator supercilii responses (change from baseline in μV; bottom panel) all as a function of picture content during conditions of seated rest, very low intensity (14 ± 2% peak power output), and low intensity (40% peak power output) cycling exercise. Data are expressed as mean (± S.E.).

### 3.4. Manipulation checks

Mean SAM ratings in response to pleasant, neutral, and unpleasant pictures on day 1 were collapsed across slide shows. The sample ratings did not differ from normative data for college women. SAM ratings of arousal, valence, and dominance during the exercise and seated rest conditions are shown in Table 2. As expected, there were no differences between exercise conditions for SAM ratings of arousal, valence, or dominance prior to each exercise condition. The analysis of arousal ratings during the exercise conditions showed a significant exercise × time interaction [$F(4, 92) = 6.681$, $P = 0.001$, $\eta^2 = 0.225$, $\varepsilon = 0.719$] and significant main effects for exercise [$F(2, 46) = 10.008$, $P < 0.001$, $\eta^2 = 0.303$] and time [$F(2, 46) = 22.167$, $P < 0.001$, $\eta^2 = 0.491$, $\varepsilon = 0.649$]. Arousal: (1) increased from pre- to min 5 through min 20 during very low intensity cycling; (2) increased significantly through min 5 and from min 5 to min 20 during low intensity cycling; and (3) was significantly higher during low intensity cycling compared with during seated rest and very low intensity cycling.

Table 2
Self-assessment Manikin ratings (1–9) of valence, arousal and dominance during the exercise and seated rest conditions

| | Pre-exercise | Min 5 | Min 20 |
|---|---|---|---|
| *Valence* | | | |
| Seated rest | 7.1 (1.6) | 6.8 (1.4) | 6.4 (1.6) |
| Very low intensity | 7.0 (1.6) | 7.0 (1.6) | 6.3 (1.5) |
| Low intensity | 7.2 (1.4) | 6.2 (1.7) | 5.4 (1.6) |
| *Arousal* | | | |
| Seated rest | 2.9 (2.1) | 3.0 (2.1) | 3.5 (2.0) |
| Very low intensity | 2.6 (1.9) | 3.3 (2.2) | 4.3 (2.1) |
| Low intensity | 3.0 (2.3) | 4.2 (2.0) | 5.5 (2.0) |
| *Dominance* | | | |
| Seated rest | 5.8 (2.4) | 5.9 (2.4) | 5.6 (2.4) |
| Very low intensity | 6.5 (2.2) | 6.3 (2.2) | 6.0 (2.1) |
| Low intensity | 6.5 (2.2) | 5.8 (1.7) | 5.0 (2.0) |

The analysis of valence ratings during the exercise conditions showed a significant exercise × time interaction [$F(4, 92) = 5.397$, $P = 0.002$, $\eta^2 = 0.190$] and significant main effects for exercise [$F(2, 46) = 4.157$, $P = 0.022$, $\eta^2 = 0.153$] and time [$F(2, 46) = 20.845$, $P < 0.001$, $\eta^2 = 0.475$, $\varepsilon = 0.711$]. Valence ratings were significantly lower (more unpleasant) at min 20 during low intensity exercise compared with all time points during the seated rest and very low intensity exercise conditions. The analysis of dominance ratings during the exercise conditions showed a significant exercise × time interaction [$F(4, 92) = 2.761$, $P = 0.045$, $\eta^2 = 0.107$] and a significant main effect for time [$F(2, 46) = 4.399$, $P = 0.036$, $\eta^2 = 0.161$, $\varepsilon = 0.633$]. After adjustment for multiple comparisons, dominance ratings did not differ significantly across time or among exercise conditions.

The descriptive data for HR, ratings of perceived exertion, and leg muscle pain ratings are shown in Table 3. HR was not different prior to each exercise condition and exercise intensity dependent increases in HR were observed. Ratings of perceived exertion increased with exercise intensity and were significantly different across exercise conditions throughout the 20-min test session. In addition, there were no differences in state anxiety scores prior to each test session, and VAS ratings of attention to the pictures did not differ significantly between exercise conditions.

