Published paper
Mata, J.L., Rodriguez-Ruiz, S., Ruiz-Padial, E., Turpin, G., Vila, J. (2009) *Habituation and sensitization of protective reflexes: dissociation between cardiac defense and eye-blink startle*, Biological Psychology, 81 (3), pp. 192-199
http://dx.doi.org/10.1016/j.biopsycho.2009.04.006

Biological Psychology 81 (2009) 192-199

HABITUATION AND SENSITIZATION OF PROTECTIVE REFLEXES: DISSOCIATION BETWEEN CARDIAC DEFENSE AND EYE-BLINK STARTLE

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Abstract

We examined the habituation and recovery of two protective reflexes, cardiac defense and eye-blink startle, simultaneously elicited by a white noise of 500 ms as a function of the time interval between stimulus presentations. Participants were 90 volunteers (54 women) randomly distributed into 6 inter-trial interval (ITI) conditions. They all received three presentations of the stimulus with a time interval of 30 min between the first and third noise. The timing of the second noise was manipulated in six steps, using a between-group design, in order to increase the ITI between trials 1-2 and symmetrically decrease the ITI between trials 2-3. Cardiac defense showed fast habituation at the shortest ITI (2.5 min), but reduced habituation and increased recovery at the longest ITI (27.5 min). In contrast, eye-blink startle showed sensitization irrespective of the ITI. This pattern of findings highlights dissociations between protective reflexes when simultaneouly examined. The results are discussed in the context of the cascade model of defense reactions.

KEY WORDS: Cardiac defense, eye-blink startle, habituation, sensitization, defense cascade.

1. Introduction

Defense reactions are essential mechanisms for survival. They are not only important in traumatic, life-threatening situations (Schauer, Neumer & Elbert, 2005) but also in everyday life. Thus, protection of the body surface from collision is a daily defense mechanism that requires continuous sensory-motor coordination to avoid potentially dangerous objects (Graziano & Cooke, 2006). Defense reactions are closely linked to fear and anxiety, the typical emotional responses to the presence of danger or threat. Advances in the neurophysiology of fear and anxiety have primarily derived from animal research into defense responses such as freezing, startle and escape-attack behaviours (Davis, 1992; LeDoux, 2000; Blanchard & Blanchard, 1988; Fanselow, 1994).

Defense reactions do not constitute separate entities. Rather, a dynamic sequence or cascade of defense responses, from increased attention to protective actions, seems to take place depending on the type and severity of the danger, its spatial and temporal proximity and the success or failure of the initial responses to cope with it (Gallup, 1977; Marks, 1987; Gray; 1988; Blanchard & Blanchard, 1988; Fanselow, 1994; Lang, et al., 1997; Bracha, 2004; Facchinetti et al., 2006). In non-human mammals, a sequence of four defense responses has been well established as a function of proximity of the danger and availability of escape: *attentive freezing* (when danger is first encountered at a safe distance), *flight* (when danger is approaching and an escape route is available), *fight* (when attack is imminent and no escape route is available), and *tonic immobility* (when a dominant predator has already made direct physical contact with the prey).

Although there is increasing evidence that the same sequence applies to humans (Marx et al., 2008), research on defense reactions has traditionally focused on somatic and autonomic protective reflexes such as motor startle and cardiac defense. This tradition is rooted in the work of Pavlov and Cannon. Pavlov (1927) used the term *defense reflex* to refer to unconditioned responses elicited by noxious stimulation such as hand withdrawal to an electric shock or eye-blink to a puff of air. Cannon

(1929) used the term *fight or flight* to refer to a sympathetically-mediated cardiovascular response to emergency situations aimed at facilitating adaptive behaviours such as attack or escape. Since then, motor startle and cardiac defense have been extensively studied in both animals and humans (Strauss, 1929; Landis & Hunt, 1939; Bond, 1943; Sokolov, 1963; Graham & Cliffton, 1966; Davis, 1984; Turpin, 1986; Graham, 1992; Lang, 1995; Ramírez et al., 2005; Vila et al., 2007) and have made a widely recognised contribution to knowledge on the neurophysiology and psychology of fear and stress (Davis, 1992; Koolhaas, et al., 1999; LeDoux, 2000; Lang & Davis, 2006; Lissek et al., 2007; Walters et al., 2008).

The eliciting stimuli used in the first descriptions of motor startle and cardiac defense were unexpected loud sounds of a pistol shot just behind the individual's or animal's head (Strauss, 1929; Bond, 1943). Strauss, a German psychiatrist, was one of the first to systematically study the startle reflex in humans, which involved a quick closing of the eyes accompanied by stiffening of the head, dorsal neck, body wall, and limbs, as if to protect from a predator (see Graziano & Cooke, 2006). This response pattern rapidly habituates on repeated stimulus presentations, although some components of the reflex, such as the eye-blink, tend to persist and habituate more slowly. As regards cardiac defense, Bond (1943), a student of Cannon, was the first to describe in cats and dogs a complex response pattern to intense noises (*made by a pistol shot or by hitting a table top with an iron rod several times in less than 2 seconds*) characterized by an initial heart rate acceleration that lasted for 4 to 6 sec, followed by a sudden fall and then a second acceleration with a more gradual slope reaching its peak between 20 and 40 sec. This response pattern also showed habituation with stimulus repetition (faster for the second acceleration), although in separate sessions the response was *remarkably constant and repeatedly demonstrable for the individual* (Bond, 1943, page 89).

