

BRIEF REPORT

Communalities and differences in fear potentiation between cardiac defense and eyeblink startle

MARÍA B. SÁNCHEZ,^a PEDRO GUERRA,^a MIGUEL A. MUÑOZ,^a JOSÉ LUÍS MATA,^b
MARGARET M. BRADLEY,^c PETER J. LANG,^c AND JAIME VILA^a

^aDepartment of Personality, University of Granada, Granada, Spain

^bDepartment of Psychology, University of Jaén, Jaén, Spain

^cUniversity of Florida, Gainesville, Florida, USA

Abstract

This study examines similarities and differences in fear potentiation between two protective reflexes: cardiac defense and eyeblink startle. Women reporting intense fear of animals but low fear of blood or intense fear of blood but low fear of animals viewed pictures depicting blood or the feared animal for 6 s in 2 separate trials in counterbalanced order. An intense burst of white noise, able to elicit both a cardiac defense response and a reflexive startle blink, was presented 3.5 s after picture onset. Both cardiac and blink responses were potentiated when highly fearful individuals viewed fearful pictures. However, differences appeared concerning picture order. This pattern of results indicates communalities and differences among protective reflexes that are relevant for understanding the dynamics of emotional reflex modulation.

Descriptors: Fear, Emotion, Defense, Startle, Heart rate, Eyeblink

Fear potentiation of the acoustic startle reflex in the processing of fear-evoking stimuli has been consistently demonstrated in both animals (Davis, 1992) and humans (Globisch, Hamm, Esteves, & Öhman, 1999; Sabatinelli, Bradley, & Lang, 2001). Blink potentiation has been interpreted as reflecting a priming of the organism's defensive reflexes when subcortical structures involved in defensive behavior, including the amygdala, are activated, in accordance with the *motivational priming hypothesis* (Lang & Davis, 2006). Presentation of an intense acoustic stimulus also prompts a cardiac defense response that has a complex pattern with two accelerative/decelerative components in humans, one of short latency (peak around 3 s) and one of long latency (peak between 30 and 40 s); the first accelerative component is sometimes identified with cardiac startle (Turpin, Schaefer, & Boucsein, 1999).

Using an acoustic stimulus capable of eliciting both cardiac defense and the reflexive blink response, Sánchez et al. (2002) found emotional modulation of both cardiac defense and eyeblink startle when participants viewed affective pictures. The potentiated cardiac response when viewing unpleasant pictures was characterized by a single large and prolonged acceleration, with no subsequent deceleration. These parallels in affective modulation between cardiac defense and eyeblink startle have

been found using masked or unmasked fearful pictures (Ruiz-Padial, Mata, Rodríguez, Fernández, & Vila, 2005; Ruiz-Padial & Vila, 2007). However, these studies also reported a dissociation between the reflexes in relation to the trial in which the fear potentiation occurred, finding a larger potentiated response for cardiac defense in the first trial and for eyeblink startle in subsequent trials, which calls into question the proposed parallelism between these reflexes (see Grillon & Cornwell, 2007).

Cardiac defense and eyeblink startle correspond to two different response systems (cardiovascular and motor) that have distinct sensitivities to experimental manipulation (see Ramírez, Sánchez, Fernández, Lipp, & Vila, 2005; Vila et al., 2007). For instance, acoustic stimulation can elicit both reflexes, but the optimal parametric characteristics of the eliciting stimulus differ (500 ms with any rise time for cardiac defense and 50 ms with very short rise time for eyeblink startle). Response habituation is another differential characteristic. Cardiac defense habituates rapidly, and the full response pattern almost disappears after the first stimulus presentation, whereas eyeblink startle, when simultaneously elicited with cardiac defense, tends to show either no habituation (Ramírez et al., 2005) or sensitization (Mata, Rodríguez, Ruiz-Padial, Turpin, & Vila, 2009). This differential habituation limits cardiac defense tests to a few trials, hence reducing the possibility of balancing perceptual characteristics of the pictures by experimental control or random distribution. It may also explain the dissociation between cardiac defense and eyeblink startle with respect to the time course of the *motivational priming effect*.

