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Psychophysiological correlates of chronic worry: Cued versus non-cued fear reaction

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ABSTRACT

Worry has been defined as a chain of thoughts and images that promote mental attempts to avoid anticipation of potential threats. From this perspective worry can be conceptualized as a state of anticipatory anxiety or non-cued fear reaction. The present study examines high and low chronic worriers during cued and non-cued defense reaction paradigms and during resting and self-induced worry periods. The non-cued procedure was based on the cardiac defense paradigm, whereas the cued procedure was based on the startle probe paradigm using pleasant, neutral and unpleasant pictures as cues. High worriers, compared to low worriers, showed (a) a greater cardiac defense response in the non-cued fear response paradigm, (b) no differences in eye-blink in the startle probe paradigm, (c) reduced skin conductance reactivity during the startle probe paradigm and (d) reduced Respiratory Sinus Arrhythmia, accompanied by increased respiratory rate and decreased expiratory period, during the resting period. These results support the notion of chronic worry as a state of anticipatory anxiety, accompanied by indices of reduced vagal control, that modulates non-cued defense reactions.

1. Introduction

Worry has been conceptualized as a chain of thoughts and images, negatively affect laden and relatively uncontrollable, that promotes mental attempts to avoid anticipation of potential threats (Borkovec, 2002). This conceptualization emphasizes a key aspect of worry: anticipation of threat. Anticipation of threat activates defense reactions, either the fight–flight response or the freezing response. Continuous activation of this type of defense reactions is a form of being permanently stressed and vigilant to emotional negative information, thus increasing the risk of physical and mental problems (Brosschot et al., 2006; Knepp and Friedman, 2008).

The psychophysiological correlates of worry have been investigated in a number of studies using as participants non clinical high trait worriers and patients with generalized anxiety disorder (GAD) (Hoehn-Saric et al., 1993; Dua and King, 1987; Borkovec et al., 1983, Borkovec and Roemer, 1995; Karteroliotis and Gil, 1987; Lyonfields et al., 1995; Thayer et al., 1996, 2000; Segerstrom et al., 1999; Wilhelm et al., 2001; Davis et al., 2002; Brosschot et al., 2003; Hofmann et al., 2005; Jönsson, 2007; Conrad et al., 2008). Although the data are not totally consistent, the two most repeated findings are the absence of sympathetic hyper-reactivity (indexed mainly by skin conductance measures) and the presence of reduced vagal control (indexed by HR variability measures) in high worry people. Confirming previous findings, Thayer and Brosschot (2008) have recently reported increased HR and decreased HR variability, monitored over a 24 h period, associated with daytime worry and the subsequent nighttime. As regards brain mechanisms, a recent study using functional MRI in GAD patients (Oathes, 2008) found greater amygdala activation during anticipation of emotional pictures, but hypo-reactivity in the same region during the actual presentation of the pictures.

A relevant psychophysiological research area, insufficiently investigated in relation to chronic worry, is the modulation of defense reactions. As mentioned above, a central feature of chronic worry is anticipation of threat. Therefore, it should be expected that anticipation of threat in high worriers would modulate defense reactions. Two specific defense reactions, eye-blink startle and cardiac defense, have been widely investigated in recent years in the context of fear and anxiety research (for a review, see Lang et al., 2000; Grillon, 2002; Bradley and Lang, 2007; Vila et al., 2007). The startle reflex in humans involves a quick closing of the eyes accompanied by stiffening of the head, dorsal neck, body walls, and limbs, as if to protect from a predator (Graziano and Cooke, 2006). Cardiac defense, on the other hand, refers to the heart rate response to intense or aversive stimulation. It consists of a complex response pattern, observed within the 80 s after stimulus onset, with a short latency acceleration/deceleration (peak around second 3), followed by a long latency acceleration/deceleration (peak between 30 and 40 s) (Vila et al., 2007).

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Cardiac defense and eye-blink startle belong to two different response systems (cardiovascular and motor) with different sensitivities to experimental manipulations (see Ramírez et al., 2005). However, both reflexes can be elicited by intense acoustic stimulation and can show affective modulation. Blink startle is potentiated when viewing unpleasant pictures and inhibited when viewing pleasant ones, compared to neutral pictures (Bradley et al., 1990; Lang et al., 2000). Cardiac defense is also potentiated when viewing unpleasant and fearful pictures, compared to neutral and pleasant ones (Sánchez et al., 2002; Sanchez et al., 2009). In this context, an interesting distinction has been made between cued and non-cued (contextual) fear in order to differentiate fear from anxiety. Non-cued fear, identified with anxiety, refers to the aversive expectation of potential future danger, whereas cued fear refers to the aversive emotional reaction elicited by specific danger.

