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Affective modulation of eyeblink reactions to noxious sural nerve stimulation: A supraspinal measure of nociceptive reactivity?

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Abstract

Research suggests affective picture-viewing modulates subjective and physiological reactions to noxious stimulation (pain report, heart rate acceleration, skin conductance response, nociceptive flexion reflex). Because the nociceptive flexion reflex (a spinal reflex) is modulated by picture-viewing, this suggests affective processes are able to modulate afferent nociception at spinal levels. This highlights the importance of assessing nociceptive reactivity from physiological measures mediated at different levels of the neuraxis (spinal vs. supraspinal) to help elucidate the mechanisms associated with pain regulation. The present study examined whether affective pictures modulate eyeblink reactions (a supraspinal reflex) to noxious stimulation. Healthy men and women (N=23) were recruited from the psychology subject pool to participate. Pictures (attack, loss, neutral, food, erotica) that manipulated affective valence and arousal were presented and noxious electrodermal stimulations were delivered to the sural nerve. Picture duration (500-ms vs. 6-s) was also manipulated, balanced across picture content. Results suggested affective valence and arousal contributed to the modulation of eyeblinks. Eyeblinks were larger during highly arousing unpleasant pictures (attack) than highly arousing pleasant pictures (erotica), but low arousal pictures (loss, food) did not lead to significant modulation. Affective modulation was independent of picture duration or the perceived painfulness of noxious stimulation. This study suggests eyeblink reactions can serve as a supraspinal outcome in procedures used to study affective modulation of pain and nociception.

Keywords: Motivation; Defensive; Appetitive; Emotion; Eyeblink; Nociception; Shock

1. Introduction

The perception of pain is determined by the afferent pain signal (nociception), but also endogenous inhibitory and facilitatory processes that continuously modulate (regulate) nociception. Thus, understanding pain modulatory processes is important to our understanding of pain. Our laboratory has used motivational priming theory (MPT) as a theoretical framework to conceptualize and study pain modulation. MPT proposes that affective processes are organized around two opponent motive systems: one appetitive and one defensive (Bradley et al., 2001; Lang, 1995; Lang and Davis, 2006). The appetitive system is primarily activated in contexts that advance survival (e.g., sustenance, procreation), and appetitive activation promotes the subjective experience of positive affect. In contrast, the defensive system is activated in contexts involving threat (e.g., predation, attack), and defensive activation promotes the subjective experience of negative affect. MPT predicts that defensive responses should be inhibited by appetitive activation (positively valenced affect) and facilitated by defensive activation (negatively valenced affect). Supporting this, numerous studies have suggested that priming the defensive system using unpleasant stimuli results in larger startle reflexes (a defensive response), whereas priming the appetitive system with pleasant stimuli generally results in smaller startle reflexes (e.g., Grillon et al., 1993; Jansen and Frijda, 1994; Lang et al., 1990; Vrana et al., 1988).

Pain and pain-related reactions are considered defensive responses (e.g., Donaldson et al., 2003). Noxious stimuli (harmful or potentially harmful stimuli that activate Aδ and/or
C-fibers) elicit a repertoire of motoric (e.g., withdrawal reflexes), autonomic (e.g., HR acceleration), neuroendocrine (e.g., epinephrine release), and subjective (e.g., pain) reactions that motivate escape and protect an organism from harm. Thus, MPT predicts that affective processes should also modulate pain. Indeed, findings suggest positive affect inhibits pain and negative affect facilitates it (de Wied and Verbaten, 2001; Meagher et al., 2001; Zillmann et al., 1996). One limitation of this pain research, however, has been the reliance on subjective pain report and/or voluntary pain behaviors (e.g., latency of arm removal from cold water) as the primary dependent variables. While these outcomes are important, they can be biased by experimental demands. Based on these earlier studies alone, it is unclear whether affect modulates nociception or whether affect biases voluntary pain report/behaviors. To address this concern, recent pain research has included the measurement of involuntary physiological reactions to noxious stimulation to assess nociceptive processing.

For example, our laboratory has studied the influence of affective picture-viewing on the nociceptive flexion reflex (NFR). The NFR is a spinally-mediated withdrawal reflex elicited by activation of Aδ fibers (typically achieved by electrodermal stimulation over the sural nerve at the ankle) and measured from the biceps femoris of the thigh using EMG (France et al., 2002; Sandrini et al., 2005; Skljarevski and Ramadan, 2002; Wiesenfeld-Hallin et al., 1984; Willer, 1977). Because Aδ fibers (pain receptors) are necessary for its elicitation, and because the magnitude of this spinal reflex correlates with subjective pain intensity, the NFR has been used to assess spinal nociceptive processes (Chan and Dallaire, 1989; Guieu et al., 1992; Wiesenfeld-Hallin et al., 1984; Willer, 1977). Prior studies have shown that the NFR can be influenced by psychological factors, presumably via brain-to-spinal cord modulatory circuitry (Danziger et al., 1998; Kiernan et al., 1995; Willer et al., 1979). Thus, we predicted that affective processes could modulate the NFR. In the first study, Rhudy and colleagues (2005) presented erotic, neutral, and attack pictures during which unpredictable noxious stimulations were delivered to elicit pain and the NFR. Results suggested pain ratings and NFR magnitudes were facilitated during attack pictures and inhibited during erotic pictures (Rhudy et al., 2005). These findings were replicated and extended by a subsequent study designed to examine the unique influences of affective valence, affective arousal, and affective picture duration (Rhudy et al., 2007a). In brief, Rhudy et al. (2007a) found a valence-by-arousal interaction characterized the affective modulation of pain and NFR. Pain and NFR were inhibited by pleasant pictures and facilitated by unpleasant pictures, but only highly arousing pictures (erotic and attack) led to significant modulation relative to neutral pictures. These effects were produced by 6-s and 500-ms pictures, indicating even brief affective stimuli can modulate pain and nociception.

