



# The effect of conscious and non-conscious presentation of biologically relevant emotion pictures on emotion modulated startle and phasic heart rate

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## ARTICLE INFO

### Article history:

Received 17 June 2010

Received in revised form 17 November 2010

Accepted 1 December 2010

Available online 9 December 2010

### Keywords:

Startle

Heart rate

Emotion

Non-conscious

## ABSTRACT

Many researchers have proposed an emotion regulation circuit that includes the prefrontal cortex and the amygdala. LeDoux (1996) has proposed that there are two pathways by which emotion information is processed. A fast route that bypasses the prefrontal cortex to allow rapid response to potential threat, and a slower route that includes the prefrontal cortex and allows modulation of bottom-up inputs. We investigated these pathways and their peripheral manifestations using emotional pictures presented for either 30 milliseconds or 6 seconds. 36 female participants were randomly assigned to view pleasant, unpleasant, and neutral pictures that varied in viewing time while the eyeblink startle magnitude and phasic heart rate (HR) responses were recorded. Significant Group by Valence interactions were found for both startle and heart rate responses. For the 6 second condition the expected emotion modulated startle effect was found with a larger startle for unpleasant and smaller startles for pleasant foregrounds relative to neutral pictures. For HR, the D1 component was larger for pleasant and unpleasant foregrounds compared to the neutral and the A1 component was larger for the unpleasant compared to the pleasant and the neutral. For the 30 millisecond condition, startle magnitudes were larger for the pleasant and unpleasant compared to the neutral. Whereas the HR response showed the expected tri-phasic profile there were no significant between valence differences. These results suggest that briefly presented emotion stimuli access the fast route of emotion recognition perhaps via the amygdala. The 6 second presentations allow the prefrontal cortex to modulate the bottom up inputs and thus produce a context appropriate response.

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## 1. Introduction

The efficient processing of biologically relevant information is critical for effective human functioning and survival. Numerous neural structures including the amygdala and the prefrontal cortex have been implicated in the neural circuitry that supports emotional information processing. LeDoux (1996) has proposed that there are two pathways by which emotion information is processed. A fast route that involves the amygdala but bypasses the prefrontal cortex to allow rapid response to potential threat, and a slower route that includes the prefrontal cortex and allows the modulation of bottom-up inputs. However it is still not entirely clear whether the specialized rapid response pathways are specific to threat information or are more generally tuned to biologically relevant stimuli of both an appetitive as well as an aversive nature (Davis and Whalen, 2001; Cunningham et al., 2008).

One approach that has been used successfully to study the rapid response pathway has been the use of briefly presented and backward masked stimuli for which individuals find it difficult to consciously report awareness of the nature of the stimulus (Öhman, 1997; Öhman and Mineka, 2001). Whereas several peripheral physiological responses such as the startle reflex, electrodermal responses, and cardiac responses have been used to investigate emotional information processing and to draw inferences about the related neural circuitry most studies of briefly presented and potentially non-consciously perceived stimuli have used electrodermal activity such as the skin conductance response (SCR) to verify the processing of these stimuli. However the SCR is relatively non-specific and may index a more general orienting response (OR) to biologically relevant stimuli regardless of valence or it may represent a specific defensive reaction (DR) to threatening stimuli (Graham, 1979). Thus the use of additional peripheral physiological responses may help to clarify the nature of the processing of stimuli presented outside of conscious awareness.