Subsequent research on cardiac defense in humans has confirmed Bond's findings and advanced knowledge on many aspects of this response pattern, including individual differences, characteristics of the eliciting stimulus and its physiological and psychological functionality (see Vila et al., 2007 for a review). Nevertheless, although a fundamental issue in the theoretical debate on cardiac reflexes, the rapid habituation of this response has been inadequately investigated (Graham, 1979, 1992). According to Graham's classic model, cardiac defense is differentiated from cardiac startle in the transient/sustained characteristics of the eliciting stimulus and their response habituation rate, among other criteria. Transient high-intensity stimuli are reported to elicit startle whereas sustained high intensity stimuli are said to elicit defense, and the defense reflex is said to be more resistant to habituation in comparison to startle.

Graham's model has been widely investigated (see, for instance, Kimmel, Van Olst, & Orlebeke, 1979; Siddle, 1983; Lang, Simons, & Balaban, 1997; Dawson, Schell, & Böhmet, 1999) but has also been the subject of continuous debate and reformulation (Barry & Maltzman, 1985; Cook & Turpin, 1997; Graham & Hackley, 1991; Graham, 1997; Öhman, Hamm, & Hugdahl, 2000; Turpin, Schaefer, & Boucsein, 1999; Vossel & Zimmer, 1992; Barry, 2006). As regards startle and defense, the data have never supported the assumed higher resistance to habituation of cardiac defense (Turpin and Siddle, 1978, 1983; Turpin, Schaefer & Boucsein, 1999; Ramírez et al., 2005). In fact, when eye-blink and cardiac defense were simultaneously examined using an acoustic stimulus capable of eliciting both reflexes, a faster habituation was observed for cardiac defense than for eye-blink startle (Ramírez et al., 2005; Fernández et al., 2008).

Besides the theoretical implications of the differential habituation of cardiac defense and eye-blink startle, it also poses methodological difficulties for research into the relationship among protective reactions in relation to the above-mentioned defense cascade. It has been suggested (Turpin et al., 1999; Vila et al., 2007) that the complex response pattern that characterizes cardiac defense reflects the succession of two defensive phases: an attentional protective phase linked to short-latency acceleration/deceleration and a motivational defensive phase linked to long-latency acceleration/deceleration. The attentional protective phase would be equivalent to a *startle/freezing* response (interruption of ongoing activity and heightened attention to the potential danger), whereas the motivational protective phase would be equivalent to the *fight/flight* response (preparation for active defense, either escape or attack). This idea is supported by data showing that cardiac defense (a) also includes a decelerative component after the first acceleration (Vila et al. 1992), (b) is positively correlated with attentional tasks of sensory intake (Vila et al., 1997; Pérez et al., 2000; Fernández & Vila, 1989a), and (c) is physiologically mediated by both vagal and sympathetic mechanisms: the first acceleration/deceleration is controlled by parasympathetic influences, whereas the second acceleration/deceleration is controlled by reciprocal sympathetic and parasympathetic influences (Bond, 1943; Fernández & Vila, 1989b; Reyes del Paso et al., 1993, 1994).

Confirmation of this attention-motivation model of cardiac defense requires comparative studies with other protective reflexes differentially associated with attention (e.g., startle) or motivation (e.g., fight/flight). In the case of startle, however, the fast habituation of cardiac defense limits the test to a few trials, reducing the possibilities of comparative studies with experimental procedures (e.g., the pre-pulse inhibition or the startle probe paradigms) that use large numbers of trials and a repeated-measures design. Examination of the differential habituation/recovery of cardiac defense and eye-blink startle within a single laboratory session is therefore a relevant theoretical target, but numerous methodological difficulties must be overcome.

Cardiac defense and eye-blink startle belong to two different response systems (cardiovascular and motor) with different sensitivities to experimental manipulations. Thus, acoustic stimulation can elicit both reflexes but the optimal parametric characteristics of the eliciting stimulus are different (see Ramírez et al., 2005). Whereas the whole pattern of cardiac defense requires an eliciting stimulus of long duration (around 500 ms and over) but no specific rise time (even long rise times of around 240 ms can evoke cardiac defense), motor startle can be elicited with short and long stimulus durations (50 ms and over) but requires very short rise times (less than 24 ms). The response latency is a further differential characteristic that affects the interval between stimulus presentations. Cardiac defense requires a minimum of 80 s for the response to be fully developed, whereas eye-blink startle is initiated and completed within a window of 20-150 ms. Therefore, the short inter-trial intervals (10-20 s) typically used in eye-blink startle studies would rule out examination of cardiac defense. Hence, the simultaneous examination of both reflexes necessarily implies using experimental procedures that might be optimal for one reflex but not for the other.

The aim of the present study was to examine similarities and differences in habituation and recovery between cardiac defense and eye-blink startle by using a single laboratory session. We followed an optimal experimental procedure to study the habituation and recovery of cardiac defense: three presentations of an intense white noise of 500 ms duration and instantaneous rise time, capable of eliciting both reflexes, within a time interval of 30 min. The interval between stimulus presentations was manipulated, using a between-group design, by increasing the time interval between the 1st and 2nd stimulus, from 2.5 to 27.5 min, and symmetrically decreasing the time interval between the 2nd and 3rd stimulus. It was hypothesized that increasing the time interval between the 1st and 2nd stimulus would reduce habituation, whereas increasing the time interval between the 2nd and 3rd stimulus would facilitate recovery.

2. Method

Participants

Participants were 90 volunteer university students, 36 men and 54 women aged between 17 and 34 yrs old (M = 20.37; SD = 2.54). No participant was undergoing psychological or pharmacological treatment or had auditory or cardiovascular problems.