Pain modulation is a complex process mediated by multiple mechanisms that exert influence at different levels of the neuraxis (Fields and Basbaum, 1999; Watkins and Mayer, 1986). Our studies suggest affect engages descending circuits to modulate the afferent nociceptive signal at spinal levels before it is transmitted to supraspinal centers to be evaluated and experienced as pain (Rhudy et al., 2005). This would explain why the NFR and subjective pain are modulated in parallel. However, affect could engage cortico-cortical circuits that influence supraspinal nociception and subjective pain without necessitating modulation of spinal nociception. Indeed, another study was conducted by our laboratory that used the same picture-viewing methodology, except that half of the participants were randomly assigned to receive predictable (preceded by a cue) noxious stimulations during pictures (Rhudy et al., 2006). Whereas the results from participants receiving unpredictable noxious stimuli replicated our other studies (NFR and pain were inhibited by pleasant but facilitated by unpleasant pictures), the results from participants receiving predictable noxious stimuli did not. When noxious stimuli were predictable, subjective pain was inhibited by pleasant pictures and facilitated by unpleasant pictures; however, the NFR was not influenced by picture-viewing. This suggests descending circuits that modulate spinal nociception can be disengaged at the same time other mechanisms (e.g., cortico-cortical circuits) that modulate the evaluative component of nociception (subjective pain) are engaged. But because pain ratings are subjective, the possibility that pain report was biased by experimental demands in this study cannot be ruled out.

The results of Rhudy et al. (2006) highlight the importance of assessing multiple physiological reactions to noxious stimulation that are mediated by circuits organized at different levels of the neuraxis (e.g., spinal vs. supraspinal). Doing so could provide information about pain modulation pathways in humans, such as the level of the neuraxis where modulation is exerted. Moreover, our studies suggest the affective picture-viewing paradigm may be used to test the integrity of processes that modulate pain. Currently, there are no non-invasive procedures available to pain researchers that can be used to engage inhibitory and facilitatory processes in the same paradigm. Such a paradigm would be valuable, because some chronic pain disorders (e.g., fibromyalgia, headache) are thought to be initiated or maintained by dysfunctional pain modulation mechanisms. For example, underactive inhibitory processes may fail to keep nociceptive signals controlled (e.g., Lautenbacher and Rollman, 1997; Piels et al., 2005), or overactive facilitatory processes may amplify signals to cause persistent hyperalgesia (enhanced pain sensitivity) (e.g., Porreca et al., 2002). It is currently unknown whether mechanisms associated with affective modulation contribute to chronic pain, and if so, at what level of the neuraxis these modulatory mechanisms exert their effects. If altered modulatory patterns are detected in persons with chronic pain (e.g., attenuated pleasure-induced inhibition and/or augmented displeasure-induced facilitation), this could contribute to our understanding of the pathophysiology of the disease given that the circuitry responsible for affective modulation is becoming elucidated (Lang and Davis, 2006).

In an attempt to assess nociceptive reactivity more comprehensively, our laboratory has also measured autonomic reactions (heart rate [HR] acceleration, skin conductance response [SCR]) to noxious sural nerve stimulation during affective picture-viewing. These reactions were modulated by...
affective pictures in the same manner as pain and the NFR (Rhudy et al., 2007a; Rhudy et al., 2007c). In the present study, we assessed affective modulation of another response to noxious sural nerve stimulation: the eyeblink reflex measured from orbicularis oculi EMG. This measure was chosen because it has been used effectively within the picture-viewing paradigm and it is mediated by a supraspinal circuit (Aramideh and Ongerboer de Visser, 2002; Blumenthal et al., 2005; Koch, 1999; Lang and Davis, 2006).

1.1. Affective modulation of the startle eyeblink response

To our knowledge, every study that has examined affective modulation of the eyeblink reflex has used a non-noxious stimulus (also called a probe) to elicit the eyeblink component of the startle response (e.g., Blumenthal et al., 2005; Grillon and Baas, 2003). Nonetheless, the results of this research generally mirror what we have observed in our pain studies. Eyeblinks are facilitated by defensive activation (positive affect) and inhibited by appetitive activation (negative affect) (for reviews, Bradley et al., 1999; Grillon and Baas, 2003), although inhibition by appetitive activation is less robust (Grillon and Baas, 2003; Jackson et al., 2000). This modulation by affective valence1 (the unpleasantness–pleasantness dimension of affect) is most reliable when the probe onset is between 3 and 5 s after onset of the affect-inducing stimulus (3 to 5-s lead interval) (Bradley et al., 1993b, 2006), unless the task instructions are altered (e.g., Dichter et al., 2002; Vanman et al., 1996). Valence modulation during a 3 to 5-s lead interval has even been found for affective pictures presented for 300 to 500-ms (Codispoti et al., 2001; Larson et al., 2005).

Affective intensity/arousal also contributes to startle eyeblink modulation (Cuthbert et al., 1996). For example, Bradley and colleagues (2001) used 18 picture contents to manipulate affective valence and arousal. They found the degree of eyeblink inhibition by pleasant pictures was correlated with picture-evoked arousal (r = −.77; higher arousal = greater inhibition), and the degree of eyeblink facilitation by unpleasant pictures was correlated with picture-evoked arousal (r = .86; higher arousal = greater facilitation). This implies a valence-by-arousal interaction also characterizes affective modulation of the startle eyeblink reflex (Bradley et al., 2001; Cuthbert et al., 1996). However, the statistical significance of the valence-by-arousal interaction has not been directly tested in the startle eyeblink literature.