Whereas electrodermal and cardiac responses have been used for many decades to investigate emotional responses much recent research has focused on the startle response (SR). A detailed understanding of the

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neural circuitry underlying the affective modulation of the startle reflex has been developed. Much of this knowledge has been based on animal models but recent brain lesion and neuroimaging studies in humans have also advanced our understanding of the affective modulation of the startle reflex. The motivational model of Lang hypothesizes that the emotion-modulated startle effects are the result of emotional priming of largely subcortical aversive and appetitive circuits (Lang, 1995). Davis and associates have investigated the acoustic startle reflex circuit in great detail in rats although less is known about humans (Davis et al., 1999). Based on this rat model they have hypothesized that a primary, spinal circuit that shows habituation and a secondary circuit that does not readily habituate involving the central nucleus of the amygdala forms the basis of the startle reflex circuitry. Importantly, this second circuit allows the startle reflex to be repeatedly modulated (without habituation) by activity of the central nucleus of the amygdala. The resistance to habituation of the affective modulation of the startle reflex in humans supports this model (see Bradley et al., 1999) and highlights the importance of the central nucleus of the amygdala in the affective modulation of the startle reflex. Similarly, the report of a person with damage to the right amygdala showing a reduced acoustic startle reflex and a failure to show affective startle modulation suggests that the neural circuitry identified in rats may generalize to humans (Angrilli et al., 1996). In summary, the neural circuitry of the startle reflex suggests that the affective modulation is achieved via a pathway involving the central nucleus of the amygdala.

Phasic HR responses have also been used to investigate emotion related information processing (Bradley et al., 2001; Johnsen et al., 1995; Thayer et al., 2000; Vico et al., 2010). An initial HR deceleration following stimulus presentation has been consistently linked with the orienting response (Sokolov, 1963; Graham and Clifton, 1966). Whereas an initial HR acceleration has been associated with a defense response to fear-related pictures in phobic people (Hare, 1973; Thayer et al., 2000), a pronounced HR deceleration has also been associated with a vagally mediated defense response to highly arousing unpleasant pictures (Bradley and Lang, 2000). In addition, highly arousing pleasant pictures show, after an initial deceleration, a brief heart rate acceleration (Bradley and Lang, 2000; Vico et al., 2010). Thus, in the context of viewing affective pictures, a more complex pattern of phasic heart rate changes has been reported. These phasic HR responses have been found to be primarily vagally mediated (Somsen et al., 1983). Moreover, vagally mediated HR responses have been linked to the prefrontal cortex (Ahern et al., 2001). Specifically, it was found that pharmacological blockade of the prefrontal cortex (PFC) was associated with increased HR and decreased vagally mediated HRV suggesting that the PFC tonically inhibits cardioacceleratory circuits. Further specification of the PFC structures associated with HR regulation suggests that the ventromedial PFC may be particularly important (Thayer et al., 2010). Across different laboratories, tasks, and imaging modalities we found that pregenual (BA 32) and ventral subgenual ACC (BA 25) blood flow were positively correlated with vagally mediated HRV. Wager et al. (2009) found more dorsal regions to be associated with HR increases. Relatedly, a recently proposed medial PFC-brainstem network has been implicated in the regulation of a variety of peripheral physiological processes (Lane and Wager, 2009). Thus, the combined examination of startle responses and phasic HR responses may provide information about neural processing involving the amygdala and the prefrontal cortex, respectively.

It has been hypothesized that the fast route proposed by LeDoux (1996) can take place without conscious processing. Recent research on the processing of stimuli at different levels of conscious awareness implicates a network of neural structures involving the amygdala and the prefrontal cortex (Williams et al., 2006). Importantly, whereas the same network was found to be active during both conscious and non-conscious presentations of fearful facial expressions, the functional connectivity differed as a function of level of conscious awareness. These

researchers used the SCR to verify the influence of the fearful facial expressions, whether consciously perceived or not, on physiological responses. However, as noted above, this leaves unresolved the nature of the response. That is, whether the response represents an attentional orienting response to all biologically relevant stimuli or a defensive response to aversive stimuli. This difference has important implications for whether this circuit is activated only by threatening stimuli or by biologically relevant stimuli regardless of valence.