Design

All participants received three presentations of an intense acoustic stimulus with appropriate characteristics to elicit both cardiac defense and eye-blink startle (Ramírez et al., 2005): white noise of 105 dBA, 500 ms duration, and instantaneous rise time. This long stimulus duration, compared to the typical startle noise (50 ms), is the key methodological factor to simultaneously elicit both reflexes. Time between 1st and 3rd stimulus presentation was 30 min for all participants. The timing of the second presentation was manipulated in a between-group design following an increasing/decreasing symmetrical distribution of the 30 min into two intervals: Inter-Trial Interval between 1st and 2nd presentation (ITI1) and Inter-Trial Interval between 2nd and 3rd presentation (ITI2). Six ITI Conditions were generated (see Fig. 1): Condition 1 (2.5 min ITI1/27.5 min ITI2,), Condition 2 (7.5/22.5 min), Condition 3 (12.5/17.5 min), Condition 4 (17.5/12.5 min), Condition 5 (22.5/7.5 min), and Condition 6 (27.5/2.5 minutes min). The shortest ITI condition (2.5 min) was based on the time interval typically used in previous studies that reported a fast habituation of cardiac defense (Vila et al., 2007). The remaining ITI conditions were formed by successive 5 min increments. The 90 participants were randomly assigned to the six conditions (6 men and 9 women per condition).

Insert Figure 1

Psychophysiological Test

The psychophysiological test had the following sequence: (a) 10 minutes of rest period; (b) three trials of stimulus presentation under the corresponding ITI conditions with no warnings; and (c) a final rest period of 120 s. Each trial consisted of a 15-s pre-trial recording period, 500-ms stimulus presentation, and 80-s post-trial recording period. Participants were instructed to rest, breathe normally, keep their eyes open, and look at a fixed point at a distance of 2 m in front of their eyes. They were monitored by a TV camera to ensure that none of them fell asleep or became excessively distressed during the test.

Instruments and measures

White noise. A Coulbourn V85-05 audio system with IMQ Stage Line amplifier was used to generate the white noise, which was presented binaurally through earphones (Telephonic TDH Model- 49). The intensity of the sound was calibrated using a sonometer (Bruel & Kjaer, model 2235) and artificial ear (Bruel & Kjaer, model 4153).

Physiological measures. Physiological data were collected on a Pentium 2 computer running VPM software with a PCL812PG card (Cook, 1997). The *Cardiac defense response* was obtained from the Electrocardiogram (EKG), which was recorded using standard Ag/AgCl electrodes filled with electrode paste in lead II configuration (right arm and left leg with ground electrode on right leg) using a Grass 7P4 EKG preamplifier with a band-pass filter set at 10-35 Hz. Weighted averaged second-by-second heart rate was obtained from the R-R interval of the EKG signal (beat-to-beat heart period), with a resolution of 1 ms, using the VPM software (Cook, 1997). The 80 heart rate values after onset of the acoustic stimulus were then expressed in terms of the difference in scores with respect to the 15-s pre-trial period. The statistical analysis was facilitated by using a summary technique previously applied by our group, reducing the 80 sec-by-sec heart rate values for each participant to 10 heart rate values corresponding to the median values of 10 progressively longer intervals: two of 3 s, two of 5 s, three of 7 s, and three of 13 s (Pérez et al., 2000; Vila et al., 1992, 1997, 2007). The *Startle blink reflex* was obtained from the Electromyogram (EMG) recorded by placing two miniature Ag/AgCl electrodes filled with electrode paste over the left orbicularis oculi muscle: one beneath the pupil and the second at around one cm lateral to the outer cantus of the eye. EMG activity was recorded with a Coulbourn V75-25 bio-amplifier using a frequency band of 90-1000 Hz and sampled at 1000 Hz from 500 before to 1000 ms after noise onset. The raw EMG signal was rectified and integrated online using a Coulbourn V75-04 integrator with a time constant of 75 ms. 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 the Balaban procedure (Balaban et al., 1986). Magnitude was scored as zero for trials with no detectable peak.

Self-Report Measures. Participants completed a post-experimental questionnaire that assessed the intensity and unpleasantness of the three noises on a scale from 0 to 100 (0 = not at all intense/unpleasant, 100 = extremely intense/unpleasant).

Procedure

Each participant attended a single laboratory session of approximately 60 min. Upon arrival, the participant was seated in an armchair, received information about the experimental session, signed the informed consent form, and completed a personal interview to confirm that selection criteria were met. The participant was informed that the purpose of the experiment was to record physiological data during rest and during presentations of brief loud noises. Instructions did not mention the aversive nature of the stimulus. In line with the standard cardiac defense testing procedure, the noise was not presented prior to the test. The electrodes were then attached, the signals checked, the earphones placed on the participant's head and the participant was left alone in a semi-darkened room. After the test, the experimenter removed the earphones and the electrodes and the participant completed the post-experimental questionnaire. The participant was then debriefed and awarded the credits for his/her participation.

Statistical Analysis

Cardiac and blink responses were analyzed using mixed between-group ANOVAs with repeated measures. Greenhouse Geisser corrected values are reported for main effects and interactions involving repeated-measures factors with more than two levels. In analyses of the cardiac response, the between-group factor was Condition (the 6 ITI conditions) and the repeated-measures factors were Trial (3 trials) and Time (10 median heart rate values). For the blink response and self-report measures, the between-group factor was again Condition and the repeated-measures factor was Trial. Significant interaction effects were analyzed by following Keppel's procedure (Keppel, 1991). We first identified the levels of interacting factors that explained significant effects (simple effects analysis) and then examined habituation and recovery in the three trials by means of orthogonal trend analysis and/or multiple pairwise comparisons using Bonferroni test. Quantification of habituation (or sensitization) and recovery was based on statistically significant decreases and/or increases along the three trials. In addition, Pearson's product-moment correlations between the startle response and the short- and long latency acceleration of cardiac defense were calculated using median 1 as index of the short latency acceleration, and medians 5, 6, 7, and 8 as index of the long latency acceleration. Correlations were also calculated for the difference score between trials in both reflexes. The level of significance was set at .05 for all analyses.

