Biological Psychology 93 (2013) 334–341

Contents lists available at SciVerse ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

# Association between respiratory sinus arrhythmia and reductions in startle responding in three independent samples

Stephanie M. Gorka[a], Sarah Kate McGowan[a], Miranda L. Campbell[a], Brady D. Nelson[a], Casey Sarapas[a], Jeffrey R. Bishop[b], Stewart A. Shankman[a,*]

[a] University of Illinois at Chicago, Department of Psychology, 1007 West Harrison Street (M/C 285), Chicago, IL 60657, United States
[b] University of Illinois at Chicago, College of Pharmacy, Department of Pharmacy and Practice, Chicago, IL 60612, United States

ARTICLE INFO

*Article history:*
Received 22 May 2012
Accepted 11 March 2013
Available online 23 March 2013

*Keywords:*
Respiratory sinus arrhythmia
Habituation
Startle

ABSTRACT

Evidence suggests that respiratory sinus arrhythmia (RSA) may be an important indicator of physiological flexibility. However, few studies have examined the relation between RSA and defensive habituation, a process contingent on physiological flexibility. In three independent samples, habituation was defined as the time course of 9 startle responses. In Sample one and two, startle was recorded (1) while shock electrodes were attached to participants' and (2) before a threat-of-shock task. In Sample three, startle was recorded without these two components. In the first two samples, startle magnitude significantly decreased over time but in Sample three, startle only decreased at a trend level. Further, low RSA was associated with less of a reduction in startle magnitude over time within the first two samples, but was unrelated to startle reduction in the third. This suggests that low RSA is associated with less habituation to contextual anxiety, which may reflect difficulties regulating anxiety.

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## 1. Introduction

For the past several decades, researchers have been interested in understanding the psychophysiological mechanisms underlying habituation. Habituation is defined as the gradual decrease in physiological responding to a stimulus over time, and is considered to be an adaptive response style to an ongoing, non-threatening stimulus (Groves and Thompson, 1970; Herry et al., 2010; Rankin et al., 2009). Within the affective literature, there has been a particular emphasis on understanding deficits in habituating to aversive stimuli. A slower rate (or lack) of response reduction to a non-threatening stimulus has been considered to be maladaptive and an index of heightened vigilance (Oken et al., 2006), difficulty in flexibly responding to environmental challenges (Raskin, 1975; Siddle, 1991), and/or deficits in inhibitory learning (Craske et al., 2008).

According to Foa and Kozak (1986) classic "information model," in response to an aversive stimulus, neural structures that underlie fear learning are initially activated. Subsequently, information signaling that the aversive stimulus does not pose actual danger or threat is learned. Studies have suggested that this subsequent learning occurs through interactions within the medial prefrontal-amygdala circuit (Myers and Davis, 2007) as well as activation of the temporal lobe memory system and structures involved in conscious awareness (LeDoux, 1996). Although researchers are beginning to challenge aspects of Foa and Kozak's theory (Craske et al., 2008), it is thought that the result of habituation processes include diminished defensive responding, decreased scanning of the environment for threatening cues, and lower levels of anxiety.

Some empirical evidence indicates that individuals with anxiety disorders exhibit lower rates of habituation. For example, individuals with panic disorder have exhibited decreased habituation of startle responses and skin conductance in response to aversive auditory tones (Ludewig et al., 2002; Roth et al., 1990). Similarly, it has been demonstrated that individuals with posttraumatic stress disorder (PTSD) have less skin conductance habituation to trauma cues compared with individuals without PTSD (Rothbaum et al., 2001), and socially anxious individuals have evidenced less skin conductance and heart rate habituation while giving impromptu speeches compared with low-anxious controls (Eckman and Shean, 1997). Although these studies suggest that individuals with anxiety disorders indeed display habituation deficits, it is important to note that methodological and operational heterogeneity within the habituation literature has lead to some mixed findings (Hoenig et al., 2005; Ross et al., 1989).

Another physiological indictor associated with flexible responding to the environment is respiratory sinus arrhythmia (RSA). RSA is the rhythmic fluctuation of heart rate during the respiratory

* Corresponding author. Tel.: +1 312 355 3812.
*E-mail addresses:* sgorka2@uic.edu (S.M. Gorka), smcgow4@uic.edu (S.K. McGowan), mnelso26@uic.edu (M.L. Campbell), bnelso7@uic.edu (B.D. Nelson), csarap2@uic.edu (C. Sarapas), jbishop@uic.edu (J.R. Bishop), stewarts@uic.edu (S.A. Shankman).

