# The rhythm of the heart in the blink of an eye: Emotion-modulated startle magnitude covaries with heart rate variability

ELISABETH RUIZ-PADIAL,<sup>a</sup> JOHN J. SOLLERS, III,<sup>b</sup> JAIME VILA,<sup>c</sup> and JULIAN F. THAYER<sup>b</sup>

<sup>a</sup>Department of Psychology, University of Jaén, Jaén, Spain

<sup>b</sup>The National Institute on Aging, Baltimore, Maryland, USA

<sup>c</sup>Department of Clinical Psychology, University of Granada, Granada, Spain

# Abstract

Emotion-modulated startle is a robust phenomenon that has been demonstrated in a wide range of experimental situations. Similarly, heart rate variability (HRV) has been associated with a diverse range of processes including affective and attentional regulation. The present study sought to examine the relationship between these two important measures of affective behavior. Ninety female participants viewed pleasant, neutral, and unpleasant pictures while exposed to acoustic startle stimuli. The eyeblink startle was recorded both during the affective foregrounds and during intertrial intervals. HRV was assessed during a resting baseline and relationships between HRVand startle magnitudes examined. Results indicated that resting HRV was inversely related to startle magnitude during both intertrial intervals and affective foregrounds. In addition, the participants with the highest HRV showed the most differentiated emotion-modulated startle effects, whereas those with the lowest HRV, compared to those with the highest HRV, showed significantly potentiated startle to neutral foregrounds and marginally potentiated startle to pleasant foregrounds. The findings are consistent with models that posit that prefrontal cortical activity modulates subcortical motivation circuits. These results have important implications for the use of startle probe methodology and for HRV in the study of emotional regulation and dysregulation.

Descriptors: Startle, Heart rate variability, Emotion, Parasympathetic

The startle probe methodology has revolutionized the study of emotional processes. This technique can be used with both human and nonhuman investigations of emotion as well as in studies of information processing and attention. Importantly, an emotion-modulated startle effect has been demonstrated. Lang and colleagues have developed a motivational priming model that proposes aversive and appetitive motivational systems that either match or mismatch with the defensive startle response elicited by a sudden, intense stimulus. Relative to neutral foreground stimuli, foreground stimuli that engage the aversive motivational system potentiate startle responses whereas foreground stimuli that engage the appetitive motivational system inhibit startle responses (Bradley, Cuthbert, & Lang, 1999).

The factors that influence the modulation of the startle reflex have been extensively investigated. Bradley et al. (1999) have recently summarized this vast literature. Affective modulation of the startle reflex has proven to be one of the most robust phenomena in psychophysiology. Whereas the affective modulation of the startle reflex has been most often demonstrated in the context of picture viewing, it has also been demonstrated in the context of viewing of films (Jansen & Frijda, 1994), listening to sounds (Bradley & Lang, 2000), and the smelling of odors (Miltner, 1994). In addition, a wide range of characteristics of the foreground stimuli and of the startle probe have been investigated. Affective modulation of the startle reflex has been demonstrated using picture durations range from brief (150 ms) to relatively long (6 s) durations as well as during affective imagery, and does not depend on the actual perceptual presence of the foreground stimulus (Bradley & Lang, 2000). In addition, the affective modulation effect appears very rapidly after picture onset (within 300 ms; Codispoti, Bradley, & Lang, 2001). Moreover, the affective modulation of the startle reflex has been used to investigate a wide range of individual difference variables including persons with specific phobias, anxiety disorders, schizophrenia, psychopathology, and depression (Allen, Trinder, & Brennan, 1999) as well as the personality of the participants (Corr et al., 1995). Furthermore, stimulation of right hemisphere neural structures has been consistently associated with greater acoustic startle potentiation in the context of aversive compared to appetitive stimuli (Bradley et al., 1999). In summary, the emotionmodulated startle effect has shown itself to be remarkably consistent in a wide variety of research settings.

The detailed understanding of the neural circuitry underlying the affective modulation of the startle reflex helps to explain the

Address reprint requests to: Julian F. Thayer, NIA/GRC/LPC, 5600 Nathan Shock Drive, Baltimore, MD 21224, USA. E-mail: thayer@lpc.grc.nia.nih.gov.

robustness of this phenomenon. Much of this knowledge has been gained in the investigation of 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. Davis and associates have investigated the acoustic startle reflex circuit in great detail in rats although less is known about humans (Davis, Walker, & Lee, 1997). 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.

The study of heart rate variability (HRV) has also demonstrated a far-reaching utility in the study of emotional, attentional, and autonomic processes. Due to the differential frequency characteristics of the sympathetic and parasympathetic neural modulation of heart rate, spectral analysis of the heart rate time series allows us to examine these neural influences. Modulation at frequencies from 0.03 to 0.15 Hz corresponds primarily to baroreceptor-mediated regulation of blood pressure. This low frequency component involves modulation by both sympathetic and parasympathetic (vagal) influences. Modulation at frequencies greater than 0.15 Hz are respiration modulated and are defined as the high-frequency (HF) component. As the vagus is the only autonomic influence known to exist at this frequency, the resulting modulation of heart rate reflects almost exclusively cardiac vagal activity and parasympathetic neural modulation. From the large number of both time- and frequency-domain indices of HRV, the time domain measure of the mean successive differences (MSD) and the frequency domain measure of HF spectral power have been shown to most closely reflect parasympathetic influences.