Several procedures, based on the modulation of protective reflexes, have been used to examine cued and non-cued fear reactions. The startle probe (Lang, 1995), the threat-of-shock (Grillon et al., 1993), and the cardiac defense (Vila et al., 2007) paradigms are well-established experimental procedures to study specific (cued) and contextual (non-cued) fear in laboratory settings. It has been shown, for instance, that diagnosed anxiety patients with more general anxiety reactions (Panic and Post-traumatic Stress Disorder), compared to patients with more focal fear reactions (Specific Phobias and Social Phobia), exhibit more eye-blink startle potentiation when no cues are presented (i.e., during resting baseline) than during specific cue presentation (i.e., visualization of unpleasant or fearful pictures) (Cuthbert et al., 2003; Grillon et al., 1994, 1998). Thus, since chronic worry is considered an example of anticipatory anxiety (contextual fear), it can be expected that chronic worriers would be characterized by a general sensitivity to react defensively when no specific cues allow identification of danger, rather than by a sensitivity to react defensively to specific fearful cues.

The modulation of protective reflexes can also help to advance knowledge on the physiological mechanisms underlying chronic worry. As mentioned above, the most consistent findings suggest a reduced vagal control, rather than an increased sympathetic activation, in chronic worriers. Reduced vagal control, indexed by low heart rate variability or low Respiratory Sinus Arrhythmia (RSA), has been postulated as a major determinant of psychological and physiological dysfunction (Thayer and Siegel, 2002; Thayer and Brosschot, 2005; Porges, 2007). However, most studies suggesting reduced vagal control in high worriers have not controlled for respiratory changes. The link between excessive worry and vagal control, based solely on RSA data, remains therefore questionable, since it is well known that changes in respiration can easily reduce the amplitude of RSA independently of vagal tone changes (Grossman and Kollai, 1993; Grossman and Taylor, 2007).

The modulation of cardiac defense and eye-blink startle can provide additional evidence in favour or against the assumed vagal control mechanism underlying chronic worry. On one hand, both reflexes can be examined concurrently with the skin conductance response, an index of sympathetic activation. In the startle probe paradigm, skin conductance should show an arousal effect: greater response to emotional pictures (both pleasant and unpleasant) than to neutral ones (Bradley and Lang, 2007). On the other hand, the cardiac defense response has components that are differentially mediated by sympathetic and parasympathetic mechanisms: the short latency component is controlled by vagal influences, whereas the long latency component is controlled by reciprocal sympathetic and parasympathetic influences (Fernández and Vila, 1989; Reyes del Paso et al., 1993, 1994). Thus, the finding of significant differences in any of these indices can help to identify the specific physiological mechanism underlying chronic worry.

The aim of the present study was twofold. First, was to examine the modulation of defensive reflexes in high and low chronic worriers under cued and non-cued defense reaction paradigms. It was hypothesized that high chronic worriers, compared to low chronic worriers, would exhibit (a) greater defense reaction under a non-cued than under a cued defense reaction paradigm, (b) reduced indices of vagal control in the non-cued defense reaction paradigm, and (c) reduced indices of sympathetic activation (skin conductance) in both paradigms. Second, was to examine in the same people whether the reported reduced indices of vagal control (RSA) in high worriers during resting/worry periods can be explained by changes in respiration. It was hypothesized that high chronic worriers, compared to low chronic worriers, would exhibit during both periods reduced RSA amplitude independently of respiratory parameters (respiratory rate, inspiratory period and expiratory period).

2. Method

2.1. Participants

Participants were 70 female volunteer university students, age ranged between 18 and 24 years old. They were selected from an initial pool of 438 students who completed the Penn State Worry Questionaire (PSWQ, Meyer et al., 1990). High worry participants were 48 students who scored within the top fifth (20%) of the PSWQ distribution ($M = 69.9$, $SD = 3.6$, range 63–77), whereas low worry participants were 22 students who scored within the bottom fifth of the distribution ($M = 35.5$, $SD = 6.7$, range 19–43). No participant was undergoing psychological or pharmacological treatment, or had auditory or cardiovascular problems. They were all screened using the ADIS-IV (Brown et al., 1994) to guarantee that none of them suffered from generalized anxiety disorder. Six participants had physiological artifacts or missing data on some measures (three in questionnaires, one in skin conductance in the non-cued defense paradigm, four in eye-blink startle and six in skin conductance in the cued defense paradigm, one in RSA, and two in respiratory parameters). They were excluded from the specific analyses concerning those measures.[1]

2.2. Psychophysiological test

The psychophysiological test had the following sequence: (a) baseline resting period: 8 min of rest with physiological recording during the last 5 min; (b) non-cued defense paradigm (Vila et al., 2007): after a baseline of 15 s, an intense white noise of 105 dB intensity, 500 ms duration, and instantaneous risetime, capable of eliciting cardiac defense, was presented through earphones, followed by 80 s extended recording period; (c) cued defense paradigm (Lang, 1995): 30 pictures selected from the *International Affective Picture System* (Lang et al., 2008) according to the Spanish normative ratings (Moltó et al., 1999; Vila et al., 2001) were presented during 6 s; the pictures differed both in valence and arousal scores (10 highly pleasant, 10 neutral, and 10 highly unpleasant); each picture was accompanied by an acoustic startle (the same white noise previously presented but reduced to 50 ms duration); three additional non-cued acoustic startles were also presented interspersed among the picture trials; and (d) worry period: 5 min of self-induced worry with physiological recording. The transition from one period to the next was unsignaled except for the worry period that was indicated by a written instruction projected on the screen in front of the participant during 5 s.