1.2. Eyeblink reactions to noxious stimulation: a supraspinal measure of nociceptive reactivity

Taken together, research on affective modulation of the startle eyeblink reflex suggests eyeblinks evoked by noxious sural nerve stimulation may also be modulated by affective picture-viewing. Further, the modulation pattern should parallel what our laboratory has observed for other nociceptive reactions (pain, NFR, HR acceleration, SCR). Specifically, eyeblinks elicited by noxious sural nerve stimulation should be modulated by 500-ms and 6-s pictures according to a valence-by-arousal interaction (Rhudy et al., 2007a). If true, eyeblinks may be a viable supraspinal measure of nociceptive reactivity.

There may be important differences between startle eyeblinks evoked by non-noxious stimuli and eyeblinks evoked by noxious electrodermal stimulation, however. For example, it is unclear whether the same supraspinal circuit mediates both responses. Whereas the startle eyeblink reflex is believed to be mediated by a circuit that includes the caudal pontine nucleus of the reticular formation (Lang and Davis, 2006), electrically-evoked eyeblinks may involve other brainstem circuitry (Aramideh and Ongerboer de Visser, 2002; Blumenthal et al., 2001; Sarno et al., 1997). While it is reasonable to assume the reflex arc of electrically-evoked eyeblinks involves a supraspinal circuit (Aramideh and Ongerboer de Visser, 2002; Koch, 1999), it is not known whether this circuit is modulated by affective processes. In addition, it is not clear whether shock exposure will sensitize the circuit in the same way that shock has been shown to sensitize the startle circuit (Greenwald et al., 1998). If the circuit does become sensitized, this could create a ceiling effect such that affective modulation cannot be observed.

An additional issue that must be addressed is whether electrodermal stimulation of the sural nerve (which allows elicitation of the spinally-mediated NFR) can evoke reliable eyeblinks. Although a number of laboratories have used electrically-evoked eyeblinks to study nociceptive reactivity, most stimulate nerves in the face/head (Drummond, 2003; Ellrich et al., 1997; Ellrich, 2002; Katsarava et al., 2003; Kaube et al., 2000; Sarno et al., 1997; Schmolesky et al., 1996; Willer et al., 1985). To evoke reliable eyeblinks from stimulation sites more distal, intense noxious stimuli may be necessary. Two studies found that non-noxious electric stimuli delivered to nerves of the arms and legs (including the sural nerve) did not evoke reliable eyeblinks in healthy subjects (Miwa et al., 1995; Miwa et al., 1998). In another study, electric stimuli set 50% above pain threshold only evoked eyeblinks in 35% of trials (Blumenthal et al., 2001). However, eyeblinks were evoked in 69% of trials (vs. 79% for 95 dB noise) when intense, 150 V, electric stimuli were delivered to the arm (Blumenthal and Swerdlow, 2002). But even then, the magnitude of electrically-evoked eyeblinks was considerably smaller than noise-evoked eyeblinks (Blumenthal and Swerdlow, 2002). Thus, to maximize response probability in the current study we delivered stimulations directly over a nerve and set the stimulus intensity above participants’ physiologically-determined nociceptive thresholds (120% NFR threshold), rather than a subjectively-determined criteria (pain threshold). These steps should have ensured that stimuli were noxious and sufficiently intense to elicit reliable eyeblinks.

1.3. The present study

In the present study, erotic, food, neutral, loss, and attack picture contents were presented in pseudorandom order. During
50% of pictures and 10 inter-picture intervals, noxious electric stimulations set at 120% NFR threshold were delivered to the sural nerve, and orbicularis oculi EMG was measured to assess eyelblink reactions. Half of the pictures were presented for 6-s and half for 500-ms. This study design allowed us to test the unique influences of affective valence, affective arousal, and affective picture duration on eyelblink reactions to noxious stimulation.

The primary goal of this study was to determine whether eyeblinks elicited by noxious electrodermal stimulation of the sural nerve were modulated by affective picture-viewing. In addition, two secondary goals were assessed. The first examined whether perceived painfulness (low vs. high) of electric stimulations moderated affective picture modulation. Although stimulus intensity was equivalent across individuals in its capacity to evoke spinal nociception (120% NFR threshold), research has demonstrated there are individual differences in the subjective painfulness of this stimulus level (e.g., France et al., 2002; Rhudy et al., 2007b). Such differences in painfulness could influence mood during picture-viewing, and negative mood has been shown to reverse (moderate) the typical pattern of valence modulation (Grüsser et al., 2007). The last goal was to examine if eyeblinks evoked during inter-picture intervals showed significant habituation like eyeblinks evoked by non-noxious probes (e.g., Bradley et al., 1993a; Ornitz and Guthrie, 1989a). These two exploratory analyses were conducted to inform how eyeblinks to noxious stimulation could be used in future studies of nociceptive reactivity.

1.3.1. Hypotheses
Consistent with studies of startle eyelink and nociceptive reactions (e.g., Bradley et al., 2001; Cuthbert et al., 1996; Rhudy et al., 2007a), it was predicted that the magnitude of eyblink reactions to noxious stimuli would be influenced by affective valence and arousal. Specifically, eyeblink magnitude was expected to be facilitated by unpleasant pictures and inhibited by pleasant pictures. However, pictures eliciting greater arousal were expected to show stronger modulation: attack pictures were expected to elicit the greatest facilitation and erotic pictures the greatest inhibition. Further, brief (500-ms) and longer (6-s) pictures were both expected to modulate eyeblinks (Codispoti et al., 2001; Larson et al., 2005; Rhudy et al., 2007a). Finally, onset latency was expected to be shorter during unpleasant pictures than pleasant pictures, because similar effects have been noted in studies of startle eyelink (Bradley et al., 1990, 1993b; Bradley et al., 2006). No directional hypotheses were made for exploratory analyses.