Functionally, vagally (parasympathetic) mediated HRV is positively associated with good psychological and physiological functioning. Thayer and Lane (2000) have recently presented a model of neurovisceral integration in which a network of neural structures associated with attentional, emotional, and physiological regulation has been related to HRV via the sympathetic and parasympathetic innervations to the heart. Recent studies have shown vagally mediated HRV to be associated with phasic cardiac indices of attention and emotion (Thayer, Friedman, Borkovec, Johnsen, & Molina, 2000), and attentional bias and inhibition as indexed by the emotional Stroop (Johnsen et al., in press). Specifically, high levels of vagally mediated HRV were associated with larger orienting responses but faster habituation to nonthreat stimuli whereas low HRV was associated with a failure to habituate to nonthreat stimuli (hypervigilence) and defensive reactions (HR acceleration) to the presentation of threat-related words (Thayer et al., 2000). In another study, dental phobics with higher levels of vagally mediated HRV compared to those with lower levels of HRV showed greater inhibition of prepotent responses in a Stroop paradigm as indexed by faster reaction times to color-incongruent and threatrelated words (Johnsen et al., in press). These results suggest that high levels of vagally mediated HRV are associated with better affective and attentional regulation.

The neural origins of cardiac autonomic modulation have also been investigated. A set of neural structures termed the central autonomic network (CAN) has been identified. Structurally, the CAN includes the anterior cingulate, insular, and ventromedial prefrontal cortices, the central nucleus of the amygdala, the paraventricular and related nuclei of the hypothalamus, the periaquaductal gray matter, the parabrachial nucleus, the nucleus of the solitary tract (NTS), the nucleus ambiguus, the ventrolateral medulla, the ventromedial medulla, and the medullary tegmental field. The primary output of the CAN is mediated through the preganglionic sympathetic and parasympathetic neurons. Importantly, these neurons innervate the heart via the stellate ganglia and the vagus nerve. The interplay of these inputs to the sino-atrial node of the heart is the source of the complex variability that characterizes the heart rate time series (Saul, 1990). Thus, the output of the CAN is directly linked to HRV.

Recent neuroimaging and pharmacological blockade studies have investigated the neural origins of HRV. Lane, Reiman, Ahern, and Thayer (2001) have presented evidence that medial prefrontal activity is associated with HRV. Vagally mediated HRV is considered to reflect antagonism of sympathoexcitatory influences (Uijtdehaage & Thayer, 2000). To explore its central neural substrates, a spectrally derived index of vagally mediated HRV (HF-HRV) was correlated with measures of cerebral blood flow (rCBF) derived from positron emission tomography (PET) in 12 healthy women during emotion and neutral conditions. During the emotion minus neutral conditions, HF-HRV correlated with rCBF in the medial prefrontal cortex and the left posterior orbitofrontal and anterior insular cortices. More specifically, emotional arousal was associated with a decrease in HRV and concomitant decreases in brain activation in these regions. These findings are consistent with a general inhibitory role for the medial prefrontal cortex via the vagus as suggested by Ter Horst (1999).

Skinner (1985) has suggested that an intact frontal cortex may tonically inhibit subcortical (amygdala) activity that, in turn, is associated with autonomically mediated defensive behavior. Direct and indirect pathways by which the frontal cortex modulates parasympathetic activity via subcortical inputs have been identified (Ter Horst, 1999; Ter Horst & Postema, 1997). Human evidence for the inhibitory role of the frontal cortex comes from a recent study of HR and HRV before and after right and left side intracarotid sodium amobarbital (ISA) injection (the Wada test; Ahern et al., 2001). Qualitatively similar changes in HR were observed during each hemisphere's pharmacological blockade. During 10-min inactivations of either hemisphere, HR increased, peaked at about minute three, and gradually declined toward baseline values. These data support the notion that cortical activity tonically inhibits brainstem sympathoexcitatory circuits. However, differential hemispheric effects appeared, with larger and faster HR increases during right hemisphere inactivations. Concomitant with these HR increases, vagally mediated HRV decreased, mirroring the HR changes with respect to

differential hemispheric effects. Specifically, vagally mediated HRV decreases were greater in the right hemisphere inactivations. These results support the anatomical and physiological findings that right hemispheric autonomic inputs to the heart are associated with greater cardiac chronotropic control. It is important to note in this context that affective acoustic startle modulation is also greater with right hemisphere stimulation (Bradley et al., 1999).

The effects of the ISA test are largely restricted to anterior neural structures, which include the orbital and medial prefrontal cortices (Ahern et al., 1994; Hong et al., 2000). These areas have been broadly associated with biopsychological functions such as affective, attentional, and autonomic regulation (Thayer & Lane, 2000). In addition, these structures are linked with inhibitory control of behavior in general (Roberts & Wallis, 2000) and cardiac behavior in particular (Verberne & Owens, 1998). Importantly, direct and indirect pathways connect these areas with parasympathetic (vagal) motor output regions (Ter Horst, 1999). A number of researchers have hypothesized inhibitory cortical-subcortical circuits (Benarroch, 1993, 1997; Davidson, 2002; Drevets, 1999; Hariri, Bookheimer, & Mazziotta, 2000; Masterman & Cummings, 1997; Mayberg et al., 1999; Spyer, 1989); however, our group has been the first to tie these circuits to heart rate variability (Ahern et al., 2001; Thayer & Lane, 2000, 2002). The results of the ISA test experiment provide compelling evidence that cortical structures tonically inhibit sympathoexcitatory circuits and that this inhibition is via vagal mechanisms. One important implication of these studies is that cortical activity appears to modulate subcortical circuits associated with basic motivational behavior. Specifically, Davidson (2002) and Drevets (1999) have both proposed models and provided data that show that activity of the prefrontal cortex is inversely related to amygdala activity.