Previous studies from our group have examined either startle responses (Ruiz-Padial and Vila, 2007) or phasic cardiac responses (Ruiz-Padial et al., 2005) to a stimulus eliciting a defense response while fearful participants were viewing non-consciously presented fear-relevant stimuli. However to date no studies have been published in which simultaneously measured startle and phasic cardiac responses to brief masked emotional pictures have been reported together. The concurrent examination of the startle and cardiac responses may allow us to investigate more clearly, using peripheral measures, the amygdala–cortical circuitry identified by Williams et al. (2006) as important for the modulation of emotional responses at different levels of conscious awareness. To manipulate awareness we presented pictures for either 30 milliseconds (non conscious) or 6 s (conscious).

We expected to replicate the robust emotional modulation effect for the startle reflex in the conscious group with a potentiated startle reflex to the unpleasant pictures and an inhibited startle reflex to the pleasant pictures relative to the neutral ones (Lang, 1995). With respect to the cardiac responses we expected to find differential multi-phasic cardiac responses as a function of valence as has been previously reported (Bradley et al., 2001; Thayer et al., 2000). For the non-conscious group we predicted something different. Öhman (1997) used the SCR as an index of OR, but as Graham (1979) notes, the SCR is an ambiguous index of OR because it is also related to the defense reflex. So, it is not clear if the preattentively elicited SCR is indicative of orienting, defense or both. Moreover, Öhman focused on the emotion of fear and threatening stimuli, but he has not investigated if the presentation (without conditioning) of appetitive biologically relevant stimuli generates an OR preattentively in normal subjects (not phobics). Thus by using the phasic cardiac response we may be able to disentangle the OR (initial HR decrease) from the DR (initial HR increase) as well as examine the responses to positively valenced biologically relevant stimuli. Consistent with our previous studies we expected that non-consciously presented positive and negative biologically relevant stimuli would be associated with modulated startle responses relative to neutral stimuli.

## 2. Materials and method

### 2.1. Participants

36 female (mean age = 20.36, sd 2.13) students from a large Spanish university comprised the final sample. None were undergoing psychiatric or pharmacological treatment, and none presented visual or auditory deficits. They were randomly distributed to 2 experimental groups. Data from 1 participant were excluded because of equipment failure. The experimental protocol was approved by the University ethics committee and all participants gave their written informed consent prior to the start of the experiment.

### 2.2. Materials and design

Forty-five pictures (fifteen pleasant, fifteen neutral and fifteen unpleasant) were selected from the International Affective Picture System slides on the basis of their valence and arousal ratings from the Spanish norms (Moltó et al., 1999). The pleasant and unpleasant pictures differed from each other on valence but did not differ on arousal (valence:  $F(1,42) = 401.00$ ,  $p < 0.001$ ; arousal:  $F(1,42) = 1.20$ ,

**Table 1**

Mean and standard deviations for subjective ratings of the pictures.

	Pleasant	Neutral	Unpleasant
Valence	6.90 (0.55) <sup>b,c</sup>	5.14 (0.30) <sup>a,c</sup>	2.22 (0.91) <sup>a,b</sup>
Arousal	6.71 (0.40) <sup>b</sup>	3.18 (0.48) <sup>a,c</sup>	6.92 (0.68) <sup>b</sup>

<sup>a</sup> Significant difference from pleasant pictures.<sup>b</sup> Significant difference from neutral pictures.<sup>c</sup> Significant difference from unpleasant pictures.

$p=0.28$ ). Both pleasant and unpleasant pictures differed from the neutral pictures on both valence and arousal (see Table 1). The pleasant pictures were all depictions of nude couples, the unpleasant pictures were all depictions of mutilations, and the neutral pictures were all depictions of household objects. Thus, all of the pleasant and unpleasant slides depicted biologically relevant stimuli involving depictions of humans. Each picture was presented for either 6 s or 30 ms depending on the independent grouping variable Duration. The pictures were followed by a 100 milliseconds mask consisting of a brilliant image that did not contain a recognizable object.