3. Results

Cardiac defense

The average cardiac defense response elicited by the three presentations of intense noise is shown as a function of ITI in Figure 2. In all conditions, the response to the first stimulus reproduced the expected response pattern: an initial acceleration/deceleration with peak between seconds 1-3 (median 1) and a second acceleration/deceleration with peak between seconds 20-40 (medians 5-8). Figure 2 also illustrates the fast habituation tendency of the second accelerative component, evident in Condition 1, when the ITI1 was the shortest (2.5 min). Some evidence of recovery is also observed in this condition, when the ITI2 was the longest (27.5 min). The opposite tendency is observed in Condition 6 (symmetrically opposed to Condition 1), with some evidence of delay habituation with the longest ITI1 (27.5 min), and reduced recovery with the shortest ITI2 (2.5 min).

This impression was confirmed by the 6 x (3 x 10) ANOVA, Condition x (Trial x Time), which revealed main effects of Trial, $F(2, 168) = 11.15$, $p = .001$, $\eta p^2 = .117$, and Time, $F(9, 756) = 52.91$, $p = .0001$, $\eta p^2 = .386$, and interaction effects of Trial x Time, $F(18, 1512)=3.70$, $p = .001$, $\eta p^2 = .042$, and Condition x Trial x Time, $F(90, 1512) = 1.63$, $p = .003$, $\eta p^2 = .089$. The significant Condition x Trial x Time interaction was followed by separate ANOVAs for each Condition. The (3 x 10) ANOVAs (Trial x Time) only yielded significant Trial x Time interactions for Conditions 1, $F(18, 252) = 3.96$, $p =.003$, $\eta p^2 = .22$, and 6, $F(18, 252) = 2.20$, $p = .05$, $\eta p^2 = .136$. Subsequent analyses were centred on comparisons between these two conditions for each Time level (median values).

Insert Figure 2

Results showed significant Condition x Trial interaction for medians 1 (first acceleration), 6 (second acceleration), and 10 (second deceleration). Trend analysis of this interaction indicated that the two Conditions differed in the quadratic trend. Condition 1 had a significant quadratic trend (V form) in medians 1 ($p = .01$) and 6 ($p = .05$), indicating a larger reduction of first and second acceleration in Trial 2 than in Trial 3 (i.e., greater habituation and greater recovery), whereas Condition 6 had a significant linear trend in medians 1 ($p = .02$) and 6 ($p = .02$) indicating smaller reductions of the first and second accelerations in Trial 2 than in Trial 3 (lesser habituation and lesser recovery). With regard to median 10 (second deceleration), the opposite quadratic trend (inverted V form) was found in Condition 1 ($p = .002$) indicating a larger reduction of the deceleration in Trial 2 than in Trial 3 (more habituation and more recovery), whereas a linear trend was found in Condition 6, indicating a smaller reduction of the deceleration in Trial 2 than in Trial 3 (lesser habituation and lesser recovery).

Pairwise comparisons confirmed these findings: (a) in Condition 1, significant heart rate reductions were found in Trial 2 versus Trials 1 and 3, with no significant differences between Trials 1 and 3 ($p < .05$ in all cases); (b) in Condition 6, significant heart rate reductions was found in Trial 3 versus Trial 1, with no significant differences between Trials 1 and 2 ($p < .05$ in all cases).

No significant differences were found among the remaining Conditions (Conditions 2 to 5. These Conditions showed intermediate reponse patterns concerning habituation and recovery (see Figure 3), the maximum habituation and minimum recovery being found at the shortest ITI (2.5 min).

Eye-blink startle.

Figure 3 shows the average eye-blink response elicited by the three presentations of intense noise as a function of the ITI. In general, the response magnitude increases rather than decreases after the first stimulus presentation. In condition 1 (shortest ITI1 and longest ITI2), there was an increase from trial 1 to trial 2 and then from trial 2 to trial 3. In condition 6 (longest ITI1 and shortest ITI2), there was an increase from trial 1 to trial 2 and then a relative decrease from trial 2 to trial 3. The remaining conditions tend to show a similar pattern to condition 6 with the exception of condition 3, in which no change was detected along the three trials.

Statistical analysis using a 6 x (3) ANOVA, Condition x (Trial), yielded a significant Trial effect, $F(2, 168) = 5.87$, $p = .005$, $\eta p^2 = .065$. Neither Condition nor Condition x Trial interaction were significant ($p > .21$). Trend analysis showed significant linear ($p = .02$) and quadratic ($p = .02$) trends, and pairwise comparisons (Bonferroni test) revealed significant differences between trials 1 and 2 ($p = .01$) and marginally significant differences between trials 1 and 3 ($p = .057$). No significant differences were observed between trials 2 and 3 ($p = .97$).