Each trial in the cued defense paradigm ensued as follows: (a) 3 s of baseline data collection; (b) 6 s picture presentation with a startle

[1] Participants were unequally distributed in the high and low worry groups because high worry participants, after the present study, were going to be split into two groups, equivalent in number to the low worry group, in order to participate in a controlled intervention study. Results of the second part of the study are being reported elsewhere.

auditory stimulus presented randomly at a point between 2.5 s and 4.5 s; (c) 3 s post-picture, extended data collection; and (d) a randomly varied 4 to 7.5 s inter-trial interval. Pictures were presented in random order. The 10 highly pleasant pictures belonged to the category of erotic couples (5) and sports (5), the 10 neutral pictures belonged to the category of household objects, and the 10 highly unpleasant pictures belonged to the category of animal/nature threat (5) and human threat (5). Pleasant and unpleasant pictures were matched in high arousal. Spanish norms for those pictures (scale 1–9) were: pleasant (valence: $M=7.32$, $SD=.52$; arousal: $M=7.40$, $SD=.41$), neutral (valence: $M=5.37$, $SD=.32$; arousal: $M=2.48$, $SD=.30$), unpleasant (valence: $M=2.07$ $SD=.71$; arousal: $M=7.52$, $SD=.33$). All pictures were presented in random order for each participant.[2]

2.3. Measures and instruments

2.3.1. Self-report measures

In addition to the PSWQ, participants completed the following questionnaires: (a) Beck Depression Inventory (BDI; Beck et al., 1979); (b) Trait scale of the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970); (c) Positive and Negative Affect Schedule (PANAS; Watson et al., 1988); and (d) Subjective Health Complaints (SHC; Ericksen et al., 1999). The SHC consists of 29 items concerning severity and duration of several health complaints during the last two weeks (musculoskeletal pain and headache, gastrointestinal problems, pseudoneurological complaints such as dizziness and anxiety, and allergy, flu and common cold). Finally, participants also assessed the degree of success achieved during the 5 min worry period using the "Self-induced worry questionnaire", a scale ranging from 0 to 10, 0 meaning not success at all and 10 complete success.

2.3.2. Cardiac defense

A Grass polygraph (Rps 7c 8b) was used to record the heart rate, which was derived from the EKG (configuration II) using a 7P4 preamplifier. R–R intervals were measured in milliseconds and transformed into second-by-second heart rate using a weighted averaging method (Reyes del Paso and Vila, 1998). Then, the cardiac defense response in the non-cued defense paradigm was obtained following the procedures outlined by Vila et al. (1992): The 80 second-by-second HR values after the onset of the defense evoking stimulus were expressed as differential scores from a baseline and reduced to the medians of 10 intervals progressively longer with midpoints at seconds 2, 3, 9, 14, 20, 27, 34, 44, 57, and 70. The cardiac defense pattern is indicated by the presence of a significant cubic trend (Graham and Slaby, 1973): from first acceleration to first deceleration, from first deceleration to second acceleration and from second acceleration to second deceleration. Matlab software Ecglab (Carvalho et al., 2002) and KARDIA (Perakakis et al., 2008) were used for R peak detection and R interval analysis, respectively.

2.3.3. Eye-blink startle

The startle response was measured by recording EMG activity from the orbicularis oculi region beneath the left eye using small Sensormedic Ag/AgCl electrodes filled with electrolyte paste. The raw EMG signal was amplified and integrated using Coulbourn bioamplifiers V75-04 and V76-23, respectively. The signal was filtered using a frequency band of 90–1000 Hz and integrated using a time constant of 75 ms. The startle reflex magnitude to the acoustic stimulus in the cued defense paradigm was defined as the difference in microvolts between the peak of the integrated response and the response onset occurring between 20 and 100 ms following the initiation of the startle evoking stimulus. Finally, the startle magnitude values were log transformed (log[DATA + 1]) in order to normalize the data. Affective modulation of eye-blink startle is indicated by the presence of a significant linear trend (Bradley et al., 1990): smaller response to pleasant than to unpleasant pictures, with the response to neutral pictures occupying an intermediate position.