2. Materials and methods

2.1. Participants
The data analyzed for the present study were collected during our most recent study of affective modulation of nociceptive reactions (Rhudy et al., 2007a). In the last 23 participants recruited (of 29 total), orbicularis oculi EMG was measured. Participants (10 male, 13 female) 18 years of age and older were recruited from the psychology department. A healthy population was targeted by excluding participants for: self-reported cardiovascular, neurological, and/or circulatory problems, or Raynaud’s disease. Participants were also excluded for recent use of analgesic, anxiolytic, or antidepressant medication, recent psychological trauma, or specific phobia of snakes or spiders. Inclusion/exclusion criteria were assessed using a questionnaire designed specifically by our lab to assess for these criteria (Rhudy et al., 2005, 2007a). Two participants were excluded because a nociceptive flexion reflex could not be obtained (see Noxious Electrodermal Stimulation section below). One additional participant was excluded for equipment problems. The remaining 20 participants completed the study and are included in the analyses. Of the 20 participants, most were female (65%), White non-Hispanic (55%), single (95%), and worked less than 40 h per week (90%). Average age was 20.74 yrs (SD=1.88). All procedures were approved by The University of Tulsa ethics review board and all participants provided informed consent prior to participation.

2.2. Apparatus
Stimulus presentation and data acquisition were computer controlled using a PC equipped with dual monitors, A/D board (PCI-6036E; National Instruments, Austin, TX), and LabVIEW software. Noxious electrical stimuli were delivered using a Grass Instruments stimulator (Model S88, West Warwick, RI), stimulus isolation unit (Model SIU8T), constant current unit (Model CCU1), and a bipolar stimulating electrode (Nicolet, 019-401400, Madison, WI) attached to the left ankle over the retromalleolar pathway of the sural nerve. The maximum intensity was set at 40 mA. Psychophysiological signals were collected/filtered with a Grass Instruments Model 15LT Bipolar Amplifier with Quad AC (15A54) and Dual DC (15A12) modules. Before stimulating and recording electrodes were applied, the skin was prepared by first degreasing with alcohol and then gently abrading using NuPrep gel to reduce impedances below 10 KΩ. Orbicularis oculi EMG was amplified ×20,000 and bandpass filtered (30 to 1 KHz). EMG was sampled at 1000 Hz for 3-s before and 8-s after picture onset. The experimenters monitored the participants by video from an adjacent room. Questionnaires were presented by computer and responses were made by a computer mouse positioned on a lap desk.

2.3. Picture-viewing: affect-induction
Affect was induced by presenting pictures chosen from the International Affective Picture System (IAPS) (CSEA, 1999; Lang et al., 1999). Pictures were chosen to create 5 picture contents (erotic, food, neutral, loss, attack) that would independently manipulate valence and arousal. To do so,
pleasant pictures were chosen to create a high arousal picture set of 12 pictures (couples in erotic poses) and a low arousal picture set of 12 pictures (food). Unpleasant picture sets were also created with high arousal (12 attack scenes) and low arousal (12 loss/grief). A set of 12 neutral, low arousal pictures (household objects, mushrooms) was also used. A total of 60 pictures were presented. Mean normative valence and arousal ratings for each picture content were: erotic couples (valence: \( M = 6.66 \), arousal: \( M = 6.10 \)), food (valence: \( M = 6.88 \), arousal: \( M = 4.82 \)), neutral (valence: \( M = 4.93 \), arousal: \( M = 2.53 \)), loss (valence: \( M = 2.65 \), arousal: \( M = 4.56 \)), and human and animal attack (valence: \( M = 3.00 \), arousal: \( M = 6.35 \)). Thus, these picture contents allowed us to test the unique effects of valence (pleasant vs. unpleasant) and arousal (low vs. high) and a Valence × Arousal interaction. Pictures were presented on a computer monitor positioned approximately 5 m in front of the participant. Picture order was randomized with the limitation that no more than 2 pictures of the same content were shown consecutively. Intervals between pictures (inter-trial intervals, ITI) varied randomly from 12 to 22 s. Half of the pictures were presented for 500 ms and half for 6 s, balanced across picture content. Noxious stimuli were delivered 3 to 5 s following the onset of 50% of pictures (balanced across content and duration).

2.4. Manipulation checks: Self-Assessment Manikin ratings

The Self-Assessment Manikin (SAM; Bradley and Lang, 1994) consists of two sets of five pictographs depicting affective valence/pleasure (unpleasant—pleasant) and arousal (calm—excited). Affective responses to picture-viewing were rated using a computerized version of the SAM (Rhudy et al., 2005). Participants moved an indicator on or between any of the figures and submitted their answers by pressing a button. This yielded ratings between 1 and 9 for each dimension (higher scores = greater pleasure or arousal). SAM ratings were made immediately following each picture (Bradley et al., 2001; Schupp et al., 1997).

2.5. Noxious electrodermal stimulations

The level of noxious stimulation was calibrated to a percentage (120%) of the participant’s nociceptive threshold as assessed by NFR threshold (for a description of NFR recording methods, see Rhudy et al., 2005). Given the NFR is elicited by activation of Aδ fibers (nociceptors) (Sandrini et al., 2005; Skljarevski and Ramadan, 2002), stimulus intensities above NFR threshold are noxious. Electric stimulations were delivered with a bipolar stimulating electrode attached over the retromalleolar pathway of the left sural nerve. Stimulations consisted of 5 rectangular wave pulses of 1 ms duration at 250 Hz. To determine the stimulus intensity used during picture-viewing, stimulations were presented in an ascending/descending staircase starting at 0 mA and increasing by 1.5 mA steps (8 to 12-s intervals) until a NFR was detected, and then decreased in .75 mA steps until the NFR was no longer present. Starting at this intensity, the staircase was repeated twice with .5 mA steps. The average intensity of the last two peaks and troughs was increased by 20% and used as the stimulus intensity throughout picture-viewing. The average intensity used was 8.52 mA (SD = 6.33). Two stimulations were delivered at this intensity to assess baseline subjective pain before beginning the picture-viewing phase. Stimulations were delivered during 50% of pictures, balanced across picture content and duration. Stimulations were also delivered during 10 random inter-picture intervals to prevent participants from associating pictures with stimulations and to examine eyeblink habituation.