Previous data provided evidence that vagally mediated (HF) HRV is associated with activity of the prefrontal cortex. The evidence reviewed above suggests that prefrontal activity is inversely associated with activity of subcortical sympathoexcitatory circuits including the amygdala. Given that the presence of an intact amygdala is associated with startle magnitude and affective modulation of the startle reflex (Angrilli et al., 1996) it was hypothesized that HRV would be associated with the magnitude of the startle reflex and with the affective modulation of the startle reflex. To date, no studies have examined the relationship between startle modulation and HRV. As part of a larger study, this relationship was examined using the picture-viewing paradigm of Lang and colleagues. It was hypothesized that HRV would be negatively correlated with startle magnitude during both intertrial intervals (base) and affective foregrounds reflecting the cortical modulation of subcortical defensive circuits associated with the amygdala. Furthermore, it was hypothesized that individuals with high vagally mediated HRV would show a pattern of highly differentiated emotion-modulated startle whereas those with low HRV would show evidence of faulty affective modulation of the startle reflex due to tonic activation of the amygdala via a lack of inhibitory input from the prefrontal cortex.

As part of a larger study examining picture presentation time, 90

female University of Granada students, randomly distributed to

#### Method

#### **Participants**

six experimental groups, comprised the final sample. They were given course credit for their participation. None was undergoing psychiatric or pharmacological treatment. All participants had normal or corrected-to-normal vision and hearing. Data from 3 participants were excluded due to equipment failure.

#### Materials and Design

Forty-five pictures (15 pleasant, 15 neutral, and 15 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).<sup>1</sup> Each picture was presented for either 6 s, 1 s, 150 ms, three presentations of 150 ms, 30 ms, or three presentations of 30 ms depending on the independent grouping variable duration.<sup>2</sup> The pictures were followed by a 100-ms mask consisting of a brilliant image that did not contain a recognizable object. The first four viewing durations were considered to be supraliminal, and the latter two viewing durations were thought to be subliminal.

There were 54 trials distributed as follows: 33 pictures (11 of each valence) with acoustic startle probe (occurring between 3 and 4 s after the onset of the picture in the 6-s group and randomly between 870 ms and 1,870 ms after the end of the mask in the other groups), 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 passed through a IMQ Stage Line amplifier. The sounds were presented through research grade earphones (TDH Model-49; Telephonics) that were calibrated with a Bruel & Kjaer sonometer (model 2235) using an artificial ear (model 4153).

# Physiological Recording and Data Reduction

A Coulbourn polygraph (Model Link) was used to record electromyographic (EMG) and the electrocardiographic (EKG) data. 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 EMG data were analyzed using VPM (Cook, 1994), and startleblink amplitudes were recorded in microvolts. EKG data were

<sup>&</sup>lt;sup>1</sup>The IAPS pictures used in this study were: pleasant: 4607, 4608, 4631, 4652, 4653, 4658, 4659, 4660, 4664, 4670, 4672, 4680, 4690, 4800, 4810; neutral: 7000, 7002, 7010, 7025, 7030, 7035, 7040, 7050, 7060, 7170, 7205, 7207, 7217, 7224, 7820; unpleasant: 3010, 3030, 3053, 3060, 3110, 3140, 3160, 3190, 3210, 3230, 3250, 3300, 3350, 3400, 9400. The pleasant [mean (*SD*) = 6.9 (0.56)], neutral [mean (*SD*) = 5.14 (0.30)], and unpleasant [mean (*SD*) = 2.2 (0.91)] slides differed significantly from each other on valence, pleasant versus neutral, F(1, 42) = 56.8, p < 0.001, pleasant versus unpleasant, F(1, 42) = 4010.0, p < .001, neutral versus unpleasant, F(1, 42) = 155.9, p < .001. Both the pleasant [mean (*SD*) = 3.2 (0.48)] slides, F(1, 42) = 324.3, p < .001, and F(1, 42) = 364.9, p < .001, respectively, but did not differ from each other, F(1, 42) = 1.2, p = .28.

 $<sup>^{2}</sup>$ For the conditions in which multiple presentations of pictures occurred, the sequence of events was as follows: picture onset (for 30 ms or 150 ms), mask (for 100 ms), blank screen (for 870 ms or 750 ms, respectively) with this sequence repeated for each of three successive seconds.

recorded using three Ag-AgCl electrodes placed as follows: one electrode on the right arm, one on the left leg, and a ground on the right leg. Beat-to-beat heart rate data were extracted, and a semiautomated process was used to correct these data for artifacts within VPM. This process yielded baseline time series of 2 min duration.

Time and frequency domain analyses were performed on these data using a custom HRV package. Time domain analysis provided estimates of root mean square successive difference in milliseconds (MSD) and heart rate in beats per minute (HR). Spectral analyses using an autoregressive algorithm following the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (1996) guidelines were performed. The frequency domain measure of high frequency (HF: 0.15-0.4) power that has been associated with respiratory-modulated parasympathetic outflow was used to index vagally mediated HRV. MSD and HF power were highly correlated in the present study, r(85) = .89, p < .0001. The central frequency of the high frequency component (HFF) has been shown to correlate with strain gauge measures of respiration frequency (Thayer, Peasley, & Muth, 1996; Thayer, Sollers, Ruiz-Padial, & Vila, 2002) and was used as an index of respiration in the present study.<sup>3</sup> Spectral estimates of power (in milliseconds squared per hertz) were transformed logarithmically (base 10) to normalize the distribution of scores.