There were 54 trials distributed as follows: 33 (11 of each valence) picture with acoustic startle probe (occurring randomly between 3 and 4 s after the onset of the picture in the 6 seconds group and randomly between 870 ms and 1870 ms after the end of the mask in the other group), 12 picture only (buffer trials) and 9 startle only (base startle) trials presented in one of three orders. The interval between trials was between 19 and 26 s. Two Kodak Ektapro 9000 projectors were used to display the slides and the mask. The images were displayed on the wall 2.5 m in front of the participant with viewing dimensions of  $100 \times 70$  cm.

The acoustic startle probe stimulus was a white noise burst (105 dB, 50 ms duration with a virtually instantaneous rise-time) delivered via a Coulbourn V85-05 white-noise generator passing through a IMQ Stage Line amplifier. The sounds were presented through research grade earphones (TDH Model-49; Telephonics) that were calibrated with a Bruel and Kjaer sonometer (model 2235) using an artificial ear (model 4153).

### 2.3. Physiological recording and data reduction

A Coulbourn polygraph (Model Link) was used to record electromyographic (EMG) and the electrocardiographic (EKG) data using two separate channels. Data acquisition (sampling rate = 1000 Hz) and stimulus control were monitored by VPM 10 (Cook, 1994).

Facial EMG was recorded from two Ag–AgCl electrodes placed over the orbicularis oculi muscle of the left eye. The raw electromyographic signal was recorded at 1000 Hz and was rectified and integrated by using a 75-millisecond time constant using VPM (Cook, 1994). Startle blink amplitudes were defined as the difference in microvolts between peak and onset of the integrated response. EKG data were recorded using 3 Ag–AgCl electrodes placed as follows: one electrode on the right arm, one on the left leg and a ground on the right leg. HR was recorded as 1/2 second averages within VPM for 3 s before the picture onset (baseline) and for 15 s following the picture onset (task). Phasic HR responses were scored as task minus baseline change scores in 1/2 second increments.

### 2.4. Procedure

Prior to the experimental session, we conducted an interview in order to ascertain age, visual or auditory deficits, health, and pharmacological or psychiatric treatment. After receiving instructions and having the electrodes and earphones attached, participants were left alone in a darkened room. A two-minute resting baseline preceded the presentation of the stimuli.

Once the experimental phase was over, participants completed a subjective reaction questionnaire that included ratings of intensity and unpleasantness of the auditory stimuli, and a recognition

**Table 2**

Recognition questionnaire results.

	Targets		Distractors	
	Correct answer	False negative	False positive	Correct rejection
Conscious group	89.12% (9.96)	10.88%	4.44% (6.81)	95.56%
Non-conscious group	3.40% (4.54)	96.6%	4.18% (4.72)	95.82%

(Mean value of confidence rating in parenthesis when the participants said to have seen those pictures).

questionnaire with questions about the pictures comprised of 5 sheets with a total of 90 pictures (45 targets and 45 distracters). The recognition questionnaire included a forced-choice recognition test where the participants indicated whether they recognized the picture (yes or no). If they said to have “seen” a picture, they also had to add the confidence associated with that judgment where 0 indicated a complete lack of confidence and 10 indicated complete certainty.

### 2.5. Design and statistical analysis

For the EMG data a factorial design was used with one between-groups factor (Duration of Picture) and two repeated measures factors (Valence and Trial).<sup>1</sup> For the phasic HR data a factorial design was used with one between-groups factor (Duration of Picture) and two repeated measures factors (Valence and Time). Time represents the 1/2 second HR data for the 15 second post-slide onset.<sup>2</sup> For clarity we examined simple effects for the tri-phasic HR components D1 (first deceleration following slide onset: average half seconds 3 and 4) and A1 (first acceleration following the slide onset: linear trend over half seconds 4–8). Pre-planned contrasts compared these components among the three valences for the 6-second and 30-millisecond groups separately. For clarity in the HR figures we show only the data for the first 6 s post-slide onset.