2.6. Eyeblink reactions to noxious stimulation

Eyeblink reactions to noxious stimuli were recorded from the left orbiculare oculi muscle with two Ag/AgCl electrodes placed 1 cm inferior to the median of the eye and the distal corner of the eye (Blumenthal et al., 2005). A common ground electrode was placed on the lateral epicondyle of the left femur (Rhudy et al., 2005). In post-processing, eyeblink magnitude and onset latency was scored from orbicularis oculi EMG after the signal was full-wave rectified and integrated using a second order Butterworth filter (10-ms time constant). Magnitude was scored by subtracting the mean of the 60-ms prior to shock onset from the maximum integrated response in the 60 to 200-ms post-stimulation interval. This eyeblink magnitude scoring interval was chosen to accommodate response delay due to nerve conduction from the sural nerve to the neck (Dowman, 1992; Vogel et al., 1986). Trials with clear movement artifact or excessive baseline activity (e.g., spontaneous eyblinks) were rejected (3.5% were rejected). Trials without a visible eyeblink were scored zero and reported.

2.7. Pain ratings of noxious stimuli

Following each electric stimulation, participants were presented with a computerized numerical rating scale (NRS) to rate the intensity of the stimulus to their ankle. The scale ranged from 0 at the bottom to 100 at the top with labels of 0 (no sensation), 1 (just noticeable), 25 (uncomfortable), 50 (painful), 75 (very painful), and 100 (maximum tolerable). Participants used a computer mouse to drag an indicator to specify the intensity of the stimulus, and submitted their answer by pushing a button. For the present study, pain ratings were used to assess for differences in perceived painfulness of the noxious stimuli in exploratory analyses.

2.8. Procedure

Participants’ eligibility and health status were assessed using a health questionnaire and brief interview. Electrodes were applied to eligible participants and they were familiarized with rating scales to be used during the study (SAM, NRS pain rating scale). There were two phases to the experiment. Phase 1 (NFR threshold assessment) involved sending electric pulses to the ankle to determine the level of stimulation used during picture-viewing. Phase 1 concluded by delivering 2 baseline electrical stimuli at 120% NFR threshold. During phase 2, pictures were presented on the computer screen and electric stimulations were delivered during and in between pictures. Stimulations were randomly delivered during half of the pictures 3 to 5 s after picture onset (balanced across picture content and duration) and during 10...
2.9. Data analysis

Valence ratings, arousal ratings, eyelblink magnitude, and onset latency were averaged by Picture Content (attack, loss, neutral, food, erotica) and Picture Duration (500-ms, 6-s). Before averaging, eyelblink magnitude was converted to z scores. Analyses for SAM valence, SAM arousal, eyelblink magnitude, and eyelblink onset latency were conducted using 5 (Picture Content) × 2 (Picture Duration) repeated measures ANOVAs. In addition, eyelblink magnitude was analyzed using a 2 (Valence: unpleasant vs. pleasant) × 2 (Arousal: low vs. high) × 2 (Picture Duration: 500-ms vs. 6-s) repeated measures ANOVA to directly assess for a significant interaction of valence and arousal as suggested by previous studies (e.g., Bradley et al., 2001; Cuthbert et al., 1996; Rhudy et al., 2007a). To conduct the 2 × 2 × 2 analysis, responses to the neutral picture content had to be omitted. Fisher’s LSD tests were used for a priori hypotheses (follow-up tests for the main effect of Picture Content for SAM ratings, eyelblink magnitude, onset latency) whereas Bonferroni corrections were used for post-hoc comparisons (all other follow-up tests). Polynomial trend analyses were also assessed as a follow-up to 5 × 2 ANOVAs. In these analyses, picture contents were ordered from greatest defensive activation to greatest appetitive activation (attack, loss, neutral, food, erotica). Thus, the linear trend tests the influences of valence and arousal (e.g., attack > loss > neutral > food > erotica), the quadratic trend represents the arousal effect (e.g., attack > loss > neutral < food < erotica), and the cubic trend can represent the valence effect (e.g., attack = loss > neutral > [food = erotica]). All three of these trends were assessed to determine which explained the most variance in each DV. Greenhouse-Geisser corrections were used to overcome sphericity violations when needed, and epsilon (ɛ) values were reported. Partial eta-squared (η^2) was used as the effect size for F tests and Cohen’s d was used for mean comparisons. Cohen (1977) provides guidelines for interpreting η^2 (small = .01, medium = .06, large = .14) and d (small = .2, medium = .5, large = .8).

3. Results

3.1. Preliminary analyses

9.76% of orbicularis oculi EMG trials following noxious stimulation of the sural nerve were scored zero (i.e., eyelinks not observed). The mean NRS ratings of baseline electric stimulations was 57.31 (SEM = 6.25), suggesting participants found the stimuli to be painful on average.