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http://dx.doi.org/10.1016/j.biopsycho.2013.03.003

cycle and is considered a non-invasive measure of the extent to which the vagus nerve mediates parasympathetic influences on the heart (Porges, 1995, 1997, 2007). Importantly, RSA is often conceptualized as an individual difference factor that reflects one's ability to maintain homeostasis and adaptively respond to environmental demands (Porges, 1995, 2007; Thayer and Lane, 2000). Consistent with this conceptualization, low RSA has been shown to be associated with difficulty regulating emotional states and attention (Berntson et al., 1998; Demaree et al., 2004; Frazier et al., 2004; Porges et al., 1994; Weinstein and Quigley, 2006).

It is also important to note that a large body of evidence indicates that individuals with anxiety disorders exhibit low levels of RSA (see Friedman, 2007 for a review). Numerous studies have shown that individuals with PTSD, panic disorder, and generalized anxiety disorder all exhibit lower RSA during resting periods as well as during symptom provocation (e.g., trauma cues for PTSD; Blom et al., 2010; Cohen et al., 1998; Jovanovic et al., 2009; Thayer et al., 1996; Yeragani et al., 1993).

Given that the startle eyeblink response (i.e., the rapid contraction of the orbicularis oculi muscle below the eye) is a useful measure of aversive emotional states (Bradley et al., 1999; Lang, 1995), several studies have begun to explore the relation between individual differences in RSA and startle reactivity. For instance, Ruiz-Padial et al. (2003) demonstrated that low RSA is associated with greater startle reactivity during the viewing of affective pictures, and Melzig et al. (2009) found that low RSA was related to greater startle reactivity during threat-of-shock. Notably, however, these studies did not examine whether RSA was related to the pattern of startle responding over time. Instead, both of these prior studies (and much of the affective science literature at large) collapsed across startle responses to create an average startle reactivity measure. Therefore, it is currently unclear whether low RSA is associated with greater average reactivity, less habituation over time, or both. This distinction is noteworthy given that dysfunctional reactivity and responding over time may reflect different mechanisms.

In sum, extant research and theory would predict that low RSA would be associated with less habituation. However, to our knowledge, no study has specifically examined this question. The primary aim of the current study was to examine whether individual differences in RSA were associated with habituation, defined as the decrease in startle responding over time. To explore this question, we used data from three independent samples, during which participants' reflexive eyeblinks were measured in response to nine acoustic startle probes presented over the course of 2.5 min.

There were several important differences among the samples that allowed for a thorough analysis of the RSA-habituation association. The first two samples of participants had shock electrodes attached to their wrists and subjects were told that right after the 2.5 min period, they would partake in a threat-of-shock task. As these two factors likely increased the contextual anxiety of the startle assessment (Baas et al., 2002; Grillon and Ameli, 1998) and likely confounded data interpretation, we conducted a third experiment, with the aim of reducing contextual anxiety and isolating the relation between RSA and habituation to startle probes.

Additionally, although the protocols for the first two samples were almost identical, the second employed a different decibel level of the startle probe than the first sample. As the magnitude of startle response may vary as a function of decibel level (Blumenthal, 1988, 1996; Blumenthal et al., 2005), the second sample provided evidence as to the robustness of the findings from Sample 1. Lastly, in Sample 1, RSA was exclusively collected during the startle habituation task. Although one could argue that RSA may best be understood as interactions between autonomic regulation and environmental demands (Bertsch et al., 2012; Casadei et al., 1996), it is possible that RSA may have been confounded by the state effects of the task. As such, within Sample 2 and Sample 3, RSA was collected during a pre-task resting period and during the startle task. In sum, these important differences between sample methodologies were designed to elucidate the relation between RSA and habituation, with and without contextual anxiety.