Insert Figure 3

Self-report Measures

In general, participants rated the first noise as more intense (*M* = 78.14, *SD* = 20.19) than the second (*M* = 71.02, *SD* = 20.34) and third (*M* = 68.87, *SD* = 22.96) noises. Likewise, the first noise was rated as more unpleasant (*M* = 65.77, *SD* = 30.62) than the second (*M* = 58.29, *SD* = 29.54) and third (*M* = 57.81, *SD* = 30.65) noises. The 6 x (3) ANOVA, Condition x (Trial), yielded a significant Trial effect for both intensity ($F(2, 168) = 9.76$, $p = .001$, $\eta p^2 = .104$) and unpleasantness ($F(2, 168) = 8.42$, $p = .001$, $\eta p^2 = .091$). Pair wise comparisons showed significant differences between trial 1 and the other two trials ($p < .005$), with no significant differences between trials 2 and 3 ($p > .50$).

Correlations between Cardiac Defense and Eye-Blink Startle

Significant correlations were found between the short latency acceleration of cardiac defense in trial 1 and the magnitude of the startle responses in trials 1 ($r = .361$, $p = .001$, $N = 90$), 2 ($r = .285$, $p = .007$, $N = 90$) and 3 ($r = .328$, $p = .002$, $N = 90$). No correlation was found with long latency acceleration in any trial ($p > .14$). Finally, no significant correlation in the change score along the three trials was found between startle magnitude and cardiac defense ($p > .09$).

4. Discussion

In this study, cardiac defense and eye-blink startle differed in their tendency to manifest habituation and sensitization as a function of the time intervals between the three presentations of the acoustic stimulus. Cardiac defense showed (a) the expected pattern of heart rate response to the first stimulus presentation, with two accelerative/decelerative components; (b) the expected habituation tendency of the two accelerative components –faster for the second acceleration- when the stimulus was repeated after a short inter-trial interval (2.5 min), and (c) the expected reduced habituation and increased recovery with a longer time interval between stimulus repetitions. These effects were clearly observed when the two extreme ITI conditions were compared. In contrast, no habituation of eye-blink startle was observed, with a sensitization of this response after the first stimulus presentation across all ITI conditions. This effect was more evident in trial 2 than in trial 3. No significant differences were observed as a consequence of the ITI manipulation. The correlational analysis confirmed the dissociation between cardiac defense and eye-blink startle in habituation and sensitization along the three trials. Nevertheless, significant positive correlations were found between the short-latency acceleration in trial 1 and the magnitude of the startle response in trials 1, 2 and 3.

This differential habituation tendency of cardiac defense and eye-blink startle is consistent with previous findings. The repeated presentation of a stimulus capable of eliciting both reflexes under different stimulus and task conditions (manipulation of stimulus duration from 50 to 1000 ms [Ramírez et al., 2005], habituation of startle reflex before defense trials [Fernández et al., 2008] or visualization of unpleasant and fearful pictures immediately prior to defense stimulus [Sánchez et al., 2002]) has consistently shown faster habituation for cardiac defense than for eye-blink startle. In Ramírez et al.'s study, a 50-ms white noise, identical to the stimulus used in the present study, was delivered five times with intertrial intervals similar to our short ITI. The novelty of the present findings is (a) the sensitization effect observed in eye-blink startle, which questions the assumption that eye-blink startle shows habituation under all conditions (Grillon & Cornwell, 2007), and (b) the reduced habituation and increased recovery of cardiac defense with longer time interval between stimulus repetitions.

There are various possible explanations for the sensitization effect observed in our study. Groves and Thompson's dual-process habituation theory predicts sensitization during the initial presentations of high-intensity stimuli at a slow presentation rate (Groves & Thompson, 1970; Thompson et al., 1973). Some conditions of our study might also have facilitated the sensitization process. First, no test stimulus was presented before the physiological procedure. Second, participants were informed that brief intense noises would be presented after a rest period of several minutes, but no warning signal was given and instructions did not mention the aversive nature of the stimulus.

Consequently, participants were more aroused after the first stimulus presentation than before it. Finally, only three stimulus presentations were applied, reducing the expected spontaneous decay of the sensitization process after initial stimulus presentation.

A further explanation may be provided by the *motivational priming hypothesis,* which attributes potentiation of the eye-blink startle response during processing of unpleasant and fearful stimuli to the congruence between the emotional state induced by the stimuli (aversive) and the type of reflex being elicited (defensive) (Lang, 1995; Lang, Davis & Öhman, 2000). Hence, the sensitization of eye-blink startle observed in our study might have resulted from motivational priming after the first defense trial. This experience might have left the participant in an aversive motivational state that potentiated the startle response in subsequent trials. However, subjective rating of intensity and unpleasantness of the noise decreased, rather than increased, from the first to the second and third trial, thus arguing against this explanation. Alternatively, the sensitization effect might be due to anticipation of the aversive stimulus after the first noise presentation, as in the *threat of shock paradigm* (Grillon et al., 1993). Greenwald, Bradley, Cuthbert & Lang (1998) reported dramatic sensitization effects on eye-blink startle after exposure to electric shock. This effect was largest in the first startle test after the shock exposure. A similar sensitization effect has been reported in animals (Davis, 1989). To the extent that our first noise presentation can be considered similar to a shock experience, as suggested by the participants' subjective ratings, the *anticipatory process* hypothesis represents a plausible explanation.

The dissociation in habituation and sensitization between cardiac defense and eye-blink startle has methodological and theoretical implications. The rapid habituation of cardiac defense is a well-documented phenomenon (see Vila et al., 2007). The long latency acceleration practically disappears after the first stimulus presentation, even under priming conditions when the response is elicited during the viewing of unpleasant and fearful pictures (Sánchez et al., 2002; Ruiz et al., 2005). Thus, the *motivational priming effect* on cardiac defense appears to be restricted to the first trial, which limits the possibilities of comparative studies with other protective reflexes such as eye-blink startle. Moreover, if the affective picture visualization paradigm is used to examine the priming effect and habituation is found for cardiac defense and sensitization for eye-blink startle, this dissociation would determine the trial in which the potentiated response is observed (cardiac defense in the first trial and eye-blink startle in the second or third). This research limitation may be mitigated by the approach proposed in the present study, in which the habituation of cardiac defense could be delayed and its recovery increased by manipulating the inter-trial interval within a time window suitable for a single laboratory session.