The analysis of HR showed a significant exercise × time interaction [$F(4, 92) = 132.294$, $P < 0.001$, $\eta^2 = 0.852$] and significant main effects for Exercise [$F(2, 46) = 110.559$, $P < 0.001$, $\eta^2 = 0.828$] and time [$F(2, 46) = 235.679$, $P < 0.001$, $\eta^2 = 0.911$]. HR significantly increased from rest by min 5 during the very low intensity and low intensity exercise conditions, and significantly increased from min 5 to min 20 during the very low intensity and low intensity exercise conditions by 6 and 14 beats × $\min^{-1}$, respectively. As expected, mean HR was significantly higher during the low intensity exercise condition (136 beats × $\min^{-1}$) compared with the seated rest (81

Table 3
Mean (±S.D.) HR, ratings of perceived exertion, and leg muscle pain ratings before and during (min 5 and 20) seated rest, very low (14±2% peak power output) and low (40% peak power output) intensity cycling exercise

| | Pre-exercise | Min 5 | Min 20 |
|---|---|---|---|
| *Heart rate (beats × min$^{-1}$)* | | | |
| Seated rest | 77 (12) | 79 (11) | 82 (14) |
| Very low intensity | 81 (16) | 98 (13) | 104 (14) |
| Low intensity | 81 (14) | 129 (19) | 143 (22) |
| *Ratings of perceived exertion (6–20)* | | | |
| Seated rest | 6.0 (0.0) | 6.0 (0.0) | 6.0 (0.2) |
| Very low intensity | 6.0 (0.0) | 7.0 (0.6) | 8.4 (1.0) |
| Low intensity | 6.0 (0.0) | 8.7 (1.9) | 11.7 (1.9) |
| *Leg muscle pain ratings (0–10)* | | | |
| Seated rest | 0.0 (0.1) | 0.0 (0.1) | 0.0 (0.1) |
| Very low intensity | 0.0 (0.0) | 0.1 (0.3) | 0.4 (0.4) |
| Low intensity | 0.0 (0.1) | 0.8 (1.6) | 1.4 (1.2) |

beats × min$^{-1}$, $P < 0.05$) and very low intensity exercise conditions (101 beats × min$^{-1}$, $P < 0.05$).

Ratings of perceived exertion significantly increased from min 5 to min 20 during the very low intensity and low intensity exercise conditions (1.4 and 2.9 units, respectively). Ratings of perceived exertion were significantly higher at min 20 during low intensity exercise compared with during seated rest and very low intensity exercise ($P < 0.05$). Leg muscle pain intensity ratings increased from pre-exercise to min 20 during very low intensity cycling and increased from pre-exercise to min 5 during low intensity cycling ($P < 0.05$). The mean (±S.D.) state anxiety scores prior to testing on day 1 and prior to EMG preparation for the seated rest, very low intensity, and low intensity cycling conditions were 31.3 (±5.7), 33.2 (±9.6), 31.8 (±9.7), and 31.6 (±8.2), respectively. The mean (±S.D.) VAS ratings of attention for the seated rest, very low intensity, and low intensity cycling conditions were 90 (±7), 89 (±9), and 88 (±10) mm and did not differ significantly between exercise conditions. Attention ratings were obtained from 13 participants only.

## 4. Discussion

The novel finding from this experiment was that very low and low intensity cycling exercise did not affect the magnitude of the acoustic startle eyeblink response or corrugator supercilii EMG responses during the viewing of pleasant, neutral, and unpleasant pictures. In addition, baseline orbicularis oculi EMG activity did not change significantly during very low and low intensity exercise; however, baseline corrugator supercilii EMG activity significantly increased during low intensity exercise compared with during the control condition.

### 4.1. Baseline EMG

Baseline orbicularis oculi EMG activity did not significantly change during very low and low intensity exercise compared with during seated rest. However, baseline corrugator supercilii EMG activity significantly increased during very low intensity exercise compared with during the control condition. The results for baseline orbicularis oculi activity did not confirm the hypothesis that baseline orbicularis oculi activity would be increased during the exercise conditions. However, the hypothesis that baseline corrugator activity would be increased during exercise was confirmed.

### 4.2. Startle magnitude

These findings are in opposition to a study in rats that indicated startle magnitude decreased during movement (Wecker and Ison, 1986). However, Wecker and Ison did not address the potential effect on the startle response of the appetitive nature of the movements they observed (such as eating and grooming). Studies in humans: (1) have not examined affective modulation of the startle response during movement; (2) have not quantified exercise intensity; (3) have not manipulated physical activity much above a resting metabolic rate; but (4) have examined the effects of static contractions on startle responses. Dynamic contractions may more closely mimic real situations that may evoke movement toward a pleasant stimulus or away from an aversive stimulus. One ethical constraint and drawback of the laboratory environment is that the evocation of the full breadth of appetitive and defensive emotional activation is not possible. The viewing of emotional pictures does not elicit overt appetitive and defensive behavior. Nevertheless, several authors have postulated that active avoidance of threat may engage the appetitive motivational system and thus reduce defensive emotional responsiveness (Fowles, 1980; Patrick and Berthot, 1995; Miller et al., 1999). Lang and colleagues have also emphasized that emotions are action dispositions, and furthermore, that emotions are often the result of inhibited reflexive action in response to environmental events that cue appetitive or defensive motivational circuits (Lang et al., 1998). Based on these views, one could hypothesize that active engagement of muscles involved in moving one away from threat may diminish reflexive responses to aversive stimuli (such as the aversive startle probe). Nevertheless, this effect was not observed. The startle response was not decreased in magnitude during cycling, and furthermore, startle magnitude was modulated effectively by the affective foreground content of picture stimuli. These findings are consistent with those reported by Miller et al. (1999) in which finger tapping had no effect on fear-potentiated startle. This experiment extends this work by manipulating the activity of muscles more likely engaged in a response to threat at an intensity measurably above a resting metabolic rate, and also an intensity not distinctly pleasant or unpleasant to perform.