## 2. Methods

### 2.1. Participants

Data for the first two samples came from two independent, larger studies examining factors associated with startle potentiation to predictable and unpredictable threat-of-shock (Nelson and Shankman, 2011; Sarapas et al., under review). Data for the third sample was collected exclusively to elucidate findings from Samples 1 and 2. All participants were undergraduates and data collection took place between 2007 and 2012 at a midsize urban university. Demographic information including age, sex, and ethnicity were collected from all participants. In Samples 2 and 3, psychiatric medication was an exclusion criterion as prior evidence has shown that many psychotropic medications affect startle modulation to threat (e.g., Grillon et al., 2006) and RSA (Licht et al., 2009). Medication use was not assessed in Sample 1. Eligibility for all three samples included right handedness and no history of head trauma. Each sample protocol was approved by the University Institutional Review Board and informed consent was obtained prior to participation. The *N*'s for the three samples were 69, 110, and 51, respectively. However, to ensure data quality, within Sample 1 three individuals were excluded for not producing at least 5 of 9 possible startle responses (final $N = 66$). Within Sample 2, two were excluded for missing resting RSA data, three for missing RSA data during the task, three for current psychotropic medication use, and eight for not producing at least 5 of 9 possible startle responses (final $N = 94$). Within Sample 3, two individuals were excluded due to technical equipment failure (final $N = 49$). Participant characteristics for the final three samples are presented in Table 1.

### 2.2. Procedure

The procedures were largely the same for Sample 1 and Sample 2. First, participants provided written informed consent and were told that they would receive electric shocks during a computerized task later in the laboratory visit. Next, shock electrodes were placed on the participants' left wrist and they were seated in an electrically shielded, sound-attenuated booth where they completed the startle task. Participants were told that during this task, they would only hear loud tones through headphones and not receive any shocks. They were directed to sit still and look at the computer monitor in front of them, which displayed a fixation cross. Participants were not told when the task would end or how many startle probes they would hear. Over the course of 2.5 min, participants were administered 9 acoustic startle probes with probe-to-probe intervals of 15–20 s (average time between startle probes = 17.22 s [$SD = 2.22$]). Eyeblink reflexes in response to each startle probe were recorded. Electrocardiogram (ECG) data was simultaneously collected throughout the task.

Given that the designs of Sample 1 and Sample 2 likely elicited contextual anxiety, Sample 3 was designed to examine the relation between RSA and habituation in the absence of (or at least reduced) contextual anxiety. To this end, Sample 3 did not include a shock task (and thus, participants were not informed of an upcoming shock task) and shock electrodes were not attached participants' wrist during data collection. Sample 3 participants did, however, complete the exact same startle task as the prior two samples (e.g., same probe-to-probe intervals, etc.). The task instructions provided to participants was also the same as the prior two samples.

**Table 1**
Demographics and descriptive statistics for each sample.

| | Sample 1 ($N = 66$) | Sample 2 ($N = 94$) | Sample 3 ($N = 49$) |
|---|---|---|---|
| % Female | $76.9\%_a$ | $59.6\%_b$ | $77.6\%_a$ |
| Age (*SD*) | $19.83\ (2.76)_a$ | $19.39\ (2.25)_{a,b}$ | $18.82\ (1.41)_b$ |
| Task RSA (*SD*) | $6.44\ (1.38)_a$ | $6.91\ (1.01)_b$ | $6.74\ (1.34)_{a,b}$ |
| Rest RSA (*SD*) | – | $6.66\ (1.05)_a$ | $6.93\ (1.46)_a$ |
| Ethnicity | | | |
| African American | $12.3\%_a$ | $8.5\%_a$ | $12.2\%_a$ |
| Caucasian | $41.5\%_a$ | $34.0\%_a$ | $26.5\%_a$ |
| Asian | $23.1\%_a$ | $21.3\%_a$ | $22.4\%_a$ |
| Hispanic/Latino | $15.4\%_a$ | $27.7\%_a$ | $30.6\%_a$ |
| Other | $7.7\%_a$ | $8.5\%_a$ | $6.1\%_a$ |

*Note.* Means or percentages with different subscripts across rows were significantly different in pairwise comparisons ($p < .05$, chi-square test for categorical variables and Tukey's honestly significant difference test for continuous variables). *SD* = standard deviation; RSA = respiratory sinus arrhythmia; Task RSA = RSA during the startle task; Rest RSA = RSA collected at rest prior to the startle task.

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