## 3. Results

### 3.1. Recognition questionnaire results

As can be seen in Table 2, the conscious group recognized correctly most of the targets with a high confidence (89.1%, 9.96 mean value confidence rating) while the non conscious group recognized very few (3.4%, 4.54 confidence). Moreover, the non conscious group “recognized” a similar amount of distracters as targets (4.18%, 4.72 confidence), which means that they really could not distinguish any pictures and were answering at random.

### 3.2. EMG results

Results of the  $2 \times 3$  ANOVA (Group  $\times$  Valence) showed a significant main effect of Valence ( $F(2,54) = 13.89$ ,  $p = 0.00011$ , epsilon = 0.75)

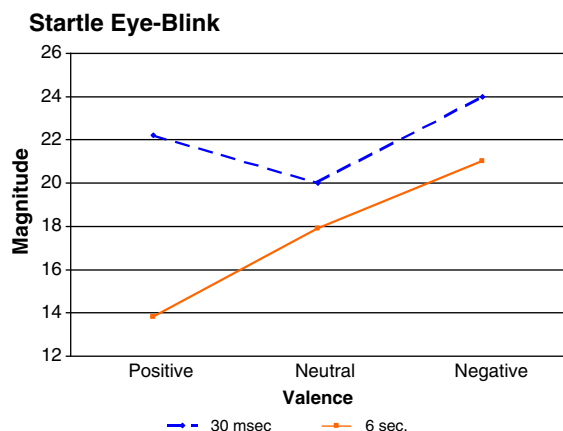
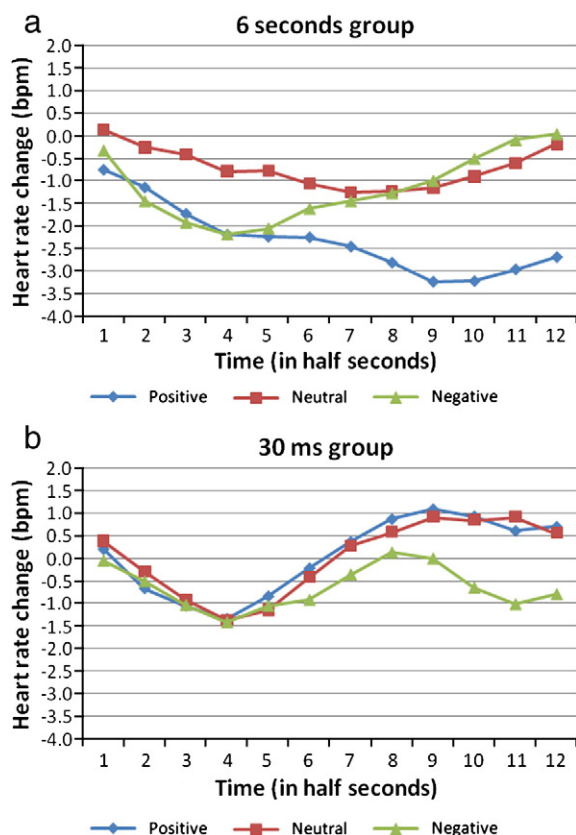
<sup>1</sup> Preliminary analyses to examine the Trials effect on EMG grouped the eleven trials into groups comprised of the first four trials (Early), the middle three trials (Middle), and the last four trials (Late). The Viewing Duration (6 s and 30 ms) by Valence by Trials (Early, Middle, and Late) ANOVA revealed that Trials did not interact with any other factor. However, the expected main effect of Trials was evident [ $F(2,154) = 63.94$ ,  $p < 0.0000001$ , epsilon = 0.81]. We therefore collapsed over Trials for all subsequent analyses. These results are consistent with the well-replicated finding that whereas the absolute startle magnitude decreases over time, the emotion-modulated startle effect is resistant to habituation (Bradley and Lang, 2000).