The aim of the present study was twofold: first, to replicate the fear potentiation of cardiac defense and eyeblink startle using an

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Address reprint requests to: Jaime Vila, Facultad de Psicología, Campus La Cartuja s/n. 18011 Granada, Spain. E-mail: jvila@ugr.es

improved methodology to control for perceptual characteristics of the fearful and nonfearful pictures and, second, to confirm the above-mentioned dissociation by examining intercorrelations between eyeblink startle and cardiac defense (both short- and long-latency components). To use identical pictures as fearful and nonfearful stimuli, we selected individuals who were specifically fearful of one object but not of the other and vice versa. In addition, the test was restricted to two trials, one for the fearful and the other for the nonfearful stimulus, counterbalanced across participants in terms of order of presentation. We expected cardiac defense and eyeblink startle to be potentiated when fearful participants viewed fearful versus nonfearful pictures. We also expected to find that the potentiated response would be larger for cardiac defense in the first trial and for eyeblink startle in the second trial. The dissociation would be confirmed by correlation analysis.

Method

Participants

Participants were 40 female university students selected from a pool of 600 students according to their scores on three questionnaires: the Spider Questionnaire (SPQ), the Snake Questionnaire (SNAQ), and the Mutilation Questionnaire (MQ; Klorman, Weerts, Hastings, Melamed, & Lang, 1974). Twenty participants reported intense fear of animals (10 of spiders and 10 of snakes) and low fear of blood/mutilation, and 20 participants reported intense fear of blood/mutilation and low fear of animals (10 of spiders and 10 of snakes). The three questionnaires are formed by 30–31 true or false items with a maximum score of 30–31. Participants had scores higher than 17 ($M = 21.3$, $SD = 2.8$) for their intense-fear object and lower than 6 ($M = 3.38$, $SD = 1.7$) for their low-fear one. The age range of participants was 18–44 years ($M = 22.78$, $SD = 5.4$) with no significant differences among the groups ($F < .01$). The vision of all participants was normal or corrected to normal, and no participant had auditory problems or was undergoing psychiatric or pharmacological treatment. Participants received course credits for their participation.

Materials and Design

A spider picture (International Affective Picture System [IAPS] number 1220), snake picture (IAPS number 1030), and blood picture (IAPS number 3250) that were equivalent in valence and arousal were selected from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2005; Moltó et al., 1999). All participants saw one blood and one animal picture; half of the subjects in each fearful group viewed the animal picture first and then the blood picture, and the other half viewed the pictures in the opposite order. The acoustic stimulus used to elicit the defensive reflexes was a white noise of 105 dB, instantaneous rise time, and 500 ms duration. This long-stimulus duration in comparison to the usual startle noise of 50 ms is the key methodological factor to simultaneously elicit both reflexes (Ramirez et al., 2005). Each picture–noise trial consisted of a 15-s prepicture period, a 6-s picture presentation, and an 80-s postpicture period. The acoustic stimulus was presented at 3.5 s after the picture onset, and the intertrial interval was 120 s.

A Coulbourn audio system was used to generate the white noise, which was presented binaurally through earphones. Pictures were projected on the wall in front of the subject at a distance of 3

m, using a Kodak Ektapro 9000 slide projector. Physiological data were collected using VPM software (Cook, 1997).

Cardiac defense response. Electrocardiography was recorded using lead II configuration (right arm and left leg with ground electrode in right leg) and sampled at 1000 Hz by means of a Grass polygraph with 7P4 preamplifier. Band-pass filters were set at 10–35 Hz. Weighted averaged second-by-second heart rate was obtained from the beat-to-beat heart period recorded from the electrocardiogram using the VPM software. The 80 heart rate values after onset of the acoustic stimulus were expressed as difference scores with respect to the 15-s prepicture period and reduced to 10 heart rate values, that is, the medians of 10 progressively longer intervals: two of 3 s, two of 5 s, three of 7 s, and three of 13 s (Vila, Fernández, & Godoy, 1992).