## Procedure

Prior to the experimental session, an interview was conducted 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 2-min 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 questionnaire with questions about the pictures. The results of the ratings and recognition questionnaire will not be reported here.

## Design and Statistical Analysis

The data for this study were taken from a larger experiment designed to examine different picture-viewing durations. For this larger study, a factorial design was used, with one betweengroups factor (Duration of Picture) and two repeated measures factors (Valence and Trial). Six independent groups were created that differed in picture viewing duration. All of the subjects viewed 11 target trials of each of three different categories of pictures (pleasant, neutral, and unpleasant).

For the present analyses, to investigate the effects of HRV on startle magnitude, Pearson correlations between indices of cardiac function and startle magnitudes were calculated. For the intertrial interval startle responses, the first trial, the average of the first three trials, and the average of all nine trials were examined. Consistent with previous research, the relationship was expected to be strongest in the early trials (Bradley et al., 1999). For the affective foreground startle responses, the average of all 11 startle trials was examined.<sup>4</sup> In addition, independent groups of participants were formed on the basis of their baseline logHF. To investigate the interaction of HRV on emotion-modulated startle magnitude a one between-group (HRV quartile) and one within-group (valence) analysis of variance (ANOVA) design was used.<sup>5</sup>

## Results

# Correlations between HRV and Startle Magnitude

Intertrial interval startle magnitude was significantly correlated with indices of neural cardiac control. Intertrial interval startle magnitude was positively correlated with mean HR and negatively correlated with MSD and logHF. This was especially true for the early presentations. Similar relationships were found between startle magnitude and cardiac indices for all three emotional foreground conditions (see Table 1).

#### Analysis of HRV Quartiles

As a manipulation check, ANOVA and follow-up tests on the mean HR, MSD, and logHF values indicated that each HRV quartile group differed significantly from each other group.

<sup>&</sup>lt;sup>3</sup>Respiration was measured in the present study via a mercury strain gauge. The correlation between strain gauge respiration frequency and the HFF index was .80, and the average difference between the measures was .3 breaths per minute in the present study. Thus the HFF measure was shown to be a reliable and accurate index of respiration frequency in the present study.

<sup>&</sup>lt;sup>4</sup>Preliminary analyses to examine the Trials effect grouped the 11 trials into groups comprised of the first four trials (early), the middle three trials (middle), and the last four trials (late). The Viewing Duration (supraliminal and subliminal) × Trials (early, middle, and late) × HRV Quartiles × Valence ANOVA revealed that Trials did not interact with any other factor. However, the expected main effect of Trials was evident, F(2,154) = 63.9, p < .001, epsilon = .81. Subsequent analyses were performed collapsing over Trials. These results are consistent with the well-replicated finding that the emotion-modulated startle effect is resistant to habituation (Bradley & Lang, 2000).

<sup>&</sup>lt;sup>5</sup>Preliminary analyses showed that the pattern of correlations was similar for both the supraliminal and the subliminal groups. When crossed with HRV quartiles, the Viewing Duration factor produced cell sizes that were too small for examination of the interaction of HRV Quartiles and Valence. We therefore grouped all of the supraliminal groups together and all of the subliminal groups together. Using supraliminal versus subliminal as a grouping variable we performed a 2 (Viewing Duration: supraliminal and subliminal)  $\times$  HRV quartile  $\times$ Valence (pleasant, neutral, and unpleasant) ANOVA. There was a significant main effect of valence and significant two-way interactions of HRV quartile × Valence, and Viewing Duration × Valence. Preplanned contrasts were performed to examine this latter interaction. For the supraliminal group, unpleasant startle magnitudes were significantly greater than both neutral, F(1, 83) = 6.6, p = .012, and pleasant, F(1,83) = 56.4, p < .001, trials. In addition, neutral startle magnitudes were greater than pleasant trials, F(1, 83) = 57.5, p < .001. For the subliminal group, unpleasant startle magnitudes were significantly greater than neutral trials, F(1, 83) = 6.2, p = .015. The pleasant startle magnitudes did not differ significantly from the neutral, F(1, 83) = 1.5, p = .22, or the unpleasant conditions, F(1, 83) = 1.7, p = .20. The cell sizes were too small to examine the HRV Quartiles × Valence interaction for the subliminal group alone. However, the results reported for the total sample were virtually identical to those found in the supraliminal group alone. Specifically, linear trends were found for both the highest and the lowest HRV quartiles, F(1, 51) = 24.6, p < .001 and F(1, 51) = 7.6, p = .008, respectively. However, whereas the highest HRV quartile failed to show a quadratic trend, the lowest HRV quartile showed a quadratic trend, F(1,51) = 10.2, p = .002, that was even larger than the linear effect. In addition, whereas the highest HRV quartile showed differences between each emotion condition, [pleasant versus neutral: F(1, 51) = 240.0, p = .001; neutral versus unpleasant: F(1, 51) = 6.1, p = .016], the lowest HRV quartile failed to show a difference between the neutral and the unpleasant pictures [pleasant versus neutral: F(1, 51) = 20.4, p < .001; neutral versus unpleasant: F(1, 51) = .03, p = .86]. This pattern of results is identical to those found in the total sample.