<sup>2</sup> Data were the averages of the 11 trials as we found no main [ $F(2,27) = 0.159$ ,  $p = 0.854$ ] or interaction effects involving Trial. Preliminary analyses revealed significant main effects for Valence [ $F(2,68) = 3.69$ ,  $p = 0.038$ , epsilon = 0.84] and Time [ $F(29,986) = 9.64$ ,  $p < 0.00001$ , epsilon = 0.19] and significant interaction effects for Group  $\times$  Valence [ $F(2,68) = 6.49$ ,  $p = 0.0046$ , epsilon = 0.84], Group  $\times$  Time [ $F(29,986) = 4.06$ ,  $p = 0.0012$ , epsilon = 0.19], and Group  $\times$  Valence  $\times$  Time [ $F(58,1972) = 2.22$ ,  $p < 0.01$ , epsilon = 0.19].

**Table 3**

Mean and standard deviations for startle magnitude.

	Pleasant	Neutral	Unpleasant
6 s	13.43 (13.30) <sup>b,c</sup>	17.92 (15.39) <sup>a,c</sup>	21.21 (17.58) <sup>a,b</sup>
30 ms	22.32 (12.40) <sup>b</sup>	20.01 (11.65) <sup>a,c</sup>	24.08 (13.76) <sup>b</sup>

<sup>a</sup> Significant difference from pleasant pictures.<sup>b</sup> Significant difference from neutral pictures.<sup>c</sup> Significant difference from unpleasant pictures.**Fig. 1.** Startle magnitude in microvolts during the three categories of pictures for the 6 second and 30 ms groups.**Fig. 2.** Heart rate changes to the three categories of pictures for the first six seconds post-picture onset in half second increments. a. six seconds group. b. 30 ms group.

and an interaction effect of Group  $\times$  Valence ( $F(2,54) = 7.69$ ,  $p = 0.00329$ ,  $\epsilonpsilon = 0.75$ ). The main effect for Group was not significant.

The Valence main effect showed a startle reflex magnitude potentiated in the aversive picture trials versus the neutral and the positive. The pre-planned simple effects tests indicated that for the conscious group the responses to all the categories of slides were different from each other, a linear trend was found but no quadratic trend. For the non-conscious group we found significant differences between pleasant and neutral pictures and between unpleasant and neutral pictures. We found no evidence for a linear trend but there was a significant quadratic trend (see Table 3).

Fig. 1 shows the interaction of Group  $\times$  Valence. No significant differences were found between groups for any category of valence.

### 3.3. HR results

Pre-planned simple effects analyses examined the D1 and A1 HR components for the conscious and non-conscious groups separately. For the non-conscious group the traditional tri-phasic response was found. However, there were no differences among the three valences for either the D1 or the A1 components. For the conscious group, there were significantly greater D1 components for the pleasant and the unpleasant conditions compared to the neutral condition ( $p = 0.002$  and  $p = 0.007$ , respectively). The D1 for the pleasant and the unpleasant conditions did not differ. In addition, the A1 component was significantly greater for the unpleasant condition compared to both the pleasant and the neutral conditions ( $p = 0.047$  and  $p = 0.015$ , respectively). The A1 component did not differ between the pleasant and the neutral conditions. These results are displayed in Fig. 2.

## 4. Discussion

By the inclusion of both startle responses and phasic cardiac responses the results of present study added significantly to our understanding of the nature of responses to biologically relevant stimuli presented outside of conscious awareness. When participants were consciously aware of the stimuli the expected emotional modulation effect on the startle eyeblink response, with potentiated SR for the unpleasant pictures and attenuated SR for the pleasant pictures versus the neutral pictures was found. In addition, the conscious group responded with a greater cardiac deceleration to the pleasant and unpleasant pictures compared to the neutral pictures, which indicates a larger orienting D1 component to the affective pictures. After this first cardiac deceleration, there was a steeper cardiac acceleration to the unpleasant pictures compared to the neutral or the pleasant pictures. This finding is consistent with those reported by Thayer et al. (2000) for Generalized Anxiety Disorder (GAD) patients and Hare and Blevings (1975) for participants with a specific phobia, suggesting a motivated inattention to biologically relevant aversive stimulation (Jennings, 1986). This cardiac acceleration after an initial OR has been interpreted as a type of perceptual defense against a feared or threatening stimulus (Lacey, 1967). Thus the results of the conscious group replicate and extend previous research to specifically biologically relevant stimuli.