Startle blink reflex. Electromyography (EMG) activity was recorded by placing two miniature Ag/AgCl electrodes filled with electrode paste over the left orbicularis oculi muscle and using a GRASS 7P3 EMG-Integrator preamplifier. The raw EMG signal, recorded with a band-pass filter set at 10–1000 Hz, was rectified and integrated using the analogue section of the preamplifier with a time constant of 75 ms. Sampling rate was 1000 Hz. Startle reflex magnitude was defined as the difference in microvolts between the peak of the integrated response and the onset of the response, initiated between 20 and 100 ms after stimulus onset following Balaban's procedure (Balaban, Losito, Simons, & Graham, 1986). The magnitude was scored as 0 for trials with no detectable peak (only 1 participant scored 0 in both trials and was excluded from the statistical analysis). Finally, the data were square-root transformed to normalize the distribution.

Self-report measures. The unpleasantness and arousal of the animal and blood pictures were retrospectively rated after the test on a scale of 0 to 100 (0 = *not at all unpleasant or arousing*; 100 = *extremely unpleasant or arousing*).

Statistical Analysis

Animal- and blood-fearful participants were collapsed into a single fearful group because a preliminary analysis showed no significant group differences in cardiac and blink responses. Both responses were then analyzed using mixed analyses of variance (ANOVAs) in which the Greenhouse–Geisser epsilon correction was used for repeated measures factors with more than two conditions. In analyses of the cardiac response, the between-subject factor was Picture Order (fearful/nonfearful and nonfearful/fearful), and the repeated measures factors were Picture Content (fearful and nonfearful) and Time (10 heart rate medians). For the blink response, the between-subject factor was again Picture Order and the repeated measures factor was Picture Content. Simple effects analysis tested (a) whether the fearful picture elicited greater cardiac defense and eyeblink startle than the nonfearful picture and (b) whether the difference was a function of the order of picture presentation. For cardiac defense, separate tests were performed for short-latency (average of Medians 1 and 2) and long-latency (average of Medians 4, 5, 6, and 7) accelerations. Finally, correlation between cardiac defense (short- and long-latency acceleration) and eyeblink startle was separately evaluated (Pearson's product moment correlation) for each picture order. This analysis used both the direct score and the differential score of the responses to the fearful and non-

fearful pictures. The level of significance was set at .05 for all analyses.

Results

Cardiac Defense

Figure 1 illustrates the cardiac defense response elicited by intense noise when participants were viewing the fearful and nonfearful pictures as a function of Picture Order. A high and prolonged heart rate acceleration was observed when participants were viewing the fearful picture presented first (top panel). The differences were smaller when the fearful picture was presented second (bottom panel).

The 2 (Picture Order) \times 2 (Picture Content) \times 10 (Time) ANOVA yielded significant effects for Picture Content, $F(1,38) = 8.36$, $p < .006$, $\eta_p^2 = .180$, Picture Order \times Picture Content, $F(1,38) = 5.67$, $p < .02$, $\eta_p^2 = .130$, Time, $F(9,342) = 9.37$, $p < .0001$, $\eta_p^2 = .198$, $\varepsilon = .332$, Picture Content \times Time, $F(9,342) = 3.59$, $p < .007$, $\eta_p^2 = .086$, $\varepsilon = .468$, and Picture Order \times Picture Content \times Time, $F(9,342) = 5.78$, $p < .0001$, $\eta_p^2 = .132$, $\varepsilon = .468$. Follow-up of the triple interaction for each Picture Order indicated that heart rate differences between fearful and nonfearful pictures were only significant when participants viewed the fearful picture first: Picture Content, $F(1,19) = 13.99$, $p < .001$, $\eta_p^2 = .424$, and Picture Content \times Time, $F(9,171) = 6.75$, $p < .0001$, $\eta_p^2 = .262$, $\varepsilon = .403$. Significant differences were found for short- ($p < .004$) and long-latency ($p < .001$) acceleration. In the reverse

order, only the Time factor was significant: Time, $F(9,171) = 5.1$, $p < .002$, $\eta_p^2 = .211$, $\varepsilon = .384$.