2.3.4. Skin conductance (SC)

Skin conductance was registered from two standard Sensormedic Ag/AgCl electrodes filled with isotonic electrolyte paste (0.29 g NaCl per 100 ml water) placed on the left hypothenar eminence. The signal was recorded using the Coulbourn bioamplifier V75-23. The SC response to the defense noise in the non-cued defense paradigm was obtained following identical procedure applied to cardiac defense. The response pattern is indicated by the presence of a significant quadratic trend: progressive increase followed by progressive decrease. The SC response to the pictures in the cued defense paradigm was obtained following the procedure used by Bradley et al. (1990) and Keil et al. (2008). First, average changes in microSiemens were obtained every half second during 6 s after picture onset, expressed as deviations from a baseline period of 1 s preceding the picture. SC magnitude to the pictures was then defined as the maximum change between 1 and 4 s after picture onset. Finally, the magnitude values were log transformed (log[DATA + 1]) in order to normalize the distribution. Affective modulation of skin conductance is indicated by the presence of a significant quadratic trend (Bradley et al., 1990): greater response magnitude to pleasant and unpleasant pictures than to neutral ones.

2.3.5. Respiration

Respiratory measures were recorded using a pneumographic transducer around the participant's chest, at the xiphoid cartilage level, connected to a Coulbourn respiration amplifier V75-25A. The following breathing parameters were obtained during baseline and worry periods: mean respiratory rate, inspiratory period and expiratory period.

2.3.6. Respiratory Sinus Arrhythmia (RSA)

RSA amplitudes during the resting and the self-induced worry period were obtained using the peak-to-trough method (Reyes del Paso et al., 1993). The amplitude was defined as the difference in milliseconds between the minimum heart period found during inspiration (when vagal activity is inhibited) and the maximum heart period found during expiration (when vagal activity is present), adjusting the temporal phase relation between the cardiac and respiratory activity according to Eckberg (1983). When the minimum heart period during inspiration was longer than the maximum heart period during expiration, an amplitude of zero was assigned to that respiratory cycle.

2.3.7. Experimental control

The sequence of stimuli presentations and the acquisition and analysis of physiological data were controlled by the VPM software program (Cook, 1994) using the *Advantech*-PCL812PG A/D converter and input–output data card run by a Pentium 4 computer.

2.3.8. Picture presentation

Pictures were presented using a Canon LV-53 projector. The projector presented 145 × 95 cm images 3 m away from the participant.

2.3.9. White noise

A Coulbourn audio system model V85-05 with an 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 and Kjaer, model 2235) and an artificial ear (Bruel and Kjaer, model 4153).

[2] IAPS codes for pictures were: pleasant 4561, 4669, 4670, 4672, 4676, 8178, 8179, 8185, 8370, 8496; neutral 5531, 7002, 7009, 7025, 7175, 7207, 7224, 7233, 7235, 7705; unpleasant 1050, 1525, 2683, 2800, 2981, 6244, 6555, 8480, 8485, 9410.

2.4. Procedure

Participants after completing the PSWQ were contacted by phone and invited to attend a single laboratory session that lasted 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 the selection criteria. The participant was informed that the purpose of the experiment was to record physiological data during rest, during presentations of brief loud noises and pictures, and during a final 5 min period in which they had to worry about the things they usually worry. After instructions were given and any doubts were clarified, the electrodes were attached, the signals checked, the earphones placed on the participant's head, and the participant was left alone in a semi-darkened room. Following the test, the experimenter removed the earphones and the electrodes and the participant completed the self-report questionnaires. Finally, participants were clinically assessed, using the ADIS-IV structured interview, to discard diagnosis of generalized anxiety disorder. None of the participants were excluded due to this reason.

2.5. Statistical analysis

Physiological data that included repeated measures factors were analyzed by means of repeated measures ANOVAS using the multivariate test statistic (*Wilks' lambda*) generated by SPSS. This method is free of sphericity assumptions and thus is more suitable for repeated measures designs (O'Brien and Kaiser, 1985). Results are presented reporting the *F* value associated to the *Wilks' lambda* statistic. Self-report measures and physiological data that did not include repeated measures factors were analyzed by means of analysis of variance (ANOVA) with a single between group factor: high and low worry groups. In the non-cued defense paradigm, the predicted pattern of differences between groups in the response pattern (Time factor) was tested by trend analysis: a significant cubic trend for cardiac defense, and a significant quadratic trend for skin conductance, assuming a significant Group × Time interaction. In the cued defense paradigm, the predicted pattern of eye-blink and skin conductance responses across the three picture categories was also tested by trend analysis: a significant linear trend for blink startle, with the smallest response to the pleasant pictures, and a significant quadratic trend for skin conductance, with the smallest response to the neutral pictures. Additionally, multiple pairwise comparisons to examine differences between picture categories in the cued defense paradigm were performed using *Holm test* (Glantz, 2005). Finally, the independency between RSA and respiration was tested by mean of (a) Pearson's bivariate correlations and (b) analysis of covariance using Group as independent variable, RSA as dependent variable, and the three respiratory parameters as covariates. The level of significance was set at .05 for all analyses.