To date, few studies have examined startle or cardiovascular responses to emotion stimuli presented outside of conscious awareness. For the non-conscious group, the startle eyeblink variable showed a potentiated startle to the pleasant and unpleasant pictures compared to the neutral pictures consistent with recent suggestions that the amygdala is sensitive to both appetitive and aversive biologically relevant stimuli (Cunningham et al., 2008; Davis and Whalen, 2001; Ruiz-Padial & Vila 2007). Moreover, whereas this group showed the expected tri-phasic HR response, unlike the conscious group they failed to show any differential response as a function of the valence of the



pictures. This result replicates the cardiac response to pictures presented for 500 ms found by Codispoti et al. (2001). Given the importance of the prefrontal cortex in the context appropriate modulation of cardiac responses (Thayer et al., 2010), these results suggest that, in non phobic participants, access to the context setting prefrontal cortex is necessary to produce differentiated cardiac responses to biologically relevant affective stimuli. Taken together, these results suggest that the cardiac response was sensitive to the perception of a briefly presented stimulus whereas the SR was sensitive to the biologically relevant content of the stimulus. This helps to clarify the findings of Öhman (e.g., Öhman and Mineka, 2001) and Williams et al. (2006) with respect to the nature of the SCR responses that these groups have reported to non-consciously presented stimuli. Based on the present combined startle and cardiac data it seems that the response to non-consciously presented stimuli involves an attention related OR that is sensitive to biological relevance but relatively insensitive to whether the stimulus is aversive or appetitive.

The startle responses while participants view non-consciously presented emotional stimuli replicate and extend previous findings from our group (Ruiz-Padial and Vila, 2007). In a similar paradigm we reported that non-conscious presentation of pleasant and unpleasant stimuli produced emotion-modulated startle effects. In that study it was found that pleasant stimuli (nudes) had the smallest magnitude startle responses. However, in neither the conscious nor the non-conscious groups did the SR during the pleasant stimuli differ significantly from the neutral stimuli. In the present study during the conscious presentations the SR to the pleasant stimuli were significantly smaller than during the neutral stimuli. During the non-conscious presentations however the SRs were larger than the neutral and did not differ from the unpleasant (mutilation) stimuli. Whereas the previous study and the present study are consistent in showing that emotion modulated startle effects can be found using non-conscious presentations the exact nature of that modulation especially for positively valenced stimuli (increased or decreased SR) remain unclear. Future research will be needed to further explicate this phenomenon. Importantly both findings suggest that the amygdala is sensitive to biologically relevant stimuli regardless of valence.

Contrary to our previous results (Ruiz-Padial et al., 2005) the phasic heart rate responses to the non-conscious stimuli do not differ as a function of valence. Several reasons may account for this inconsistency. First, participants in our previous study were highly fearful to spiders and were presented with a masked phobic picture. In the present study, participants have not been selected for any fear criteria and the negative stimuli included depictions of mutilations. Second, we found differential heart rate responses to the masked biologically relevant stimuli in a cardiac defense paradigm, which involves some important methodological differences compared to the startle study reported here. To evoke the cardiac defense response a 500 millisecond white noise was used (compared to a 50 millisecond startle stimulus), very few trials could be measured (2 defense trials compared to 33 startle trials) and, importantly, heart rate changes were analyzed during 80 s (compared to 15 s in the present study). The longer cardiac defense response, showing modulation over an 80 second time period, might allow various regions of the prefrontal cortex to gather and use more information to shape the responses more than is possible with the information available in the first few seconds such as we examine in this startle paradigm. In addition having a fear response connected to the stimulus, such as is the case with spider phobic responses to spider stimuli, may facilitate the non-conscious responding at several levels, including heart rate. All these factors indicate the necessity of future studies for clarifying the conditions under which heart rate responses can reflect the non-conscious processing of biologically relevant stimuli.