 Table 1. Correlations between Startle Magnitude and Cardiac Indices

	HR	MSD	LogHF
First startle	.236*	205*	311**
Avg. startles $1 - 2 - 3$	.252**	194*	227*
Avg. all 9 startles	.227*	172	162
Avg. all 11 pleasant trials	.222*	230*	235*
Avg. all 11 neutral trials	.256**	231*	234*
Avg. all 11 unpleasant trials	.184*	187*	147

\*p < .05, one-tailed; \*\*p < .01, one-tailed.

These results confirmed successful recruitment of a sample with a wide range of HRV values.<sup>6</sup>

There was a significant interaction between logHF quartiles and valence, Rao's R (6,160) = 2.47, p = .026. Preplanned simple effects tests examined this interaction for each of the quartiles. Significant linear trends and nonsignificant quadratic trends were found for each quartile except the group with the lowest logHF. For this latter group, significant linear and quadratic trends were found. Mean startle amplitudes for each quartile for each emotion condition are presented in Table 2.

Preplanned tests were done to examine these effects for the extreme groups. For the lowest quartile, there was a significant difference between startle magnitude for the pleasant and neutral conditions and for the pleasant and unpleasant conditions, but no significant difference between neutral and unpleasant conditions. For the highest quartile, significant differences were found between each emotion condition. The other two quartiles fell as expected between the extreme groups such that only the highest HRV quartile group showed significant differences between each emotion condition (see Table 2).

Additional tests indicated that whereas there was no difference in startle magnitude between the quartile with the lowest HRV and the highest HRV for the unpleasant condition, startle magnitudes for the lowest HRV quartile compared to the highest HRV quartile were significantly higher for the neutral condition, t(40) = 2.28, p < .05, and marginally higher for the pleasant condition, t(40) = 1.55, p < .10 (see Figure 1).

#### Discussion

The results of the present study supported the hypotheses and indicated that the magnitude of the intertrial interval acoustic startle response was inversely related to indices of HRV and positively associated with HR. This finding was also evident during each of the affective foregrounds. Importantly, those individuals in the highest quartile of HRV showed the expected startle potentiation to unpleasant pictures and the expected startle inhibition to the pleasant pictures compared to the neutral pictures whereas those in the lowest quartile failed to show this expected pattern. In addition, the persons in the lowest quartile compared to persons in the highest HRV quartile showed potentiated startle responses to the neutral and pleasant (marginally significant) pictures.

The manipulation check revealed that the groups differed significantly from each other on indices of vagally mediated HRV. Individuals in the lowest quartile of this unselected sample had values similar to individuals in our previous research with anxiety disorders. Lang and colleagues have recently reported that a general startle sensitivity may characterize individuals with high negative affect. Lang, Davis, and Ohman (2000) have speculated that these individuals may be especially vigilant and apprehensive in novel situations. They further suggest that enhanced startle may be associated with sustained anxious apprehension. It has been repeatedly shown that individuals with anxiety disorders and others high in anxiety have decreased HRV relative to nonanxious persons (see Friedman & Thayer, 1998, for a review). Furthermore, it has been shown that persons with generalized anxiety disorder failed to habituate to nonthreat stimuli (Thayer et al., 2000). These individuals fit the description proposed by Lang and colleagues showing high degrees of anxious apprehension and low levels of vagally mediated HRV. In addition, both animal and human studies have suggested that anxiety is associated with decreased prefrontal cortical activity (see Thayer & Friedman, 2002). In the present study, there was an inverse relationship between startle magnitude and vagally mediated HRV. In addition, compared to those persons with high HRV, individuals with low HRV show potentiated startle during neutral foregrounds and marginally significantly potentiated startle during pleasant foregrounds. These results suggest a general startle sensitivity in persons with low HRV and perhaps a preattentive bias toward vigilance and apprehension in novel situations. Similarly, the present results are consistent with recent conceptualizations that propose that the amygdala plays a role in the hypervigilance of anxiety disorders (Davis & Whalen, 2001) and that social phobics show exaggerated amygdala responses to neutral stimuli (Birbaumer et al., 1998). It is proposed that the general startle sensitivity observed by Lang and colleagues may



Figure 1. Mean startle amplitude as a function of baseline HRV and valence. Startle amplitudes are in microvolts.

<sup>&</sup>lt;sup>6</sup>The distribution of HRV scores across the different viewing conditions (groups) did not differ significantly. Thus the six different groups did not differ significantly on any of the HRV indices. Some authors have argued that measures of HRV need to be corrected for respiration, but recent research suggests that the uncorrected measures of HRV are at least as good an index of vagally mediated modulation of cardiac chronotropy as those corrected for respiration rate, tidal volume, or PCO<sub>2</sub> (Houtveen, Rietveld, & de Geus, 2002). In the present experiment, HFF was a nonsignificant covariate, F(1, 107) = .03, p = .865, in an analysis of covariance with HRV quartiles as the independent variable and logHF power as the dependent variable. Thus it is extremely unlikely that respiration influenced the findings of the present experiment.