3. Results

3.1. Self-report measures

Table 1 presents the mean and standard deviation of self-report measures for each group. Participants in the high worry group, compared to the low worry group, scored significantly higher in depression ($F(1, 66) = 19.07$ $p < .0001$), trait anxiety ($F(1, 67) = 84.07$, $p < .0001$), negative affect ($F(1, 67) = 31.8$, $p < .0001$), and subjective health complaints ($F(1, 65) = 14.42$, $p < .0001$). They also scored significantly lower in positive affect ($F(1, 67) = 13.11$, $p < .001$). Finally, as regards the self-induced worry questionnaire, participants in both groups rated their performance during the worry period as moderately successful, no significant differences being found between the high and low worry groups ($F(1, 65) = .31$, $p > .58$).

Table 1
Mean and (standard deviation) scores in the Self-Report Measures for High and Low worry participants.

	High worry group	Low worry group	*p* differences
Depression (BDI)	10.0 (6.9)	2.95 (3.3)	<.0001
Trait anxiety (STAI-T)	32.52 (10.0)	11.19 (5.4)	<.0001
Health complaints (SHC)	16.54 (8.9)	8.42 (4.4)	<.0001
Negative affect (PANAS-N)	24.08 (7.4)	14.57 (3.5)	<.0001
Positive affect (PANAS-P)	28.46 (6.3)	33.95 (4.4)	<.001
Self-induced worry	6.0 (1.7)	6.3 (1.6)	NS

3.2. Non-cued defense reaction paradigm

3.2.1. Cardiac defense

Fig. 1 (left panel) represents the cardiac defense response to the intense white noise in the high and low worry group. Both groups show the expected short and long latency acceleration. High worriers, however, display a reduced heart rate deceleration both after the short latency acceleration and after the long latency acceleration. Confirming this impression, the 2 × (10), Group × Time, ANOVA yielded significant effects of Time ($F(9, 60) = 17.87$, $p < .0001$) and Group × Time interaction ($F(9, 60) = 2.12$, $p < .04$). Trend analysis (without including the baseline zero point) only showed a highly significant cubic trend in this interaction ($F(1, 68) = 7.37$, $p < .008$). As seen in Fig. 1, groups differed in the decelerative phases of the cubic trend.

3.2.2. Skin conductance

Fig. 1 (right panel) represents the skin conductance response to the intense noise in the non-cued defense paradigm. Both groups display a large response, with peak around second 9, which does not recover within the recording period after stimulus onset. The amplitude of the response in the high worry group appears smaller than in the low worry group. However, the 2 × (10), Group × Time, ANOVA did not confirm this impression. Neither the Group ($F(1, 67) = 1.10$, $p > .30$) nor the Group × Time interaction ($F(9, 59) = 1.20$, $p > .31$) was significant. The only significant effect was Time ($F(9, 59) = 11.14$, $p < .0001$), with a highly significant quadratic trend ($F(1, 67) = 53.51$, $p < .0001$).

3.3. Cued defense reaction paradigm

3.3.1. Eye-blink startle response

The 2 × (3), Group × Valence, ANOVA yielded a significant main effect of Valence ($F(2, 63) = 17.68$, $p < .0001$). Group and Group × Valence were not significant. Trend analysis on the Valence factor revealed a highly significant linear trend ($F(1, 64) = 34.17$, $p < .0001$). Fig. 2 represents the eye-blink startle magnitude to the white noise when participants were viewing the pleasant, neutral, and unpleasant pictures. As expected, the magnitude when viewing pleasant pictures was the smallest followed by the neutral and then the unpleasant ones. Pairwise comparisons using *Holm test* revealed significant differences between pleasant and neutral ($p < .025$) and pleasant and unpleasant ($p < .017$) categories, but not between neutral and unpleasant. Trend analysis for each group separately also revealed significant linear trend for the high, ($F(1, 45) = 39.10$, $p < .0001$) and low ($F(1, 19) = 10.17$, $p < .005$) worry groups, the low worry group also showing a significant quadratic trend ($F(1, 19) = 5.48$, $p < .03$). Pairwise comparisons using *Holm test* revealed significant differences between the three picture categories in the high worry group (all $p < .05$). In the low worry group, significant differences only appeared between pleasant and neutral ($p < .025$) and pleasant and unpleasant ($p < .017$) categories.