Very low intensity exercise and low intensity exercise, not exceeding 50% of maximal aerobic capacity, were employed in the present investigation in order to manipulate physical activity while minimizing the experience of an unpleasant affective state. This experimental design permitted an examination of the independent effect of motor activity on emotional responsiveness within a dimensional (i.e. valence-arousal) theory of emotion. The fact that low intensity physical activity had no effect on emotional responsiveness suggests that physical activity alone is not sufficient to augment either approach or withdrawal related emotional responsiveness to emotional foreground stimuli in normally physically active, healthy college females.

The main effect for the exercise conditions on startle response magnitude was marginally non-significant. Inspection of Fig. 1 suggests that the very low intensity exercise condition may be driving this potential effect. Nevertheless, when the difference in startle magnitude between neutral and unpleasant pictures was compared across exercise conditions (reflecting the effect of unpleasant pictures over neutral pictures for each exercise condition), the main effect was non-significant ($P = 0.18$) and the contrasts between exercise conditions were non-significant (seated rest vs. very low intensity, $P = 0.26$; seated rest vs. low intensity, $P = 0.48$). The most straightforward interpretation is that any difference between the exercise conditions occurred by chance.

It has been emphasized that attenuation and potentiation of the startle response is governed primarily by an interaction between the valence and arousal components of the emotional stimulus (Lang et al., 1998). It is plausible, however, that non-physically active individuals with elevated scores for trait anxiety or depression may respond differently to low intensity exercise paired with affective pictures than normally physically active, non-anxious, non-depressed individuals. In two recent studies, depressed females did not exhibit normal startle attenuation during pleasant picture viewing, and dysphoric females responded with an initial increase in corrugator activity during the viewing of happy faces (Allen et al., 1999; Sloan et al., 2002). Increased physiolgical arousal through exercise could augment responsiveness to pleasant stimuli, especially in those who may exhibit significant psychomotor retardation.

Another testable hypothesis along these lines is that an arousing, high intensity, exercise stimulus that also alters an individual's affective state, for example by evoking feelings of unpleasantness and pain (Cook et al., 1997), may be more likely to alter defensive emotional responsiveness. This same view could be applied to the findings in rats reported by Wecker and Ison (1986), in which motor activity related to appetitive-type behaviors resulted in an inhibition of acoustic startle responses.

A small amount of prior research has examined the influence of manipulating motor activity on the acoustic startle eyeblink response. Two studies have examined startle responses during dynamic and static muscle contractions. Research by Aniss et al. (1998) examined the effect of simultaneous static contractions of unknown intensity in several muscle groups, however no clear effects were observed on startle EMG responses measured in the orbicularis oculi or limb muscles. Sanes (1984) examined electrically elicited (trigeminal nerve) eyeblink reflexes during dynamic and static forearm contractions, requiring a force of 10 kg for a duration of 4 s. Blink reflexes (R2) were attenuated 50 ms after the initiation of dynamic contractions but returned to baseline 4000 ms after these contractions were initiated. Blink reflexes were attenuated at 50 and 100 ms after initiation of the static contractions (likely reflecting pre-pulse inhibition; Blumenthal, 1996) and were not different from baseline during the remaining 3200 ms after the initiation of the static contractions. The muscular contractions performed in these studies were brief and were not performed relative to each individual's maximum. Many physiological and perceptual responses to exercise are more closely related to the exercise intensity when it is expressed as a percentage of a person's maximum compared with when it is expressed as an absolute intensity, such as force in Newtons.

Comparisons of the present experiment with previous work should be made cautiously. The aforementioned investigations did not employ an activity mode, intensity, or duration that would be expected to increase the metabolic rate much above a resting level or did not examine affectively modulated reflexes. Aniss et al. (1998) examined the effect of simultaneous static concentric contractions of several muscle groups. No clear effects were observed for either increased or decreased startle EMG responses when measured in the limb muscles. Despite inadequate control over the exercise stimulus, the authors reported that the amplitude of the acoustic startle eyeblink response was consistently increased during these static contractions. It is plausible that static muscular contractions from several body regions contributed to this effect, whereas in the present study, dynamic muscular contractions of the legs were manipulated primarily.