Quartiles	HR (bpm)	MSD (ms)	LogHF (ms <sup>2</sup> /Hz)	Pleasant (mV)	Neutral (mV)	Unpleasant (mV)
1 (Low HRV)	90.40	22.80	2.17	17.48 <sup>a</sup>	20.61 <sup>b</sup>	20.01
	(13.18)	(5.75)	(0.33)	(14.05)	(14.13)	(14.04)
2	84.19	34.52	2.66	16.82 <sup>a</sup>	17.93 <sup>c</sup>	20.55
	(8.21)	(8.14)	(0.07)	(12.92)	(13.68)	(15.07)
3	76.38	42.14	2.91	17.14 <sup>a</sup>	18.54 <sup>6</sup>	19.38
	(9.35)	(6.62)	(0.10)	(10.93)	(11.16)	(11.66)
4 (High HRV)	70.09	82.38	3.48	15.36 <sup>a</sup>	17.50 <sup>6,c</sup>	20.68
	(9.13)	(32.37)	(0.27)	(13.15)	(11.85)	(14.40)

 Table 2. Means and Standard Deviations for Cardiovascular and Emotion-Modulated Startle

 Variables by Heart Rate Variability Quartiles

Significant within HRV quartile differences, p < .05: <sup>a</sup>pleasant versus unpleasant; <sup>b</sup>pleasant versus neutral; <sup>c</sup>unpleasant versus neutral.

better be understood as reflecting both decreased prefrontal cortical modulation of the amygdala and an associated decreased vagal autonomic nervous system modulation.

The pattern of startle responses in the lowest HRV group was also similar to the pattern reported by Patrick, Bradley, and Lang (1993) for incarcerated psychopaths. These results have been taken to suggest that psychopaths exhibit emotional dysregulation. The exact nature of the dysregulation in the Patrick et al. study cannot be discerned because absolute startle magnitude data were not presented. However, other researchers have reported low HRV in psychopaths (Beauchaine, 2001). The present results extend previous findings on the emotional Stroop (Johnsen et al., in press) and phasic HR responses (Thayer et al., 2000) indicating poor emotional and attentional regulation in persons with low HRV. Conversely, it has been suggested that vagally mediated HRV is positively associated with good emotional regulation (Porges, 1992; Thayer & Lane, 2000). In the present study, only the highest HRV group showed clear emotional differentiation of each foreground condition from each other. This high degree of emotional differentiation and complexity has been suggested as the hallmark of an adaptive, flexible, and healthy organism, and may reflect a highly specific modulation of brainstem appetitive and aversive circuits by the prefrontal cortex (Friedman & Thayer, 1998; Thayer & Friedman, 2002; Thayer & Lane, 2000).

The present results are also consistent with a recently proposed model of neurovisceral integration (Thaver & Lane, 2000). In this model, a network of neural structures associated with emotional, attentional, and autonomic regulation was identified. These structures have considerable overlap with the neural structures identified by Davis et al. (1997) with anxiety and fear, and implicated by Lang et al. (2000) as the neural basis of the enhanced startle found in individuals with persistent anxious apprehension. Numerous studies in both animals and humans suggest that the presence of an intact amygdala is associated with larger magnitude startle and with emotionmodulated startle (Aggleton & Young, 2000; Angrilli et al., 1996). A related structure, the bed nucleus of the stria terminalis, has been implicated in the general startle sensitivity associated with anxiety in rats and negative affect in humans (Bradley & Lang, 2000). Relatedly, Davidson (2000) has reported that medial prefrontal cortex activity is inversely associated with activity of the amygdala and is involved in the modulation of the startle response. It has recently been reported that medial prefrontal activity is positively correlated with vagally mediated HRV (Lane et al., 2001) and that inactivation by intracarotid sodium amytal of frontal cortex is associated with a disinhibition of subcortical sympathoexcitatory circuits associated with defensive behavior (Ahern et al., 2001). In addition, it has recently been reported that individuals with low vagally mediated HRV show enhanced cortisol reactivity to mild psychological challenge compared to individuals with high levels of vagally mediated HRV, thus further suggesting cortical modulation of subcortical defensive circuits (Johnsen, Hansen, Sollers, Murison, & Thayer, 2002). The present results provide further support for the notion that activity of the prefrontal cortex is inversely related to activity of structures associated with defensive behaviors and serves to modulate interactions with the environment (Thayer & Lane, 2000). These results also further suggest that HRV can be used to index activity in this network of neural structures associated with emotional regulation, and that this regulation may be preferentially lateralized to the right hemisphere, as both HRV modulation and affective startle modulation show evidence of right hemisphere lateralization.

The present study is not without its limitations. First, only female participants were studied. Females have been reported to have higher levels of vagally mediated HRV compared to males (Rossy & Thayer, 1998) and to show greater right hemisphere cortical modulation of vagally mediated HRV during pharmacological cerebral inactivation (Ahern et al., 2001). However, the affective modulation of the startle reflex has not proven to differ between males and females (Bradley et al., 1999). Whereas it is unlikely that these results are specific to females, replication with equal numbers of males and females is called for. Second, some of the picture viewing durations used in the present study were shorter than have previously been investigated. However, exclusion of those conditions did not alter the present findings. Moreover, if anything, inclusion of those conditions worked against the hypotheses. Finally, measures of individual differences among the participants such as trait anxiety or personality were not included. These measures would help to characterize potential psychological differences among the HRV quartile groups. Clearly, future research should include measures of the psychological status of the participants to more fully illuminate the psychological and physiological underpinnings of the observed effects.