3.3.2. Skin conductance

The 2 × (3), Group × Valence, ANOVA yielded significant main effects of Group ($F(1, 62) = 8.74$, $p < .004$) and Valence ($F(2, 61) = 12.27$,

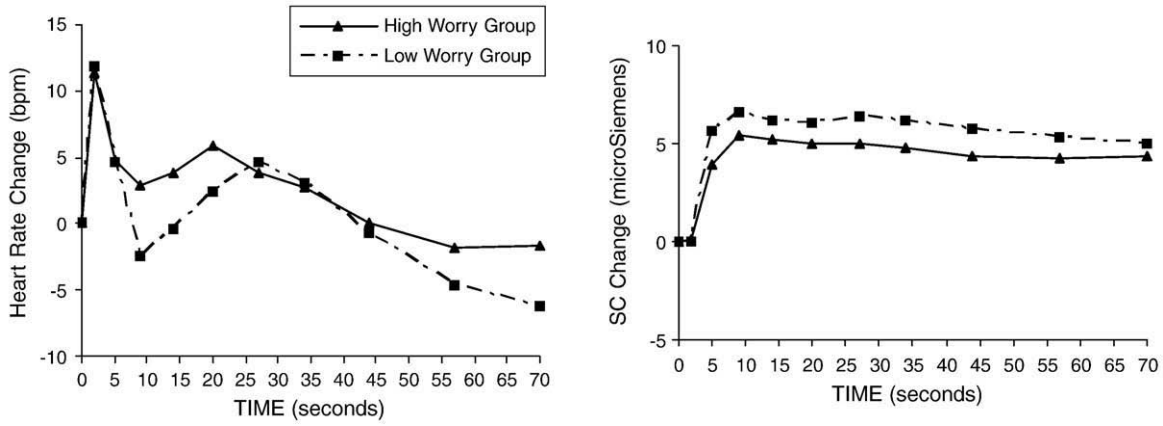

Fig. 1. Cardiac defense (left panel) and skin conductance (right panel) response in the non-cued defense reaction paradigm.

$p < .0001$). The Group × Valence interaction was not significant. Trend analysis for the Valence effect revealed a highly significant quadratic trend ($F(1, 62) = 24.86$, $p < .0001$). Fig. 3 represents the skin conductance magnitude to the pleasant, neutral, and unpleasant pictures. As expected, the magnitude to the neutral pictures was the smallest with the pleasant and unpleasant ones showing higher magnitudes. The significant main effect of Group indicates that the high worry group exhibited a smaller skin conductance response to all pictures, irrespective of affective category. As regards the Valence effect, pairwise comparisons using *Holm test* revealed significant differences between pleasant and neutral ($p < .017$) and unpleasant and neutral ($p < .025$) categories, but not between pleasant and unpleasant. Trend analysis for each group separately also revealed significant quadratic trends for the high ($F(1, 44) = 10.30$, $p < .002$) and low ($F(1, 18) = 10.94$, $p < .004$) worry groups. Pairwise comparisons using *Holm test* revealed significant differences for both groups between pleasant and neutral (High worriers: $p < .017$; Low worriers: $p < .025$), and between the unpleasant and neutral (High worriers: $p < .025$; Low worriers: $p < .017$) categories, with no differences between the pleasant and unpleasant ones.

3.4. Resting period

3.4.1. Respiratory Sinus Arrhythmia (RSA) and heart rate

RSA during the 5 min resting period was significantly lower in high worriers ($M = 59.43$, $SD = 31.92$) than in low worriers ($M = 80.03$, $SD = 53.75$; $F(1, 67) = 3.96$, $p < .05$). No significant differences were found in heart rate ($F(1, 65) = 2.04$, $p > .15$) between high worriers ($M = 81.7$, $SD = 12.0$) and low worriers ($M = 77.4$, $SD = 9.6$).

3.4.2. Respiratory measures

Two respiratory parameters showed significant differences between high and low worriers during resting period: respiratory rate ($F(1, 67) = 3.96$, $p < .05$) and expiratory period ($F(1, 66) = 5.78$, $p < .02$). High worriers exhibited higher respiratory rate ($M = 16.47$, $SD = 3.17$) and shorter expiratory period ($M = 1.62$, $SD = .50$) than low worriers (respiratory rate: $M = 14.48$, $SD = 3.26$; expiratory period: $M = 1.94$, $SD = .53$).

3.4.3. Skin conductance

No significant difference was found between high and low worriers in skin conductance during the 5 min resting baseline (High worriers: $M = 5.06$, $SD = 6.47$; Low worriers: $M = 5.75$, $SD = 4.0$; $F(1, 63) = .20$, $p > .65$).

3.5. Change from resting to worry period

3.5.1. Respiratory sinus arrhythmia and heart rate

Respiratory Sinus Arrhythmia during the 5 min worry period did not significantly change from the resting period. Heart rate did not significantly change either. The significant differences in RSA between the high and low worry groups during resting disappeared during the worry period ($F(1, 67) = 2.46$, $p > .12$).