The differential habituation/sensitization of cardiac defense and eye-blink startle questions the classic theory proposed by Sokolov (1963) and Graham (1992) that defense is more resistant to habituation than startle. In Graham's model, both startle and defense are indexed by the first heart rate acceleration and the difference derives from their peak latency (0-2 s for startle and 3-6 s for defense). Difficulties in the unambiguous application of this criterion led Turpin (1986) and Turpin et al. (1999) to suggest a differentiation between cardiac startle and defense based on the two accelerative components of the response, with startle corresponding to the short latency acceleration (peak at around 3 s) and defense to the long latency acceleration (peak at 30-40 s). This distinction is consistent with our findings of a higher resistance to habituation of the short latency acceleration and its positive correlation with eye-blink. It also implies that the complex pattern of heart rate changes characteristic of cardiac defense might represent two successive defense responses with different functionalities. Thus, the first acceleration would serve the protective function of startle (disengagement from ongoing activity and heightened attention to potential danger) and the second would serve the protective function of the traditional *fight and flight* response (preparation for active defense).

The rapid habituation of cardiac defense may be explained within this conceptual framework. Hence, the fast habituation of the long-latency acceleration/deceleration may follow disconfirmation of danger after the first noise presentation. An intense unexpected noise, in addition to arousing the organism, signals a potential danger that requires attention and preparation for quick action. If the potential danger is not confirmed after the first stimulus presentation, in subsequent presentations of the acoustic stimulus within a short time period there will be no need for further response mobilization (second acceleration/deceleration). However, this rapid habituation can be expected to diminish with a lengthening of the time interval between stimulus presentations, which would explain the delayed habituation and recovery observed in our study. It has been postulated that the physiological mechanism underlying rapid habituation involves a resetting of the baroreflex threshold after evocation of the full response pattern (Fritsch et al., 1989; Reyes del Paso et al., 1994; Turpin, 1986). This baroreceptor threshold resetting is also expected to disappear with a longer time interval between stimulus repetitions.

The sensitization of eye-blink startle when elicited simultaneously with cardiac defense may be explained by various mechanisms, as reported above. However, none of those mechanisms explains why manipulation of the time interval between stimulus presentations did not influence the sensitization effect, as it did not affect the subjective ratings of the noise either. Two obvious limitations of our study –small number of participants per ITI condition and retrospective character of the self-report measures- must be considered as possible contaminating factors that should be controlled in future studies.

In summary, these data confirm the complex pattern of heart rate changes that characterizes cardiac defense. They also confirm the differential habituation tendencies of cardiac defense and eye-blink startle when simultaneously elicited by an intense acoustic stimulus. In addition, manipulation of the inter-trial interval within a 30-min time window produced delayed habituation and greater recovery of cardiac defense but had no effect on sensitization of the eye-blink startle response or on subjective ratings of the noise.

Acknowledgements

This research was supported by two granst from the Spanish Ministry of Science and Education and the European Union (Projects: BSO2001-3211; SEJ2004-07956).