In summary, the results of the present experiment provided evidence that HRV may modulate startle magnitude during both intertrial intervals and affective foregrounds. In addition, individuals with low HRV may be particularly likely to show signs of a general startle sensitivity and emotional dysregulation. These findings have important implications for the use of both the startle probe methodology and HRV in the study of emotion and for understanding emotional regulation and dysregulation.

## REFERENCES

- Aggleton, J. P., & Young, A. (2000). The enigma of the amygdala: On its contribution to human emotion. In R. D. Lane & L. Nadel (Eds.), *Cognitive neuroscience of emotion* (pp. 106–128). New York: Oxford University Press.
- Ahern, G. L., Labiner, D. M., Hutzler, R., Osburn, C., Talwar, D., Herring, A. M., Tackenberg, J. N., Weinand, M. E., & Oommen, K. J. (1994). Quantitative analysis of the EEG in the intracarotid amobarbital test: I. Amplitude analysis. *Electroencephalography & Clinical Neurophysiology*, 91, 21–32.
- Ahern, G. L., Sollers, J. J., Lane, R. D., Labiner, D. M., Herring, A. M., Weinand, M. E., Hutzler, R., & Thayer, J. F. (2001). Heart rate and heart rate variability changes in the intracarotid sodium amobarbital (ISA) test. *Epilepsia*, 42, 912–921.
- Allen, N. B., Trinder, J., & Brennan, C. (1999). Affective startle modulation in clinical depression: Preliminary findings. *Biological Psychiatry*, 46, 542–550.
- Angrilli, A., Mauri, A., Palomba, D., Flor, H., Birbaumer, N., Sartori, G., & di Paola, F. (1996). Startle reflex and emotion modulation impairment after a right amygdala lesion. *Brain*, 119, 1991–2000.
- Beauchaine, T. (2001). Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology*, 13, 183–214.
- Benarroch, E. E. (1993). The central autonomic network: Functional organization, dysfunction, and perspective. *Mayo Clinic Proceedings*, 68, 988–1001.
- Benarroch, E. E. (1997). The central autonomic network. In P. A. Low (Ed.), *Clinical autonomic disorders* (2nd ed., pp. 17–23). Philadelphia: Lippincott-Raven.
- Birbaumer, N., Grodd, W., Diedrich, O., Klose, U., Erb, M., Lotze, M., Schneider, F., Weiss, U., & Flor, H. (1998). FMRI reveals amygdala activation to human faces in social phobics. *NeuroReport*, 9, 1223– 1226.
- Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1999). Affect and the startle reflex. In M. E. Dawson, A. Schell, & A. Boehmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science* and clinical science (pp. 242–276). Stanford, CA: Stanford University Press.
- Bradley, M. M., & Lang, P. J. (2000). Measuring emotion: Behavior, feeling, and physiology. In R. D. Lane & L. Nadel (Eds.), *Cognitive neuroscience of emotion* (pp. 106–128). New York: Oxford University Press.
- Codispoti, M., Bradley, M. M., & Lang, P. J. (2001). Affective reactions to briefly presented pictures. *Psychophysiology*, 38, 474–478.
- Cook, E. W., III. (1994). VPM reference manual. Birmingham, Alabama: Author.
- Corr, P. J., Wilson, G. D., Fotiadou, M., Kumari, V., Gray, N. S., Checkley, S., & Gray, J. A. (1995). Personality and affective modulation of the startle reflex. *Personality and Individual Differences*, 19, 543–553.
- Davidson, R. J. (2000). The functional neuroanatomy of affective style. In R. D. Lane & L. Nadel (Eds.), *Cognitive neuroscience of emotion* (pp. 106–128). New York: Oxford University Press.
- Davidson, R. J. (2002). Anxiety and affective style: Role of prefrontal cortex and amygdala. *Biological Psychiatry*, 51, 68–80.
- Davis, M., Walker, D. L., & Lee, Y. (1997). Roles of the amygdala and bed nucleus of the stria teminalis in fear and anxiety measured with the acoustic startle reflex: Possible relevance to PTSD. Annals of the New York Academy of Sciences, 821, 305–331.
- Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilace and emotion. Molecular Psychiatry, 6, 13–34.
- Drevets, W. C. (1999). Prefrontal cortical-amygdalar metabolism in major depression. Annals of the New York Academy of Sciences, 877, 614–637.
- Friedman, B. H., & Thayer, J. F. (1998). Autonomic balance revisited: Panic anxiety and heart rate variability. *Journal of Psychosomatic Research*, 44, 133–151.