3.5.2. Respiratory measures

The respiratory parameters did not change from baseline to worry. The significant differences in respiratory rate and expiratory period

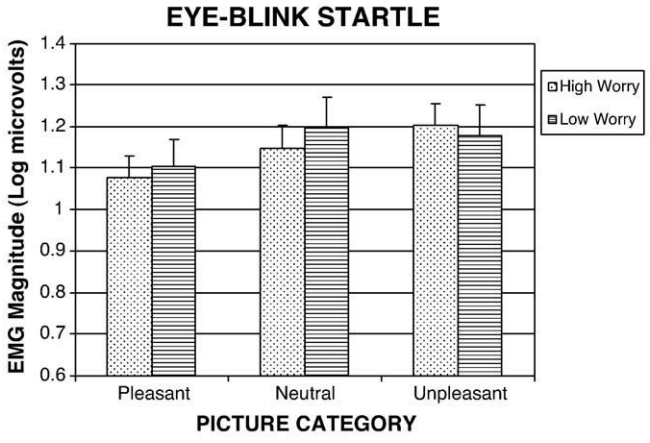

Fig. 2. Eye-blink startle response during visualization of pleasant, neutral, and unpleasant pictures in the cued defense reaction paradigm (bars are standard error of the mean).

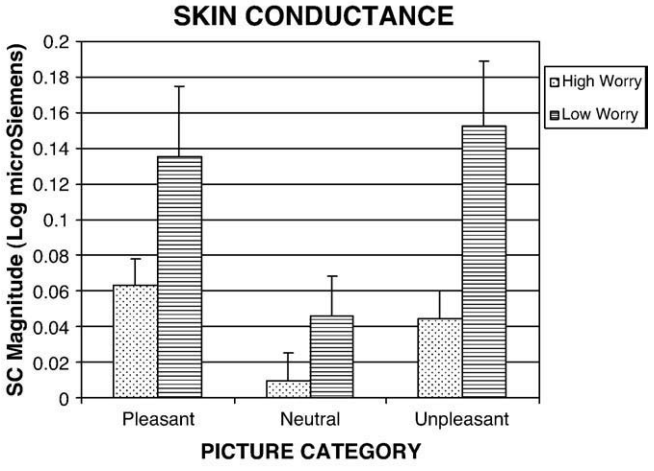

Fig. 3. Skin conductance response during visualization of pleasant, neutral, and unpleasant pictures in the cued defense reaction paradigm (bars are standard error of the mean).

were maintained during the worry period: respiratory rate ($F(1, 66) = 4.3$, $p < .04$) and expiratory period ($F(1, 66) = 5.73$, $p < .02$).

3.5.3. Skin conductance

Skin conductance was increased during the 5 min worry period compared to the resting period ($F(1, 63) = 19.55$, $p < .0001$), but this increase was not significantly different in high and low worry groups (High worriers in worry period: $M = 5.94$, $SD = 6.79$; Low worriers in worry period: $M = 7.36$, $SD = 4.47$; $F(1, 63) = 1.64$, $p > .20$).

3.6. Relations between RSA and respiration

3.6.1. Bivariate correlations

A significant negative correlation was found between RSA amplitude and respiratory rate during resting ($r = -.537$, $p < .001$) and self-induced worry ($r = -.487$, $p < .001$). Significant positive correlations were also found between RSA amplitude and inspiratory period (resting: $r = .440$, $p < .001$; self-induced worry: $r = .244$, $p < .05$) and between RSA amplitude and expiratory period (resting: $r = .372$, $p < .002$; self-induced worry: $r = .365$, $p < .002$).

3.6.2. Analysis of covariance

Analysis of covariance on RSA amplitude values using respiratory parameters as covariates eliminated the significant Group differences found during the resting period ($F(1, 63) = .796$, $p > .37$).

4. Discussion

The major findings of our study can be summarized as follows. In the *non-cued defense reaction paradigm*, high worriers display (a) a cardiac defense response more accelerative than low worriers, the differences being located during the first and second deceleration, and (b) no differences in the skin conductance response, compared to low worriers. In the *cued defense reaction paradigm*, high worriers, compared to low worriers, show (a) no differences in the modulation of the eye-blink startle response, and (b) significant lower skin conductance response to the affective pictures, irrespective of content. During the *resting baseline*, high worriers have (a) a lower Respiratory Sinus Arrhythmia and (b) higher respiratory rate accompanied by a reduction in expiratory period. Finally, during the *worry period*, compared to resting baseline, high worriers maintain the respiratory differences but the RSA differences disappear.

The significant differences in the cardiac defense response during the non-cued defense paradigm and the lack of differences in the eye-blink response during the cued defense paradigm confirm our first hypothesis. The differences observed in the non-cued defense paradigm between the high and low worry groups suggest that the emotional state in which chronic worriers are during the test prior to the presentation of the defense stimulus–as a consequence of their different traits–is the key factor explaining the observed differences. A similar emotional priming effect with no cue presentation has been found in anxiety patients with more general anxiety reactions, compared to patients with more focal fear reactions or controls (Grillon et al., 1998; Oathes, 2008). The motivational priming hypothesis proposed by Lang and colleagues (see Cuthbert et al., 2003) explains this effect as due to the congruence between the existing emotional state of the organism (aversive) and the type of reflex being elicited (defensive). The hypothesis further postulates that the potentiation of defense reactions by contextual (non-cued) fear is mediated by the *bed nucleus of stria terminalis*, whereas the potentiation of defense reactions by explicit fear cues is mediated by the *central nucleus of the amygdala* (Davis, 1998).