3.2. Manipulation checks: Self-Assessment Manikin (SAM) ratings

SAM data are illustrated in Fig. 1. The main effect of Picture Content was significant for valence/pleasure ratings [F(4, 76) = 65.17, p < .001, ɛ = .51, η^2 = .77]. The linear [F(1, 19) = 99.27, p < .001, η^2 = .84] and cubic [F(1, 19) = 71.40, p < .001, η^2 = .79] trends explained more variance than the quadratic trend [F(1, 19) = 5.00, p = .037, η^2 = .21]. Pleasure ratings were similar for attack and loss pictures (p = .29, d = .29), but were lower than neutral pictures (ps < .001, ds > 2.86). Erotic and food pictures had similar pleasure ratings (p = .16, d = .25), but were higher than neutral pictures (ps < .002, ds > 1.44). These effects were qualified by a Picture Content × Picture Duration interaction [F(4, 76) = 6.30, p < .01, ɛ = .61, η^2 = .25]. The only significant simple effect for Picture Duration was in response to loss pictures, with 6-s pictures eliciting lower pleasure ratings than 500-ms pictures (p = .001, d = .80). The main effect of Picture Duration was not significant (p = .24, d = .11).

The main effect of Picture Content was significant for arousal ratings [F(4, 76) = 18.92, p < .001, ɛ = .71, η^2 = .50]. The quadratic trend was significant and explained the greatest variance, F(1, 19) = 47.83, p < .001, η^2 = .72. Attack pictures were more arousing than loss (p < .001, d = 1.02), and erotic pictures were more arousing than food (p = .01, d = .53). Food and loss pictures were more arousing than neutral pictures (ps < .001, ds > 1.00). Food and loss pictures were similar in arousal ratings (p = .87, d = .04), but attack pictures were slightly more arousing than erotic pictures (p = .03, d = .56). The Picture
Duration main effect and the interactions that included the Picture Duration IV were all non-significant for arousal ratings ($p_s > .23$, $\eta^2 < .07$).

In sum, erotic and food pictures were pleasant, and loss and attack pictures were unpleasant. However, erotic and attack pictures were more arousing than food and loss pictures. Thus, picture contents successfully manipulated affective responses so that the unique effects of valence and arousal on eyeblink could be tested.

### 3.3. Eyeblink reactions to noxious stimulation

The $5 \times 2$ ANOVA suggested that there was a significant main effect of Picture Content for eyeblink magnitude, $F(4,76) = 2.77, p = .046, \varepsilon = .79, \eta^2 = .13$ (Fig. 2). The linear trend was significant and explained the greatest amount of variance in eyeblink magnitude, $F(1,19) = 6.11, p = .02, \eta^2 = .24$. Mean comparisons suggested that eyeblinks were significantly larger during attack pictures than erotic pictures ($p = .01, d = 1.01$). Other mean comparisons were non-significant ($p_s > .08$). The main effect of Picture Duration [$F(1,19) = 1.82, p = .19, \eta^2 = .087$] and the Picture Content $\times$ Picture Duration interaction [$F(4,76) = .67, p = .57, \varepsilon = .22, \eta^2 = .03$] were both non-significant for eyeblink magnitude.

The only significant effect obtained from the $2 \times 2 \times 2$ ANOVA on eyeblink magnitude was a significant Valence $\times$ Arousal interaction, $F(1,19) = 2.05, p = .01, \eta^2 = .30$. To decompose this interaction, the simple effect of valence was examined at each level of arousal. The simple effect of valence was significant for high arousal pictures (erotic vs. attack, $p = .009$), but not for low arousal pictures (loss vs. food, $p = .75$). Highly arousing unpleasant pictures (attack) led to larger blink magnitudes than highly arousing pleasant pictures (erotic), but low arousal pictures did not lead to significant modulation (were not significantly different). The main effects of Valence, Arousal, and Picture Duration and the interactions including the Picture Duration IV were all non-significant in this analysis ($p_s > .05$). Together, these analyses suggest affective modulation of eyeblinks in reaction to noxious stimulation is characterized by a valence-by-arousal interaction.

Eyeblink onset latencies were not influenced by Picture Content, Picture Duration, or the Picture Content $\times$ Picture Duration interaction ($p_s > .25, \eta^2 < .08$) (Table 1).

### 3.4. Exploratory analyses

Two exploratory analyses were conducted to further clarify the characteristics of nociception modulation and electrically-evoked eyeblinks.

#### 3.4.1. Moderation by perceived painfulness

All participants received noxious electric stimulations (120% NFR threshold), however, not all participants rated the stimulations as painful (NRS rating $\geq 50$). Thus an analysis was conducted to determine if a perception of the stimulus as “painful” or not altered nociception modulation. Participants with a mean baseline NRS rating $\geq 50$ (painful) were labeled as “high pain” ($n = 11$) and those with a mean <50 were labeled as “low pain” ($n = 9$). The $5 \times 2$ ANOVA for eyeblink magnitude was repeated with painfulness (high pain vs. low pain) entered as a between-subjects variable [$5$ (Picture Content) $\times 2$ (Picture Duration) $\times 2$ (Painfulness)] repeated measures ANOVA). Results were the same as those reported above, and no significant main effects or interactions involving perceived painfulness were found ($p_s > .08$). This suggests affective modulation was not moderated by perceived painfulness.

#### 3.4.2. Blink habituation

Eyeblinks elicited by non-noxious stimuli show significant habituation (e.g., Bradley et al., 1993a; Ornitz and Guthrie, 1989a). An exploratory analysis was conducted to determine if habituation was present for eyeblinks to noxious electric stimulation. A random mixed models repeated measures ANOVA was used to assess for habituation of ITI eyeblinks with trial (ITI: 1–10) and painfulness (low vs. high) entered as fixed IVs and participant number as a random IV. The effects of trial, painfulness, and Trial $\times$ Painfulness interaction were all non-significant ($p_s > .74$). This suggests that eyeblinks did not significantly habituate over the 10 ITI trials.