Eyeblink Startle

The 2 (Picture Order) \times 2 (Picture Content) ANOVA yielded a significant main effect for Picture Content, $F(1,37) = 6.59$, $p < .01$, $\eta_p^2 = .151$. The Picture Content \times Picture Order interaction was marginally significant, $F(1,37) = 3.05$, $p < .09$, $\eta_p^2 = .076$. Blink magnitude was larger when participants were viewing the fearful versus nonfearful picture. However, examination of the differences in blink magnitude as a function of order of picture presentation only revealed significant differences when the fearful picture was presented second (fearful: $M = 12.4$, $SD = 5.4$; nonfearful: $M = 10.1$, $SD = 4.6$), $F(1,18) = 6.84$, $p < .02$, $\eta_p^2 = .275$. In the reverse order, the difference was not significant ($p > .49$; fearful: $M = 10.7$, $SD = 5.8$; nonfearful: $M = 10.2$, $SD = 4.9$).

Correlation Analysis

No significant correlations were found between cardiac defense (short- or long-latency component) and eyeblink startle when participants viewed the fearful picture first and the nonfearful picture second (all $p > .2$). When participants viewed the fearful picture second, significant positive correlations were found between cardiac defense (both short- and long-latency component) in Trial 1 and eyeblink response in Trial 2: $r = .632$, $p < .004$, for short-latency component and $r = .666$, $p < .002$, for long-latency component. Analysis of the differential score (response while viewing the fearful picture minus response while viewing the nonfearful picture) also showed a significant negative correlation for the same Picture Order participants between cardiac defense (long-latency component) and eyeblink startle ($r = -.499$, $p < .03$).

Self-Report Measures

The picture of the participants' specific feared material was rated as more unpleasant ($M = 71.0$, $SD = 25.9$) and arousing ($M = 67.8$, $SD = 24.9$) compared with the nonfearful picture (unpleasantness: $M = 31.9$, $SD = 27.8$; arousal: $M = 39.1$, $SD = 30.1$). These differences were confirmed by the significant main effect of Picture Content for both pleasure, $F(1,38) = 50.62$, $p < .0001$, $\eta_p^2 = .571$, and arousal, $F(1,38) = 32.81$, $p < .0001$, $\eta_p^2 = .463$.

Discussion

Cardiac defense and eyeblink startle were significantly potentiated in fearful participants when they viewed the fear-relevant picture in comparison to the nonrelevant picture. The potentiation of eyeblink startle replicates a well-documented phenomenon (Globisch et al., 1999; Hamm et al., 1997; Lang, Bradley, & Cuthbert, 1990; Sabatinelli et al., 2001). The simultaneous potentiation of cardiac defense also confirms previous findings. A greater cardiac defense response has been reported when participants view unpleasant versus neutral or pleasant pictures (Sánchez et al., 2002). A similar response pattern has also been observed when a fearful (e.g., spider) versus nonfearful (e.g., flower) picture is presented to fearful participants in masked or unmasked conditions (Ruiz-Padial et al., 2005, 2007). However, the specific physical content of the pictures was not controlled in the above studies, limiting unambiguous interpretation of the fear potentiation. The present results provide stronger evidence