The differences found between high and low worriers in the two decelerative components of cardiac defense suggest that high worriers manifest a lower vagal activation during the evocation of the defense response, thus confirming our second hypothesis. Using indirect indices of sympathetic and parasympathetic activation, Fernández and Vila (1989), Reyes del Paso et al. (1993, 1994) have shown that the short latency acceleration/deceleration is controlled exclusively by vagal influences, whereas the long latency acceleration/deceleration is controlled by sympathetic and parasympathetic influences working reciprocally. Therefore, the differences observed in the first deceleration between high and low worriers suggest a reduced vagal activation in high worriers. The differences observed in the second deceleration may be due to reduced vagal activation or increased sympathetic activation, or both. However, given the skin conductance results (our sympathetic index), with no differences between high and low worriers in the non-cued defense paradigm, and with a significant skin conductance reduction for high worriers in the cued defense paradigm, it is unlikely that the reduced second deceleration in high worriers could be explained by increased sympathetic activation.

The finding of a reduced skin conductance response to the pictures, irrespective of affective content, in the high worry group during the cued defense paradigm is also consistent with our third hypothesis. However, such effect was not present in the non-cued defense reaction paradigm. Previous studies showing sympathetic hypo-reactivity or absence of sympathetic hyper-reactivity in high worriers (Hoehn-Saric and McLeod 1988; Hoehn-Saric et al., 1989; Thayer et al., 1996) have not used procedures based on the modulation of protective reflexes. To our knowledge, this is the first study showing that high and low worry people do not differ in the pattern of modulation of the skin conductance response in the startle probe paradigm–since both groups show the expected quadratic trend–but that they do differ in the general level of sympathetic activation induced by the pictures, the high worriers showing a significant lower skin conductance reactivity.

The second aim of our study was to examine whether the reduced indices of vagal control (HR variability and RSA) reported in high worriers (Thayer et al., 1996; Hofmann et al., 2005; Thayer and Brosschot, 2008) could be explained by changes in respiration. Our findings do confirm the presence of a reduced RSA amplitude in high worriers during the resting period–not present during the self-induced worry period–but also the simultaneous presence of respiratory changes that are known to reduce RSA amplitude: faster respiratory rate and shorter expiratory period. The correlations between RSA and the respiratory parameters were all statistically significant (negative for respiratory rate and positive for inspiratory and expiratory period). Furthermore, when the RSA amplitude was analyzed including the respiratory parameters as covariates, the difference between high and low worriers disappeared. Thus, interpretation of reduced indices of vagal control in high worriers, based exclusively on absolute RSA values, is questionable given not only the own RSA limits (e.g., presence of residual inspiratory vagal activity) but also the demonstrated contamination of respiratory changes (Grossman and Kollai, 1993; Grossman and Taylor, 2007).

The implications of our findings should be evaluated taking into consideration some methodological limitations. First, our control group was a low worry group and not a 'normal' moderate worry group. This was done to maximize the effect of worry, assuming that chronic worry, assessed by the PSWQ, is a continuous dimension with a normal distribution. Future research will have to confirm such assumption by also examining intermediate groups along the worry dimension. Second, the low worry group had a smaller number of participants than the high worry group. Although a similar sample size has proved to be sufficient to reliably demonstrate modulation of both cardiac defense (Ramírez et al., 2005) and eye-blink startle (Bradley et al., 1996), the smaller size in the low worry group might have differentially affected the statistical power of some analyses or the results of some experimental manipulations, such as the random distribution of the pictures in the cued defense paradigm. Third, our measurement of respiratory parameters did not allow to reliably

assess indices of respiratory amplitude and ventilation. These are important parameters needed to conclude whether the faster respiratory rate and shorter expiratory period found in high worriers do indicate a tendency to hyperventilation, a relevant finding that future research will have to address.

Keeping these limitations in mind, the results of our study provide new evidence confirming that chronic worry can be conceptualized as a state of contextual fear or anxiety, in contrast to specific fear (Grillon, 2002). Such a state was demonstrated by the modulation of cardiac defense in high worriers under the non-cued defense paradigm–reduction of the short and long latency decelerations–and the absence of differences under the cued defense paradigm. Contextual fear or anticipatory anxiety, as a key characteristic of chronic worry, is also suggested by the significant differences observed in cardio-respiratory measures between high and low worriers during the resting baseline: a reduced heart rate variability, indexed by RSA, accompanied by an enhanced respiratory rate and a shorter expiratory period. Although these later findings do not justify by themselves any conclusion on the physiological mechanism underlying chronic worry, the presence in high worriers of additional indices of reduced vagal control–in the cardiac defense paradigm–and reduced sympathetic activation–in the startle probe paradim–supports the view that chronic worry is associated with poor autonomic regulation accompanied by an increased sensitivity to react defensively to unexpected danger.

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