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**Table 1** Eyeblink onset latencies averaged by picture content and picture duration

<table>
<thead>
<tr>
<th>Picture Duration</th>
<th>Attack</th>
<th>Loss</th>
<th>Neutral</th>
<th>Food</th>
<th>Erotica</th>
</tr>
</thead>
<tbody>
<tr>
<td>500-ms</td>
<td>105.72</td>
<td>107.11</td>
<td>107.69</td>
<td>107.92</td>
<td>105.86</td>
</tr>
<tr>
<td>SEM</td>
<td>7.52</td>
<td>8.14</td>
<td>7.95</td>
<td>6.87</td>
<td>8.10</td>
</tr>
<tr>
<td>6-s</td>
<td>110.56</td>
<td>99.42</td>
<td>102.65</td>
<td>101.83</td>
<td>103.31</td>
</tr>
<tr>
<td>SEM</td>
<td>7.27</td>
<td>5.97</td>
<td>5.31</td>
<td>6.33</td>
<td>5.98</td>
</tr>
<tr>
<td>Total</td>
<td>108.14</td>
<td>103.26</td>
<td>105.17</td>
<td>104.88</td>
<td>104.59</td>
</tr>
<tr>
<td>SEM</td>
<td>6.66</td>
<td>6.42</td>
<td>6.16</td>
<td>5.51</td>
<td>6.48</td>
</tr>
</tbody>
</table>

Note: Latencies are reported in milliseconds. SEM=standard error of the mean.

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4 The results from this analysis were the same if NRS pain ratings were entered as a continuous, rather than dichotomous, measure.

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**Fig. 2** Standardized ($z$ score) eyeblink magnitude elicited by noxious electrodermal sural nerve stimulation averaged by picture content. The linear trend explained 24% of the variance in eyeblink magnitude. Eyeblinks were significantly larger during attack pictures compared to the erotic pictures. Error bars are SEM.
4. Discussion

This study examined the influence of affective picture-viewing on eyeblink reactions to noxious stimulation. To do so, noxious electrodermal stimuli were delivered to the sural nerve during pictures varying in valence, arousal, and duration. Manipulation checks suggested picture contents successfully manipulated affective responses to allow an investigation of the unique effects of valence and arousal on eyeblink magnitude. Erotica and food pictures elicited pleasure, whereas loss and attack pictures elicited displeasure. However, attack and erotic pictures elicited greater arousal than loss and food pictures.

Analyses suggested a valence-by-arousal interaction characterized the affective modulation of eyeblink magnitude. Eyeblinks were significantly larger during attack pictures than erotic pictures, whereas loss and food pictures did not lead to significant modulation. When pictures were ordered from greatest defensive activation to greatest appetitive activation (i.e., attack, loss, neutral, food, erotic) the linear trend for the picture content main effect explained 24% of the variance in eyeblinks. And finally, the analysis that omitted neutral pictures to directly test the Valence × Arousal interaction found it was significant. As a result, these data are consistent with those of studies that assessed the unique contribution of affective valence and arousal in the modulation of startle eyeblink (Bradley et al., 2001; Cuthbert et al., 1996) and nociceptive reactions (Rhudy et al., 2007a).

Although it is currently unclear why no affective picture content led to significant modulation relative to neutral pictures, this could have been influenced by low power. We only delivered 3 electrical stimuli per cell of the study design to minimize participant stress (40 unpredictable noxious stimuli during picture-viewing can be stressful). Therefore, only 3 eyeblinks were averaged for each cell of the Picture Content × Picture Duration interaction. Although studies have averaged as few as 2 eyeblinks (e.g., Bradley et al., 2001), averaging so few eyeblinks can decrease the stability of sample means (increase error variance) making it difficult to achieve statistical significance. This problem is exacerbated if cells have missing data (rejected trials) or statistical outliers. Fortunately, there were few rejected trials and outliers in our study. This issue suggests though, that our failure to find a significant Picture Content × Picture Duration interaction should be interpreted with caution. However, there should be greater confidence in the picture content main effect given that 6 eyeblinks contributed to each of those 5 means. Nonetheless, future studies are needed to replicate these findings with a greater number of responses to average per cell.

Eyeblink magnitudes did not significantly habituate over the 10 inter-picture trials (regardless of the perceived painfulness of the stimuli). This lack of habituation is inconsistent with studies of startle eyeblink evoked by non-noxious stimuli (e.g., Bradley et al., 1993a; Ornitz and Guthrie, 1989b), but also contrasts another study of eyeblinks evoked by 150 V electric stimuli to the arm (Blumenthal and Swerdlow, 2002). A few potential reasons why our findings are discrepant with these prior studies are worth considering. For one, our analysis of habituation could have been statistically underpowered. Our sample was small (N=20) and we only examined 10 trials. However, Blumenthal and Swerdlow (2002) were able to detect significant eyeblink habituation over 9 trials in a sample as small as 24 participants. Alternatively, we may have needed more trials to observe habituation because our stimuli were more noxious/intense than these prior studies. Our stimuli were certainly more noxious than auditory startle probes; but, it is unclear whether our stimuli were more noxious than those used by Blumenthal and Swerdlow (2002). They reported using single, 5-ms, 150 V, constant-voltage pulses. Unfortunately, with constant-voltage stimuli, changes in stimulating electrode impedance (resistance) can affect the current output as determined by Ohm’s law (Current = Voltage/Resistance). They did not report electrode impedance in that study, and without knowing impedance we cannot directly compare stimulus intensity. On the other hand, our stimulus density was larger (5 × 1-ms pulses vs. 1 × 5-ms pulse). Additionally, we set our stimulus intensity 20% above the participant’s physiologically-determined nociceptive threshold and stimulated over the sural nerve. Thus, these procedural differences may have contributed to the differences from Blumenthal and Swerdlow (2002).