### 4.3. Corrugator responses

There was no effect of exercise-induced activity on corrugator supercilii responses. Previous investigations that have examined the startle response during a manipulation of motor activity have not assessed corrugator responses. The current findings for corrugator responses are consistent with the findings for startle response magnitude and suggest that the neural system governing responses to aversive stimuli is relatively unaffected during low intensity cycling. Thus, the measurement of corrugator responses during affective picture viewing can be accomplished during low intensity cycle ergometry exercise.

### 4.4. Manipulation checks

As planned there were significant mean differences in arousal among the three exercise conditions as evidenced by SAM arousal ratings, HR, and ratings of perceived exertion. Low intensity cycling exercise was employed in order to manipulate motor activity while maintaining a similar affective valence associated with the performance of each exercise condition. Nevertheless, the present sample evidenced differences in SAM valence ratings and leg muscle pain intensity ratings between the exercise conditions. SAM ratings of valence reflected less pleasantness at min 20 during the low intensity cycling condition compared with during seated rest and very low intensity cycling exercise. Despite this difference, the mean rating of 5.4 at min 20 during low intensity cycling corresponds most closely with the neutral figure on the SAM (feelings described as neither pleasant nor unpleasant). Leg muscle pain intensity ratings increased from pre-very low intensity cycling (a mean rating of 0.0) to min 20 during very low intensity cycling (a mean rating of 0.4, most closely associated with the verbal anchor “very faint pain [just noticeable]”). Leg muscle pain intensity ratings also increased from pre-low intensity cycling to min 5 and then remained stable through min 20 during low intensity cycling (mean ratings of 0.8 and 1.4, respectively, both most closely associated with the verbal anchor “weak pain”). Thus, although valence ratings reflected more unpleasantness during low intensity cycling and faint or weak leg muscle pain was present during both of the cycling conditions, these differences were small and did not indicate that the cycling exercise was remarkably unpleasant.

### *4.5. Conclusion*

The present study, using experimental manipulations of increased motor activity, replicated several findings from prior research conducted while participants were seated at rest. Acoustic startle eyeblink response magnitude and corrugator responses varied significantly with the emotional content of the pictures (greater during unpleasant compared with during pleasant pictures; Bradley et al., 1990; Vrana et al., 1988). Self-assessment Manikin ratings were also obtained in response to the exercise during the maximal exercise test. As exercise intensity increased the participants rated their feelings during exercise as more aroused, more unpleasant, and less dominant. These findings are consistent with our previous observations of SAM ratings during maximal exercise testing and during exercise at 40 and 70% $\dot{V}O_{2peak}$ (Smith et al., 2002). The SAM appears to be a useful measure for assessing subjective emotion during exercise. The findings from this and our previous experiment (Smith et al., 2002) add to its established validity as a self-report measure of dimensional emotion (Bradley and Lang, 1994).

In conclusion, very low intensity and low intensity exercise-induced activity had no effect on startle responses and corrugator supercilii responses during the viewing of pictures designed to evoke pleasant, neutral and unpleasant emotions. Baseline orbicularis oculi activity increased, but did not significantly change during exercise, while baseline corrugator supercilii activity significantly increased during low intensity exercise. Despite increases in baseline muscular tension, valid measures of emotional responsiveness, as assessed by the acoustic startle eyeblink response and corrugator supercilii EMG responses during the viewing of pleasant, neutral, and unpleasant pictures, may be obtained in healthy college females during mildly painful, low intensity cycling exercise.

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## Appendix A: IAPS Slides Employed

Slide Show 1: pleasant, 8190, 4533, 5830, 8490, 8040, 4680, 5629*; neutral, 7006, 5533, 7050, 9210, 7560, 2720, 7170*; unpleasant, 3030, 9300, 6200, 9250, 3120, 9420, 6350*. Slide Show 2: pleasant, 8034, 4660, 8180, 4532, 8470, 1710, 5626*; neutral, 7550, 7002, 5532, 2690, 7150, 7040, 9070*; unpleasant, 9530, 3170, 6260, 9810, 3080, 6540, 1120*. Slide Show 3: pleasant, 4531, 4650, 8033, 5623, 8460, 2080, 8170*; neutral, 7035, 8260, 5531, 7000, 7140, 7500, 2410*; unpleasant, 9500, 6510, 3150, 3071, 9600, 6230, 2710*. Slide Show 4: pleasant, 4510, 8130, 8030, 8370, 4609, 2050, 5470*; neutral, 2210, 7233, 7830, 7030, 7100, 5520, 6150*; unpleasant, 6212, 3060, 9410, 6370, 9910, 3140, 9430*.

* Denotes slides that were presented without a noise stimulus.

Note: slide identification numbers for each slide show are grouped by valence and are not listed in the order in which they appeared during viewing.

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