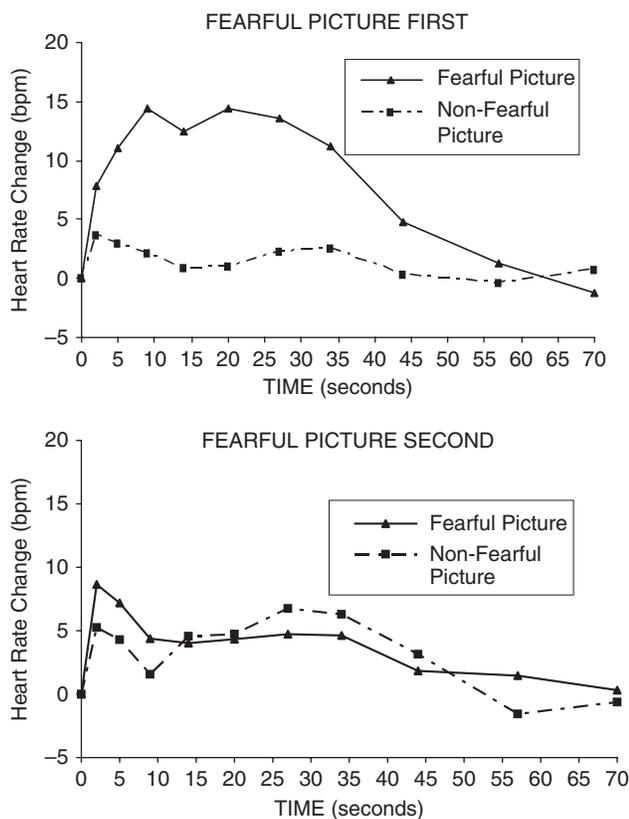


Figure 1. Cardiac defense elicited when participants were viewing the fearful and nonfearful picture as a function of picture order. Top: fearful picture first and nonfearful picture second. Bottom: nonfearful picture first and fearful picture second.

in favor of the *motivational priming hypothesis* and its extension to cardiac defense.

Besides the above communalities, we found a difference in the effect of picture order between the autonomic and somatic defensive reflexes under study, as previously reported (Ruiz-Padial et al., 2005; Ruiz-Padial & Vila, 2007). Whereas potentiated cardiac responses were stronger for fearful stimuli presented in the first trial, fear modulation of the blink component was stronger in the second trial. This dissociation was confirmed by the significant correlations found between cardiac defense and eyeblink startle when participants viewed the fearful picture second. A larger decrease (habituation) in cardiac defense (the long-latency acceleration) was accompanied by a larger increase (sensitization) in eyeblink startle. Similarly, participants with greater cardiac acceleration response in Trial 1 (both short- and long-latency component) also had a greater eyeblink startle response in Trial 2. These correlations were not found when participants viewed the fearful picture first.

Because responses were measured in only two trials, numerous mechanisms might explain this dissociation. One hypothesis is that habituation more strongly affects cardiac defense whereas sensitization is more characteristic of the blink response, especially when elicited using an experimental procedure designed to study cardiac defense (intense and long startling noises, ex-

tended intertrial period, small number of trials). Groves and Thompson's (1970) dual process of habituation predicts sensitization during the initial presentations of high-intensity stimuli at a slow presentation rate. Sensitization may also occur due to *motivational priming* after the first defense trial. After this aversive experience, the organism is in a defensive motivational state that potentiates the subsequent startle response (Lang et al., 1990). Alternatively, sensitization might result from anticipation of the aversive stimulus after the first noise presentation, as in the *threat of shock paradigm* (Grillon, Ameli, Merikangas, Woods, & Davis, 1993). Consequently, if sensitization of eyeblink startle occurred, the expected fear potentiation would only be evident when the fearful stimulus was presented second. In the reverse order, the fear potentiation in Trial 1 and the sensitization effect in Trial 2 would mask each other. This might explain the lack of significant correlations between eyeblink startle and cardiac defense when the fearful picture was presented first.

In summary, the data reported here replicate the extension of the *motivational priming hypothesis* to protective autonomic reflexes such as cardiac defense. In addition, new correlational data confirmed differences between eyeblink startle and cardiac defense with respect to the time course of the fear potentiation phenomenon.

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