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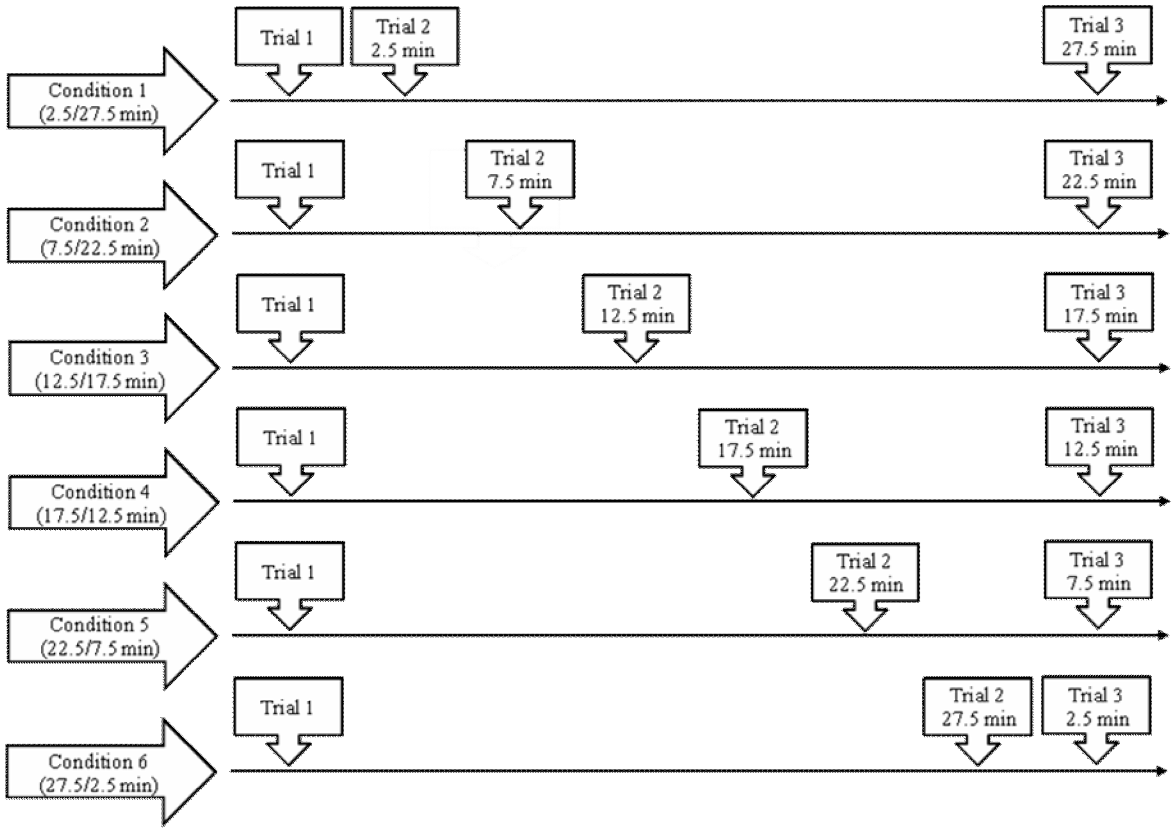
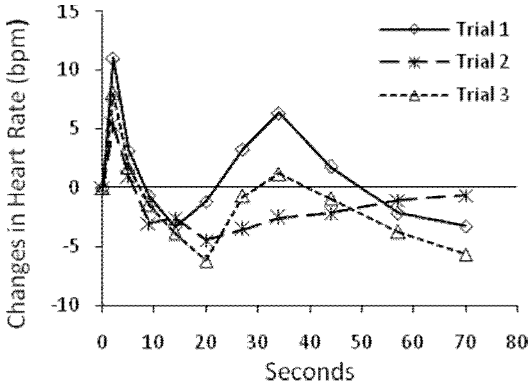
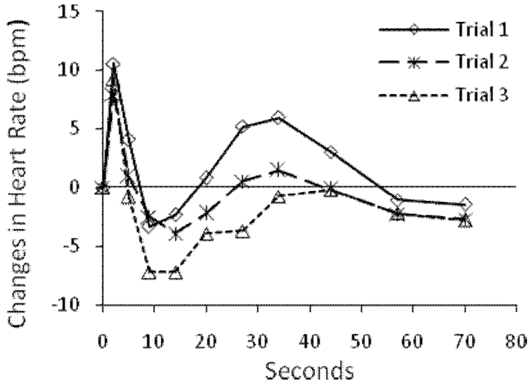

Condition 1
(2.5/27.5 min)
Trial 1
Trial 2
2.5 min
Trial 3
27.5 min
Condition 2
(7.5/22.5 min)
Trial 1
Trial 2
7.5 min
Trial 3
22.5 min
Condition 3
(12.5/17.5 min)
Trial 1
Trial 2
12.5 min
Trial 3
17.5 min
Condition 4
(17.5/12.5 min)
Trial 1
Trial 2
17.5 min
Trial 3
12.5 min
Condition 5
(22.5/7.5 min)
Trial 1
Trial 2
22.5 min
Trial 3
7.5 min
Condition 6
(27.5/2.5 min)
Trial 1
Trial 2
27.5 min
Trial 3
2.5 min

Figure 1

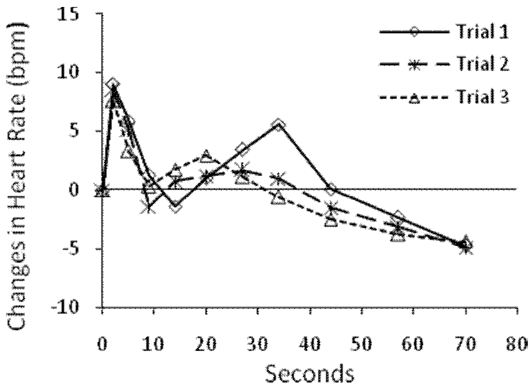
Condition 1
ITI 1 = 2.5 min ITI = 27.5 min



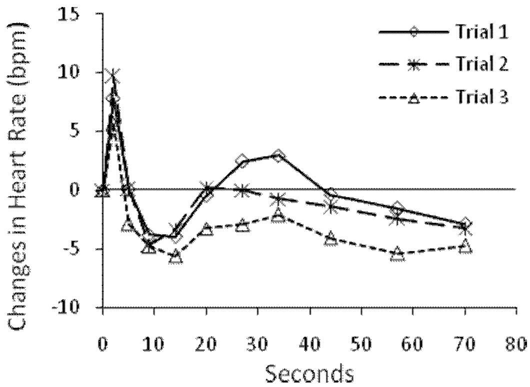
Condition 2
ITI 1 = 7.5 min ITI 2 = 22.5 min



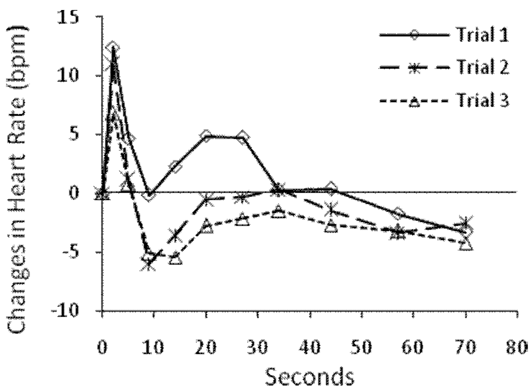
Condition 3
ITI 1 = 12.5 min ITI 2 = 17.5 min