The neural underpinnings of responses to stimuli presented outside of conscious awareness have been recently examined by Williams et al. (2006). These researchers used functional connectivity analysis to examine amygdala and prefrontal cortical responses to fearful stimuli

presented outside of conscious awareness. Importantly they reported that the same prefrontal-amygdala circuits were activated for both aware and unaware stimuli but that the functional connectivity differed as a function of awareness. During the awareness of pictures of fearful faces a negative connection between both cortical and subcortical pathways to the amygdala was found. However during unawareness the responses to the fearful stimuli were supported by a positive connection in a direct subcortical pathway to the amygdala. They interpreted these results to suggest that reentrant feedback may be necessary for the context appropriate response to biologically relevant stimuli that are consciously perceived. However, they suggest that excitatory feedforward connections along this same pathway are sufficient for responses to fearful stimuli presented outside of conscious awareness. The present findings are consistent with this idea and further suggest that reentrant feedback is necessary for the inhibitory “sculpting” of context appropriate responses that has been suggested for the prefrontal cortex in the neurovisceral integration model (Thayer and Lane, 2000; 2009).

Whereas the excitatory feedforward responses of the amygdala would be sufficient to produce the relatively undifferentiated startle and cardiac responses found in the present study when the stimuli were presented outside of conscious awareness, the reentrant feedback would be needed to “sculpt” these undifferentiated responses into the context appropriate startle and cardiac responses found when the stimuli were presented consciously. In particular Williams et al. (2006) noted a negative connectivity between the amygdala and the ventral ACC regardless of the level of awareness. However, they found fewer amygdala-cortical connections during the non-conscious conditions which suggested to them that the degree of connectivity was related to the level of conscious awareness. It is interesting to note that the amygdala and ventral ACC activations reported by Williams et al. (2006) to be involved in both conscious and non-conscious fear overlap nearly identically with activations we have found to be associated with vagally mediated heart rate variability (HRV) in a recent meta-analysis of neuroimaging and HRV studies (Thayer et al., 2010). It is well known that phasic heart rate responses are largely vagally mediated and thus the present results comport well with the findings of Williams et al. (2006). However, future studies that combine neuroimaging with multiple peripheral physiological measures including the SCR, the SR, and phasic cardiac responses would help to further clarify the nature of this neural circuitry.

One limitation of the present experiment was that only female participants were studied. Some researchers have found significant gender differences in startle as well as cardiac responses to affective pictures (Bradley et al., 2001). However, this effect may vary by picture category. For example, Bradley et al. (1999) found no differences in the startle responses of males and females to mutilation or erotic couple pictures. This would suggest that our results may be similar in males. Thus by using only females we may have reduced some variability in the responses but we cannot unambiguously generalize these results to males. Second, it is not clear that household items represent biologically relevant neutral stimuli. However the vast majority of neutral pictures in the IAPS set are of household items and we did not select items not included in the standardized IAPS picture set. Given that we did not use pictures of facial expressions where a neutral expression is possible, the use of household items as neutral stimuli may represent a limitation. Therefore, future studies similar to the Öhman and Williams studies which include positively valenced facial expressions may be useful. However, we have reported that happy and angry (and perhaps) fearful faces differ with respect to arousal level as well as valence (Johnsen et al., 1995) so these studies would not be absolutely conclusive either.

As noted by Darwin (1872) more than one hundred years ago when commenting on the work of the French physiologist Claude Bernard, the heart and the brain are intimately connected such that any action of either the heart or the brain will be reflected in the brain and the heart, respectively. The present study further supports this

intimate connection and suggests that peripheral physiological responses such as the SR and phasic cardiac responses can inform us about important activities of the brain including the modulation of responses to biologically relevant stimuli.

## Acknowledgments

The present research was supported by a grant from the Spanish Ministry of Science and Innovation (PSI2009-12368).

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