- Hariri, A. R., Bookheimer, S. Y., & Mazziotta, J. C. (2000). Modulating emotional responses: Effects of a neocortical network on the limbic system. *NeuroReport*, 11, 43–48.
- Hong, S. B., Kim, K. W., Seo, D. W., Kim, S. E., Na, D. G., & Byun, Y. S. (2000). Contralateral EEG slowing and amobarbital distribution in Wada test: An intracarotid SPECT study. *Epilepsia*, 41, 207–212.
- Houtveen, J. H., Rietveld, S., & de Geus, E. J. C. (2002). Contribution of tonic vagal modulation of heart rate, respiratory drive, respiratory depth, and respiratory frequency to RSA during mental stress and physical exercise. *Psychophysiology*, 39, 427–436.
- Jansen, D. M., & Frijda, N. H. (1994). Modulation of the acoustic startle response by film-induced fear and sexual arousal. *Psychophysiology*, 31, 565–571.
- Johnsen, B. H., Hansen, A. L., Sollers, J. J., III, Murison, R., & Thayer, J. F. (2002). Heart rate variability is inversely related to cortisol reactivity during cognitive stress [abstract]. *Psychosomatic Medicine*, 64, 289.
- Johnsen, B. H., Thayer, J. F., Laberg, J. C., Wormnes, B., Raadal, M., Skaret, E., Kvale, G., & Berg, E. (in press). Physiological and attentional characteristics of patients with fear of dental treatment. *Journal of Anxiety Disorders*.
- Lane, R. D., Reiman, E. M., Ahern, G. L., & Thayer, J. F. (2001). Activity in medial prefrontal cortex correlates with vagal component of heart rate variability during emotion. *Brain and Cognition*, 47, 97–100.
- Lang, P. J., Davis, M., & Ohman, A. (2000). Fear and anxiety: Animal models and human cognitive psychophysiology. *Journal of Affective Disorders*, 61, 137–159.
- Masterman, D. L., & Cummings, J. L. (1997). Frontal-subcortical circuits: The anatomical basis of executive, social and motivated behaviors. *Journal of Psychopharmacology*, 11, 107–114.
- Mayberg, H. S., Liotti, M., Brannan, S. K., McGinnis, S., Mahurin, R. K., Jerabek, P. A., Silva, J. A., Tekell, J. L., Martin, C. C., Lancaster, J. L., & Fox, P. T. (1999). Reciprocal limbic-cortical function and negative mood: Converging PET findings in depression and normal sadness. *American Journal of Psychiatry*, 156, 675–682.
- Miltner, W. (1994). Emotional qualities of odors and their influence on the startle reflex in humans. *Psychophysiology*, *31*, 107–110.
- Moltó, J., Montañés, S., Poy, R., Segarra, P., Pastor, M. C., Tormo, M. P., Ramírez, I., Hernández, M. A., Sánchez, M., Fernández, M. C., & Vila, J. (1999). Un nuevo método para el estudio experimental de las emociones: El Internacional Affective Picture System (IAPS). Adaptación española. *Revista de Psicología General y Aplicada*, 52, 55–87.
- Patrick, C. J., Bradley, M. M., & Lang, P. J. (1993). Emotion in the criminal psychopath: Startle reflex modification. *Journal of Abnormal Psychology*, 102, 82–92.
- Porges, S. W. (1992). Autonomic regulation and attention. In B. A. Campbell, H. Hayne, & R. Richardson (Eds.), Attention and information processing in infants and adults (pp. 201–223). Hilldale, NJ: Erlbaum.
- Roberts, A. C., & Wallis, J. D. (2000). Inhibitory control and affective processing in the prefrontal cortex: Neuropsychological studies in the common marmoset. *Cerebral Cortex*, 10, 252–262.
- Rossy, L. A., & Thayer, J. F. (1998). Fitness and gender-related differences in heart period variability. *Psychosomatic Medicine*, 60, 773–781.
- Saul, J. P. (1990). Beat-to-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *News in Physiological Science*, 5, 32– 37.
- Skinner, J. E. (1985). Regulation of cardiac vulnerability by the cerebral defense system. *Journal of the American College of Cardiology*, 5, 88B–94B.
- Spyer, K. M. (1989). Neural mechanisms involved in cardiovascular control during affective behavior. *Trends in Neuroscience*, 12, 506– 513.

- Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation*, 93, 1043–1065.
- Ter Horst, G. J. (1999). Central autonomic control of the heart, angina, and pathogenic mechanisms of post-myocardial infarction depression. *European Journal of Morphology*, 37, 257–266.
- Ter Horst, G. J., & Postema, F. (1997). Forebrain parasympathetic control of heart activity: Retrograde transneuronal viral labeling in rats. *American Journal of Physiology*, 273, H2926–H2930.
- Thayer, J. F., & Friedman, B. H. (2002). Stop that! Inhibition, sensitization and their neurovisceral concomitants. *Scandinavian Journal of Psychology*, 43, 123–130.
- Thayer, J. F., Friedman, B. H., Borkovec, T. D., Johnsen, B. H., & Molina, S. (2000). Phasic heart period reactions to cued threat and non-threat stimuli in generalized anxiety disorder. *Psychophysiology*, 37, 361–368.
- Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders*, 61, 201–216.

- Thayer, J. F., & Lane, R. D. (2002). Perseverative thinking and health: Neurovisceral concomitants. *Psychology and Health*, 17, 685–695.
- Thayer, J. F., Peaseley, C., & Muth, E. R. (1996). Estimation of respiratory frequency from autoregressive spectral analysis of heart period. *Biomedical Sciences Instrumentation*, 32, 93–99.
- Thayer, J. F., Sollers, J. J., III, Ruiz-Padial, E., & Vila, J. (2002). Estimating respiratory frequency from autoregressive spectral analysis of heart period. *IEEE Engineering in Medicine and Biology Magazine*, 21, 41–45.
- Uijtdehaage, S. H. J., & Thayer, J. F. (2000). Accentuated antagonism in the control of human heart rate. *Clinical Autonomic Research*, 10, 107–110.
- Verberne, A. J. M., & Owens, N. C. (1998). Cortical modulation of the cardiovascular system. *Progress in Neurobiology*, 54, 149–168.

(RECEIVED August 20, 2001; ACCEPTED September 25, 2002)