Condition 4
ITI 1 = 17.5 min ITI 2 = 12.5 min



Condition 5
ITI 1 = 22.5 min ITI 2 = 7.5 min



Condition 6
ITI 1 = 27.5 min ITI 2 = 2.5 min

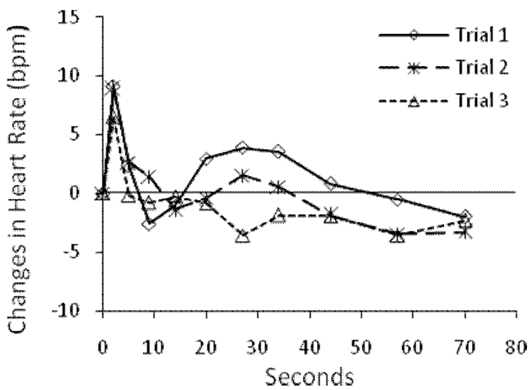


Figure 2

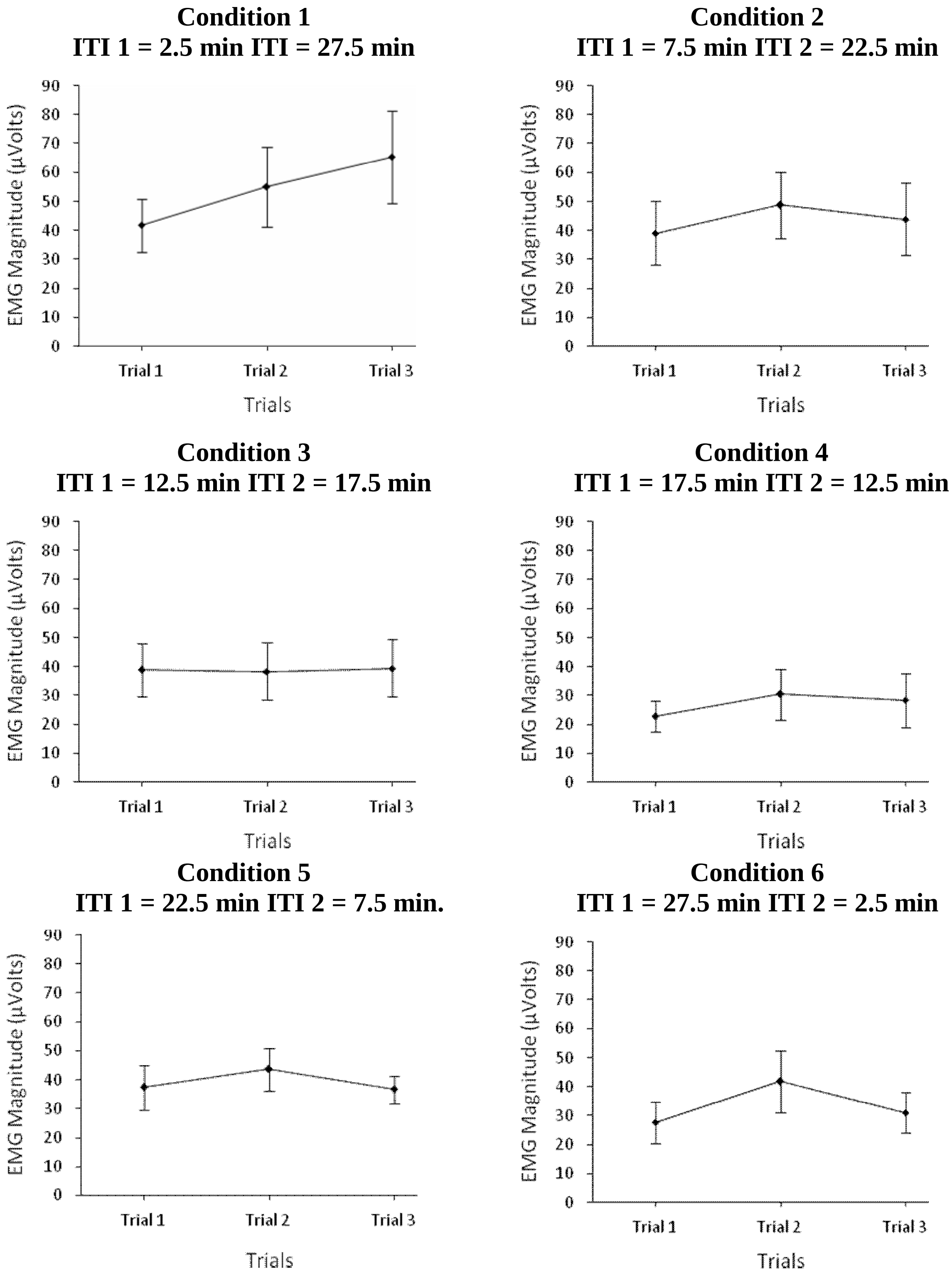

Condition 1
ITI 1 = 2.5 min ITI = 27.5 min
Condition 2
ITI 1 = 7.5 min ITI 2 = 22.5 min
Condition 3
ITI 1 = 12.5 min ITI 2 = 17.5 min
Condition 4
ITI 1 = 17.5 min ITI 2 = 12.5 min
Condition 5
ITI 1 = 22.5 min ITI 2 = 7.5 min.
Condition 6
ITI 1 = 27.5 min ITI 2 = 2.5 min
EMG Magnitude (µVolts)
0
10
20
30
40
50
60
70
80
90
Trial 1
Trial 2
Trial 3
Trials

Figure 3

FIGURE LEGENDS

Figure 1. Diagram showing the six Inter-Trial Conditions. Numbers within boxes indicate the time in minutes between Trials

Figure 2. Cardiac defense: Heart rate response to the three noise presentations as a function of ITI conditions

Figure 3. Eye-blink startle: Blink magnitude to the three noise presentations as a function of the six ITI conditions (bars are standard error of the mean)