A final explanation for the lack of habituation could be that eyeblinks elicited by noxious sural nerve stimulation are neurobiologically and/or functionally distinct from startle eyeblink. Under some conditions, electrically-evoked eyeblinks are directly tied to nociception rather than startle. Kaube and colleagues (2000) have shown that stimulation of the trigeminal nerve using a concentric electrode reliably evokes eyeblinks only when nociceptors (Aδ and C-fibers) are intact. If nociceptors were blocked by a local anesthetic, eyeblinks were almost completely abolished (Kaube et al., 2000). In addition, the magnitude of electrically-evoked eyeblinks covaries linearly with the intensity of noxious stimulation and pain ratings (Bromm and Scharein, 1982). Regrettably, the current study cannot determine the biological/functional underpinnings of the eyeblinks elicited by noxious sural nerve stimulation. In fact, the present results could have been obtained from eyeblinks emanating from startle or nociceptive circuitry; because a similar pattern of modulation has been observed for startle eyeblinks evoked by non-noxious acoustic stimuli (Bradley et al., 2001; Cuthbert et al., 1996; Lang et al., 1997) and reactions that are nociceptive in origin (pain, NFR) (Rhudy et al., 2005, 2006, 2007a). Furthermore, startle and nociception may be modulated by a common circuit (e.g., amygdala & PAG) (Crown et al., 2000; Davis, 1997; Fanselow, 1994; Fendt et al., 1994; Lang and Davis, 2006; Manning and Mayer, 1995; McLemore et al., 1999). Although additional research is needed to address this issue, our data suggest eyeblinks elicited by noxious electric stimulation of the sural nerve do not habituate as quickly as eyeblinks elicited by acoustic startle probes or electrical probes delivered to the arm.

In the present study eyeblinks were reliably evoked (>90% response probability) by noxious sural nerve stimuli. In contrast, other studies that have attempted to evoke eyeblinks using electric stimuli delivered to limbs find much lower response probabilities (Blumenthal et al., 2001; Miwa et al., 1995; Miwa et al., 1998) or fail to report response probabilities (Bromm and Scharein, 1982;
Dowman, 1992). One study did note a probability of 69% when a 150 V constant-voltage stimulus was delivered (Blumenthal and Swerdlov, 2002). Unfortunately, as noted above, it is difficult to make comparisons with this study, because current level and/or electrode impedance were not reported. Constant-current is generally preferred over constant-voltage (as used in the Blumenthal and Swerdlov study), because magnitude estimates of constant-voltage can vary as electrode impedance changes (Tursky, 1974). Therefore, it is possible that our increased response probability resulted from our use of a constant-current stimulus. This methodology may have ensured that the stimulus was stable throughout testing. Additionally, our use of noxious stimuli delivered directly over a nerve and set above the physiologically-determined nociceptive threshold may have improved response probability.

An exploratory analysis suggested affective modulation was present regardless of the perceived painfulness of the noxious stimuli. We were concerned that perceived painfulness of the noxious stimuli may have influenced mood during testing and thus altered affective modulation (Grussler et al., 2007). However, our data suggest perceived painfulness does not alter the pattern of affective modulation. This indicates eyelink reactions can be used to test affective modulation of nociceptive reactivity in future studies, because modulation is present despite individual differences in the subjective experience of the evoking stimulus.

We have found that affective picture-viewing reliably modulates other nociceptive reactions to noxious stimulation (i.e., subjective pain report, NFR, skin conductance, HR acceleration) (Rhudy et al., 2005, 2006, 2007a,c). We argue that the procedures to study affective modulation of nociception can be used to study individual differences in modulation that might contribute to chronic pain (Rhudy et al., 2007a). The present study implies eyelink reactions to noxious stimuli are also modulated by picture-viewing, thus eyelinks can serve as a supraspinal response to complement the spinal-mediated NFR. Ultimately, when assessed in the same study, NFR and eyelink could provide information about nociceptive processing at spinal and supraspinal levels, respectively. However, additional research is needed to elucidate the circuitry that mediates eyelinks resulting from noxious stimuli and to determine the circumstances under which the NFR and eyelink reactions converge/diverge. If divergence is observed in pain-free individuals, it could represent normal modulatory functioning that provides flexibility in the way afferent pain signals are modulated. For example, it can be adaptive to inhibit the nociceptive flexion reflex during fight-or-flight, because this withdrawal reflex can interfere with active fleeing or fighting. However, inhibition of supraspinal reflexes may not be necessary under these circumstances. Indeed, research on healthy animals (Illich et al., 1995; King et al., 1996) and humans (France et al., 2002; Rhudy et al., 2006) has found that supraspinal and spinal measures of nociceptive reactivity are not always modulated in parallel. In other circumstances, divergence of spinal and supraspinal reflexes may represent a localized dysfunction in pain modulation, perhaps contributing to hyperalgesia in persons with chronic pain (Sandrini et al., 2006). Studies are currently underway to examine these issues.

It is worth mentioning that onset latencies for eyelinks following sural nerve stimulation were longer on average (~105-ms onset) than eyelinks evoked by auditory and visual stimuli, as well as other somatosensory stimuli delivered to the head (for a review, Blumenthal et al., 2005). However, our observed onset latencies are reasonable when the extra distance required for neural transmission from the ankle to the neck/head is considered (Vogel et al., 1986). Although it is unclear why onset latencies were not modulated by affective pictures in the current study, this extra distance may have contributed additional error variance to latency measurements. Alternatively, affective modulation of onset latencies may not be a robust phenomenon (e.g., Bradley et al., 1991).

In summary, this study found that eyelinks were reliably evoked by noxious sural nerve stimulation and showed a pattern of affective modulation similar to nociceptive reactions (pain, NFR, SCR, HR acceleration) and eyelinks elicited by non-noxious probes (e.g., acoustic probes). Together, these data suggest eyelink reactions to noxious stimulation can be measured together with subjective pain, NFR, SCR, and HR acceleration to study the modulation of reactions to nociceptive input.

References


Erlbaum, Hillsdale, NJ, pp. 97


